EPIDEMIOLOGY, PATHOGENESIS AND MANAGEMENT
OF ENDOMYOCARDIAL FIBROSIS

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Abstract

This thesis investigates Endomyocardial Fibrosis (EMF), the commonest form of restrictive cardiomyopathy affecting children and young adults from poor developing countries. EMF is the second cause of admission for acquired cardiovascular disease in these age groups in endemic areas and has an unclear etiology and pathogenesis. It has no specific treatment and carries a poor prognosis.

The methodology followed for this investigation was the implementation of large-scale epidemiological studies on incidence, prevalence and determinants of EMF in an endemic area of Mozambique, using echocardiography. New classification and scoring system for diagnosing and assessing EMF severity was evolved and applied in community and hospital-based studies. Concomitantly, characterization of the clinical and biological profile of EMF in its different stages was done, as well as investigation of the genetic susceptibility to the disease. Finally, new surgical procedures based on the specific pathophysiology were developed and applied, with attempts at optimizing the timing of surgery and its results.

This work has established that echocardiographic screening is able to detect early and asymptomatic stages of EMF, and that it is a useful tool in assessing its progression, identifying patients that can benefit from surgery and monitoring response to treatment. It also shows that novel surgical approaches based in tailored techniques to the specific components of the disease can improve the outcome. The use of the standardized criteria in prospective studies may improve knowledge of the natural history and pathogenesis of EMF allowing better management of EMF and identification of new therapeutic targets.
Declaration of Originality

The contents of this thesis are original material except where the material has been recreated from referenced sources.

This thesis is solely my own work, except where indicated in the case of laboratorial work done in collaboration with others. I carried out all epidemiological and clinical work, and have enjoyed the assistance of collaborators, for some of the laboratorial work namely the characterization of eosinophils (Dr Patricia Silva), tissue immunostaining (Dr Padmini Sarathchandra, Dr Lorraine Lawrence), Western Blotting for antiheart antibodies (Dr Najma Latif), Multiplex tests for cytokine profile (Dr John Smith), ELISA tests for measurement of eotaxin levels (Dr Gill Martin and Dr Dolores Conroy), ELISA tests for prothrombotic factors (Mrs Kiran Parmar), HLA typing (Mr Peter Bolton and Dr Juliette Brown), and immunofluorescence IT-04 tests (Dr Gabi Margos). Professor Sir Magdi Yacoub carried out the surgical procedures of all EMF patients operated during the research programme.
Acknowledgements

Mozambique, the country where I come from, is among the poorest in the world. Studying at Imperial College and being able to research into a subject that is so important for my own country was for me a unique privilege. I feel humbled and extremely fortunate to have been given this opportunity, and would like to thank the Harefield Research Foundation and the Magdi Yacoub Institute for funding this research.

I am deeply grateful to my supervisor Professor Sir Magdi Yacoub for his inspiration, enthusiasm and constant guidance throughout the duration of my studies, and for giving me the freedom and support to develop my own ideas. I also acknowledge Professor Daniel Sidi who advised and encouraged me to embrace this fascinating subject. Special thanks are due to my mentor Professor Sir Leszek Borysiewicz, and supervisors Professor Timothy Williams and Dr Adrian Chester for their continuous support over these years.

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<tbody>
<tr>
<td>A wave</td>
<td>second wave of the diastolic mitral flow</td>
</tr>
<tr>
<td>AB</td>
<td>alcian blue</td>
</tr>
<tr>
<td>AB-SR</td>
<td>alcian blue-sirius red</td>
</tr>
<tr>
<td>BEMF</td>
<td>bilateral endomyocardial fibrosis</td>
</tr>
<tr>
<td>BSA</td>
<td>body surface area</td>
</tr>
<tr>
<td>CD</td>
<td>cluster of differentiation</td>
</tr>
<tr>
<td>Ch2R</td>
<td>carbol chromatotrope technique</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>CT</td>
<td>Computed Tomography</td>
</tr>
<tr>
<td>EDD</td>
<td>end-diastolic dimension of the left ventricle on M-mode</td>
</tr>
<tr>
<td>EDTA</td>
<td>ethylenediaminetetraacetic acid</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>EMF</td>
<td>endomyocardial fibrosis</td>
</tr>
<tr>
<td>EOS</td>
<td>eosinophils</td>
</tr>
<tr>
<td>EOT</td>
<td>eotaxin</td>
</tr>
<tr>
<td>ESD</td>
<td>end-systolic dimension of the left ventricle on M-mode</td>
</tr>
<tr>
<td>ESR</td>
<td>erythrosedimentation rate</td>
</tr>
<tr>
<td>EVG</td>
<td>elastin van Gieson</td>
</tr>
<tr>
<td>E wave</td>
<td>Initial wave of the diastolic mitral flow</td>
</tr>
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>H&amp;E</td>
<td>Hematoxillin and Eosin</td>
</tr>
<tr>
<td>HES</td>
<td>hypereosinophilic syndrome</td>
</tr>
<tr>
<td>HIV</td>
<td>human immunodeficiency virus</td>
</tr>
<tr>
<td>HLA</td>
<td>Histocompatibility Leukocyte Antigens</td>
</tr>
<tr>
<td>HuEot</td>
<td>Human eotaxin</td>
</tr>
<tr>
<td>ICAM</td>
<td>intercellular adhesion molecule</td>
</tr>
<tr>
<td>Ig</td>
<td>immunoglobulin</td>
</tr>
<tr>
<td>IL</td>
<td>Interleukin</td>
</tr>
<tr>
<td>KD</td>
<td>kilodalton</td>
</tr>
<tr>
<td>LA</td>
<td>left atrium</td>
</tr>
<tr>
<td>LDS</td>
<td>laser detection system</td>
</tr>
<tr>
<td>LEMF</td>
<td>left-sided endomyocardial fibrosis</td>
</tr>
<tr>
<td>LL</td>
<td>lateral-lateral</td>
</tr>
<tr>
<td>LV</td>
<td>left ventricle</td>
</tr>
<tr>
<td>MAB</td>
<td>monoclocal antibody</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic Resonance Imaging</td>
</tr>
<tr>
<td>MT</td>
<td>Masson Trichrome</td>
</tr>
<tr>
<td>NYHA</td>
<td>New York Heart Association</td>
</tr>
<tr>
<td>PAI</td>
<td>Plasminogen activator inhibitor</td>
</tr>
<tr>
<td>PBS</td>
<td>Phosphate buffer solution</td>
</tr>
<tr>
<td>RA</td>
<td>right atrium</td>
</tr>
<tr>
<td>RV</td>
<td>right ventricle</td>
</tr>
<tr>
<td>REMF</td>
<td>right-sided endomyocardial fibrosis</td>
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RHD          rheumatic heart disease
SDS-PAGE     sodium dodecylsulphate polyacrylamide gel electrophoresis
SF           shortening fraction, calculated by the formula (EDD-ESD)/EDD
SI           supero-inferior
SMA          smooth muscle actine
SR           Sirius Red
sTF          soluble tissue factor
TGF          Transforming Growth Factor
TF           Tissue factor
VEGF         vascular endothelial growth factor
VG           van Gieson
WB           Western Blot
Chapter 1

INTRODUCTION AND RESEARCH AIMS
1.1. Background

Summary

Endomyocardial Fibrosis (EMF) is a progressive disease that, in its established stage, is characterized by fibrosis in the ventricular cavities affecting particularly the apex and the subvalvar regions (Davies, 1960). This definition of the disease, based on the knowledge about its clinical and pathology findings, applies entirely to the advanced phases of this condition, and allows easy clinical and echocardiographic diagnosis in the presence of heart failure, mainly in patients with predominant right ventricular disease. In contrast, there is lack of diagnostic criteria for early stages of EMF.

EMF is a restrictive cardiomyopathy of unclear etiology and pathogenesis affecting mainly children and adolescents from tropical regions of Africa, South America and Asia (Bukhman et al, 2008). Its pathophysiology associates restriction to diastolic filling caused by endocardial fibrosis, with atrioventricular valve regurgitation that results from involvement of the leaflets and the subvalvar apparatus in the fibrotic process.

There is no specific treatment for Endomyocardial Fibrosis. Advanced forms of EMF carry high morbidity and mortality, with death occurring due to progression to chronic heart failure or as a result of arrhythmia and thromboembolism. Surgery can correct some structural and functional abnormalities in patients with EMF, but has been associated with high morbidity and mortality (Valiathan et al, 1987), as well as uncertain long-term outcome.
1.1.1. History of EMF

Arthur Williams (1938) reported for the first time two hearts with large endocardial patches of fibrosis in Uganda. The same findings were described some years later by other authors (Bedford, 1946), but Jack Davies (1948) was the first to describe EMF as a distinct pathological entity and the correlation between the pathology features and the clinical signs (Davies, 1960), allowing the diagnosis of endomyocardial fibrosis in life.

Several designations have been used to describe EMF namely tropical endomyocardial disease, endocarditis parietalis fibroplastica, endocardial fibrosis, constrictive endocarditis, endocardial fibroelastose and Davies disease. The expression “Heart of Africa” was also used to designate this entity due to the similarity of the heart shape in right ventricular EMF with retraction of the apical region to the map of Africa, and because the entity occurred frequently in Uganda, a country located in a central position in Africa. However, the term “Endomyocardial Fibrosis” is the one that best describes the abnormalities found in this condition, and has been adopted worldwide. In 1965 the World Health Organization classified EMF as a restrictive cardiomyopathy of unclear etiology (Hutt et al, 1965).

Research on EMF has been largely concentrated in description of clinical features, characterisation pathology changes and results of surgical management. Most publications came from Uganda, Nigeria, Ivory Coast, India and Brazil, but there have been reports of cases outside the tropics in all regions of the world. Few studies have looked at mechanisms of the disease and the various etiological hypotheses have been incompletely investigated.
1.1.2. Epidemiology

The majority of literature available describes the advanced stages of EMF seen in hospital. Data on incidence, rate of progression and prevalence are scarce.

EMF is thought to be the most widely prevalent form of restrictive cardiomyopathy (Somers, 1990). In endemic areas of Africa, EMF is as common a cause of cardiac failure as rheumatic heart disease and accounts for 25% of all cardiac necropsies (Somers, 1989). It has also been stated that undiagnosed EMF may also be common in populations living in areas from where patients have been reported (Mayosi, 2007).

EMF is very prevalent near the Equator (Figure 1.1) and high prevalence has been reported from several tropical countries, namely: Uganda (Davies, 1948; Connor et al., 1967; Shaper, 1967; Shaper, 1968), Kenya (Somers, 1989), Zambia (Lowenthal, 1978), Nigeria (Abrahams, 1962; Ikeme, 1972; Falase et al., 1976; Andy et al., 1981; Jaiyesimi, 1982), Ghana and Ivory Coast (Dienot, 1981; Bertrand, 1993) in Africa; Brazil, Colombia, Venezuela and Mexico in South America; and India (Cherian et al., 1982; Vijayaraghavan et al., 1983; Valiathan et al., 1987). More recently, cases have been reported from Zimbabwe (Hakim et al., 1996), Mozambique (Bijlsma, 1979; Ferreira et al., 1992; Ferreira et al., 2002), Egypt (Rashwan et al., 1995), Guine Equatorial (Perez et al., 2005), South Africa (Beck and Schirire, 1972), Saudi Arabia (Graham et al., 1981), USA (Eterovic et al., 1979; Gonzalez-Lavin et al., 1982), Korea (Yie et al., 2003), England (Wiseman et al., 1986), Spain (Estornell et al., 2003), Switzerland (Schneider et al., 1998), Japan (Niino et al., 2001) and China (Yin, 2000).
Variations in distribution of EMF have been noted in different countries where the disease has been reported to be endemic (Kartha, 1995). In India the prevalence of EMF is highest in the coastal areas of Kerala (Kutty et al, 1996) with very few cases reported from the northern areas of the country. In Nigeria, it is endemic in the South-West but rare in the hot and dry north (Parry, 1964; Ijaola and Falase, 1988). Several studies from Uganda reported preponderance of the condition in patients from Rwanda and Burundi that immigrate to the South-West (Connor et al, 1967; Shaper 1972). More recently, in Mozambique, an analysis of referrals to a cardiovascular unit over a 10-year period showed a striking high attack rate in Inharrime, a rural coastal area in the south of Mozambique (Figure 1.2) (Ferreira et al, 2002).

Figure 1.1. World map showing endemic areas for EMF worldwide (Buckman, 2008)
Figure 1.2. Map of Mozambique in the south coast of Africa

It is not clear whether the reported variations in prevalence and incidence of EMF are related to environmental or genetic factors (Mayosi and Somers, 2007). Several environmental factors have been implicated in the origin of the disease, namely geography (Mayanga-Kizza et al, 2000; Kutty et al, 1996), social deprivation (Rutakingirwa et al, 1999), infectious diseases (Shaper et al, 1968) and diet (Ojo, 1970; McKinney, 1975; Jaiyesimi, 1980; Sezi, 1996a; Sezi 1996b). Ethnicity is also thought to play a role in pathogenesis, since in Uganda it affects almost exclusively individuals from
certain ethnics groups of immigrants from Rwanda and Burundi (Somers, 1973). Familial occurrence of the disease has also been noted in some clinical series (Patel et al., 1971; Lowenthal et al., 1978).

While EMF may be found in any age group, hospital-based series show that it affects predominantly children and adolescents (Jaiyesimi, 1982; Somers, 1990), with a peak incidence at the ages 11-15 years in both sexes (Somers, 1968). In most series women show a second peak between 26 and 30 years (Rutakinguirwa et al., 1999). Although most patients are under 15 at the time of first presentation, there are reports of the disease in infants (Bijlsma, 1976; Jatene et al., 2003).

There is no clear gender preponderance in prevalence of EMF. Whereas in Uganda there seems to be female preponderance with a female: male ratio of 2:1 (Connor et al., 1967; Shaper, 1972), in Nigeria no sex difference was found (Parry, 1964), and in some countries a male preponderance has been noted (Brockington and Edington, 1972; Bijlsma, 1976).

The only epidemiological survey performed in Africa aiming at determining the prevalence of EMF was performed in the Inharrime district of Mozambique (Ferreira, 2001). In this study 948 inhabitants between 4 and 45 years had cardiac auscultation and those with abnormal findings were submitted to echocardiography. A prevalence of 8.9% was found, suggesting that EMF was a major public health problem in this rural area.
1.1.3. Natural History

EMF appears to evolve in three successive stages after an unknown stimulus or trigger. Initial endocardial necrosis would be followed by thrombosis, and later fibrosis would develop in the area adjacent to the thrombus leading to the characteristic lesions found in advanced disease (Brockington and Olsen, 1973; Olsen, 1990) (Diagram 1.1). This sequence of events has been difficult to prove for several reasons: (1) initial and acute EMF are rarely reported in hospital-based studies; (2) research has been based in clinical series, which included only patients with advanced disease; (3) studies on the natural history of EMF have been done retrospectively based on follow-up of patients from clinical series; (4) most research on this issue took place before the advent of echocardiography making it difficult to document and monitor progression of intracardiac lesions in vivo.

The mode and rate of progression of EMF to advanced forms is variable (Parry, 1965; Andy 1998) (Diagram 1.2). After the initial insult most patients have a rapidly progressing heart failure that leads to death within 2 years (Sliwa et al, 2005). Some patients, however, have a history of slowly progressive chronic heart failure with several admissions to hospital or have a steady period without any deterioration after an initial febrile episode with or without heart failure (Somers, 1972b).

The reported mean survival time after the onset of symptoms is two years, but isolated or predominant LEMF determines earlier fatality due to complications of pulmonary hypertension and systemic thromboembolism (Somers 1978). Death results usually from
complications of progressive chronic heart failure (protein-losing enteropathy, cardiac cirrhosis, hepatic failure), but can sometimes be sudden due to pulmonary embolism or arrhythmias (Diagram 1.3).

Diagram 1.1. The natural history of EMF (as described by Olsen)
Chapter 1 Introduction and Aims

Triggers

↓

Subclinical illness ← Initial illness → “Burnt out case”
(Systemic illness) No residual heart disease

Pancarditis, eosinophilia

↓

Thrombotic Phase

↓

Restrictive Cardiomyopathy
due to Endomyocardial Fibrosis

↓

Death

Diagram 1.2. Natural history and progression of EMF (by Parry, Andy and Jaiyesimi)
Poverty

↓

**Low protein cassava diet**  →  Stunted body growth

↓

Low plasma aminoacid profiles due to overuse for growth and pregnancies

↓

Plasma aminoacid profiles below critical levels

Most prominent at the apices due to poor apical endomyocardial supply and to maximal mechanical stress and sluggish flow at the apices

↓

*Failure of cardiac cell repair*

(because the endocardium receives a poorer blood supply from end arteries)

↓

*Endomyocardial degeneration*

(associated with cerium and cassava)

↓

*Endomyocardial Fibrosis*

↓

Mural thrombosis and Thromboembolism

**Diagram 1.3.** Proposed pathophysiology of endomyocardial fibrosis (adapted from Sezi)
1.1.4. Etiology and Pathogenesis

The cause of EMF is unknown and the mechanisms involved in its pathogenesis are incompletely understood. None of the hypotheses studied - including geochemical actors, infectious agents, dietary factors, hypersensitivity, autoimmunity, hypereosinophilia, genetic susceptibility and ethnicity - can explain the occurrence of EMF worldwide. Overall, there seems to be a preponderance of environmental factors playing a role in the pathogenesis of EMF (Mayosi and Somers, 2007).

Hypereosinophilia

Eosinophils have been considered an etiological factor in the pathogenesis of EMF (Andy, 1998; Patel et al, 1977) and some authors consider the condition to be a variant of the Hypereosinophilic Syndrome (HES) seen in temperate climate, owing to the fact that the late fibrotic lesions in both condition are identical (Brockingham and Olsen, 1973). Eosinophilia from 10 to 30% of the total WBC counts, presenting sometimes over several months or even years, is a frequent finding in EMF (Somers, 1972b) and its level seems to be inversely related to the duration of the illness (Andy, 1998).

Hypereosinophilia could probably be induced by parasitic infections by helminths (Andy, 1998), schistosomiasis (Rashwan et al, 1995), microfilaria loa-loa (Beck and Schrire, 1972), or filariasis (Ive, 1967), but this theory is difficult to establish because eosinophil count peaks during larval migration returning to normal thereafter. On the other hand several studies failed to show an increased prevalence of these infections in EMF patients.
when compared to the general population (Brockington, 1974; Connor et al, 1967; Carlisle et al, 1972; Brockington and Olsen, 1973). In the Ugandan experience eosinophilia did not affect patients with EMF any more than the population at large (Patel et al, 1977), nor has eosinophilia been recognized in the thrombotic and fibrotic endocardial lesions studied in biopsy (Somers, 1971) and necropsy specimens (Connor et al, 1968). Another study showed no difference between the mean eosinophil counts in patients with EMF and controls from the general population; degranulated eosinophils were absent in the blood and bone marrow aspirates of the patients; the mean concentrations of eosinophil granule basic proteins were not different in the two groups (Urhoghide and Falase, 1987). However, some authors argue that these studies were done in patients with advanced disease and, assuming that cardiac damage in EMF is triggered by parasite induced eosinophilia, a raised eosinophil count need not to be present in chronic disease (Jaiyesimi, 1982; Andy et al, 1981). If parasite-induced eosinophilia is related to cardiac damage, the eosinophilia in EMF should be sought in early disease. Although few studies looked at this issue, one suggested that there is a highly significant inverse relationship between hypereosinophilia and the duration of symptoms in EMF patients (Andy, 1998).

The fibrotic lesions characteristic of EMF, considering this hypothesis of hypereosinophilia as the pathogenic mechanism, would represent the end result of an acute eosinophilic endocardial injury. The cardiotoxicity of the eosinophil has long been demonstrated (Spry et al, 1983). Human eosinophil granule basic proteins have been shown to injure heart cells in rats, and eosinophils in patients with an eosinophilia are metabolically and functionally more effective than normal. Why is the endocardium so
susceptible to eosinophil dependent injury and what determines the rate at which endocardial disease progresses to the late fibrotic stages is not known.

The possibility of hypereosinophilia being an independent risk for EMF not attributable to parasitism was also postulated (Rutakingirwa et al, 1999).

**Infectious agents**

Several infectious agents have been studied in an attempt to establish the etiology and pathogenesis of EMF. The role of infectious agents appears plausible in view of climatic restrictions of the disease and of the reports of sporadic cases in foreigners after short visits to endemic areas (Brockington et al, 1967; Beck and Schrire, 1972). Plasmodium species (Shaper et al, 1968), Schistosoma (Barbosa et al, 1998; Victor et al, 1996), Microfilaria (Berenguer et al, 2003), Helminths (Andy, 1998), Toxoplasma (Ludman and Somers, 1966) and Coxsackie B virus (Ijaola and Falase, 1990), have been implicated as possible causes or triggers for disease, but none has been proven to cause EMF.

In Africa EMF is endemic where malaria is also prevalent (Sliwa et al, 2005). The finding of endomyocardial fibrotic lesions mimicking human disease in mice infected with *Plasmodium berghei* (Eling et al, 1988) supports the hypothesis of malaria being involved in the pathogenesis of this condition, and provides one of the best models of the disease. This theory could also be reinforced by reports of increased eosinophil activity in acute *Plasmodium falciparum* infection (Kurtzhals et al, 1998; Shanks et al, 1992).
Autoimmunity

The role of autoimmunity has been postulated to be of importance in EMF (Van der Geld et al, 1966; Shaper et al, 1968) but this remains to be firmly established. An immunological syndrome consisting of high titres of anti-malarial antibody associated with the presence of IgM and circulating autoantibodies to the heart has been identified in populations with high prevalence of EMF (Shaper et al, 1968; Jayesimi et al, 1984). Moreover, EMF patients have been shown to have high prevalence of antimalarial and antiheart antibodies (Van der Geld et al, 1966) that seem to correlate (Shaper 1974). Whether these antibodies are the cause or the result of EMF is not clear, but these findings led to the hypothesis that the presence of tissue autoantibodies associated with an unusual response to malarial infection may play a role in the pathogenesis of EMF (Shaper et al, 1967). Pathological studies however failed to confirm the existence of large numbers of immunologically competent cells at the endomyocardial junction (Connor et al, 1968) and antiheart antibodies (IgG, IgM and IgA) were not detected in Indian patients with angiographic diagnosis of EMF and matched voluntary blood donors controls, when analysed by indirect immunoflorescent technique (Kartha et al, 1984; Vijayaraghavan and Sadanandan, 1984).

Geochemical factors

The equatorial distribution of EMF mentioned previously stimulated several theories for the causation of the disease. One of the main factors that characterize the equatorial regions is the intensity of the ultraviolet radiation. Since plants can synthesize vitamin D
on exposure to sunlight, because they contain pro-vitamin D2 (ergosterol), it was thought that the practice, common to poorer people in the tropics, of drying staple foods in the sun would increase the levels of vitamin D in plants (particularly roots of cassava), leading to increase levels of calcium in people eating these plants (Davies, 1990). Vitamin D-induced calcinosis would be the basis for cellular hyperplasia and excessive production of collagen and elastin in the endomyocardium, and would explain the fibrosis and calcification characteristic of this entity. Initial research on this issue showed that EMF patients in Kerala were found to have high blood levels of calcium (Valiathan et al., 1986), but no further studies confirmed this hypothesis.

Children have high needs of Mg for growth and development. It is thought that poor children from tropical regions are prone to develop magnesium deficiency because of insufficient intake, or chronic diarrhoea due to malnutrition. In Kerala (Valiathan et al., 1993) it was shown that the deficiency of magnesium promotes the absorption of cerium and enhances its toxicity, which could form the basis for the initial injury of the heart in EMF. Interestingly, the cardiac tissue of EMF patients showed high levels of cerium (a major constituent of monazite, which is found in the soil of tropical regions in high concentrations) and low concentrations of magnesium. Both, hypomagnesemia and EMF are widely prevalent in children from low-income groups in the equatorial regions of India (Eapen et al., 1993), Africa (Cadell 1967) and Brazil (Cerci Neto, 2006).
Chapter 1 Introduction and Aims

Dietary factors

Because EMF affects mainly poor people from deprived rural areas, initial studies on pathogenesis concentrated on the role of nutritional deficiencies with deceiving results (Falase, 1983). Despite the high prevalence of malnutrition in some areas of the world, there is no correlation between the prevalence of the two diseases, since nutritional deficiencies neither explain the confinement of EMF to certain areas of these low-income countries, nor the differences among various ethnic groups. Furthermore, EMF has been found in well-nourished Caucasians resident in tropical Africa (Brockington, 1967; Beck and Schrire, 1972).

Vitamin E deficiency also produces cardiac enlargement and fibrosis similar to EMF in malnourished animals (Lee et al, 1960; Ferrans, 1985). This is due to auto-oxidation of unsaturated lipids with deposition of ceroid bodies in the tissues, that later become fibrosed leading to endocardial fibrosis when the heart is affected. However, measurement of blood levels of vitamin E was normal in EMF patients (Jaiyesimi, 1978). The fact that tropical EMF occurs near the equator and afflicts relatively poor people whose staple diet is tuberous (roots of cassava) has led to the hypothesis that such a diet, rich in vitamin D, could be the critical background to EMF (Davies, 1990). However, in countries like Mozambique, both endemic and non-endemic areas for EMF have cassava dried through sunlight as the basis of their diet (Figures 3 and 4).
Plantains and bananas which contain high levels of serotonin (5-hydroxytryptamine) are consumed in large quantities in some communities from India where EMF is common. Because excess of serotonin in the blood stream produces a cardiac lesion which some authors felt was similar to EMF (Ball, 1957), it was suggested that the disease could result from excessive ingestion of plantains. However, there is no correlation between the geographical distribution of EMF and the plantain-eating habits of the African population.

**Figure 1.3.** Cassava plants (left) and their roots (right), which are usually dried at sunlight, constitute the basic food in this area

In summary, all these association studies searching for factors potentially involved in the etiology of EMF did not prove any of the hypotheses.
Experimental models

Both animal models and in vitro studies were performed aiming to clarify the role of several factors in the etiopathogenesis of EMF.

a. Animal models

In a study that attempted at developing an animal model for EMF (Kartha and Gupta, 1993) different degrees of magnesium deficiency were induced in rats through dietary means. The effects of cerium supplementation in the same animals were explored through autopsy and measurement of the levels of the two elements in the tissues. The results included myocardial lesions consisting of interstitial haemorrhages or myocarditis, and
increase in calcium levels in the heart tissue while there was a decrease of calcium levels in bones. Cerium appeared to intensify the cardiac histologic lesions in the presence of magnesium deficiency. Although unsuccessful in creating the animal model, this experiment has provided supportive evidence for a hypothesis on the possible connection of EMF with myocardial levels of cerium and magnesium.

Another experiment was performed to test the effects of protein deficient cassava diet in African green monkeys *Cercopithecus aethiops* (Sezi, 1996a). Three monkeys were fed on uncooked banana diet while other three were fed on uncooked cassava. The hearts were harvested for histology whenever the animal health deteriorated. Later five other monkeys were fed on cassava for longer periods. The histological findings in monkeys fed on cassava included some features of EMF namely endocardial thickening, interstitial fibrosis, fibrous septa formation, papillary muscle fibrosis and apical fibrosis in the left ventricle. Animal on banana diet, which also lacked protein, did not develop similar lesions. It was concluded that EMF could be experimentally induced in the monkey and that protein deficient cassava diet could be one of the causes of the disease.

The role of malaria in the pathogenesis of EMF was also explored through animal experiments. C57B1/Rij mice infected with *Plasmodium berghei* developed progressive endocardial edema and extensive endocardial thrombosis with a predilection for the right half of the heart (Eling *et al*, 1984). Mural thrombus grew out until death of the host or until chemotherapy cleared the infection, in which case there was evolution to fibrosis of the affected areas.
b. *In vitro* studies

The increased production of collagen that is characteristic of EMF can be due to hyperfunction of individual cells or to increase in number of cells. An interesting study using non-myocardial rat heart cells and human cardiac explants evaluated the influence of high levels of cerium and low levels of magnesium on heart cell proliferation in vitro (Nair *et al.*, 1993) and suggested that low levels of cerium may have a stimulatory effect on heart cell proliferation.

The relationship between magnesium deficiency and the expression of cerium toxicity was examined in more detail in a study aiming to understand the molecular basis of endomyocardial damage induced by cerium (Shivakumar and Prakash Kumar, 1993). Very high concentrations of cerium had an inhibitory effect on protein biosynthesis in cultured cardiac myocytes, the effect being more pronounced in cells exposed to a magnesium-deficient medium. At levels comparable with those found in the cardiac tissue in EMF, cerium was found to stimulate collagen and non-collage protein synthesis in cardiac explants and fibroblasts. This study suggested that magnesium deficiency may amplify the cardiotoxicity of cerium, and that stimulation of collagen synthesis by low levels of cerium may contribute to the accumulation of collagen observed in EMF.

### 1.1.5. Pathophysiology and Progression

Endomyocardial Fibrosis appears to start as a febrile episode triggered by unknown factors, followed by ventricular thrombosis which appear to be associated with
pancarditis, hypereosinophilia, facial swelling, body itching, urticaria and neurological features caused by thromboembolism (Parry and Abrahams, 1965; Andy, 1998). Later, mural and valvar thrombosis evolves to organization of the thrombus and endocardial fibrosis.

The sites most frequently affected by EMF lesions are RV “apex”, the left ventricular apex and the recess behind the posterior leaflet of the mitral valve (Connor et al, 1967). Endocardial fibrosis of these areas reduces ventricular cavity size and impedes adequate filling leading to restrictive physiology. On the other hand fibrosis of the endocardium affecting the papillary muscles, chordae and/or leaflets causes valve distortion, and usually leads to severe atrioventricular regurgitation. The combination of these two abnormalities is responsible for the typically small ventricles with severely dilated atria found in this entity.

The sustained low cardiac output results in finger and toe clubbing, growth retardation, testicular atrophy, failure to develop male secondary sexual characters and cachexia (Jaiyesemi and Falase, 1976; Somers, 1993; Bolarin and Andy, 1982; Abrahams, 1962).

In right ventricular EMF, the most common form of presentation either in isolation or as part of biventricular disease, the chronic systemic venous hypertension is responsible for the exophthalmos, elevated jugular pressure, gross hepatomegaly and congestive splenomegaly (Abrahams, 1962). Some distinctive clinical features of EMF cannot be explained solely by low cardiac output and retrograde congestion. For instance, there is still some controversy regarding the pathophysiology of the central cyanosis and the
voluminous ascites that can lead to ventral or inguinal hernia in the absence of pedal edema (Jayiesimi, 1982).

### 1.1.6. Diagnosis

The clinical picture of EMF depends on the ventricle affected, the duration of disease and the presence of active disease. Although not clearly defined there are three clinical forms of the endomyocardial fibrosis that have been usually described namely acute, chronic and steady (Guimaraes, 1993). Acute EMF is suggested by fever, abdominal distension, facial or periorbital swelling, body itching, urticaria, and neurological features, associated with hypereosinophilia and myopericarditis (Andy, 1998). Clinical features of chronic EMF include finger and toe clubbing, growth retardation, testicular atrophy, failure of the development of male secondary sexual characters and cachexia (Andy, 1998; Abrahams, 1962). Patients are said to be in a steady phase when there is neither amelioration nor deterioration of the clinical signs during several years (Bertrand, 1993).

By the time of clinical presentation patients with EMF are usually at an advanced stage (Somers, 1990). Few patients recall a febrile illness at the beginning of the disease, and most present a poor clinical history particularly in lone or predominant right EMF. Interestingly, patients are mildly symptomatic while having severe structural abnormalities detected on echocardiography (Ikeme, 1972; Salemi et al, 2005).
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Right Ventricular EMF

Patients with REMF often present with exophtalmus, jaundice, peripheral cyanosis, finger clubbing, atrial fibrillation and ascitis in the absence of pedal edema (Freers et al., 1996a) (Figure 1.5). The dilated and pulsatile right ventricular outflow tract causes a heave at the left upper parasternal area. Cardiac murmurs are often absent, but a third sound is usual. In advanced disease with free atrioventricular regurgitation cardiac auscultation can be normal (Somers, 1990).

Left Ventricular EMF

In LEMF the patients are usually in a better general status and have shorter duration of the disease. There is usually a systolic murmur that is typically soft, short and confined to early systole, associated with a delayed opening snap, a combination that is unique to left endomyocardial fibrosis (Somers, 1989). A loud second sound, indicating increased pulmonary pressures is also a common feature.
Figure 1.5. Ascites without pedal edema is a distinctive finding of REMF

Laboratory

The biological profile of EMF patients is unspecific and does not reveal evidence of infection or parasitism (Kartha, 1995). The presence of high eosinophil counts is variable (Somers, 1972b). High anti-streptolysin O and anti-malaria antibodies titres have been shown in some series, and in advanced cases mild hypoproteinemia is common (Somers, 1972b; Shaper et al, 1967).

The effusions, particularly the ascitic fluid, are typically exudates having more leukocytes (predominantly lymphocytes) and higher protein content than expected in right heart failure (Freers et al, 1996a).
**Electrocardiography**

The electrocardiogram shows no constant pattern. Atrial fibrillation is present in many cases at first admission (Somers, 1972). In advanced disease there are low voltage QRS complexes, non-specific ST-T wave changes and conduction disturbances (Zabsonre et al, 2000). In advanced REMF the electrocardiogram shows a tall and broad right atrial wave, a characteristic “qr” pattern in the leads V3R or V1, and delayed right ventricular conduction can also occur (Jaiyesimi, 1982; Abrahams, 1962) (Figure 1.6). The ECG in left EMF shows signs of left trial hypertrophy and pulmonary hypertension.

**Chest X-Ray**

The chest x-ray in REMF (Figure 1.7) shows severe right atrial enlargement, a bulge over the left heart border due to dilatation of the infundibulum, and hypoperfused lungs.

![Electrocardiogram of a patient with right EMF with atrial flutter and the characteristic qr pattern in lead V1.](image)

**Figure 1.6.** Electrocardiogram of a patient with right EMF with atrial flutter and the characteristic qr pattern in lead V1.
Figure 1.7. Chest radiography shows cardiomegaly due to enlargement of the right atrium. Notice the bulge over the left heart border due to dilatation of the infundibulum, and hypoperfused lungs are usual findings in REMF (left). LEMF causes less impressive cardiomegaly, main pulmonary artery dilatation, pulmonary changes of hypertension in the lung fields and LA enlargement (right).

In LEMF there is prominent main pulmonary artery, exaggeration of the blood vessels in the lung fields and left atrial enlargement (Figure 1.7).

Echocardiography

Echocardiography is the main tool for the diagnosis of EMF, by demonstrating dense endocardial echos along different parts of the ventricular wall, the atrioventricular valve and the subvalvar apparatus, associated with a restrictive filling pattern. This diagnostic technique has added precision to the non-invasive diagnostic of EMF, being essential for recognition of involvement of the contralateral ventricle in dominant univentricular
disease, and allowing comprehensive pre- and postoperative care (Venkitachalam et al., 1993; Berensztein et al., 2000; Okerere et al., 1991; Freers, 1996b).

REMF is diagnosed in the presence of thickening of the endocardium at the apex and obliteration of the trabecular portion of the right ventricle. In advanced cases there is a shrunken RV cavity with an apical notch, free tricuspid regurgitation, signs of spontaneous contrast, right atrial thrombi and pericardial effusion (Figure 1.8).

Echocardiography of LEMF shows patchy enhancement of the endocardial echo and thickening of the endocardium of the LV apex that can be obliterated. The posterior mitral leaflet is plastered-down causing eccentric mitral regurgitation. There is rapid early filling followed by restriction, and left atrial enlargement (Figure 1.8). Moderate to severe tricuspid regurgitation is usually present allowing the confirmation of the presence of pulmonary hypertension.

The main echocardiographic criteria used for the diagnosis of EMF are summarized in Table 1.1.
**Figure 1.8.** Transthoracic echocardiography showing obliteration of the left apex (arrow) in a patient with bilateral disease. Retraction of the right ventricle and aneurismal right atria in REMF cause compression of the left cavities (right).

**Computed Tomography**

This technique is seldom used in clinical practice since it adds little to the echocardiography. Computed Tomography accurately depicts morphologic and dynamic features of EMF, by allowing direct visualisation and mapping of fibrosis in the endocardium and within the myocardial wall. The presence of a linear calcification distal to the pericardium (along the inner border of the myocardium) suggests EMF at conventional and spiral computed tomography (Mousseaux *et al.*, 1996).
Table 1.1. Usual findings on echocardiography of endomyocardial fibrosis (EMF) as reported in the literature (after Venkytachalam et al, 1993, Hassan et al, 2005; Freers, 1996; Wiseman, 1986; Vijayaraghavan et al, 1983)

<table>
<thead>
<tr>
<th>RIGHT VENTRICULAR EMF</th>
<th>LEFT VENTRICULAR EMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>. Enhanced endocardial echos in the right ventricle usually in septal and apical portions</td>
<td>. Enhanced endocardial echos in the left ventricle usually in the apex and posterior wall</td>
</tr>
<tr>
<td>. Adherent tricuspid valve apparatus</td>
<td>. Plastered posterior mitral leaflet</td>
</tr>
<tr>
<td>. Obliterated trabecular portion of the right ventricle</td>
<td>. Obliterated left ventricular apex or posterior papillary muscle recess</td>
</tr>
<tr>
<td>. Tricuspid regurgitation</td>
<td>. Mitral regurgitation</td>
</tr>
<tr>
<td>. Endocardial calcification on the right ventricle</td>
<td>. Endocardial calcification on the left ventricle</td>
</tr>
<tr>
<td>. Pulmonary valve diastolic opening and dilated hypercontractile right ventricular outflow tract</td>
<td>. M-movement of interventricular septum and/or posterior wall</td>
</tr>
<tr>
<td>. Tall E wave on tricuspid Doppler flow</td>
<td>. Tall E wave and small A wave (E/A =&gt; 2)</td>
</tr>
<tr>
<td>. Dilated right atrium</td>
<td>. Dilated left atrium</td>
</tr>
<tr>
<td>. Large pericardial effusion</td>
<td>. Pulmonary Hypertension</td>
</tr>
<tr>
<td>. Spontaneous contrast or intra-cavitary thrombi</td>
<td>. Spontaneous contrast or intra-cavitary thrombi</td>
</tr>
<tr>
<td>. Apical notch or shrunken right ventricle</td>
<td>. Left ventricle reduced longitudinal dimension</td>
</tr>
</tbody>
</table>
Magnetic Resonance Imaging

Magnetic Resonance Imaging (MRI) has many advantages over other diagnostic techniques, mainly in patients with severe structural abnormalities leading to distorted anatomy. This technique confirms the existence of thrombus or calcifications, allows an exact deliniation of hypoperfused areas that correspond to fibrosis (Estornell et al., 2003), and provides information hemodynamic information through the cine mode. Myocardial suppression scans, acquired 10’ after injection of gadolinium, also allow exact appreciation of disease extension by delayed hyperenhancement of the pathologic areas (Estornell et al., 2003; Smedema et al., 2004).

MRI is therefore the ideal diagnostic tool for management, allowing monitoring of the response to medical and surgical treatment (Smedema et al., 2004). Unfortunately it is a technique that is expensive and therefore not available in most endemic areas for EMF (Cury et al., 2005).

Cardiac catheterisation

Before the advent of echocardiography cardiac catheterisation was the only diagnostic method available to confirm the clinical diagnosis of EMF (Tharakan et al., 1993; Abrahams, 1962; Hutt et al., 1965). However, this technique is not adequate for diagnosis of localized or mild forms of disease, and can be technically challenging and dangerous in advanced disease. Cardiac catheterisation is associated with (1) bleeding from venous puncture sites due to high venous pressure; (2) difficulty in entering the right ventricle
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and pulmonary artery due to markedly dilated right atrium, tricuspid regurgitation, small right ventricular cavity, and distorted and displaced right ventricular outflow tract; (3) high risk of pulmonary thromboembolism from right atrial thrombi; (4) considerable risk of acute hemodynamic deterioration, mainly in severe REMF with ascitis and low basal cardiac output, but also due to stimulation of atrial tachyarrhythmia.

In REMF pressures in the right atrium, right ventricle, and pulmonary artery are equal both in form and amplitude. Angiograms show flattened apex, loss of trabeculated pattern, dilated and hypercontractile infundibulum (with contractility of the remaining parts of the ventricle varying from normal to severely impaired), free tricuspid reflux, large right atrium and dilated cava veins (Hassan et al, 2005).

LEMF is characterised by very high left ventricular end-diastolic pressure with dip-plateau pattern, and pulmonary hypertension variably damped by the presence of the RV disease. The angiogram shows left apical obliteration with varying degree of mitral regurgitation. The increased distance from coronary arteries to the contrast inside the ventricular cavity demonstrates the obliteration of the apices.

Endomyocardial Biopsy is not essential for the diagnosis and management of EMF, and can be misleading when tissue is obtained from unaffected sites. EMB is technically difficult in areas of dense endocardial fibrous thickening and, due to the imbalance between the information obtained and the risk of the procedure, is rarely used.
1.1.7. Differential Diagnosis

In endemic areas for EMF it is important to differentiate this condition from Rheumatic Heart Disease, Dilated Cardiomyopathy, apical type of Hypertrophic Cardiomyopathy, Constrictive Pericarditis and Tuberculous Pericarditis. Amyloidosis, hemochromatosis, myocardial sarcoidosis and neoplastic infiltration from lymphomas are other entities that must be considered in the differential diagnosis.

1.1.8. Pathological Spectrum

The basic lesion in EMF is ventricular endocardial fibrosis without primary involvement of extracardiac organs. The macroscopic appearance and the sites of involvement in the ventricle are distinctive and differentiate this condition from any other form of cardiac disease. The end result is restriction of ventricular filling and distortion of the papillary muscles, chordae and leaflets, resulting in restrictive cardiomyopathy with dysfunction of the atrioventricular valves.

The classical descriptions of the pathology of EMF have been based on autopsies done on two sets of patients: those with advanced disease dying from complications of long-standing heart failure or those with acute EMF who died as a result of febrile episodes with rapidly progressive heart failure (Connor et al, 1967; Connor et al, 1968; Shaper et al, 1968). Few studies, mainly case reports, have documented the histological findings in vivo using endomyocardial biopsies in patients submitted to cardiac catheterization (Chopra et al, 1990) or surgery (Santos et al, 2001; Saraiva et al, 1999). However,
because facilities for cardiac catheterization and open-heart surgery are not available in most endemic areas knowledge about the macroscopic and microscopic features of intermediate stages of EMF is scarce.

**Macroscopical appearance**

At opening of the pericardial sac adhesions between the parietal and visceral layers are frequent. A “right border notch” is frequently visible in hearts with right ventricular disease causing retraction. There is usually cardiomegaly due to dilatation of the atria but the heart is not very heavy.

Thrombosis and fibrosis are characteristically prominent in the ventricular apices, and the posterior wall of the left ventricle behind the posterior leaflet of the mitral valve. In the right ventricle the scar tissue may be massive, engulfing and fusing the trabeculae carneae, obliterating the apex of the right ventricle, fixing and obliterating the papillary muscles, chordae tendineae, and leaflets of the tricuspid valve. The tricuspid annulus is usually severely dilated. In the left ventricle the lesions affect mainly the posterior wall of the left ventricle; the thrombus and scar tissue may engulf and obliterate the posterior leaflet of the mitral valve, its attached chordae and the posterior papillary muscle (Figure 1.9). The left ventricular apex is frequently scarred and thrombosed, but the left ventricle is never contracted at the apex. The semilunar valves and the great vessels are not involved.
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Microscopy

All three layers of the heart appear to be involved (Farrer-Brown, 1972; Andrade and Teixeira, 1973) but the hallmark of EMF is endocardial thickening due to acellular fibro-collagen tissue deposition underneath the endothelial layer of the endocardium (Connor et al, 1968; Chopra et al, 1990; Seth 2004). A marked degree of myocardial loss is not found, although myocytolytic lesions are present in the subendocardium. Intense inflammatory infiltrates, including eosinophils, are conspicuously absent. Small vessel disease is unusual.

A study of 42 autopsies and 89 biopsies from patients submitted to surgery (Kartha et al, 1993) failed to detect a dominant lesion such as necrosis, inflammation, thrombosis or small vessel lesions to explain the pathogenesis of the disease. It revealed increase in interstitial cellularity, even in ventricles that macroscopically had no lesions, a finding that has also been reported from other studies (Connor et al, 1968; Somers, 1971). These findings did not confirm the theories about eosinophil mediated myocardial injury inducing necrosis and setting the stage for endocardial thrombosis that would heal and lead to fibrosis. Also, no support was found for the theory of endocardial fibrosis being secondary to subendocardial myofibre atrophy due to coronary arterial changes of an unknown etiology, a theory previously proposed (Connor et al, 1968). Several other studies have contributed little to support either of the two above views or the contention that EMF is a sequel to a pancarditis of undetermined etiology.

To date there is consensus that EMF is a disease where the cardinal feature is abnormal stimulation of cardiac fibroblasts leading to enhanced collagen synthesis. It may be a
reactive stromal change, and progressive interstitial fibrosis a result of cardiac fibroblast growth, with or without proliferation and enhanced collagen synthesis. It would seem that EMF is the outcome in circumstances where an unidentified form of cardiac injury results in a response mediated mainly or entirely by the interstitial tissue and cardiac fibroblasts, rather than the myocardial cell or the microvasculature. Entry of trophic factors into the interstitium could be mediated by injury to microcirculation and alteration of permeability. Myocytolysis seen in EMF is a minor component and could be caused by entrapment by fibrosis and toxicity by the same factors, which produce the interstitial injury.
Figure 1.9. Photograph of the heart of a patient with bilateral EMF. There is gross dilatation of the right atrium that contained a thrombus. The interatrial septum has irregularities that result from thickening of the septum in some areas as well to recoil of the aneurismal septum that bulged to the left ante-mortem. The right ventricular endocardium is white, opaque and thick in the admission chamber and the trabecular part, where it forms the floor of a retracted ventricle with obliteration of the trabeculae. The tricuspid valve annulus is severely dilated, and its leaflets are thin although some chordae were thickened and stretched. The papillary muscles were completely encased in the fibrous endocardium. The left ventricle has a small area of apical fibrosis.
1.1.9. Management

There is no specific therapy for EMF. Symptomatic medical therapy with corticosteroids, diuretics, ACE-inhibitors, digitalis, β-blockers and warfarin is used in the management of these patients aiming to control acute disease, heart failure and arrhythmias, as well as to prevent thromboembolism.

The overall results of medical therapy are poor. Patients presenting with advanced disease need frequent admissions to hospital to treat episodes of heart failure, despite the large doses of drugs administered. Admissions to hospital are also needed to perform invasive procedures for alleviation of effusions (paracentesis, thoracocentesis, pericardiocentesis) and treatment of arrhythmias (electrical cardioversion).

The use of short courses of oral corticosteroids in patients with hypereosinophilia and other signs of activity is not based on systematic studies of the effects of these drugs on the progression of the disease.

Surgery of EMF

The unsatisfactory response of patients with symptomatic EMF to medical treatment has prompted the search for surgical correction of the structural and functional abnormalities characteristic of this condition.

The initial procedures were intended to be palliatives. Pericardiectomies and valve pericardio-peritoneal shunts have been tried for massive and recurrent pericardial effusions (Adebonojo, 1977), and later, connections between the right atrium and the
pulmonary arteries were also used for palliation.

The first attempts to corrective surgery consisted of total stripping of the endocardial fibrous membrane from the myocardium, excision of the damaged papillary muscles, and replacement of the damaged atrioventricular valves. The main complications of these extensive procedures were low cardiac output syndrome and complete atrioventricular block. In the medium and long term complications of valve prostheses were very important with both, documented valve thrombosis and uncontrolled bleeding being frequent, due to poor compliance and inadequate control of anticoagulation. The immediate surgical mortality was around 18 % (Dubost et al, 1989; Moraes et al, 1999), remaining high thereafter: 21,7% within 30 days and 13% during the first postoperative year (Valiathan et al, 1987). This scenario was improved with the introduction of changes to the operative technique including avoidance of atrioventricular block by subtotal endocardial resection on the RV (Metras et al, 1982), the use of mitral valve repair to treat mitral incompetence (Metras et al, 1983) and improvements in myocardial protection (Moraes et al, 1993).

The rational for surgical treatment of EMF is based on several important facts: (1) The disease is fatal if untreated; (2) Severe hemodynamic derangement caused by restriction of the diastolic filling and atrioventricular regurgitation can be corrected; (3) In most cases the myocardium remains healthy and unaffected and endocardectomy is feasible through a well preserved cleavage plan; (4) Evidence of recurrence is controversial and low rates have been reported, even for those patients that go back to their areas of origin (4,5-5.8%). All these factors contribute to the acceptance of the superiority of surgical
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treatment of EMF in patients in NYHA class III and IV (Valiathan et al, 1993; Barretto et al, 1993).

Although it is accepted that surgery must be indicated in all symptomatic patients with significant severe hemodynamic lesions (Dubost et al, 1989) the high morbidity and mortality associated with it, led to a general preference for medical treatment for patients in NYHA class I/II. Also, due to the infrequent diagnosis of EMF in western countries and the lack of human and material resources for open-heart surgery in most areas where EMF is endemic, the surgical experience is growing slowly. However, surgery has been shown to increase survival and quality of life (Cherian et al, 1982b), when compared to medical therapy, and the low incidence of recurrence after surgery suggests that it should be performed before irreversible cardiac and hepatic damage occur (Gonzalez-Lavin et al, 1982).

There is need for further systematic studies of different phases of the disease and its progression, coupled with accurate definition of structure-to-function relationship using modern imaging techniques. This could lead to the development of targeted approaches to surgical treatment followed by adequate follow up using integrated approaches that could lead to evidence-based guidelines for the management of the disease.
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1.2. Research Aims

Almost seven decades after it was first described, there are many gaps in our knowledge of EMF and it can be considered a neglected disease. Data on epidemiology are scarce, the etiology remains unknown and the pathogenesis is incompletely understood, resulting in the inexistence of standardized methods for prevention, systematic programmes for early detection or consensus about management, including the timing of surgery. To date no genetic studies have been done in order to investigate the role of heritability in determining susceptibility and phenotypic variability of this entity.

In this context of prevalent disease with no adequate treatment operational research combining both basic and clinical aspects of the disease is mandatory.

Large-scale community studies can now be done using portable echocardiography to detect early stages of the disease, which are probably associated with specific immune system activation. The description of these changes in early stages would allow better understanding of the mechanisms involved in the pathogenesis of EMF, and description of its natural history.

The great variability in the mode of presentation and rate of progression of EMF is not accurately demonstrated by using the current simplistic classifications of the disease. We believe that a comprehensive classification can be achieved by using detailed clinical, laboratory and echocardiographic criteria, resulting in improvement of the management of the disease and the surgical outcome. Also knowledge of the pathogenesis can help identify new therapeutic targets for the disease preventing its progression to advanced...
and deforming stages, and therefore improving the prognosis.

We therefore designed this research aiming at

(1) Determine the prevalence of EMF in a known endemic area (Inharrime)

(2) Detect early cardiac changes using echocardiography

(3) Evolve a comprehensive classification of EMF using clinical, biological and echocardiographic criteria

(4) Establish a national registry to determine the prognosis of the disease, search for prognostic indicators, and help in design and implementation of therapeutic trials in the future

(5) Describe the immune response in blood and cardiac tissue through measurement of pro-inflammatory cytokines, pro-thrombotic factors, antiheart antibodies and malaria mimicry

(6) Investigate the role of genetic susceptibility for EMF through HLA-typing

(7) Establish the role and timing of surgery in management, and evolve tailored approaches to specific types of structural and functional defects with optimal follow up through the database
1.3. Layout

The work presented in this thesis has been divided in twelve chapters, starting with the current introductory chapter.

Chapter 2 details the design of research and the procedures used to implement the three branches of the research programme, in three different sites: Maputo (at the Heart Institute of Mozambique), Inharrime (at the Research Centre) and London (at the Imperial College and other laboratories).

Chapter 3 presents a new classification of EMF evolved by the researcher and tested in two different situations: in large-scale echocardiographic screening in a rural area of Mozambique, and as a diagnostic and management tool in the clinical setting. The issues related to relevance and accuracy of the criteria are discussed.

In Chapter 4 we present the work related to determination of prevalence of EMF in Inharrime district using echocardiographic screening of the population. In the first part, a description of the sampling method, issues related to preparation and implementation of a prevalence study in general population, and the results found are presented. In the second part of this chapter, the procedures for the creation of a cohort of children from Inharrime to be used in follow-up studies are present.
Chapter 5 describes the clinical profile of EMF patients who present to hospitals. The major signs of established disease are described and discussed.

The pathological features of established EMF are presented in Chapter 6, which involves macroscopic evaluation of the cardiac lesions, as well as detailed description of histology and immunochemistry on tissue obtained during surgery and at autopsy. Echocardiographic findings are compared with intraoperative features and, finally, a clinicopathological correlation is attempted.

Chapter 7 describes the pattern of immune activation in chronic established EMF, including cellular and humoral changes in blood and cardiac tissue. Particular emphasis is given to changes in eosinophils and its related cytokines, namely IL-5 and Eotaxins. First, the results of eosinophil studies in the rural community and at the hospital are presented. Then, tissue studies are described and the adaptation of the technique of detection of eotaxins in the lung is described and applied to cardiac tissue of patients with EMF. The cytokine profile of these patients is explored and, finally, the presence and level of antiheart antibodies is determined. The results of these studies are discussed together.

Chapter 8 presents and discusses the results of investigation on the presence of prothrombotic factors, endothelial activation and inflammation markers in recent onset EMF. The implications of the results to management of the disease are highlighted.
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Chapter 9 describes the results of a multifaceted investigation on the role of malaria in pathogenesis of EMF, involving studies of prevalence of the infection in individuals from the rural area studied and patients from the clinical registry, the determination of the rate of concurrence of EMF and Malaria, and also the presence of cardiac damage in severe and complicated malaria. An interpretation of the results is described and new strategies for research in this topic are proposed.

Genetic susceptibility to EMF is explored through determination of HLA-typing in EMF patients and ethnically matched controls, whose results are presented and discussed in Chapter 10.

Chapter 11 presents the different therapeutic options available for the management of EMF. A new surgical approach has been evolved and applied and its results are described, detailing the procedures used pre- and intra-operatively. The major issues on the post-operative period and those raised in the mid-term follow up are also presented and discussed.

The last chapter summarizes the conclusions of our work and proposes new research lines into the mechanisms of this cardiomyopathy.
Chapter 2

METHODS
2.1. Design of the Investigation

Our research project included three main branches, namely community-based studies, hospital-based research and laboratory work (Diagram 2.1).

The investigator was based in Maputo-Mozambique, where the clinical research took place. This included the establishment and management of the National EMF registry at the Heart Institute in Maputo, the site of referral for patients from all regions of the country.

Frequent trips to the rural area endemic for EMF (Inharrime-Inhambane Province) were necessary to prepare and implement the epidemiological research. During these visits the researcher worked in close collaboration with the local authorities to organize the fieldwork, establish facilities for clinical follow up at the village, and train the local personal involved in the epidemiological surveys.

Significant amount of time was spent in the United Kingdom where the researcher did several transferable skill courses, including some training on the laboratorial techniques and methods used in the various studies, and worked with collaborators in performing the tests.
(1) Community Work (Inharrime District – Inhambane Province, Mozambique)

- Training of local people
- Design and Installation of Research Facilities
- Epidemiology studies
- Prevalence study (January 2005 -May 2005)
- Establishing cohort of school children (October 2006)
- Follow-up of participants in epidemiology and clinical studies

(2) Clinical Research (Instituto do Coração – Maputo, Mozambique)

- Establishment of a National EMF Registry
- Clinical follow-up of patients
- Organization and participation in surgical missions
- Pre- and post-operative management
- Blood and tissue collection and storage

(3) Laboratory Studies (United Kingdom)

- Participation in laboratory work

Diagram 2.1. Work undertaken by the investigator in the three main sites of research
2.1.1. Epidemiology research

Inharrime District - Mozambique

Mozambique is a country located on the east coast of Southern Africa (Figures 2.1, 2.2, 2.3). It has a total surface of 799 380 km$^2$ and a coast of over 2500kms. The population reaches 20 million and is predominantly rural (71.4%). Given that previous studies in Mozambique revealed that Inharrime district, in the south of Inhambane province was an endemic area for EMF, we chose this rural district as the area for our community studies.

Study Area

Inharrime is a coastal district in Mozambique with a total surface area of 2744Km$^2$, situated 400Kms from the main referral units for cardiovascular diseases, which are located in Maputo (Figure 2.4). It has a total population of 76518 inhabitants. Some areas of the district are located more than 100kms from the village (Inharrime Sede).

The district is divided into five main zones called Localidade that are in turn subdivided into smaller administrative areas under the administration of traditional leaders, the Povoados. There are 59 “Povoados” in Inharrime. The community of this rural area is primarily composed of very low-income people working in agriculture, and living in small houses made of local materials (Figures 2.5, 2.6). Electricity and pipe-borne water supply are available for less than 1% of the households. There are no conventional roads
Figure 2.1. A map of Africa where Mozambique is shown in blue.

Figure 2.2. Mozambique has 20,366,795 inhabitants distributed by 10 provinces; 43.1% of the population is under 15 years and the life expectancy is 47.6 years.
**Figure 2.3.** Inhambane province, although being the least accessible of the 3 southern provinces of Mozambique, was the origin of most EMF patients assisted at the national referral units.
Figure 2.4. Inharrime is one of the 14 districts of Inhambane province, and is the district from where most patients are referred.
Figure 2.5. Typical houses and schools in Inharrime are built using local materials.

Figure 2.6. Mandioc, the basis of diet in Inharrime, is produced and sold by the families.
(Figure 2.7) apart from the main national road linking the north and the south of the country, a conventional road that crosses the district.

Our community research consisted in determining the prevalence of EMF in the population and establishing a cohort of school children to be followed for future studies on incidence and rate of progression of Endomyocardial Fibrosis. Given that there were no facilities to allow accommodation of the research team for fieldwork, clinical assessment and follow-up of participants in the studies, and storage of blood samples collected in the community, the initial phase of the research was the design and construction of the Inharrime Research Centre in the village of Inharrime.

Research Facilities

The research facilities are located next to the Public Health Centre in Inharrime District (Figure 2.8). They accommodate five divisions: (1) a room for clinical observation of the participants with portable electrocardiography and echocardiography machines; (2) small laboratory for blood preparation and conservation at -80°C; (3) bedroom for researchers; (4) a room for keeping the files; (5) and a kitchen.

From these facilities we were able to organize our daily visits to the several areas and schools involved in the studies, recharge the electrical equipment used during the day, and preserve the blood collected. The facilities were also used for follow-up of the participants in community studies, clinical evaluation of EMF patients (Figure 2.9), and training of collaborators from the Health and Education Departments that were involved in the research.
Figure 2.7. Inharrime district has no conventional roads.

Figure 2.8. The research facilities, seen in this picture, were also used for accommodation of the research team during community work.
Preparation of Fieldwork

The preparation of the fieldwork involved the local authorities in Inharrime, namely the community leaders for the prevalence study and the teachers for the work at schools. The local authorities agreed to participate in the teams to guide the researcher and introduce her to the population and parents. They were trained by the researcher regarding the procedures of the studies, namely the information to the participants and the informed consent, and were involved in the process of randomisation (Figure 2.10).

The community leaders and teachers of the randomly selected areas and schools were introduced to the project, trained on the procedures of the fieldwork and helped with the translation of the informed consent to the local languages. Teachers in charge of the selected classrooms were trained to identify symptoms and signs of cardiac disease, register them using a standardized protocol, and refer the children for evaluation by the researcher during the periods in between the visits to the schools (Figure 2.11).

The schedule of fieldwork was agreed with these authorities that had the responsibility of informing their communities of the dates for the studies. Periodic follow-up meetings were scheduled to allow the feedback to the leaders and the community.

Prevalence Study

For the prevalence study we used clustering at two levels selecting 33 “povoados” out of the 59 in Inharrime district, and then including all members of families randomly selected in the area, in order to achieve clusters of at least 30 people. The families were visited in
Chapter 2 Methods

their houses, where epidemiological data were collected and transthoracic echocardiography performed.

Figure 2.9. Accommodation of the team and storage of material in the research facilities

Figure 2.10. Community leaders assisting in randomization of povoados and houses
**Chapter 2 Methods**

**Cohort of School Children**

A cohort of school children randomly selected from Inharrime district was established for future studies on pathogenesis and rate of progression of EMF. The selection of these children was done using clustering at two levels. First, 10 schools were randomly selected from a total of 54 in the district. Then, all children from second grade in these schools were invited to participate in the study, and were submitted to clinical evaluation and transthoracic echocardiography.

These children will be followed through clinical examination, echocardiography and blood sampling every 12 months, in order to evaluate the incidence of EMF and natural history of the disease.

This approach was chosen due to the logistical and financial aspects related to such long follow-up of children in this remote area. Financial constraints make it more feasible to follow-up children at their schools with the help of their teachers. It is expected that children at the second year of school will be available for follow up for the next two years, since “schooling” is compulsory until the 5\textsuperscript{th} degree and the highest rates of withdrawal are reported in the first year.

**Diagnosis and classification of EMF**

Transthoracic echocardiography using a portable *Vivid-i* (GE) machine was performed to all members of the selected families and school classes (Figure 2.12). The examinations were recorded for re-evaluation by two blinded experienced cardiologists. The diagnosis of EMF was accepted when there was agreement of at least two cardiologists.
Chapter 2 Methods

Figure 2.11. The teachers from the selected schools (left) were trained and participated in planning and implementation of the research in each school (right).

Figure 2.12. Echocardiography performed by the researcher inside a selected house.
Based on knowledge of advanced disease and pathology findings of early stages described in post-mortem studies we defined major and minor criteria for the diagnosis of EMF that were used to make the diagnosis and to classify the disease. A score was attributed to each criterion. Diagnosis was made in the presence of two major criteria, or one major criterion associated with two minor criteria. According to the location of structural lesions EMF was classified as: (1) Biventricular when lesions were equally distributed in both ventricles without predominance of one side; (2) Right ventricular when lesions affected in isolation or were predominant in the right ventricle; (3) Left ventricular when lesions were confined to or affected predominantly the left ventricle. A scoring system that considered the extension and severity of the structural and functional changes of the heart was also developed allowing classification of EMF in mild, moderate, severe and advanced.

Further management of cases detected in the community

Participants with EMF detected during community studies were included in the registry and evaluated further at the Inharrime Research Centre (described above) to be treated according to the stage of disease. The follow up of these patients was done through monthly visits to the site by the researcher (Figure 2.13). Participants with severe or advanced disease needing surgery were offered surgical treatment at the Heart Institute in Maputo at the expenses of the project.
2.1.2. Clinical research

Heart Institute, Maputo - Mozambique

The Maputo Heart Institute is the main referral unit for cardiovascular diseases in Mozambique, and the only hospital with capacity of performing invasive cardiology and open-heart surgery in the country. The hospital-based research included the establishment of a national registry of Endomyocardial Fibrosis and clinical follow-up of patients referred from all regions of the country, including participants in community studies.

National EMF Registry

The registry was started at the beginning of the research project in order to insure the systematization of information regarding epidemiology data, clinical characterization and management of the patients. Standardized procedures for medical attention prior and after surgery were elaborated (Diagram 2.2).

The diagnosis of Endomyocardial Fibrosis was established or confirmed by using transthoracic echocardiography. Classification of the disease was done using the criteria that will be detailed in next chapter. The researcher assessed all patients clinically, and was responsible for the medical management and follow up, including performing invasive procedures like pericardial drainage, paracenthesis, and cardiac catheterization.
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Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis

Diagram 2.2. Flowchart for clinical investigation in EMF patients
Chapter 2 Methods

Surgical Programme

The researcher was also responsible for the selection of patients to surgery, as well as for the pre-operative preparation and post-operative care. Health professionals from different surgical groups that operate in Mozambique, who agreed to participate in this project, composed the surgical team (Figure 2.14). They worked under the leadership of the main supervisor of this research project, Professor Magdi Yacoub. The researcher was present in the operating room to describe the intraoperative findings, and receive and prepare the cardiac tissue for pathology studies.

2.1.3. Laboratory Research

Mozambique and United Kingdom

Laboratory work related with clinical diagnosis was done in Mozambique, namely routine blood studies, inflammation markers (erythrosedimentation rate, C-reactive protein), urinalysis, stool examination and histology of cardiac tissue.

Due to lack of human and material resources at the local laboratory, blood and tissue samples were periodically transferred to the UK using specialized mailing services. In the UK the researcher was involved in several steps of the laboratory work designed to understand the pathogenic mechanisms involved in EMF, which was done essentially by collaborators from Imperial College, the Kings College London, and the National Blood Service. These studies included the assessment of (1) the immune activation (eosinophil
studies, cytokine profile, autoimmunity), (2) the presence of endothelial activation and hemostatic dysfunction and (3) genetic susceptibility (HLA-typing).

**Figure 2.13.** Follow-up of participants in community studies at the research facilities

**Figure 2.14.** Surgical procedure performed at the Heart Institute in Mozambique
Chapter 2 Methods

2.2. Statistical Analysis

The statistical methods used are discussed in the individual chapters.

2.3. Ethical issues

Every participant was informed of all procedures of the research project. Eligible participants were invited to participate in the studies and to sign a written informed consent (or finger-print whenever the participant or parent was not capable of giving a signature). The informed consent was typed in Portuguese (the official language of Mozambique) but its information was translated to the local language before obtaining the consent.

No charges were made to participants for clinical visits or laboratory exams done during the study. The best available management was warranted to every participant, using the local health care facilities in the National Health System and the Heart Institute, and following the rules for referral of patients at the various levels of health care defined by the Ministry of Health of Mozambique.

In order to achieve sustainability and obtain acceptance of this long research programme both official and traditional authorities were involved in all phases of preparation and implementation of the research programme. The authorities belonging to the selected zones were invited to participate or send their representatives to the fieldwork.

Ethical approval was obtained from the National Bioethical Committee in Mozambique. The research project has an international registration number (IRB 00002657).
Chapter 5 Clinical Characterisation

Chapter 3

EVOLVING AND APPLYING NEW CRITERIA FOR DIAGNOSIS AND CLASSIFICATION OF ENDOMYOCARDIAL FIBROSIS
3.1. Introduction

Great variability in mode of presentation and echocardiographic features is found in EMF patients that seek medical attention. However, no attempt has been made to systematically define the disease and classify the patients.

Echocardiography has the potential for detection of earlier stages of EMF (Wiseman et al 1986; Vijayaraghavan et al, 1983) and for detailed characterization of the structural and functional changes that occur in this condition. Portable echocardiography is a potentially useful tool for large-scale community studies, to identify initial stages of the disease and to describe its natural history through imaging and hemodynamic changes.

We hypothesized that detailed characterization of echocardiographic abnormalities would allow early diagnosis and comprehensive classification of EMF. After extensive review of literature we systematized the available criteria for diagnoses of established disease and identified echocardiographic signs that could represent early stages of this condition.

The aim was to evolve echocardiographic criteria for early diagnosis that could be used for screening purposes, and propose a new classification of EMF to be used on follow-up of individuals participating in large-scale community studies and for clinical management of patients.
3.2. Population and Methods

3.2.1. Population

The criteria selected were applied to study individuals from the endemic community (Inharrime District) randomly selected from villages and schools, as well as patients from the national EMF registry seen at the referral unit in Maputo.

3.2.2. Evolving Echocardiographic Diagnosis and Classification

The diagnosis of endomyocardial fibrosis was based on transthoracic echocardiography using a portable ultrasound machine (Vivid I, General Electrics) for community studies and a HP 5500 (Philips) for the clinical research. Both machines have the possibility to do 2D, M-mode and Doppler (continuous, pulsed and colour) evaluation. The echocardiographic exams were recorded and kept as part of the database and can be recalled. The collection of echocardiographic data was standardized (Appendix 1).

3.2.2.1. Diagnosis

The hallmark of established EMF is the presence of thickened endocardium, but we wanted to detect initial phases of the disease using echocardiographic screening. Applying knowledge obtained from pathological examination of hearts from EMF patients who died or were submitted to catheterization (Connor et al, 1967; Kartha et al, 1993; Kinare and Deshpande, 1993), from surgical series (Moraes et al, 1993; Metras, 1993; Valiathan and Shyamkrishnan, 1993; Moraes et al, 1999) and echocardiographic
studies (Hassan et al., 2005; Freers et al., 1996b; Wiseman et al., 1986; Vijayaraghavan et al., 1983; Venkitachalam et al., 1993) we defined criteria for diagnosis and classification of EMF.

The features used for diagnosis were classed as major and minor criteria, according to their specificity for the diagnosis, and their importance for management and prognosis. The diagnosis of EMF was made in the presence of two major criteria or one major criterion associated with one or more minor criteria (Table 3.1). For echocardiographic diagnosis of EMF endocardial plaque or thickening had to be present.

### 3.2.2.2. Echocardiographic measurements

In community studies one echocardiographic examination was considered for the diagnosis of EMF for each individual. All measurements were recorded for offline re-evaluation by the investigator, blinded to the epidemiological and clinical data. In cases where doubts persisted after full examination two other cardiologists blinded for demographic data and to the results of the main investigator, were asked to review the recorded exams using the same criteria and the scoring system to classify the disease.

Regarding the patients from the clinical registry we had scheduled one echocardiographic examination every three months, but some patients had more exams done due to sudden aggravation of their clinical condition and the need to adapt the medical therapy.
Table 3.1. New criteria for Diagnosis and Assessment of Severity of EMF

<table>
<thead>
<tr>
<th>MAJOR CRITERIA</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endomyocardial plaques (2 or more mm in thickness)</td>
<td>2</td>
</tr>
<tr>
<td>Thin endomyocardial “patches” affecting more than one ventricular wall</td>
<td>3</td>
</tr>
<tr>
<td>Obliteration of the right ventricular or left ventricular apices</td>
<td>4</td>
</tr>
<tr>
<td>Thrombi/Spontaneous contrast without severe ventricular dysfunction</td>
<td>4</td>
</tr>
<tr>
<td>Retraction of the right ventricular apex (right ventricular apical notch)</td>
<td>4</td>
</tr>
<tr>
<td>Atrioventricular valve dysfunction due to adherent valvar apparatus to the ventricular wall</td>
<td>1-4*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>MINOR CRITERIA</th>
<th>SCORE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thin endomyocardial “patches” localised to one ventricular wall</td>
<td>1</td>
</tr>
<tr>
<td>Restrictive flow pattern across mitral or tricuspid valves</td>
<td>2</td>
</tr>
<tr>
<td>Pulmonary valve diastolic opening</td>
<td>2</td>
</tr>
<tr>
<td>Diffuse thickening of the anterior mitral leaflet</td>
<td>1</td>
</tr>
<tr>
<td>Enlarged atrium with normal sized ventricle</td>
<td>2</td>
</tr>
<tr>
<td>M-movement of the interventricular septum and/or posterior wall observed by m-mode echo</td>
<td>1</td>
</tr>
<tr>
<td>Enhanced density of the moderator or other intraventricular bands.</td>
<td>1</td>
</tr>
</tbody>
</table>

**Definite case:** presence of 2 major or 1 major associated with 2 minor criteria.

The severity score applies as follows

*Mild EMF* score < 8;

*Moderate EMF* score 8-15;

*Severe EMF* score 16-23;

*Advanced EMF* score >24.
Evaluation of the Left Atrium

The left atrium maximal linear dimensions were measured in three plans: anteroposterior, lateral and superoinferior at the end of left ventricular systole. The left atrial anteroposterior diameter was measured by bidimensional-guided M-mode echocardiography from the parasternal short-axis view at the base of the heart (or the parasternal long-axis), from the trailing edge of the posterior aortic wall to the leading edge of the posterior left atrial wall (Lang et al, 2005). The lateral and superoinferior dimensions were measured by bidimensional echocardiography from the apical 4 chambers view using an inner-edge-to-inner-edge measurement. The left atrial area was measured by planimetry of the inner contour from the apical 4 chambers view, excluding the confluences of the pulmonary veins and the left atrial appendage from the tracing. Table 3.2 indicates the criteria used for definition of left atrial enlargement. Additionally an sphericity index for the left atrium was calculated as the ratio between lateral and superoinferior diameters.

Due to the frequent distortion of the left cavities shape in patients with REMF or BEMF the volumes were not consistently measured. However, whenever possible for clinical follow up of patients submitted to surgery, the left atrial volume was determined using the Simpson’s rule from the apical 4-chamber view at the end-systole, from the frame preceding mitral opening. The plane of mitral annulus was taken as the inferior left atrial border.

For participants from the clinical registry left atrial volume and area were indexed to body surface area, while in community studies the area was indexed to age.
Evaluation of the Left Ventricle

Left ventricle end-systolic diameters, left ventricle end-diastolic diameters, septal and posterior wall thickness at end-diastole were measured in the parasternal long axis view, using bidimensional-guided M-mode echocardiography according to current recommendations for chamber quantification (Lang et al, 2005). An sphericity index was calculated as the ratio between lateral and superoinferior diameters in 4-chambers view. The criteria used for classification of left ventricular reduction are presented in Table 3.3.

For follow up of patients submitted to surgery and without severe distortion of the left ventricular shape, the left ventricular end-systolic and end-diastolic volumes and ejection fraction were determined from the apical 4-chamber view according to the modified Simpson’s rule.

In patients with heart cavity distortion the left ventricular systolic function was evaluated through a visual semi-quantitative scale (good contractility, +++; moderate dysfunction, ++ ; and poor contractility, +) using bidimensional guided M-mode in several incidences (4-chambers, parasternal long axis, parasternal short axis and sub-costal views).

The left ventricular inflow velocities were recorded using the apical four-chamber view. The pulsed-wave Doppler sample volume was placed at the level of the leaflets tips of the mitral valve, where the highest peak velocity was recorded. Peak flow velocities of the left ventricular inflow in early diastole (E) and late diastole with atrial contraction (A) were measured. E/A velocity ratios were calculated for each cardiac cycle. The duration of the Isovolumetric Relaxation Time (IVRT) and Deceleration Time (DT) was also
### LA DILATATION

<table>
<thead>
<tr>
<th>Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild: LA area increased but less than the LV area</td>
</tr>
<tr>
<td>Moderate: LA area up to 1.5 times the LV area with sphericity index 0.75-1</td>
</tr>
<tr>
<td>Severe: LA area &gt; 1.5 the LV area with sphericity index &gt; 1</td>
</tr>
<tr>
<td>There is compression of right cavities</td>
</tr>
</tbody>
</table>

#### Table 3.2. Classification of left atrial (LA) enlargement

### LV REDUCTION

<table>
<thead>
<tr>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild: Obliteration or occupation by thrombus of less than 10% the LV cavity area measured in two planes</td>
</tr>
<tr>
<td>Moderate: Obliteration/occupation by thrombus of 10-25% the LV cavity area measured in two planes and/or reduction of the longitudinal diameter with sphericity index 0.50-0.75</td>
</tr>
<tr>
<td>Severe: Obliteration/occupation by thrombus of &gt;25% the LV cavity area measured in two planes and/or reduction of the longitudinal diameter with sphericity index &gt; 0.75</td>
</tr>
</tbody>
</table>

#### Table 3.3. Classification of left ventricular (LV) reduction
measured. Restriction to the left ventricular filling was defined by the presence of any of the following: (1) E/A ration > 2; (2) IVRT < 160ms; or (3) DT < 120ms (Mandinov, 2000).

The aortic root dimension on diastole, left atrial dimension, left atrial end-systolic and end-diastolic dimension, left ventricular posterior wall thickness and septal thickness were measured by M-mode according to the American Society of Echocardiography recommendations (Lang et al, 2005).

Left ventricular dimensions were indexed to body surface area for participants from the clinical registry, and for age in participants in community studies and compared to normal values.

Evaluation of the Mitral Valve

The evaluation of the mitral valve was done using bidimensional in several planes, using the M-mode along the leaflets to confirm their thickening and measuring the length of the leaflets. The dimensions of the mitral annulus were measured from both 4-chambers and long axis views. Colour Doppler evaluation was used for semi-quantitative estimation of mitral regurgitation severity according to previous validated criteria (Table 3.4), taking into account the width and depth of regurgitant jets from different views (4-chambers, short-axis, parasternal). The relation between jet size and left atrial size was assessed by visual integration of the information obtained from all the different transducer positions, to ensure exploration of the entire receiving chamber.
Mitral Regurgitation | Visual evaluation of the regurgitant jet in the LA  
---|---  
**Mild (grade 1)** | jet width visually judged to be $\leq 1/3$ LA width  
(or less than $1/3$ the anteroposterior LA dimension);  
**Moderate (grade 2)** | jet width visually judged to be between $1/3$ and $< 1/2$ LA width (and not reaching the roof of the atrium)  
**Severe (grade 3)** | jet width greater than one half of LA width  
(or jet reaching the roof of the atrium)  

**Table 3.4. Classification of mitral regurgitation**

*Evaluation of the Right Atrium*

The lateral and superoinferior dimensions of the right atrium were measured by bidimensional echocardiography from the apical 4-chamber view using an inner-edge-to-inner-edge measurement. The right atrial area was measured by planimetry of the inner contour from the apical 4-chamber view, excluding the confluences of the inferior vena cava from the tracing. The plane of tricuspid annulus was taken as the inferior right atrial border. The right atrial area was indexed to body surface area, for participants from the clinical registry. Additionally, an sphericity index was calculated as the ratio between lateral and superoinferior diameters.

The classification of right atrial enlargement was done using the criteria presented on **Table 3.5**.
RA DILATATION | Features
---|---
Mild | RA area increased less than 1.5 times the RV area
Moderate | RA area between 1.5 and 2 times the RV area + sphericity index between 0.75-1.0
Severe | RA area is greater than twice the RV area; dilated RA compresses the left cavities; dilated IVC; sphericity index > 1

**Table 3.5.** Classification of right atrial (RA) enlargement

**Right Ventricle**

The lateral and superoinferior dimensions of the right ventricle were measured by bidimensional echocardiography from the apical 4-chamber view using an inner-edge-to-inner-edge measurement. An sphericity index was calculated as the ratio between lateral and superoinferior diameters in 4-chambers view.

The right ventricular end-systolic and end-diastolic areas were determined by planimetry of the inner contour from the apical 4-chamber view. In few cases for patients submitted to surgery the area-shortening fraction was used to estimate the ejection fraction of the RV since it is not influenced by the ventricular geometry. It was calculated using the following formula:

\[
\text{Area Shortening Fraction} = \frac{(\text{End diastolic area} – \text{End systolic area})}{\text{End diastolic area}}.
\]
The right ventricular systolic function was evaluated through a visual semi-quantitative scale (+++ good contractility; ++ moderate dysfunction; + poor contractility) as previously done by Cherian et al (1982) using bidimensional guided M-mode in several incidences (4-chambers, parasternal long axis, parasternal short axis and sub-costal views).

The diastolic function parameters were measured through evaluation of the tricuspid inflow by pulsed Doppler, namely E wave peak, A wave peak, and E/A ratio.

RV dimensions were indexed to body surface area for participants from the clinical registry, and age in participants in community studies. The criteria used to classify reduction in right ventricular size are presented in Table 3.6.

<table>
<thead>
<tr>
<th>RV REDUCTION</th>
<th>Criteria on 4-chambers view</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild</td>
<td>RV trabecular portion obliteration with cavity reduction &lt; 25%</td>
</tr>
<tr>
<td>Moderate</td>
<td>RV trabecular portion obliteration with cavity reduction 25-50%; Absence of RV apical notch; sphericity index 0.75-1.0</td>
</tr>
<tr>
<td>Severe</td>
<td>RV wall retraction (apical “notch”) or obliteration with cavity reduction &gt; 50%; sphericity index &gt;1.0</td>
</tr>
</tbody>
</table>

Table 3.6. Classification of reduction of right ventricular (RV) cavity size
Evaluation of the Tricuspid Valve

The evaluation of the tricuspid valve was done using bidimensional mode in several planes, M-mode along the leaflets to confirm their thickening and also by measuring the length of the anterior and septal leaflets. The dimensions of the tricuspid annulus were measured from both the 4-chambers and short axis views. The antero-posterior diameter of the right ventricular outflow tract was measured by bidimensional-guided M-mode echocardiography from the parasternal short-axis view at the base of the heart, from the trailing edge of the anterior RV wall to the leading edge of the aortic valve.

Colour Doppler evaluation of tricuspid regurgitation was used for semi-quantitative estimation of tricuspid regurgitation severity taking into account the width and depth of regurgitant jets from different views (4-chambers, short-axis and sub-costal). The relation between jet size and right atrial size was assessed by visual integration of the information obtained from all the different transducer positions, to ensure exploration of the entire receiving chamber. Another criteria used to define severe tricuspid regurgitation was the lack of aliasing of the jet (indicating absence of turbulence across the annulus) and its large width at origin, associated with severe distortion of the tricuspid valve leaflets that causes non-cooptation.
Table 3.7. Classification of tricuspid regurgitation (TR)

<table>
<thead>
<tr>
<th>DEGREE OF TR</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mild (grade 1)</td>
<td>Jet width $\leq \frac{1}{4}$ RA width</td>
</tr>
<tr>
<td>Moderate (grade 2)</td>
<td>Jet width between $\frac{1}{4}$ and $1/2$ of the RA width</td>
</tr>
<tr>
<td>Severe (grade 3)</td>
<td>Non-turbulent jet or free tricuspid regurgitation</td>
</tr>
</tbody>
</table>

3.2.2.3. Definition of Echocardiographic Features

The echocardiographic features were divided in major or minor criteria according to their specificity for diagnosis, relevance for decision on the management option and importance for prognosis.

Major criteria

Echocardiographic features that are distinctive or pathognomonic of endomyocardial fibrosis and that have a major impact on management were considered major criteria. An individual score was attributed to each feature according to its relevance for structural and hemodynamic changes. The following were considered major criteria for diagnosis of EMF:

(1) Endocardial plaque of at least 5mm width or endocardial thickening greater than 1mm
(2) Patchy endocardial thickening in two or more ventricular walls

(3) Obliteration of ventricular apices or valve recesses

(4) Ventricular thrombi or spontaneous contrast in the absence of ventricular dysfunction

(5) Reduction of the RV cavity volume due to exclusion of the trabecular portion

(6) Restriction of AV valve leaflet movements due to adherence to the ventricular walls

We defined endocardial plaques as areas of endocardial thickening of more than 5mm width and thicker than 1mm as a major criterion for diagnosis of EMF. The term large plaque was used when endocardial thickening exceeded 10 mm in width and 2mm in depth.

**Minor criteria**

The echocardiographic findings that are not specific but may suggest EMF were classified as minor criteria. Although commonly found in patients with EMF, these features are not exclusive to this condition and do not define the disease if found in isolation. They were considered to have little influence on management and prognosis, when compared to major criteria previously described. The following were considered minor criteria for the diagnosis of EMF:

(1) Patchy endomyocardial thickening localized to one ventricular wall

(2) Ventricular restrictive filling pattern

(3) Diffuse thickening of the atrioventricular valve leaflets

(4) Enhanced density of the moderator band or intraventricular trabeculae
(5) Abnormal M-movement of the interventricular septum and/or posterior LV wall
(6) Enlarged atrium with normal-sized homolateral ventricle
(7) Presence of thickened “false tendon” of the LV

Patchy endocardial thickening corresponded echocardiographically to small areas of enhanced density of the endocardium with less than 1mm in depth and evenly distributed in the ventricular walls.

3.2.2.4. Classification

The echocardiographic appearance of EMF varies both in terms of distribution and severity of structural lesions and hemodynamic abnormalities.

3.2.2.4.a) Distribution of lesions

We used the criteria described above to describe lesions in each side of the heart. The scoring system was applied to each ventricle separately, and according to the distribution of the cardiac lesions we classified the disease in three types REMF, LEMF and BEMF. REMF was defined when characteristic lesions affected exclusively or predominantly the right side of the heart, with a score that was greater than the score of left heart lesions by more than 3 points. LEMF designated patients with isolated or predominant affection of the left side of the heart, with a score greater than that of right-sided lesions by more than 3 points. Finally, BEMF designated patients with no clear predominance of lesions in one side of the heart, in which the difference of scores between the two sides of the heart was 3 points or less.
3.2.2.4.b) Severity of EMF

The assessment of the severity of the disease was done through a scoring system to quantify the structural and hemodynamic lesions (Table 3.1). Four progressive grades of severity were defined in view of management and prognosis namely:

**Grade I (or Mild EMF)**

Endocardial plaque or patchy thickening associated with thickening of the atrioventricular valve leaflets without any other structural or hemodynamic abnormality.

**Grade II (or Moderate EMF)**

Large endocardial plaques, apical valve recess ventricular obliteration, and mild to moderate atrioventricular valve regurgitation. There is mild to moderate atrial dilatation, mild ventricular cavity reduction, and preserved myocardial function.

**Grade III (or Severe EMF)**

Large endocardial plaques, moderate reduction of ventricular cavity dimensions, marked atrial dilatation and severe atrioventricular valve dysfunction. Myocardium is visible underneath the endocardial thickening and ventricular function is nearly normal.

**Grade IV (or Advanced EMF)**

Presence of large endocardial plaques associated with severe reduction of the ventricular size and compression of the contralateral cavities by the severely dilated atrium.
Endocardial calcification, poor ventricular contractility, and large persistent effusions (pericardial, peritoneal and/or pleural) are other features of advanced EMF.

3.2.3. Staging and Mode of Progression

The staging and characterisation of the mode of progression of EMF needed a minimum six months follow up with monthly visits and, for logistical reasons was only used for patients from the clinical registry. This classification aimed at distinguishing activity and chronicity, which are thought to influence management and prognosis.

Activity was defined as the finding of two or more of the following signs in a patient with echocardiographic diagnosis of EMF:

1. unexplained fever, recurrent facial edema, urticaria or asthma-like episodes
2. severe hypereosinophilia (absolute eosinophil count > 1.5 x 10^9/L)
3. ventricular thrombi not attributable to severe myocardial dysfunction
4. evidence of pancarditis with acute heart failure
5. increased C-Reactive Protein and/or erythrosedimentation rate

The persistence of signs of activity and the progression of structural and/or functional abnormalities during the first 6 months after diagnosis, defined three distinct stages of the disease: Active with remission, Active persistent and Rapidly progressive.

Active EMF with remission refers to patients that had signs of activity at diagnosis that regressed during the first six months of follow up, while Active persistent disease signified persistence of signs of activity for 6 months or more. Finally, we used the
Chapter 5 Clinical Characterisation

expression Active rapidly progressive to define patients with persistence of signs of activity after the initial episode of heart failure, who progressed to death or a higher degree of severity in less than six months.

Patients with history of recent onset of symptoms (less than 6 months) were considered to have acute disease. The definition of chronicity was the presence of signs and symptoms attributable to EMF for more than six months and echocardiographic features of established EMF on admission. Depending on the occurrence of recrudescence of disease during the first six months of follow up the patients were classified as having quiescent or recurrent disease. Patients who develop recrudescence of active disease in the course of follow up, going back to a quiescent stage thereafter, were considered to have chronic recurrent disease.
3.3. Results

We performed transthoracic echocardiography on 1534 individuals between November 2004 and October 2007: 1063 individuals of all ages randomly selected from the community, 296 school children randomly selected in Inharrime district, and 175 patients from the national clinical registry established at the Heart Institute – Maputo (Table 3.8). The patients from the clinical registry were submitted to several echocardiographic examinations according to their clinical needs. A total of 411 individuals with the diagnosis of Endomyocardial Fibrosis were studied, of which 236 detected in the community.

During the prevalence study 184 eligible individuals were not present at home (99 men who worked in the neighboring countries and represented 53% of the eligible participants) and 2 had psychiatric problems with uncontrolled behavior that prevented their evaluation. Regarding the school children cohort, 36 eligible students were not examined: 24 were absent, 7 did not have the informed consent, and 5 abandoned the school during the period between preparation of fieldwork with signature of the informed consent and the visit to school to perform the echocardiographic evaluation.

The mean age of the different populations varied between 8 and 22 years (Table 3.9). There were slightly more males than females on the clinical registry, while in the community more females were studied.

Of the 236 individuals with echocardiographic signs of EMF found during the community research only 52 (22.0%) had complaines or recalled any particular disease,
confirming the high occurrence of clinical-echocardiographic dissociation in EMF (Table 3.10).

<table>
<thead>
<tr>
<th>Table 3.8. Summary of the population studied</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Period</strong></td>
</tr>
<tr>
<td>Nov04 – Oct07</td>
</tr>
<tr>
<td>Jan06-May06</td>
</tr>
<tr>
<td>Oct06 -</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Participants</strong></th>
<th><strong>Clinical Registry</strong></th>
<th><strong>Prevalence Study</strong></th>
<th><strong>Prospective Study</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cases of EMF</strong></td>
<td>175</td>
<td>211</td>
<td>25</td>
</tr>
<tr>
<td><strong>Mean age (range) years</strong></td>
<td>15.7 (3-64)</td>
<td>22.6 (0-96)</td>
<td>8.6 (6-16)</td>
</tr>
<tr>
<td><strong>Female/Male ratio</strong></td>
<td>83/92</td>
<td>611/452</td>
<td>157/139</td>
</tr>
<tr>
<td><strong>Severe Malnutrition</strong></td>
<td>25/175</td>
<td>3/211</td>
<td>0/296</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3.9. Epidemiological features and prevalence of evidence of malnutrition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Individuals with EMF</strong></td>
</tr>
<tr>
<td>----------------------------</td>
</tr>
<tr>
<td><strong>Presence of symptoms/signs of disease</strong></td>
</tr>
</tbody>
</table>

Table 3.10. Number of individuals with EMF who were symptomatic
According to the distribution of lesions in the heart cavities, bilateral disease was the most frequent form of affection (Table 3.11).

<table>
<thead>
<tr>
<th>Echocardiographic Features</th>
<th>Clinical Registry Prevalence Study</th>
<th>Community Studies Prospective Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>BEMF</td>
<td>95 (54.3%)</td>
<td>117 (55.45%)</td>
</tr>
<tr>
<td>REMF</td>
<td>55 (31.4%)</td>
<td>59 (27.96%)</td>
</tr>
<tr>
<td>LEMF</td>
<td>25 (14.3%)</td>
<td>35 (16.59%)</td>
</tr>
</tbody>
</table>

**Table 3.11.** Distribution of EMF lesions in individuals from all studies

**Abnormalities of the Left Side of the Heart**

Early left EMF, usually detected in the community, was characterized by fibrosis of the *false tendons*, thickening of the mitral leaflets (Figure 3.1), apical thrombus (Figure 3.2), and/or obliteration of the apex or the recess between the posterior leaflet and the posterior wall (Figure 3.3). In two cases, thrombi were found in the subvalvar apparatus (Figure 3.2) involving the free edges of both papillary muscles. Flow across the mitral valve showed usually a tall E wave and small A wave, corresponding to early diastolic filling followed by restriction (Figure 3.4). There was no dilatation of the left atrium.

In established LEMF thickening of the endocardium was prominent in the apex (Figure 3.5) and posterior wall behind the recess of the posterior leaflet of the mitral valve (Figure 3.6). The ventricle assumed a spherical shape being hypercontractile in its basal
Figure 3.1. Mild EMF. There is patchy apical thickening of the endocardium, the moderator band on the right ventricle, the false tendon on the left ventricle, and the leaflets of both atrioventricular valves.

Figure 3.2. Early EMF. Left ventricular thrombi are usually located at the apex and subvalvar apparatus, and are associated with septal endocardial thickening on the left side and diffuse thickening of the mitral leaflets.
Figure 3.3. Apical obliteration in LEMF associated with a plaque of fibrosis and mild mitral regurgitation

Figure 3.4. LEMF with marked diastolic dysfunction. There is a tall E wave, short isovolumetric relaxation and deceleration times and the ratio E/A is greater than 3.
Figure 3.5. Echocardiography of moderate LEMF, presenting apical and septal endocardial thickening, spherical left ventricle, thickening of the anterior leaflet of the mitral valve, and increased echos at the posterior papillary muscle.

Figure 3.6. This image of moderate BEMF shows obliteration of the posterior recess of the mitral valve, septal wall endocardial thickening and left atrial dilatation. On the right side thickening of the moderator band and right atrial dilation are visible.
portion. At this stage most cases had a competent mitral valve, but in some cases from the clinical registry there was progression of the lesion in the posterior leaflet with restriction of its movement with appearance of an eccentric mitral regurgitation and passive pulmonary hypertension, that progressed from mild to severe during the six months follow up period. Another common finding in predominant left forms was the so-called M-movement of the interventricular septum (Figure 3.7). The most characteristic picture of advanced LEMF was the association of septal and apical fibrosis with severe eccentric mitral regurgitation due to fusion of posterior leaflet to the wall, disproportionally small left ventricle, aneurismal left atrium and severe pulmonary hypertension (Figures 3.8). Even in patients with extensive endocardial thickening there was never retraction of the LV apex.

The most frequent lesions affecting the left side of the heart are listed in Table 3.12.

Abnormalities of the Right Side of the Heart

Initial lesions consisted of thickening of the moderator band associated with turbulent blood flow inside the trabecular portion of the ventricle between this structure and the ventricular wall, as assessed by color flow Doppler. In the longitudinal view of the right ventricle (short axis of the left ventricle at the level of the aorta) there seemed to be a stretched moderator band separating the inflow and outflow tracts from the trabecular portion (Figure 3.9).
### Table 3.12. Comparison of frequencies of echocardiographic features on the left side of the heart in EMF patients in clinical and community studies

<table>
<thead>
<tr>
<th>Echocardiographic features</th>
<th>Community Studies</th>
<th>Clinical Registry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickening of the AML</td>
<td>121 (51.3%)</td>
<td>148 (84.5%)</td>
</tr>
<tr>
<td>Thickening of the PML</td>
<td>117 (49.6%)</td>
<td>102 (58.3%)</td>
</tr>
<tr>
<td>Endocardial thickening of the septal wall</td>
<td>106 (44.9%)</td>
<td>140 (80.0%)</td>
</tr>
<tr>
<td>Restrictive filling pattern</td>
<td>81 (34.3%)</td>
<td>57 (24.2%)</td>
</tr>
<tr>
<td>Enhanced density of the papillary muscles</td>
<td>78 (33.1%)</td>
<td>130 (74.3%)</td>
</tr>
<tr>
<td>Apical endocardial thickening</td>
<td>55 (23.3%)</td>
<td>57 (32.6%)</td>
</tr>
<tr>
<td>Thickening of the false tendon</td>
<td>47 (19.9%)</td>
<td>11 (6.29%)</td>
</tr>
<tr>
<td>Obliteration of the recess behind the PML</td>
<td>27 (11.4%)</td>
<td>30 (17.1%)</td>
</tr>
<tr>
<td>Mitral regurgitation</td>
<td>26 (11.0%)</td>
<td>107 (61.1%)</td>
</tr>
<tr>
<td>Severe dilatation of the left atrium</td>
<td>13 (5.5%)</td>
<td>87 (49.7%)</td>
</tr>
<tr>
<td>Apical thrombus/obliteration</td>
<td>4 (1.7%)</td>
<td>13 (7.4%)</td>
</tr>
<tr>
<td>Non-apical intraventricular thrombus</td>
<td>1 (0.4%)</td>
<td>5 (2.9%)</td>
</tr>
<tr>
<td>Left atrial thrombus</td>
<td>0 (0%)</td>
<td>7 (4.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>236</td>
<td>175</td>
</tr>
</tbody>
</table>

PML posterior mitral leaflet; AML anterior mitral leaflet
Figure 3.7. Typical M-movement of the interventricular septum, found in some patients with predominant LEMF. There is thickening and abnormal movement of the posterior leaflet of the mitral valve.

Figure 3.8. Severe LEMF endocardial thickening and organized thrombus are visible (arrow). Mitral regurgitation is usually directed to the posterior atrial wall, and there is dilatation of the right cavities due to passive pulmonary hypertension.
Figure 3.9. This picture of the longitudinal view of the right ventricle shows reduction of ventricular cavity size, which in the initial stages is due to obliteration of the trabecular portion of the right ventricle (arrow).

Figure 3.10. Restriction of the movements of tricuspid valve leaflets (arrow) is the mechanism that explains regurgitation in initial phases of EMF, but later on progressive enlargement of the right atrium leads to dilatation of the tricuspid ring and free regurgitation. The atrium and the ventricle work as a single chamber and spontaneous contrast is usually seen in both cavities in advanced disease (right).
RV trabecular cavity obliteration was usually associated with mild to moderate tricuspid regurgitation caused by restriction to the movement of the anterior and septal leaflets of the tricuspid valve. The leaflets had some attachments to the wall leading to an echocardiographic picture similar to the “Ebstein Malformation”, with dilatation of the tricuspid annulus (Figure 10), and usually tricuspid regurgitation with the jet originating inside the ventricular cavity, from the trabecular portion of the ventricle.

Severe REMF was characterized by retraction of the ventricular cavity due to elimination of the trabecular portion of the cavity (Figure 3.11). Severe tricuspid regurgitation with almost no turbulence was associated with restriction of leaflet movements caused by involvement of the papillary muscles in the fibrotic process, as well as to dilatation of the annulus that followed severe right atrial dilatation (Figure 3.11). Most patients had spontaneous contrast inside the right atrium extending in anterograde fashion to both the inflow tract of RV and retrograde to the inferior vena cava and dilated supra-hepatic veins. These did not usually show the normal respiratory changes, indicating increased systemic venous pressure.

The reduction of the right ventricular cavity size was partially compensated for by dilation of the outflow tract. In patients with advanced REMF free tricuspid regurgitation was the rule due to a completely adherent valve apparatus and aneurismal RA, and was usually associated with dynamic intracavitary echos or large thrombi (Figure 3.12). There was equalization of high-pressure in the atrium, ventricle and pulmonary artery, diastolic opening of the pulmonary valve could be noticed. The aneurismal right atrium
caused heart distortion and compression of the left cavities making it difficult to evaluate the presence of mitral dysfunction. Abundant pericardial effusion and compression of left cavities prevented adequate evaluation of the left ventricular function in very advanced cases of REMF, as shown in Figure 3.13. The structural features of REMF were distinctive and are summarized in Table 3.13.

Figure 3.11. Severe REMF has fibrous plaques separating the trabecular portion of the right ventricle from its admission chamber (left) or retraction of the right ventricle with the characteristic “apical notch” (arrow), right atrial thrombi and pericardial effusion.

Figure 3.12. Advanced EMF. Severe cavity distortion, intra-atrial thrombi and large pericardial effusion are common features.
### Echocardiographic features

<table>
<thead>
<tr>
<th>Echocardiographic features</th>
<th>Community</th>
<th>Registry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thickening of the moderator band</td>
<td>141 (59.7%)</td>
<td>28* (16.0%)</td>
</tr>
<tr>
<td>Apical endocardial thickening</td>
<td>115 (48.7%)</td>
<td>143 (81.7%)</td>
</tr>
<tr>
<td>Tricuspid regurgitation</td>
<td>49 (20.7%)</td>
<td>87 (49.7%)</td>
</tr>
<tr>
<td>Apical /obliteration of trabeculae</td>
<td>32 (13.6%)</td>
<td>49 (28.0%)</td>
</tr>
<tr>
<td>Apical notch</td>
<td>24 (10.2%)</td>
<td>14 (8.0%)</td>
</tr>
<tr>
<td>Apical retraction</td>
<td>4 (1.6%)</td>
<td>37 (21.1%)</td>
</tr>
<tr>
<td>STL fusion to the septal wall</td>
<td>27 (11.4%)</td>
<td>53 (30.3%)</td>
</tr>
<tr>
<td>Severe dilatation of the right atrium</td>
<td>20 (8.5%)</td>
<td>66 (37.7%)</td>
</tr>
<tr>
<td>Intraventricular thrombus</td>
<td>14 (5.9%)</td>
<td>6 (3.4%)</td>
</tr>
<tr>
<td>Distended IVC</td>
<td>6 (2.5%)</td>
<td>52 (29.7%)</td>
</tr>
<tr>
<td>Right atrial thrombi</td>
<td>0 (0%)</td>
<td>33 (18.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>236</td>
<td>175</td>
</tr>
</tbody>
</table>

STL septal tricuspid leaflet; IVC inferior vena cava

**Table 3.13.** Comparison of echocardiographic features most frequently found on the right side of the heart in patients with EMF in clinical and community studies
Echocardiographic findings in Bilateral EMF

Individuals with initial BEMF had usually fibrosis of the moderator band, fibrosis of the false tendon, thickening of both atrioventricular valves and occasionally, left apical thrombus (Figure 3.14). In established disease the heart had a peculiar aspect of enlarged atria with small ventricles, the so-called “Mickey mouse heart” in the two-dimensional four chambers view (Figure 3.15).

In patients with severe BEMF coexistence of mitral and tricuspid regurgitation and biventricular size reduction was notorious (Figure 3.16). The affection of the right ventricle, by reducing the right ventricular output and pulmonary perfusion, partially reduced the effects of the pulmonary venous hypertension caused by left ventricular disease, favoring a better outcome than in pure LEMF.

Severity of EMF

While the majority of individuals with EMF in the community have mild to moderate disease (224/236; 94.9%) those that seek medical attention have predominantly severe to advanced (94; 57.3%), as shown in Table 3.14. Remarkably, six out of 12 individuals with severe disease found in the community denied having any symptoms and did not present any clinical signs of the disease.
Figure 3.13. Advanced EMF is associated with multiple effusions (pericardial, pleural and peritoneal). Pericardial effusion further impairs diastolic function and precipitate low cardiac output syndrome.

Figure 3.14. Mild BEMF. Right ventricular obliteration coexists with endocardial thickening of the left side of interventricular septum (arrow).
Figure 3.15. Progression of BEMF is characterized by bilateral apical fibrosis (arrows), ventricular size reduction and atrial enlargement, resulting in the classical “Mickey mouse heart”.

Figure 3.16. Right ventricular cavity obliteration progresses to fibrosis but there is normal myocardium underneath it (arrow).
Follow-up and Staging

Signs of activity were present in 90 (51.4%) patients from the clinical registry. The most frequent clinical features associated with laboratory signs of active disease were facial edema in 54 patients and hypereosinophilia present in 43.

Sixty-six (37.7%) patients had recent onset EMF with signs of activity at time of diagnosis. Of these, 10 had rapidly progressive disease and died within 2 months of diagnosis. In all cases there were intraventricular thrombi and myocardial dysfunction. There was persistence of activity in 18 patients.

Table 3.14: Distribution of EMF by severity in the three populations studied
3.4. Discussion

The most important outcome of this study was the improvement in echocardiographic assessment of EMF patients, including the possibility of diagnosis of early stages in large-scale community research, and risk stratification of patients in the clinical setting. By applying these new diagnostic criteria we were able to standardize the diagnosis of EMF in asymptomatic individuals in the community, and describe systematically the structural and hemodynamic abnormalities in the clinical setting, contributing in this way to a comprehensive classification and tailored management of EMF patients.

The clinical diagnosis of EMF can only be done when structural changes are advanced and lead to hemodynamic abnormalities. On the other hand, there is no knowledge of a single biological marker for the disease. Echocardiography is a non-invasive diagnostic tool for cardiovascular diseases that has become widely available in developed countries. Despite its use being currently restricted to some referral centres of urban areas in most developing countries, this technique has the potential to be increasingly used in health structures from these poor areas. Moreover, the use of new portable user-friendly and battery-operated machines can potentially increase the accessibility of echocardiographic diagnosis for people from rural areas of developing countries that are endemic for EMF. We therefore concentrated our efforts in developing echocardiographic criteria that could be applied for early diagnosis, follow-up and management of this condition in endemic areas.
Echocardiographic features: implications for diagnosis and management

Endocardial thickening was the hallmark of established EMF on echocardiography, forming large plaques or having a patchy distribution in one or both ventricles. Mural endocardial plaques interfered mainly with diastolic ventricular function by preventing normal relaxation of the underlying myocardium. When endocardial fibrosis was extensive, besides impeding normal filling of the ventricles due to marked reduction of the ventricular cavity size, there was also variable compromise of the systolic function leading to further reduction in cardiac output. We therefore considered the extension of endocardial fibrosis an important marker of the severity of the disease.

Ventricular thrombosis or “spontaneous contrasts”, echocardiographic findings usually associated with stasis and severe myocardial dysfunction, were frequently seen in otherwise normal ventricles. Most left ventricular thrombi were apical, but there were also thrombi involving the subvalvar apparatus and the left ventricular outflow tract. On the right side the trabecular portion of the ventricle was the area most prone to thrombi. A high score was attributed to the presence of ventricular thrombi because they pose an enormous vital risk to the patient and are a major determinant of management and prognosis. Interestingly, most patients with intraventricular thrombi claimed being asymptomatic and did not recall any illness that could be related to thromboembolism.

Obliteration of the ventricular cavity causes reduction of the effective volume of the affected cavity. When obliteration involves the valve apparatus it additionally leads to restriction of the leaflet and chordae movements that may progress to its fusion to the ventricular wall, resulting in severe atrioventricular valve dysfunction. Right ventricular
obliteration, consisting of partial or complete exclusion of the trabecular portion of the right ventricle from the circulation, was frequent in asymptomatic individuals from the community. Although organization and fibrosis of the mural thrombus and its adjacent endocardium has been said to be the main mechanism for ventricular retraction (Brockington et al., 1967; Connor et al., 1967), in view of our findings in initial phases of the disease in the community, progressive fusion of the obliterated trabeculae following its exclusion from the circulation seems to be the most plausible process leading to cavity retraction. On the right ventricle this process is associated to compaction of the trabecular portion that in severe cases results in thinning of the apical wall and the creation of an “apical notch”, which is a distinctive feature of advanced right endomyocardial fibrosis (Connor et al., 1967).

Left ventricular obliteration affected usually the apex and/or the posterior leaflet recess excluding these parts from the ventricular cavity. This abnormality is associated with increased thickness of the apex and seemed to correspond to early phases of left affection. Although there was never retraction of the apical wall, individuals with large apical plaques had thin wall with a spherical left ventricle, due to considerable reduction of its longitudinal diameter with dilatation of the basal portion when it was spared from the fibrous process. The restricted movement of the fibrotic apex of the left ventricle and its obliteration are associated with a compensatory contractile mechanism that results in exaggerated and distinctive motion of the basal portion of the left ventricle in early diastole (Dienot et al., 1981; Acquatella, 1983). On M-mode the septal wall can assume
an M-shaped movement on the basal portion of the ventricle, a finding that was common only in advanced lone or predominant left EMF. On the posterior wall on mode-M there is a rapid increase in left ventricular dimensions in early diastole, which is not followed by any further variation in dimensions through the remaining of the diastole (Dienot et al, 1981).

Patchy endocardial thickening was extremely frequent in individuals from the community, and was considered a feature that suggested the diagnosis of EMF in the presence of other minor or major criteria. The low score attributed to this characteristic was due to the fact that patchy endocardial thickening may be present in patients with other cardiac pathology affecting the endocardium, although it is also true that most of that pathology afflicts adults and not children and adolescents as is the case in EMF. On the other hand, no large-scale echocardiographic screening studies have been performed in African populations. It can therefore be argued that these small-thickened areas in the endocardium constitute a normal phenotype in these populations, highlighting the need for further research in the communities to determine the spectrum of normal variants in African hearts.

The most common ventricular filling pattern was the characteristic brisk early diastolic filling with poor filling in the remainder of diastole, the absence of respiratory changes and the presence of a normal pericardium, which enabled distinction from constrictive pericarditis. The tricuspid filling pattern was difficult to evaluate since most patients with severe diastolic dysfunction had free tricuspid regurgitation, and the right cavities acted
physiologically as a single chamber due to severe restriction of the tricuspid leaflets movement.

Diffuse irregular thickening of the atrioventricular valve leaflets associated with thin chordae was a common finding of EMF. This contrasted with the more prominent thickening of the free borders of the leaflets extending to the chordae that is typical of rheumatic valve disease, constituting one of the pitfalls in differentiating the two diseases (Hassan et al, 2005), which are almost equally prevalent in some areas of Africa (Freers et al, 1996b). The presence of diffusely thickened atrioventricular valve leaflets, obliteration of left ventricular apex, small or normal-sized ventricle with severe atrial dilatation, and significant pulmonary hypertension should favour the diagnosis of EMF.

Enhanced densities of moderator band and/or left ventricular false tendons were considered the initial echocardiographic features of EMF, and constituted the most common mural changes in mild or grade I disease. The moderator band is a cordlike structure crossing at mid-right ventricle between septum and free walls, which contains the right bundle branch conduction tissue. The left ventricular false tendons are fibrous or fibromuscular bands that stretch across the left ventricle from the septum to the free wall. They can also tether to a papillary muscle, but unlike the chordae tendineae, do not connect to the mitral leaflets, being considered anatomic variants. Thickening of these structures was frequently associated with patchy endocardial thickening and the association was thought to correspond to early phases of EMF, an hypothesis that needs to be confirmed in follow up studies.
Most echocardiographic criteria for assessing ventricular size and function have been
developed for the left ventricle, but the ability of two-dimensional apex echocardiography
to evaluate right ventricular and right atrial size has also been shown in children with
congenital heart defects (Fontana et al, 1982). Our criteria for assessment of left
ventricular function did not include the conventional volume measurements, which are
difficult to apply in severe and advanced EMF owing to the fact that there is marked heart
cavity distortion. The Simpson’s method assumes an ellipsoid shape of the left ventricle,
which is not the case in most cases of EMF. In LEMF there is reduction of the
longitudinal dimension of the ventricle that assumes a round shape, while in REMF the
bulging of the interventricular septum compresses and deforms the left ventricle, which
assumes a “banana” shape.

The evaluation of myocardial function in EMF is biased by the presence of endocardial
fibrosis, which restricts the function of the underlying myocardium, even if the
myocardial texture seems to be maintained. We therefore frequently evaluated the
ventricular function using the visual semi-quantitative scale, a tool that has been
proposed for estimation of myocardial function in EMF (Cherian et al, 1982). However,
in mild and moderate disease with no paradoxical movement of the interventricular septum, it seems reasonable to use the shortening fraction of the left ventricle.

Another important part of our study was to develop and apply criteria for determination
of the degree of cavity distortion and estimation of ventricular function, based on
measurements of cavity dimensions, area-shortening fraction and sphericity index. The
area-shortening fraction of the left ventricle, despite its well-known limitations and
disadvantages, was considered a reasonable tool for assessment of its systolic function in EMF patients and was used in the clinical setting, mainly for follow-up of patients submitted to surgery.

The evaluation of the right ventricle, the ventricle more frequently affected in EMF (Somers et al., 1978), is still the subject of debate among the medical community. The irregular geometry of the right ventricle, does not allow the use of parameters used for the left ventricle. Also, remodeling of the right ventricle seems to be a complex process that may include unique elements not observed in left ventricular remodeling (Kret and Arora, 2007). However, it has been proven that two-dimensional echocardiography, with apical and four-chambered view, enables accurate visualization of the right atrium and ventricle (Bommer et al., 1979). The right ventricular outflow tract shortening fraction, a simple and noninvasive measure of systolic function (Lindqvist et al., 2003), does not seem adequate to assess the global right ventricular function in EMF, owing to the presence of compensatory mechanisms leading to dilatation and hypercontractility of the right ventricular outflow tract as a result of trabecular cavity obliteration or retraction.

The sphericity index is a measure of ventricular size and shape that has been used to assess changes that occur in non-ischemic cardiomyopathy from valvular or diffuse myocyte origin, and is a way to quantify the abnormal geometrical changes that accompany heart failure in dilated failing left ventricles (Di Donato et al., 2006). The global measurements of sphericity index may be useful for global disease but fails to detect regional apical shape abnormalities in dilated cardiomyopathy. We think that due to geometrical changes associated with EMF the sphericity index may be good tool for
measurements of abnormalities of ventricular shape and volume associated with the severity of this condition.

A major limitation of the sphericity index is that it relies on analysis of the entire chamber obtained by the global axis ratio, which corresponds to a single plane ratio, reflecting a linear alteration in the two axes of the entire chamber. This is of particular importance in EMF where regional changes seem to be present in all phases of the disease in a high proportion of patients. Its use needs to be further tested, validated and eventually widespread.

The use of the new criteria of diagnosis and classification of EMF in the clinical setting allowed identification of patients who can benefit from surgery. We think that patients with mild or grade I EMF do not benefit from surgery at this stage, while structural abnormalities characteristic of advanced or grade IV disease are very often unsuitable for corrective surgery. This unsuitability is usually related to the severity of heart abnormalities, such as extensive endocardial thickening with disappearance of the atrioventricular valve apparatus, thick endocardium without reasonable amount of myocardium visible underneath it, extensive calcification, severe myocardial dysfunction.

For patients whose abnormalities can be total or partially corrected by surgery (grades II/III) the new classification allows the planning of tailored surgical techniques and the anticipation of post-operative complications. The addition of a scoring system to the traditional topographic classification improved the management of EMF patients by identifying those that can benefit from surgery and by attempting the stratification of their operative risk.
We found remarkable differences in the frequency of several echocardiographic features used for diagnosis and classification of EMF, when comparing the participants in the community studies with patients from the clinical registry. We here discuss some of these differences.

In severe and advanced disease, which constitutes the major group of patients from the clinical registry, the posterior mitral leaflet was engulfed in the fibrous tissue of the posterior wall, thus explaining the low prevalence of thickening of the posterior leaflet in the first group, which had preponderance of mild disease. For similar reasons, there was high frequency of thickening of false tendons in the LV and the moderator band on the RV in the community, which contrasts with the low frequency in patients of the national registry, who presented mostly severe and advanced forms of the disease. We think that false tendons and the moderator band are involved in process that leads to obliteration and retraction of the ventricle, becoming part of the left ventricular apical fibrosis or the floor of the RV obliterated cavity respectively. The evaluation of the restrictive filling pattern was conditioned by the existence of severe atrioventricular valve regurgitation in severe and advanced EMF, more frequent in the clinical setting.

Ventricular thrombi were more frequent in the community group, probably representing early stages of the disease, while atrial thrombi occurred mainly in patients with advanced stages found in the clinical series.

Atrioventricular valve regurgitation, a feature that follows distortion of the subvalvar apparatus and the fusion of leaflets to the ventricular walls, was a major determinant of clinical symptoms, being therefore more common in the clinical series.
The size of the left atrium has been shown to correlate with the exercise capacity in EMF patients (Mady et al, 2005). In a large cohort of patients with hypertrophic cardiomyopathy from a nationwide registry, a marked increase in left atrial dimension was predictive of long-term outcome, independent of co-existent atrial fibrillation or outflow obstruction (Nistri et al, 2007). This study concluded that left atrial dimension is a novel and independent marker of prognosis in hypertrophic cardiomyopathy, particularly relevant to the identification of patients at risk for death related to heart failure.

We consider that systematic echocardiographic evaluation, in addition to serum markers of diastolic dysfunction such as NT-proBNP (Mady et al, 2008), could help identifying prognostic indicators in EMF patients. However, at this stage of our research we were not able to carry out research aiming at identification of such indicators.
**Conclusions**

These studies confirm the need for using echocardiographic screening in epidemiological research on EMF, since most individuals affected by the disease in the community were asymptomatic. Research aiming at characterization of the spectrum of normal features on echocardiography of the African population is mandatory, since it can be argued that some echocardiographic features considered being mild or initial EMF lesions represent in fact variants of the normal features in this population.

The use of criteria for standardization of the diagnosis and classification of EMF is useful for better understanding of the pathogenesis and pathophysiology, and will allow comparison between series in different endemic areas. The usefulness of these new criteria and scoring system needs validation on follow-up studies.

The usual assumptions made when calculating parameters for assessment of systolic and diastolic function of both ventricles are not present in a considerable number of individuals with moderate, severe and advanced disease. Therefore, non-conventional measurements and indexes must be used to evaluate left and right ventricular function in EMF.

Tissue Doppler Imaging has a great potential for research on early stages of EMF and must be envisaged in the future, since it could probably uncover early regional myocardial changes on echocardiography, improving the diagnosis of the initial phases of the disease.
The addition of new markers of diastolic dysfunction like NT-proBNP to the above-described criteria may eventually improve the pre-clinical diagnosis of EMF, and be used in community research.
Chapter 4

EPIDEMIOLOGY OF EMF IN INHARRIME
4.1. **Background**

In Mozambique, EMF has been reported from clinical series (Bijlsma, 1979; Ferreira *et al* 1992). An analysis of referrals to a cardiovascular unit over a 10-year period showed a striking high attack rate in the district of Inharrime (*Figure 4.1a*) (Ferreira *et al*, 2002). Subsequent research, using clinical screening followed by echocardiographic confirmation of diagnosis, showed a prevalence of 8.9%, indicating that EMF is a major form of heart disease in that region (Ferreira, 2001). It was thought that these figures represent an underestimation of the problem since cardiac auscultation can be normal in a considerable number of EMF patients with severe echocardiographic abnormalities (Chimenti *et al*, 2001; Salemi *et al*, 2005).

Because most studies on EMF have been retrospective and looked at patients in advanced stages of the disease, there is no clear understanding of the natural history of the disease, and its initial stages have not yet been characterized.

We designed two studies using systematic sampling of the community coupled with detailed echocardiographic examination of all selected individuals. The first aimed at determining the prevalence of EMF in Inharrime and investigating its determinants, while the second established a cohort of children selected by echocardiography aiming at determining the incidence and describe the natural history of the disease. Research facilities were implanted in Inharrime village to allow the implementation of such large-scale studies in this remote area (*Figure 4.1b*).
Figure 4.1a. Inharrime’s main village, in the main National Road.

Figure 4.1b. Inharrime Research Centre is located in the main village (Vila Sede)
4.2. *A population study of Endomyocardial Fibrosis in Inharrime*

**Study area – Inharrime District**

Inharrime is a district of Inhambane province in the South East of Mozambique. It is a coastal district with 76518 inhabitants, mostly peasants that rely on subsistence agriculture and small-scale fishing for food supply. This district has two Posto Administrativo (Inharrime and Mocumbi), divided in several Localidades that are in turn subdivided into Povoados. The distribution of the population of Inharrime by Localidades is as follows:

<table>
<thead>
<tr>
<th>Localidade</th>
<th>Inhabitants</th>
<th>Povoados (Selected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vila Sede</td>
<td>3970</td>
<td>8(5)</td>
</tr>
<tr>
<td>Nhanombe</td>
<td>19603</td>
<td>11(7)</td>
</tr>
<tr>
<td>Chacane</td>
<td>12175</td>
<td>7(4)</td>
</tr>
<tr>
<td>Dongane</td>
<td>19169</td>
<td>8(5)</td>
</tr>
<tr>
<td>Mahalamba</td>
<td>13420</td>
<td>14(7)</td>
</tr>
<tr>
<td>Nhapadiane</td>
<td>8181</td>
<td>15(5)</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>76518</strong></td>
<td><strong>63(33)</strong></td>
</tr>
</tbody>
</table>

There are in total 63 Povoados in Inharrime that belong to the 5 Localidades of the district namely: Vila Sede, Nhanombe, Dongane, Chacane, Mahalamba and Nhapadiane.
In the *Posto Administrativo* of Inharrime the *povoados* randomly selected for the prevalence study were: Cambula, Mafassane, Doropa, Chichacha, Nhamuessa, Macupulane, Chemane, Chinhembue, Conge, Sihane, Mazonda, Calangane, Muchipa, Ngulela, Chirreu, Mavunjane, C Aviação, Nhamibe II, 7 De Abril Chilengue -Sede, 7 De Abril Nhacondo-Sede, and 25 De Junho.

The *povoados* randomly selected for the prevalence study in the *Posto Administrativo* of Mocumbi were: Cove, Nhapadiane, Muenda, Malene, Marrucula, Mahalamba, Boquisso, Cuaiaia, Mavela, Naila, Chambe, and Chitava.

The population density varies from high in the main village (Vila Sede) to extremely low in Nhapadiane (where many families have their nearest neighbour as far as 0.5 to 1 Km from their house). There is no electricity or tap water, except in the main village (Vila Sede) where the main administrative headquarters and the Research Centre are located.

### 4.2.1. Population and Methods

*Sample size calculation*

The sample size for the prevalence study in the general population was calculated using an adaptation of the two-stage cluster sampling approach. For practical and logistic reasons we opted for a cluster size of 30 subjects. Calculation of the number of clusters was done using the following formula (Bennet *et al.*, 1991):
\[ C = p (1-p) \frac{D}{s^2 b} \]

**p**: expected prevalence of the condition = 7% (lower prevalence in a previous study)

**D**: study design effect (with a rate of homogeneity of 0.02) = 1.58

**s**: the required standard error of the estimated prevalence, taken to be 1%

**b**: cluster size

Using the formula we arrived at a sample size of 33 clusters, corresponding to a minimum of 990 subjects.

**Sample selection**

Selection of the sample was done in two stages: *povoados* and households. Since there was no data on population size of individual *povoados*, we could not use probability proportional to size sampling. We therefore selected 33 villages by simple random sampling (*Figure 4.2a, 4.2.b*).

The community leaders from the selected villages were asked to create a list of all households of each *povoado*. By this mean we were able to get the number of families in each area of the district (*Table 4.1*) from the community leaders. The first household to be studied in each *povoado* was then selected by random sampling. Second stage clustering was done after arrival to the index household.

A household was defined as a group of people living together for more than three months and sharing the same food on a daily basis. Upon arrival at the index household informed consent was obtained (*Figure 4.3a, 4.3b, 4.3c, 4.3d*). An identification card was issued to the whole family and each member was assigned a unique identification number.
Figure 4.2a. *Posto Administrativo de Inharrime-Sede* with 4 localidades: NHANOMBE and VILA SEDE crossed by the main road, CHACANE at 25 kms from the main road and DONGANE at 27 kms.

Figure 4.2b. *Posto Administrativo de Mocumbi* with 2 localidades (MAHALAMBA at 25 km and NHAPADIANE at 60 km from the national road)
The exact location of the house was registered using a GPS system (Garmin e-trex Legend) to enable future follow up.

<table>
<thead>
<tr>
<th>Localidade</th>
<th>Eligible Participants (families observed)</th>
<th>Percentage from the total sample (%)</th>
<th>Female/Male ratio</th>
<th>Mean Age (years)</th>
<th>Absents (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chacane</td>
<td>167 (28)</td>
<td>13.37</td>
<td>95/72</td>
<td>22.5</td>
<td>13.2</td>
</tr>
<tr>
<td>Dongane</td>
<td>152 (26)</td>
<td>12.17</td>
<td>78/74</td>
<td>21.7</td>
<td>22.4</td>
</tr>
<tr>
<td>Nhanombe</td>
<td>298 (50)</td>
<td>23.86</td>
<td>135/105</td>
<td>22.6</td>
<td>9.6</td>
</tr>
<tr>
<td>Vila Sede</td>
<td>221 (41)</td>
<td>17.69</td>
<td>153/145</td>
<td>21.9</td>
<td>16.4</td>
</tr>
<tr>
<td>Nhapidiane</td>
<td>171 (27)</td>
<td>13.69</td>
<td>91/80</td>
<td>23.8</td>
<td>15.2</td>
</tr>
<tr>
<td>Mahalamba</td>
<td>240 (45)</td>
<td>19.22</td>
<td>130/91</td>
<td>23.2</td>
<td>14.5</td>
</tr>
<tr>
<td>All</td>
<td>1249 (217)</td>
<td>100.0</td>
<td>682/567</td>
<td>22.6</td>
<td>14.9</td>
</tr>
</tbody>
</table>

Table 4.1. Eligible participants, families observed, sex distribution, mean age and percentages of absent people by *localidade* in Inharrime District.
Figure 4.3a. The team had the help of local people to transport material from the nearest point accessible by car to the selected houses

Figure 4.3b. Collection of epidemiological data was done at arrival to the selected houses
Figure 4.3c. GPS registration was performed in all selected houses

Figure 4.3d. Preparation of the houses for echocardiographic examination
Households surrounding the index family within the limits of the village were noted. A number was attributed to each of them for simple random selection of the next household to be visited. This procedure was repeated for the following families until we reached a minimum of 30 subjects.

**Data Collection**

Demographic data from every subject of the selected family were collected in the form of a questionnaire (Appendix 2A). A cardiologist then performed detailed transthoracic echocardiography using a hand-carried echocardiography battery-operated system (Vivid I, General Electrics) with M-mode, two-dimensional and Doppler (pulsed, continuous and colour). The following views were obtained: apical two-, four-, and five-chamber views; parasternal long-axis and short-axis at the level of papillary muscles and aortic valve; subcostal views; and suprasternal views (parallel to the aortic arch). Hard copies with detailed location and type of lesions (Appendix 3) and electronic records of relevant data were kept.

**EMF Diagnosis and Classification**

We defined major and minor criteria for the diagnosis of EMF and used them to define the disease and evaluate its severity (Chapter 3). Two blinded experienced cardiologists reviewed the records. The diagnosis of EMF was accepted when there was agreement of at least two cardiologists, including the researcher.
Detailed clinical examination was performed in all subjects with echocardiographic features of EMF.

Statistical analysis

Frequencies are given as absolute numbers and percentages or proportions; continuous data are reported as mean (standard deviation). We used the chi-square test to compare percentages between groups and logistic regression to test for trends in percentages with a continuous variable. Association between continuous variables was measured using the Pearson correlation coefficient, with a t-test for zero correlation. Analysis of variation within the family was carried out using mixed model analysis of variance (ANOVA). The “±” sign indicates the range of a 95% confidence interval.

Analyses were performed using Minitab, Release 13 (Minitab Inc, State College, PA) and SAS, Release 8.02 (SAS Institute, Cary, NC).
4.2.2. Results

Characterisation of the population

We visited 217 households/families of all selected areas in the district. The average family size was 5.8 (range 1 to 19); the mean number of members per family varied between 5.6 in Mahalamba and 4.4 in Chacane (Table 4.2).

There was an acceptable proportional distribution of the individuals observed in the two main Administrative Councils: 838 (67.09%) in Inharrime Sede and 411 (32.91%) in Mocumbi, which have 53 658 (70.12%) and 22 860 (29.88%) inhabitants, respectively. GPS marking confirmed the random distribution of the households selected which is presented in Figure 4.4a, 4.4b, 4.4c).

The mean age for each Localidade varied from 21.7 to 23.2 years. In all areas the ratio female/male was greater than 1. The mean age, sex ratio and percentage of individuals submitted to echocardiography out of the eligible ones after randomisation is shown in Table 4.2.

Characterisation of the Absents

One hundred and eighty-six eligible individuals were absent and therefore not observed (14.9%). The distribution of the absent people by Localidade varied from 9.6 in Mahalamba to 22.4% in Dongane. The mean age of these people was 22.6 years (Se = 0.5). From the 186 eligible people who did not participate, 99 were adult male who lived
in the neighbouring country (South Africa) working as miners; 79 other selected people were not at home but the family denied any disease as the reason for their absence; and 6 people had travelled to hospitals outside the community searching for treatment, but no clear diagnosis of cardiac disease was suggested by the history given by the family. Echocardiography was not performed to two participants because they had psychiatric problems showing uncontrolled behaviour. There were no refusals to participate.

**Characteristics of Participants with EMF**

We screened 1063 subjects using transthoracic echocardiography. The mean age (±SE) of all screened subjects was 22.5 ± 0.7 years, and 611(57.5±3.0%) were female. All but four were black.

Of the 1063 subjects observed, 211 (19.8 ± 2.4%) had EMF. The prevalence of EMF was higher in Nhapadiane (35/145; 24.1%), while the lowest prevalence was found in Chacane (20/145; 13.8%), as shown in Table 4.2. The distribution of the disease inside the district varied between different povoados, with the highest prevalences being found in the following povoados: Muenda-Mahalamba (50.0%), Cove-Mahalamba (44.4%), Mavela-Nhanombe (39.4%), Chemane-Nhanombe (32.4%) and Cambula-Dongane (32.1%).

There was not significant evidence that the prevalence differed between the different *Localidade* in the district (p=0.32) (Table 4.2). However, the proportion of individuals with EMF did differ by *Povoado* within the Localidade (p=0.009, mixed model ANOVA) and between families within a *povoado* (p=0.039, mixed model ANOVA).
### Table 4.2. Distribution of subjects observed by *localidade*

<table>
<thead>
<tr>
<th>Localidade</th>
<th>Subjects Observed</th>
<th>EMF cases</th>
<th>EMF Prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chacane</td>
<td>145</td>
<td>20</td>
<td>13.8</td>
</tr>
<tr>
<td>Dongane</td>
<td>118</td>
<td>26</td>
<td>22.0</td>
</tr>
<tr>
<td>Nhanombe</td>
<td>249</td>
<td>49</td>
<td>19.7</td>
</tr>
<tr>
<td>Vila Sede</td>
<td>189</td>
<td>34</td>
<td>18.0</td>
</tr>
<tr>
<td>Nhapadiane</td>
<td>145</td>
<td>33</td>
<td>22.8</td>
</tr>
<tr>
<td>Mahalamba</td>
<td>217</td>
<td>49</td>
<td>22.6</td>
</tr>
<tr>
<td><strong>All</strong></td>
<td><strong>1063</strong></td>
<td><strong>211</strong></td>
<td><strong>19.9</strong></td>
</tr>
</tbody>
</table>

**Figure 4.4a.** GPS location of houses with cases of EMF
**Figure 4.4b.** GPS location of houses where no echocardiography was done

**Figure 4.4c.** GPS location of houses with no case of EMF
EMF was biventricular in 117 subjects (55.5 ± 6.7%); right-sided in 59 (28.0 ± 6.1%) and left-sided in 35 (16.6 ± 5.0%). Most subjects studied (163/211 = 77.2%) had mild lesions, 39/211 = 18.5% had moderate affection, and 9/211 = 4.3% had advanced EMF. The most frequent lesions in mild disease were apical obliteration of the right ventricle, diffuse thickening of the mitral valve, and mild mitral or tricuspid regurgitation.

**Echocardiographic Findings**

According to the scoring system used 181 (85.8%) participants had mild EMF, 29 (13.7%) had moderate lesions, and 1 (0.5%) presented severe structural abnormalities on echocardiography. The most frequent lesions were plaques of fibrosis in the right ventricular apex (present in 108 individuals, 51.18%), thickening of the anterior leaflet of the mitral valve (107, 50.71%), plaques of fibrosis in the interventricular septum (103, 48.82%), thickening of the posterior leaflet (102, 48.34%) and fibrosis of the moderate band (78, 36.97%). *(Table 4.3).*

**Clinical-echocardiographic correlation**

Among the 211 subjects with echocardiographic findings of EMF only 48 (22.7%) had symptoms or showed abnormalities on physical examination. Eight (16.6%) individuals complained of having atypical chest pain or palpitations. The most common findings on physical examination were abnormalities in auscultation (third sound, cardiac murmur and premature beats) present in 21 subjects, periorbital edema in 6 and jugular vein distension in 4 *(Table 4.4).*
### Table 4.3. Distribution of specific lesions in individuals with EMF found during the study

<table>
<thead>
<tr>
<th>Echocardiographic features</th>
<th>N&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>RV apical endocardial thickening and enhanced density</td>
<td>108</td>
<td>51.18</td>
</tr>
<tr>
<td>Thickening of the AML</td>
<td>107</td>
<td>50.72</td>
</tr>
<tr>
<td>Enhanced density of the left side of the IVS</td>
<td>103</td>
<td>48.82</td>
</tr>
<tr>
<td>Thickening of the PML</td>
<td>102</td>
<td>48.34</td>
</tr>
<tr>
<td>Fibrosis of the moderator band</td>
<td>78</td>
<td>36.97</td>
</tr>
<tr>
<td>Restrictive filling of the LV</td>
<td>64</td>
<td>30.33</td>
</tr>
<tr>
<td>Endocardial thickening of the right side of the IVS</td>
<td>63</td>
<td>29.86</td>
</tr>
<tr>
<td>Tricuspid regurgitation</td>
<td>49</td>
<td>23.22</td>
</tr>
<tr>
<td>LV apical endocardial thickening or enhanced density</td>
<td>42</td>
<td>19.91</td>
</tr>
<tr>
<td>Enhanced density of the MV posterior papillary muscle</td>
<td>42</td>
<td>19.91</td>
</tr>
<tr>
<td>Enhanced density of the false tendon</td>
<td>39</td>
<td>18.48</td>
</tr>
<tr>
<td>Enhanced density of the TV septal papillary muscle</td>
<td>31</td>
<td>14.69</td>
</tr>
<tr>
<td>Enhanced density of the MV anterior papillary muscle</td>
<td>29</td>
<td>13.74</td>
</tr>
<tr>
<td>Reduction of TV leaflet mobility</td>
<td>27</td>
<td>12.80</td>
</tr>
<tr>
<td>LVPW endocardial thickening and enhanced density</td>
<td>27</td>
<td>12.80</td>
</tr>
<tr>
<td>Mitral regurgitation</td>
<td>26</td>
<td>12.32</td>
</tr>
<tr>
<td>Retraction of the RV trabecular portion (“apex”)</td>
<td>20</td>
<td>9.48</td>
</tr>
<tr>
<td>Obliteration of the RV trabecular portion (“apex”)</td>
<td>17</td>
<td>8.06</td>
</tr>
<tr>
<td>LV thrombus</td>
<td>4</td>
<td>1.90</td>
</tr>
</tbody>
</table>

<sup>a</sup> Number of individuals presenting the specific feature out of the 211 participants diagnosed with EMF

RV right ventricle; LV left ventricle; AML anterior mitral leaflet; PML posterior mitral leaflet; IVS interventricular septum; MV mitral valve; TV tricuspid valve; LVPW left ventricular posterior wall

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*Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis*
Possible determinants

Age: The percentage of subjects with EMF differed between the various age groups (p=0.001) but there was no evidence for systematic increase or decrease with age (p=0.95) as shown in Figure 4.5. The prevalence was highest in the 10-19 years old group (28.1 ± 5.5%). Left-sided EMF was more common among those over 30 (32.7% versus 11.3%, p=0.001).

Gender: The prevalence of EMF was significantly higher in men (23.0% vs. 17.5%, p=0.026), the main difference occurring in the 20-29 age group. The percentage of each type of EMF lesions (left, right and bilateral) did not differ between the two sexes (p=0.29) (Table 4.5).

Familial predisposition: Out of 214 families, 99 had no EMF cases, 63 had one case and 52 had more than one case. There was no correlation between the percentage of subjects with EMF in a family and the observed family size (correlation = 0.095; p = 0.17). However, the chance of having the disease was higher when other members of the family had it (p=0.001, using a logistic regression model). If EMF status was independent of other members of the family, the prevalence among families with at least one case would be approximately 28%. The observed prevalence was approximately 32% when another member of the family had the disease, and reached 39% for subjects with three or more family members having the condition.
Table 4.4. Clinical findings in 48 individuals with echocardiographic features of EMF

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>N</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abnormal auscultation</td>
<td>21</td>
<td>43.75</td>
</tr>
<tr>
<td>Periorbital edema</td>
<td>6</td>
<td>12.50</td>
</tr>
<tr>
<td>Jugular vein distension</td>
<td>4</td>
<td>8.33</td>
</tr>
<tr>
<td>Cyanosis</td>
<td>3</td>
<td>6.25</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>3</td>
<td>6.25</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>3</td>
<td>6.25</td>
</tr>
</tbody>
</table>

N = Number of individuals presenting the clinical feature

Table 4.5. Distribution of frequencies and percentages of EMF by gender.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Bilateral EMF</th>
<th>Right EMF</th>
<th>Left EMF</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>57(53.3%)</td>
<td>28(26.2%)</td>
<td>22(20.6%)</td>
<td>107</td>
</tr>
<tr>
<td>Male</td>
<td>60(57.7%)</td>
<td>31(29.8%)</td>
<td>13(12.5%)</td>
<td>104</td>
</tr>
<tr>
<td>All</td>
<td>117(55.5%)</td>
<td>59(28.0%)</td>
<td>35(16.6%)</td>
<td>211</td>
</tr>
</tbody>
</table>
**Figure 4.5.** Age distribution of EMF cases detected in the community

dark bar = all    slashed bar = male    blank bar = female
4.2.3. Discussion

A high prevalence of EMF was found in this rural community using echocardiographic screening. We found a non-uniform distribution of the prevalence of EMF inside the district with 12 out of the 33 povoados reaching prevalence of more than 25%, while others had less than 5% of people affected (Mazonda-Chacane and Malene-Mahalamba with 3.6% and 3.5% respectively). Inharrime district is crossed by several rivers and is part of the Mozambican Coast (Indian Ocean), and it is interesting to notice that the areas with higher prevalence of EMF were located mainly near the lakes or rivers. Weather this is of relevance in search for an environmental factor like a vector for infectious disease as the trigger of EMF is an issue that needs to be further studied.

Mild asymptomatic EMF was the most common form in contrast with findings from clinical series, which reveal predominance of severe debilitating disease (Freers et al, 1996b; Tharakan et al, 1993; Venkitachalam et al, 1993). An extreme phenotypic variability of EMF was found in this community, with EMF lesions varying from patchy endocardial fibrosis without any hemodynamic changes, to extensive mural and atrioventricular valve endocardial fibrosis with resulting structural and hemodynamic changes. Our findings suggest that patients seeking medical attention represent a minority of the affected individuals, stressing the importance of community research to uncover the natural history and pathogenic mechanisms of this condition.

The most frequent type of EMF in the community was BEMF. There was predominance of structural lesions on the right side of the heart, either in isolation or as part of bilateral disease. Interestingly LEMF, was the less common form of disease, and affected
predominantly adults over 30 years. Since LEMF is known to be the most lethal form of
disease in children, these adults could represent a particular subset of patients affected in
their childhood, who had mild abnormalities and entered a long steady period (without
progression of the disease), reaching adulthood without major cardiac abnormalities.

Inharrime, like other areas of Mozambique, is an area with high prevalence of rheumatic
heart disease. The use of standard criteria and a scoring system to diagnose and classify
EMF in the community was of extreme relevance in differential diagnosis. In the absence
of large plaques of fibrosis the distinction between left-sided EMF and mitral
regurgitation due to Rheumatic Heart Disease relied on specific echocardiographic
features. The diagnosis of EMF was favoured when echocardiography revealed diffuse
thickening of the anterior mitral leaflet, areas of endocardial thickening involving the
posterior wall and the papillary muscles, and obliteration of either the trabecular portion
of the right ventricle or the recess behind the posterior mitral leaflet.

This study showed that EMF in the community affects mainly children and young
adolescents, corroborating findings from in vivo hospital-based studies (Parry, 1964;
Somers, 1990) or autopsies (Bijlsma, 1976; Shaper et al, 1968). Females present a second
peak of prevalence in the fourth decade of life (Figure 4.5). The reason for this bimodal
age distribution in women that has been reported previously in some clinical series
(Shaper, 1972; Rutakingirwa et al, 1999) is not fully understood. The second peak during
reproductive years may be related to the increased number of pregnancies and prolonged
time of breast-feeding in women from these poor settings, which would become more
prone to reactivation of a quiescent disease acquired early in life.
The higher frequency of male individuals found in our study suggests that gender is a determinant of the disease in this particular area. The results of gender distribution of EMF patients in several clinical series have not been consensual with some showing male (Brockington an Edington, 1972; Jaiyesimi, 1982) or female (Connor et al, 1967; Shaper, 1972) preponderance, while others show no difference between sexes (Parry, 1964). Our findings are important since male predominance in hospital setting in poor endemic areas could be interpreted as being due to cultural believes and misconceptions that would determine selective referral of male children to medical care in detriment of girls.

The familial occurrence of EMF in the community that was found in our study needs to be further explored. There is the possibility of this pattern being linked to genetic susceptibility or to several environmental factors affecting people who live together, like exposure to the same transmissible diseases or poisoning agent. Although we did not systematically collect data on the characteristics of the environment, dietary factors and economic status of the families, there was no evidence of variation in the standard of living inside the same area, which would explain the difference in prevalence between families from the same povoado.

The prevalence of endomyocardial fibrosis was higher than that found in a previous survey in Inharrime using clinical screening followed by echocardiographic confirmation of those individuals with abnormal auscultation (Ferreira, 2001). This is understandable since EMF can course with few or no auscultatory signs, particularly in right forms of the disease when there is free tricuspid regurgitation, and in mild EMF where there are no hemodynamic changes. Our results show that echocardiographic screening adds
sensitivity and precision to the diagnosis of EMF, by detecting early disease and asymptomatic individuals.

There are some limitations to this study. Some participants had echocardiographic findings suggesting EMF but did not reach the necessary scores to be considered definite cases. Whether these individuals represent early stages of the disease or have forms of mild disease that will persist in quiescent stage forever are questions that cannot be answered with certainty now. The clarification of these important issues requires cohort studies looking at incidence and mode of progression of the disease in this endemic area, and should be facilitated by use of the new standard criteria for diagnosis. Such follow up studies would also be used to validate our classification.

We acknowledge that 186 (14.9%) eligible individuals were absent and therefore were not submitted to echocardiographic screening. This value should be considered acceptable in a large-scale community study like ours. Importantly, the results of the analysis of the demographic characteristics of these individuals did not show any difference to that obtained for the whole sample of the population.

In conclusion, this study showed a high prevalence of EMF in a rural community of Mozambique and demonstrated that echocardiography detects and characterizes early and asymptomatic stages of the disease. In this population the disease appeared to affect predominantly children, with higher prevalence in males. The newly described scoring system has the potential to evaluate the mode, mechanisms and rate of progression of EMF. Further research is needed to determine the role of genetic susceptibility in determining the different phenotypes and in explaining the familial occurrence.
The large population of subjects affected offers unique opportunities for studying the basic molecular mechanisms responsible, which could help in evolving strategies for prevention and treatment of the disease, and could be valuable for understanding other forms of cardiomyopathy and diseases involving fibrosis.
4.3. Establishing a cohort of school children in Inharrime

4.3.1. Population and Methods

Sample selection and inclusion criteria

We randomly selected 10 out of 50 schools from Inharrime (Figure 4.6, Table 4.6). In each school all children from the second level were invited to participate in the study, through meetings between the parents and the teachers, who had been trained by the researcher. Parents had to give informed consent for their children to be included in the study.

Data collection

Between July and September 2006 teachers collected demographic data from every selected child by filling in a standardized questionnaire (Appendix 2B). A unique identification number was attributed to all participant children. During the month of October 2006 detailed transthoracic echocardiography was performed to all children using a hand-carried echocardiography battery-operated system (Vivid I, General Electrics). We obtained M-mode, two-dimensional and Doppler (pulsed, continuous and color) images. The following views were obtained: apical two-, four-, and five-chamber views; parasternal long-axis and short-axis at the level of papillary muscles and aortic valve; subcostal views; and suprasternal views (parallel to the aortic arch). Hard copies
with detailed location and type of lesions (Appendix 3) and electronic records of relevant data were kept.

Figure 4.6. Pictures of 2 classes selected for the prospective studies in different schools
<table>
<thead>
<tr>
<th>SCHOOLS</th>
<th>STUDENTS</th>
<th>TEACHERS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Senduza II</td>
<td>31</td>
<td>Armando Francisco</td>
</tr>
<tr>
<td>Cuaiaia</td>
<td>43</td>
<td>Armando António/Gaspar Languisso</td>
</tr>
<tr>
<td>Mahalamba</td>
<td>118</td>
<td>P. Mahique/ Olga Malate/ R. Cumbi/C. Lisure</td>
</tr>
<tr>
<td>Inhantumbo</td>
<td>47</td>
<td>Teresa Júlio/Luis Nhiuane</td>
</tr>
<tr>
<td>Mahessa</td>
<td>35</td>
<td>Jerónimo Muholiciane/Ernesto Hele</td>
</tr>
<tr>
<td>Coche</td>
<td>47</td>
<td>Julião Paulo/Francelino Deve</td>
</tr>
<tr>
<td>Magula</td>
<td>38</td>
<td>Domingas Fernando/Mariamo Abdula</td>
</tr>
<tr>
<td>Incuane</td>
<td>58</td>
<td>Horácio Xavier/Arlindo Afonso Cumbi</td>
</tr>
<tr>
<td>Cambula</td>
<td>33</td>
<td>Lídia Machava/João Chambe</td>
</tr>
<tr>
<td>Chilorane</td>
<td>40</td>
<td>Basílio Zango/Rafael Augusto</td>
</tr>
</tbody>
</table>

**Table 4.6.** List of the 10 schools randomly selected from a total of 50. All students from second level were invited to participate in the cohort follow-up study. The teachers responsible for each group of selected students are also included in the table.
Blood collection and Assays

Blood samples were collected from every fifth child included in the study (following the code number), by puncture of the antecubital vein. A rapid test for malaria antigen was performed on site. Blood smears were prepared for leukocyte count, eosinophil count and search for malaria parasites.

Statistical analysis

Frequencies are given as absolute numbers and percentages or proportions; continuous data are reported as mean (standard deviation). We used the chi-square test to compare percentages between groups and logistic regression to test for trends in percentages with a continuous variable. Association between continuous variables was measured using the Pearson correlation coefficient, with a t-test for zero correlation. Analysis of variation within family was carried out using mixed model analysis of variance (ANOVA). The “±” sign indicates the range of a 95% confidence interval.

Analyses were performed using Minitab, Release 13 (Minitab Inc, State College, PA) and SAS, Release 8.02 (SAS Institute, Cary, NC).

Ethical Issues

Signed informed consent was obtained from parents of all children participating in the study. Children with cardiac disease were offered free treatment and follow-up in Inharrime Research Centre or, if surgery was indicated, at the Heart Institute in Maputo. Oral treatment with Arinate® was provided to all children with malaria infection.
4.3.2. Results

There were 363 eligible children. The mean age of participants was 8 (± 1) years, and 195 (53.7%) were girls.

Two hundred ninety-six children were included in the study: 268 (90.5%) had normal hearts; 25 (8.5%) had endomyocardial fibrosis; and 3 (1.0%) had rheumatic heart disease. Children with EMF were found in all schools.

Blood was collected from a sample of 72 children randomly selected showing that most children (49; 68.1%) had malaria infection, although denying any symptoms. The mean eosinophil count was $1.2 \times 10^9$/L (± 2.4).

There was no statistically significant difference in any of the measured parameters between the participants with and without malaria infection. Also no difference was found when comparing participants with and without hypereosinohilia.

Classification of Endomyocardial Fibrosis (25 children)

Most children (16; 64.0%) had BEMF, 7 had REMF (28.0%) and the remaining 2 (8.0%) had LEMF. Regarding the severity 12 (48.0%) children had mild lesions, 10 (40.0%) had moderate lesions and 3 (12.0%) had severe EMF.
Comparison between participants with and without EMF

There was no significant difference in age (p=0.69), sex (p=0.69), leukocyte count (p=0.99), malaria infection (p=0.34) and eosinophil count (p=0.54) between children with and without EMF.

Other Diseases

We found 3 children with rheumatic heart disease (1%) and 2 children had Atrial Septal Defect.
4.3.3. Discussion

The prevalence of EMF in school children was high, but corresponded only to nearly half of that found in the general population, in concordance with the fact that in the whole population of Inharrime the disease affects mostly older children than the group selected for this study. However, since EMF in its severe forms is a very debilitating disease, we must also consider the possibility of children with severe EMF being symptomatic and therefore absent from school during this study.

The prevalence of malaria, an infection that has been implicated in the pathogenesis of EMF, was also very high in these children, a finding that can be explained by the fact that Inharrime District has the highest transmission rate for malaria in Mozambique. The finding of almost no symptomatic malaria might be explained by acquisition of immunity due to frequent inoculation of the parasite in early years of life, a common event in endemic areas (Riley et al., 1994). There is need to test the immune response in these children to corroborate this hypothesis and explain this interesting result. Considering that malaria and EMF are highly prevalent in Inharrime we think that the occurrence of both diseases in 3 children is explained by chance.

Blood hypereosinophilia was common in school children in this district, but no statistically significant difference was detected when comparing children with EMF from those without the disease. Hypereosinophilia is a common finding in people from endemic areas for EMF as reported from studies in Nigeria (Ijaola and Falase, 1988; Urhoghide and Falase, 1987). Blood films and stool specimens of primary and secondary
school children from two rural communities (one an endemic area for EMF and one a non-endemic area for EMF) areas were examined, and the results showed that the mean eosinophil counts and the parasitic infection rates were similar in both communities (Ijaola and Falase, 1988).

Several parasitic infestations are endemic in Mozambique (Dgedge et al., 2001) and could be the reason for such high prevalence of high eosinophil counts in children from Inharrime, namely helminths, schistosomiasis and filariasis. The burden of schistosomiasis, for instance, is significant and affects over 80% of children in school surveys carried out in parts of the country (Gujral et al., 2000; Traquinho et al., 1998). In our study the presence of parasitic infestations was not investigated for logistical reasons. Inharrime, like other rural districts of Mozambique has a high of dropout rate among school children, which has been shown to be more important in the first year. By selecting children in their second years of studies we expect to lose few children participating in our prospective studies.

The involvement of teachers and parents at the beginning of this long-term project was of paramount importance. They participated as partners of the research team and helped with the organization of the fieldwork. They have been trained for detection of signs of cardiovascular disease, and will be integrated in the prospective studies.

All children will be followed through clinical and echocardiographic evaluation every 18 month. While the follow up of children with EMF is expected to help to understand the natural history of this condition and identify predisposing factors, the establishment of a cohort of 271 children without EMF will allow to determine the incidence and rate
of progression of the disease, including the assessment of the value of the minor lesions as predictors of EMF.

This group of children will constitute a cohort that will be followed by serial echocardiographic screening for determination of the incidence and rate of progression of EMF in the community.
4.4. Conclusions

This epidemiological research showed a high prevalence of EMF in a rural community of Mozambique and demonstrated that echocardiography detects and characterizes early and asymptomatic stages of the disease. In the population studied EMF affects predominantly children, with higher prevalence in males. Further work is required to evaluate the mode, mechanisms and rate of progression of EMF, as well as the role of genetic susceptibility in determining the different types and familial occurrence.

The large population of subjects affected who were identified and echocardiographic phenotype was extensively characterized offers unique opportunities for studying the basic molecular mechanisms responsible for EMF, which could help in developing strategies for prevention and treatment of the disease. This knowledge could also be valuable for understanding other forms of cardiomyopathy and diseases involving fibrosis.
Chapter 5

CLINICAL CHARACTERISATION OF ESTABLISHED EMF


5.1. Background

EMF has been classified according to the mode of presentation, the type of evolution of the disease and the distribution of lesions inside the heart, but the different types seem to overlap. Designations such as acute, chronic, steady (Abrahams, 1962; Guimaraes, 1993) and active (Andy, 1998) have been used without any clear definition of their meaning. Some clinical features of EMF are striking and have stimulated a lot of publications. Advanced stages of REMF have distinctive clinical features like the typically enormous ascitis with no pedal edema, central cyanosis, exoftalmus, and clinical feminization of male patients (Abrahams, 1962; Bolarin and Andy, 1982). The pathophysiology of ascitis in EMF patients remains the subject of controversy since not fully explained by heart failure and retrograde congestion (Jaiyesimi, 1982). Similarly, the origin of cyanosis has been debated, since not always related to the presence of a persistent foramen ovale or low cardiac output syndrome.

Describing the clinical profile of EMF patients in this era of use of echocardiography and the possibility of early recognition of the disease is of paramount importance. We therefore designed a study to characterize the clinical, echocardiographic and biological profile of EMF patients who seek medical care in Mozambique.
5.2. Population and Methods

A national registry for EMF patients was established at the Heart Institute. Tertiary and quaternary referral hospitals from Mozambique were contacted and asked to transfer all patients with the diagnosis of EMF to be evaluated and included in the registry. In addition, we assessed all patients already diagnosed and assisted at the Heart Institute. A standard flowchart was designed (Diagram 5.1) and clinical, echocardiographic and laboratory data were collected.

Clinical examination

Complete physical examination was performed in all patients. Scoring systems were developed and applied for the evaluation of ascitis, hepatomegaly and splenomegaly (Tables 5.1, 5.2, 5.3).

Cardiovascular diagnostic techniques

Electrocardiography, chest radiography and echocardiography were performed to all patients. Computed Axial Tomography of the chest and cardiac catheterization were indicated when there was severe distortion of the cardiac anatomy with lesions that seemed suitable for surgery. The 24h monitoring of the cardiac rhythm was indicated in patients with suspicion of paroxysms of atrial or ventricular arrhythmia.
Classification of EMF

Patients were classified as detailed in Chapter 3, using the following flow chart:

Diagram 5.1: Flowchart used to assess and manage EMF patients included in the registry
### Table 5.1. Classification used for assessment of the severity of the ascitis

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absence of ascitis</td>
</tr>
<tr>
<td>1</td>
<td>Small (changes with position)</td>
</tr>
<tr>
<td>2</td>
<td>Moderate (easily detected and allowing palpation of abdominal organs)</td>
</tr>
<tr>
<td>3</td>
<td>Severe (difficult palpation of abdominal organs)</td>
</tr>
<tr>
<td>4</td>
<td>Giant (under tension, palpation of abdominal viscera impossible)</td>
</tr>
</tbody>
</table>

### Table 5.2. Classification used for assessment of the degree of hepatomegaly

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Absence of hepatomegaly</td>
</tr>
<tr>
<td>1</td>
<td>Lower liver border palpable at costal border or only on inspiration</td>
</tr>
<tr>
<td>2</td>
<td>Lower liver border palpable between costal border and umbilicus</td>
</tr>
<tr>
<td>3</td>
<td>Lower liver border palpable at the level of the umbilicus</td>
</tr>
<tr>
<td>4</td>
<td>Lower liver border palpable below the level of the umbilicus</td>
</tr>
</tbody>
</table>

### Table 5.3. Classification used for assessment of the degree of splenomegaly

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No palpable spleen</td>
</tr>
<tr>
<td>1</td>
<td>Spleen border at the level of the costal border</td>
</tr>
<tr>
<td>2</td>
<td>Spleen border palpable between the costal border and the umbilicus</td>
</tr>
<tr>
<td>3</td>
<td>Spleen border palpable at the level of the umbilicus</td>
</tr>
<tr>
<td>4</td>
<td>Spleen border palpable below the level of the umbilicus</td>
</tr>
</tbody>
</table>
Other Investigations

The laboratory workout included a complete hematological evaluation, measurement of inflammatory markers (C-reactive protein and eritrosedimentation rate), renal function, liver function, urinalysis and stool examination. Blood smears were prepared for all patients for manual count and characterization of eosinophils (using May-Grunvald staining).

Hypereosinophilia was defined as an absolute eosinophil count of $1.5 \times 10^9/L$ or greater.

Patients were also submitted to a screening for infectious diseases with known high prevalence in our setting, namely malaria, tuberculosis, schistosimiasis, helminths and HIV.

The inflammation markers and eosinophil count were repeated every three months and whenever there was recrudescence of heart failure or clinical signs of activity.

Statistical Analysis

Frequencies are presented as absolute numbers and percentages or proportions; continuous variables are reported as means (± SD) or medians (IQR range) according to the distribution of the values. The ‘±’ sign indicates the range of a 95% confidence interval. We used the chi-square test to compare percentages between groups; p values $<0.05$ were considered for significance.

Analyses were performed using SPSS software.
5.3. Results

Patients

There were 64 EMF patients already followed at the Heart Institute at the beginning of the research programme; 98 new patients were referred from other hospitals after the starting of the project; 8 were detected in the community and referred to the Heart Institute for further follow-up; and 5 had the diagnosis done at the Heart Institute for the first time without being referred. The registry had 175 patients who were submitted to the investigations described above in Figure 5.1.

Epidemiological profile

The patients were referred from all regions of the country. They came mostly from tertiary hospitals (91; 52.0%) and from the quaternary hospital in Maputo (69; 39.4%).

Origin: Most patients 101 (57.7%) were originally from Inhambane (one of the 10 provinces of Mozambique) where Inharrime district is located.

Age and Gender: The median age at diagnosis was 13 years (IQ range 8-20), ranging from 3 to 64. The age distribution of patients at the time of diagnosis is shown in Figure 5.2. Ninety-two patients were males (52.6%).
Clinical findings on presentation

Most patients (155; 88.6%) presented in classes III and IV of the NYHA (Figure 5.3). The median BMI was 17.0 (IQR range 14.9-20.0) and 19 patients (10.9%) had extreme

Figure 5.1: The 175 patients were referrals from all regions of the country
Age groups are 1(0-9 years), 2(10-19 years), 3(20-29 years), 4(30-39 years), 5(>40 years)

**Figure 5.2.** Distribution of EMF patients by age at diagnosis

**Figure 5.3.** NYHA functional class of EMF patients at presentation
cachexia. The children and adolescents were in general small for age and presented poor
development of the secondary sexual characters.

Sixty-eight patients (38.9%) had severe ascitis (Figure 5.4). Periorbital edema was
present in 54 (30.9%) patients (Figure 5.5); cyanosis in 89 (50.9%); and proptosis in 38
(21.7%). Severe splenomegaly was found in 25 patients (14.3%).

Investigations at time of diagnosis

The median cardio-thoracic index at admission was 74.0% (IQR range 67 to 86). Examples
of chest radiographies of EMF patients are shown in Figures 5.6 - 5.9). Patients with
REMF had the higher cardio-thoracic indexes than those with LEMF or BEMF,
sometimes ratios approaching 1. In certain patients the evaluation of the cardiothoracic
was difficult due to the presence of pleural effusion.

Rhythm disturbances were common (47; 26.9%) mainly atrial fibrillation which was
found in 35 patients (20.0%). Figure 5.10 shows some of the most common
electrocardiographic features in patients with EMF. In advanced REMF there is
commonly low voltage, broad tall right atrial p waves, qr pattern in V1 and T wave
changes. Atrial flutter and fibrillation are the most common arrhythmia but ventricular
ectopic beats are also frequent in late stages of the disease.

Inflammation markers that are considered laboratory signs of activity (high CRP or ESR
>30) were present in 87 (49.7%) patients. The mean eosinophil count was 1.2 (range 0-
8.8); 43 patients (25.4%) had absolute eosinophilia greater than 1.5 x 109/L.
Characterization of eosinophils was performed in 95 patients; degranulation was found in
50 (52.6%) with a median percentage of 8% (IQR range 0-32%).
Figure 5.4. Large ascitis and evidence of severe emaciation

Figure 5.5. Facial edema, a common finding both in initial and late phases of EMF
Figure 5.6. The chest radiography in BEMF shows cardiomegaly mainly due to enlargement of atria and mild pulmonary congestion.

Figure 5.7. BEMF with predominance of right lesions with aneurysmal right atrium and mild left pleural effusion.
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Figure 5.8. The chest radiography in predominant LEMF presents cardiomegaly due to bi-atrial dilatation and pulmonary congestion

Figure 5.9. Pericardial effusion is a common finding in both early and late stages of the disease and further impedes ventricular filling
Chapter 5 Clinical Characterisation

The median hemoglobin level was 11.5g% (IQrange 10.4-13.1); 97 patients (58.4%) had hemoglobin below 12g%. The median platelet count was 207 (IQrange 151-283). Hypoproteinemia was present in 45 (25.7%) patients, liver dysfunction in 31 (17.7%) and renal dysfunction in 5 (2.9%). Two patients had kwashiorkor.

Associated Conditions

Malaria infection was present in 13 (7.4%) patients at diagnosis, while active tuberculosis was found in 6 (3.4%). Three patients (1.7%) had hepatic schistosomiasis and three (1.7%) had HIV infection. None of our patients had associated infective endocarditis. There was association of EMF with Myasthenia Gravis in two patients. In six patients (3.4%) there was association with Rheumatic Heart Disease. Episodes of bronchospasm occurred in 45 (25.7%) patients.

Classification

Echocardiography revealed that most patients had biventricular EMF (95; 54.3%), 55 (31.4%) had REMF and the remaining 25 (14.3%) had LEMF (Figure 5.11). Only 20 (11.4%) had minimal lesions for which there was no clear benefit of surgery. Ninety-four patients (53.7%) had severe or advanced disease, meaning grades II or IV of severity (Figure 5.12). Seventy patients (40.2%) presented with intracavitary thrombi and 12 (6.9%) had ventricular calcification when first diagnosed.
Figure 5.10: The ECG in advanced REMF commonly shows low voltage, broad tall right atrial p waves, qr pattern in V1 and T wave changes (top). Atrial fibrillation is the most common arrhythmia but ventricular ectopic beats are also frequent in late stages of the disease (bottom).
Figure 5.11. Distribution of types of EMF

Figure 5.12. Distribution of grades of severity of EMF from mild (I) to advanced (IV)
Most patients (153; 87.4%) had cardiac lesions that needed surgical correction. However, 63 (36%) had advanced lesions or other medical conditions that were considered contraindications to surgery, mainly severe and persistent ascitis (49; 26.9%), severe pulmonary hypertension (4; 2.3%) and extreme cachexia (19; 10.9%).

**Determinants for type and severity of EMF**

The features that differed between the BEMF, REMF and LEMF were the age (p=0.014) and the cardiothoracic ratio (p<0.001). Bilateral EMF was diagnosed later in life when compared to REMF (at 18 years versus 13 years for REMF and LEMF). Patients with REMF presented the highest cardiothoracic index.

The parameters that differed according to the severity of EMF were the body mass index (p=0.01) lower in patients with grade IV and the cardiothoracic index (p<0.001) that was also higher in patients with Grade IV.

The mean hemoglobin levels were higher in both extremes of the severity classification, 11.9 and 12.3 g/dL for mild and advanced EMF respectively (p=0.046).

The demographic and clinical characteristics of patients according to the type and severity of EMF are presented in **Tables 5.4 and 5.5**.
Follow-up

Staging and Mode of Progression

Of the 175 patients from the National Clinical Registry 11(6.3%) did not complete the six months follow up immediately after diagnosis. The mean follow-up of patients was 21 months (range 0.2 to 78 months).

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>BEMF (N 95)</th>
<th>REMF (N 55)</th>
<th>LEMF (N 25)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18 ± 12</td>
<td>13 ± 8</td>
<td>13 ± 6</td>
<td>0.014</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>17.9 ± 4.4</td>
<td>18.5 ± 3.0</td>
<td>16.5 ± 4.1</td>
<td>0.145</td>
</tr>
<tr>
<td>CTRatio (%)</td>
<td>72.1 ± 13.1</td>
<td>82.5 ± 12.9</td>
<td>68.4 ± 12.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Hemoglobin (g/dl)</td>
<td>11.4 ± 1.8</td>
<td>12.1 ± 1.7</td>
<td>11.5 ± 2.3</td>
<td>0.129</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>36.8 ± 5.4</td>
<td>38.6 ± 5.0</td>
<td>36.5 ± 6.3</td>
<td>0.133</td>
</tr>
<tr>
<td>Eosinophils (x10⁹/L)</td>
<td>1.02 ± 1.5</td>
<td>1.20 ± 1.4</td>
<td>1.55 ± 1.6</td>
<td>0.306</td>
</tr>
<tr>
<td>ESR (mm/1h)</td>
<td>37 ± 39</td>
<td>31 ± 30</td>
<td>36 ± 27</td>
<td>0.532</td>
</tr>
</tbody>
</table>

ESR erythrosedimentation rate

Table 5.4. Demographic and clinical characterization of patients according to EMF type
The majority of patients had chronic disease which was either quiescent (64;36.6%) or with recurrence of activity within the period of follow up (34;19.4%). Considering the patients with signs of activity at diagnosis 38 (21.7%) had remission, 18 (10.3%) had persistence of activity and in 10 (5.7%) there was progression of the disease to a higher degree of severity during the first 6 months after diagnosis. The results of the first 6 months of follow up are presented in Figure 5.13.

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Mild (Grade I)</th>
<th>Moderate (Grade II)</th>
<th>Severe (Grade III)</th>
<th>Advanced (Grade IV)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>21 ± 14</td>
<td>15 ± 12</td>
<td>15 ± 8</td>
<td>14 ± 11</td>
<td>0.133</td>
</tr>
<tr>
<td>BMI</td>
<td>20.7 ± 5.5</td>
<td>16.7 ± 3.8</td>
<td>18.0 ± 3.6</td>
<td>18.4 ± 3.5</td>
<td>0.001</td>
</tr>
<tr>
<td>CTR (%)</td>
<td>53.9 ± 12.0</td>
<td>71.2 ± 11.4</td>
<td>79.3 ± 10.8</td>
<td>83.5 ± 12.6</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Hb (g/dl)</td>
<td>11.9 ± 2.1</td>
<td>11.1 ± 2.1</td>
<td>11.8 ± 1.7</td>
<td>12.3 ± 1.6</td>
<td>0.046</td>
</tr>
<tr>
<td>Htc (%)</td>
<td>37.4 ± 5.6</td>
<td>36.4 ± 6.4</td>
<td>37.4 ± 4.8</td>
<td>39.1 ± 4.6</td>
<td>0.326</td>
</tr>
<tr>
<td>Eos (X10^9/L)</td>
<td>0.5 ± 0.6</td>
<td>1.4 ± 1.8</td>
<td>1.3 ± 1.5</td>
<td>0.8 ± 1.1</td>
<td>0.112</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>28 ± 26</td>
<td>39 ± 30</td>
<td>34 ± 29</td>
<td>37 ± 31</td>
<td>0.603</td>
</tr>
</tbody>
</table>

BMI body mass index; CTR cardiothoracic ratio; Hb hemoglobin; Htc hematocite; Eos absolute eosinophil count; ESR erythrosedimentation rate

Table 5.5 Demographic and clinical characteristics of EMF patients according to severity
Twenty-nine patients were lost for follow up, since they did not appear for evaluation in the last 12 months.

**Mortality**

Twenty-three patients (13.1%) died during the period of the study. Most had chronic disease with frequent recurrences of activity (19; 39.1%) or active disease without remission of activity and rapid progression of structural changes (6; 26.1%), as shown in Figure 5.14. The survival rate of patients with EMF from the clinical registry was 70% at 5 years after diagnosis (Figure 5.15).

![Figure 5.13: EMF patients by clinical stage.](image_url)

Chronic quiescent (CQ) (64; 36.6%), active with remission (AR) (38; 21.7%); chronic recurrent (CR) (34; 19.4%); active persistent (AP) 18 (10.3%); active rapidly progressive (ARP) 10 (5.7%); patients not classified 11 (6.3%).
Figure 5.14. Number of deaths according to the stage of the disease.

Figure 5.15. The survival curve of EMF patients from the clinical registry shows that nearly 70% of patients are alive five years after the diagnosis.
5.4. Discussion

The establishment of a National Registry for EMF was important for improvement in management and follow up of patients from around the country. The distribution of patients according to the region of the country is related to the geographical accessibility to the Heart Institute in Maputo. More than ¾ of the patients are residents in one of the three provinces of the South of Mozambique, and interestingly more than half (100; 57.5%) are originally from Inhambane province. This is remarkable since, of the three southern provinces, Inhambane is the one that is located farther from the Heart Institute. These findings corroborate data from Ferreira et al. (2002), who studied the geographical distribution of EMF in Mozambique in another referral centre and showed a striking high prevalence area in Inhambane province, particularly in Inharrime. The closer provinces, Maputo and Gaza, referred respectively 19 (10.9%) and 17 (9.8%) patients.

The rate of lost for follow up of 16.5% is acceptable, if we consider that only 17 (9.7%) patients were referred from areas within 200km from the referral centre. Importantly, 22.3% of patients were sent from the seven provinces located between 1000 and 2000km from the Heart Institute. Some of the patients lost for follow up may have died, but because their houses are located in remote areas so far from the Institute and the families do not have a mean of contact, we were not able to contact any next to keen.

The Mozambican health system is based on nursing personal for assistance at the primary level of health care (Health Posts and Health Centres). There are approximately 500 doctors for 20 millions people, with more than 75% of them concentrated in the Southern...
region, near the capital Maputo. Each province has a tertiary level hospital where doctors specialized in Pediatrics and Internal Medicine send patients that need further assistance from Cardiologists to the quaternary hospitals located in each of the three main geographical regions (South, Centre and North), which have one cardiologist each.

Once the National Registry was started most EMF patients were referred directly from tertiary hospitals or by cardiologists from the quaternary hospitals. This can be partially explained by the absence of knowledge of the real importance of the disease in the country that leads to underdiagnosis but can also surely related to the difficulty of diagnosing EMF on the basis of the clinical examination. Even in endemic areas with high index of awareness the diagnosis of early phases of this condition needs echocardiography and warrants a certain level of clinical knowledge and laboratory capacity to differentiate it from diseases that are also prevalent in the same communities. In Mozambique these include rheumatic heart disease, constrictive and tuberculous pericarditis, tuberculous peritonitis, intestinal schistosomias.

The male predominance found in this clinical series has been attributed to cultural misperceptions that influence the access of girls from remote rural areas to treatment at referral units. However, gender seems to be an independent determinant for EMF, as suggested by our epidemiological studies (Chapter 4).

More than 50% of patients included in our registry were diagnosed between 8 and 20 years of age. Despite their young age most had chronic and severe disease, associated with signs of malnutrition, stunted growth, long-standing heart failure, atrial fibrillation.
and presence of endocardial calcification. This suggests that considerable amount of time had passed between the initial injury and presentation to medical institutions, confirming that initial injury occurs early in life, and most importantly attests for the malignant nature of EMF.

Approximately ¾ of our patients had right lesions either in isolation or as part of bilateral EMF. These patients presented acceptable tolerance to exercise and had persisted relatively asymptomatic for several months, despite severe disease associated with cardiomegaly and intermittent pericardial effusion. This unexpected exercise capacity has also been reported in studies from Nigeria, where patients with REMF without severe heart failure had considerable lack of disability and relative longevity, associated with the capacity to increase cardiac output and slightly decrease the right atrial pressure on exercise (Abrahams, 1962).

Ascitis was a peculiar finding in EMF patients irrespective of the ventricle affected, but more prominent in REMF. Accumulation of fluid in the peritoneal space could not be fully explained by congestion due to right heart failure since not associated to pedal edema in most cases. The pathophysiology of this peculiar feature has been the subject of controversy (Jaiyesimi, 1982). Its origin has been attributed to protein losing enteropathy due to inflammatory changes in the bowel and peritoneum (Freers et al, 2000), which would cause both peritoneal inflammation and decreased reabsorption of peritoneal fluid. Cachexia was another very distinctive clinical feature in Mozambican patients with EMF. In severe and advanced disease it could be explained by the sustained low cardiac output.
However, the body mass index of patients was low for all degrees of severity with no particular trend. One possible explanation for these findings comes from a study in Uganda that demonstrated the presence of skeletal muscle fibrosis in biopsies samples from EMF patients suggesting a generalized fibrous process that would explain the remarkable skeletal muscle atrophy common in these patients (Freers et al., 2000).

The mean levels of hemoglobin were above the normal for our population, which has a high prevalence of anemia due to repeated episodes of malaria infection and intestinal parasitic diseases (Dgedge et al., 2001). Indeed the mean levels of hemoglobin in EMF patients were higher than those found usually in children from these rural areas without any the disease. Interestingly, and linked to this finding, central cyanosis was frequent in our EMF patients, mostly those with right-sided disease. Similarly, central cyanosis occurred in about 50% of patients with REMF in Nigeria (Jaiyesimi and Falase, 1976; Parry and Abrahams 1963; Somers et al., 1968). The pathophysiology of cyanosis remains incompletely understood since it occurs in the absence of atrial septal defect or patent foramen ovale. There were attempts to relate the arterial desaturation to intracardiac shunts, chronic hepatic dysfunction and azygos-pulmonary venous shunt (Parry and Abrahams, 1963) but were soon disproved (Cockshot, 1965). Later, in studies of lung function in EMF patients it was shown that vital capacity and arterial saturation were inversely related to cardiothoracic ratio (Jaiyesimi, 1977), and that after hyperoxia arterial oxygen tension increased significantly in these patients, but not in children with cyanotic congenital heart disease. It was concluded that ventilation/perfusion imbalances
caused by the large heart were in the origin of the arterial desaturation, but further research is needed to unveil the pathophysiology of cyanosis in these patients. Cardiomegaly, measured by the cardiothoracic ratio, increased with the severity of EMF lesions related to severe atrial dilatation and/or pericardial effusion. Although atrial enlargement is one of the most important pathophysiological adaptative mechanisms in restrictive cardiomyopathy playing an important role in maintaining cardiac output, it has been associated with impairment in exercise capacity in patients with BEMF (Mady et al, 2005). Owing to the fact that cardiopulmonary exercise testing is of limited clinical application in patients with EMF due to presence of advanced heart failure, ascitis and cachexia, the left atrial dimension has been proposed to estimate the functional capacity (Mady et al, 2005). On the other hand, the presence of pericardial effusion further impairs the diastolic function, already compromised by the endocardial thickening, further decreasing exercise capacity.

A remarkable finding of the study was that despite the poor social conditions of the patients, the high frequency of cutaneous and other infections, and the need for several invasive procedures, none of the patients in our registry had infective endocarditis. This is in contrast with findings from an autopsy study in Uganda (Shaper et al, 1968a) in which infective endocarditis was found in 10 out of 173 cases of EMF. However, in that study of the 10 patients with EMF 7 had concurrence of rheumatic heart disease. When compared to its occurrence in rheumatic heart disease infective endocarditis was rare in EMF patients, this despite the higher prevalence of invasive procedures performed in these patients. The explanation for this phenomenon might be in the characteristic
pathological findings, which consist of the presence of thickened fibrous avascular endocardium separating the subendocardial vessels from the circulating blood (Connor et al., 1968).

We found a frequent association between EMF and RHD (12/175). There is lack of data on the epidemiology of these two conditions in our population and it is therefore difficult to say whether this finding results from a particular association between the two diseases or occurs just by chance. In Uganda EMF and RHD a higher than expected prevalence of concomitant disease in the same patients was found, but no clear explanation to the findings could be given (Shaper et al., 1968a). From Brazil there was also the description of a mitral valve disease with rheumatic appearance in the presence of left ventricular endomyocardial fibrosis (Saraiva et al., 1999).

At 70%, survival at 5-years follow up was better than the 2 years mean survival reported from Uganda (Somers, 1990). This may be explained by improvements in therapies for heart failure and its complications, mainly in the control of arrhythmias and prevention of thromboembolism, from which our patients have been benefiting.

Most hospital deaths were related to active disease, as defined in Chapter 3. Despite using the same criterion used for definition of hypereosinophilic syndromes (absolute eosinophil count over $1.5 \times 10^9/L$) we were intrigued to verify that only the heart was primarily affected in EMF. Multi-organ affection was absent in any of the patients, with death resulting from either episodes of severe myocardial dysfunction with recrudescence of heart failure or complications of thromboembolism, both in patients with active persistent or rapidly progressive disease. We also noticed that eosinophil counts varied
between normal levels and hypereosinophilia without any apparent cause (parasitic infection).

The control of hypereosinophilia in Hypereosinophilic Syndromes with intracavitary thrombus causes remodeling of the heart (Lofiego et al, 2005). Evaluation of the effects of new available anti–hypereosinophilia drugs must be carried out in patients with EMF and severe hypereosinophilia. This approach may be of value in changing the natural history of the subset of EMF patients who have recurrent hypereosinophilia, by inducing favorable long-term ventricular remodeling.

We acknowledge the lost of 29 patients for follow up, since having no contact with them for more than 12 months. A considerable number of families of EMF patients are poor and reside in areas of Mozambique with no access to telephone land lines or mobile phones making it impossible to actively search patients who do not come for follow up, while living in remote areas.
5.5. Conclusions

Most EMF patients presenting to medical attention are males in the second decade of life. The majority of patients from the seen in the referral centre reside in Inhambane province. Bilateral affection is the commonest phenotype in EMF patients, who usually have advanced heart failure, severe cardiac lesions and poor general condition. The survival of our patients is good. It seems to be negatively influenced by persistence of hypereosinophilia and clinical signs of activity. In the absence of consensual criteria to define activity and monitor progression we suggest periodical assessment of these clinical and laboratory signs of activity in order to detect recrudescence of disease and use adequate therapies for controlling hypereosinophilia.
Chapter 6

EVALUATION OF PATHOLOGICAL CHANGES
6.1. Introduction

The pathological findings of advanced EMF are well characterized. Most classical
descriptions have been based on autopsy studies of patients who died with complications
of long-standing heart failure or following episodes of rapidly progressive heart failure
(Connor et al, 1967; Shaper et al, 1968a). The scarcity of facilities for cardiac
catheterisation and open-heart surgery in most endemic areas for EMF has prevented the
studies in vivo. On the other hand, EMF patients usually seek medical attention with signs
of advanced disease and poor general condition, being therefore contraindicated for
cardiac catheterisation or surgery. Few studies have documented the histology findings in
vivo using endomyocardial biopsies obtained during cardiac catheterisation (Chopra et al,
1990; Andrade and Teixeira, 1973) or surgery (Santos et al, 2001; Saraiva et al, 1999).
The most salient pathological feature of established EMF is sub-endocardial deposition of
fibrous tissue, mainly type I collagen fibers (Radhakumary et al, 2001), which leads to
restriction of ventricular filling and distortion of the atrioventricular valve apparatus,
resulting in restrictive cardiomyopathy with atrioventricular valve dysfunction.

We evaluated the heart of EMF patients on autopsy and during surgery, and obtained
cardiac tissue from necropsies and excisional biopsies, aiming at describing the pathology
features, and relate them to clinical, echocardiographic and intraoperative abnormalities.
6.2. Patients and Methods

6.2.1. Autopsy Examination

The autopsy examinations were done in another institution, the Central Hospital of Maputo, since the Heart Institute does not have facilities for post-mortem examination. Permission for autopsy was obtained for two patients who died at the Heart Institute. The autopsy examination was performed in the presence of the investigator. Due to work overload pathologists only agreed to perform detailed examination of the heart and lungs. Tissue samples were obtained from the posterior wall of the left ventricle, left ventricular apex, trabecular right ventricular and atrioventricular valves. Liver, lungs and kidneys were also sampled.

6.2.2. Surgical studies

Patients from the Maputo Heart Institute’s EMF Registry were examined on echocardiography and classified as described in Chapter 3. All patients with moderate (Grade II) and severe (Grade III) EMF were considered for surgery.

Preparation for Surgery

Pre-operative care included medical management of (1) heart failure with diuretics and ACE-inhibitors; (2) atrial arrhythmias with digoxin and β-blockers; (3) thrombotic risk with oral anticoagulation; and (4) hypereosinophilia through short courses of oral
prednisolone. All patients were followed up for at least 3 months prior to surgery in order to monitor changes in eosinophil counts, treat concomitant infections, improve general condition through high protein diet and correct anemia. In patients with refractoriness to medical therapy drainage of pericardial, pleural and peritoneal effusion was considered. Cardiac catheterisation was considered in patients with severe distortion of the heart not allowing accurate echocardiographic evaluation of cavity dimensions and pulmonary pressures.

_Intra-operative evaluation_

The presence and extent of the mural and valvular changes was evaluated by the surgeon and the investigator, and systematically annotated. Details of the distribution of the intracardiac lesions were noted on a standard manner. Intra-operative photography was done using a Sony digital camera (model DSC-T9).

_Tissue sampling_

Targeted incisional endomyocardial biopsies were obtained in all patients from one or both ventricles during surgery for valve repair and/or endocardial resection performed via atriotomy. Biopsies of the left ventricle were taken from fibrotic areas of the posterior wall and the area surrounding the papillary muscles, whilst for the right ventricle tissue was collected at the admission chamber and the trabecular area. In patients who required endocardial resection the excised fibrous tissue was also made available for histology and immunostaining. Mitral valve tissue was obtained from one patient who underwent valve
replacement. Atrial tissue from patients who underwent atrial reduction was also examined.

6.2.3. Sample processing and staining

All specimens, obtained by surgery or during autopsy, were fixed in 10% buffered formalin, routinely processed for light microscopy, embedded in paraffin wax blocks and cut into 4 to 5 μm thick sections. These were stained for cell and for tissue morphology (Hematoxylin and Eosin, H&E) and collagen content, elastic fibers and mucins (Masson’s Trichrome, Verhoeff Elastic-Van Gieson, Alcian Blue-Sirius Red). The was used to identify eosinophils.

Immunostaining

Immunohistochemistry using the Avidin-Biotin peroxidase complex technique was done for identification of leucocytes (CD45), macrophages (CD68), endothelial cells (CD31), vessels (CD34) and myofibroblasts (Smooth Muscle Actin, SMA). All the primary antibodies were supplied by Dako. Paraffin wax 5μm-thick sections were dewaxed and rehydrated through xylene and graded alcohols to water, washed in phosphate buffered saline (PBS) for 5 minutes before blocking for endogenous peroxidase using 0.3% hydrogen peroxide in PBS for 15 minutes. Sections were then washed twice in PBS and blocked with 3% bovine serum albumin (BSA) in PBS for 30 minutes. Details of antigen retrieval, dilutions, and incubation times are given in Table
6.1. Negative control sections were incubated in 3% BSA only in PBS. Excess primary antibody was removed by washing the sections 3 times in PBS followed by a second layer of biotinylated goat anti-mouse immunoglobulins (GAM IgG-Vector laboratories) diluted 1/250 in PBS for 1 hour. Sections were then washed 3 times in PBS before 1-hour incubation with Avidin-Biotin Complex ABC-Vector laboratories). Reactivity was detected using diaminobenzidine tetrahydrochloride (DAB tablets-Sigma) (25mg/ml) and hydrogen peroxide (0.01% W/V). Sections were then counterstained with haematoxylin, dehydrated, mounted and viewed on a Zeiss Axioskop microscope. Photomicrographs were taken using Nikon DMX1200 camera.

6.2.4. Evaluation of cardiac tissue

The tissue was assessed for endothelial integrity, presence of inflammation, evidence of myofibroblastic proliferation, neovascularization, fibrosis, myocyte characterization, microvascular abnormalities, and fibrosis. This microscopic evaluation of the cardiac tissue was performed using several definitions that are discussed below.

Inflammatory changes

We assessed the presence of inflammatory cells. When it was present the phenotype and density of inflammatory cells was evaluated. According the its distribution in the tissue inflammation was classified as (1) Focal, when small foci of inflammation were found in parts of the tissue evaluated; or (2) Diffuse, when inflammation was diffusely present in all areas of the tissue evaluated. The intensity of the inflammation was classified as (1) rare when there were less than 5 inflammatory cells per/field observed at high power.
magnification; (2) mild when the number of cells varied between 5 and 10; and (3) intense when more than 10 inflammatory cells were present per field of high power magnification.

**Table 6.1.** List of primary antibodies (all supplied by DAKO©) used for immunostaining. This table shows the antibodies used for biopsy evaluation, their specificity, dilutions used, method of antigen retrieval, and the incubation times).

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Specificity</th>
<th>Dilution</th>
<th>Antigen retrieval</th>
<th>Incubation time/T°</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD45</td>
<td>Leukocytes</td>
<td>1/50</td>
<td></td>
<td>0.1 molar citrate buffer</td>
</tr>
<tr>
<td>CD68</td>
<td>Macrophages</td>
<td>1/50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD31</td>
<td>Endothelium</td>
<td>1/40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD34</td>
<td>Immature Fibroblasts</td>
<td>1/100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SMA</td>
<td>Smooth Muscle Cells</td>
<td>1/200</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Endocardial fibrosis**

Endocardial fibrosis was classified as (1) Focal when fibrous tissue was present in certain areas of the endocardium obtained; or (2) Diffuse, when all the extension of the endocardium in the specimen had fibrous tissue.

The type of fibroblastic response was also assessed and classified as (1) Early when there was a predominance of cells on the background of loose fibrous tissue, or (2) Hyaline when there was plaque-like collagen with little cellular response.
The measurement of the endocardium was done at the site of maximal thickening using the JULIA software. Endocardial thickening was considered mild (<1000 μm), moderate (1000-3000 μm) or severe (> 3000 μm).

Myocardial fibrosis
The presence of excessive collagen in the myocardium was classified according to its distribution as (1) interstitial – in the intercellular spaces that separated individual myofibers; (2) septate – extension of subendocardial fibrosis forming septa that separated groups of myofibers; (3) perifascicular – fibrosis entrapping several myofibers that become isolated from the rest of myocardium; and (4) replacement scars – presence scars inside the myocardium expressing the loss of myocardial fibers that are replaced by fibrous tissue. Table 6.2 presents a summary of the parameters evaluated, the staining methods used to detect them and the scoring system applied to each characteristic.

6.2.5. Clinical-pathological correlation
In the absence of a gold standard for early or active Endomyocardial Fibrosis, clinical and laboratory signs described earlier were used (Chapter 3) to classify the patients and evaluate whether in “acute and active fibrosis” there were signs of tissue inflammation, thrombus formation and intense fibroblastic response. The criteria compared were the following:
Clinical diagnosis versus operative findings

We compared the EMF stage and grade (using clinical, laboratory and echocardiographic criteria) with the operative pathology findings. The description of the echocardiographic features was compared to the lesions found at surgery, particularly the following: (1) LV plastered posterior mitral papillary muscle and leaflet; (2) fibrosis of the posterior wall; (3) apical fibrosis; (4) thickening of leaflets; (5) RV trabecular obliteration; (6) RV apical retraction; (7) size of the RV; (8) presence of intra-cardiac thrombus.

Clinical classification versus severity of pathology findings

EMF stage and grade were compared to findings in histology and immunochemistry, specially the presence, type and intensity of tissue inflammation (edema, inflammatory infiltrates, eosinophilia) and vascular lesions (arteritis and mural thickening). The severity of EMF assessed by echocardiography was also related to the intensity of endocardial fibrosis, presence of myocardial fibrosis, pattern of fibrosis and presence of myofibroblastic proliferation.
Table 6.2. Summary of the parameters evaluated, the staining methods and the scoring system applied for evaluation of the ventricular tissue samples. For the atrial samples evaluation of the epicardium was added, looking mainly for presence of inflammation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Staining</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Endocardium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Integrity of the endothelial layer</td>
<td>CD31</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Presence of elastin fibers</td>
<td>EVG</td>
<td>Present/absent</td>
</tr>
<tr>
<td><strong>Subendocardium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myofibroblastic proliferation</td>
<td>SMA</td>
<td>Yes/no</td>
</tr>
<tr>
<td>Presence, type of fibroblastic response</td>
<td>SR, MT</td>
<td>Hyaline/cellular,</td>
</tr>
<tr>
<td>Distribution of the fibrosis</td>
<td>SR, MT, VG</td>
<td>Focal/diffuse</td>
</tr>
<tr>
<td>Fibrous septae extending into myocardium</td>
<td>SR, MT, VG</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Neovascularisation</td>
<td>H&amp;E, CD34</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Presence and distribution of inflammatory cells</td>
<td>H&amp;E,</td>
<td>Yes/No,</td>
</tr>
<tr>
<td>Type of inflammatory cell</td>
<td>H&amp;E, Ch2R, CD45, CD68</td>
<td>focal/diffuse, Cell type</td>
</tr>
<tr>
<td><strong>Myocardium</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocyte changes (necrosis, hypertrophy, degeneration)</td>
<td>H&amp;E</td>
<td>Present/absent</td>
</tr>
<tr>
<td>Patterns of fibrosis (scars, interstitial, perivascular)</td>
<td>SR, MT, VG</td>
<td>Yes/No</td>
</tr>
<tr>
<td>Changes in myocardial microvasculature</td>
<td>H&amp;E</td>
<td>Yes/No,</td>
</tr>
<tr>
<td>Presence and distribution of inflammatory cells</td>
<td>H&amp;E, Ch2R, CD45,</td>
<td>focal/diffuse</td>
</tr>
<tr>
<td>Type of inflammatory cell</td>
<td>CD68</td>
<td>Cell type(s)</td>
</tr>
</tbody>
</table>

H&E hematoxillin-eosin; EVG elastin van Gieson; SMA smooth muscle actine; MT Masson Trichrome; Ch2R carbol chromatotrope technique; Vg Van Gieson;
6.3. Results

Two autopsy examinations were performed. Twenty-nine patients were included in operative studies.

6.3.1. Autopsy studies

Eleven patients died in hospital. From these, we obtained family consent for autopsy for two patients, which clinical characteristics are summarized in Table 6.3.

Patient 1

This boy was a 15-years old with chronic heart failure due to bilateral disease with predominance of lesions in the right side of the heart. The severity of the disease was graded III. Despite being admitted for intensive medical therapy including anticoagulants he had chronic pulmonary thromboembolism and refractory NYHA class IV heart failure. His body weight was 31kg and the height 136 cm.

On autopsy examination the body presented generalized edema, with bilateral hydrothorax and ascitis. At the opening of the chest there was a dilated pericardial sac with effusion of approximately 350ml. The pericardial fluid was dark yellow and was an exudate with predominant lymphocytes (70%) and high protein content.

The heart weight was 268g. On external observation of the heart there was right atrial dilatation, retraction of the right ventricle border originating a “notch” and dilatation of its outflow tract (Figure 6.1).
Chapter 6 Pathological Findings

The most striking feature on examination of the heart cavities was the presence of white thickened endocardium in both ventricles that was very prominent in the right side and did not have its trabecular portion (Figure 6.2). The fibrous process also involved the atrioventricular valves; on the left side it formed gross nodules on the leaflets and chordae (Figure 6.3), while the tricuspid valve had the leaflets and chordae less involved but presented great dilatation of the annulus and fusion of the papillary muscles to the fibrosis of the trabecular portion with tethering of the valve (Figure 6.4). Despite the absence of gross myocardial lesions, it presented penetrating spurs of fibrous tissue extending from the subendocardium in several areas. There were no macroscopic lesions of the semilunar valves.

The lungs presented signs of chronic pulmonary embolism and areas of atelectasia on the lower lobe of the right lung. There were signs of chronic congestion of the liver. The other organs did not present any abnormalities other than congestion.

Table 6.3. Clinical characteristics and diagnosis of the two patients that were autopsied

<table>
<thead>
<tr>
<th>Age/Sex</th>
<th>Weight</th>
<th>Height</th>
<th>EMF Classification</th>
<th>RPD</th>
</tr>
</thead>
<tbody>
<tr>
<td>15/M</td>
<td>31 Kg</td>
<td>136 cm</td>
<td>REMF III</td>
<td>RPD</td>
</tr>
<tr>
<td>22/F</td>
<td>41Kg</td>
<td>163</td>
<td>BEMF III</td>
<td>CQD</td>
</tr>
</tbody>
</table>

RPD rapidly progressive disease; CQD chronic quiescent disease

Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis
**Figure 6.1.** External aspect of a specimen at autopsy. The sulcus between the ventricles corresponds to the “notch” seen on echocardiography. Notice an aneurysmal right atrium.

**Figure 6.2.** The right ventricle shows extensive endocardial fibrosis forming an artificial floor that excludes the trabecular portion from the “functional ventricular cavity”.

*Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis*
Figure 6.3. Nodular aspect of the borders of the mitral leaflets with thickened chordae and whitening of the endocardium covering and surrounding the papillary muscles (top). Notice the diffuse whitening of the endocardial surface of the endocardium with thick fibrous tissue visible in the apex. The whitening of the endocardium extends through the outflow up to the valves but spares the aortic cusps, which are normal (bottom).
**Patient 2**

This patient was a 22-years old female who had been operated on nine days before for correction of BEMF with predominant left-sided lesions. She died as a result of accidental perforation of the heart during pericardial drainage to relieve pericardial tamponade. The examination of the chest did not show any apparent macroscopic complications of the surgery. There was cardiomegaly and the pericardial sac was filled with clots. A laceration 18mm long was identified in the anterior wall of the right ventricle, near the apex. There were no coronary lesions. The weight of the heart is not available.

The internal lesions of the heart were altered by the recent surgery that included left endocardial resection, and mitral and tricuspid repair with the use of bands of Goretex material (**Figure 6.5**). Fibrous plaques were visible in the left ventricular apex and the posterior wall of the left ventricle. The right ventricle was dilated with thin wall (5mm) and had scattered foci of fibrosis in the endocardium of the inflow tract. There were no lesions of the semilunar valves.

There was pulmonary and liver congestion, and the tissue samples from all main organs of the body showed no signs of disease apart from changes related to chronic passive congestion.
Figure 6.4. The tricuspid annulus was usually dilated, there is tethering of the leaflets due to fibrosis of the papillary muscles and partial fusion of the leaflets to the walls (bottom) starting at the level of the annulus and leading to an Ebstein-like abnormality of the tricuspid valve.
6.3.2. Operative studies

 Characteristics of patients

15 patients had grade II EMF whereas 14 had grade III lesions. EMF was confined to the left ventricle in 13 patients (LEMF), to the right ventricle in 4 (REMF) and was biventricular in the remaining 12 (BEMF). Their mean (± SD) age was 12 (± 4.6) years. Seventeen 17/29 were male. The details of the relevant clinical and laboratory findings in these patients are presented in Table 6.4. Twenty-eight patients were in NYHA functional class III-IV. Mean body mass index was 15.7 (± 2.4) kg/m². Sixteen patients had signs of active disease as defined by raised C-reactive protein (CRP) levels, increased erythrocyte sedimentation rate (ESR) and/or peripheral blood hypereosinophilia (EOS). The mean (±SD) ESR was 31.8 (± 28.0) mm/h and the mean absolute eosinophil count was 1.6 (± 1.5) x 10⁹/L.

At sternotomy there was cardiac enlargement in all patients with a mild to moderate pericardial effusion in 8. The pericardial fluid was yellow and constituted an exudate in all cases with high protein content (mean 52g/dL) and increased cellularity with predominance of lymphocytes (mean 61%). None tested positive for tuberculosis.
### Table 6.4.
Clinical data from the 29 patients operated from whom tissue was collected (this and next pages)

<table>
<thead>
<tr>
<th>Code</th>
<th>Tissue</th>
<th>Age/Sex</th>
<th>BMI</th>
<th>ESR</th>
<th>CRP</th>
<th>NYHA</th>
<th>Ex10^9/L</th>
<th>EMF Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>0029</td>
<td>LV</td>
<td>17/M</td>
<td>17.3</td>
<td>High</td>
<td>N</td>
<td>IV</td>
<td>0.3</td>
<td>LEMF II</td>
</tr>
<tr>
<td>0085</td>
<td>LV</td>
<td>6/F</td>
<td>14.2</td>
<td>High</td>
<td>N</td>
<td>IV</td>
<td>1.8</td>
<td>LEMF III</td>
</tr>
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M male; F female; NYHA New York Heart Association; E eosinophil count
BMI body mass index; ESR erythro sedimentation rate; CRP C reactive protein
Chapter 6 Pathological Findings

Figure 6.5. Specimen from a patient died after surgery for EMF, showing the aspect of the endocardium of the left ventricle. This patient benefited from mitral repair using Goretex bands with no endocardial resection.

Figure 6.6. This specimen reveals whitening of the endocardium with occasional strands of fibrosis, atrial hypertrophy and thrombus fixed to the atrial appendage.
All patients had dilatation of one or both atria. Atriotomy of the dilated atria revealed thickening of the wall due to edema and muscle hypertrophy. No major plaques of fibrosis were seen, but in 7/29 cases there was patchy whitening and thickening of the endocardium (Figure 6.6).

In the typical case of REMF the tricuspid valve annulus was markedly dilated, affording wide access to the right ventricular cavity (Figure 6.7). Left atriotomy showed a moderately dilated mitral valve annulus, and in all but one patient assessment of the distribution and extent of subvalvar and wall involvement by EMF was achieved through gentle retraction of the valve apparatus (Figure 6.8). The exception was a patient with a large calcified thrombus involving the papillary muscles and chordae of the mitral valve as well as important left apical fibrosis, for whom left ventriculotomy was required.

The most striking finding in the ventricles was the presence of fibrotic plaque-like lesions in the inflow tracts, the left ventricular apex, the recess behind the posterior mitral leaflet and covering the trabecular portion of the right ventricle. When the right ventricle was affected it was in the obliterative stage necessitating reopening of the cavity. Mural and valvular abnormalities coexisted in varying proportions, but all patients had severe valve dysfunction needing surgery. Mural involvement was present to a variable extent causing reduction of the ventricular cavity and effacement of the components of the valve apparatus, which were fused to the mural plaques. The distribution of plaques in these patients is shown in diagrams 2 (left ventricle) and 3 (right ventricle). A more detailed description of the changes in each side of the heart ventricle is now given.
Figure 6.7. Intraoperative visualization of lesions on REMF was made through right atriotomy and using retraction of the dilated tricuspid valve ring

Figure 6.8. Intraoperative evaluation of LEMF lesions through left atriotomy. Notice the complete fusion of the posterior leaflet to the posterior wall.
a) Left Cavities
In LEMF the mitral valve leaflets invariably showed diffuse irregular thickening, with fibrotic nodules in some cases. The chordae were frequently thickened but not fused. In most cases the posterior leaflet, its chordae and the posterior papillary muscle were partially or totally fused to the posterior wall (Figure 6.8). The anterior papillary muscle was usually fused to the wall, with variable restriction of its mobility, contributing significantly to mitral regurgitation. In cases of extensive involvement of the anterior papillary muscle there was restriction of the movement of the anterior leaflet leading to an additional component of mitral stenosis.

The left atrium was variably dilated, in some cases being aneurismal.

b) Right Cavities
In REMF all right ventricles were in the obliterative phase. In these cases a layer of endocardial fibrosis 2-3 mm thick appeared to start just below the level of the tricuspid annulus and extended downwards into the ventricular cavity. The fibrotic area was firmly attached to the ventricular wall and usually spared the tricuspid leaflet and chordae. However, in more advanced cases, these structures were embedded in fibrous tissue extending along segments of the circumference of the annulus. The fibrosis extended all round the inflow tract to the level of its junction with the trabecular right ventricle where it formed an “artificial floor” isolating the trabecular right ventricle from the rest of its cavity (Figure 6.7). The trabecular cavity was obliterated due to separation of the
entrapped trabeculae from the main cavity and their fusion together and to the muscular septum, while being spared by the fibrotic process.

The right atrium was markedly dilated and mural thrombi were present in three cases.

In the 12 patients with BEMF the above findings were observed in varying proportion.

As a result of prolonged elevation in pulmonary arterial pressure there was hypertrophy and dilatation of the right ventricle in both LEMF and BEMF with predominance of LEMF. Two cases with severe pulmonary hypertension had thin-walled right ventricles, raising the suspicion of cardiomyopathy that was later excluded on histology. There was usually balanced bi-atrial dilatation.

### 6.3.3. Microscopic findings

**a) Ventricles**

Biopsies from 25 left ventricles and 12 right ventricles were evaluated. Adequate amount of myocardial tissue was available for evaluation in 26 of the 29 patients studied. The summary of the main abnormalities on histology of the 29 ventricles is given in Table 6.5 while Table 6.6 presents the frequency and intensity of fibrosis (6.6a) and inflammation (6.6b) according to the echocardiographic scoring system.

The main finding in the ventricular tissue from these patients was endocardial thickening due to deposition of hyaline and cellular collagen in varying proportions (Figure 6.9a),
beneath a layer of apparently normal endocardial endothelial cells. The thickened sub-
endocardium consisted of a superficial layer of collagen usually with hyalinization
(calcification was found in only one case), and a deeper, loose spongy layer of fibrous
tissue with numerous capillary channels and scanty mixed inflammatory infiltrates.
Small amounts of fragmented elastic tissue were mixed with the fibrous tissue. There was
mild proliferation of smooth muscle cells in the superficial subendocardium, usually
forming a single thin layer with fibroblast above and below it.

Deep in the sub-endocardium there were foci of intense neovascularisation associated
with chronic inflammation the latter composed mainly of mononuclear cells, namely
lymphocytes, macrophages and plasma cells (Figure 6.9b). These findings contrasted
with the usually absent or very mild myocardial inflammation. In 2 cases there was a
mixed inflammatory infiltrate with increased number of eosinophils. Significant
endocardial and myocardial eosinophilia was found in only one patient (Figure 6.10).

Strands of fibrous tissue penetrated the inner myocardium, seemingly tethering the
subendocardium and the myocardium together and being in continuity with the
perivascular spaces. In 23/29 cases an increase in fibroblasts in the interstitial spaces was
noticed. Nine patients had foci of replacement fibrosis in the myocardium.

Degenerative changes of the myocardium were usual including myocyte hypertrophy,
enlarged bizarre nuclei and myocardial (but not myofibril) disarray often in relation to
scars. The myocardial vessels showed thickening of the media in most cases.
Table 6.5. Histological abnormalities in ventricular tissue samples obtained surgically (this and next pages)

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<th>Endocardium Thickness</th>
<th>Collagen Type</th>
<th>Sub-endocardium Inflammation</th>
<th>New vessels</th>
<th>Myocardium Pattern of Fibrosis</th>
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ESS= EMF severity score; ?= unknown; Thickness in µm; IF= interstitial fibrosis; WS=wide septa; RF=replacement scars; PF=perivascular fibrosis
Table 6.6. Distribution and intensity of fibrosis and inflammation in ventricular tissue samples according to the echocardiographic severity score (ESS). The stromal changes were quantified without subtracting the SMA positive cells.

6.a) Fibrosis

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* Two grade III and one grade II not evaluated; some patients with more than one pattern

b) Inflammation

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<tr>
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Figure 6.9a. Microscopic findings in the ventricle affected by EMF include marked endocardial thickening (E) and fibrosis that affects the inner part of the myocardium stained in red (5X Van Gieson, top left). Vessels are seen in the endomyocardial interface, mainly in strands of fibrosis that penetrate the myocardium (top right, H&E 5x). The bottom picture amplifies and area of the myocardium that reveals large bands of fibrosis (F) separating groups of myofibers (M).
Figure 6.9b. Endocardial thickening (E) and inflammatory infiltrates (I) in the interface between the endocardium and the myocardium (x5 H&E staining, top left), which is also
rich in vessels (V) with a thickened wall (5X H&E staining, top right). The inflammatory infiltrates were constituted mainly of lymphocytes (bottom).

**Figure 6.10.** The endocardium in EMF patients had little mucopolisacharides that stain in blue in Alcian Blue staining (top left, x10). Mixed inflammatory infiltrates were also
frequently found (x5 H&E, top right) in some patients. Tissue eosinophilia was found in one patient with moderate LEMF (x40HE)

\textit{b) Atria}

Atrial tissue from 6 patients was examined. The histopathological findings on atrial tissue are presented in Table 6.7.

\begin{table}[h]
\centering
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\hline
EMF grade & Age (years) & NYHA Class & Endocardial thickness (µm) & Fibrosis pattern & Inflammation \\
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0068 BEMF III & 9 & III & 760 & Patchy & Mild chronic multifocal \\
0059 LEMF II & 12 & IV & 450 & Rare & No \\
0064 LEMF II & 12 & III & 1315 & Patchy & Mild mixed perivascular \\
0048 LEMF II & 11 & IV & 1804 & Patchy* & Mild mixed multifocal \\
0163 BEMF IV & 10 & IV & 929 & Rare & No \\
0167 BEMF IV & 10 & IV & 499 & Rare & Mild chronic multifocal \\
\hline
\end{tabular}
\caption{Microscopic findings in 6 EMF patients from whom atrial tissue was obtained}
\end{table}

* Patchy with replacement fibrosis
The tissue showed diffuse irregular thickening of the endocardium to a much lesser extent, if compared to that observed for the ventricles. In two patients the left atrial tissue presented considerable endocardial thickening (more than 1mm) and there was deposition of fibrous tissue and moderate hypertrophy of the smooth cells in between the endocardium (Figure 6.11).

The evaluation of the atrial myocardium revealed no areas of fibrosis with overlying endocardial thickening, the myocardium being often involved without apparent endocardial lesions. Moderate hypertrophy of nuclei and contraction band necrosis, representing signs of reperfusion probably surgery-related were frequent. In some cases there was also focal myocardial hemorrhage related to the surgical trauma. There was usually mild interstitial fibrosis. Replacement fibrosis was present in one case. Inflammation affected randomly all layers of the atrial wall, and was more intense in the subendocardium, the epicardium and surrounding the intramyocardial vessels. Mixed inflammatory infiltrates were the rule with predominance of lymphocytes and few eosinophils.

Vascular changes occurred in most cases, the most frequent being in the muscularis of the subendocardial arterioles (medial focal proliferation in medium size arteries), which was replaced by a mixture of hyalinized collagen and elastic fibers (Figure 6.12). The majority of these vessels were surrounded by excessive fibrous tissue. In no case intimal proliferation was observed.
Figure 6.11. Atrial tissue showed fibrosis (red) in all three layers of the wall (Masson Trichrome, x5). Thickening of the atrial endocardium is also noticed (E) but to lesser extent when compared to the ventricles. Perivascular inflammation (I) was more prominent in the epicardium in areas surrounding the vessels (V) as shown in top right and bottom pictures.
Figure 6.12. Immunostaining for CD34 showed medial proliferation in medium size arteries of ventricular tissue (x10 left, x20 right)

Figure 6.13. Microphotograph showing mitral valve tissue from an EMF patient with predominant left lesions (H&E, x25).
c) Valves

There was thickening of the leaflets due to deposition of fibrous tissue in the fibrosa layer. No inflammatory infiltrates or neovascularization were found in the leaflets or chordae. The spongiosa, auricularis and ventricularis were normal (Figure 6.13).

d) Immunohistochemistry

Immunostaining of the tissue samples allowed characterization of the inflammatory infiltrates, evaluation of the endothelial integrity, determination of the presence of smooth muscle cells and immature fibroblasts.

In all samples the cells present in the inflammatory infiltrates were predominantly CD45 and CD68 positive (Figure 6.14).

CD31 staining of ventricular and atrial tissue showed a preserved endothelial lining in all samples, as well as in the blood vessels located in the subendocardium and myocardium (Figure 6.15).

The positive staining for SMA was confined to one single layer of cells inside the thickened sub-endocardium. There is no smooth muscle cells proliferation (Figure 6.16).

The thickened sub-endocardium stained positive for CD34 in all samples (Figure 6.17).
Figure 6.14. CD45 immunostaining confirmed the presence of few granulocytes (left) while CD68 staining confirmed the presence of macrophages (right).

Figure 6.15. CD31 staining of ventricular tissue of an EMF patient (left) and negative control (right). Notice concomitant staining of monocytes and macrophages inside the vessels in the patient’s sample.
Figure 6.16. SMA (smooth muscle actine) positive cells in normal amounts in the vessel wall in areas of with increased vascularization (left, x5). Strands of SMA positive cells were encased by fibroblasts in the subendocardium (right, x20).

Figure 6.17. CD 34 positive cells are seen in the subendocardium of trabeculae of the right ventricle of one patient with REMF, forming longitudinal strands. In a patient with LEMF (right, x5) smaller strands of CD34 positive cells were found.
6.3.4. Clinicopathological correlation

a) Concordance between echocardiographic diagnosis and surgical findings

In 24 of the 29 patients the echocardiographic description correlated well with the intraoperative findings, with absolute concordance for fusion of papillary muscle to the posterior wall, fusion of the posterior leaflet to the wall, left ventricular apical fibrosis, thickening of the atrioventricular leaflets, right ventricular obliteration, right ventricular retraction and ventricular thrombi (Table 6.8).

Echocardiography overdiagnosed fibrosis of the left ventricular septum and posterior wall, and underdiagnosed abnormalities of chordae and the anterior wall including the anterior papillary muscle.

In one case of left ventricular EMF the echocardiography did not reveal the extension of involvement of the anterior papillary muscle, which on surgery was completely fused to the anterolateral wall. In two cases severe fibrosis of the anterior wall was assessed as moderate on echocardiography. In one case of bilateral EMF there was an associated congenital malformation of the tricuspid valve (double orifice) not diagnosed on echocardiography.

An 8-years old boy with obliteration of the trabecular portion of the right ventricle and history of disease for less than 6 months was found to have more fibrosis than anticipated by echocardiography. No signs of thrombus were found in the obliterated part of the ventricle either on macroscopy or in histological examination. The fibrosis formed a false
floor that isolated the fused trabeculae. This could be separated, leading to reopening of
the right ventricular cavity.

Table 6.8. Comparison of the structural abnormalities detected by echocardiography with
the findings during intra-operative evaluation of the operated hearts

<table>
<thead>
<tr>
<th>Characteristic findings</th>
<th>Echocardiography</th>
<th>Surgery</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusion papillary muscle-posterior wall</td>
<td>10</td>
<td>10</td>
<td>100%</td>
</tr>
<tr>
<td>Fusion posterior leaflet-posterior wall</td>
<td>10</td>
<td>10</td>
<td>100%</td>
</tr>
<tr>
<td>Fusion anterior papillary muscle to wall</td>
<td>8</td>
<td>11</td>
<td>73%</td>
</tr>
<tr>
<td>Fibrosis of the LV posterior wall</td>
<td>18</td>
<td>17</td>
<td>&gt;100%</td>
</tr>
<tr>
<td>Fibrosis of the LV septum</td>
<td>5</td>
<td>3</td>
<td>&gt;100%</td>
</tr>
<tr>
<td>LV apical fibrosis</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>Thickening of mitral or tricuspid leaflets</td>
<td>16</td>
<td>16</td>
<td>100%</td>
</tr>
<tr>
<td>RV Obliteration</td>
<td>10</td>
<td>10</td>
<td>100%</td>
</tr>
<tr>
<td>RV apical retraction</td>
<td>2</td>
<td>2</td>
<td>100%</td>
</tr>
<tr>
<td>Thickening or fusion of chordae</td>
<td>3</td>
<td>5</td>
<td>80%</td>
</tr>
<tr>
<td>Ventricular Thrombus</td>
<td>1</td>
<td>1</td>
<td>100%</td>
</tr>
<tr>
<td>Ventricular calcification</td>
<td>1</td>
<td>2</td>
<td>50%</td>
</tr>
</tbody>
</table>
Table 6.9. Presence of tissue inflammation according to EMF staging

<table>
<thead>
<tr>
<th>Tissue</th>
<th>EMF Type and Grade</th>
<th>Sub-endocardium</th>
<th>EMF Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inflammation</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>New vessels</td>
<td></td>
</tr>
<tr>
<td>0029</td>
<td>LV</td>
<td>LEMF II</td>
<td>Chronic +</td>
</tr>
<tr>
<td>0085</td>
<td>LV</td>
<td>LEMF III</td>
<td>Chronic +</td>
</tr>
<tr>
<td>0064</td>
<td>LV</td>
<td>LEMF II</td>
<td>No</td>
</tr>
<tr>
<td>0123</td>
<td>LV</td>
<td>LEMF II</td>
<td>Chronic ++</td>
</tr>
<tr>
<td>0009</td>
<td>LV</td>
<td>BEMF III</td>
<td>Mixed ++</td>
</tr>
<tr>
<td>0048</td>
<td>LV</td>
<td>LEMF III</td>
<td>Mixed ++</td>
</tr>
<tr>
<td>0072</td>
<td>LV</td>
<td>LEMF II</td>
<td>Chronic +</td>
</tr>
<tr>
<td>0125</td>
<td>LV</td>
<td>LEMF III</td>
<td>Chronic ++</td>
</tr>
<tr>
<td>0128</td>
<td>LV</td>
<td>LEMF III</td>
<td>Chronic +</td>
</tr>
<tr>
<td>0144</td>
<td>LV</td>
<td>LEMF II</td>
<td>Chronic ++</td>
</tr>
<tr>
<td>0068</td>
<td>LV RV</td>
<td>LEMF II</td>
<td>Chronic +</td>
</tr>
<tr>
<td>0062</td>
<td>LV RV</td>
<td>BEMF III</td>
<td>Chronic ++</td>
</tr>
<tr>
<td>0142</td>
<td>LV</td>
<td>BEMF III</td>
<td>Chronic ++</td>
</tr>
<tr>
<td>0146</td>
<td>LV RV</td>
<td>BEMF III</td>
<td>Chronic ++</td>
</tr>
</tbody>
</table>
### Table 6.9

<table>
<thead>
<tr>
<th>Tissue</th>
<th>EMF Type and Grade</th>
<th>Sub-endocardium</th>
<th>EMF Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Inflammation</td>
<td>New vessels</td>
</tr>
<tr>
<td>0065</td>
<td>LV RV</td>
<td>LEMF II</td>
<td>Mixed +</td>
</tr>
<tr>
<td>0059</td>
<td>LV</td>
<td>LEMF II</td>
<td>Chronic +</td>
</tr>
<tr>
<td>0138</td>
<td>LV</td>
<td>BEMF III</td>
<td>Chronic +</td>
</tr>
<tr>
<td>0139</td>
<td>LV</td>
<td>BEMF II</td>
<td>Chronic +</td>
</tr>
<tr>
<td>0179</td>
<td>LV RV</td>
<td>BEMF II</td>
<td>No</td>
</tr>
<tr>
<td>0180</td>
<td>LV RV</td>
<td>BEMF II</td>
<td>No</td>
</tr>
<tr>
<td>0169</td>
<td>LV</td>
<td>BEMF III</td>
<td>No</td>
</tr>
<tr>
<td>0163</td>
<td>LV RV</td>
<td>BEMF II</td>
<td>No</td>
</tr>
<tr>
<td>0052</td>
<td>RV</td>
<td>REMF III</td>
<td>Chronic +</td>
</tr>
<tr>
<td>0167</td>
<td>LV RV</td>
<td>BEMF III</td>
<td>No</td>
</tr>
<tr>
<td>0152</td>
<td>RV</td>
<td>REMF III</td>
<td>No</td>
</tr>
<tr>
<td>0110</td>
<td>RV</td>
<td>REMF III</td>
<td>Chronic ++</td>
</tr>
<tr>
<td>0151</td>
<td>RV</td>
<td>REMF II</td>
<td>No</td>
</tr>
<tr>
<td>0107</td>
<td>LV</td>
<td>LEMF II</td>
<td>Acute ++</td>
</tr>
<tr>
<td>0140</td>
<td>LV</td>
<td>BEMF II</td>
<td>No</td>
</tr>
</tbody>
</table>
b) Concordance between clinical classification and microscopic findings

Table 6.9 summarizes the way disease activity correlated with tissue inflammation and neovascularization. There was no clear correlation between clinically quiescent disease and absence of inflammation in the sub-endocardium and the myocardium: 5/9 had signs of mild chronic inflammation and the remaining 4 had no tissue inflammation at all. Of the 12 patients with severe blood hypereosinophilia, only one had mild tissue eosinophilia in atrial tissue. Conversely, eosinophilic infiltrates were found in ventricular tissue of a patient without severe eosinophilia (absolute eosinophil count $0.9 \times 10^9/L$) and who had only patchy deposition of cellular collagen in the sub-endocardium (Figure 6.10).

As shown in Table 6.6 and Figure 6.18 severe or Grade III EMF was more frequently associated with intense endocardial fibrosis than Grade II.
Figure 6.18. Graph showing the relation between the severity of EMF as assessed by echocardiography and the degrees of endocardial thickening on histology (mild < 1000 µm, moderate 1000-1999 µm; severe > 2000 µm.)
6.4. Discussion

The pathological features of EMF were distinctive and allowed easy differential diagnosis from other heart conditions.

On external examination of the heart the most striking findings were the aneurysmal atria contrasting with small ventricles and, in severe REMF, the “right apical notch”.

The pathological hallmark of established EMF was focal or diffuse endocardial whitening and thickening, with a smooth and shiny surface, involving mainly the ventricles. Microscopically these lesions corresponded to deposition of dense fibrous tissue in the subendocardium, with variable involvement of the inner myocardium. The fibrous tissue markedly diminished the volume and compliance of the affected ventricles, and distorted the mitral and tricuspid valves, producing a restrictive functional abnormality associated with severe atrioventricular regurgitation, both resulting in extremely dilated atria.

The mechanism of cavity retraction and apical notch in patients with severe REMF was obliteration of the right ventricular trabecular portion causing its exclusion from the circulation and subsequent fusion of the trabeculae. In the obliterative phase there was compaction of the trabeculae between the thickened endocardium and the epicardium, with no thrombus or blood in between them. Although progressive fusion of trabeculae leads to retraction of the ventricular wall towards endocardial fibrotic floor resulting in the typical right apical notch (Figure 6.1), the myocardium between the thickened endocardium and the epicardium was viable (Figure 6.2). In all hearts the moderator
band was lost, engulfed in the endocardial fibrosis, and there was the constitution of a false floor linking the admission chamber to the right ventricular outflow tract.

There were no macroscopic signs of inflammation in mural and atrioventricular valve endocardium, even in patients with clinical and biological features of inflammation. Moreover, on microscopy the outer regions of the affected endocardium were avascular with inflammation being located essentially in the endomyocardial interface. This lack of vessels in the outer endocardium could explain why bacterial endocarditis did not occur in any patient with EMF from our series. Although most patients had chronic inflammation none could be considered as having severe endocarditis, myocarditis or pericarditis. Our results corroborate early findings in Uganda (Shaper et al, 1968a) where the degree of neovascularisation of the valves has been used as a pathological criterion to differentiate RHD from EMF, as well as to diagnose the concurrence of both diseases in the same heart.

Fibrosis affected primarily the ventricular and, to a lesser extent, the atrial endomyocardium where it presented a patchy pattern more prominent in dilated and hypertrophied atria. Despite most authors referring to EMF as being a disease that affects the ventricles, fibrosis of the atrial endomyocardium has been noted in 19-60% of the cases (Ball et al, 1954; Edington and Gilles, 1976). This atrial patchy fibrosis is thought to constitute the structural basis for atrial fibrillation, rather than the atrial dilatation (Jaiyesimi, 1982). Unfortunately, we did not systematically obtain atrial tissue at the beginning of the studies and cannot comment on the relation between atrial fibrosis and rhythm disturbances. Evaluation of atrial tissue collected from patients submitted to atrial
reduction showed macroscopic and microscopic abnormalities and therefore we started collecting atrial tissue samples from all EMF patients submitted to surgery.

The distribution of endocardial lesions was variable with predominance of valvar lesions in some patients, mural abnormalities in others, or a mixture of both. An attempt at classifying the pathological patterns of distribution of endomyocardial lesions was done by researchers in Uganda (Shaper et al, 1968a). They defined five patterns of endocardial lesions: type 1 affecting only the apex; type 2 extending from the apex to the valve; type 3 affecting only the valvular region; type 4 affecting separately the valves and the apex with areas of unaffected endocardium between them; and finally, type 5 when there was patchy involvement of areas away from the apex and the valve. Considering this classification our patients did fall mostly in intermediate or combined patterns, and we did not find the classification useful since it did not include any clinical-pathological correlation.

Mural thrombosis and endocardial fibrosis affected mainly the left ventricular apex, the recess behind the posterior leaflet of the mitral valve, and the trabecular portion of the right ventricle, and areas of poor blood supply from the end coronary arteries. This consistent distribution of thrombotic and fibrotic lesions in EMF has been attributed to the sluggish blood flow at these regions, which would result in relative hypoxia and increased susceptibility to injury, creating conditions for thrombotic damage and local fibrosis (Sezi, 1996a). Research using a model of the ventricle to study the diastolic flow patterns concluded that this distribution corresponded apparently to regions of low wall shear stress (Shaper and Bellhouse, 1973). Interesting for the exploration of the
mechanisms of EMF is the fact that these regions have recently been found to modulate
the expression of genes encoding basic fibroblast growth factor (b-FGF) and platelet-
derived growth factor (PDGF) in the endothelium (Malek et al, 1993).
The most salient histological abnormality in the hearts of patients with EMF was the
increase in the number of fibroblasts in the subendocardium, responsible for endocardial
fibrous thickening of variable intensity in different hearts and also varying from one area
to the other in the same heart. There was also variation in the pattern of extension of the
subendocardial fibrous tissue to the myocardial compartment. The most common pattern
was the formation of fibrous septa that penetrated the inner portion of the myocardium
separating groups of myofibers and occupying mainly the perivascular spaces.
The most frequent vascular changes occurred in the muscularis of the subendocardial
arterioles, which was replaced by a mixture of hyalinized collagen and elastic fibres.
These abnormalities seemed to be the extension of the sub-endocardial changes rather
than a fibroblast proliferation process, since there were not thickened sub-endothelial
areas of the vessel wall and CD34 staining was negative in the vessels. As mentioned
above the majority of these vessels were surrounded by excessive fibrous tissue.
Regarding the myocardium, the presence of inflammatory infiltrates, interstitial fibrosis
and myocardial scars could be a secondary response to ischemic injury due to vascular
wall changes that lead to low perfusion of the affected areas of the myocardium, since
these abnormalities were more prominent in areas adjacent to the subendocardial fibrosis
and abnormal vessels.
EMF appeared to be an interstitial disease, with preserved endothelial endocardium, elastic fibers and, to a certain extent, normal myocytes. The absence of smooth muscle cells proliferation, excessive mucopolisacharides or increase in elastin content differentiated EMF from fibroelastose. Our patients, who had severe disease and were submitted to surgery, had severe scarring and absence of acid mucopolysaccharides (AMP), corroborating studies that related the excessive content of AMP in cardiac tissue to short duration of the illness (Connor et al, 1968).

The concurrence of both hyaline and acellular collagen, foci of collagen necrosis, and multifocal chronic inflammation in the same patient or in different areas of the same heart, point towards an ongoing injury but could also represent the result of a process involving temporally spaced insults.

Despite our efforts to identify and characterize tissue eosinophilia, we found severe eosinophil infiltrates in only one patient. Similarly, the presence of clinical signs of disease activity did not coincide with tissue inflammation. Moreover, blood eosinophilia did not coexist with tissue eosinophilia, indicating that the insult to the heart is not entirely mediated by the eosinophil or that this cell does not cause direct damage. Eosinophil granule proteins and not the eosinophil itself could mediate cardiotoxicity of the eosinophils. Although we did not investigate this issue in our patients, previous research undertaken in Nigeria concluded that both degranulated eosinophils and significantly elevated serum levels of eosinophil granule basic proteins were not present in sera from EMF patients (Urhoghide and Falase, 1987). The results of these studies,
performed in patients with established disease, do not exclude the role of hypereosinophilia in cardiac damage, which could have occurred in earlier stages.

The heart was the sole organ primarily involved in EMF, with structural changes in all other parts of the body resulting from chronic congestion and low perfusion associated with heart failure and/or complications of thromboembolism. The reasons for this selective injury of the heart are not clear. The heterogeneity of the endothelium (Aird, 2007) could be one explanation. On the other hand, the recent discovery of tissue factor (TF) expression in the myocardium but not in other muscles types (Mackman, 2008), might explain the particular predisposition of the heart tissue to local thrombosis. The mechanism would be initiated by injury to the endomyocardium by a yet unknown mechanism that could be an ongoing infection, autoimmune mechanism or cardiotoxic eosinophil products. Endothelial cell activation would induce leakage of plasma proteins leading to local TF exposure and fibrin deposition, which would initiate the process of endomyocardial thrombosis. Indirect support for this hypothesis comes from the findings of fibrin deposits detected by immunofluorescence in hearts of EMF patients studied on autopsy (Shaper et al, 1967), and concomitant peritoneal and skeletal muscle fibrosis in EMF patients with severe muscular atrophy and ascitis (Freers et al, 2000).

Both the primary target of injury and the origin of the subendocardial fibroblasts found in heart of patients with EMF are unclear. Our patients with established disease had normal endothelial endocardium on histological examination, a finding confirmed by CD31 immunostaining. This suggests that either there is re-endothelisation after endocardial disruption associated with endocardial thrombosis described in other series looking at
patients died in earlier phases of the disease (Connor et al, 1967), or that the sequence of events leading to fibrosis in this condition differs from that described for the Hypereosinophilic Syndrome.

We found good concordance between echocardiography findings and pathological abnormalities considered as having diagnostic and prognostic value. This is of paramount importance since this suggests that echocardiography, a technique that has been made progressively more available, would ensure correct diagnosis and management of EMF in poor endemic areas, where MRI will continue to be unaffordable.

We acknowledge that obtaining informed consent from the families patients for post-mortem examination was difficult due to local cultural believes. For ethical reasons most of the cardiac tissue for evaluation was obtained from fibrotic tissue excised during surgery of EMF. For the same reason in patients in whom endocardial resection was not performed we limited the amount of tissue collected. This resulted in availability of endocardium and underlying myocardium, therefore excluding other areas of the myocardium and the epicardium, which might present specific abnormalities. Another limitation of this study is the fact that, due to our preference for valve repair, we had only one mitral valve available for evaluation.
6.5. Conclusions

EMF is an interstitial and inflammatory disease with typical distribution in the heart, affecting all layers of the ventricular and atrial walls.

Tissue eosinophilia is rare in established EMF. Fibrotic changes, chronic inflammatory infiltrates and neovascularization are more prominent at subendocardium and inner myocardium.

The features detected by echocardiography are highly concordant with those found during surgical procedures and autopsies, confirming that correct diagnosis and management of EMF is possible in the majority of patients from endemic areas using that non-invasive diagnostic tool.

The detailed intra-operative evaluation of the heart represents a powerful tool for understanding of the pathophysiology of EMF, and has allows the development and evolution of tailored techniques for the treatment of the specific lesions found in EMF patients.
Chapter 7

IMMUNE ACTIVATION IN CHRONIC ESTABLISHED EMF
7.1. Background

The study of the immune response in patients with EMF has been one of the many ways of trying to uncover the etiology and pathogenesis of this condition. Early studies demonstrated the presence of high levels of autoantibodies in African patients with EMF (Shaper et al, 1967; Van der Geld et al, 1966). This findings were difficult to interpret since not specific to EMF (Shaper et al, 1967; Shaper et al, 1968b), and no more studies deal with this issue. More recently, the pattern of serum immunoglobulins in Indian patients with EMF was described (Mathai et al, 1986; Vijayaraghavan et al, 1984).

The attention of researchers has always been concentrated on the usual finding of hypereosinophilia in EMF patients seeking medical attention (Patel et al, 1977; Andy, 1981; Andy, 1983). Normally, the eosinophils constitute a minor population (1-4%) of circulating leukocytes and, under basal conditions, are only observed in the bone marrow (where they originate) and in the gut. During parasitic infection eosinophil production by the bone marrow is up regulated, blood eosinophils are increased in number and migrate to accumulate at sites where the worms are located. Once in contact with the foreign organisms, eosinophils release their toxic granular content in order to fulfill their defensive role.
Eosinophilia from 10 to 30% of the total WBC counts, presenting sometimes over several months or even years, has been reported to be a frequent finding in EMF patients (Somers 1972), and attempts at measuring the humoral and cellular changes related to it were made by characterizing the eosinophils, and measuring the blood levels of granule basic proteins and other humoral factors in Nigerian patients (Urhoghide and Falase, 1987). Apart from studies investigating the role of eosinophilia and autoimmunity, research on the biological profile was scarce and up to date the pattern of the immune response in EMF has not been completely described.

We hypothesized that EMF patients present a singular immune system activation pattern including both cellular and humoral responses; the later involving increased circulating pro-inflammatory cytokines, eosinophil chemoattractant factors and autoantibodies. To investigate this hypothesis we designed a series of studies in patients with established EMF from our clinical registry to determine:

(1) The prevalence of eosinophilia and eosinophil-attracting chemokines in the blood and cardiac tissue;

(2) The cytokine profile, mainly the presence of pro-inflammatory cytokines and those involved in allergy; and

(3) The presence of circulating antiheart antibodies.
Chapter 7 Immune Activation

7.2. Eosinophil Studies

7.2.1. Introduction

Cardiovascular diseases in which chronic and severe eosinophilia is a prominent feature, namely Loeffler’s eosinophilic parietal endocarditis and eosinophilic collagen vascular disease, are known to be associated with endomyocardial damage (Tai et al, 1987; Spry et al, 1985). The have also been reports of the same process occurring in diseases associated with transient hypereosinophilia such as eosinophilic leukaemoide reaction, Loeffler’s eosinophilic pneumonia, trichinosis and filariasis (Roberts, 1969; Brockington and Olsen, 1973; Andy, 1977; Andy et al, 1981; Andy et al, 1998). Therefore, owing to the common finding of hypereosinophilia in patients with EMF, it was postulated that this cardiomyopathy could be the tropical variant of the hypereosinophilic syndrome (HES), an entity encountered in temperate climates (Andy, 1983; Andy, 1999) and recognized to be closely associated with overproduction of IL-5 (Fletcher and Bain, 2007). The late fibrotic lesions of the HES are identical to those of EMF (Brockington and Olsen, 1973) and, since an increase in eosinophil count resulting from frequent parasitic infestations is common in tropical Africa, there had been suggestions that EMF would be the “burnt out” phase of an eosinophilic heart disease (Andy et al, 1998). However, this has been difficult to investigate due to the usually late presentation of EMF patients seen in several regions of the world, and the rarity of diagnosis of this cardiomyopathy in its early phases.
Chapter 7 Immune Activation

We therefore planned and designed a sequence of community-based and clinical studies in order to explore this issue.

The specific objectives of our studies were to:

(1) Determine the prevalence of blood eosinophilia in children from an endemic area for EMF

(2) Determine the prevalence of blood and cardiac tissue eosinophilia in patients with established EMF;

(3) Determine the presence of Eotaxins in blood and cardiac tissue of patients with established EMF;

(4) Evaluate the relation between blood and cardiac tissue eosinophilia
Chapter 7 Immune Activation

7.2.2. Population and Methods

7.2.2.1. Blood sample collection and assays

i. Hypereosinophilia in children from Inharrime

During the epidemiological work for the establishment of a cohort of school children in Inharrime we collected blood samples from every fifth child observed in the visited schools. The samples were collected by puncture of the antecubital vein and slides were prepared for manual total leukocyte and eosinophil count.

ii. Hypereosinophilia in EMF patients with established EMF

Full leukocyte and eosinophil count were performed to patients with established EMF at admission to the clinical registry with history of more than 6 months of symptoms, who had not received steroid or other anti-inflammatory drug therapy. The eosinophil counts were repeated after 6 months of follow up. The full leukocyte count was done using the hemocoulter Symex, KX 2IN and slides were prepared for manual eosinophil count. The eosinophil count was related to the classification of EMF, specific clinical features and ESR and CRP.

In both studies direct eosinophil counts were done on slides stained using the MayGrunvald-Giemsa method. Degranulated and vacuolated eosinophils were searched for in the blood films of each subject. Whenever applicable, between 25-50 eosinophils
were studied in each slide. An eosinophil was scored as vacuolated if it contained less than 50% of the usual expected compliments of cytoplasmic granules.

**iii. Measurement of blood levels of Eotaxin -1, -2 and -3**

Venous blood was obtained by puncture of the antecubital vein and collected in heparinized tubes. Plasma was prepared by centrifugation at 3000rpm for 20 minutes at 4°C. Samples were aliquotted into Eppendorf tubes, snap frozen at -80°C and stored locally. They were transported to London on Cardice and restored at –80°C. They were thawed immediately before analysis.

The heparin-plasma samples were precipitated using high salt and low pH solution, prior to ELISA using matched antibodies purchased from *Jackson ImmunoResearch Labs Inc*. This extraction procedure is thought to be essential in Duffy positive patients since heparin interferes with ELISA measurements in this set of patients (Topping, 2003).

Plates were coated with HuEot 1 MAB320 1/125, HuEot 2 MAB343 1/500 and HuEot 3 MAB653 1/250 and incubated overnight. Block using 1%BSA and 0.02 Na Azide PBS was added. After 2h-incubation and washing, samples and standards were put in plates and incubated at 4°C overnight. The detectors (HuEot1 BAF320 1/250, HuEot 2 BAF343 1/K and HuEot 3 BAF346 1/125) on A/B 0.1%BSA + 0.05% Thimerasol were added. Incubation followed for 4h. NA/HRP was added, incubation followed for 1hr at room temperature and finally the substrate was added. H2SO4 was added to each well and plates were read at 450nm using a *Sunrise* absorbance reader.
New methodology was used after the results of this experiment using heparin samples, in which most patients had undetectable levels of eotaxins. It was decided to perform Duffy typing for all the patients to exclude the possibility of binding of the eotaxins to erythrocytes in Duffy positive patients (Topping, 2003). All patients were Duffy negative and we therefore switched from heparin to EDTA plasma samples, which do not need the extraction procedure. We therefore used plasma obtained from blood collected in EDTA tubes at the same time of the heparin samples that had been used in the first experiment. The extraction procedure was omitted in all experiments using EDTA samples (April 2005, September 2005, and October 2005). Two dilutions (undiluted and at ½ dilution) were used each time. For the second experiment we included some of the 18 samples previously tested and two new samples (undiluted or at ½ dilution). A third experiment was performed using the remaining 34 plasma samples at ½ dilution.

7.2.2.2. Tissue collection and preparation

i. Histology

Targeted endomyocardial biopsies were obtained intra-operatively in EMF patients who were submitted to surgery. The cardiac tissue was immediately fixed in 10% buffered formalin, routinely processed for light microscopy and embedded in paraffin blocks.

Four μ thick sections were prepared, and Hematoxylin-Eosin (H&E) and Carbol chromatotrope (Ch2R) staining were used to identify eosinophils.
ii. Immunostaining for eotaxins

Sections from the paraffin blocks were dewaxed using Histoclear and alcohol. Endogenous peroxidases were blocked by incubating sections in 1% Hydrogen Peroxide in methanol. Antigen retrieval used MAB 320 for Eotaxin-1, MAB 343 for Eotaxin-2 and P156 for Eotaxin-3.

All primary antibodies were used at 10 and 25 μg/ml (there were 2 sections, 1 for each concentration). The diluent was 5%BSA. Immunoglobulin controls were used on the negative controls at the same concentrations. All sections were incubated in primary antibody or Immunoglobulin in humidified chamber at 4°C overnight.

Biotinylated secondary antibody was used. For MAB 320 and MAB 343 this was anti-mouse (Jackson ImmunoResearch Labs inc. Biotin –SP-conjugated affinipure F(ab’) fragment donkey anti-mouse. Cat no 715-066-150) used at 1:500. For P156 this was anti-goat (from Jackson labs. Biotin-SP-conjugated Affinipure Fab’ fragment donkey anti-goat-cat no 705-066-147) was used at 1:250. Human adenoid was used as positive control.
Chapter 7 Immune Activation

7.3. Results

We collected blood from 71 school children: 65 were healthy students and 6 had EMF. The mean (±SD) age of children was 8 years (±1.6), and there were 37 female (52.1%). The mean leukocyte count was $8.0 \times 10^9/L$ (± 2.4). The age of children with hypereosinophilia did not differ from that of children with normal eosinophil counts (p=0.58 two-sample t-test), and there was no significant evidence that the incidence of severe hypereosinophilia differed between girls and boys (p=0.96; chi square test).

Prevalence of Eosinophilia in Children from Inharrime

Hypereosinophilia was common in school children from Inharrime district 19/71 (26.8%). The mean absolute eosinophil count was $1.2 \times 10^9/L$ (± 0.9) and the mean percentage of eosinophils was 14.5% (± 9.0; range 0-44). The mean percentage of degranulated eosinophils was 15.8% (± 14.3; range 0-52), while the mean percentage of vacuolized eosinophils was 7.8% (range 0-45). Eosinophilia in children with EMF (1.5 ± 2.0) did not differ from that of healthy children (1.1 ± 0.8; p=0.54 Mann-Whitney test). Of the 71 children for whom eosinophil count was obtained 6 (8.5%) had EMF; of these only one had hypereosinophilia. There was no significant difference in eosinophila in healthy children (17/65) when compared to children with EMF (1/6) (p=1, Fisher exact test). The numbers and percentage of severe eosinophilia according to EMF status are presented in Table 7.1.
Table 7.1. Eosinophil counts in children from Inharrime schools according to EMF status

<table>
<thead>
<tr>
<th></th>
<th>Healthy</th>
<th>EMF</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Eosinophil Count</td>
<td>48 (90.6%)</td>
<td>5 (9.4%)</td>
<td>53 (100%)</td>
</tr>
<tr>
<td>Hypereosinophilia (&gt;1.5x10^9/L)</td>
<td>17 (94.4%)</td>
<td>1 (5.6%)</td>
<td>18 (100%)</td>
</tr>
<tr>
<td>All</td>
<td>65 (91.5%)</td>
<td>6 (8.5%)</td>
<td>71 (100%)</td>
</tr>
</tbody>
</table>

No significant difference in hypereosinophilia levels was found when comparing participants with (1.3 ± 1.3) and without malaria infection (1.0 ± 0.7); (p=0.19, Mann Whitney test). Of the 48 participants with positive blood smear for malaria, 13 had severe hypereosinophilia, while 6 healthy children out of the 23 who were negative for malaria also had severe hypereosinophilia. There was no statistically significant difference in the presence of hypereosinophilia in participants with malaria when compared to those without the infection (p=0.93; chi-square test). Table 7.2 presents the relation between eosinophil count and malaria infection.

Hypereosinophilia was frequent in children from both sexes (10/37; 27% for females, and 9/34; 26.5% for males) with no statistically significant difference between the sexes. The distribution of hypereosinophilia according to gender is shown in Table 7.3.
Table 7.2. Eosinophil counts in children from Inharrime schools according to presence of malaria infection

<table>
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<tr>
<th></th>
<th>No Malaria</th>
<th>Malaria</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Eosinophil Count</td>
<td>17 (32.70%)</td>
<td>35 (67.3%)</td>
<td>52 (100%)</td>
</tr>
<tr>
<td>Hypereosinophilia (&gt;1.5x10⁹/L)</td>
<td>6 (31.6%)</td>
<td>13 (68.4%)</td>
<td>19 (100%)</td>
</tr>
<tr>
<td>All</td>
<td>23 (32.4%)</td>
<td>48 (67.6%)</td>
<td>71 (100%)</td>
</tr>
</tbody>
</table>

Table 7.3 Eosinophil counts in children from Inharrime schools according to gender

<table>
<thead>
<tr>
<th></th>
<th>No hypereosinophilia</th>
<th>Hypereosinophilia</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>27 (73.0%)</td>
<td>10 (27.0%)</td>
<td>37 (100%)</td>
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<tr>
<td>Male</td>
<td>25 (73.5%)</td>
<td>9 (26.5%)</td>
<td>34 (100%)</td>
</tr>
<tr>
<td>All</td>
<td>52 (73.2%)</td>
<td>19 (26.8%)</td>
<td>71 (100%)</td>
</tr>
</tbody>
</table>

Prevalence of Eosinophilia in Patients with established chronic EMF

We determined eosinophil counts from 169 patients out of the 175 from our registry. Severe hypereosinophilia was present in 44 (26.0%) at entry to the registry. The mean absolute eosinophil count was 1.2 x10⁹/L (range 0-8.9) and the mean percentage of
eosinophils was 11% (range 0 to 64). The mean percentage of eosinophils that were
degranulated and vacuolated was 16% (range 0-32) and 5.7%, respectively.

As shown in Table 7.4 among the 43 patients with severe hypereosinophilia
intraventricular thrombosis was present in 17 patients (39.5%), periorbital edema was
found in 17 (39.5%), while asthma–like episodes where seen in 10 (23.2%) patients.

**Table 7.4.** Clinical features according to the presence of hypereosinophilia

<table>
<thead>
<tr>
<th>Clinical Features</th>
<th>Hypereosinophilia (N 43)</th>
<th>No hypereosinophilia (N126)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intraventricular Thrombus</td>
<td>17 (39.5%)</td>
<td>50 (29.5%)</td>
</tr>
<tr>
<td>Facial or periorbital edema</td>
<td>17 (39.5%)</td>
<td>36 (21.3%)</td>
</tr>
<tr>
<td>Asthma-like episodes</td>
<td>10 (23.2%)</td>
<td>7 (4.1%)</td>
</tr>
</tbody>
</table>

There was a trend to increased frequency of eosinophil-related clinical abnormalities such
as ventricular thrombosis, facial edema and asthma-like episodes in patients with
hypereosinophilia.

Only 9 were submitted to short course of oral corticoids, since the remaining patients had
contraindications. The main contraindications for steroids were severe respiratory
infections or malnutrition with high risk of reactivation of tuberculosis.
There was not a specific trend in the variation of blood eosinophilia in EMF patients. Out of the 24 consecutive patients tested on admission to the registry and six months after diagnosis that did not receive steroids and did not reveal any parasitic infestation during follow up, eosinophilia decreased in 11, remained at the same level in 1, but increased in 12 (Figure 7.1).

_Eotaxin blood levels_

The results of the first experiment, involving the determination of eotaxin levels in 18 patients with EMF are shown in Table 7.5a. The levels of eotaxin-1 were below the level of detection of the test in 16 out of 18 patients (the two measurable values were 122.7 and 177.3 pM). Regarding eotaxin-2 it was detected in all samples and the mean levels, at 114.3pM (± 47.7) ranging 62.4 to 205.4. The levels of eotaxin-3 were below the limit of detection of the assay for all but one sample (73.4 pM in a patient with absolute eosinophil count of 8.5 x 10^9/L).
Figure 7.1. Variation in percentage of eosinophils in 24 consecutive patients (P1 to P24) for whom eosinophil counts were performed at diagnosis (T0) and 6 months after follow up (T1), for whom no steroids had been administered.
**Table 7.5a.** Eotaxin levels of 15 EMF patients measured in heparin plasma samples

<table>
<thead>
<tr>
<th>EMF patients</th>
<th>Eotaxin-1 pM (pg/ml)</th>
<th>Eotaxin-2 pM (pg/ml)</th>
<th>Eotaxin-3 pM (pg/ml)</th>
</tr>
</thead>
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<tr>
<td>0008</td>
<td>*</td>
<td>81.9</td>
<td>*</td>
</tr>
<tr>
<td>0017</td>
<td>*</td>
<td>169.5</td>
<td>*</td>
</tr>
<tr>
<td>0020</td>
<td>*</td>
<td>99.0</td>
<td>*</td>
</tr>
<tr>
<td>0029</td>
<td>*</td>
<td>78.1</td>
<td>*</td>
</tr>
<tr>
<td>0030</td>
<td>*</td>
<td>114.7</td>
<td>*</td>
</tr>
<tr>
<td>0033</td>
<td>*</td>
<td>63.2</td>
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<td>*</td>
<td>205.4</td>
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<tr>
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<td>*</td>
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<td>*</td>
</tr>
<tr>
<td>0054</td>
<td>*</td>
<td>73.4</td>
<td>*</td>
</tr>
<tr>
<td>0062</td>
<td>177.3</td>
<td>200.4</td>
<td>73.4</td>
</tr>
<tr>
<td>0065</td>
<td>*</td>
<td>119.4</td>
<td>*</td>
</tr>
<tr>
<td>0070</td>
<td>*</td>
<td>105.3</td>
<td>*</td>
</tr>
<tr>
<td>0073</td>
<td>*</td>
<td>86.6</td>
<td>*</td>
</tr>
</tbody>
</table>

* Below the levels of detection of the assay
Using EDTA samples the levels of eotaxin-1 were below the level of detection of the assay in 11 out of 66 samples tested. Regarding eotaxin-2 one sample had the levels above the limit of the assay. In contrast the levels of eotaxin-3 were below the level of detection of the assay for all 51 samples that were tested for this cytokine. The results obtained were not reproducible for the 12 samples that were re-tested. The results of the tests performed for EDTA samples are presented in Tables 7.5b,c.

Comparing the results of 49 EMF patients (46 with pure EMF and 3 with concurrence of RHD) with 5 controls with RHD we found that there was no clear relationship between the eotaxin levels and absolute eosinophil counts (Figure 7.2), and that no statistically significant difference existed between eotaxins levels in any of the three groups of patients (Figures 7.3).

**Tissue eosinophilia**

Tissue eosinophilia was rare in the excisional biopsies obtained from 29 patients. Out of the 12 patients with blood hypereosinophilia only one had marked tissue eosinophilia in the atrium. Remarkably, only one patient had severe ventricular eosinophilia (Figure 10 Chapter 6) but did not have blood eosinophilia and was classified as mild EMF characterized by patchy mural fibrosis and predominant mitral valve affection.


**Table 7.5b.** Eotaxin-1 and eotaxin-2 levels of 15 EMF patients measured in undiluted and ½ diluted EDTA plasma samples

<table>
<thead>
<tr>
<th>Code</th>
<th>Eotaxin-1 undiluted PM</th>
<th>Eotaxin-1 ½ diluted PM</th>
<th>Eotaxin-2 undiluted pM</th>
<th>Eotaxin-2 ½ diluted pM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0023</td>
<td>7.8</td>
<td>2.9</td>
<td>75.7</td>
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<td>11.1</td>
<td>6.1</td>
<td>34.7</td>
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</tr>
<tr>
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<td>8.0</td>
<td>4.3</td>
<td>37.9</td>
<td>75.7</td>
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<td>5.9</td>
<td>57.6</td>
<td>149.4</td>
</tr>
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<td>0040</td>
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<td>2.8</td>
<td>43.9</td>
<td>99.8</td>
</tr>
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<td>6.6</td>
<td>24.9</td>
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<tr>
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<td>4.1</td>
<td><strong>223.1</strong></td>
<td><strong>125.7</strong></td>
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</table>
Table 7.5c. Blood levels of Eotaxins-1, -2 and -3 in 34 EMF patients measured in EDTA plasma samples at ½ dilution.

<table>
<thead>
<tr>
<th>Code</th>
<th>Eotaxin-1 pM</th>
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<th>Eotaxin-3 pM</th>
</tr>
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<td>&lt; min</td>
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<td>13.0</td>
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(continuation of Table 7.5c from previous page)

<table>
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<tr>
<th>Code</th>
<th>Eotaxin-1 (pM)</th>
<th>Eotaxin-2 (pM)</th>
<th>Eotaxin-3 (pM)</th>
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<td>18.7</td>
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</tr>
<tr>
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<td>8.7</td>
<td>86.5</td>
<td>&lt; min</td>
</tr>
<tr>
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<td>&lt; min</td>
</tr>
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<td>29.7</td>
<td>&lt; min</td>
</tr>
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<td>26.1</td>
<td>&lt; min</td>
</tr>
<tr>
<td>0070</td>
<td>&lt; min</td>
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<td>&lt; min</td>
</tr>
<tr>
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<td>&lt; min</td>
<td>10.7</td>
<td>&lt; min</td>
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<tr>
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<td>0073</td>
<td>&lt; min</td>
<td>&lt; min</td>
<td>&lt; min</td>
</tr>
</tbody>
</table>

*<min = below the level of detection*
Figure 7.2 Blood levels of eotaxin-1 (left) and eotaxin-2 (right) in 49 patients with EMF show no clear correlation with the absolute eosinophil count in the blood.

Figure 7.3. Blood levels of eotaxin-1 and eotaxin-2 in patients with EMF (emf), rheumatic heart disease (rhd) or concomitance of RHD and EMF (mixed).
Eotaxins in cardiac tissue

Eotaxins were detected in cardiac tissue from 2 patients with EMF, 2 patients with Rheumatic Heart Disease (RHD) and 2 patients with Congenital Heart Disease (CHD). Immunostaining showed that the chemoattractant was predominantly inside mononuclear inflammatory CD68 positive cells (macrophages or monocytes), but were also present in other cell types (Figure 7.4). The presence of eotaxins in the cardiac tissue was not associated with eosinophil recruitment.

Figure 7.4. Several types of inflammatory cells stained for Eotaxin-2 (top left), Eotaxin-1 (top right) and Eotaxin-3 (bottom) inside and outside the vessels. The cells that stained more strongly were macrophages, monocytes and lymphocytes.
7.3. Cytokine profile

7.3.1. Introduction

Cytokines participate in a wide range of biologic processes and their effects on cardiovascular system include promotion of inflammation, intravascular coagulation, oxidative stress, cardiac structural and functional abnormalities, endothelial injury and cardiomyocyte or endothelial apoptosis (Dinarello, 2000; Mann et al, 1994). Cytokines that are involved in allergic reactions might also be increased in EMF patients.

Among many cytokines, Interleukin 5 (IL-5) and Eotaxins (Eot) modulate eosinophil function. IL-5 plays a critical role in the differentiation, proliferation, migration, and activation of eosinophils (Chung, 2002). Eotaxins (-1, -2, -3) are chemotatic cytokines (chemokines) selective for eosinophils that act via CCR3 receptors (Jose et al, 1994). They can induce both the local recruitment of eosinophils from the microcirculation and rapid mobilization of eosinophils from the bone marrow, in synergy with IL-5 (Palframan et al, 1998). Animal models of pulmonary inflammation show that local eotaxin generation correlates with eosinophil accumulation (Humbles et al, 1997; Li et al, 1997). IL-5 and Eotaxins have been recently identified as therapeutic targets for diseases associated with hypereosinophilia. In this context the clarification of their role and that of the eosinophil in the pathogenesis of EMF is of paramount importance.

The objective of the study was to determine the presence of cytokines IL1α, IL1β, IL3, IL4, IL5, IL9, IL10 and IL13 in patients with EMF.
7.3.2. Population and Methods

**EMF patients and controls**

Sixteen consecutive patients who had not been medicated with anti-inflammatory drugs were recruited from our EMF registry between October and December 2004 when coming for their appointments. At the time of participation in the study, these patients were in different stages of the disease with medical treatment consisting of diuretics, ACE-inhibitors, digitalis and β-blockers in most cases. Eight patients were taking oral anticoagulation.

**Blood sample collection and storage**

Blood samples were collected by puncture of the antecubital fossa and collected into heparin-tubes. Plasma was prepared by centrifugation at 3000rpm for 20 minutes at 4°C. Samples were aliquoted into Eppendorf tubes and snap frozen, at -80°C and stored locally. They were transported to London on Cardice, restored at −80°C and thawed immediately before analysis.

**Blood Assays**

Cytokine levels were measured on heparin-plasma samples after defrosting at room temperature (RT), using protocol A of the Beadlyte® Human Multi-Cytokine Beadmaster Kit. The kit was customized for IL1α, IL1β, IL2, IL4, IL5, IL9, IL10 and IL13.
The standards were re-suspended in tissue culture media provided in the kit and serial
dilution was done. Each well was filled with 50µl of standard/sample and 25µl of bead solution supplied, and incubated for 2 hours in dark while shaking; 25µl of reporter solution were added to each well, mixed gently using a voretex and incubated for 1.5 hour in dark at RT. Then 25µl of diluted streptavidin-phycoerythrin solution were added and left in dark for 30 min. Stop Solution was added and left for 5 min in dark at room RT. Reading was done using Luminex® 100™ system.
7.3.3. Results

Several cytokines were below the limit of detection of our assay. Th2 cytokines had more detectable values when compared to the other cytokines. Remarkably all patients had measurable levels of IL-5. The mean level of IL-5 was 3.0 (range 2.1 to 9.3).

There was no clear correlation between the eosinophil counts and the levels of cytokines in most patients. High levels of pro-inflammatory cytokines and hypereosinophilia coexisted in 2 out of 7 patients with high absolute eosinophil counts. Table 7.6 summarizes the clinical and echocardiographic findings, eosinophil counts and the blood levels of the cytokines measured in 16 patients with EMF.

Following these results suggesting problems in the lower part of the standard curves we decided to introduce a second wash in experiment in order to increase sensitivity. During the second experiment we also changed the patients with associated RHD, replacing them with healthy subjects. Unfortunately, due to excessive fibrin content in the plasma samples from EMF patients the reading procedure by the Luminex ® 100™ machine was impeded by blockage of the machine.
**Table 7.6.** Summary of the clinical-echocardiographic findings, eosinophil counts and measured levels of the cytokines IL-1α, IL-1β, IL-3, IL-4, IL-5, IL-9, IL-10 and IL-13 in 16 patients with EMF

<table>
<thead>
<tr>
<th>Code</th>
<th>Age (y)</th>
<th>EMF Classif</th>
<th>Eos (x10⁹/L)</th>
<th>IL1α</th>
<th>IL1β</th>
<th>IL3</th>
<th>IL4</th>
<th>IL5</th>
<th>IL9</th>
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<td>-</td>
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<td>-</td>
<td>2.8</td>
<td>-</td>
</tr>
<tr>
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<td>11</td>
<td>B GIII</td>
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<td>-</td>
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<td>-</td>
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<tr>
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<tr>
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<td>-</td>
<td>-</td>
<td>2.4</td>
<td>2.3</td>
<td>-</td>
<td>2.3</td>
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</tr>
<tr>
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<td>1.9</td>
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<tr>
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<td>-</td>
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<td>1.2</td>
<td>-</td>
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<td>2.1</td>
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<td>12</td>
<td>B GIII*</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>2.2</td>
<td>-</td>
<td>1.4</td>
<td>-</td>
</tr>
<tr>
<td>0070*</td>
<td>12</td>
<td>L GII*</td>
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<td>-</td>
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<td>-</td>
<td>2.2</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Y = years* Associated RHD; L left; R right; B bilateral; G grade; EOS absolute eosinophil count; IL Interleukin; - Below level of detection; E Error
7.4. Antiheart antibodies

7.4.1. Introduction

The epidemiological and clinical similarities between EMF and RHD generated the hypothesis of the two conditions being different expressions of the same pathological process (Shaper et al., 1968a). Despite the pathogenesis of RHD being not completely understood, it is accepted that the response against Streptococcal infection of the upper airways and probably skin, causes the typical heart lesions through mechanisms immunologically mediated.

EMF patients have been shown to have high prevalence of antiheart antibodies (Van der Geld et al., 1966; Shaper et al., 1968). The demonstration of bound Ig to the sarcolemmal and sub-sarcolemmal sites in myofibers (Van der Geld et al., 1966) was considered an indicator of autoimmune mechanism existing in EMF.

More recently immunological studies undertaken in India had divergent results. In one study EMF patients and matched controls had both raised immunoglobulin levels, although a higher proportion of patients had markedly raised serum levels of IgG, Ig A, Ig M and Ig E (Vijayaraghavan et al., 1984). However, antimyocardial antibodies were not detected in sera and no bound immunoglobulins were observed in myocardial tissue of EMF patients, suggesting that there was no autoimmune reactivity in that series of patients (Karthra et al., 1984). Interestingly, the tropical immunological syndrome prevalent in Africa was absent in India (Vijayaraghavan et al., 1984).
We therefore designed a study to assess the presence, frequency and specificity of circulating anti-myocardial antibodies of IgM and IgG classes in EMF patients from Mozambique.

### 7.4.2. Patients and Methods

Serum was obtained from 56 consecutive EMF patients with chronic disease from our registry between November 2004 and October 2005.

**Characteristic of patients**

The mean age of the patients was 18 years (SD 11, range 6 to 49), and 35 (62.5%) were females. Thirty-two patients (57.1%) had biventricular disease, 20 (35.8%) had right ventricular disease and 4 (7.1%) had left ventricular EMF. The mean age was 18 years (range 6 to 49), and 35 (62.5%) were females. The majority of patients were in functional class III or IV of the NYHA (45; 80.3%) and 18 (32.1%) had atrial fibrillation. The mean eosinophil count was $0.4 \times 10^9$/L (IQ range 0.2-1.2). All patients had a negative blood smear for malaria at time of blood collection. The main characteristics of the patients are presented in Table 7.7.

**Control subjects**

Ten blood donors from the same population were used as controls. Their mean age was 20 (SD 2, range 18 – 23) and 5 (50%) were females.


**SDS-PAGE and Western Blotting**

Normal ventricular myocardium was obtained from a donor heart and immediately frozen in liquid nitrogen. It was pulverized while still frozen and homogenized in 1% SDS. A protein assay was carried out using the dye-binding procedure of Bradford. Samples (25ug) were solubilosed and denatured by heating at 70°C for 10 minutes in LDS sample buffer (Invitrogen). The samples were loaded onto 10% tris-bis gels and run at 60mA/gel until the tracking dye reached the end of the gel. Molecular weight markers (Amersham) were concomitantly run on each gel. The proteins were electrophoretically transferred to nitrocellulose at 30V for 1 hour.

**Detection of antiheart antibodies**

Nitrocellulose strips carrying separated lanes of myocardial proteins separated by SDS-PAGE were blocked for 1h with 3% w/v nonfat dried milk (Marvel) in phosphate buffered saline containing 0.05% w/v Tween-20 (blocking solution). Strips were then incubated with patient’s serum, diluted 1:100 in blocking solution, and agitated for 1h at room temperature. After thorough washing in PBS-Tween strips were incubated for a further 1h in either peroxidase-conjugated rabbit antihuman IgG (Dako) or IgM (Dako) at a dilution of 1:500 in blocking solution. The strips were then washed thoroughly in PBS. Protein bands to which antiheart antibodies had bound were visualized using enhanced chemiluminescence detection system (ECL, Amersham). Blots were incubated with the detection reagent for 1 min and then exposed to Hyperfilm (Amersham) for 1, 2 and 5 mins. The films were developed using an automated radiograph developer.
Data Analysis

Quantitative data are given as means ± SD. The Fischer exact test was used to compare the positive results in patients and controls. A p value of less than 0.05 was considered statistically significant.

Table 7.7. Characteristic of patients participating in the study on antiheart antibodies

<table>
<thead>
<tr>
<th>Variable</th>
<th>Value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>18 ± 11</td>
</tr>
<tr>
<td>Male/Female</td>
<td>21/35</td>
</tr>
<tr>
<td>NYHA class I-II/III-IV</td>
<td>8 (14.3)</td>
</tr>
<tr>
<td>II</td>
<td>3 (5.4)</td>
</tr>
<tr>
<td>III</td>
<td>22 (39.3)</td>
</tr>
<tr>
<td>IV</td>
<td>23 (41.0)</td>
</tr>
<tr>
<td>Severity of EMF Mild (I)</td>
<td>13 (23.2)</td>
</tr>
<tr>
<td>Moderate (II)</td>
<td>8 (14.3)</td>
</tr>
<tr>
<td>Severe (III)</td>
<td>18 (32.1)</td>
</tr>
<tr>
<td>Advanced (IV)</td>
<td>17 (30.4)</td>
</tr>
<tr>
<td>Active/Quiescent disease</td>
<td>8 (14.3)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>18 (32.1)</td>
</tr>
</tbody>
</table>
7.4.3. Results

Detection of IgG and IgM

Fifty-four (96.4%) EMF patients tested positive for anti-myocardial antibodies of the IgG class whilst 33 (58.9%) tested positive for those of the IgM class. Endomyocardial Fibrosis patients presented more circulating anti-myocardial antibodies in the serum when compared to healthy controls. The sera from control subjects showed minimal or no reactivity against myocardial proteins (Figures 7.5 and 7.6).

The frequency of IgG class anti-myocardial antibodies in patients with endomyocardial fibrosis and control subjects is presented in Table 7.8. Higher frequencies for IgG class were found in patients with EMF when compared with healthy controls. These differences were statistically significant for proteins of the following molecular weights: 42 kD (33; 58.9% in patients versus 0 in controls; p=0.0009), 35 kD (32; 57.1% in patients vs 0 in controls; p= 0.0009) and 70 kD (23; 41.1% in patients versus 0 in controls; p=0.011). Patients had also higher frequencies of antiheart antibodies against proteins of 60 kD (18; 32.1% versus 0) and 90 kD (19; 33.9% versus 0) molecular weights, which were statistically nearly significant with p values of 0.051 and 0.052, respectively.
Figure 7.5. IgG measured by WB in patients with EMF (left) and healthy controls from the same population matched for age and ethnics (right).

Figure 7.6. IgM and IgG measured by WB in patients with EMF.
Table 7.8. Comparison of frequency of IgG class antiheart antibodies of different molecular weights in patients with established EMF and controls

<table>
<thead>
<tr>
<th>Protein Molecular Weight (kD)</th>
<th>EMF N+ (%)</th>
<th>EMF N−</th>
<th>Controls N+ (%)</th>
<th>Controls N−</th>
<th>Fisher Exact Test</th>
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<td>9</td>
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<tr>
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<td>10</td>
<td>0.19</td>
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<tr>
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<td>0 (0)</td>
<td>10</td>
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<tr>
<td>42</td>
<td>33 (58.9)</td>
<td>23</td>
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<tr>
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<td>0 (0)</td>
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<tr>
<td>60</td>
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<td>70</td>
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<td>0 (0)</td>
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<tr>
<td>80</td>
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<td>41</td>
<td>2 (20)</td>
<td>8</td>
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Regarding antibodies of the IgM class the difference in frequencies between EMF patients and control subjects was not statistically significant. All controls were negative for any of the IgM class antibodies. The proteins more frequently detected in EMF patients were 46 kD (17; 30.4% versus none in controls, p=0.053) and 80 kD (12; 21.4% versus none in controls, p=0.19). Table 7.9 shows the frequency of anti-myocardial antibodies of the IgM class in patients with endomyocardial fibrosis and control subjects.
Table 7.9. Comparison of frequency of IgM class antiheart antibodies of different molecular weights in patients with established EMF and controls

<table>
<thead>
<tr>
<th>Protein Molecular Weight (kD)</th>
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<th>Controls 10</th>
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<tbody>
<tr>
<td></td>
<td>N+ (%)</td>
<td>N+</td>
<td>N+</td>
</tr>
<tr>
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<td>80</td>
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<td>90</td>
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<td>100</td>
<td>6 (10.7)</td>
<td>0</td>
<td>10</td>
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</table>

Reactivity according to disease activity and severity

Clearly the mean (± SD) number of antibodies was higher for left EMF (14.0 ± 9.1), than for BEMF (8.5 ± 6.0) and REMF (8.4 ± 4.2), but there were only 4 patients in the former group. There is not significant evidence that the mean number of antibodies differed between the types (p = 0.18, analysis of variance).
There were 35 patients with severe (grades 3 and 4) and 21 with mild-moderate disease (grades 1 and 2), and the mean (± SD) number of antibodies were respectively 9 ± 5 and 9 ± 7. There was not significant evidence that the mean number of antibodies differed between less and more severe patients (p = 0.68, t-test).

The characteristics of the 8 patients with active disease are shown in Table 7.10. Four had BEMF, 2 had REMF and the remaining 2 had LEMF. According to the severity of cardiac lesions 2 patients had Mild, 3 had Moderate, 1 had Severe and 2 had Advanced EMF. The other patients had quiescent disease. There was strongly significant evidence that the mean number of antibodies is greater for patients with active disease (19.6 ± 3.7) compared to those with quiescent disease (7.1 ± 3.3) with p < 0.001.

Table 7.10. Comparison of frequency of IgM class antiheart antibodies of different molecular weights in patients with established EMF and controls

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<th>0068</th>
<th>0066</th>
<th>0056</th>
<th>0048</th>
<th>0031</th>
<th>0030</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMF type</td>
<td>LEMF</td>
<td>LEMF</td>
<td>BEMF</td>
<td>BEMF</td>
<td>BEMF</td>
<td>BEMF</td>
<td>REMF</td>
<td>REMF</td>
</tr>
<tr>
<td>EMF severity</td>
<td>II</td>
<td>II</td>
<td>II</td>
<td>I</td>
<td>I</td>
<td>III</td>
<td>IV</td>
<td>IV</td>
</tr>
<tr>
<td>Number IgG</td>
<td>10</td>
<td>14</td>
<td>14</td>
<td>10</td>
<td>11</td>
<td>11</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>Number IgM</td>
<td>6</td>
<td>8</td>
<td>0</td>
<td>10</td>
<td>9</td>
<td>7</td>
<td>4</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td>16</td>
<td>22</td>
<td>14</td>
<td>20</td>
<td>20</td>
<td>18</td>
<td>13</td>
<td>18</td>
</tr>
</tbody>
</table>
7.5. Discussion

Blood Eosinophilia

At the time of diagnosis 26% of patients with established EMF had hypereosinophilia at levels that are used to define the hypereosinophilic syndrome. Similar results have been reported from Nigeria (Manyanja-Kizza et al., 2000) and Uganda (Somers et al., 1972b; Freers et al., 1993).

The characterization of eosinophils in patients with hypereosinophilia showed increased degranulation. During the episodes of hypereosinophilia most patients had clinical features that could be related to eosinophilic damage of the heart (intraventricular thrombosis) or recrudescence of heart failure with appearance of signs of congestion, asthma-like episodes and periorbital edema. The asthma-like episodes occurred in children with no previous personal or family history of asthma and could not be associated to any particular parasitic agent or allergen. We think that they may reflect the effects of increased eosinophils and their related products in the lungs, since eosinophil accumulation in the lungs also occurs in non-allergic asthma (Jose et al., 1994).

The eosinophil counts in our patients with EMF varied without a particular trend during 6 months follow up. Despite our efforts to identify parasitic infestations that are associated with severe eosinophilia, which are known to be prevalent in our community (namely schistosomiasis, helminthiasis and filariasis) the changes in eosinophil counts did not seem to be associated with new parasitic infestations. Similarly, neither the previous
history nor the presenting symptoms and signs allowed a diagnosis of allergy that would explain this increase in eosinophil counts.

In our population there was no difference of either the previous history of parasitic infestation or its prevalence at presentation when comparing patients with hypereosinophilia from those with normal eosinophil counts. Although there have been reports of patients who developed EMF after parasitic infestations (Brockington et al, 1967; Ive et al, 1967; Brockington, 1974; Andy et al, 1981), results similar to ours were obtained in previous attempts to establish a relation between parasitism, eosinophilia and chronic EMF in endemic areas in Africa. Eosinophilia was as common in EMF patients as in controls in a study that concluded that degranulated eosinophils and significantly elevated levels of eosinophil granule basic proteins were not present in EMF (Urhoghide and Falase, 1987). A poor correlation between eosinophilia and parasitic infestations has also been reported in other series (Patel et al, 1977), and more recently, it was concluded that neither absolute/relative eosinophilia, nor the presence of helminthic infestation could be the only contributing factor to the pathogenesis of EMF (Ijaola and Falase, 1988). Authors from Uganda proposed absolute eosinophilia as being a putative cause of EMF, and suggested that hypereosinophilia is an independent risk for EMF not attributable to parasitism (Rutakingirwa et al, 1999). However, it must be mentioned that when looking at hypereosinophilia in EMF patients with recent onset of disease, one study found a highly significant inverse relationship between hypereosinophilia and the duration of symptoms (Andy, 1998), suggesting that the frequency of hypereosinophilia in our patients would be higher had we detected them earlier.
We did not find a significant difference in the levels of eosinophilia in school children between those with and without EMF. Similarly, in Ugandan experience eosinophils do not affect patients with EMF any more than the population at large (Patel et al., 1977), nor has eosinophilia been recognized in the thrombotic and fibrotic endocardial lesions studied in biopsy (Somers et al., 1971) and necropsy specimens (Connor et al., 1967; Connor et al., 1968). In Nigeria degranulated eosinophils were absent in the blood and bone marrow aspirates of EMF patients, and the mean concentrations of eosinophil granule basic proteins were not different in patients and control subjects (Urhoghide and Falase, 1987).

The role of hypereosinophilia in determining the cardiac damage is the hypothesis more frequently explored to explain the pathogenesis of EMF, because of the knowledge that exists of the Hypereosinophilic Syndrome in which the final damage to the heart is similar to that found in established EMF (Olsen, 1990). However, owing to results of several studies on EMF we must also consider the possibility of hypereosinophilia being part of the body response to an unknown insult that also results in cardiac damage seen in these patients, the two responses being independent. Prospective studies looking at hypereosinophilia and its determinants in individuals from endemic areas for EMF are therefore needed.

**Eosinophil-related cytokines**

It has been accepted that eosinophils are largely under the control of IL-5 and eotaxins (Heath et al., 1997). These modulators of eosinophil function have been identified as
therapeutic targets for diseases associated with hypereosinophilia, hence our interest in investigating their presence in EMF patients.

Although some studies were performed looking at the levels of IL-5 in EMF patients, no research has been undertaken trying to determine whether the level of eotaxins are elevated in EMF patients. In fact, there are no such studies in populations from tropical regions with high prevalence of eosinophilia. However, despite several problems faced by us during the setting up of the technique for determination of eotaxin blood levels, some changes to procedures of blood collection and extraction improved the results obtained by the assay.

There is no agreement on the levels of eotaxins in healthy individuals, and no studies were done to determine the level of eotaxins in the Mozambican population. A wide range of eotaxin levels has been reported from studies in different populations from Europe and Asia, varying from 5 to 75 pM in plasma from normal subjects (Topping, 2003; Fujisawa et al, 2002; Economou et al, 2001). The blood levels found in the majority of our patients are therefore within the normal ranges. Interestingly, high levels were found for all eotaxins in few patients with severe blood hypereosinophilia.

The determination of the cytokine profile using the multiplex technique was jeopardized by the high content of fibrin in the plasma samples from our patients. Therefore the results obtained will not be discussed in detail. WeIt is however of interest to mention that we were able to show that the blood levels of IL-5 in our patients, half of which had severe hypereosinophilia, were not increased.
Chapter 7 Immune Activation

An important consequence of the finding of abnormally high levels of fibrin in the plasma samples was the design of another study aiming at determining the presence of an abnormal pro-thrombotic factors and hemostatic dysfunction in patients with early onset EMF, which we present in Chapter 8.

Tissue Eosinophilia

Cardiac tissue eosinophilia was rare in established EMF, despite the presence of eotaxins in cardiac tissue. Out of the 25 patients studied, cardiac tissue eosinophilia was detected in 2, of which one had normal blood eosinophilia. These results are similar to those reported in other series of EMF patients (Connor et al, 1968; Somers et al, 1971; Chopra et al, 1989), and are in contrats with the usual pathology features in Hypereosinophilic Syndrome. Characteristically, in HES there are deposits of activated eosinophils and secreted eosinophil granule proteins in the necrotic and thrombotic lesions (Tai et al, 1987), as well as persisting tissue eosinophilia and degranulation of eosinophils in the fibrotic stage (Frustaci et al, 1989).

To our knowledge the study we designed was the first attempt at determining the presence of eotaxins in cardiac tissue of EMF patients and trying to correlate its presence with eosinophil recruitment and accumulation. The rational behind this research was the knowledge that in animal models of pulmonary inflammation local eotaxin generation correlates with eosinophil accumulation (Humbles et al, 1997, Li et al, 1997; Matthews et al, 1998). Interestingly, eotaxins where detected in cardiac tissue from EMF patients as
well as in those with RHD and CHD, suggesting that these cytokines may not have yet unidentified physiology roles.

Although the number of patients involved in our pathology studies was small, their results suggest that eosinophils and eotaxins do have neither an essential nor an exclusive role in established chronic EMF. They might however be involved in the pathogenesis in earlier stages of this condition. The temporal relationship between blood eosinophilia, blood levels of IL-5 and eotaxins, and structural abnormalities of the heart needs to be demonstrated on cohort studies.

We acknowledge that the number of patients included in the studies is low and that the technique for testing eotaxins in cardiac tissue is yet being established. We think that an improvement to the assays for determination of eotaxins in humans is warranted to prove or discard the relevance of these cytokines in the pathogenesis of EMF. Also, the role of hypersensitivity, suggested from pathology studies (Connor et al, 1968) and the finding of markedly raised levels of IgE’s in some patients with EMF (Mathai et al, 1986) was not assessed in our studies.

Autoimmunity

Antiheart antibodies were detected in blood of EMF patients providing evidence for the role of autoimmunity in this cardiomyopathy. It seems however that this phenomenon is not exclusive to EMF since it was shown to occur in African patients irrespective of the nature of the heart condition (Shaper et al, 1967; Shaper et al, 1968b). Regarding the Indian experience, in a study using direct immunofluorescence on myocardial tissue
obtained at surgery or at autopsy from EMF patients no deposits of IgG, IgM or IgA were found in the myocardium, suggesting that there was no autoimmune reactivity in that series of patients (Mathai et al, 1986).

The Western Blotting procedure, the technique used by us to detect IgG and IgM class antibodies in our studies, is the most sensitive and gives results that are easier to interpret (Dunn et al, 1991). Autoantibodies of the IgG class were more prevalent than those of IgM class, corroborating previous studies, which found IgG class immunoglobulins in larger numbers in patients with EMF (Carlisle et al, 1972).

The significance of heart-reactive antibodies in cardiac disease has been the subject of intense debate. Some authors who point out that these antibodies occur commonly following several types of cardiac injury including surgery and myocardial infarction (Akl et al, 1992; Van der Geld et al, 1964; Latif et al, 1993) suggest a non-pathogenic role, a theory that had been supported by initial failure of attempts to demonstrate their in vitro and in vivo cytotoxicity (Thompson and Halbert, 1971). However, in recent years a cause-and-effect relationship was demonstrated between the presence of human cardiac myosin autoantibodies and impairment of myocyte contractility (Warraich et al, 2006), supporting the circumstantial evidence that implicates the participation of heart-reactive antibodies in certain forms of heart disease such as Rheumatic Heart Disease (Eichbaum et al, 1995; Shastry et al, 1988), cardiomyopathies (Das et al, 1973), postmyocardial infarction (Dressler’s) syndrome, and postpericardiotomy syndrome (Akl et al, 1992). Moreover, it has been recognized that antiheart antibodies play a major role in rejection events following transplantation (Warraich et al, 2000) and that immunosuppressive
therapy results in decrease in levels of these antibodies that is associated with improvement in clinical condition.

The molecular weights of 35 kD, 42 kD, and 70 kD correspond to Tropomyosin, Actine and Heat Shock Protein (HSP)-70, respectively. The first two are major components of the contractile apparatus of the myocyte whereas HSP-70 is a marker of cardiac damage. We could speculate as to the importance of the finding of antibodies to the 46 kD protein in nearly one third of our EMF patients. The 46 kD corresponds to the molecular weight of an integral membrane protein, component of specialized intercellular junctions, that serves as a receptor for Coxsackievirus (Baldwin et al, 2005), a virus commonly associated with myocarditis and cardiomyopathy (Takata et al, 2004; Cihakova et al, 2004) and studied as one of the possible factors in the pathogenesis of EMF (Ijaola and Falase, 1990).

Additionally the molecular weight of 42 kD is known to correspond to Connexin43. Considering that the primary target of the disease is not known its relevance cannot be excluded and therefore further investigation on its possible role is warranted.

It has been suggested that antiheart antibodies are a non-invasive marker of early disease in some forms of cardiomyopathy (Caforio et al, 1997). They bind to their specific cardiac tissue in later stages of cardiomyopathy, explaining the reduction in circulating levels in advanced stages (Das et al, 1974). Follow-up studies on EMF patients diagnosed in earlier stages are needed to define the temporal changes that occur in the autoimmune response in this condition, and clarify the role of autoimmunity in its pathogenesis.
Chapter 7 Immune Activation

There are some limitations to our study. The pieces of normal ventricular myocardium used for building the nitrocellulose strips also contain endothelial components of the vessels and elements of the extracellular matrix, which molecular weights may yet be unknown. We therefore acknowledge that the proteins identified cannot be attributed exclusively to the myocardium, and that is why we use the term “antiheart”.

Although the primary target of injury is not yet identified in EMF, the most prominent lesions occur in the interstitium and affect the endocardium and subendocardial region. There is therefore need for further research aiming at identification of the sub-classes of immunoglobulins involved and the proteins targeted by these antibodies.

The present work shows that immune markers are found in only a proportion of patients with EMF, suggesting that there would be some patients in whom endomyocardial damage is mediated by mechanisms other than autoimmunity.

Most of our patients were in grade III/IV heart failure, and as mentioned above several studies relate seropositivity for autoantibodies to heart failure rather than to any particular pathologic process (Akl et al, 1992; Van der Geld et al, 1964; Latif et al, 1993). Inclusion of a control group of patients with heart failure of other etiology would help clarify this issue.

Another limitation of the interpretation of the significance of these antiheart antibodies is that the cardiac, skeletal and smooth muscle may share antigenic determinants, and therefore the antibodies may not be entirely organ specific.
7.6. Conclusions

The conclusions of these studies exploring the immune response in EMF are the following:

1. Severe blood eosinophilia is a common finding in both early onset and chronic EMF. It seems to be a major determinant of clinical signs in a subset of these patients, but does not correlate with the blood levels of eotaxins.

2. Eotaxins are frequently detected in cardiac tissue while cardiac tissue eosinophilia is rare in established EMF, meaning that the presence of these chemoattractants is not associated with eosinophil recruitment or accumulation in the heart.

3. Autoimmunity is present in a large subset of patients with established EMF. Autoimmune markers may provide adjunct tools for diagnosis and staging of EMF, potentially contributing to improve the management of EMF patients, by identifying those in whom immunosupression is of potential benefit.

Further research is needed to clarify the role of eosinophils and their related cytokines, inflammatory cytokines and autoantibodies in EMF, since they represent excellent therapeutic targets for altering the natural history of EMF.
Chapter 8

ENDOTHELIAL ACTIVATION AND HAEMOSTATIC DYSFUNCTION IN RECENT ONSET EMF
8.1. Introduction

The endothelium is a multifunctional organ and biologically active interface between the blood components and the vascular smooth muscle. When activated it is a critical factor in many pathological conditions (Kurivila and Kartha, 2003) and could be involved in EMF, a disease characterized by clinical features of hypercoagulability. On the other hand, although the role of inflammation and the usefulness of measuring serum levels of inflammatory cytokines in cardiovascular diseases is well established (Nadar et al, 2004; Libby et al, 2002), no attempts at characterizing the biological profile of EMF patients have been made.

Endothelial activation is characterized by a shift of function of the endothelium towards a proinflammatory state, reduced vasodilatation and prothrombotic properties and could be implicated in the pathogenesis of EMF. Edema and spontaneous intraventricular thrombi, findings suggesting impairment of endothelial function (Chong et al, 2004), are frequently observed in EMF patients (Chapter 5), and plasma obtained from EMF patients in our series seems to have abnormally high fibrin content (Chapter 7).

We hypothesized that the endocardial injury resulting in endocardial activation and procoagulant changes might play a role in early phases of EMF, and designed a study to assess the presence of circulating markers of endothelial cell activation, inflammation and haemostasis disturbances, in EMF patients recruited soon after diagnosis.
8.2. Population and Methods

Patient details
We studied 35 patients recruited less than 6 months after the initial symptoms of disease with confirmed echocardiographic diagnosis of EMF. Twenty-seven patients were self-referrals or referred from schools or medical units in Inharrime, whereas 8 patients were referred to the Mozambique Heart Institute from other endemic areas in Mozambique.

Controls
Due to the ethical issues related to the obtention of informed consent for blood collection from healthy children controls were young adults from a pre-university school in the same geographic region of the Heart Institute (12) and recruited from “blood donors” (12) who are friends or family members of non-EMF patients asked to donate blood to their relatives pre-operatively. These controls were non-smokers, not taking medication and resident in Maputo for more than two years. They were unmatched for age and sex.

Study period and inclusion criteria
From June 2006 to December 2006 we evaluated 43 patients who had EMF diagnosed within the previous six months. Eight patients were excluded because they were already receiving anticoagulants or anti-inflammatory medication, leaving 35 to evaluate. Malaria was excluded by a blood smear assessment.
Clinical evaluation of patients

The clinical evaluation of the patients included the assessment of several parameters namely: weight, height and heart rhythm at admission, New York Heart Association (NYHA) functional class of heart failure, the presence and the degree of splenomegaly, and the presence of signs of “active disease”. The degree of splenomegaly was determined by palpation and graded by using a system, which states five degrees of spleen enlargement (Chapter 5). Active disease was defined by the presence of unexplained fever, facial or periorbital swelling, body itching/urticaria and severe hypereosinophilia (absolute eosinophil count > 1.5 x 10^9), criteria used in previous studies (Andy, 1998).

All individuals had transthoracic echocardiography to evaluate the presence and distribution of the fibrotic lesions in the endocardium and intraventricular thrombosis. On echocardiography endocardial thickening, the hallmark of the disease, had to be present for diagnosis, either in the wall or affecting the atrioventricular valves. Patients with EMF were classified according to the classification presented in Chapter 3.

The main characteristics of patients and controls are summarized in Table 8.1. As previously stated the controls were significantly older. The deleterious effects of the disease on habitus were assessed by measuring the BMI of patients. Twenty eight (80%) of patients had one or more signs of active disease. Three quarters of the patients had splenomegaly with the majority of them having massive enlargement of the spleen.
Table 8.1. Clinical characteristics of recent onset EMF patients and healthy controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>EMF (n = 35)</th>
<th>Controls (n = 32)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median (Interquartile range)</td>
<td>Median (Interquartile range)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>13 (9-17)</td>
<td>17 (13-24)</td>
<td>0.004</td>
</tr>
<tr>
<td>Gender F/M</td>
<td>9/26</td>
<td>14/18</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>28 (19-38)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>131 (114-148)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>16.2 (13.8-32.3)</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>11.4 (10.5-13.3)</td>
<td>11.4 (10.6-13.8)</td>
<td>0.78</td>
</tr>
<tr>
<td>Hematocrit (%)</td>
<td>37 (35-42)</td>
<td>37.1 (34-41)</td>
<td>0.59</td>
</tr>
<tr>
<td>Platelets (x10⁹/L)</td>
<td>269 (179-321)</td>
<td>285 (228-344)</td>
<td>0.23</td>
</tr>
<tr>
<td>Leukocytes (x10⁹/L)</td>
<td>7.3 (6.2-10)</td>
<td>7.1 (6.0-13.8)</td>
<td>0.37</td>
</tr>
</tbody>
</table>

NA not available

A summary of the clinical profile of EMF patients according to the location of cardiac lesions is presented in Table 8.2.
**Table 8.2.** Comparison of the clinical profile of patients with the different types of EMF

<table>
<thead>
<tr>
<th>Variables</th>
<th>BEMF</th>
<th>LEMF</th>
<th>REMF</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>No of patients</td>
<td>12</td>
<td>10</td>
<td>13</td>
<td></td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>29.0 (18.0-82.4)</td>
<td>27.5(17.0-48.0)</td>
<td>20.0 (9.6-78.5)</td>
<td>0.93</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>127 (106-166)</td>
<td>134 (111-167)</td>
<td>125 (73-169)</td>
<td>0.33</td>
</tr>
<tr>
<td>Body Mass Index</td>
<td>27.8 (24.4-42.3)</td>
<td>28.3(23.5-32.7)</td>
<td>27.4 (21.7-40.9)</td>
<td>0.92</td>
</tr>
<tr>
<td>Ventricular dysfunction</td>
<td>2(16.7%)</td>
<td>5(50%)</td>
<td>4(30.8%)</td>
<td>0.25</td>
</tr>
<tr>
<td>Active EMF</td>
<td>10(83.3%)</td>
<td>7(70%)</td>
<td>11(84.6%)</td>
<td>0.56</td>
</tr>
<tr>
<td>Haemoglobin (g/dL)</td>
<td>11.6 (10.1-20.3)</td>
<td>10.9(5.2-15.1)</td>
<td>12.5(9.7-15.6)</td>
<td>0.13</td>
</tr>
<tr>
<td>Haematocrit (%)</td>
<td>39.6(29.8-65.6)</td>
<td>36.3(29.4-45.5)</td>
<td>39.3(32.0-48.7)</td>
<td>0.38</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>81.7(64.6-89.4)</td>
<td>80.9(72.5-87.0)</td>
<td>82.7(69.8-101.1)</td>
<td>0.64</td>
</tr>
<tr>
<td>Platelets (x10^9/L)</td>
<td>250(146-366)</td>
<td>229(123-482)</td>
<td>319(148-391)</td>
<td>0.55</td>
</tr>
<tr>
<td>Leukocytes (x10^9/L)</td>
<td>7.9 (5.5-12.5)</td>
<td>6.9 (4.3-16.9)</td>
<td>6.6(2.4-12.9)</td>
<td>0.19</td>
</tr>
<tr>
<td>ESR (mm/h)</td>
<td>12.5 (2.0-46.0)</td>
<td>15.75(1.0-48.0)</td>
<td>30.7(1.0-127)</td>
<td>0.92</td>
</tr>
<tr>
<td>C-Reactive Protein (mg/L)</td>
<td>2.4 (0.1-18.9)</td>
<td>3.6 (0.3-11.1)</td>
<td>2.0 (0.3-7.8)</td>
<td>0.81</td>
</tr>
</tbody>
</table>

MCV mean corpuscular volume; ESR erythro sedimentation rate
Blood sample collection and storage

Using a tourniquet, blood samples were collected after puncture of the antecubital fossa; the tourniquet was then loosened to obtain the blood samples. Blood was collected into siliconised glass tubes (Bectin Dickinson) either containing 1/10 by volume of 3.8% trisodium citrate or EDTA. Plasma was prepared by centrifugation at 3000rpm for 20 minutes at 4°C. Samples were aliquoted into Eppendorf tubes and snap frozen, at -80°C and stored locally. They were transported to London on Cardice and restored at –80°C. They were thawed immediately before analysis. The analysing laboratory was blinded to the samples.

Assays

A full blood count including white blood cell count, eosinophil count, haemoglobin concentration, hematocrit, erythrocyte mean corpuscular volume and platelets was performed for patients and all controls except the 20 adult blood donors. C-reactive protein and erythrocyte sedimentation rate were measured for patients. Enzyme linked immunosorbant assays (ELISA) were used to measure endothelial, haemostatic and inflammatory markers. The assays were commercially based; solid-phase ELISA’s whereby a specific monoclonal antibody for the antigen in question was pre-coated onto micro-test wells. Markers of endothelial dysfunction included soluble P-selectin, (R&D Systems - UK), and soluble intercellular adhesion molecule ICAM (R&D Systems - UK). Inflammatory markers included interleukin-5 (IL 5) and interleukin-6 (IL6), (both from R&D Systems - UK). Haemostatic markers included plasminogen...
activator inhibitor-1 (PAI-1), prothrombin fragment 1+2 (PF 1+2) (Dade Behring, Sysmex (UK)), soluble tissue factor (TF) (ADI, Axis- Shield - UK) and D-Dimers (Bio Pool, Trinity Biotech - UK). The intra assay and interassay coefficients of variation for ICAM were 3.6% and 7.4%; for P-Selectin they were 5.2% and 8.9%; for TF they were 6.0% and 5.0%; for PAI-1 were 3.3% and 2.9%; for PF1+2 they were 6.0% and 9.0%; for D-Dimers they were 3% and 4%; for IL-5 they were 4.2% and 7.1%; and for IL-6 they were 4.2% and 5%.

Statistical analysis

The results were not normally distributed even after logarithmic transformation. Thus they are presented using the median (and the interquartile range or range). The differences between the groups were assessed using the Mann-Whitney rank sum and the Kruskal-Wallis tests. Correlations were analysed using Spearmans rank order correlation. P value less than 0.05 was considered significant. Data were analysed using the statistical package Minitab.
8.3. Results

Thirteen patients (38%) had right EMF, 12(34%) had bilateral EMF, and 10(28%) had left disease. Almost one third (11/35) had intraventricular thrombosis, 6 on the right side and 5 on the left ventricle. There were no significant differences in body mass index, signs of active disease, and full blood count or erythrocyte sedimentation rate between these three groups.

The medians of hemoglobin (11.4g%) and hematocrit (37%) were the same for patients and controls. This finding may be explained by the high prevalence of anemia in this tropical population and by the presence of cyanotic polycytemia in 3 patients with REMF due to reopening of the foramen ovale by increased right atrial pressure.

Both erythrocyte sedimentation rate and the C-reactive protein tended to be raised, with the levels being very high in some patients.

The details of the haemostatic factors, markers of inflammation and endothelial dysfunction assessed in these patients are presented in Table 8.3. The Figures 8.1 and 8.2 show the same results presented graphically. The patients had increased levels of eosinophils (median 1.2x10⁹/L, Interquartile range [0.4-1.9]) when compared to controls (0.3x10⁹/L; [0.2-0.5], p<0.006). As expected, the eosinophil counts were probably underlying protozoal and worm infestations.

The levels of D-Dimers were significantly raised in patients with a median of 304ng/ml [116-574] versus the controls with 119ng/ml [61-226] with a p value of 0.001.
Soluble TF levels were significantly increased in EMF patients (263 pg/ml [221-394] compared to controls (201pg/ml; [158-269], p=0.004). Equally, the serum levels of PAI-1 in patients (68 ng/ml; [31-123]) were higher than in controls (40ng/ml; [27-78]), with a p value of 0.047.

Regarding ICAM, its levels were significantly increased in patients compared to controls, median 368ng/ml versus 152ng/ml, with interquantile (IQ) ranges of 251-470 and 91-193 respectively (p<0.0001). ICAM levels correlated with PAI-1 (p<0.001), PF1+2 (p=0.017), sTF (p=0.004) and D-Dimers (p=0.005). There was an inverse correlation with the weight (p=0.029), the height (p=0.002) and the body mass index (p=0.001).

Figure 1 also shows that patients with Endomyocardial Fibrosis had higher levels of IL6 (4.4pg/ml; [2.7-7.3]) when compared to controls (1.9pg/ml; [1.4-2.7]), p=0.0001.

The levels of P-Selectin, PF 1+2 and IL5 did not differ between patients and controls. The median (IQ range) levels of patients were for P-Selectin 84ng/ml[62-102], for PF 1+2 0.4mmol/L [0.2-0.7] and for IL5 13 pg/ml [8-16] when compared to controls that had 73ng/ml[53-112; p=0.31], 0.5mmol/L[0.3-1.0; p=0.21] and 12pg/ml[7-20; p=0.89] respectively.

The results of all parameters measured for patients according to the type of EMF are presented in Tables 8.4. The results are expressed in median (range).

A multivariate analysis suggested that the two most important variables differentiating patients from controls are soluble Tissue Factor (p=0.01) and IL6 (p=0.005).
Figure 8.1. Endothelial activation and hemostatic factors in EMF (cases) and controls
Figure 8.2. Inflammation markers measured in EMF patients (cases) and controls

*Figure 8.2* Inflammation markers measured in EMF patients (cases) and controls
Table 8.3. Comparison of the medians for the parameters measured in EMF patients and controls. The results in parentesis correspond to the interquantile ranges.

<table>
<thead>
<tr>
<th>Parameter (normal range)</th>
<th>Patients N(35)</th>
<th>Controls N(32)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophilia (&lt; 0.5x10^3/µL)</td>
<td>1.2 (0.4-1.9)</td>
<td>0.3 (0.2-0.5)</td>
<td>&lt; 0.006</td>
</tr>
<tr>
<td>D-dimers (&lt; 145 ng/ml)</td>
<td>304 (116-574)</td>
<td>119 (61-226)</td>
<td>0.001</td>
</tr>
<tr>
<td>PF1+2 (0.2-1.2 nmol/l)</td>
<td>0.4 (0.2-0.7)</td>
<td>0.5 (0.3-1.0)</td>
<td>0.21</td>
</tr>
<tr>
<td>sTF (40-300 pg/ml)</td>
<td>262 (221-394)</td>
<td>201 (158-269)</td>
<td>0.004</td>
</tr>
<tr>
<td>PAI-1 (0-33 ng/ml)</td>
<td>68 (31-123)</td>
<td>40 (27-78)</td>
<td>0.047</td>
</tr>
<tr>
<td>ICAM (117-311 ng/ml)</td>
<td>368 (251-470)</td>
<td>152 (91-193)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>P-selectin (22-34 ng/ml)</td>
<td>84 (62-102)</td>
<td>73 (53-112)</td>
<td>0.31</td>
</tr>
<tr>
<td>IL-6 (0.4-6.2 pg/ml)</td>
<td>4.4 (2.7-7.3)</td>
<td>1.8 (1.4-2.7)</td>
<td>&lt; 0.0001</td>
</tr>
</tbody>
</table>
Table 8.4. Comparison of the results of all parameters measured for patients with different types of EMF. The results are expressed in median (range).

<table>
<thead>
<tr>
<th>Parameters</th>
<th>BEMF (12)</th>
<th>LEMF (10)</th>
<th>REMF (13)</th>
<th>p-value (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophilia (x10^3/µL)</td>
<td>1.4 (0.22-4.90)</td>
<td>1.2 (0.12-9.60)</td>
<td>1.2 (0.19-1.78)</td>
<td>0.51</td>
</tr>
<tr>
<td>D-dimers (ng/ml)</td>
<td>213.5 (27.4-1212.3)</td>
<td>394 (30.0-2271.0)</td>
<td>347 (99.0-3722)</td>
<td>0.51</td>
</tr>
<tr>
<td>PF1+2 (nmol/l)</td>
<td>0.44 (0.07-29.30)</td>
<td>0.49 (0.06-14.60)</td>
<td>0.31 (0.12-6.90)</td>
<td>0.77</td>
</tr>
<tr>
<td>sTF (pg/ml)</td>
<td>327.8 (225.2-470.2)</td>
<td>255.6 (216.5-587.9)</td>
<td>236.2 (158.1-603.5)</td>
<td>0.48</td>
</tr>
<tr>
<td>PAI-1 (ng/ml)</td>
<td>67.1 (22.2-189.3)</td>
<td>102.2 (42.6-175.5)</td>
<td>36.7 (18.8-186.7)</td>
<td>0.039</td>
</tr>
<tr>
<td>ICAM (ng/ml)</td>
<td>371.5 (16.2-680.8)</td>
<td>372.8 (192.8-650.1)</td>
<td>285.1 (129.5-529.4)</td>
<td>0.55</td>
</tr>
<tr>
<td>P-selectin (ng/ml)</td>
<td>76.9 (57.7-218.5)</td>
<td>99.9 (58.6-138.5)</td>
<td>72.3 (4.9-347.3)</td>
<td>0.14</td>
</tr>
<tr>
<td>IL-5 (pg/ml)</td>
<td>13.9 (4.5-41.6)</td>
<td>13.4 (8.4-32.0)</td>
<td>11.6 (4.6-35.3)</td>
<td>0.61</td>
</tr>
<tr>
<td>IL-6 (pg/ml)</td>
<td>3.6 (1.8-41.6)</td>
<td>4.8 (2.5-614.9)</td>
<td>4.4 (1.5-48.4)</td>
<td>0.35</td>
</tr>
</tbody>
</table>
8.4. Discussion

We have shown that patients with recent onset EMF have increased levels of IL-6, endothelial cell activation and increased fibrinolysis, strongly suggesting that inflammation, endothelial injury and pro-coagulant changes play an important role in the early phases of this condition. These patients usually present acute heart failure associated with unexplained fever, cough, progressive breathlessness, facial swelling and severe hypereosinophilia.

Soluble ICAM was used as a marker of endothelial cell activation since it is up regulated during the process of endothelial cell activation, with subsequent shedding into the plasma. PAI-1, the major inhibitor of tissue plasminogen activator, is produced by the endothelium and released by activated platelets; its plasma levels are greatly increased during endothelial cell activation. The generation of adhesion molecules and production of PAI-1 participate in the inflammatory response and contribute to a prothrombotic state. Our patients had high levels of circulating D-dimers indicating increased fibrin degradation related to break down of fibrin clot. Patients with EMF had increase levels of Soluble Tissue Factor (sTF), a marker of up-regulation of Tissue Factor. The levels of PF1+2, produced when prothrombin is activated to thrombin and therefore highly sensitive to coagulation turnover, did not differ in patients and controls. Also there was no difference in P-selectin levels and platelet counts in the two groups, suggesting that it is a better marker of platelet activation, more than a marker of endothelial cell activation.
The association of intracavitary thrombi with increased markers of endothelial cell activation suggests that there may be an active ongoing process in the endocardium. Although we cannot be certain if the increased markers of endothelial cell activation represent activation of the endothelial endocardium or a more widespread process, we can speculate as to the cause of these biological changes, which can be due to an ongoing infection, be part of an autoimmune mechanism, or be related to the cardiotoxicity of the eosinophil.

Despite the higher levels of eosinophil counts found in our patients, only few could be considered as having severe hypereosinophilia for the standards of our setting, where there is a high prevalence of parasitic infections in the general population. We were surprised to find no correlation between the degree of eosinophilia, and endothelial cell and hemostatic activation. EMF and hypereosinophilic syndromes are thought to have similar pathogenic mechanisms (Brockington and Olsen, 1973; Andy, 1999), and patients with both conditions have eosinophils with unusual morphology, metabolically and functionally more effective than normal, with a large number showing evidence of degranulation (Rutakingirwa et al, 1999).

The cardiotoxicity of the eosinophil is mediated by activation of heart mast cells by eosinophil granule proteins (Patella et al, 1996) and secretion of pro-fibrotic mediators (Swartz et al, 2004). In our study patients had higher levels of eosinophils in the blood when compared to controls. Our results confirm previous data reporting a highly significant inverse relationship between hypereosinophilia and the duration of symptoms in EMF patients (Andy 1998), and support suggestions that failure to show difference in
eosinophilia between EMF patients and controls has been related with the fact that most studies are done in patients with chronic established disease. Indeed, assuming that cardiac damage in EMF is triggered by parasite induced eosinophilia, a raised eosinophil count need not to be present in chronic disease. Further, helminth associated hypereosinophilia peaks during larval migration and tends to return to normal thereafter, implying that if parasite-induced eosinophilia is related to cardiac damage, the eosinophilia in EMF should be sought in early disease.

We also measured the blood levels of IL-5, and showed that although they did not differ significantly between EMF patients and controls, they correlated with eosinophil count in the subgroup of patients (p=0.006). Due to its selectivity in the differentiation, activation, recruitment and survival of human eosinophils, IL5 is currently a target for drug therapy of eosinophil-mediated diseases (Garret et al, 2003; Sutton et al, 2005). Therefore, further research must be done in order to clarify this issue that could have an impact in management of EMF.

The criteria currently available for diagnosis of EMF rely on confirmation of endocardial fibrous thickening, which occurs late in its natural history. Echocardiography allows the diagnosis of Endomyocardial Fibrosis in its thrombotic phase in patients with less pronounced fibrosis, but its specificity to identify small thrombus is relatively low. This study is the first attempt at characterizing the biomarker profile of recent onset EMF, which knowledge would be useful for population screening and identification of recrudescence on follow-up, particularly after surgery.
Our findings could also have implications for the management of Endomyocardial Fibrosis, since they suggest that modulation of both inflammation and hemostasis might be a new therapeutic target in Endomyocardial Fibrosis, and might alter the natural history of the disease. Prednisone, Ace-inhibitors, β-blockers and appropriate anticoagulation have been shown to reduce fibrous thickening of the endocardium following apical obliteration by thrombus, to improve the clinical outcome and increase survival in Loeffler endocarditis (Lofiego et al, 2005; Davies et al, 1983) and could also be tested in EMF patients detected with hypereosinophilia and intraventricular thrombosis.

Although supporting the role of endothelial activation in pathogenesis of endomyocardial fibrosis our results do not clarify, either the selective injury of the endocardial endothelium or the particular distribution of the lesions which are concentrated in the left ventricular apex, the recess behind the posterior leaflet of the mitral valve, and the trabecular portion of the right ventricle. The poor blood supply from the end coronary arteries to the apices and the sluggish blood flow at the same regions would contribute to increase the susceptibility of the endocardium to injury, creating conditions for apical thrombosis and subsequent fibrosis. The heterogeneity of the endothelium may also play a role (Aird, 2007).

We aimed at studying patients early in the course of their disease, by selecting patients with symptoms for less than 6 months but acknowledge that clinical manifestations of Endomyocardial Fibrosis can appear long after the initial insult. This clinical-echocardiographic dissociation has been recognized particularly in REMF (Salemi et al,
2005), and is one of the reasons contributing to late presentation of patients to medical attention.

We also acknowledge that the majority of our patients had heart failure at presentation, and that this may have partially influenced some results as endothelial dysfunction and damage have been reported in congestive heart failure, using parameters different from those used by us (Chong et al, 2004).

High circulating levels of ICAM have been demonstrated in patients with heart failure and suspected myocardial inflammation (Klein et al, 1998). Although not proved, if Endomyocardial Fibrosis is caused by myocardial inflammation and damage, as it would be the case with the autoimmunity hypothesis, the levels of this marker could be solely the result of this insult.
8.5. Conclusions

Patients with recent onset EMF have increased levels of IL-6, endothelial cell activation and increased fibrinolysis, strongly suggesting that inflammation, endothelial injury and pro-coagulant changes play an important role in the early phases of this condition. The results suggest also that an insult to the endocardium may be involved in the pathogenesis of EMF.

Further research is needed to confirm these findings since they can have important therapeutical implications. The biomarkers identified could potentially be used on community studies for early detection and follow up.
Chapter 9

MULTIFACETED INVESTIGATION ON THE ROLE OF MALARIA
9.1. Background

The epidemiology of malaria and EMF are not absolutely superimposed. However, endemic regions for Endomyocardial Fibrosis (EMF) are also areas of high prevalence of malaria in Africa (Sliwa et al, 2005). Mozambique is an endemic area for Plasmodium falciparum malaria, the leading cause of morbidity and mortality in the country. The Inharrime District has the highest transmission rate of malaria in the country (Cuamba, 2003) and is an area where EMF is endemic (Ferreira et al, 2002).

The first studies relating malarial infection to EMF were done in Uganda, where the possibility of an immune mechanism was postulated. Several studies (Van der Geld et al, 1966; Shaper et al, 1967; Shaper et al, 1968; Patel et al, 1971) showed the presence of an immunological syndrome consisting of high titers of malarial antibody, high titers of IgM and circulating antiheart antibodies in populations with high prevalence of EMF. The prevalence of antimalarial and antiheart antibodies did correlate (Shaper 1974), but it was not clear if the antibodies were the cause or the result of EMF.

Mice infected with murine malaria parasite Plasmodium berghei (Eling et al, 1984; Eling et al, 1988) developed endomyocardial fibrotic lesions that mimic human disease, providing one of the best animal models for EMF. From the model of murine malaria there seems to be an indirect involvement of the malaria parasite in pathogenesis and the fibrotic lesions are related to long lasting and/or repeated episodes of parasitemia (Eling et al, 1983; Eling et al, 1984).
Myocardial injury has been demonstrated in human severe and complicated malaria by *P. falciparum*, and does not seem to result from lowered hemoglobin levels (Ehrhardt *et al.*, 2004; Ehrardt *et al.*, 2005). We hypothesize that an autoimmune lesion due to molecular mimicry between *Plasmodium* and human myosins might cause myocardial injury, and be involved in the pathogenesis Endomyocardial Fibrosis.

Malarial infection is among the hypotheses that have been explored to explain the occurrence of Endomyocardial Fibrosis. Endemic regions for Endomyocardial Fibrosis in Africa are also areas of high prevalence of malaria (Sliwa *et al.*, 2005), and in Mozambique this endemic area for Endomyocardial Fibrosis (Ferreira *et al.*, 2002) has the highest transmission rate for *P. falciparum* malaria in Mozambique (Cuamba, 2003).

However, despite suggestions of its role in pathogenesis from an animal model (Shaper *et al.*, 1967; Shaper *et al.*, 1968b, Eling *et al.*, 1988), the hypothesis of human malaria being involved in the pathogenesis of this cardiomyopathy has never been proven.

In order to investigate the role of malaria in pathogenesis of Endomyocardial Fibrosis we designed a series of studies to determine:

1. The prevalence of *P. falciparum* malarial infection in children, the age group most frequently affected by EMF;
2. The presence of anti-malarial antibodies in the sera of patients with EMF and healthy controls from the same population;
3. The binding of sera from EMF patients to mouse heart; and
4. The presence of markers of myocardial injury in children from the same population during episodes of severe or complicated *P. falciparum* malaria.
9.2. Population and Methods

9.2.1. Design of the research

The studies on the role of malaria in pathogenesis of malaria took place in Maputo city and in the remote rural district of Inharrime, extensively described in previous chapters. The recruitment of patients was done during the high transmission season, which occurs from October to April.

Between October and December 2004, we studied 6 consecutive EMF patients from our registry who were resident in Inharrime. We then selected 12 controls: 6 children matched for age and sex attending the local health centre for acute respiratory infections, and the 6 mothers of the previously selected children. All were submitted to echocardiographic examination to exclude EMF.

The prevalence of malaria infection was measured in Inharrime District during the month of October 2006. The determination of myocardial damage in children with severe or complicated malaria was undertaken in a hospital serving a suburban area of Maputo between October 2007 and January 2008.

9.2.2. Population

The study for the establishment of a cohort of school children determined the prevalence of malaria infection in school children. From the 323 schoolchildren eligible for the study, 296 had an informed consent signed by their parents and were therefore included.
in the study. Blood samples were obtained from every fifth participant, meaning that tests for malaria were performed in 71 children.

At Inharrime district we also studied 6 EMF patients and 12 controls from the same population for detection of circulating antibodies to Plasmodium falciparum and reactivity of patient’s sera with mouse heart tissue.

Finally, we determined the presence of markers of myocardial injury in severe malaria studying children between 5 and 15 years old admitted to a local hospital. Only children with the diagnosis of severe and/or complicated malaria (WHO 2000) were invited to the study. We selected 47 consecutive children fulfilling the inclusion criteria, namely confirmed *P. falciparum* monoinfection in absence of any other known disease, and absence of blood transfusion or anti-malarials prior to blood collection. Severe anemia was defined as hemoglobin of 8g/dL or less.

**9.2.3. Blood collection and conservation**

Blood samples were obtained by puncture of the antecubital vein. The blood was collected into siliconised glass tubes (Bectin Dickinson) either containing 1/10 by volume of 3.8% trisodium citrate or EDTA. Plasma was prepared by centrifugation at 3000rpm for 20 minutes at 4°C. Samples were aliquoted into Eppendorf tubes and snap frozen, at –80°C and stored locally. They were transported to London on Cardice and restored at –80°C. For immunofluorescence, sera were thawed immediately prior to analysis and aliquots diluted 1:500 and 1:1000 in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA). The remaining serum was aliquoted and frozen at –80°C.
to be used for the Western Blotting analysis. The analysing laboratory did not know the status of individual sera (EMF patients/controls).

4. Assays

a) Immunofluorescence on mixed and synchronized *P falciparum* asexual blood stage parasites

**Preparation of parasites**

*P falciparum* clones IT04 of the Ituxi strain and C10 were cultured under standard conditions as described (Webb *et al*, 1996; Margos *et al*, 2004). Thin films of C10 were prepared on glass and dried under stream of air. Slides were fixed in cold acetone (-20°C) for 10 minutes, air-dried and stored at -20°C until further use. IT04 parasites were synchronised by sorbitol lyses. Schizonts were concentrated on a 66%v/v Percoll cushion (Dluzewski *et al*, 1984), washed in culture medium then allowed to grow for further 30-60 minutes at 37°C before being collected for Western blot analysis. For this, IT04 parasites were centrifuged at 1,000xg for 5 minutes. The supernatant was removed, protease inhibitor cocktail (Sigma) and 1 mM EDTA were added, and the parasite pellet stored at -20°C.

**Immunofluorescence microscopy**

Acetone-fixed smears of mixed stages of *P falciparum* strain C10 were probed for 1 h at room temperature with human immune serum from patients with EMF (1:1000) and the
twelve controls. The slides were washed in PBS, and then incubated with FITC-conjugated goat anti-human antibody (Abcam, UK), diluted in 1:200 in PBS-BSA. Nuclei were counterstained with diamidino phenyl-indole (DAPI) (1μg/ml). Following washes with PBS, slides were mounted using ProLong mounting medium (Molecular Probes Europe BV, Leiden, The Netherlands). Cells were viewed using a Nikon Optiphot microscope equipped with differential interference contrast microscopy and interchangeable filter blocks for fluorescence. A cooled, monochromatic CCD camera was used to enhance and give artificial colour to the images that were assembled using Adobe Photoshop 7.0.

*P. falciparum* asexual parasites express stage-specific proteins. To establish whether sera from patients or controls react differently with parasite stages, in the microscopic analysis we distinguished between ring stages (R), early schizonts (S), late (budding) schizonts (LS), free merozoites (mz), and staining of the red blood cell (rbc) itself.

*b) Western Blotting on Plasmodium falciparum mixed asexual stages using human sera*

IT04 parasites were solubilized in sample buffer for SDS-PAGE and proteins were separated on 10% polyacrylamide gels using Introgen NuPAGE® Bis-Tris MOPS buffer system and blotted on to microcellulose. The blots were blocked with PBS-1% skimmed milk and probed for 1 h with primary antibodies. Following washed with PBS, blots were visualized with a 1:3000 dilution HRP-conjugated goat anti-human-Ig (Sigma, UK).
c) Immunofluorescence using human sera on mouse heart tissue (confocal immunohistochemistry)

Adult mouse hearts from Balb/c strain frozen by immersion in liquid nitrogen were cut (15 µm cryosections) and fixed in 4% paraphormaldehyde in PBS (pH 7.3) for 5 minutes at 4°C prior to staining. Sections were blocked and primary antibody (sera from EMF patients using dilutions from 1:100 to 1:4000) was applied. Then the secondary antibodies diluted in PBS (1:75 and 1:200) were applied, sections were mounted in Vectashield (Vector Laboratories) and analyzed using Leica TCS-NT confocal laser scanning microscope.

d) Diagnosis of malaria in the community studies

Venous blood from each patient was collected into EDTA in sterile vacutainers during fieldwork.

Rapid tests to detect the presence of HRPII (histidin-rich protein II) antigen of *P falciparum* in the blood were performed. Five µl of whole blood was applied to the sample well and instructions for the use of the ICT Malaria P.f. Cassette Test were followed. Interpretation of the results was done according to manufacturer’s recommendations (ICT Diagnostics).

Parasitemia was assessed by counting asexual forms of *P falciparum* on thin blood smears under a light microscope after staining with 10% Giemsa solution, and was expressed as percentage of infected red blood cells or using the system of crosses (0 to 5+).
e) Myocardial injury

Blood for the rapid tests for the measurement of Troponin-T levels was obtained through puncture of a peripheral vein, using tubes with lithium heparin. Semi-quantitative determination of cardiac troponin T (cTnT) was done using the CARDIAC reader instrument (Roche). The upper limit of normal cTnT blood levels was defined as 0.1µg/l (Immer et al, 1997). Table 9.1 shows the interpretation of the semi-quantitative determination of cTnT.

Table 9.1. Interpretation of the troponin levels using the cardiac reader

<table>
<thead>
<tr>
<th>Troponin T blood levels</th>
<th>CARDIAC Reader</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 0.03 ng/ml</td>
<td>NEGATIVE</td>
<td>Low risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myocardial lesion absent</td>
</tr>
<tr>
<td>0.03 ng/ml to 0.1 ng/ml</td>
<td>LOW &lt; 0.1 ng/ml</td>
<td>Moderate risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myocardial lesion possible</td>
</tr>
<tr>
<td>0.1 ng/ml to 2 ng/ml</td>
<td>Quantitative between 0.1 and 2 ng/ml</td>
<td>High risk</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myocardial lesion present</td>
</tr>
<tr>
<td>&gt; 2 ng/ml</td>
<td>HIGH</td>
<td>Massive myocardial lesion</td>
</tr>
</tbody>
</table>

5. Ethical issues

Parents or participants (when older than 18 years) were informed of all procedures of the research project. All those included in the studies had given a written informed consent for the studies (or finger-print whenever the participant/parent was illiterate).
9.3. Results

Prevalence of malaria in school children

The prevalence of malaria infection in school children of Inharrime was 67.6% (48/71). All children denied any symptoms. Malaria coexisted with EMF in 3 children out of 6 with EMF (Table 9.2). There was no statistically significant evidence that the percentages of EMF detected differed whether malaria was present or not (p=0.34, chi-square test).

Table 9.2. Prevalence of malaria infection and EMF in schoolchildren

<table>
<thead>
<tr>
<th></th>
<th>No EMF</th>
<th>EMF</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Malaria negative</td>
<td>20 (87.0%)</td>
<td>3 (13.0%)</td>
<td>23 (100.0%)</td>
</tr>
<tr>
<td>Malaria positive</td>
<td>45 (93.8%)</td>
<td>3 (6.2%)</td>
<td>48 (100.0%)</td>
</tr>
<tr>
<td>All</td>
<td>65 (91.6%)</td>
<td>6 (8.4%)</td>
<td>71 (100.0%)</td>
</tr>
</tbody>
</table>

Anti-Plasmodium falciparum antibodies in sera of patients with EMF

Anti-Pf antibodies were present in sera of both EMF patients and controls. The results of the microscopic analysis on immunofluorescence are shown in Table 9.3. There was no obvious difference in reactivity of sera to the different parasite stages between sera of EMF patients and controls.
Table 9.3. Stages of *P. falciparum* found in both patients with EMF and controls

Recognition pattern of sera from Mozambique on acetone-fixed *P. falciparum* IT04

<table>
<thead>
<tr>
<th>Name</th>
<th>Serum dilution</th>
<th>Pos/negative</th>
<th>Stages positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMF7</td>
<td>1:1,000</td>
<td>++</td>
<td>LS, Mz, R</td>
</tr>
<tr>
<td>EMF8</td>
<td>1:1,000</td>
<td>+</td>
<td>LS, Mz, rbc</td>
</tr>
<tr>
<td>EMF9</td>
<td>1:1,000</td>
<td>+</td>
<td>LS (+), Mz (+)</td>
</tr>
<tr>
<td>EMF10</td>
<td>1:1,000/1:500</td>
<td>-/+</td>
<td></td>
</tr>
<tr>
<td>EMF11</td>
<td>1:1,000</td>
<td>+(+)</td>
<td>S (+), Mz (+)</td>
</tr>
<tr>
<td>EMF12</td>
<td>1:1,000</td>
<td>++</td>
<td>S, Mz, R, rbc</td>
</tr>
<tr>
<td>EMF13</td>
<td>1:1,000</td>
<td>+</td>
<td>S</td>
</tr>
<tr>
<td>EMF14</td>
<td>1:1,000</td>
<td>++</td>
<td>S, R, rbc, Mz</td>
</tr>
<tr>
<td>EMF15</td>
<td>1:1,000</td>
<td>++</td>
<td>S, Mz, R</td>
</tr>
<tr>
<td>EMF16</td>
<td>1:1,000</td>
<td>+</td>
<td>LS, Mz</td>
</tr>
<tr>
<td>EMF17</td>
<td>1:1,000</td>
<td>+</td>
<td>S, Mz</td>
</tr>
<tr>
<td>EMF18</td>
<td>1:1,000</td>
<td>++</td>
<td>S, Mz, R</td>
</tr>
<tr>
<td>E0008</td>
<td>1:1,000</td>
<td>++</td>
<td>S, Mz, R</td>
</tr>
<tr>
<td>E0176</td>
<td>1:1,000</td>
<td>++</td>
<td>S, Mz</td>
</tr>
<tr>
<td>E018</td>
<td>1:1,000</td>
<td>+</td>
<td>S (+), Mz, R</td>
</tr>
<tr>
<td>E020</td>
<td>1:1,000</td>
<td>+</td>
<td>S</td>
</tr>
<tr>
<td>E030</td>
<td>1:1,000</td>
<td>+</td>
<td>S, rbc</td>
</tr>
<tr>
<td>E034</td>
<td>1:1,000</td>
<td>++</td>
<td>S, Mz</td>
</tr>
</tbody>
</table>

Symbols indicate intensity of staining (- = negative; + = positive; ++ = strong positive). Parasite stages are abbreviated as following: R = ring stage; Mz = merozoite; S = schizont; LS = late schizont; rbc = red blood cell.
Figure 9.1 presents different stages of the parasite identified by immunofluorescence in patients and controls. In young trophozoites (or ring stages) malaria pigment is not visible while late trophozoites/early schizonts have some malaria pigment in their pigment vacuoles. Since there was no nuclear staining which would have allowed estimating the number of nuclei in the parasites, it is difficult to distinguish late trophozoites and early schizonts. Those parasites most likely to be trophozoites/early schizonts (judging from parasite size, the absence or amount of pigment) showed hardly any fluorescent staining but the red blood cell harbouring the parasite showed dotted staining which may perhaps be staining of proteins associated with Maurer's clefts or other parasite structures in the red blood cell. More developed schizonts show peripheral fluorescent staining. Mature schizonts showed fluorescent staining of internal structures probably labelling the periphery of developing merozoites. Rupturing schizonts showed fluorescent staining of surface and internal structures of individual merozoites. Free merozoites showed internal fluorescent staining (probably apical) reminiscent of apical complex structures (rhoptries, micronemes) or cytoskeletal structures. Details of these features are revealed in Figure 9.2.

Binding of sera from EMF patients with mouse heart

The Western Blotting showed binding of sera to different mouse heart proteins with no specific pattern in any of the two groups (Figure 9.3). At the higher concentration of secondary antibody there was a noticeable background and most of the staining resulted from this.
Figure 9.1. Stages of the Plasmodium falciparum identified by immunofluorescence in some control subjects
Figure 9.2. Composite of the different asexual stages of *Plasmodium falciparum* identified by immunofluorescence, namely trophozoits/early schizonts (a), developed schizonts (b), mature schizonts (c), rupturing schizont (d), free merozoite (e and f)
**Figure 9.3.** Western Blotting of sera of six EMF patients and twelve controls.
Markers of myocardial injury in severe/complicated Plasmodium falciparum malaria

Parents of one child refused to participate. One child tested negative for the presence of *P. falciparum* antigen HRPII and was excluded from the study. We therefore studied 45 children. The clinical features of these patients are presented in the Table 9.4. All patients had fever, hyperpyrexia and chills, and 18 (20.0%) had seizures.

Table 9.4. Frequency of clinical symptoms and signs in patients with severe and complicated malaria

<table>
<thead>
<tr>
<th>Symptoms and signs</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever, Chills, Hyperpyrexia</td>
<td>45</td>
<td>100%</td>
</tr>
<tr>
<td>Seizures</td>
<td>18</td>
<td>40.0%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>17</td>
<td>37.8%</td>
</tr>
<tr>
<td>Severe anemia</td>
<td>10</td>
<td>26.7%</td>
</tr>
<tr>
<td>Impairment of consciousness</td>
<td>5</td>
<td>11.1%</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>2</td>
<td>4.4%</td>
</tr>
</tbody>
</table>

The mean age (± SD) was 7 (± 3.5) years, and 27 (60%) were females. Eleven children (28.9%) had severe blood parasitemia (greater than 0.5%). Eighteen (40%) patients had
cerebral malaria and 2 (4.4%) had heart failure thought to be due to severe anemia. Two-thirds of the children had anemia (30; 66.7%), which was severe in 8 patients. The mean (± SD) hemoglobin level was 9.3 g/dL (± 2.2). Only two children (4.4%) had splenomegaly greater than grade I.

All children evaluated had undetectable (negative) levels of circulating cTnT indicating that myocardial injury did not occur in any of these patients with severe and complicated \textit{P. falciparum} malaria.

The electrocardiograms were all normal without signs suggestive of ischaemia. Systolic and diastolic function was preserved in all cases, but LV dimensions indexed for body surface area were abnormal in two children with severe anemia (2; 4.4%).

All children were treated with intravenous quinine. There was no mortality.
9.4. Discussion

The prevalence of malaria infection in school children of Inharrime is very high. Despite malaria being endemic in this rural area we did not expect to find active infection in more than 2/3 of the examined children. Of interest is the fact that these children had asymptomatic malaria, probably related to partial immunity, due to frequent biting by infecting mosquitos (Nielsen et al., 2002), considering that Inharrime is the area with the highest transmission rate in Mozambique.

Our results indicate that strong immunity against malaria infection by *P. falciparum* is present in the population of this district, independent of the presence of EMF. There was no specific pattern of reactivity of sera from EMF patients and controls on both immunofluorescence and Western Blotting, with the groups presenting no difference in terms of stages of the parasites detected.

The results of the search for the presence of antimyosin antibodies in blood using Western Blotting were negative. Since myosins from different species show high homology, we expected anti-human myosin activity to be detectable using human sera against mouse heart. Several bands were detected in both patients and controls, but none corresponded to the molecular weight of myosin, suggesting that patients with EMF do not have circulating anti-myosin antibodies.

In the literature there are conflicting results regarding the prevalence of myocardial damage in *P. falciparum* malaria. Circulating concentrations of cardiac proteins have been shown in patients with complicated and uncomplicated *P. falciparum* malaria (Ehrhardt et
al, 2004; Ehrardt et al, 2005). Myocardial damage in *P falciparum* malaria was rare when assessed by cTnT (Gunther et al, 2003) in a retrospective study of patients from Ghana of unspecified age and severity of *P falciparum* malaria. Our study included blood film microscopic diagnosis, confirmation of infection by rapid test for antigen HRP-2, strict criteria of severity of malaria, and echocardiographic examination confirmed the absence of myocardial dysfunction. For detection of myocardial damage we used cTnT, a marker that has high specificity and sensitivity in the assessment of myocardial damage in children (Immer et al, 1997). Since Troponin-T was not detected in any child studied during an episode of severe and/or complicated malaria, we conclude that severe and complicated *P falciparum* malaria is not associated with myocardial damage.

**Limitations**

There are some limitations to our studies on the role of malaria in pathogenesis of EMF. The numbers involved are small considering the extent of both diseases in this particular area.

The rational for our studies was the knowledge that *P falciparum* has multiple myosins that are part of its motile apparatus and some are similar to mammalian myosins (Chaparro-Olaya et al, 2005). We therefore hypothesized that *P falciparum* has some myosins that would share homology with the human myosin. However, we used mouse heart in our studies and, therefore must acknowledge that the protein(s) involved in molecular mimicry could be those that are different in the two species (human and mouse) and therefore would not be detected using human sera against mouse tissue.
Chapter 9 Investigating the Role of Malaria

Reciprocal testing of highly specific anti-\textit{P falciparum} antibodies against human cardiac tissue and human heart proteins against \textit{Plasmodium falciparum} purified myosins would help clarify this issue.

On the other hand a certain amount of time is needed for the humoral response to be set up after the acute malaria episode. It can therefore be argued that myocardial injury might be detectable days or weeks following the acute episode of malaria, and not concomitant with the infection. Thus, for follow-up of children with severe and complicated malaria would be necessary to detect this response. We therefore agree with those who postulate that respective studies on immune response against malaria should include serial measurements of anti-malarial antibodies, markers of myocardial damage, electrocardiography or echocardiography (Franzen \textit{et al}, 1992).

Experimental work on murine malaria showed that infection of C57B1 mice with \textit{Plasmodium berghei} (Eling \textit{et al}, 1984; Eling \textit{et al}, 1988) results in endomyocardial fibrotic lesions that mimic human disease, providing one of the best animal models for EMF. Several features of the condition produced in C57B1 mice infected with \textit{P. berghei} were similar to those observed in humans: the predilection of lesions for the right half of the heart, genetic factors involved in the disease, the absence of hypereosinophilia syndrome, the supposed role of thrombi covering endothelial lesions, as well as the role of recurrent inflammatory processes in the pathogenesis of the disease.

From the model of murine malaria it would be concluded that the lesions are related to long lasting and/or repeated episodes of parasitemia. This work by Eling (1984) also suggested an indirect involvement of the malaria parasite. Despite the close correlation...
between development of lesions and parasitemia, parasitized erythrocytes were not usually present in the affected areas (Eling et al., 1984), leading to the hypothesis of the initial event being the activation and release of macrophages through phagocytosis, or the effect of the products released by this activation. Indeed, early changes included sticking of macrophages to the endocardial endothelium, and migration to subendothelial areas associated with leakage and edema. Subsequently, subendothelial infiltrates of lymphocytes, neutrophil granulocytes, macrophages and fibroblasts were found (Eling et al., 1983; Eling et al., 1984). These endothelial lesions grew out until death of the host or until chemotherapy cleared the infection. Chemotherapy of the infection rapidly arrested thrombus formation and growth and initiated resorption of thrombotic material, resulting in fibrosis of the affected areas.

More recently the attention of researchers has been directed to the importance of coagulation and hemorrhage disturbances in the molecular mechanisms of malaria (van der Heyde et al., 2006), since almost all clinical studies of P falciparum patients indicate a procoagulant state. Patients with severe falciparum malaria exhibit prolonged bleeding times, PT and PTT, and impaired platelet aggregation, and these clinical measurements of coagulopathy correlate well with the severity of malaria (Rojanasthien et al., 1992).

Another recent knowledge acquired in recent years was the publication of reports of immune mimicry in malaria (MacDonald et al., 2001). The secretion of translational controlled tumor protein (TCTP), a homolog of histamin-releasing factor, by Plasmodium falciparum was demonstrated in vitro and in vivo. This protein causes histamine release from basophils and IL-8 secretion from eosinophils, substances that have been reported to
be elevated in patients with malaria (Kurtzhals et al, 1998; Shanks and Wilairatanaporn, 1992). Reports of rebound eosinophilia after malarial infection in Thailand (Sliwa et al, 2005) also reinforce the need to explore the relationship between this parasitic infection and EMF, a disease that shares many similarities with the hypereosinophilic syndromes.

In summary, the possibility of other mechanisms of *P falciparum* infection being involved in the pathogenesis of EMF must not be overlooked. Particularly, the role of endothelial dysfunction, inflammatory changes and pro-coagulant abnormalities must be addressed through experimental research using animal models and *in vitro* studies.
9.5. Conclusions

The prevalence of malaria is high in this rural community where EMF is also endemic. EMF patients and healthy individuals from this community have increased humoral response to Plasmodium falciparum as measured by immunofluorescence and Western Bloting. Malaria affects equally children with and without EMF.

There is no evidence of early myocardial damage in Plasmodium falciparum severe and complicated malaria in children. Our results are against an early myocardial lesion in malaria and do not support the role of molecular mimicry between products of the Plasmodium falciparum and the human myosins. However, they cannot completely exclude the role of malaria in the pathogenesis of EMF.

In the light of the recent progress in identification of markers of endocardial activation and myocardial damage, cohort studies involving larger number of participants are needed. These should include long-term monitoring of the pattern of antibody responses to Plasmodium falciparum malaria, as well as determination of the presence and kinetics of markers of endocardial activation, hemostatic dysfunction and myocardial damage following episodes of acute malaria.
Chapter 10

EXPLORING IMMUNOGENETIC HETEROGENEITY IN EMF
10.1. Background

EMF presents great phenotypic variability in terms of the distribution and severity of the lesions. The disease affects one or both sides of the heart in variable proportion, and its severity and mode of progression vary in different individuals, suggesting the possible influence of host factors in the establishment and outcome of EMF.

In Ugandan experience EMF was more common among immigrants from neighboring Rwanda and Burundi, with poor socio-economic conditions, who had settled in a specific geographic district of the country (Connor et al., 1967; Connor et al., 1968; Rutakingirwa et al., 1999). However, the disease also occurred in native Ugandans as well as in Europeans who have lived in tropical Africa (Brockington et al., 1967; Beck and Schrire, 1971), suggesting that the condition could not be explained solely on the basis of ethnic factors or social deprivation.

Some clinical series from Uganda and Zambia reported familial cases of EMF in endemic regions (Adi, 1963; Patel et al., 1971). This finding was confirmed in epidemiological performed in the rural community we studied in Mozambique (Chapter 4). Using echocardiographic screening of the general population for diagnosis no correlation was found between the percentage of subjects with EMF in a family and the family size, but for each individual the chance of having the disease was higher when other members of the family had the disease. Although some authors suggest that there is no compelling evidence for supporting the contribution of inherited factors to the pathogenesis of EMF.
(Mayosi and Somers, 2007), the familial occurrence of EMF could be explained by either genetic or environmental factors (Sliwa et al, 2005).

In recent years, both infectious and autoimmune diseases have been associated with increased frequencies of various specific histocompatibility leukocyte antigens (HLA) that may be linked to immune response genes. Common diseases of presumed viral origin, which have been linked to HLA specificities, include lupus erythematosus, juvenile diabetes mellitus, rheumatoid arthritis, multiple sclerosis and rheumatic heart disease (Maharaj et al, 1987; Guedez et al, 1999).

We hypothesize that hereditary factors, possibly immunological in nature, may contribute to genetic susceptibility in EMF, and that host-related factors may explain the dissimilarities in apparent clinical features.

The aim of this study was to determine the allelic and haplotypic diversity of the HLA-A, HLA-B, HLA-C, HLA-DR and HLA-DQ loci in patients from the EMF registry of Mozambique and ethnically matched controls, to determine whether any HLA determinants could be implicated in susceptibility to or protection against the development of EMF, and to investigate the possible influence of genotype in determining distribution and severity of the cardiac lesions.
10.2. Population and Methods

Study period and patients
Consecutive patients with EMF seen at the Heart Institute Mozambique between Nov 2004 and August 2006 were submitted to clinical evaluation and echocardiographic evaluation for diagnosis and classification of EMF. The patients were referred from all regions of the country, but remarkably 43 (53.8%) came from one single province Inhambane. The median age was 15 years (IQ range 10-20). There were 44 (55.0 %) males. All patients were black.

At admission 67 (83.8%) were in NYHA functional classes III and IV. Twenty patients (25.0%) were in atrial fibrillation. Most had abnormalities in both sides of the heart (Bilateral EMF) 43 (53.8%), 27 (33.7%) had lone or predominant right-sided EMF and the remaining 10 (12.5%) had left-sided disease. According to the severity of structural lesions only 14 (17.5%) patients had mild lesions.

The most frequent blood group among the patients was Orh+ 34 (56.7%). Two patients had associated rheumatic heart disease and one had concomitant myasthenia gravis.

Controls
The controls were 85 ethnically matched individuals from Mozambique who were blood donors.
Blood sample collection and storage

Blood was collected into siliconised glass tubes (Bectin Dickinson) containing EDTA and centrifuged for plasma preparation. After removal of the plasma the remaining cells were aliquoted into eppendorf tubes and snap frozen, at -80°C and stored locally. They were transported to London on Cardice and restored at –80°C, being thawed immediately before DNA extraction. The analysing laboratory was blinded to the samples.

DNA extraction

DNA was extracted from the blood samples using a BioRobot® M48 workstation and MagAttract® DNA extraction kits (Qiagen). The DNA was stored at -40°C.

HLA typing

Testing for HLA-A, -B, -C, -DRB1 and –DQB1 types was performed using commercial reverse PCR-SSOP kits (Reli™, Invitrogen). Briefly, DNA is amplified using locus specific biotinylated primers and the resulting amplicons are hybridised to arrays of sequence specific oligonucleotide probes immobilised on nylon strips. The presence of an amplicon/probe complex is detected using a colorimetric reaction and appears as a blue line. The strips are scanned and the probe pattern is analysed and interpreted to give an HLA type.
Chapter 10 Immunogenetic Heterogeneity

Ethics

The study was performed at the Maputo Heart Institute (Mozambique) in accordance with the ethical standards laid down in an updated version of the 1964 Declaration of Helsinki and after approval by the National Bioethical Committee of Mozambique.

Statistical analysis of results

Results were analysed using software developed by J D’Amaro et al (1995). Control data were tested for deviation from Hardy-Weinberg equilibrium expectations. The differences in frequency of HLA types between patients and controls were tested for significance by means of the chi-square test (without Yates correction). Relative risk was calculated according to the method of Svejgaard et al (1986).

The frequency of each A, B, C, DR and DQ type was compared between subjects with BEMF, REMF and LEMF using ANOVA.
10.3. Results

In the control group all HLA loci tested (HLA-A, -B, -C, -DRB1 and –DQB1) conformed to random expectations of combinations of HLA types at each locus with all p values greater than 0.1. The percentage phenotype frequencies of HLA-A, HLA-B, HLA-C, HLA-DR and HLA-DQ types of patients with endomyocardial fibrosis and the control subjects are shown in Tables 10.1 to 10.5.

In Tables 10.2, 10.3 and 10.5 the HLA alleles detected are shown as the equivalents of HLA specificities. Therefore in Table 10.2 HLA-B*1401 has been interpreted as HLA-B*64, HLA-B*1402 as HLA-B*65, HLA-B*1501 as HLA-B*62, HLA-B*1503 as HLA-B*72, HLA-B*1510 as HLA-B*71, HLA-B*1516 as HLA-B*63, and HLA-B*1521 as HLA-B*75. In Table 10.3 HLA-Cw*0303/11-13 has been interpreted as HLA-Cw9 and HLA-Cw*0302/04-08 has been interpreted as HLA-Cw10. Finally, in Table 10.5 HLA-DQB1*0301/ has been interpreted as HLA-DQ7, HLA-DQB1*0302/ as HLA-DQ8 and HLA-DQB1*0303/ as HLA-DQ9.

In these 165 Mozambicans the HLA specificities with the highest frequencies in each of the HLA classes were: HLA-A*30 (67; 40.6%), -A*29 (40; 24.3%), -A*68 (35; 21.2%); -B*58 (37; 22.4%), -B*72 and -B*44 (both 29; 17.5%), -B*42 (25; 15.2); -Cw*07 (96; 58.2%), -Cw*04 (55; 33.3%), -Cw*06 (46; 27.9%); -DRB1*11 (69; 41.8%), -DRB1*13 (51; 30.9%), -DRB1*15 (44; 26.7%); -DQB1*0301 or *07 (62; 37.6%); -DQB1*06 (63; 38.2%) and -DQB1*02 (55; 33.3%).
The HLA types HLA-A*25, -A*26, -A*31, -A*69, -B*37, -B*38, -B*52, -B*54, -B*55, -B*56, -B*59, -B*67, -B*73 and -DRB1*16, although tested were not found in any of the patients and the controls.

Table 10.1 shows a comparison of the frequencies of HLA-A types for controls and patients, including patients with mild to moderate disease (grades I and II), and those with severe and advanced disease (grades III and IV).

The frequency of HLA-A*23 was significantly reduced in the patients (8/80, 10%) when compared to the normal control subjects (20/85, 23.5%; p=0.018). When the patients are divided according to severity of the disease only the frequency of HLA-A*23 in patients with mild and moderate disease is significantly different. When comparing individuals with severe disease and normal control subjects we found differences in frequencies of HLA-A*33 (17.5% vs 5.9%; p=0.037).

The comparison of frequencies of HLA-B types for controls, all patients, patients with grades I+II and grades III+IV EMF is shown in Table 10.2. The frequency of HLA-B*58 is raised in the patients compared to controls (27.5% vs 17.6%) but we found no statistically significant difference. While in patients with less severe disease the frequency is the same as that of controls, in patients with more severe disease it is significantly higher (37.5% vs 17.6; p=0.015). Most patients with grade IV EMF (6 out of 11; 54.5%) were positive for HLA-B*58.
Table 10.1. HLA-A phenotype frequencies in EMF patients and controls. Patients are subdivided in two groups mild and moderate (EMF 1+2) or severe and advanced (EMF 3+4).

<table>
<thead>
<tr>
<th>HLA-A type</th>
<th>Controls N=85 % (n)</th>
<th>All patients N= 80 % (n)</th>
<th>Patients EMF 1 + 2 N= 40 % (n)</th>
<th>Patients EMF 3+4 N= 40 % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*01</td>
<td>5.9 (5)</td>
<td>7.5 (6)</td>
<td>2.5 (1)</td>
<td>12.5 (5)</td>
</tr>
<tr>
<td>A*02</td>
<td>34.1 (29)</td>
<td>26.2 (21)</td>
<td>22.5 (9)</td>
<td>30.0 (12)</td>
</tr>
<tr>
<td>A*03</td>
<td>4.7 (4)</td>
<td>10.0 (8)</td>
<td>7.5 (3)</td>
<td>12.5 (5)</td>
</tr>
<tr>
<td>A*11</td>
<td>1.2 (1)</td>
<td>1.2 (1)</td>
<td>2.5 (1)</td>
<td>0</td>
</tr>
<tr>
<td>A*23</td>
<td>23.5 (20)</td>
<td>10.0 (8)</td>
<td>7.5 (3)</td>
<td>12.5 (5)</td>
</tr>
<tr>
<td>A*24</td>
<td>3.5 (3)</td>
<td>5.0 (4)</td>
<td>5.0 (2)</td>
<td>5.0 (2)</td>
</tr>
<tr>
<td>A*29</td>
<td>23.5 (20)</td>
<td>25.0 (20)</td>
<td>22.5 (9)</td>
<td>27.5 (11)</td>
</tr>
<tr>
<td>A*30</td>
<td>42.3 (36)</td>
<td>38.7 (31)</td>
<td>50.0 (20)</td>
<td>27.5 (11)</td>
</tr>
<tr>
<td>A*32</td>
<td>2.3 (2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>A*33</td>
<td>5.9 (5)</td>
<td>8.7 (7)</td>
<td>0</td>
<td>17.5 (7) *</td>
</tr>
<tr>
<td>A*34</td>
<td>1.2 (1)</td>
<td>6.2 (5)</td>
<td>5.0 (2)</td>
<td>7.5 (3)</td>
</tr>
<tr>
<td>A*36</td>
<td>3.5 (3)</td>
<td>2.5 (2)</td>
<td>2.5 (1)</td>
<td>2.5 (1)</td>
</tr>
<tr>
<td>A*43</td>
<td>0</td>
<td>2.5 (2)</td>
<td>5.0 (2)</td>
<td>0</td>
</tr>
<tr>
<td>A*66</td>
<td>4.7 (4)</td>
<td>2.5 (2)</td>
<td>0</td>
<td>5.0 (2)</td>
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<tr>
<td>A*68</td>
<td>20.0 (17)</td>
<td>22.5 (18)</td>
<td>32.5 (13)</td>
<td>12.5 (5)</td>
</tr>
<tr>
<td>A*74</td>
<td>9.4 (8)</td>
<td>15.0 (12)</td>
<td>10.0 (4)</td>
<td>20.0 (8)</td>
</tr>
</tbody>
</table>

*versus control, p=<0.05
Table 10.2. HLA-B phenotype EMF frequencies in patients and controls. Patients are subdivided into two groups mild and moderate (EMF 1+2) or severe and advanced (EMF 3+4).

<table>
<thead>
<tr>
<th>HLA-B type</th>
<th>Controls N=85 % (n)</th>
<th>All patients N=80 % (n)</th>
<th>Patients EMF 1+2 N=40 % (n)</th>
<th>Patients EMF 3+4 N=40 % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>B*07</td>
<td>9.4 (8)</td>
<td>6.3 (5)</td>
<td>7.5 (3)</td>
<td>5.0 (2)</td>
</tr>
<tr>
<td>B*08</td>
<td>11.8 (10)</td>
<td>10.0 (8)</td>
<td>5.0 (2)</td>
<td>15.0 (6)</td>
</tr>
<tr>
<td>B*13</td>
<td>5.9 (5)</td>
<td>3.8 (3)</td>
<td>5.0 (2)</td>
<td>2.5 (1)</td>
</tr>
<tr>
<td>B*1401 (64)</td>
<td>1.2 (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B*1402 (65)</td>
<td>9.4 (8)</td>
<td>11.3 (9)</td>
<td>12.5 (5)</td>
<td>10.0 (4)</td>
</tr>
<tr>
<td>B*1501 (62)</td>
<td>2.4 (2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B*1503 (72)</td>
<td>20.0 (17)</td>
<td>15.0 (12)</td>
<td>12.5 (5)</td>
<td>17.5 (7)</td>
</tr>
<tr>
<td>B*1510 (71)</td>
<td>12.9 (11)</td>
<td>17.5 (14)</td>
<td>25.0 (10)</td>
<td>10.0 (4)</td>
</tr>
<tr>
<td>B*1516 (63)</td>
<td>0</td>
<td>3.8 (3)</td>
<td>2.5 (1)</td>
<td>5.0 (2)</td>
</tr>
<tr>
<td>B*1521 (75)</td>
<td>1.2 (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B*18</td>
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<td>5.0 (4)</td>
<td>7.5 (3)</td>
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</tr>
<tr>
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<td>2.5 (1)</td>
<td>0</td>
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<td>B*35</td>
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<td>7.5 (6)</td>
<td>12.5 (5)</td>
<td>2.5 (1)</td>
</tr>
<tr>
<td>B*39</td>
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<td>1.3 (1)</td>
<td>2.5 (1)</td>
<td>0</td>
</tr>
<tr>
<td>B*42</td>
<td>18.8 (16)</td>
<td>11.3 (9)</td>
<td>15.0 (6)</td>
<td>7.5 (3)</td>
</tr>
<tr>
<td>B*44</td>
<td>16.5 (14)</td>
<td>18.8 (15)</td>
<td>20.0 (8)</td>
<td>17.5 (7)</td>
</tr>
<tr>
<td>B*45</td>
<td>8.2 (7)</td>
<td>7.5 (6)</td>
<td>10.0 (4)</td>
<td>5.0 (2)</td>
</tr>
<tr>
<td>B*49</td>
<td>8.2 (7)</td>
<td>6.3 (5)</td>
<td>5.0 (2)</td>
<td>7.5 (3)</td>
</tr>
<tr>
<td>B*50</td>
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<td>2.5 (1)</td>
<td>2.5 (1)</td>
</tr>
<tr>
<td>B*51</td>
<td>3.5 (3)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>B*53</td>
<td>10.6 (9)</td>
<td>16.3 (13)</td>
<td>15.0 (6)</td>
<td>17.5 (7)</td>
</tr>
<tr>
<td>B*57</td>
<td>7.1 (6)</td>
<td>5.0 (4)</td>
<td>5.0 (2)</td>
<td>5.0 (2)</td>
</tr>
<tr>
<td>B*58</td>
<td>17.6 (15)</td>
<td>27.5 (22)*</td>
<td>17.5 (7)</td>
<td>37.5 (15)*</td>
</tr>
<tr>
<td>B*81</td>
<td>7.1 (6)</td>
<td>10.0 (8)</td>
<td>5.0 (2)</td>
<td>15.0 (6)</td>
</tr>
</tbody>
</table>

*versus control, p=<0.05
Tables 10.3, 10.4 and 10.5 show the frequencies of HLA-C, -DR and -DQ types for controls and patients (subdivided in grade 1+2 and 3+4). There was no statistically significant difference between any of the four groups.

Table 10.3. HLA-B phenotype frequencies in EMF patients and controls

<table>
<thead>
<tr>
<th>HLA-C type</th>
<th>Controls N=85 % (n)</th>
<th>All patients N= 80 % (n)</th>
<th>Patients EMF 1 + 2 N= 40 % (n)</th>
<th>Patients EMF 3+4 N= 40 % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cw*01</td>
<td>1.2 (1)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cw*02</td>
<td>18.8 (16)</td>
<td>16.3 (13)</td>
<td>12.5 (5)</td>
<td>20.0 (8)</td>
</tr>
<tr>
<td>Cw*0303 (9)</td>
<td>2.4 (2)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Cw*0302/04 (10)</td>
<td>14.1 (12)</td>
<td>16.3 (13)</td>
<td>27.5 (11)</td>
<td>5.0 (2)</td>
</tr>
<tr>
<td>Cw*04</td>
<td>32.9 (28)</td>
<td>33.8 (27)</td>
<td>35.0 (14)</td>
<td>32.5 (13)</td>
</tr>
<tr>
<td>Cw*05</td>
<td>0</td>
<td>2.5 (2)</td>
<td>5.0 (2)</td>
<td>0</td>
</tr>
<tr>
<td>Cw*06</td>
<td>28.2 (24)</td>
<td>27.5 (22)</td>
<td>22.5 (9)</td>
<td>32.5 (13)</td>
</tr>
<tr>
<td>Cw*07</td>
<td>60.0 (51)</td>
<td>56.3 (45)</td>
<td>50.0 (20)</td>
<td>62.5 (25)</td>
</tr>
<tr>
<td>Cw*08</td>
<td>10.6 (9)</td>
<td>13.8 (11)</td>
<td>12.5 (5)</td>
<td>15.0 (6)</td>
</tr>
<tr>
<td>Cw*12</td>
<td>4.7 (4)</td>
<td>2.5 (2)</td>
<td>2.5 (1)</td>
<td>2.5 (1)</td>
</tr>
<tr>
<td>Cw*14</td>
<td>1.2 (1)</td>
<td>6.3 (5)</td>
<td>5.0 (2)</td>
<td>7.5 (3)</td>
</tr>
<tr>
<td>Cw*15</td>
<td>1.2 (1)</td>
<td>2.5 (2)</td>
<td>2.5 (1)</td>
<td>2.5 (1)</td>
</tr>
<tr>
<td>Cw*16</td>
<td>10.6 (9)</td>
<td>5.0 (4)</td>
<td>7.5 (3)</td>
<td>2.5 (1)</td>
</tr>
<tr>
<td>Cw*17</td>
<td>23.5 (20)</td>
<td>12.5 (10)</td>
<td>15.0 (6)</td>
<td>10.0 (4)</td>
</tr>
<tr>
<td>Cw*18</td>
<td>14.1 (12)</td>
<td>11.3 (9)</td>
<td>7.5 (3)</td>
<td>15.0 (6)</td>
</tr>
</tbody>
</table>

p = not significant for all comparisons
Table 10.4. HLA-DR phenotype frequencies in patients and controls

<table>
<thead>
<tr>
<th>HLA-DR type</th>
<th>Controls N=85 % (n)</th>
<th>All patients N= 80 % (n)</th>
<th>Patients EMF 1 + 2 N= 40 % (n)</th>
<th>Patients EMF 3+4 N= 40 % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRB1*01</td>
<td>11.8 (10)</td>
<td>7.5 (6)</td>
<td>10.0 (4)</td>
<td>5.0 (2)</td>
</tr>
<tr>
<td>DRB1*0301</td>
<td>18.8 (16)</td>
<td>22.5 (18)</td>
<td>22.5 (9)</td>
<td>22.5 (9)</td>
</tr>
<tr>
<td>DRB1*0302</td>
<td>15.3 (13)</td>
<td>17.5 (14)</td>
<td>17.5 (7)</td>
<td>17.5 (7)</td>
</tr>
<tr>
<td>DRB1*04</td>
<td>2.4 (2)</td>
<td>5.0 (4)</td>
<td>2.5 (1)</td>
<td>7.5 (3)</td>
</tr>
<tr>
<td>DRB1*07</td>
<td>9.4 (8)</td>
<td>13.8 (11)</td>
<td>15.0 (6)</td>
<td>12.5 (5)</td>
</tr>
<tr>
<td>DRB1*08</td>
<td>7.1 (6)</td>
<td>12.5 (10)</td>
<td>7.5 (3)</td>
<td>17.5 (7)</td>
</tr>
<tr>
<td>DRB1*09</td>
<td>1.2 (1)</td>
<td>3.8 (3)</td>
<td>5.0 (2)</td>
<td>2.5 (1)</td>
</tr>
<tr>
<td>DRB1*10</td>
<td>5.9 (5)</td>
<td>2.5 (2)</td>
<td>2.5 (1)</td>
<td>2.5 (1)</td>
</tr>
<tr>
<td>DRB1*11</td>
<td>43.5 (37)</td>
<td>40.0 (32)</td>
<td>40.0 (16)</td>
<td>40.0 (16)</td>
</tr>
<tr>
<td>DRB1*12</td>
<td>10.6 (9)</td>
<td>12.5 (10)</td>
<td>10.0 (4)</td>
<td>15.0 (6)</td>
</tr>
<tr>
<td>DRB1*13</td>
<td>31.8 (27)</td>
<td>30.0 (24)</td>
<td>30.0 (12)</td>
<td>30.0 (12)</td>
</tr>
<tr>
<td>DRB1*14</td>
<td>0</td>
<td>3.8 (3)</td>
<td>2.5 (1)</td>
<td>5.0 (2)</td>
</tr>
<tr>
<td>DRB1*15</td>
<td>31.8 (27)</td>
<td>21.3 (17)</td>
<td>25.0 (10)</td>
<td>17.5 (7)</td>
</tr>
</tbody>
</table>

p = not significant for all comparisons
Chapter 10 Immunogenetic Heterogeneity

**Table 10.5.** HLA-DQ phenotype frequencies in patients and controls

<table>
<thead>
<tr>
<th>HLA-DR type</th>
<th>Controls N=85 % (n)</th>
<th>All patients N= 80 % (n)</th>
<th>Patients EMF 1 + 2 N= 40 % (n)</th>
<th>Patients EMF 3+4 N= 40 % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DQB1*02</td>
<td>29.5 (25)</td>
<td>37.5 (30)</td>
<td>37.5 (15)</td>
<td>37.5 (15)</td>
</tr>
<tr>
<td>DQB1*0301</td>
<td>35.5 (30)</td>
<td>40.0 (32)</td>
<td>37.5 (15)</td>
<td>42.5 (17)</td>
</tr>
<tr>
<td>DQB1*0302</td>
<td>2.4 (2)</td>
<td>5.0 (4)</td>
<td>2.5 (1)</td>
<td>7.5 (3)</td>
</tr>
<tr>
<td>DQB1*0303</td>
<td>2.4 (2)</td>
<td>1.3 (1)</td>
<td>0</td>
<td>2.5 (1)</td>
</tr>
<tr>
<td>DQB1*04</td>
<td>15.3 (13)</td>
<td>18.8 (15)</td>
<td>20.0 (8)</td>
<td>17.5 (7)</td>
</tr>
<tr>
<td>DQB1*05</td>
<td>32.9 (28)</td>
<td>28.8 (23)</td>
<td>30.0 (12)</td>
<td>27.5 (11)</td>
</tr>
<tr>
<td>DQB1*06</td>
<td>58.8 (20)</td>
<td>53.8 (43)</td>
<td>55.0 (22)</td>
<td>52.5 (21)</td>
</tr>
</tbody>
</table>

p = non significant for all comparisons

The comparison between HLA-A and HLA-B phenotype frequencies in patients with severe EMF and healthy controls are shown in **Tables 10.6a and 10.6b**, which also presents the p values for these comparisons. There were no statistically significant differences in frequencies between these two groups.

Regarding the HLA-B*58 type both patients and controls had the same frequencies of either B*5801/04 or B*5802/06. **Table 10.7** shows the distribution of these allele groups in patients with EMF grades III+IV and in controls. Out of the 15 patients with EMF 3+4 who were HLA-B*58, 9 were 5801/04, 6 were 5802/06 and 1 had both alleles (5801/04 and 5802/06). There were 7 patients with mild to moderate EMF who had the HLA-B*58 type; 5 were 5801/04, 3 were 5802/06 and 1 had both alleles (5801/04 and 5802/06).
Regarding the controls, out of the 15 who were HLA-B*58, 8 were 5801/04, 7 were 5802/06, with one having both alleles. Only one subject, who had grade III EMF, was positive for both HLA-A*33 and HLA-B*58.

Table 10.6a. HLA-A phenotype frequencies in patients with severe EMF and controls

<table>
<thead>
<tr>
<th>HLA-A type</th>
<th>Severe EMF N= 40 % (n)</th>
<th>Controls N=85 % (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A*01</td>
<td>12.5 (5)</td>
<td>5.9 (5)</td>
<td>0.177</td>
</tr>
<tr>
<td>A*02</td>
<td>30.0 (12)</td>
<td>34.1 (29)</td>
<td>0.669</td>
</tr>
<tr>
<td>A*03</td>
<td>12.5 (5)</td>
<td>4.7 (4)</td>
<td>0.101</td>
</tr>
<tr>
<td>A*11</td>
<td>0 (0)</td>
<td>1.2 (1)</td>
<td>0.767</td>
</tr>
<tr>
<td>A*23</td>
<td>12.5 (5)</td>
<td>23.5 (20)</td>
<td>0.162</td>
</tr>
<tr>
<td>A*24</td>
<td>5.0 (2)</td>
<td>3.5 (3)</td>
<td>0.596</td>
</tr>
<tr>
<td>A*29</td>
<td>27.5 (11)</td>
<td>23.5 (20)</td>
<td>0.610</td>
</tr>
<tr>
<td>A*30</td>
<td>27.5 (11)</td>
<td>42.3 (36)</td>
<td>0.111</td>
</tr>
<tr>
<td>A*32</td>
<td>0 (0)</td>
<td>2.3 (2)</td>
<td>0.544</td>
</tr>
<tr>
<td>A*33</td>
<td>17.5 (7)</td>
<td>5.9 (5)</td>
<td>0.037</td>
</tr>
<tr>
<td>A*34</td>
<td>7.5 (3)</td>
<td>1.2 (1)</td>
<td>0.058</td>
</tr>
<tr>
<td>A*36</td>
<td>2.5 (1)</td>
<td>3.5 (3)</td>
<td>0.896</td>
</tr>
<tr>
<td>A*43</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>A*66</td>
<td>5.0 (2)</td>
<td>4.7 (4)</td>
<td>0.824</td>
</tr>
<tr>
<td>A*68</td>
<td>12.5 (5)</td>
<td>20.0 (17)</td>
<td>0.667</td>
</tr>
<tr>
<td>A*74</td>
<td>20.0 (8)</td>
<td>9.4 (8)</td>
<td>0.087</td>
</tr>
</tbody>
</table>
Table 10.6b. HLA-B phenotype frequencies in patients with severe EMF and controls

<table>
<thead>
<tr>
<th>HLA-B type</th>
<th>Severe EMF</th>
<th>Controls</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N=40</td>
<td>N=85</td>
<td></td>
</tr>
<tr>
<td></td>
<td>% (n)</td>
<td>% (n)</td>
<td></td>
</tr>
<tr>
<td>B*07</td>
<td>5.0 (2)</td>
<td>9.4 (8)</td>
<td>0.542</td>
</tr>
<tr>
<td>B*08</td>
<td>15.0 (6)</td>
<td>11.8 (10)</td>
<td>0.570</td>
</tr>
<tr>
<td>B*13</td>
<td>2.5 (1)</td>
<td>5.9 (5)</td>
<td>0.508</td>
</tr>
<tr>
<td>B*1401 (64)</td>
<td>0 (0)</td>
<td>1.2 (1)</td>
<td>0.767</td>
</tr>
<tr>
<td>B*1402 (65)</td>
<td>10.0 (4)</td>
<td>9.4 (8)</td>
<td>0.837</td>
</tr>
<tr>
<td>B*1501 (62)</td>
<td>0 (0)</td>
<td>2.4 (2)</td>
<td>0.544</td>
</tr>
<tr>
<td>B*1503 (72)</td>
<td>17.5 (7)</td>
<td>20.0 (17)</td>
<td>0.777</td>
</tr>
<tr>
<td>B*1510 (71)</td>
<td>10.0 (4)</td>
<td>12.9 (11)</td>
<td>0.695</td>
</tr>
<tr>
<td>B*1516 (63)</td>
<td>5.0 (2)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B*1521 (75)</td>
<td>0 (0)</td>
<td>1.2 (1)</td>
<td>0.767</td>
</tr>
<tr>
<td>B*18</td>
<td>2.5 (1)</td>
<td>3.5 (3)</td>
<td>0.896</td>
</tr>
<tr>
<td>B*27</td>
<td>0 (0)</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>B*35</td>
<td>2.5 (1)</td>
<td>9.4 (8)</td>
<td>0.184</td>
</tr>
<tr>
<td>B*39</td>
<td>0 (0)</td>
<td>5.9 (5)</td>
<td>0.114</td>
</tr>
<tr>
<td>B*42</td>
<td>7.5 (3)</td>
<td>18.8 (16)</td>
<td>0.110</td>
</tr>
<tr>
<td>B*44</td>
<td>17.5 (7)</td>
<td>16.5 (14)</td>
<td>0.832</td>
</tr>
<tr>
<td>B*45</td>
<td>5.0 (2)</td>
<td>8.2 (7)</td>
<td>0.590</td>
</tr>
<tr>
<td>B*49</td>
<td>7.5 (3)</td>
<td>8.2 (7)</td>
<td>0.969</td>
</tr>
<tr>
<td>B*50</td>
<td>2.5 (1)</td>
<td>3.5 (3)</td>
<td>0.896</td>
</tr>
<tr>
<td>B*51</td>
<td>0 (0)</td>
<td>3.5 (3)</td>
<td>0.276</td>
</tr>
<tr>
<td>B*53</td>
<td>17.5 (7)</td>
<td>10.6 (9)</td>
<td>0.253</td>
</tr>
<tr>
<td>B*57</td>
<td>5.0 (2)</td>
<td>7.1 (6)</td>
<td>0.746</td>
</tr>
<tr>
<td>B*58</td>
<td>37.5 (15)</td>
<td>17.6 (15)</td>
<td>0.015</td>
</tr>
<tr>
<td>B*81</td>
<td>15.0 (6)</td>
<td>7.1 (6)</td>
<td>0.140</td>
</tr>
</tbody>
</table>
Table 10.7. Distribution of haplotype HLA-B*58 in EMF patients and controls

<table>
<thead>
<tr>
<th>HLA-B*58</th>
<th>5801</th>
<th>5802</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients N15</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Controls N15</td>
<td>9</td>
<td>7</td>
</tr>
</tbody>
</table>

Comparison between patients with different types of EMF

We compared the frequencies of all HLA types detected between the different clinical types of EMF according to the distribution of structural lesions in the heart. No difference in frequency was significantly different. We classified 78 patients according to the distribution of the lesions in the heart in three groups: BEMF (42), REMF (27) and LEMF (9). Table 10.8 shows the frequency of HLA-A*02, HLA-A*23, HLA-A*33 and HLA-B*58 in the different groups. HLA_B*58 is more frequent in the patients with bilateral and right EMF than in those with LEMF (11/42; 26.2% for BEMF; 9/27; 33.3% for REMF; and 1/9; 11.1% for LEMF), but this result is not statistically significant. REMF differed from BEMF only for HLA-A*02 (11/27 vs 8/42, respectively; p=0.047). Comparisons between the frequencies of haplotypes for patients with LEMF vs BEMF and LEMF vs REMF were not statistically significant.
Table 10.8. Distribution of haplotype HLA-B*58 and HLA-A*33 in EMF patients according to the type of disease

<table>
<thead>
<tr>
<th></th>
<th>BEMF</th>
<th>REMF</th>
<th>LEMF</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLA-A*33</td>
<td>3/42 (7.1)</td>
<td>4/27 (14.8)</td>
<td>0/9 (0)</td>
</tr>
<tr>
<td>HLA-B*58</td>
<td>11/42 (26.1)</td>
<td>9/27 (3.3)</td>
<td>1/9 (11.1)</td>
</tr>
</tbody>
</table>

Comparison between controls and patients with different types of EMF

There was no difference in frequency of any of the haplotypes when comparing patients with BEMF and LEMF with controls. When comparing patients with REMF to control subjects, we found statistically significant difference in the frequency of HLA-Cw*8 (7/27 vs 9/76; p=0.041).
10.4. Discussion

The frequency of HLA-B*58 was increased in patients with severe EMF. More than half the patients with advanced EMF were positive for this haplotype, for which 15 different alleles have been recognized (www.ebi.ac.uk/cgi-bin/imgt). Of these, two contributed to the increase in the frequency of B*58 found in our population, namely 5801/04 and 5802/06. Proportionately, the allele 5801/04 was slightly increased.

Despite no statistically significant difference between the frequency of HLA-A*33 haplotype in EMF patients and controls, we also found an association between this HLA type and the presence of severe and advanced EMF (p=0.037).

The association of the haplotypes HLA-A*33 and HLA-B*58 is frequent in the African-American population of the United States of America (www.bioinformatics.nmdp.org). However, in the Mozambican series only one patient who had severe EMF out of the 80 patients expressed both HLA-A*33 and HLA-B*58. Also, in the control subjects the association of HLA-A*33 and HLA-B*58 was not present in more individuals than would be expected by chance. Similar finding has been reported from a study of another southern African population (from Botswana) (Novitsky et al, 2001). This absence of simultaneous occurrence of these two HLA types may be due to African-American ascendance being mainly from West Africa.

The subanalysis of frequencies of the different haplotypes according to the type of EMF did not show any significant differences. The lower frequency of HLA-A*58 found in LEMF compared to that of BEMF and REMF, was not statistically significant probably
due to the small number of patients with isolated LEMF in our study. Whether this finding is of relevance in the view of the difference in natural history and prognosis of LEMF is an issue that needs further investigation involving larger groups of patients and controls.

There are no studies on HLA typing in Mozambican populations. Despite the relatively small number of patients and controls included in our study we identified a high concordance of HLA class I antigen specificities with high frequencies with those found in the native population from Botswana (Novitsky et al, 2001), in which HLA-A*30, -A*02, -A*23, -A*68, -B*58, -B*72, -B*42 and -B*48 were the HLA class I specificities observed at the highest frequency (Table 10.8). Regarding HLA class II antigen specificities a similar pattern was found with the highest frequencies being HLA-Cw*07, HLA-Cw*02, HLA-Cw*17 and HLA-Cw*06. DR and DQ specificities were not tested in that study. Our results therefore do not support those from Cao et al (2004) which showed remarkable allelic and haplotypic diversity in HLA class I loci between four sub-Saharan populations (Kenya, Uganda, Mali, Zambia), and stressed the need for characterization of HLA systems in the different population of all regions of sub-Saharan Africa.

Evidence suggesting a role of infections in EMF has come from reports of sporadic cases of EMF in foreigners after short visits to endemic areas (Brockington et al, 1967; Beck and Schrire, 1972), and is supported by the climatic and geographic restrictions of the disease (Buckman et al, 2008). Several infectious agents might be implicated namely Plasmodium (Eling et al, 1988), Schistosoma (Rashwan et al, 1995), Microfilaria...
(Jaiyesimi, 1980), and Helminths (Andy, 1983), but none explains the occurrence of EMF in all regions where it has been described. Although not firmly established, autoimmunity has also been postulated to be of importance in the pathogenesis of EMF. Molecular mimicry between heart structures and proteins secreted by infectious agents that are targets for the host immune response has been suggested (Shaper et al, 1968b), but there is also the possibility of aberrant humoral, cellular and immunoregulatory responses, similar to those involved in pathogenesis of Chagas disease (Dos Reis et al, 2005). In recent years, both susceptibility to infections and autoimmune diseases have been associated with increased frequencies of various specific HLA haplotypes in populations from southern Africa (Lombard et al, 2006). We had therefore hypothesized that in our population HLA molecules were linked with susceptibility to or protection against the development of EMF and could also determine its severity.

Our results are interesting in view of the reported association of HLA-B*58 haplotype with increased susceptibility to HIV infection in the African population of Botswana (Novitsky et al, 2001). Furthermore, a study of Rwandan population infected with HIV-1 subtype A showed association of HLA-B*5802 with increased viral loads and rapid disease progression (Lazaryan et al, 2006), confirming the unfavorable effect this allele on viral load previously reported for HIV-1 subtype C in South Africa (Kiepela et al, 2004).

The significance of the association of HLA-A*33 and HLA-B*58 positivity with the severity of EMF lesions at presentation, is difficult to interpret in view of the incomplete understanding of its natural history. While some patients from our clinical series have a
Chapter 10 Immunogenetic Heterogeneity

steady disease with little or no progression after initial presentation, other reveal progression of structural and functional abnormalities on follow-up. We therefore think that systematic evaluation of HLA types must be extended to patients in earlier stages of EMF found in community-based studies to better correlate findings from HLA typing with disease susceptibility, severity and natural history.
Conclusion

This study shows evidence of immunogenetic heterogeneity in patients with EMF. HLA-B*58 type is associated with severe EMF in the Mozambican population. Studies on HLA typing of the Mozambican population will allow better interpretation of the results obtained. Also, further research is needed to uncover the role of genetics in determining the type and mode of progression of EMF.
Chapter 11

MANAGEMENT OPTIONS
11.1. Background

Currently, there is no specific treatment aimed at arresting or reversing the structural defects present in the heart of patients with EMF. Medical therapy is aimed at treating acute disease, heart failure and its complications, while surgery partially corrects some of the structural and hemodynamic abnormalities.

The medical treatment has been used with little success, an is associated to frequent admissions to hospital. EMF patients present usually in advanced stages needing very large doses of drugs to treat heart failure. The control of arrhythmias and thromboembolic episodes is also poor. Short courses of oral corticosteroids are currently used in EMF patients with hypereosinophilia and/or other signs of activity, but this has not been validated by clinical trials or systematic studies of the effects of the drugs on progression of the disease.

The surgical treatment of EMF is currently based on four important facts (Metras, 1993): (1) severe disease is fatal if untreated; (2) the severe hemodynamic derangement caused by restriction of the diastolic filling and atrioventricular regurgitation can be corrected; (3) In most cases the myocardium remains healthy and unaffected and endocardectomy is feasible through a relatively well preserved cleavage plan; (4) Evidence of recurrence is controversial and low rates have been reported. The high morbidity and mortality associated with surgery (Valiathan et al, 1989; Moraes et al, 1999; Dubost, 1990), has led to a general preference for medical treatment for patients in NYHA classes I and II, and
due to the lack of human and material resources for open-heart surgery in most areas where EMF is endemic, there has been little improvement in surgical techniques. However, surgery has been shown to increase survival and quality of life (Metras et al., 1982; Cherian et al., 1982; Gonzalez-Lavin et al., 1983), when compared to medical therapy.

In Mozambique, open-heart surgery was performed for the first time in 2001 with the help of surgeons from European hospitals who have been working within a humanitarian programme mainly directed to pediatric surgery. Initially, patients with EMF would be excluded from surgery. However, with the starting of the research programme it was felt that, at least for some EMF patients with predominant left valvular disease, there was room for surgery and some surgeons started to operate those cases. At the beginning of my research project, with the institution of the National Registry, standardized diagnostic and management procedures including selection criteria for surgery were introduced.

In this chapter we present and discuss the different therapeutic strategies applied for the management of EMF in Mozambique. First, we describe the drugs and medical procedures used for controlling heart failure and its complications. A second section of the chapter is dedicated to the description of the results of surgical technique performed in the institution, outside the EMF research programme. Finally, we describe a new management strategy evolved and applied by us, including both drug therapy and surgical options.
Characterization of patients from the National Registry

Of the 175 patients from the clinical registry, which were all symptomatic, 20 (11.4%) patients had mild EMF while 21 (12.0%) presented advanced EMF, and were therefore not considered for surgery. EMF patients with grade II (61; 34.3%) and grade III (73; 41.7%) severity were all considered for surgery (Diagram 11.1).

![Diagram 11.1. Flowchart for selection of patients for surgery](image-url)
11.2. Descriptive study of the medical therapy and invasive procedures

11.2.1. Methods
We evaluated the medical files of all patients from the clinical registry and collected data on the drugs administered and invasive procedures performed, as well as the number of hospitalizations and deaths of patients under exclusive medical therapy.

11.2.2. Results
Of the 175 patients that are included in the registry, only 3 had no treatment. The remaining 172 received drugs for the management of heart failure, prevention and treatment of arrhythmias and thromboembolism, control of hypereosinophilia and superimposed infections, as well as for the correction of nutritional deficiencies, mainly vitamins. The drugs most commonly used were the diuretics and β-blockers as shown in Table 11.1.

Management of Heart Failure
The anti-Heart Failure therapy involved the use of diuretics in all patients, vasodilators (mainly ACE-inhibitors) and β-blockers (mainly propranolol) in those with maintained blood pressure.

The treatment of episodes of hypereosinophilia was done using immunosuppressive therapy with steroids. In total 43 patients had severe hypereosinophilia. In 21 of these
corticosteroids were not used due to concurrent bacterial infections. In the 12 patients in whom a 10-days course of oral steroids at 1mg/kg/d was used, hypereosinophilia was reduced in only 6, did not change in 3 and increased in the other 3.

The most frequent invasive procedures performed to alleviate heart failure were drainage of ascitic fluid (58 for 40 patients), pericardial fluid (21 for 18 patients) and pleural fluid (8 for 8 patients). Five patients in severe heart failure at diagnosis needed simultaneous pericardial and peritoneal drainage.

Table 11.1. Drugs most commonly used for management of EMF patients

<table>
<thead>
<tr>
<th>Drugs</th>
<th>N of Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furosemide</td>
<td>158 (90.3%)</td>
</tr>
<tr>
<td>Spirololactone/Amiloride</td>
<td>158 (90.3%)</td>
</tr>
<tr>
<td>Propranolol</td>
<td>104 (59.4%)</td>
</tr>
<tr>
<td>Captopril/Enalapril</td>
<td>98 (56.0%)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>95 (54.3%)</td>
</tr>
<tr>
<td>Hydrochlorothiazide</td>
<td>35 (30.0%)</td>
</tr>
<tr>
<td>Warfarin</td>
<td>33 (18.9%)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>22 (12.6%)</td>
</tr>
</tbody>
</table>
Management of Arrhythmia

The management of the 47 patients with atrial arrhythmia (flutter or fibrillation) was done using mainly digoxin, propranolol, verapamil and amiodarone. Verapamil was used in 5 patients with contraindication for the use of β-blocker. Amiodarone was used in only two patients. Electrical cardioversion was used in two occasions for treatment of recent onset atrial fibrillation, associated to severe clinical deterioration. One patient submitted to electrical cardioversion had been recently operated and had developed low cardiac output syndrome associated with episodes of sustained atrial tachycardia and intermittent atrial fibrillation, which were not controlled with drug therapy.

Patients with ventricular hyperexcitability detected on routine ECG or 24h-holter monitoring were managed with oral propranolol. Two of them had advanced disease with marked ventricular fibrosis and died some weeks after the diagnosis.

Management and prevention of thromboembolism

Despite the treatment with heparin followed by adequate oral anticoagulation with warfarin, out of the 7 patients admitted for pulmonary thromboembolism 4 died in less than one month. In two patients with left ventricular thrombus we suspected systemic embolism to the gut; both patients recovered on anticoagulation therapy without needing surgical intervention. Two patients presented for the first time with signs of recent cerebral embolism.

Eighty-seven patients (49.7%) had indication for anticoagulation. The main indications were intra-cardiac thrombi and/or atrial fibrillation, which were present in 71(40.6%) and
46 (26.3%) patients respectively (Table 11.2). Several patients had more than one indication for anticoagulation.

Table 11.2. Indications for anticoagulation in EMF patients

<table>
<thead>
<tr>
<th>Indication for anticoagulation</th>
<th>N of patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intracavitary thrombus</td>
<td>71 (40.6%)</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>46 (26.3%)</td>
</tr>
<tr>
<td>Pulmonary thromboembolism</td>
<td>15 (8.6%)</td>
</tr>
<tr>
<td>Systemic thromboembolism</td>
<td>12 (6.9%)</td>
</tr>
</tbody>
</table>

Spontaneous high INR was found in 32 out of the 87 patients. Those were patients with severe disease and long-standing heart failure, presenting chronic congestive hepatomegaly, and were left without any anticoagulant therapy. Within the remaining 55 with indication for chronic anticoagulation, only 33 were eligible for oral warfarin, while the other 22 were controlled with low doses of aspirin. The criteria used to consider the eligibility for permanent oral anticoagulation were the distance from a health care unit, understanding of the risks and complications of the medication, drug availability in the region of residence, possibility of testing the INR near the residence and financial constraints.

We only had one major hemorrhagic accident due to gastrointestinal bleeding.
Management of Malnutrition

Nutritional advice was given to patients with mild to moderate hypoproteinemia. Patients with kwashiorkor were referred to Pediatric Centers from the national health system for Nutritional Recovery (through intensive nutritional therapy with oral milk-oil-sugar mixture, vitamins and minerals).

We had 12 patients with extreme cachexia, who had also severe cardiac failure and were admitted to our clinic for intensive nutritional and anti-heart failure therapy. From those, 9 had structural lesions suitable for surgery but only 2 were operated. Three died while at hospital and 4 did not recover their nutritional status, remained in extreme cardiac cachexia and were refused surgery.

Treatment of Infections

The most frequently associated infection was tuberculosis, found in 15 patients (8.6%). The prevalence of parasitic infestations in EMF patients at time of diagnosis and/or episodes of eosinophilia was as follows: 13 (7.4%) for malaria by *Plasmodium falciparum*, 13 (7.4%) for helminths, 10 (5.7%) for Scabies, 4 (2.2%) for *Schistosoma mansoni*, 2 (1.1%) for filaria by *Wuchereria bancrofti* and 2 (1.1%) for *Schistosoma Haematobium*.

None of our patients had associated infective endocarditis.
Hospital Admissions

There were 71 patients from the clinical registry (40.6%) who were never admitted to hospital. Eighty-eight (50.3%) had 1 to 3 admissions to hospital, and 16 (9.1%) had more than 3 admissions during this 3-years period. There were in total 332 hospitalizations for the 104 patients who needed hospitalization. The main causes for admission to hospital after initial diagnosis and institution of therapy were the following: (1) interruption of medical therapy (105; 16.6%); (2) need for drainage of effusions (77; 23.2%); (3) complications related to drug therapy (55; 16.6%); (4) arrhythmia (45; 13.6%); (5) progression of the disease despite correct medication (25; 7.5%); (6) thromboembolism (25; 7.5%).

Clinical amelioration

The evolution of the functional class of the 124 patients with EMF on exclusive medical therapy is shown in Figure 11.1. We excluded from this analysis the 44 patients submitted to surgery and the 4 patients who were referred for evaluation of hypereosinophilia with no specific cardiovascular symptoms. Amelioration occurred in 81 (65.4%) patients. The NYHA remained unchanged in 37 (29.8%). Three (2.4%) patients worsened during the follow up going from NYHA III to IV despite the treatment. Data were not available for 3 (2.4%) patients who were lost for follow-up. The detailed results of treatment in EMF patients from the clinical registry on exclusive medical therapy are presented in Diagram 11.2.
Diagram 11.2. Evolution of NYHA functional class of the 124 EMF patients treated medically.

There was a reduction of patients in functional class III/IV from 85.5% at diagnosis, to 47.5% after 6 months of treatment. The distribution of patients by functional class is presented in Table 11.3.
**Figure 11.1 and Table 11.3** Distribution of NYHA functional class at diagnosis and after 6 months for the 124 patients treated medically.

<table>
<thead>
<tr>
<th>NYHA class</th>
<th>I</th>
<th>II</th>
<th>III</th>
<th>IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>At diagnosis</td>
<td>12 (9.7%)</td>
<td>6 (4.8%)</td>
<td>62 (33.9%)</td>
<td>64 (51.6%)</td>
</tr>
<tr>
<td>After 6 months</td>
<td>25 (20.2%)</td>
<td>37 (29.8%)</td>
<td>38 (30.6%)</td>
<td>21 (16.9%)</td>
</tr>
</tbody>
</table>
Survival of patients treated medically

The mean follow-up time was 60.7 (SE 3.5; CI 53.8-67.7). Nineteen (15.3%) patients on exclusive medical therapy died during the follow up, of whom 10 had active disease. The mean survival of patients under medical therapy was 17.9 months (SE 3.3; CI 11.5 – 24.3). Figure 11.2 shows the survival curve of these patients, which was over 75 at 2 years and around 68% after 5 years.

Figure 11.2: Survival curve of patients submitted to medical therapy
11.2.3. Discussion

The drugs most commonly used for the treatment of EMF patients in our series were diuretics, ACE-inhibitors, Digoxin and β-blockers, which were used in more than half the patients. Adequate intensive medical therapy improved the general status and partially corrected heart failure in the majority of patients. Nevertheless, some died due to refractory heart failure and acute pulmonary thromboembolism despite maximal heart failure treatment and adequate anticoagulation, confirming the malignant nature of this condition. Resistance to medical therapy can be partially explained by the high prevalence of ascitis and intestinal wall congestion, which prevents correct absorption of drugs through the gut. However, in a considerable proportion of patients there was interruption of medication due to financial constraints, side effects of the drugs and/or cultural believes which lead to search for help from traditional healers owing to the fact that the medical therapy is perceived as ineffective in patients with severe ascitis. Thus, we cannot exclude that recrudescence of heart failure and progression of disease might be related to inadequate therapy.

Complications of medical therapy were frequent and constitute an important cause of admission to hospital. The ideal dosages for diuretics were difficult to achieve. Despite diuretics reducing pulmonary congestion, systemic blood volume, right atrial volume and pericardial constraint, they also decrease the cardiac output and cause severe electrolyte abnormalities, mainly hyponatremia.
The availability of new drugs for the treatment of heart failure has probably contributed to the improvement in the mean survival of patients with EMF submitted to medical therapy in our series, when compared to that of patients from hospital series, in which the mean survival time after the onset of symptoms was 2 years (Somers et al., 1978). The possibility of preventing thromboembolic events through a more detailed diagnosis of intra-cardiac thrombi using echocardiography might also have played a role.

The choice of specific agents in each class was determined mainly by the availability of them in the local market and the costs to the patients. The great number of patients in digoxin and propranolol was determined by the high prevalence of atrial fibrillation or supraventricular ectopics beats.

Digoxin was used in all cases with established atrial fibrillation. Although it is known that this drug has no significant effect on cardiac function in patients in sinus rhythm, due to the long distances that some patients had to cover to get to a health unit, we were forced to use it prior to development of atrial fibrillation in patients with aneurysmal atria. The rational was to block the atrioventricular node and slow the ventricular rate at the time of conversion to atrial fibrillation, an event that is usually associated with high mortality due to severe hemodynamic compromise (Somers et al., 1972a).

β-blockers were used to reduce heart rate in patients with severe diastolic dysfunction. However, several patients had contraindication for its use due to previous history of asthma, which was frequent in this series. In these cases Verapamil was the alternative.
Due to its high costs Sotalol and Amiodarone were only used for prophylaxis of recurrence of arrhythmia after electrical conversion or in the immediate post-operative period in patients with moderately dilated atria.

Oral steroids were administered in only 12 cases, a low frequency when compared to the number of patients with hypereosinophilia. The use of oral corticosteroids in patients with EMF and hypereosinophilia is not supported by clinical trials or longitudinal studies on the effects of this therapy. Several reports show that they have no or little influence on the natural course of EMF (Somers et al., 1972b; Somers et al., 1978; Somers 1990). More recently it was suggested that eosinophilia could be a putative cause of EMF independent of parasitism (Rutakingirwa et al., 1999). Therefore, the benefit of using them with no clear known advantage was balanced with the known disadvantages and risks in patients with pulmonary infections and high risk of activating tuberculosis, which is highly prevalent in our population (Dgedge et al., 2001; Salomao, 1991).

Aspirin was used in 22 (12.6%) patients mainly for its anti-platelet properties in those patients who could not afford or could not adhere to anticoagulant therapy. However, in a patient with confirmed atrial inflammation on pathology specimen obtained during surgery, we applied the drug as an adjuvant therapy to control tachycardia-induced cardiomyopathy with success. Inflammation is known to act as an initiator of atrial fibrillation (Boos et al., 2006) and, therefore we thought that the use of anti-inflammatory drugs would be recommended in this case. There were no signs of activity on physical
examination or peripheral blood in this patient, who had a favourable sustained clinical result following addition of aspirin to the anti-arrhythmic therapy. Although atrial arrhythmias have been attributed to the atrial stretch associated with the progressive dilatation and fibrosis of the atria, the possible role of atrial pancarditis in producing arrhythmia in EMF needs to be studied further as it could influence its management.

Apart from their role in the treatment of heart failure drugs acting at the Renin-Angiotensin-Aldosterone system have the potential to modulate the process of tissue fibrosis \textit{in vivo} and \textit{in vitro} (Zannad \textit{et al}, 2000; Gallego \textit{et al}, 2001; Shi \textit{et al}, 2002). We therefore privileged the use of ACE-inhibitors and spironolactone in our patients in heart failure. However, in some cases we were limited by the high costs of these drugs in our setting. There is need for clinical trials showing the benefit of using these drugs in patients with EMF. Although not subjected to randomization patients with and without chronic administration of spironolactone and captopril/enalapril, might allow ascertaining the effect of these drugs in major end-points during follow up.

Chronic anticoagulation was used to prevent systemic or pulmonary thromboembolism in patients with very dilated atria with free tricuspid regurgitation and thrombi detected in atria or ventricles on echocardiography. Due to financial restrictions, thrombolysis and low-molecular weight heparins were not available for most patients with signs of recent thromboembolism, who were treated with intravenous or subcutaneous unfractionated heparin.
Effusions were usually resistant to diuretic treatment and needed periodical drainage sometimes as an emergency procedure. Considerable clinical improvement was obtained after drainage (pericardiocentesis, paracentesis or thoracocentesis). Since the results of surgical procedures for the management of tense ascitis are not satisfactory we did not recommend them to our patients. The results of the Spitz-Holter shunt, draining the ascitis into the femoral vein, were disastrous (Jayesimi, 1982). Although it is logical to think that recurrent pericardial effusions can benefit from pericardio-pleural window or pericardioperitoneal shunt this remains to be shown in prospective studies. Our management of ascitis relied on frequent evacuation of fluid by paracenthesis. Most patients with REMF grade IV had to have repeated paracenthesis (3-6/year). Patients with very large ascitis tolerated well the evacuation of reasonable amounts of ascitic fluid. We usually evacuated 2-2.5L per procedure in children below 30 kg and up to 3-4 liters in those above 30kg body weight. When associated with reinforced diet, this approach did not aggravate albumin depletion, and was associated to noticeable improvement in response to oral medication.

Electrical cardioversion to sinus rhythm was used only in 2 patients with atrial fibrillation and rapid ventricular response not responding to drug therapy, since the majority of patients needing this procedure had intracavitary thrombi or aneurysmal atria. We therefore think that the procedure should be reserved to patients with acute hemodynamic deterioration by recent onset atrial arrhythmia. In all other patients it has a low rate of success related to severe atrial dilatation and fibrosis of the atrial wall. Also there is a high risk of dislocating clots that are not seen on echocardiography.
11.3. Initial experience of surgery for EMF in Mozambique

11.3.1. Methods

Surgery outside the research programme

The medical files of patients submitted to surgery outside the EMF research programme were evaluated. The researcher collected demographic data, clinical features, echocardiographic data, surgical techniques, and complications of surgery. The objective was to describe the results of the use of surgery in these patients.

11.3.2. Results

Characterization of patients

Nineteen patients have been submitted to surgery outside the research programme. Eleven patients were males (58.0%) and the mean age was 11 years (SD 4.8; range 4-25). The mean body mass index is 15.5 (SD 2.7; range 10-21). All patients were in NYHA functional class III/IV. The mean CTI was 72.2 (SD 10.9) ranging from 51 to 92. Twelve (12; 63.0%) had signs of activity, of which eight had severe hypereosinophilia.

The majority (16/19; 84.0%) of the patients had grade II EMF and the remaining 3 had grade III EMF. Eleven patients had bilateral EMF with predominance of left lesions, 7 had “pure” LEMF 1 had REMF. This patient with isolated REMF had a thrombus in the right atrium. RV trabecular obliteration was present in 6 patients. Only one patient had atrial fibrillation. The characteristics of patients operated are presented in Table 11.4a.
### Table 11.4a: Characteristics of the patients operated for EMF outside the research programme

<table>
<thead>
<tr>
<th>Code</th>
<th>Age-Sex</th>
<th>Weight-Height</th>
<th>BMI</th>
<th>HF Class</th>
<th>Rhythm</th>
<th>EMF Class</th>
<th>F-up</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>0010</td>
<td>5M</td>
<td>99/16</td>
<td>16.0</td>
<td>III</td>
<td>SIN</td>
<td>II B</td>
<td>42</td>
<td>No</td>
</tr>
<tr>
<td>0012</td>
<td>12M</td>
<td>135/23</td>
<td>12.8</td>
<td>IV</td>
<td>SIN</td>
<td>II B</td>
<td>14</td>
<td>Yes</td>
</tr>
<tr>
<td>0029</td>
<td>15M</td>
<td>135/32</td>
<td>17.3</td>
<td>III</td>
<td>SIN</td>
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</tr>
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<td>0059</td>
<td>12F</td>
<td>134/24</td>
<td>13.4</td>
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<td>II L</td>
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<td>11F</td>
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<td>18.2</td>
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<td>20.7</td>
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<td>II B</td>
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<td>No</td>
</tr>
<tr>
<td>0084</td>
<td>4M</td>
<td>88/16</td>
<td>13.6</td>
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<td>SIN</td>
<td>III R</td>
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<td>7F</td>
<td>109/16</td>
<td>12.9</td>
<td>IV</td>
<td>SIN</td>
<td>II L</td>
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<td>Yes</td>
</tr>
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<td>8M</td>
<td>125/20</td>
<td>12.9</td>
<td>III</td>
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<td>II L</td>
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<td>II B</td>
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<td>132/26</td>
<td>14.9</td>
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<td>SIN</td>
<td>II L</td>
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<td>152/38</td>
<td>16.4</td>
<td>III</td>
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<td>II B</td>
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<td>4F</td>
<td>100/15</td>
<td>10.0</td>
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<td>II L</td>
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<td>No</td>
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<tr>
<td>0159</td>
<td>12F</td>
<td>134/27</td>
<td>15.0</td>
<td>IV</td>
<td>SIN</td>
<td>II B</td>
<td>19</td>
<td>No</td>
</tr>
</tbody>
</table>

Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis
Mitral regurgitation was the main indication for surgery in severe LEMF, and was present in 18 patients. The other patient had advanced REMF with thrombus in the RV and severe tricuspid regurgitation.

**Surgical Technique**

Mitral repair using the Carpentier ring was the most common technique used to treat LEMF (13/18 patients). In the remaining patients a biodegradable (Kalangos®) ring was used in one, annuloplasty and implantation of Gore-Tex bands was done in other, and mitral valve replacement was performed in 3 patients. Reduction of the left atrium was added to the mitral repair in 2 patients. Endocardial resection was not used for any patient with LEMF, but tricuspid valve annuloplasty or repair was needed in 5 patients.

The patient with REMF was treated with drainage of pericardial effusion, thrombectomy, right endocardial resection, tricuspid plasty with use of Gore-Tex bands for annuloplasty, and bi-directional Glenn.

The surgical techniques used for the treatment of these 19 patients are summarized in **Table 11.4b**

**Morbidity**

The main complications of surgery were large pericardial effusion (5 patients), pericardial tamponade (1 patient), and accidents related to anticoagulation (2 patients). One patient had been taking warfarin for his mitral prosthesis and had severe urinary bleeding associated with bladder sequel of schistosomiasis. The other patient was taking...
warfarin for her atrial fibrillation and developed hemopericardium during the first postoperative days and went into pericardial tamponade.

After 12 months follow-up mitral stenosis was present in three patients. The postoperative complications are shown in Table 11.4b.

Mortality

The hospital mortality was 2/19 in this series. In one children due to incapacity to wean from the extra-corporal circulation machine and in the other due to low cardiac output syndrome. This child had pericardial effusion, severe ascitis, moderate liver dysfunction, complicated with syndrome of low cardiac output that lead to multi-organ failure, and died on day 17 after surgery.

The mortality during the first post-operative year was 2/17. One patient had had mitral and tricuspid repair and developed refractory heart failure during the first post-operative year. The other, considered to have concomitant rheumatic heart disease, had had mitral valve repair and aortic valve repair, followed by several episodes of rheumatic acute carditis, did not comply with medication and developed atrial fibrillation complicated with a stroke that lead to death.

One late death was unrelated to surgery (anaphylaxis to penicillin) in a patient in whom there was association with rheumatic heart disease. The overall mortality was 5/19 (26.0%).
**Table 11.4b**: Surgical techniques and complications in EMF patients operated outside the research programme.

<table>
<thead>
<tr>
<th>Code</th>
<th>Age-Sex</th>
<th>EMF Class</th>
<th>Indication</th>
<th>Surgical Technique</th>
<th>Complications</th>
<th>Death</th>
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<tr>
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<td>Mrepair TR</td>
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<td></td>
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<td>M12</td>
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<td>MR</td>
<td>MP TR</td>
<td>OAC</td>
<td></td>
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<td>0059</td>
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<td>MR</td>
<td>Mrepair TR Lar</td>
<td>Psychotic</td>
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<tr>
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<td>MR</td>
<td>Mrepair Bio ring*</td>
<td>MS MR</td>
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<tr>
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<td>MR</td>
<td>Mrepair Ao Plasty</td>
<td>MS AF Stroke</td>
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<td>TR THR</td>
<td>TB RVER TR Glenn</td>
<td>LCO MOF</td>
<td>D 17</td>
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<td></td>
</tr>
<tr>
<td>0123</td>
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<td>II L</td>
<td>MR</td>
<td>Mrepair**</td>
<td></td>
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<td>PT OAC</td>
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<td></td>
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<tr>
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<td>Mrepair</td>
<td>Mild MR</td>
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</tbody>
</table>

MR mitral regurgitation; TR tricuspid regurgitation; Mrepair = repair with Carpentier ring; * biodegradable ring; ** annuloplasty without ring; Ao aortic valve; TB thrombectomy; RVER right ventricular endocardial resection; MP mitral mechanic prosthesis; BC bilateral commissurotomy; OAC anticoagulant complication; AF atrial fibrillation; MS mitral stenosis; LA left atrial; LCO low cardiac output; MOF multi-organ failure; IOD intraoperative death; PE large pericardial effusion; PT pericardial tamponade; M month; D day
Follow-up

The mean follow-up of these patients was 22.8 months (SE 2.8; SD 15; range 0 to 56 months). Three patients were lost for follow-up. Three patients who survived needed re-operation: two patients developed mitral stenosis of the repaired valve and the remaining patient has partial dehiscence of the Carpentier ring with severe mitral regurgitation.

The mean survival time was 44 months (CI 33-55), limited to 59 months. The survival curve of these patients is shown in Figure 11.3.

Figure 11.3: Survival curve of patients submitted to surgery for EMF outside the research programme. The survival time is limited to 59 months.
11.3.3. Discussion

The initial experience of surgery for EMF at the Heart Institute consisted in operating mostly patients with LEMF with relatively little mural lesions and predominance of valve affection. The surgical technique was not standardized and did not include endocardial resection, even in BEMF with obliteration of the trabecular portion of the right ventricle. Correction of mitral regurgitation was done through mitral repair (15/19), which in most procedures involved the implantation of a Carpentier ring.

The clinical and echocardiographic evaluation in the immediate post-operative period showed no stenosis. However, in the mid-term results revealed mitral stenosis in three patients. We think that the use of a rigid ring in patients with a disease that primarily involves fibrosis and little dilatation of the mitral annulus has contributed to this complication. Moreover, since the patients operated were mainly children and adolescents, their growth after surgery might also have played a role in development of stenosis. Whether the use of this ring must be considered a disadvantage in surgical treatment of EMF is something that has to be assessed in long-term follow up studies.

The intraoperative death that occurred in this series was associated with incomplete diagnosis. The patient had a diagnosis of mitral regurgitation due to rheumatic heart disease when indicated for surgery. Intraoperatively, extensive endocardial fibrosis of the left ventricle was found and mitral regurgitation was in fact associated with complete absence of the posterior leaflet and severe thickening and retraction of the anterior leaflet. Mitral replacement was performed but this patient could not be weaned from
extracorporeal circulation. The death that occurred in the immediate post-operative was related to the presence of complications of long standing heart failure, severe ascitis and liver dysfunction at time of surgery in a patient with advanced REMF.

Two deaths occurred during the first post-operative year associated to refractory heart failure that seemed to be related to recurrence of atrioventricular valve regurgitation soon after surgery. The control of atrioventricular valve function immediately after surgery showed mild mitral regurgitation in both patients that increased rapidly during the follow-up. This rapid increase in valve dysfunction could be related to the persistence of marked compliance abnormality, since endocardial resection had not been performed.

Five out of the 6 patients with right ventricular trabecular obliteration developed large pericardial effusion lasting 3 to 6 months during immediately after surgery, despite diuretic and anti-inflammatory treatment. Two patients needed pericardial drainage in emergency due to pericardial tamponade. The persistence of right ventricular obliteration surely contributed to this complication and the signs of right heart failure observed in these patients.

Mitral valve replacement was performed in 3 patients (6, 7 and 15 years), of which only one survived on long term follow-up. As said before one patient had an intraoperative death, while the other died from a non-prosthesis related event.

The overall mortality was high (26%). The mean survival time at 44 months (limited to 59 months) is acceptable. There were 3 patients lost for follow up. Two out of the 3 patients awaiting re-intervention will need valve replacement for correction of postsurgical mitral stenosis.
11.4. New Tailored Approach for the Management of EMF

11.4.1. Methods

11.4.1.1. New surgical strategy

Our surgical approach started with thorough clinical and echocardiographic evaluation of the structural and functional abnormalities in patients proposed to surgery. Regarding the surgical strategy the decision was made by the researcher and the surgeon in all cases. We also used exclusion criteria and submitted all patients to intensive medical therapy prior to surgery, to correct signs of activity and optimize their clinical status. The researcher was responsible for the selection and preparation of the patients proposed to surgery. An international team lead by the main supervisor was constituted and included surgeons, anesthetists, perfusionist and intensive care nurses who contributed with their knowledge to the progression and optimization of surgical and post-surgical care.

i. Selection of patients for surgery

The pre-operative evaluation of structural changes was based on transthoracic echocardiography. Using the new diagnostic and severity criteria described in Chapter 3 we identified patients with structural lesions that would benefit from surgical correction through detailed description of the characteristics, localization and severity of the mural and valvular lesions. The most important contraindications for surgery were:
(1) large long-standing ascitis refractory to medical therapy; (2) extreme cachexia not responding to therapy; (3) fixed pulmonary hypertension; (4) extensive endocardial fibrosis or calcification with impaired myocardial function; (5) considerable shortening of leaflets in which valve replacement was anticipated.

**ii. Pre-operative Care**

The biological profile was evaluated in order to identify patients with active disease or contraindications for surgery. Prior to surgery patients were admitted to hospital for intensive medical therapy, for a period ranging from one week to one month. Multi-drug therapy was used for all including high doses of diuretics, angiotensin converting enzyme inhibitors, ferrous sulphate, vitamins and high content protein diet.

Cardiac catheterization was considered for patients with indication for surgery for which echocardiography did not fully characterized the structural and hemodynamic abnormalities, as well as pulmonary pressures/resistances and response to pulmonary vasodilators, due to heart distortion or the presence of free tricuspid regurgitation.

**iii. Intraoperative evaluation**

One surgeon (Professor MHY, the main supervisor) operated all patients. The researcher was present in the operating room to evaluate the macroscopic lesions, perform trans-esophageal echocardiography, obtain pictures of the relevant stages of surgery and handle the specimens obtained for pathology studies. The intra-cardiac echocardiographic control aimed at controlling valve repair.
iv. Postoperative follow-up

During the first two days the patients had temporary pacing, pericardial and pleural drains. Intravenous inotropes and anticoagulation were also used for the first 2 days. Clinical examination, electrocardiogram and echocardiography were performed daily during the first week, while the patients were in the hospital. After discharge weekly appointments were done for the following six weeks. These medical appointments were used to assess the appearance of pericardial effusion, atrial arrhythmia and to control the quality of anticoagulation. At the end of this period chest x-ray, hemogram and 24-h rhythm holter monitoring were performed.

The medical therapy used after surgery included diuretics, vasodilators (mainly ACE-inhibitors), anti-arrhythmic drugs, anticoagulants, aspirin, antibiotics, ferrous sulphate, and vitamins.

11.4.1.2. Surgical Techniques

i. Surgery of Right EMF

A wide longitudinal right atriotomy was performed to expose the tricuspid valve. This was usually markedly dilated affording wide access to the right ventricular cavity (Figure 11.4). The distribution and extent of pathological involvement of the tricuspid valve leaflets, chordae, right ventricular inflow tract and cavity were then carefully defined and dealt with accordingly.
Figure 11.4. The first step of the surgical intervention in BEMF and REMF was the exploration of right ventricular lesions done through a wide right atriotomy. This picture shows endocardial fibrosis, which involves the papillary muscles and thickening of chordae are visible.
Chapter 11 Management

a) Mobilization of the Trabecular Part and Tricuspid Repair

In patients with obliteration of the trabecular portion of the right ventricle endocardial resection was started near the tricuspid annulus by retracting the leaflets of the valve. Where the latter was fused in some areas, it was mobilized when feasible. Following the development of a cleavage plane by sharp dissection, a combination of sharp and blunt dissection was used to excise the thick fibrous endocardial lining (Figure 11.5). This process was continued into the ventricular cavity ensuring preservation and mobilization of the tricuspid valve chordae and papillary muscles, as well as avoidance of the conduction tissue. The part of the membrane covering the entry into the trabecular part was removed exposing the fused muscular tissue underneath it (Figure 11.6). This was followed by recreating a cavity inside the trabecular part by mainly separating the fused trabeculae but if necessary excising some muscular tissue taking care not to perforate the ventricular wall. In patients with complete fusion of the leaflets and chordae to the mural fibrosis, freeing of the tricuspid valve apparatus was performed followed by reconstruction of the valve using two bands of Gortex tubes.

b) Other procedures

Right atrial reduction was performed in patients with aneurismal right atrium. It consisted of excision of atrial appendage and variable amount of atrial tissue. Glenn procedure was performed in patients who had small right ventricular cavity after endocardectomy, and as an initial step towards intra-cardiac correction that would be done in a second surgery.
ii. Surgery of Left EMF

A wide longitudinal left atriotomy was performed to expose the mitral valve. The mitral annulus was usually moderately dilated affording adequate access to the inflow part of the left ventricle (Figure 11.7). In most cases assessment of the distribution and extent of pathological involvement of the mitral valve leaflets, chordae and papillary muscles could be carefully defined. However, in one patient with extensive involvement of the subvalvar apparatus and the apex, complete evaluation of the apical lesions was difficult, and left ventriculotomy was performed.

a) Endocardial resection and Mitral Repair

Conservative endocardectomy was started below the level of the mitral annulus (Figure 11.8), usually freeing the posterior leaflet and chordae and mobilizing the papillary muscles. Caution was taken to avoid damage of the subvalvar apparatus thus leaving some fibrotic tissue surrounding the basis of the papillary muscle. In some patients extension of chordae was needed and we performed it using Goretex material. The dilated mitral annulus was reduced through annuloplasty using Goretex bands (Figure 11.9).

b) Left Atrial Reduction

The left atrial appendage was removed in all patients. In those with aneurismal left atrium this was associated to excision of large amount of left atrial tissue (Figure 11.10).
Figure 11.5. Excision of the fibrotic tissue of the right ventricle

Figure 11.6. Release of myocardium underneath the fibrous plaque
Figure 11.7. Exploration of left ventricular lesions done through left atriotmy
**Figure 11.8** Excision of left ventricular fibrotic endocardium (F) through the mitral valve

**Figure 11.9.** Mitral valve repair included annuloplasty using Goretex bands (arrow)
Figure 11.10. Atrial appendage with several thrombi attached to the wall (top) and large portion of the atrium excised during atrial reduction (bottom)
11.4.2. Results

Using the new approach for diagnosis and management of EMF we excluded 80 patients from surgery (46%) because they presented advanced grade lesions or had concomitant medical conditions that were considered contra-indications to surgery. The most important contraindications were long-standing heart failure, refractory ascitis and fixed pulmonary hypertension (Table 11.5). Nineteen patients had already been operated. As mentioned above 20 patients had minimal lesions that did not require surgery.

At the end, 56 patients with structural lesions that could be corrected by surgery were considered for surgical treatment under this new approach. Ten patients died during preparation for surgery; the causes of death are presented in Table 11.6. Two patients refused surgery. One patient was pregnant at the time of surgery and died immediately after delivery. Nine patients are waiting for the operation and 10 were lost for follow-up during preparation for surgery. We therefore operated 24 patients using this approach.

Table 11.5. Main contraindications for surgery in EMF patients

<table>
<thead>
<tr>
<th>Contraindication for surgery</th>
<th>Number of patients</th>
</tr>
</thead>
<tbody>
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<td>Refractory ascitis</td>
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</tr>
<tr>
<td>Fixed PHT</td>
<td>5</td>
</tr>
<tr>
<td>Extreme cachexia</td>
<td>4</td>
</tr>
<tr>
<td>Chronic thromboembolism</td>
<td>2</td>
</tr>
<tr>
<td>Sequelae of Pulmonary tuberculosis</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 11.6. Causes of death in patients with EMF while in preparation for surgery

<table>
<thead>
<tr>
<th>Cause of death</th>
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</tr>
</thead>
<tbody>
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<td>Refractory heart failure</td>
<td>4</td>
</tr>
<tr>
<td>Rapidly progressive disease</td>
<td>2</td>
</tr>
<tr>
<td>Pulmonary embolism</td>
<td>1</td>
</tr>
<tr>
<td>Unknown*</td>
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</table>

* Death at home reported by the family

11.4.2.1. Characterization of patients selected for surgery

The mean age (±SD) of the patients was 12(±5) years, ranging from 6 to 22 years. There were 14 females (58.3%). The majority of patients (18; 75%) were in NYHA functional class IV. Twelve patients (50%) had BEMF, 8 (33.3%) had LEMF and 4 (16.7%) had REMF. Most patients had grade III lesions (17; 70.8%). Three patients were in atrial fibrillation. The mean cardiothoracic index was 72.5%. The mean body surface area was 1.02 (± 0.26). Table 11.7 presents the main characteristics of the 24 patients operated.

The mitral valve was severely affected in 21 cases of which one had associated stenosis. In one case there was mild MR associated with predominant REMF. The main mechanism of regurgitation was restriction of the movement or absence of the posterior mitral leaflet. Tricuspid regurgitation was present in all patients. The main mechanism of lesion was restriction of the movement of the leaflets due to involvement of the papillary muscles in the fibrotic plaque and dilatation of the annulus (16 patients), while the
remaining 8 patients had functional tricuspid regurgitation associated with annulus dilatation due to severe pulmonary hypertension caused by left sided disease.

11.4.2.2. Pre-operative Care

Four patients had β-blockers and digoxin. Five patients were under anticoagulant therapy: three had atrial fibrillation and two had severely dilated atria. Oral corticosteroids (7 to 10 days) were given to two patients with hypereosinophilia. Pericardial drainage was performed to one patient who had active EMF. Paracentesis was performed for 2 patients with REMF who also had atrial arrhythmia with rapid ventricular response and pericardial effusion.

Cardiac catheterization was performed for 2 patients. One had LEMF with aneurismal left atrium and severe heart distortion that made it possible to estimate the pulmonary pressures and evaluate the size of the right ventricle on transthoracic echocardiography. The second patient had REMF with large ascitis and free tricuspid regurgitation. The patient was proposed for a palliative procedure (Glenn procedure) and needed evaluation of the pulmonary pressures.

11.4.2.3. Intraoperative Data

We performed 26 surgeries in the 24 patients with EMF. One patient was operated in a two-staged strategy: first a Glenn procedure was done followed by right ventricular endocardial resection one year later. Another patient was re-operated after failure of the initial mitral repair, due to dehiscence of Goretex band.
Table 11.7. Characteristics of 24 patients submitted to surgery using the new tailored therapeutic approach

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<th>Rhythm</th>
<th>EMF Class</th>
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<td>14.8</td>
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<td>SIN</td>
<td>III B</td>
<td>12</td>
<td>No</td>
</tr>
<tr>
<td>0179</td>
<td>9F</td>
<td>114/16.4</td>
<td>12.6</td>
<td>IV</td>
<td>SIN</td>
<td>III B</td>
<td>12</td>
<td>No</td>
</tr>
</tbody>
</table>
The mean cardiopulmonary bypass time was 108 minutes (range 63 to 148). The mean aortic clamping time was 71 minutes (range 32-112). Cold blood cardioplegia was used for all patients. Intravenous inotropes were used in the immediate post-operative period, between 6 and 48 hours. Weaning from the ventilator was possible in all patients after 3 to 12 hours. The mean time at intensive care unit was 39 hours varying from 24 to 72, and the mean hospitalization time was 8 (± 2) days, ranging from 3 to 16 days.

We performed a two-staged surgery in 1 patient with REMF. First a Glenn procedure was performed to this 6 years old boy in NYHA IV, with large hepatomegaly and ascitis. His clinical condition improved dramatically and therefore open-heart surgery could be performed one year later in better conditions.

11.4.2.4. Valve function

Prior to surgery 21 patients had MR grade 4/4, one had grade ¼ mitral regurgitation and two did not have any mitral dysfunction. All 22 patients with mitral regurgitation improved after surgery. However, mild incompetence appeared in 2 patients treated for REMF with no intervention to the left side of the heart during surgery. On 6-months follow up 9 patients did not have mitral regurgitation, 10 had mild, two had moderate and one had important (¾) mitral regurgitation. This last patient had been treated with biventricular endocardial resection, as well as mitral and tricuspid valve repair. The echocardiography now shows non-cooptation with a jet going to the inter-atrial septum and residual endocardial fibrosis in the apex.
Modified de Vega annuloplasty with reinforcement of the annuloplasty with Gore-Tex bands was used in all cases of tricuspid valve repair. Plasty of the subvalvar apparatus was performed in the 10 patients with REMF or BEMF, and consisted of mobilization of the papillary muscles and the use of Gore-Tex chordae. On 6-months follow-up there were 3/22 patients with moderate and 7/22 with mild tricuspid regurgitation.

The follow up at 24 months was completed for 13 out of 14 patients. Eleven patients had no or mild mitral regurgitation. Equally 11 had no or mild tricuspid regurgitation. Residual moderate to severe mitral regurgitation was present in 2 patients. Moderate tricuspid regurgitation was also present in 2 patients treated for REMF, who had marked clinical improvement having only mild hepatomegaly, with the border of the liver palpable about 2cm below the costal border.

Details of valve function before and after surgery are shown in appendix 7.

11.4.2.5. Ventricular size, shape and function,

The mean left ventricular end-diastole dimensions indexed for body surface area before surgery was 49.9 (±11.2) mm, ranging from 27.2 to 67.0, and changed to 45.8 (± 6.2) mm, ranging from 31.3 to 55.8 after surgery. Regarding the end-systole dimensions they changed from a mean 29.2mm (± 6.0; range 16.9 – 43.1) to a mean of 30.4mm (± 7.1; range 22.1 – 50.0). When comparing the three types of EMF the LV dimensions were higher for the patients with BEMF with mean values indexed for body surface area were 46.9mm (± 11.0) for the lateral dimension and 56.0mm (± 12.0) for the superior-inferior
dimension. The lateral dimension was smaller for REMF (29.6 ± 2.8mm) and the superior-inferior dimension was smaller for LEMF (46.0 ± 9.8mm).

The RV function was improved in 16 patients, unchanged in 3 and worsened in one. With respect to 3 patients there was no information about the right ventricular function after surgery (1 patient died and 2 did not appear for follow up). Sixteen patients had excellent right ventricular function and only one had poor function after surgery.

The mean sphericity indexes were reduced after surgery, changing from 0.81 (± 0.19) and 1.10 (± 0.37) to 0.75 (± 0.14) and 0.87 (± 0.87), respectively for the left and right ventricles. The sphericity indexes after surgery were calculated for 20 patients, since we had two deaths in the immediate post-operative period and the follow up was not yet done for 2 patients. When comparing the different types of EMF, the sphericity indexes were higher for both the right ventricle and the right atrium before surgery, which mean values were respectively 1.34 and 1.70.

Appendix 8 presents the summary of the values of left and right ventricular dimensions indexed for body surface area and sphericity indexes for the three types of EMF.

The LV mean shortening fraction, considering the 20 patients with bilateral or predominant left EMF was 44.0% (± 7.4; range 30-59) before surgery. The ventricular function after surgery evaluated by a visual semi-quantitative scale was good in 23 out of the 24 patients. The left ventricular end-diastolic and end-systolic dimensions measured on M-mode, the systolic function and the values of E/A ratio of the transmitral flow after surgery are shown in Appendix 9.
The velocity of the E wave of the transmitral flow was above 2m/s in 7 out of the 20 patients with LEMF and BEMF. The mean value of the E/A ratio was 2.7 ± 1.2, and changed to 2.0 ± 0.8 after surgery. The E/A ratio before surgery was evaluated in 22 patients, since 2 were in atrial fibrillation. It was calculated in only 19 patients after surgery (2 patients died and there were no data for 2 patients on follow up). Right ventricular function was improved in all but 2 patients who had dilatation of the ventricle with thin wall (Appendix 10).

11.4.2.6. Atria size and shape

The mean lateral dimension of the left atrium was 55.0 mm (± 25.9; range 20.3 – 144.8) pre-operatively and was reduced to 43.2 mm (± 11.0; range 25.2 – 63.4) after surgery, while the superoinferior dimension changed from 65.2 mm before (± 15.3; range 38.9 – 94.2) to 57.0 mm (± 9.8; range 39.1 – 80.5) after surgery. The sphericity index for the left atrium was 0.85 (± 0.35) pre-operatively and decreased to 0.78 (± 0.14) after surgery. Regarding the right atrium the mean values of the lateral dimension, longitudinal dimension and sphericity index before surgery were 56.6 mm (± 22.9; range 20.6 – 107.0), 58.3 mm (± 16.7; range 33.6 – 97.2) and 0.95 (± 0.28; range 0.35 – 1.86), respectively. After surgery these mean values were 47.6 mm (± 17.5; range 24.3 – 88.6) for the lateral dimension, 51.6 mm (± 16.8; 26.4 – 88.6) for the longitudinal dimension and 0.92 (± 0.14; range 0.64 – 1.22) for the sphericity index.

The summary of the values of left and right atrial dimensions indexed for body surface area and sphericity indexes for the three types of EMF is presented in Appendix 11.
11.4.2.7. Functional improvement (NYHA class)

Prior to surgery 20 (83.3%) patients were in NYHA III/IV. The NYHA functional class reduced after surgery in all patients, with 20 of the 22 survivors being asymptomatic one year after surgery, under low doses of diuretics and ACE-inhibitors. The changes in NYHA of patients before and after surgery are shown in Figure 11.11.

![Figure 11.11] Distribution of patients by functional class pre- and post-operatively

<table>
<thead>
<tr>
<th></th>
<th>NYHA I</th>
<th>NYHA II</th>
<th>NYHA III</th>
<th>NYHA IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-operative</td>
<td>0</td>
<td>4 (16.7%)</td>
<td>3 (12.5%)</td>
<td>17 (70.8%)</td>
</tr>
<tr>
<td>Post-operative</td>
<td>20 (90.9%)</td>
<td>2 (9.1%)</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
11.4.2.8. Change in cardiothoracic ratio

The mean cardio-thoracic ratio prior to surgery was 76.4% (± 12.1) whereas it was 62.0% (± 9.3) one year after the surgeries. The distribution of the cardio-toracic ratio before and after surgery is presented in Figure 11.12. In some patients the reduction in the cardio-thoracic ratio was dramatic, as occurred in the patient with LEMF whose chest radiography is shown in Figure 11.13.

![Figure 11.12](image)

**Figure 11.12.** Evolution of the cardiothoracic ratio before (24 patients) and after surgery (22 patients)
Figure 11.13. Chest radiography of LEMF before (top) and 6 months after surgery (bottom), which included left endocardial resection, mitral repair and left atrial reduction.
11.4.2.9. Mortality and morbidity

There was no intraoperative death. The mortality rate was therefore $2/24$ (8.3%). Two intrahospitalar deaths occurred during the immediate post-operative period (days 3 and 10). Both were related to complications of pericardial drainage in emergency, for relief of pericardial tamponade that developed rapidly.

The most important complications of EMF surgery were abundant pericardial effusion and tamponade, intraventricular thrombi and transitory rhythm disturbances (Table 11.8). Several complications occurred in only one patient namely low cardiac output syndrome, subdural hematome, superior vena cava syndrome, depression and pericardium-left pleural space fistula (after pericardial drainage).

Abundant pericardial effusion developed in 14 patients, with pericardial tamponade needing drainage in 4. In all cases the effusion developed rapidly within 24 to 48 hours. In all cases of pericardial tamponade there was severe affection of the right ventricle.

Eleven patients had transitory supraventricular arrhythmia. Of these 4 presented persising atrial rhythm disturbances during the first six months, namely intermittent atrial fibrillation and paroxysms of supraventricular tachycardia. In one patient with REMF in whom right atrial reduction was performed intermittent atrial fibrillation persists after 24 months follow up. Transitory AV block lasting 24-48 hours was present in 3 patients with REMF who were submitted to endocardial resection.

The neurological complications were one subdural hematome that needed surgery and one post-operative depression.
Table 11.8. Surgical technique and complications of the tailored approach

<table>
<thead>
<tr>
<th>Code</th>
<th>EMF Class</th>
<th>Surgical Technique</th>
<th>CPB/AoC time(min)</th>
<th>Early Postoperative Complications</th>
<th>Death</th>
</tr>
</thead>
<tbody>
<tr>
<td>0065</td>
<td>II L LA</td>
<td>MR</td>
<td>62/32</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>0068</td>
<td>II B LA RA</td>
<td>MR TR LAR</td>
<td>102/68</td>
<td>PE, AT, Low cardiac output</td>
<td>No</td>
</tr>
<tr>
<td>0048</td>
<td>IV L LA RA</td>
<td>MR TR LER LAR</td>
<td>135/88</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>009</td>
<td>III B LA RA</td>
<td>MR TR LER LAR</td>
<td>94/63</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>0076</td>
<td>III L LA RA</td>
<td>MR TR LER</td>
<td>128/88</td>
<td>LVT, mild MS</td>
<td>No</td>
</tr>
<tr>
<td>0075</td>
<td>III L LA RA</td>
<td>MR TR LER</td>
<td>110/66</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>0074</td>
<td>III B LA RA</td>
<td>MR TR LER RER LAR</td>
<td>102/82</td>
<td>PE, LVT, EB, Pleural Effusion</td>
<td>No</td>
</tr>
<tr>
<td>0040</td>
<td>III B LA RA</td>
<td>MR TR LER LAR</td>
<td>102/70</td>
<td>PE/Pericardial Tamponade</td>
<td>Yes</td>
</tr>
<tr>
<td>0143</td>
<td>II B LA RA</td>
<td>MR TR LAR</td>
<td>88/57</td>
<td>Mild MS</td>
<td>No</td>
</tr>
<tr>
<td>0062</td>
<td>III B LA RA</td>
<td>MR TR LER RER LAR</td>
<td>110/91</td>
<td>PE, Stroke</td>
<td>No</td>
</tr>
<tr>
<td>0128</td>
<td>III L LA LV</td>
<td>MR LER LVT</td>
<td>140/112</td>
<td>Moderate Mitral Regurgitation</td>
<td>No</td>
</tr>
<tr>
<td>0142</td>
<td>III L LA RA</td>
<td>MR TR LER LAR</td>
<td>88/58</td>
<td>Transitory Psychosis</td>
<td>No</td>
</tr>
<tr>
<td>0144</td>
<td>III B LA</td>
<td>MR LAR</td>
<td>88/57</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>0125</td>
<td>III L LA RA</td>
<td>MR TR LER</td>
<td>89/60</td>
<td></td>
<td>No</td>
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<tr>
<td>0052</td>
<td>IV R RA</td>
<td>TR RER RAR</td>
<td>66/0</td>
<td>PE, Superior Vena Cava Syndrome</td>
<td>No</td>
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<tr>
<td>0146</td>
<td>III B LA RA</td>
<td>MR TR LER RER LAR</td>
<td>128/88</td>
<td>PE, Moderate Mitral Regurgitation</td>
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</tr>
<tr>
<td>0152</td>
<td>III R RA</td>
<td>TR RER RAR</td>
<td>130/88</td>
<td>PE, Pleural Effusion</td>
<td>No</td>
</tr>
<tr>
<td>0110</td>
<td>IV R RA</td>
<td>TR RER RAR</td>
<td>119/77</td>
<td>PE, Paroxysmal AF, RAT</td>
<td>No</td>
</tr>
<tr>
<td>0163</td>
<td>III B LA RA</td>
<td>MR TR LER RER LAR</td>
<td>98/63</td>
<td>Severe Mitral Regurgitation</td>
<td>No</td>
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<tr>
<td>0151</td>
<td>II R RA</td>
<td>TR RER RAR</td>
<td>148/79</td>
<td>PE</td>
<td>No</td>
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<tr>
<td>0169</td>
<td>III L LA RA</td>
<td>MR TR LER</td>
<td>99/69</td>
<td>PE/Pericardial Tamponade</td>
<td>Yes</td>
</tr>
<tr>
<td>0180</td>
<td>III B LA RA</td>
<td>MR TR LER RER LAR</td>
<td>135/105</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>0167</td>
<td>III B LA RA</td>
<td>MR TR LER RER LAR</td>
<td>105/65</td>
<td></td>
<td>No</td>
</tr>
<tr>
<td>0179</td>
<td>III B LA RA</td>
<td>MR TR LAR</td>
<td>137/94</td>
<td></td>
<td>No</td>
</tr>
</tbody>
</table>
Intra-ventricular thrombi in the immediate post-operative period occurred in 5 patients submitted to endocardial resection on the left ventricle. These patients were put on warfarin for three months. In one of them a subdural hematome developed despite normal INR values, and needed emergency neurosurgery for extraction of the hematome.
Table 11.9. Surgical complications and its frequency

<table>
<thead>
<tr>
<th>Complication</th>
<th>No Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large pericardial effusion</td>
<td>14 (58.3%)</td>
</tr>
<tr>
<td>Pericardial tamponade</td>
<td>4 (16.7%)</td>
</tr>
<tr>
<td>Temporary atrial tachyarrhythmia or atrial fibrillation</td>
<td>11 (45.8%)</td>
</tr>
<tr>
<td>Ventricular arrhythmia</td>
<td>5 (20.8%)</td>
</tr>
<tr>
<td>Intra-cardiac thrombosis</td>
<td>5 (20.8%)</td>
</tr>
<tr>
<td>Transitory atroventricular block</td>
<td>3 (6.4%)</td>
</tr>
<tr>
<td>Neurological complications</td>
<td>2 (8.3%)</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>2 (8.3%)</td>
</tr>
<tr>
<td>Superior Vena Cava Syndrome</td>
<td>1 (4.2%)</td>
</tr>
<tr>
<td>Pericardial-pleural fistula</td>
<td>1 (4.2%)</td>
</tr>
<tr>
<td>Permanent atrioventricular block</td>
<td>0</td>
</tr>
<tr>
<td>Infection</td>
<td>0</td>
</tr>
<tr>
<td>Residual (persistent) mitral regurgitation</td>
<td>2</td>
</tr>
<tr>
<td>Residual tricuspid regurgitation</td>
<td>1</td>
</tr>
</tbody>
</table>
11.4.2.10. Re-intervention

Re-operation was needed in one patient with BEMF who developed acute heart failure related to mitral regurgitation due to dehiscence of the suture of the Goretx-built ring to the posterior annulus. This complication developed three weeks after left endocardial resection and mitral valve repair. A new mitral valve repair was performed and the patient is now well.

11.4.2.11. Survival and Recurrence

The mean follow up of patients operated for treatment of EMF is now 25 (± 10) months, ranging from 12 to 40 months. During the follow up there was no recurrence of the disease as evaluated by the presence of echocardiographic signs of endocardial thickening in areas where endocardial resection had been performed.

The mean survival time is 38.6 months (CI 36-40). The survival curve of patients submitted to surgery using this new tailored approach is shown in Figure 11.14.
Figure 11.14: Survival curve of patients operated using the new surgical approach.
11.5. Discussion

The optimization of the management of EMF requires a correct diagnosis and includes detailed characterization of anatomical and functional abnormalities for each patient. This precision in diagnosis allows the design of tailored strategies for treatment of the existing lesions, and prevents the progression to irreversible complications.

Medical therapy was important in pre-operative care, to control heart failure, to treat superimposed pulmonary infections and also to manage complications of low cardiac output, especially cachexia. Since ascitis and pericardial effusion play a major role in determining symptoms, pre-operative care included the drainage of these effusions. Ascitis causes increased intraperitoneal pressure and consequently reduces the absorption of the intraluminal content of the bowel, including drugs administered orally. Evacuation of ascitis, despite draining considerable amounts of proteins, was associated to temporary clinical improvement and better response to medical therapy. Equally, the drainage of large pericardial effusions was associated with marked improvement probably due to alleviation of one of the components contributing to reduced atrial filling and low cardiac output. Indeed the thickened endocardial fibrous tissue causes combined systolic and diastolic dysfunction which are aggravated by the presence of pericardial effusion.

We performed cardiac catheterization in two patients to assess the volume of the RV, measure the pulmonary pressures and define the surgical strategy. This invasive procedure was indicated exclusively to patients undergoing surgery, because we feel that echocardiographic examination allows detailed evaluation of structural and hemodynamic...
abnormalities in most patients. Additionally, there is frequently an imbalance between the information obtained and the risk of the procedure, which include stimulation of arrhythmia, thromboembolism and cardiac perforation.

Reparative operations were developed and applied to target the specific components of the disease, which include: (1) fibrous plaques interfering with systolic and diastolic regional ventricular function; (2) reduction in ventricular volume caused by obliteration of the trabecular part of the right ventricle and the apical portion of the left ventricle; (3) immobilization of the papillary muscles of the mitral and tricuspid valves, which can be totally embedded in the ventricular wall; (4) chordal abnormalities; (5) fusion of the leaflets (mainly the posterior leaflet of the mitral valve) to the wall; (6) dilatation of the tricuspid and mitral annulus; (7) massive dilatation of the right and left atria.

Our initial surgical experience involved primarily the treatment of predominant or lone LEMF. With the understanding of the mechanisms of right ventricular obliteration, severe forms of right-sided disease were progressively included.

The surgical technique includes partial or extensive endocardial resection according to the location and extension of the lesions, as well as the appreciation of their contribution to the abnormalities in the global cardiac function. Also, there is usually need for mobilization of the fused papillary muscles and leaflets, atrioventricular valve repair and atrial reduction. Avoidance of complete heart block is achieved through less radical endocardectomy, while the use of atrioventricular valve repair using a non-rigid ring contributed to reduction in complications related to valve prosthesis.
We approached ventricular lesions through atriotomy. However, in a patient with LEMF with a calcified thrombus attached to the apex and subvalvar apparatus of the left ventricle, an apical incision was needed to release the mitral valve apparatus. In an attempt to improve the access to LV endocardial lesions while reducing the impact of ventriculotomy an alternative option is endocardectomy through aortotomy (Joshi et al., 2003).

Resection of the fibrous endocardium was associated with immediate improvement of the ventricular function in most cases. When apical fibrosis was resected, there was vigorous contraction of the apex with improvement in longitudinal left ventricular function immediately after surgery. In patients with right ventricular obliteration the reopening of the trabecular portion through endocardial resection and mobilization of the fused trabeculae was associated with improvement in right ventricular function and filling. In two patients increase in ventricular function unmasked a moderate mitral regurgitation previously classified as trivial.

Ventricular size and shape changed after surgery. Regarding the left ventricle the most important changes were increase in the longitudinal dimensions, leading to reduction in the sphericity index and a more normal shape of this chamber. The reduction of sphericity of the right ventricle was less marked post-operatively, since both the latero-lateral and supero-inferior dimensions increased almost proportionally.

There was reduction of the size of the dilated left atrial in all patients operated for treatment of Left or Bilateral EMF, with reduction of the sphericity. In patients with predominant unilateral disease there was reduction in the dimensions of the contra-lateral
atrium, related to reduction of the intra-atrial pressure on the treated side and consequent reduction of bulging of the inter-atrial septum.

Conventional measurements of ventricular function like shortening and ejection fraction cannot be used because the assumptions applied to calculate these parameters are not present in most EMF patients. Indeed pre-operatively in left and bilateral EMF the left ventricle, instead of an ellipsoid is converted to a round cavity due to apical fibrosis and compensatory dilatation of the basal portion, leading to a round shape that is attested by the increase in its sphericity index. Additionally, advanced right-sided lesions distort the left ventricle that assumes a “banana” shape related to compression by an aneurismal right atrium and to change in shape that is associated with the marked retraction of the apex of the right ventricle. Moreover, postoperatively the paradoxical movement of the interventricular septum does not allow the use of the formula for calculation of the shortening fraction. Therefore, despite its limitations related to the fact that it only considers two dimensions of the cavities, the linear dimensions and sphericity indexes appear to be the most acceptable tools for evaluation of the effects of surgery on EMF patients.

In patients with REMF and considerable reduction of the right ventricular size it appears that the addition of a cavopulmonary anastomosis to the procedure of endocardial resection or its use as a first stage of phased surgical treatment, improved the prognosis by reducing post-operative complications.

Our surgical results are superior to those reported earlier from Brazil, India and Ivory Coast (Moraes et al, 1993; Metras, 1993; Valiathan and Shymkrishnan, 1993). This may

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be partially explained by the improvements in myocardial protection that occurred in the recent years, which now allow longer and safer open-heart surgery. The use of new drugs for the management of intra-operative and immediate post-operative complications may also have contributed to the amelioration of the prognosis after surgery. However, although these improvements might have played an important role on recovery of the myocardial function after this long and demanding surgery, we think that the detailed characterization of disease prior to surgery and the use of the new tailored techniques played a crucial role.

We did not have any intra-operative mortality. The two post-operative deaths were due to accidents related to drainage of pericardial tamponade, the most important complication of surgery both in terms of frequency and relevance for the prognosis. Knowledge of this fact has lead to changes in the way we manage and follow this complication, preventing deaths due to this complication after an initial learning curve. In previous series mortality reached 20 to 29% (Valiathan et al, 1989; Moraes et al, 1999; Dubost, 1990) and has been related to low cardiac output state and complete atrioventricular block following extensive endocardial resection.

Complete atrioventricular block did not occur in our experience. Transitory idioventricular rhythm was seen in most patients and complete bundle branch was the most important long-term complication. Supra-ventricular arrhythmias occurred in 14 out of 24 patients, but most were transitory. At six-months follow up all patients were in sinus rhythm, except one that had been in atrial fibrillation previous to surgery.
We did not have any late deaths. Late mortality after EMF surgery in early series was also high (12.3%) during a shorter period of follow up (Valiathan et al., 1987), and has been mainly related to complications of valve prosthesis. Both, documented valve thrombosis or uncontrolled bleeding are reported to be frequent in those patients, due to poor compliance and control of anticoagulation. The uses of mitral and tricuspid repair in all patients and close follow up of complications after surgery have contributed to the low rate of complications in our series.

Recurrence of disease was not detected during follow up. In most patients the lesions found after surgery corresponded to fibrotic areas that were not accessible for endocardial resection during the surgical intervention. There are conflicting reports of recurrence of EMF after surgery. Although most authors state that recurrence is low after long follow-up, even in those patients that go back to their areas of origin, few cases have been reported on reoperation in Brazilian series and appearance of EMF lesions on the contralateral ventricle after surgery has also been reported in proportions varying from 6 to 18.8% (Moraes et al., 1999; Moraes et al., 1999). Longer follow up of patients in our series is warranted in order to clarify this issue.

All but one patient from our series were in functional class III/IV prior to surgery, despite multiple drug therapy at high doses. All surviving patients had marked clinical improvement, including those for which the echocardiographic results do not seem excellent on follow up, and had reduction of cardiomegaly as assessed by chest radiography and echocardiography. Our findings corroborate those from other studies,
which have shown that cardiomegaly is a major determinant of clinical symptoms, since leading to reduction in exercise capacity (Abrahams 1967; Mady et al, 2005).

Considering that most patients excluded from surgery had irreversible cardiac damage and/or complications of long-standing heart failure like hepatic dysfunction, fixed pulmonary hypertension and cachexia, it appears that early indication of surgery could further contribute to improved prognosis in this condition. Although the issue of timing for surgery has been debated, no consensus has been reached due to lack of knowledge about the etiology and natural history. The high mortality and morbidity associated with surgery have lead to late indication of surgical treatment of EMF, despite the several studies showing the superiority of surgery over medical therapy (Metras et al, 1982; Cherian et al, 1982; Gonzalez-Lavin et al, 1983) and the report of low recurrence (Valiathan and Shyamkrishnan, 1993; Moraes et al, 1993). Our results support the early indication of surgery to all symptomatic patients before severe cardiac damage has occurred.

This study represents the results of our initial experience with a new tailored surgical approach to EMF. Therefore, the number of patients and the follow-up period are relatively limited. Nevertheless, the lessons learned regarding disease presentation and operability of this neglected, yet highly prevalent disease are important.
11.6. Conclusions

The present results indicate that a thorough understanding of the pathophysiology of the disease and careful patient management and follow-up result in satisfactory outcomes in symptomatic patients. However, good surgical skills and adequate environment for follow up of patients after surgery are mandatory.

Based on our initial experience, complete and meticulous removal of all endocardial fibrotic tissue without compromising the conduction system or the integrity of the sub-valvular apparatus is a key element. Although no cases of EMF recurrence have yet been observed, continued clinical and echocardiographic follow-up are mandatory. Further research is necessary to identify prognostic factors and help in determining the ideal timing of surgery. We hope that continued research into the pathophysiology and the therapeutic management of this neglected condition will lead to a significant decrease in the burden of disease in children and young adults in developing countries.
Chapter 12

CONCLUSIONS AND FUTURE DIRECTIONS

We here describe a series of studies on the epidemiology, clinical-laboratory profile and management on Endomyocardial Fibrosis (EMF), a major health challenge and one of the leading neglected diseases worldwide. This condition affects several million people throughout the world with a very poor prognosis and, although known since the middle of 20th century, it has received little attention from the scientific community in the last decades and therefore did not benefit from a modern management and research approach. The evolution of the anatomical phenotype of early stages remains unclear despite major developments in imaging techniques, since these techniques are still expensive and not readily available in most endemic areas. However, the use of these modern imaging tools may uncover the mechanisms of disease, hence the need to apply them on research that should also include genetics and molecular biology.

This thesis is the first attempt at using these modern tools and innovative approaches to investigate EMF, and integrates large-scale epidemiological studies with clinical research and laboratory investigations aiming at unveiling the pathogenesis of the condition. Our main results concern epidemiology, diagnosis and management of EMF. New interrogations are raised regarding the mechanisms involved.


**EPIDEMIOLOGY**

_Echocardiographic screening and establishment of cohorts_

Echocardiographic screening has shown that EMF is common in Inharrime affecting nearly 20% of the population, and that young age, male gender and familial predisposition are important determinants of the disease. This imaging tool enables the detection of individuals in early phases of the disease whose follow up has given new insights into the natural history of this condition.

Since echocardiography uncovers the presence of asymptomatic disease we conclude that it is the most powerful tool for screening of EMF, and should be also used to assess the progression of anatomical and hemodynamic abnormalities as well the response to interventions in cohort studies.

**DIAGNOSIS**

_Phenotype characterization, evolution and surgical indications_

The use of echocardiography allows a confident non-invasive diagnosis of EMF, is essential for indication of surgery and choice of operative techniques, and has the potential to be used to define prognosis.

Our studies show that EMF presents great phenotypic variability with lesions varying from patchy endocardial thickening without any hemodynamic changes, to extensive mural and atrioventricular valve endocardial fibrosis with resulting structural and
hemodynamic changes. Although severe and debilitating forms of the disease are the most common forms of presentation to hospitals, mild asymptomatic disease is common in the community.

The most characteristic echocardiographic features of EMF are: (1) endocardial thickening affecting the walls and/or the atrioventricular valves; (2) obliteration of the trabecular portion of the right ventricle, the left ventricular apex or the atrioventricular valve recesses; (3) ventricular thrombi or spontaneous contrast in the absence of ventricular dysfunction; (4) reduction of ventricular cavity volume; and, (5) restriction of atrioventricular valve mobility due to its partial or complete adherence to the ventricular walls. However, echocardiography allowed the definition of several minor features that are suggestive of EMF and can be considered initial signs of disease. Follow up of these changes is necessary to validate this hypothesis.

EMF is a unique form of cardiomyopathy with particular pathophysiology. There is association of diastolic and systolic dysfunction, atrioventricular valve dysfunction and abnormal shape of the ventricles. Therefore, the assumptions usually made regarding ventricular shape and that are the basis for calculating parameters that assess ventricular function cannot be applied for most cases of EMF. Thus, the use non-conventional measurements and indices to evaluate ventricular function and assess the results of treatment is mandatory. We privileged ventricular and atrial linear dimensions, sphericity indices and the visual scale for evaluation of systolic function. The reduction of the sphericity index seems a useful marker of improvement after surgery for all cavities except the right ventricle, which shows variable shape post-operatively.
MANAGEMENT

Evolving new therapeutic approaches

Currently, there is no effective medical therapy for EMF. Our studies of the biological profile in EMF suggest that a subset patients present markers of inflammation, increased thrombolysis and autoimmunity. Thus, they can potentially benefit from the use of anti-inflammatory drugs, immunomodulators and anticoagulants, an hypothesis that should be tested through clinical trials.

The diagnostic and severity score system presented and applied in our work represents the first attempt at standardization of echocardiographic examination of EMF. It offers a management-driven tool that is essential for selection of patients who can benefit from surgery, as well as for design of tailored strategies and techniques for surgery. Most structural and functional abnormalities of EMF can be partially corrected through fibrous tissue resection, atrioventricular valvar repair, atrial reduction and partial cavo-pulmonary anastomosis. Late diagnosis determines poor overall prognosis in this condition, hence the need for early diagnosis and better timing of surgery.

The use of new approaches and innovative tailored surgical techniques improves the early and medium-term results of surgery. However, long-term follow up is needed to assess the rate of recurrence, survival free of major events.

Surgery, by allowing detailed examination of the heart “in vivo”, improves the understanding of the pathophysiology of EMF, and will hopefully help defining the optimal timing for surgery.
MECHANISMS OF THE DISEASE

Factors involved in pathogenesis

In an attempt to uncover the pathogenesis of EMF this thesis has searched evidence for inflammation, allergy, autoimmunity, endothelial markers, prothrombotic factors, immunogenetic susceptibility and the possible role of malaria infection in inducing EMF. Our results suggest a role of inflammation, allergy, autoimmunity, pro-thrombotic factors and endothelial dysfunction in EMF. However, the timing and relevance of each of these abnormalities, particularly their relation to clinical features of recrudescence and progression of structural abnormalities, needs further investigation.

Although no relation was found between the presence of EMF lesions and malaria infection, we think that this issue needs also to be investigated exploring the recent knowledge of the pathology of malaria, particularly endothelial dysfunction, inflammatory changes and pro-coagulant abnormalities.

The evidence for immunogenetic susceptibility in determining the severity of EMF might explain the differences in phenotypic variability, mode of progression and familial predisposition. However, larger studies characterizing the genetic profile of the unaffected individuals in Mozambique and individuals with EMF in the community are mandatory to clarify the precise role of genetic factors in the pathogenesis of this condition.
Future Directions

The epidemiological research has established cohorts of individuals who are well phenotyped by echocardiographic examination and who will be followed to assess incidence and progression of EMF. Future research should include prospective case-control studies to identify the mechanisms of the disease, and define the role of environmental factors in pathogenesis, namely parasites, viruses, diet and allergens.

The usefulness of cavity linear dimensions, sphericity indexes and visual scales for evaluation of the systolic function must be validated in larger series, as a tool for assessing the results of surgery. On the other hand research shall concentrate on evaluation of the prognostic value of features like the extent and depth of fibrosis, involvement of the papillary muscles in the fibrotic process, severe shortening of the leaflets and the presence of severe ascitis. Similarly, the influence of the surgical strategy and the techniques used must be evaluated.

In recent years we have witnessed the gain of new knowledge in several fields that are relevant to understanding EMF, namely endothelial cell biology, inflammation, hemostasis, regulation of collagen synthesis, remodeling and mechanisms of fibrosis. However, this has not been paralleled by improvement in knowledge of EMF.

The definition of the biological profile of EMF patients is of paramount importance, particularly the clarification of the role of the eosinophil. Eosinophil cationic protein (ECP) released from activated eosinophils increases the release of TGF-β1 from
fibroblasts, and this is thought to be the mechanism by which they cooperate in remodeling of the extracellular matrix leading to airway fibrosis in asthmatic patients (Zagai et al., 2007). Eotaxins, on the other hand, are chemotactic cytokines highly selective for eosinophils and are known as their most potent chemoattractant. They can induce both the local recruitment of eosinophils from the microcirculation and rapid mobilization of the bone marrow eosinophils, in synergy with IL5 (Collins et al., 1995; Palframan et al., 1998), and may be produced by many of the cells found in cardiac tissue, namely smooth-muscle cells, endothelial cells, alveolar macrophages, lymphocytes, eosinophils.

The relevance of antiheart antibodies in EMF must also be clarified. There is a possibility of homology of these heart antigens with native pathogens, and attempts must be made at identifying the nature of the specific antigens as well as assess the functional cytotoxicity of those circulating antiheart antibodies. Another challenge remains the identification of specific markers of endocardial endothelium.

Improvement in knowledge in all these areas should stimulate research aiming at testing therapeuthical interventions that can alter the natural history of the disease, preventing its progression to advanced forms. Clinical trials targeting the eosinophil and its related cytokines are recommended since drugs to control hypereosinophilia, high levels of IL-5 and eotaxins are currently available (Rothenberg et al., 2008; Sutton et al., 2005; Morokata et al., 2006). The assessement of the efficacy of the anti-inflammatory drugs in active disease is also warranted, although caution must be taken in endemic tropical areas, where the risk of infections bacterial and parasitic infestations is very high.
Although structural abnormalities are present in all components of the heart tissue, the hallmark of EMF is the sub-endocardial deposition of large numbers of fibroblasts, the predominant cellular mediators of fibrosis, which origin is still unclear. It could be the consequence of passage of fibroblast stimulatory factors from the vessels to the interstitium caused by abnormal coronary vascular permeability or the consequence of direct insult to the endocardial endothelium leading to endothelial activation, proven to be present in our studies in recent onset EMF (Chapter 8). Endothelial cell activation has the potential to induce subendocardial fibrosis, since endothelial cells can synthesize both basic fibroblast growth factor (b-FGF) and platelet-derived growth factor (PDGF/NO). Injured cells would release these factors, leading to potent growth-promoting activity for fibroblasts (Endeman and Schiffrin, 1992).

The assumption that adult fibroblasts are originally derived from embryonic mesenchymal cells and that they increase solely as a result of the proliferation of resident adult fibroblasts has been challenged. There is evidence that during fibrosis, bone marrow-derived fibroblasts and epithelial cells also contribute to fibroblast accumulation, by way of a process called endothelial-to- mesenchymal transition (Iwano et al, 2002; Zeisberg et al, 2007; Friedman, 2007; Gressner et al, 2007). This critical process to embryonic development of the heart (Eisenberg and Markwald, 1995), is thought to be regulated by inductive signals such as TGF-β and bone morphogenic proteins (Bujak and Frangogiannis, 2007), and could be involved in the pathogenesis of EMF, a hypothesis that needs to be investigated. Moreover, the concept of the irreversibility of cardiac fibrosis or scarring of the heart must be reviewed (Towbin (2007) considering the recent
of a compound that inhibits TGF-β1 endothelial-mesenchymal transition, blocks fibrosis and reduces the amount of fibrous tissue in mice (Zeisberg et al, 2007).

Another important line of research is the measurement of cardiac collagen turnover by use of serological markers, a useful tool for monitoring cardiac tissue repair and fibrosis in experimental models and clinical conditions (Zannad et al, 2001). The biological and immune profile of EMF is that of a systemic disease with cardioselectivity of fibrotic lesions, the reason for which is not known. The recent discovery of the expression of tissue factor in the myocardium but not in other muscle types (Mackman 2008; Pawlinski et al, 2007) might explain why is the heart the sole organ predisposed to primary thrombosis and subsequent fibrosis in this condition.

Research focusing on these issues should be performed using cardiac tissue and blood obtained from participants in prospective studies, since it could be of value for unveiling the sequence of events that lead to fibrosis. On the other hand, better understanding of the endocardial endothelium and identification of specific markers of its injury is mandatory. Attempts at isolation of endocardial cells and fibroblasts from the heart are needed in order to allow the design of in vitro studies looking at the effects of pro-fibrotic stimuli in those cells. Another possibility would be the development of animal models of endomyocardial fibrosis to allow the testing of insults that could lead to fibrosis of the endocardium and adjacent myocardium, as well exploration of the effects of currently available modulators of inflammation, thrombosis and fibrosis on endocardial fibrosis, myocardial fibrosis and collagen turnover.
Another area of future research will be the genetics aiming at clarification the role of heredity in determining familial predisposition and immunogenetic susceptibility found in our studies. This could be done through genetic epidemiological studies in endemic and non-endemic areas, looking for candidate genes known to have a role in fibrosis, as well as searching for genetic polymorphisms to biological markers with relevance to the mechanisms of inflammation, thrombosis and fibrosis.

Geographic variation in prevalence of EMF has been recognized within countries. We acknowledge that the results presented in this thesis reflect the situation in one specific region with high prevalence of EMF. Replication of similar programs of translational research in endemic countries might allow the validation of clinical, biological and echocardiographic criteria proposed by us for diagnosis of early disease. It is also important to implement standardized national registries/databases and identify centers of excellence in which state-of-the-art surgical management and conditions for optimum research are available. The ethical issues associated with the implementation of such large-scale screening and management programmes for a condition with unknown etiology and ineffective treatment must not be underestimated, and availability of clinical follow up in the concerned areas must be guaranteed in order to insure acceptance of these research programs by the communities.

This thesis has probably raised more questions than it has answered. However, we believe that it addresses critical issues regarding the diagnosis, contributes to better understanding and management of EMF, and will hopefully stimulate further research looking at new therapeutic targets for the disease.
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List of publications arising during this thesis

Original peer reviewed articles directly related to this thesis


Original peer reviewed articles not directly related to this thesis


Oral Presentations at conferences on subjects related to this thesis

Tropical Neglected Cardiomyopathies in Children
Florence International Course of Advances in Cardiomyopathies
5th Meeting of the European Myocardial and Pericardial Diseases Working Group of the ESC
May 2008, Florence – Italy

Recent advances in the management of Endomyocardial Fibrosis
1st Congress of the Pan African Society of Cardiology
May 2007, Nairobi – Kenya

Early results of surgery for Endomyocardial Fibrosis
South African Heart Association Congress
November 2005, Drakensberg – South Africa

Endomyocardial Fibrosis: New approaches to understanding its pathogenesis
V South African Heart Association Congress
November 2004, Durban – South Africa
Appendixes

Appendix 1. Form used for collection of echocardiographic data
Appendix 2. Form for data collection during the epidemiological studies
Appendix 3. Form for registration of detailed location and type of cardiac lesions
Appendix 4. List of the schools selected for prospective studies
Appendix 5. List of permanents members of the surgical teams operating on EMF
Appendix 6. Form for detailed description of pathology findings on surgery
Appendix 7. Valve function before and after surgery using the new tailored approach
Appendix 8. Ventricular indexed linear dimensions and sphericity index
Appendix 9. Left ventricular dimension and E/A wave ratio before and after surgery
Appendix 10. Right ventricular function before and after surgery
Appendix 11. Atrial dimensions indexed for body surface area and sphericity indexes

Papers published as first author
APPENDIX 1 (front)
APPENDIX 2A

PREVALENCE STUDY – ENDOMYOCARDIAL FIBROSIS

DATA COLLECTION

CODE________________ PSN ________________
ADRESS ______________________________________________________
CONTACT ___________________
NAME ___________________________________________________________
AGE _______ DATE OF BIRTH _________
RACE  B O SEX F M “PARENTESCO” ___________

ECOCARDIOGRAPHY
NORMAL EMF RHD CHD OTHER

4 cavities parasternal LA sub-costal

BLOOD COLLECTION YES NO

REMARKS:
_________________________________________________________________
_________________________________________________________________
DATE _______________ RESEARCHER ______________________

Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis
APPENDIX 2B

PROSPECTIVE STUDY – ENDOMYOCARDIAL FIBROSIS

DATA COLLECTION (TIME 0)

CODE_____________  TEACHER _______________

SCHOOL __________________________________________________________

ADRESS __________________________________________________________

CONTACT _________________________________________________________

STUDENT_________________________________________________________

PARENTS __________________________________________________________

AGE _______ DATE OF BIRTH _______ RACE     B/O     SEX   F   M

ECOCARDIOGRAPHY

NORMAL       EMF    RHD     CHD     OTHER

4 cavities       parasternal LA    sub-costal

BLOOD COLLECTION  YES     NO

REMARKS:

_________________________________________________________________

_________________________________________________________________

DATE _______________  RESEARCHER ____________________

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Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis
APPENDIX 3

Echocardiographic measurements used in the studies

**LEFT**

Aortic root dimensions
LVEDD/LVESD (shortening fraction)
LVEDV/LVESV (ejection fraction)
LVEDA/LVESA (area shortening fraction)
PW/Sp thickness
LA linear dimensions: AP, L, SI
Sphericity index (L/SI)
LA area, LA volume
Dimension of the mitral annulus
Doppler: E wave, A wave, E/A; IVRT, DT
Severity of MR

**RIGHT**

Linear dimensions RVOT
RV area
RVEDA/RVESV (shortening fraction)
Visual evaluation of contractility (+ to +++)
RV linear dimensions (L, SI)
AW thickness (S and D)
RA linear dimensions (L, SI)
Sphericity index (L/SI)
RA area
Dimensions of the tricuspid annulus
Doppler: E wave, A wave, E/A
Severity of TR
APPENDIX 4

LIST OF SELECTED SCHOOLS

Ten primary schools were randomly selected from a total of 50. All students from second level were invited to participate in the cohort follow-up study.

<table>
<thead>
<tr>
<th>SCHOOLS</th>
<th>N OF STUDENTS</th>
<th>TEACHERS</th>
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<tbody>
<tr>
<td>Senduza II</td>
<td>31</td>
<td>Armando Francisco</td>
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<tr>
<td>Cuaiaia</td>
<td>43</td>
<td>Armando António/Gaspar Languisso</td>
</tr>
<tr>
<td>Mahalamba</td>
<td>118</td>
<td>P. Mahique/ Olga Malate/ R. Cumbi/C. Lisure</td>
</tr>
<tr>
<td>Inhantumbo</td>
<td>47</td>
<td>Teresa Júlio/Luis Nhiuane</td>
</tr>
<tr>
<td>Mahessa</td>
<td>35</td>
<td>Jerónimo Muholiciane/Ernesto Hele</td>
</tr>
<tr>
<td>Coche</td>
<td>47</td>
<td>Julião Paulo/ Francelino Deve</td>
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<tr>
<td>Magula</td>
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<td>Domingas Fernando/Mariamo Abdula</td>
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<tr>
<td>Incuane</td>
<td>58</td>
<td>Horácio Xavier/ Arlindo Afonso Cumbi</td>
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<tr>
<td>Cambula</td>
<td>33</td>
<td>Lídia Machava/ João Chambe</td>
</tr>
<tr>
<td>Chilorane</td>
<td>40</td>
<td>Basílio Zango/ Rafael Augusto</td>
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### APPENDIX 5

**MEMBERS OF THE SURGICAL TEAMS**

From Imperial College and Magdi Yacoub Institute:

<table>
<thead>
<tr>
<th>Name</th>
<th>Role</th>
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<tbody>
<tr>
<td>Professor Sir Magdi Yacoub</td>
<td>Surgeon/Supervisor</td>
</tr>
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</table>

From IASO Genral Hospital, Athens - Greece:

<table>
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<tr>
<td>Professor Stergios Theodoropoulos</td>
<td>Surgeon</td>
</tr>
<tr>
<td>Dr Vassilis Kolias</td>
<td>Surgeon</td>
</tr>
<tr>
<td>Dr Angelos Katsianis</td>
<td>Surgeon</td>
</tr>
<tr>
<td>Elias Lazaros</td>
<td>Perfusionist</td>
</tr>
<tr>
<td>Yianna Siourdaki</td>
<td>Scrub nurse</td>
</tr>
<tr>
<td>Nicoleta Dionysiou</td>
<td>Scrub nurse</td>
</tr>
<tr>
<td>Thomas Zikopoulos</td>
<td>Scrub nurse</td>
</tr>
<tr>
<td>Kyraki Charitaki</td>
<td>ICU nurse</td>
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From Onasis Cardiac Surgery Centre, Greece:

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<tbody>
<tr>
<td>Dr Nicolaus Giannopoulos</td>
<td>Surgeon</td>
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From University Hospital Coimbra, Portugal:

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<tr>
<td>Ricardo</td>
<td>ICU nurse</td>
</tr>
<tr>
<td>António</td>
<td>ICU nurse</td>
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</table>
APPENDIX 6

SURGICAL REPORT

NAME  NID  EMF

DIAGNOSIS

(Scheme of the lesions must be schematised on the back)

LEFT VENTRICLE

- Adherence/Absence of posterior cusp
- Patchy fibrosis
- Thrombus
- Calcification
- White regular opacity/Deep scarring .inflow tract .apex .posterior wall
- Fibrosis/Shortening/Thickening/Calcification papillary muscles/chordae

RIGHT VENTRICLE

- Patchy fibrosis
- Thrombus
- Calcification
- White regular opacity/Deep scarring .inflow tract .apex .posterior wall
- Cavity dimensions Conus/Infundibulum dilatation RV atrialization
- Fibrosis/Shortening/Thickening/Calcification papillary muscles/chordae

PERICARDIUM

- Pericardial effusion/resection RV notch

ECC Time _____ min Failure to come off bypass

TECHNIQUE:___________________________________________________________

________________
SURGEON: DATE:

Biopsy: LV RV MV TV LA RA PC

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**APPENDIX 7:** Mitral and tricuspid valve function of the 24 EMF patients prior and after surgery (* Mitral Stenosis associated; † death; . follow up less than 24 months)

<table>
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<tr>
<th>Code</th>
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<td>Class</td>
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<td>TR</td>
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<td>TR</td>
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APPENDIX 8: Ventricular linear dimensions indexed for body surface area and sphericity indexes of the 24 EMF patients before and after surgery. (†death; ? unknown)

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### APPENDIX 9: Left ventricular systolic and diastolic function before and 1 year after surgery, EDD/ESD indexed for body surface area (*assessed by visual scale; †death; ? unknown).

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APPENDIX 10: Right ventricular function assessed by visual scale in the 24 EMF patients before and after surgery (†death; ? unknown)

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APPENDIX 11: Atrial linear dimensions indexed for body surface area and sphericity indexes of the 24 EMF patients before and after surgery (†death; ? unknown)

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Embel Communications

Despite the major advances in thoracic aortic surgery with DHCA, there are still major clinical challenges, including this thrombotic syndrome in the face of standard-of-care anticoagulation. Further collaborative research should advance our understanding and management of this thrombotic syndrome in the future.

References

An innovative technique for the relief of right ventricular trabecular cavity obliteration in endomyocardial fibrosis

Ana Olga Mocumbi, MD,* Daniel Sidki, MD, PhD,* Pascal Vouhe, MD, PhD,* and Magdi Yacoub, FR, FR, London, United Kingdom, Maputo, Mozambique, and Paris, France

Endomyocardial fibrosis (EMF) mainly affects persons from Africa, South America, and Asia. The pathogenesis of this condition remains unknown. In advanced forms, EMF produces marked disability and carries a poor prognosis. Although there is consensus about the potential value of surgical intervention in symptomatic patients, there is still debate regarding the exact timing and the surgical technique to be used.

EMF is characterized by endocardial fibrosis affecting the inflow tract and the apex of one or both ventricles, commonly involving the atrioventricular valves. The right ventricle is affected in most cases. In severe forms, marked reduction of ventricular volume is thought to be due to the presence of a plug of fibrotic tissue involving both the trabecular part and the apex.

We herein describe a new mechanism for special obliteration of the right ventricle in EMF. The concept was used to evolve and apply a new surgical technique to increase ventricular volume, improve contractile function by releasing the myocardium and making use of viable myocardium in the obliterated area, and correct the tricuspid regurgitation.

Endocardial resection was started near the tricuspid annulus by resecting the leaflets of the valve. If the latter was fused in some areas, it was mobilized if at all possible. After the development of a chyle plane by means of sharp dissection, a combination of sharp and blunt dissection was used to excise the thick, fibrous endocardial lining. This process was continued into the trabecular cavity, ensuring preservation and mobilization of the tricuspid valve chordae and papillary muscles. The membrane covering the entry into the trabecular part was removed (Figure 1, B), exposing the fused muscular tissue underneath. This was followed by resecting a cavity inside the trabecular part by mainly releasing the fused trabecular but, if necessary, also exciting some muscular tissue, taking care not to perforate the ventricular wall.

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Brief Communications

Figure 1. A. Echocardiogram showing obliteration of the trabecular part of the right ventricle in a case of bilateral endomyocardial fibrosis. Notice thickening in the apex of the left ventricle and dilatation of both atria. B. Through sharp dissection, a plane of cleavage is created, allowing the separation of the fibrous tissue from the myocardium. Excision of the fibrous tissue is done as a block, revealing a healthy myocardium underneath it.

Table 1. Clinical and preoperative data of the 4 patients operated on with the technique

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<td>TR</td>
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No aortic clamping was used in patient 4, who had undergone a previous Blalock procedure. NYHA, New York Heart Association; CPB, cardiopulmonary bypass; AIC, aortic crossclamp; F, female; REMF, right-sided endomyocardial fibrosis; TR, tricuspid repair; M, male; EEMF, bilateral endomyocardial fibrosis; MR, mitral repair; LVE, left ventricular endocardial resection.

In patients with complete fusion of the leaflets and chordae to the mural fibrosis, freeing of the tricuspid valve apparatus was performed. Reconstruction of the tricuspid valve with 2 bands of polytetrafluoroethylene* tubes was used in all patients.

Clinical Summary

Between February 2003 and June 2006, 4 patients with right ventricular trabecular cavity obliteration were treated by means of this operation. The clinical characteristics of these patients and the procedures used are summarized in Table 1. There was no early or late mortality. Pericardial tamponade occurred in 2 patients. Patients were kept on low doses of diuretics, aspirin, and angiotensin-converting enzyme inhibitors for 6 months. At a mean follow-up of 18 months (range, 9–48 months), all patients are asymptomatic. A two-dimensional echocardiogram showed an increase in right ventricular cavity dimensions to nearly normal levels with improvement in systolic function, acceptable compliance in all, and mild tricuspid regurgitation in 1 patient. Magnetic resonance imaging analysis in 1 patient confirmed these results.

Discussion

A new mechanism for right ventricular trabecular cavity obliteration in EMF is described. A surgical technique for its relief was developed and used with very encouraging results in terms of restoration of both structural and functional changes of the right ventricle. There was no evidence of recurrence over the relatively short follow-up period. We hope that familiarity with this technique will help to stimulate early diagnosis and timely treatment of EMF before shrinkage of the right ventricle occurs and thus have a favorable effect on the prognosis of this potentially fatal disease.

We thank the Magdi Yacoub Institute and the Chain of Hope—UK for their financial support of surgical missions to Mozambique and Drs Beatriz Ferreira and Gavin Wright for their help.

References


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MYOCARDIAL DISEASE

Neglected tropical cardiomyopathies: I. Chagas disease

Sophie Yacoub,¹ Ana Olga Mocumbi,¹,² Magdi H Yacoub¹,²

Cardiomyopathies, defined as diseases of the myocardium associated with cardiac dysfunction, are classified into dilated, hypertrophic, restrictive, and arrhythmogenic right ventricular cardiomyopathy, as well as undiagnosed. More recently, molecular classification has been suggested. Cardiomyopathies continue to be a significant cause of morbidity and mortality in the developed world. ¹² In developing countries, however, there appears to be an increased incidence of the “usual” forms of cardiomyopathy, with a modified clinical course possibly due to genetic differences or environmental factors such as malnutrition, infections and pollution.³⁴ In addition, there are specific cardiomyopathies endemic to the tropics such as Chagas and endomyocardial fibrosis, which cause a considerable amount of death and suffering and have been classified as neglected diseases. We here describe what is known about these two diseases, their antelopes, pathogenesis and management and outline directions for further research. The present article will discuss Chagas disease, and a subsequent article will address endomyocardial fibrosis.

CHAGAS DISEASE

Chagas disease is the leading cause of cardiac disease in many countries in Latin America, and the World Health Organization has estimated that 15–18 million people are currently infected and 90 million are at risk of infection.¹ Chagas disease has been classified as one of the most neglected diseases in the world,⁵ with no new drug development in the past 50 years. Yet there are still 200,000 new cases of Chagas disease reported each year and some rural communities in Latin America have seroprevalence rates as high as 49%.

EPIDEMIOLOGY

Chagas disease is caused by the protozoan parasite Trypanosoma cruzi (fig. 1), which is spread by triatomine bugs (fig. 2). The disease is mainly constrained geographically to countries where its vector is endemic, ranging from South America to Mexico and southern USA. Transmission has also been shown to occur via blood transfusions, organ transplants as well as transfusionally and by ingestion of triatomine contaminated food or drink.⁶ The bug lives in the mud walls and thatched roofs of poor quality houses in rural areas; therefore, Chagas disease is linked to socioeconomic status with the rural poor being most exposed.

With increasing immigration from Latin America to the United States there was concern relating to safety of blood donations in the USA. A study between 2006 and 2007 identified one in 6500 blood donations in three centres in California and Arizona as being positive for T. cruzi antibodies.⁷ There have been seven reported transfusion acquired infections for Chagas disease in the USA and Canada.⁸ The Food and Drug Administration has recently licensed a new T. cruzi enzyme linked immunosorbent assay (ELISA) test system to screen blood donors in the USA.⁹ Vector borne transmission of Chagas disease can also occur in the southern United States, but with five reported cases ever it remains rare, so the potential for misdiagnose is significant.¹⁰


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Figure 2 Triatoma infestans, the vector for Chagas disease.

Parasites penetrate most commonly host phagocytic cells and multiply intracellularly as amastigotes. They are then released into the blood stream as trypomastigotes able to infect a variety of host cells, most commonly myocardial cells. The trypomastigotes may also be taken up by feeding triatomine bugs, thus continuing the life cycle.  

DISEASE STAGES

The acute phase of the infection with *T. cruzi* is characterized by a self limiting syndrome lasting between 4-8 weeks, with fever, malaise, lymphadenopathy, hepatosplenomegaly and myocarditis. A chagoma may occur at the site of entry, if it is at the conjunctiva, a unilateral peripheral edema can occur called Roinhas sign. During this stage parasites can be demonstrated in the blood and there is direct invasion of myocardial fibres. Rarely, it can cause a meningoencephalitis in younger children, which carries a poor prognosis. Overall the acute infection has around a 10% mortality rate.  

In the majority of cases, though the development of immunity, the infection is controlled and the disease progresses to a latent period or indeterminate phase. Dilated cardiomyopathy constitutes the chronic phase which develops in up to 30% of infected individuals between 10-20 years later. This may present as congestive cardiac failure, symptomatic arrhythmias, thromboembolic disease or sudden death. The left ventricular wall becomes thinner, allowing the formation of an apical aneurysm, a distinctive feature of Chagas cardiomyopathy. Thrombi are often present in such aneurysms, easily explaining the common occurrence of thromboembolisms.  

Cardiac dysautonomia is a hallmark of chronic Chagas disease resulting in varying degrees of heart block in the initial stages, plus a frequent occurrence of malignant arrhythmias. Ventricular premature contractions are common. Runs of ventricular tachycardia, complete heart block, malignant arrhythmias and thromboembolic disease could all be contributing to the high prevalence of sudden death in chagasic patients. The overall 5 year mortality for patients with established Chagas cardiomyopathy is over 50%.  

EGG AND ECHOCARDIOGRAPHY FINDINGS

We studied early echo and ECG changes in patients with Chagas disease in Honduras and found 56% of asymptomatic patients infected with *T. cruzi* already had myocardial functional impairment and 29% had ECG changes, most commonly right bundle branch block (RBBB) alone or with left anterior hemiblock (fig 5).  

In the later symptomatic stage the majority of patients have grossly abnormal echocardiograms, representative of the widespread cardiac involvement including dilated ventricles, thinned walls and large left and right atria, and significant overall ventricular functional impairment (fig 4). ECG changes in these later stages are also pronounced, with both conduction disturbances and arrhythmias being found far more frequently than in dilated cardiomyopathies of other etiologies.  

DIAGNOSIS

*T. cruzi* parasites can often be detected by direct blood smear in the acute phase of the disease, but due to low levels of parasitaemia in the indeterminate and chronic phases, diagnosis has previously relied on xenodiagnosis and serological tests including ELISA and indirect immunofluorescence assay (IFA). Recently, molecular based assays using polymerase chain reaction (PCR) have been shown to be the most sensitive technique to diagnose patients with chronic Chagas disease, and has also been shown to be the most effective way for evaluation of cure following anti-trypanosome treatment.  

PATHOGENESIS

In the acute phase of the disease the myocardial tissues that develops has been associated with the parasitaemia affecting the target organ. The damage results from direct destruction due to intracellular parasitism, necrosis related to inflammation, and other cytostatic mechanisms involving CD8 T cells and, less frequently, CD4 T cells. Such cells recognize *T. cruzi* epitopes at the surface of infected cells, which...
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Figure 4 M mode echocardiogram showing a dilated left ventricle (6.38 cm is systole) of a patient with severe Chagas cardiomypathy, with pronounced hypokinesia of the thinned septum and posterior wall.

contain smasgotes, and non-infected cells, which have procesed parasite antigens.17

The histopathological picture of the myocardium in chronic Chagas disease is characterised by a chronic inflammatory process in all four chambers of the heart (with the right side often worse affected), with focal thickening, tissue destruction and interstitial fibrosis, either focal or later becoming diffusely distributed.18

The progression to this chronic stage and the factors determining why only a certain proportion of patients develop this stage remains poorly understood. Due to the scarcity of parasites in the myocardium in these later stages, and the occurrence of myocardial inflammatory infiltrate, many theories have implicated autoimmune and inflammatory processes in the pathogenesis. One of us (SY) is currently investigating the role of cytokines as mediators of disease progression in patients with different stages of Chagas disease and the role of cytokine gene polymorphisms as a predisposing factor to disease.19 20

Recent developments in molecular and immunohistochemistry have demonstrated T. cruzi antigen in the inflammatory foci in chronic Chagas hearts. This suggests that parasite persistence is driving the immune response, causing the low grade tissue destruction and chronic inflammation. However, the T. cruzi antigen remains scarce and not always associated with the inflammatory foci in the myocardium. It is likely that the pathogenesis of chronic Chagas cardiomypathy is a complex interplay between parasite and the host’s immune response. Further research is needed to investigate the mechanism of cardiac injury and why 30% of people infected progress to cardiomypathy.

PROGNOSIS

Patients with Chagas cardiomypathy have a different clinical progression to other cardiomypathies, often with a worse prognosis. A study of heart failure patients with cardiomypathies of different aetiologies in Brazil showed Chagas disease to be the most important determinant for mortality.21 A risk score for predicting mortality has recently been developed and validated for Chagas cardiomypathy.22 Of the six independent prognostic factors identified, the first three were related to functional class, cardiac myalgia and systolic dysfunction, plus demonstration of ventricular arrhythmias. Management therefore should be aimed at aggressive treatment of the heart failure and prevention of malignant arrhythmias.

MANAGEMENT

This consists of treating the different manifestations of the disease and, importantly, controlling the infection and applying preventive measures.

Cardiac failure treatment
Due to a lack of specific clinical trials for Chagas cardiomypathy patients, treatment has traditionally relied upon the same heart failure medications as have been used to treat other cardiomypathies, including diuretics, angiotensin converting enzyme (ACE) inhibitors and digoxin.23 In particular, ACE inhibitors, which have been shown to improve survival in the latter group,24 have been utilised in studies investigating the specific effect of these agents on chagasic patients. Captopril has been shown to improve cardiac function but not mortality from Chagas heart disease.25 Captopril is also an anti-inflammatory agent, acting through the immunomodulatory actions of angiotensin II and the downstream effects of bradykinin. In a mouse model of acute Chagas myocarditis, captopril significantly reduced cardiac necrosis and fibrosis without increasing parasite burden.26 β-blockers currently used in standard heart failure treatment have been postulated to be beneficial in Chagas cardiomypathy; however, data to confirm this are lacking. A recent study has demonstrated in a small cohort beneficial effect with the addition of the β-blocker carvedilol to an ACE inhibitor in cardiac function and clinical status of patients with chagasic cardiomypathy.27 Larger randomised controlled trials, including other β-blockers as well, are needed to verify these findings.

Treatment of rhythm disturbances
Due to the high frequency of atrioventricular (AV) nodal block in Chagas disease, pacemakers are often used and are a very effective treatment option, but they do not alter the progression of the disease.7 The use of these devices is life saving with a need for humanitarian organisations, individual professionals and commercial companies to donate devices to the local physicians who are willing to help deprived individuals free of charge.7

For tachyarrhythmias, antiarrhythmics such as amiodarone are used, and more recently implantable cardioverter-defibrillators (ICDs). Chagas disease patients have indications for ICD implantation similar to those of other patients.7 28 However, in patients with cardiomypathies of different aetiologies receiving ICDs, Chagas
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Transplantation

Heart transplantation for end stage Chagas cardiomyopathy has been shown to be a valuable treatment option, with old fears of reactivation of the disease in the allograft and therefore poor survival rates being disproved. In Brazil, follow-up of patients undergoing heart transplantation showed significantly better survival rates for Chagas cardiomyopathy compared to other types of cardiomyopathy. Reactivation of T. cruzi remains a concern, but improved molecular based diagnostics allows early identification of panatremia and successful treatment with antiparasitic agents.

Antiparasitic treatment

In the 1970s the antiparasitic agents benznidazole and nifurtimox were introduced for clinical treatment of Chagas disease, but high toxicity and variable efficacy, especially in the chronic phase, limited their use. More recent data suggest favourable outcomes in children and young adults treated with benznidazole in the early chronic phase of T. cruzi infection, with 83.3% showing negative seroconversion of T. cruzi antibodies at 3 year follow-up compared to controls.

In addition, a non-randomized trial in 2005 showed that the use of benznidazole in patients in indeterminate phase with no heart failure was associated with reduced progression to chronic Chagas cardiomyopathy. A Cochrane Review in 2002 also suggests potential for trypanocidal drugs in asymptomatic patients, but emphasizes the need for large randomised controlled trials including newer agents to confirm these findings.

New antiparasitic drug developments

Although there have been no new licensed antitrypanosomal drugs since the 1970s, the recent sequencing of the T. cruzi genome in 2005 opens up possibilities for the development of novel therapeutics.

Currently experimental antifungal triazoles are reported to show good trypanocidal effects in acute and chronic murine models. Trichodazone in particular has shown excellent parasitological cure in animal models and human Chagas disease. Allopurinol has also shown encouraging results in treating chronic T. cruzi infections with far fewer side effects than conventional antitrypanosomal treatments.

Other potential antiparasitic agents against T. cruzi in development include tryptopyrones, antiapoptotic clonal inhibitors and pyrazinemethane (cruzipain) inhibitors.

There is an urgent need for human clinical trials of these new therapeutic agents for the 15 million people currently infected with T. cruzi for which there are grossly inadequate treatment options.

CONTROL OF DISEASE

In the early 1990s the “southern cone initiative” included many countries in South America and saw great reductions of transmission, through vector control of Chagas disease and the development of alternative agricultural systems. More recently the Andean and Central American countries have adopted similar control programmes including the use of insecticide spraying with synthetic pyrethroids, housing improvements and health education.

In July 2007 the WHO launched a “global network for Chagas elimination” with the goal of coordinating efforts to eliminate Chagas disease by 2030. However, as T. cruzi has many animal reservoirs, completely eradicating the disease seems almost impossible. As new rural housing developments spread and insecticide spraying is labour intensive and expensive, without continued vigilance and surveillance resurgence of Chagas disease will remain a threat.

An integrated approach to prevention, diagnosis and management is badly needed.

CONCLUSION

Chagas disease is no longer a sole problem for the rural poor of South America. An estimated 10 million Latin Americans are currently resident in the USA, with up to 0.5 million thought to be infected with T. cruzi.

The management of Chagas disease depends on the stage, with patients in the acute and indeterminate phase most likely to benefit from antiparasitic treatment. Management of patients in the advanced stages should include aggressive heart failure treatments and antiarrhythmics including ICDs and heart transplantation considered for those with end stage disease.

Further research is still needed to elucidate the complex immunology underlying the pathogenesis of Chagas disease, which in turn may lead to the well needed development of novel therapeutics.
Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis

Appendices
Neglected tropical cardiomyopathies: II. Endomyocardial fibrosis

Ana Olga Mocumbi, Sophie Yacoub, Magdi H Yacoub

Epidemiology

Endomyocardial fibrosis (EMF) was described as a distinct clinicopathological entity in 1948 in Uganda. Initially several terms were used to describe the disease. These included tropical endomyocardial disease, endocarditis pannici fribroplastica, endocardial fibrosis, constrictive endocarditis and endocardial fibroelastosis.

Epidemiology

EMF is thought to be the most common type of restrictive cardiomyopathy worldwide. Although most studies have been reported from Uganda, Ivory Coast, Nigeria, Brazil and India, the disease has been occasionally encountered outside the tropics. Geographic distribution of EMF is not uniform both in Africa and Asia. In the endemic areas of Africa, EMF is the second cause of admission for acquired cardiovascular disease in children and young adults after rheumatic heart disease, accounting for up to 20% of all cases of heart failure.

To date our knowledge of the prevalence of the disease is derived from hospital based studies with absence of data from systematic studies in the community. EMF affects predominantly children and adolescents, usually from low socioeconomic background. More than half the cases are seen in the first decade of life. Male predominance was found in Kenya and Nigeria, while female predominance has been described in Brazil and Uganda. A bimodal age distribution has been reported in some studies.

Aetiology and Pathogenesis

The aetiology of EMF remains unknown. Several hypotheses have been proposed and explored including cardiotoxicity of the eosinophil, infectious agents, autoimmune processes, genetic predisposition, ethnicity, diet, climate and poverty.

Hyper eosinophilia

EMF appears to share some pathogenic mechanisms with the hypereosinophilic syndromes, which comprise a heterogeneous group of disorders characterised by peripheral eosinophilia for at least 6 months and end organ damage related to eosinophil infiltrations. These syndromes have been classified as idiopathic, familial and reactive. The similarity of mechanisms responsible for EMF and the hypereosinophilic syndromes could have exciting therapeutic implications.

Eosinophilic patients with hypereosinophilia have unusual morphology, are metabolically and functionally more effective than normal, and show a preponderance of multilobed forms and evidence of degranulation. Granule proteins mediate the cardiovascular toxicity of the eosinophils through activation of heart mast cells. Eosinophils are also capable of secreting an array of pro-fibrotic mediators, including plasminogen activator inhibitor-2 (PAI-2). This mediator has a role in plasminogen-plasmin mediated tissue destruction and remodeling and this could explain the role of eosinophils in deposition of fibrous tissue in the heart.

Severe hypereosinophilia is found in some patients with EMF and its level seems to be inversely related to the duration of the illness. Hypereosinophilia in this condition is probably induced by parasitic infections in most cases, but this link remains difficult to establish because the eosinophil count peaks during larval migration returning to normal thereafter.

Infection

Occurrence of EMF in people from Europe and North America after short stays in endemic areas supports the role of infestation in the pathogenesis of the condition. EMF has been occasionally associated with helminths, schistosomiasis, loa-loa, filariosis and malaria. However, several studies failed to show an increased prevalence of these infections in EMF patients when compared to the general population. The finding of endomyocardial fibrotic lesions mimicking human disease in mice infected with Plasmodium berghei supports the infection hypothesis and provides one of the best models of the disease.

Autoimmunity

The role of autoimmunity is suggested by the finding of high titres of malaria-specific antibodies, a high incidence of hypereosinophilic syndrome and raised anti-streptolysin titres in patients with EMF.

Genetic predisposition

Familial occurrence and the reported high incidence among certain ethnic groups in some countries also suggest genetic susceptibility.
Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis

Dietary factors
Prolonged ingestion of the tuber cassava (tapioca) in association with extreme deprivation of proteins, mainly tryptophan deficient diets, has been considered a risk factor for EMF. McKinney et al. produced an experimental model of EMF and was able to show the protective role of high protein diet in guinea pigs fed on a diet consisting largely of plantains. Animal models using African green monkeys also showed that ingestion of uncooked cassava caused endomyocardial degeneration of the ventricle, apical thickening and interstitial fibrosis.  

Geochemical factors
Increased levels of cerium derived from monazite soils were found in the hearts of people with EMF living in the coastal regions of the tropics. Cerium triggers a wound healing response in the cardiac tissue of rats leading to cardiac fibrosis, which could result from direct stimulation of subendocardial fibroblasts by this element. EMF cannot be explained by a single cause in all areas where it has been reported. The disease appears to be triggered by several environmental factors acting upon individuals with a genetic predisposition.

PATHOPHYSIOLOGY
EMF appears to have an initial stage of febrile illness and pancreatitis, frequently associated with hyperammonemia, facial and peri-oral swelling, body itching, urticaria and neurological features. This febrile episode, triggered by one or more unknown factors, is followed by ventricular thrombosis affecting usually the apices and the subvalvar apparatus. This causes obliteration of the ventricular cavity and interference with the valve mechanism that then evolves to organisation of thrombus and endocardial fibrosis typical of advanced stages of the disease.

The fibrosis of the mural and valvar endocardium impedes filling and causes valve distortion, both leading to restrictive physiology with atrioventricular regurgitation, and the typical small ventricles with severely dilated atria. The resulting heart failure with venous congestion and sustained low cardiac output explains most clinical findings, but there is still some controversy regarding the pathophysiology of central cyanosis, ascites and the absence of pedal oedema.

Finger and toe clubbing, growth retardation, testicular atrophy, failure to develop male secondary sexual characteristics, and cachexia are the result of sustained low cardiac output. Chronic systemic venous hypertension is the origin of most of the characteristic signs of right ventricular EMF—namely, exophthalmos, elevated jugular pressure, gross hepatomegaly, congestive splenomegaly, and voluminous ascites that can lead to ventral or inguinal hernia.

Death results from complications of chronic heart failure, but can occur suddenly due to thromboembolism or arrhythmia.

DIAGNOSIS
By the time of clinical presentation most patients have advanced disease with signs of longstanding heart failure, but usually give a poor history. Most patients recall a febrile episode associated with tiredness, breathlessness, cough, swelling of the face, urticaria and abdominal distension, followed by a variable interval free of symptoms—a few weeks or months in left ventricular EMF, and over 1 year in right ventricular EMF.

Clinical types
The clinical picture of EMF depends on the activity of the disease, the chamber affected and the severity of the lesions. Active disease is suggested by recent febrile episodes and the onset of heart failure, sometimes associated with urticaria and facial oedema.

Isolated or dominant left ventricular EMF
These patients have usually a good general status, pronounced dyspnea and orthopnea, and small displacement of the apex beat that is not particularly hyperdynamic. There is characteristically a soft and short systolic murmur confined to early systole. A delayed opening snap is present, due to thickening of the anterior leaflet of the mitral valve, and the pulmonary component of the second sound is loud indicating high pulmonary pressure. Occasionally, there are signs of systemic embolism.

Isolated or dominant right ventricular EMF
Most patients have proptosis, facial oedema, cyanosis, distended jugular veins and finger clubbing. Many exhibit retardation of growth and underdevelopment of secondary sexual characteristics. Some have a variable degree of jaundice, a considerable proportion present in atrial fibrillation.

A left parasternal pulsation is usually noted, and corresponds to the compensatory hypercontractile right ventricular outflow tract. The presence and intensity of the systolic murmur from the tricuspid regurgitation is variable, but a third sound is always present. Ascites is in most cases disproportionate to the almost inessential pedal oedema (fig 1), and is associated with hepato-splenomegaly.

Biventricular EMF
This group includes the majority of EMF patients. Predominance of right sided lesions is the most common form and these, by reducing pulmonary perfusion, avoid the hazards of severe pulmonary hypertension caused by left sided lesions, allowing longer survival.

Ascites is a striking feature in right ventricular EMF, but is also seen in left ventricular disease. The ascitic fluid is typically an exudate containing more protein and leukocytes (predominantly lymphocytes) than expected with right heart failure, suggesting some role of inflammation in the pathogenesis and, in advanced stages, a role of protein losing enteropathy.

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Laboratory findings
To date there is no specific laboratory test for diagnosing EMF. The presence of hypereosinophilia is variable and more frequent early in the disease. Characteristically the eosinophils show large granules, degeneration around the nucleus, vacuolization and nuclear changes. Hypoalbuminaemia is rare except in advanced disease. Similarly biochemical changes secondary to liver and/or kidney dysfunction are common. The presence of procoagulant state and activation of the immune system is currently being investigated by us in Mozambique.

Chest x ray
The chest x ray in right ventricular EMF shows right atrial enlargement and a bulge over the left heart border due to dilatation of the right ventricular outflow tract, with clear lung fields (fig 2A). Pericardial effusion when present is suggested by the presence of a large globular heart shadow. In cases of isolated left disease there is evidence of pulmonary venous congestion, an enlargement of the left atrium and a prominent pulmonary artery (fig 2B). Rarely, a linear endocardial calcification may be seen.

Electrocardiography
The ECG shows no constant abnormalities. Atrial fibrillation is present in many cases at first admission. In advanced disease there are low voltage QRS complexes, non-specific ST–T wave changes, and conduction disturbances. In predominant right forms the ECG shows a tall and broad right atrial wave and a characteristic ‘QR’ pattern in V3R or V1. In left EMF there are signs of left atrial hypertrophy and pulmonary hypertension.

Echocardiography
Echocardiography has been the main technique for the diagnosis of endomyocardial fibrosis, enabling the assessment of anatomy and cardiac function (table 1).

Criteria used for the diagnosis of right EMF are apical obliteration, reduction of the right ventricular cavity size, paradoxical movement of the interventricular septum, cleavage plane between the fibrous tissue and myocardium, parietal thrombosis, dilatation of the right ventricular outflow tract, dilatation of the right atrium,
tricuspid valve adherent to the endocardium with regurgitation, diastolic opening of the pulmonary valve, dilatation of the inferior vena cava, and pericardial effusion (fig 5A).

In left EMF the most important features are endocardial hyperdensity in the apex associated with reduction in the longitudinal diameter of the ventricle that becomes spherical or oval, with dilatation and hypercontractility of the basal portion. On Doppler evaluation there is a rapid deceleration of the E-wave with unimpressive A-wave. The posterior leaflet of the mitral valve is usually retracted or completely absent and is associated with severe anterior valve incompetence (fig 3B) and left atrial dilatation. There is usually tricuspid regurgitation of variable degree allowing confirmation of pulmonary hypertension.

We have recently evolved and used a set of echocardiographic criteria for classifying and grading the different stages of EMF (AO Macombe, unpublished data).

**Computed tomography**

To date, computed tomography (CT) has not been used for diagnosis of EMF. The disease is suggested by the presence of linear calcification parallel to the pericardium and along the inner border of the myocardium. Further studies using multislice CT may help to elucidate several structural and functional changes at different stages of the disease.

**Magnetic resonance imaging**

Magnetic resonance imaging (MRI) provides a powerful tool to define and localise many of the pathological features in the hearts of patients with EMF, including fibrosis, poor perfusion and calcification. In addition, MRI can be used to quantify and characterise three dimensional myocardial movements and flow patterns, which can be extremely valuable both for management and as a research tool. Unfortunately it is not easily available in most areas where EMF is endemic, an issue that needs to be urgently addressed.

**Cardiac catheterisation**

Invasive studies are not routinely used anymore, either for diagnosis or management, and are currently used to answer specific questions such as the degree of pulmonary hypertension and its response to pharmacological agents.
Other less frequent conditions that can be misdiagnosed as EMF are amyloidosis, haemochromatosis, myocardial sarcoidosis and neoplastic infiltration of the heart. Right ventricular EMF should be distinguished from Ebstein’s disease.

MANAGEMENT
There is no specific treatment for EMF. Medical treatment is used to control the acute disease, heart failure and arrhythmias, as well as for pre- and postoperative management. Several invasive procedures also have a role in controlling the acute and chronic complications of the disease—namely, peritoneal, pleural and pericardial drainages.

Medical treatment
During acute illness, treatment is aimed at maintaining cardiac function and suppressing the eosinophilia using short courses of oral corticosteroids, usually 7–10 days. Symptomatic treatment with diuretics has been shown to be useful, although high doses are usually required. Digoxin and β-blockers are used mainly in patients with rhythm disturbances to control heart rate. Since the population affected is typically not compliant with anticoagulation regimens and has no access to effective control of this treatment, low dose aspirin frequently replaces oral anticoagulants in patients with atrial fibrillation. Due to their known effects in reducing fibrosis, spironolactone and angiotensin converting enzyme inhibitors may be useful.

**Neglected tropical cardiomyopathies: key points**
- Chagas disease and endomyocardial fibrosis represent two examples of neglected diseases in cardiology.
- These two neglected diseases affect several million individuals in developing countries.
- Chagas disease is the most common form of cardiac disease in many countries in Latin America, causing dilated cardiomyopathy. An estimated 15 million people are currently infected.
- Endomyocardial fibrosis is thought to be the most common restrictive cardiomyopathy worldwide. In endemic areas in Africa it is responsible for 9–20% of all cases of heart failure.
- These two diseases have many specific features but share others with the usual forms of dilated and restrictive cardiomyopathy in developed countries.
- There is a need for cardiologists to be involved in the epidemiology, prevention, treatment and fundamental research into these diseases. This could not only save many lives but could also contribute to knowledge regarding other diseases.
However, their value needs to be evaluated in prospective randomised trials.

Surgery

The most commonly used surgical technique is targeted endocardial resection combined with valve repair or replacement through anastomosis. The reported early postoperative mortality has been between 15–30%, mainly due to low cardiac output syndrome and arrhythmias, and complications of prosthetic valves and permanent pacing accounted for the late mortality seen on follow-up of earlier series. Successful surgery has a clear benefit on symptoms, favourably affects survival, and gives the best long term results on isolated left ventricular EMF.

Since diagnosis is rare in western countries, and because of a lack of infrastructure for cardiac surgery in most areas where EMF is endemic, surgical experience consists of isolated episodic attempts by several groups usually involving patients with very advanced stages of the disease. A more systematic approach to surgical treatment requires accurate characterisation of the disease in each specific patient, with development of tailored surgical therapy combined with appropriate timing of the operation and intensive preoperative medical treatment. This approach is currently being tried at the Maputo Heart Institute and needs to be more widely applied in combination with integrated programmes of management.

PROGNOSIS

The overall prognosis of EMF is poor, with a high incidence of sudden death from fatal arrhythmias and thromboembolism. The prognosis of this disease can, however, be improved by early diagnosis coupled with better understanding of the pathogenesis and identification of new therapeutic targets.

Our early experience suggests that surgical treatment can be lifesaving with acceptable intermediate term results. This, however, depends critically on evolving integrated programmes for early diagnosis and management of the condition, as mentioned above.

CONCLUSIONS AND FUTURE DIRECTIONS

EMF is one of the most neglected diseases in cardiology, affecting mainly children from poor areas in developing countries. The disease has an unclear aetiology, the pathogenesis is unknown, it carries a poor prognosis, and it has no specific treatment. The availability of portable echocardiography gives an opportunity for early diagnosis and prospective studies in the affected communities, which can ultimately lead to better understanding of the natural history of the disease. Research on the mechanisms of the disease is mandatory to identify new therapeutic targets and improve the outcomes, as well as to explore preventive measures to avoid the disease or progression to advanced forms. Development of specific drugs aimed at preventing proliferation, migration and injury produced by eosinophils, combined with attempts at defining new therapeutic targets and proper application of tailored surgical procedures, should all continue to ameliorate the devastating effects of this neglected disease.

Competing interests: In compliance with EBAC/EACME guidelines, all authors participating in Education in Heart have disclosed potential conflicts of interest that might cause a bias in the article. The authors have no competing interests.

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Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis
Appendices

Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis

Images in Cardiovascular Pathology

Right ventricular endomyocardial fibrosis

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Abstract

Endomyocardial fibrosis (EMF) is a neglected tropical cardiomyopathy of unknown etiology and pathogenesis that is common in certain tropical areas of Africa, Asia, and South America. It affects predominantly children and young adults. Endomyocardial fibrosis is the hallmark of this restrictive cardiomyopathy leading to restriction to diastolic filling of the ventricles with severe atrial dilatation. Endomyocardial fibrosis carries a poor prognosis, due to the late presentation of patients. The salient features of this condition are present in the case presented here of a 14-year-old boy who died from progressive heart failure due to right ventricular EMF. These pathological findings of advanced disease highlight the need for early diagnosis and better understanding of the pathogenesis in order to improve prognosis of this debilitating and fatal disease. © 2003 Published by Elsevier Inc.

Keywords: Endomyocardial fibrosis, Restrictive cardiomyopathy, Thrombosis

EMF, and despite intensive medical treatment with diuretics, ACE inhibitors, β-blockers, and angiotensin, the boy died of refractory heart failure a few weeks later.

Fig. 1. Photograph of the excised right atrium and ventricle showing gross dilatation of the right atrium with a large hemispherical thrombus occupying most of the atrial cavity. The thrombosed valve annulus is severely dilated with preservation of the cusps and chordae. Endomyocardial fibrosis involving the mural endocardium of the right ventricle is apparent with obliteration of the apex of the right ventricle.
Appendices

Epidemiology, Pathogenesis and Management of Endomyocardial Fibrosis

myopathy and antiventricular valve regurgitation, causing the typical finding of small ventricles with enlarged atria. Mural ventricular thrombosis constitutes probably the initial lesion of EMF, whereas atrial thrombi found in advanced disease result from blood stasis related to severe atrial dilatation and arrhythmia.

The histological hallmark of this condition is marked endocardial thickening due to subendothelial deposition of dense fibrous tissue that extends to the underlying myocardium forming septa and surrounding the intramyocardial vessels [2] (Fig. 2). The thickened endocardium consists of superficial compact layer of collagen with occasional hyalinisation, and a deeper layer of loose fibrous tissue with variable amount of capillaries and lymphocytes. The myocardial fibres of the inner myocardium frequently show degenerative changes, and luminal thrombosis in intramyocardial arteries has also been described.

The management of this debilitating condition needs better understanding of the pathogenic mechanisms involved. Up till now, EMF patients present with advanced fibrotic lesions when response to medical treatment is poor and surgery (consisting of endocardial resection and valve repair or replacement) is associated with high morbidity and mortality [3]. Recent attempts to improve the outcome include epidemiological research aiming at early detection and the use of tailored approaches to the management in specialized centres of endemic areas [4].

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References

A Population Study of Endomyocardial Fibrosis in a Rural Area of Mozambique

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ABSTRACT

BACKGROUND
Endomyocardial fibrosis is the most common restrictive cardiomyopathy worldwide. It has no specific treatment and carries a poor prognosis, since most patients present with advanced heart failure. On the basis of clinical series, regional variations in distribution have been reported within several countries in Africa, Asia, and South America, but large-scale data are lacking on the epidemiologic features and early stages of the disease.

METHODS
We used transthoracic echocardiography to determine the prevalence of endomyocardial fibrosis in a rural area of Mozambique. We screened a random sample of 1063 subjects of all age groups selected by clustering. Major and minor diagnostic criteria were defined, and a severity score was developed and applied. Cases were classified according to the distribution and severity of the lesions in the heart.

RESULTS
The estimated overall prevalence of endomyocardial fibrosis was 19.8%, or 211 of 1063 subjects (95% confidence interval [CI], 17.4 to 22.2). The prevalence was highest among persons 10 to 19 years of age (26.1%, or 73 of 260 subjects [95% CI, 22.6 to 33.6]) and was higher among male than among female subjects (23.0% vs. 17.5%, P=0.05). The most common form was biventricular endomyocardial fibrosis (a prevalence of 55.5%, or 117 of 211 subjects [95% CI, 48.8 to 62.2]), followed by rightsided endomyocardial fibrosis (a prevalence of 20.0%, or 59 of 291 subjects [95% CI, 19.9 to 5.1]). Most affected subjects had mild-to-moderate structural and functional echocardiographic abnormalities. Only 48 persons with endomyocardial fibrosis (22.7%) were symptomatic. The frequency of familial occurrence was high.

CONCLUSIONS
Endomyocardial fibrosis is common in a rural area of Mozambique. By using echocardiography, we were able to detect early, asymptomatic stages of the disease. These findings may aid in the study of the pathogenesis of the disease and in the development of new management strategies.
ENDOMYOCARDIAL FIBROSIS IS A NEGLECTED TROPICAL DISEASE THAT AFFECTS AN ESTIMATED 10 MILLION PEOPLE WORLDWIDE. The disease is the most common cause of restrictive cardiomyopathy, with impaired filling of one or both ventricles caused by deposition of fibrous tissue on endocardial surfaces. This results in right heart failure, left heart failure, or both. The heart failure is associated with atrioventricular valve regurgitation. Endomyocardial fibrosis is a major cause of illness and death in areas where it is endemic, and in its severest form carries a very poor prognosis, with an estimated survival of 2 years after diagnosis. The cause and pathogenesis of the disease are poorly understood; several theories are being considered. Currently, there is no specific drug therapy, and surgical treatment of advanced cases carries a poor prognosis and is rarely performed. Most studies of endomyocardial fibrosis have been done in Uganda, Nigeria, the Ivory Coast, south India, and Brazil, in regions situated within 15 degrees of the equator. However, cases have been reported in countries outside the tropics, including industrialized countries. Regional variations in the distribution of endomyocardial fibrosis have been noted in countries where the disease is endemic.

A study of autopsies performed between 1975 and 1977 described the first five recognized cases of endomyocardial fibrosis from Mozambique, showing that the condition was not so rare as previously thought. Subsequently, clinical cases were reported, and an analysis of referrals to a cardiovascular clinic in the capital, Maputo, over a 10-year period showed that a large proportion of patients came from Inharrime, a rural coastal district in the south of Mozambique. A prevalence study carried out in this district, with cardiac auscultation as the screening method, confirmed endomyocardial fibrosis as a public health problem, with a prevalence reaching 8.9% among persons 5 to 45 years of age. Most of the literature on endomyocardial fibrosis describes clinical series of patients in advanced stages of the disease, and large-scale epidemiologic studies are lacking.

We designed a study using systematic sampling of the community coupled with detailed echocardiographic examination in randomly selected subjects. The aim was to determine the prevalence of endomyocardial fibrosis in Inharrime, Mozambique, and to describe its severity and mode of presentation in the community.

SAMPLE SIZE CALCULATION

The sample size was calculated by an adaptation of the two-stage cluster-sampling approach. For practical and logistic reasons, we opted for a cluster size of 30 subsets. The number of clusters was calculated by the formula $n = \frac{p(1-p)D}{\sigma^2} b$, where $n$ is the number of clusters, $p$ is the expected prevalence of the condition (7%), the prevalence in a previous study was lower, D is the study design effect (1.58, with a rate of homogeneity of 0.02), $\sigma$ is the required standard error of the estimated prevalence (1%), and $b$ is the cluster size. We thus arrived at a sample size of 33 clusters, corresponding to a minimum of 990 subjects.

SAMPLE SELECTION

The sample was selected at two stages, first at the povada level and second at the household level. Since there were no data on the population size of individual povadas, we used random probability-proportional-to-size sampling. We therefore selected 33 villages by simple random sampling. Community leaders from the selected villages were asked to create a list of all households in each povada. The investigators obtained written informed consent from the head of the household when they arrived at the index household. Second-stage clustering was performed after the investigators had visited the index household.

A household was defined as a group of people living together for more than 3 months and sharing food on a daily basis. The investigators obtained written informed consent from the head of the household when they arrived at the index household. An identification card was issued to the family and each member was assigned a unique identification number. The exact location of the house was registered with the use of a...
global positioning system (Garmin eTrex Legend) for follow-up visits.

The households surrounding the index household within the limits of the village were noted. A number was assigned to each, and the next household to be visited was chosen by simple random selection. This procedure was repeated for the next household until at least 50 subjects had been enrolled. The Mozambican National Bioethics Committee approved the research protocol.

**Data Collection**
Demographic data were obtained from every member of each selected family with the use of a questionnaire. A cardiologist then performed detailed transthoracic echocardiography on each family member with the use of a hand-carried, battery-operated echocardiographic system (Vivid i, General Electric) with M-mode, two-dimensional, and Doppler (pulsed, continuous, and color) imaging. The following were obtained: apical two-, four-, and five-chamber views; parasternal long-axis and short-axis views; at the level of the papillary muscles and the aortic valve; subcostal views; and suprasternal views (parallel to the aortic arch). Electronic records of relevant data were kept.

**Diagnosis and Classification of Endomyocardial Fibrosis**
We defined major and minor criteria for the diagnosis of endomyocardial fibrosis on the basis of features of advanced disease and pathologic features of early stages described in postmortem studies. Endomyocardial Fibrosis was diagnosed in the presence of two major criteria or one major criterion associated with two minor criteria (Table 1). Diagnoses were accepted after agreement of two independent, experienced cardiologists. Endomyocardial Fibrosis was classified as biventricular, right-sided, or left-sided according to whether the structural lesions involved both ventricles without predominance of one side, or predominantly the right ventricle, or only or predominantly the left ventricle, respectively. We also developed a severity-scoring system based on the type and degree of the structural and functional changes (Table 1). Possible scores ranged from 0 to 35; cases with scores of 8 or less were classified as mild, those with scores of 8 to 15 as moderate, and those with scores of 15 or more as severe. A detailed clinical examination was performed in all subjects with endomyocardial fibrosis.

**Statistical Analysis**
Frequencies are given as absolute numbers and percentages; continuous data are reported as means ±SE. We used the chi-square test to compare percentages between groups and logistic regression to test for trends in percentages of continuous variables. Association between continuous variables was expressed by the Pearson correlation coefficient, with a test for zero correlation. Variation within families was examined by mixed-model analysis of variance. Prevalence data are presented as means with 95% confidence intervals. Analyses were performed with Minitab Release 15 and SAS Release 8.22 software.

**Results**

**Study Population**
We selected 217 households to be visited. The family was absent in 3 households, and the remaining 214 households had a total of 1249 members. The mean family size was 5.84 ± 0.2 (range, 1 to 19); the mean age was 22.6 ± 0.6 years; 682 (54.6%; 95% confidence interval [CI], 51.6 to 57.4) were female; and all but 4 were black. One hundred eighty-six eligible subjects (14.9%) were not examined: 99 were adult men who were away
from home working as miners in neighboring South Africa; 79 were not at home (the families said that disease was not the reason for their absence; and 6 had traveled to hospitals outside the community in search of treatment). No dead diagnosis of cardiac disease was suggested by the history given by the family. Echocardiography was not performed in two subjects who had uncontrollable, aggressive behavior. No other subjects declined to freely participate.

PREVALENCE, TYPE, AND SEVERITY OF ENDOMYOCARDIAL FIBROSIS

We performed 1063 transthoracic echocardiographic procedures. The mean age of the screened subjects was 22.5±7.0 years, and 611 (57.5% [95% CI, 54.5 to 60.5]) were female. Two hundred eleven of the screened subjects (19.8% [95% CI, 17.4 to 22.2]) had endomyocardial fibrosis. The disease was biventricular in 117 subjects (55.5% [95% CI, 48.8 to 62.2]), rightsided in 59 (28.0% [95% CI, 21.9 to 34.1]), and left-sided in 35 (16.6% [95% CI, 11.6 to 21.6]). Of the 211 affected subjects, 163 (77.3%) had mild disease, 39 (18.5%) had moderate disease, and 9 (4.3%) had severe disease. Only 48 persons with endomyocardial fibrosis (22.7%) were symptomatic. Figure 2 shows echocardiograms illustrating different types and degrees of severity of endomyocardial fibrosis. The most frequent lesions in mild disease were apical obliteration of the right ventricle, diffuse thickening of the mitral valve, and mild mitral or tricuspid regurgitation.

The prevalence of endomyocardial fibrosis differed among age groups (P=0.001) but there was no systematic increase or decrease in prevalence with age (P=0.94 for all comparisons) (Fig. 3). The prevalence was highest among subjects 10 to 19 years of age (73 cases in 200 subjects, or 36.5% [95% CI, 22.6 to 51.6]). Left-sided endomyocardial fibrosis was more common than biventricular or right-sided endomyocardial fibrosis among subjects over 30 years of age (32.7% vs. 11.3%, P=0.001).

The prevalence of endomyocardial fibrosis was significantly higher among male than among female subjects (25.0% vs. 17.5%, P=0.03); the difference between the sexes was greatest in the group that was 20 to 29 years of age (Fig. 3). There were no significant differences between male and female subjects in the location of the principal lesions (P=0.2).

Of the 214 families studied, 99 had no cases of endomyocardial fibrosis, 65 had one case, and 52 had more than one case. There was no correlation between the percentage of persons with endomyocardial fibrosis in a family and the family size (r=0.095, P=0.17). However, the chance of a person having the disease was higher when other members of the family had it. As compared with the overall prevalence of endomyocardial fibrosis (19.8% [95% CI, 17.4 to 22.2]), the prevalence was 24.9% (95% CI, 20.6 to 27.4) among persons who had one or more other family members with endomyocardial fibrosis, 26.0% (95% CI, 23.4 to 31.2) among those with two or more affected family members, and 38.3% (95% CI, 31.2 to 46.4) among those with three or more affected family members.

DISCUSSION

In this study we used echocardiographic screening to examine the prevalence, type, and severity...
Figure 2. Echocardiograms Showing Types and Degrees of Severity of Endomyocardial Fibrosis.
Panel A shows mild left endomyocardial fibrosis with occupation of the left ventricular apex (arrow). The interventricular septum is dense, with a thickened anterior mitral leaflet. Panel B shows moderate right endomyocardial fibrosis, with obliteration of the trabecular part and fibrous floor of the right ventricular cavity (arrow). The right atrium (RA) is dilated. Panel C shows severe bilateral endomyocardial fibrosis, with obliteration of the left ventricular apex (arrow), thickened valves, and biventricular dilatation.

Figure 3. Prevalence of Endomyocardial Fibrosis According to Age Group and Sex. Error bars indicate standard errors.

of endomyocardial fibrosis in a community in Mozambique. Echocardiography is the standard technique for the diagnosis of this condition, but the criteria described in the literature apply only to the disease in the advanced stages.27,28 We describe a set of criteria for the diagnosis and classification of endomyocardial fibrosis (Table 1) that we believe will be useful in staging the disease, studying its progression, and comparing the results of different epidemiologic studies.

The prevalence of endomyocardial fibrosis in the community we studied (19.8%) was higher than the 8.9% found in a previous survey in Inharrime that used cardiac auscultation followed by confirmation with the use of echocardiography.24 The lack of association between clinical and echocardiographic findings in endomyocardial fibrosis has previously been reported29 and may partly explain the late presentation of patients in hospital-based series.7

We identified several types of endomyocardial fibrosis by echocardiography, some of which represented early stages not previously described with the use of this diagnostic tool but corresponding to the findings from autopsy studies.1 Mild endomyocardial fibrosis was common in all age groups in our study. In addition, we identified several specific features of endomyocardial fibrosis, including thrombi involving the mitral...
subvalvular apparatus with mild regurgitation, obliteration of the right ventricular trabecular cavity, and obliteration of the recess between the posterior wall and the posterior papillary muscle. We are presently conducting echocardiographic follow-up of these subjects to clarify the natural history of the disease.

In our population, left-sided endomyocardial fibrosis was more frequent among adults over 30 years of age than among younger subjects. In clinical series, by contrast, the percentage of subjects with left-sided endomyocardial fibrosis is lower in persons over 30 years of age than in younger persons, perhaps because of early death from severe pulmonary hypertension and low cardiac output.

The prevalence of endomyocardial fibrosis showed a bimodal age distribution, with the first peak occurring in the second decade of life and the second in the fourth decade (Fig. 3). This age distribution, which is also found in hospital-based studies, may reflect the age of exposure to an infection, a cohort effect of exposure to an episodic environmental factor, or exposure to the same etiologic factors early in life with delayed manifestation of the disease in some subjects.

Endomyocardial fibrosis was more frequent in male than in female subjects. There were no differences between the sexes in the relative prevalence of different types of endomyocardial fibrosis (biventricular, left-sided, or right-sided).

Our study confirms the familiar occurrence of endomyocardial fibrosis that was previously reported in clinical series, which could be due to genetic or environmental factors or both. Future studies correlating the disease with specific genetic patterns and cohort studies looking at the role of environmental factors will help to clarify this issue.

The high prevalence of endomyocardial fibrosis in this community cannot be generalized to the country of Mozambique. Local variations in the distribution of this disease have been noted in other African countries. Several factors, such as poor diet, low socioeconomic status, malnutrition, viral infection, parasitic disease, and genetic differences, may be implicated.

Our study, by using objective methods of diagnosing, classifying, and grading the severity and stage of the disease in a relatively large cohort of patients with mild as well as severe forms of the disease, can help in understanding the pathophysiology, progression, and therapeutic response of the disease.

One limitation of our study is that 14.9% of the initial sample was not available for echocardiographic evaluation. However, extensive questioning of family members and community leaders suggested that this group did not have advanced disease or other characteristics that would distinguish them from the sample studied.

This study showed a high prevalence of endomyocardial fibrosis in a rural community of Mozambique. Echocardiography, together with the use of a newly described grading system, detected and characterized early and asymptomatic stages of the disease. The prevalence was highest among persons 10 to 19 years of age and was higher among male than among female subjects. Further work is required to evaluate the mode, mechanisms, and rate of progression of endomyocardial fibrosis, as well as the role of genetic susceptibility in determining the types of the disease and its patterns of familial occurrence. The large population of subjects affected offers unique opportunities for studying the basic molecular mechanisms responsible for the disease; the results could help to develop strategies for prevention and treatment and could be valuable for understanding other forms of cardiomyopathy and diseases involving fibrosis.

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