Clinical and experimental studies in idiopathic and Crohn's-related anal fistula

By

Philip James Tozer MBBS MRCS Eng MCEM

Thesis submitted for the degree of

MD(Res)
Department of Surgery and Cancer
Imperial College London
2011

From

The Fistula Research Unit, St Mark’s Hospital
Watford Road, Harrow, HA1 3UJ
Declaration of originality

The following thesis and all studies embodied therein represent my own work including data collection and analysis, specimen retrieval, transport and processing of tissue, walk out, flow cytometry and other immunological techniques, histology, immunohistochemistry and fluorescent in situ hybridisation. Surgical procedures and colonoscopies were undertaken by the clinical staff at St Mark’s Hospital. Electron microscopy was kindly performed by the Centre for Ultrastructural Imaging at Kings College, London, and assistance in multiplex analysis was provided by the Antigen Presenting Research Group, Imperial College London. A proportion of the 3 year MRI data presented (up to 18 months follow up in around three quarters of the patients) were collected before I took over the running of the study.

All other work reported in this thesis is appropriately referenced.
Abstract

The factors leading to the creation and persistence of anal fistula in Crohn’s disease are poorly understood. As with luminal Crohn’s disease genetic, microbiological and immunological factors are implicated but the immunological and microbiological composition of Crohn’s and idiopathic anal fistulae have been obscure.

My data demonstrate a lack of clinically relevant organisms within fistula tracts, a luminally driven immune response and subtle differences in this response between Crohn’s and idiopathic fistulae which may provide the basis for diagnostic tests, interventions and further research.

Surgical treatment of anal fistula is characterised by a compromise between risk of recurrence and impairment of continence. In complex, recurrent and multiply operated anal fistulae, fistulotomy can still provide a high success rate with low additional risk of impairment of continence.

Rectovaginal fistulae are also difficult to manage both surgically and medically. In the infliximab era, successful healing of Crohn’s RVF remains disappointingly rare. Surgery for RVF requires a variety of approaches but remains a valuable tool in the treatment of both Crohn’s and non-Crohn’s tracts.

Medical treatment of Crohn’s anal fistulae with combination thiopurine and anti-TNFα agents has demonstrated good short term results. Clinical and radiological data to 3 years follow up demonstrate that around a third of patients maintain healing on infliximab, radiological healing lags behind clinical remission by around a year, and cessation may lead to recurrence in spite of a healed tract on MRI.

A treatment for anal fistula with high success and low risk of impairment of continence in complex anal fistulae eludes colorectal surgeons and gastroenterologists. A treatment combining the best aspects of current fistula management with novel elements prompted by improved aetiological understanding must be the goal for fistula surgeons and is the inspiration behind this thesis.
1 Table of Contents

3.1 1.1 A background to Crohn’s-related perianal fistulae 19

3.1.1 1.1.1 Historical context 19

3.1.2 1.1.2 The impact of perianal disease on Crohn’s 21

3.1.3 1.1.3 Factors associated with perianal Crohn’s disease 22

3.1.4 1.1.4 Hidradenitis Suppurativa 22

3.2 1.2 Aetiological factors 23

3.2.1 1.2.1 Genetic factors 25

3.2.2 1.2.2 Microbiological factors 26

3.2.3 1.2.3 Immunological factors 28

3.2.4 1.2.4 Aetiological implications of immunosuppressant therapy 31

3.2.5 1.2.5 Comparison with idiopathic fistulae 33

3.3 1.3 Assessing perianal fistulae in Crohn’s disease 34

3.3.1 1.3.1 Fistula classification 34

3.3.2 1.3.2 Assessment of perianal Crohn’s disease 34

3.4 1.4 Medical treatment of perianal Crohn’s disease 36

3.4.1 1.4.1 Corticosteroids 37

3.4.2 1.4.2 Metronidazole and ciprofloxacin 37

3.4.3 1.4.3 Immunomodulators 38

3.4.4 1.4.4 Infliximab 39

3.4.5 1.4.5 Local injection of infliximab 41

3.4.6 1.4.6 Adalimumab 41

3.4.7 1.4.7 Other medical options 43

3.4.8 1.4.8 Monitoring response to therapy 46

3.5 1.5 Surgery for perianal Crohn’s disease 48

3.5.1 1.5.1 Surgery and infliximab 49
4.3 2.3 Hypothesis and Aims 89

4.3.1 2.3.1 Hypothesis 89

4.3.2 2.3.2 Aims 89

4.4 2.4 Methods 90

4.4.1 2.4.1 Patient selection 90

4.4.2 2.4.2 Sample collection 90

4.4.3 2.4.3 Surgery 90

4.4.4 2.4.4 Colonoscopy 91

4.4.5 2.4.5 Proctitis 91

4.5 2.5 Microbiology methods - Quantification of Fistula Tract and Anal/Rectal Mucosa-Associated Microbiota using Fluorescent In Situ Hybridisation 92

4.5.1 2.5.1 Patients 92

4.5.2 2.5.2 Anal/rectal and fistula tract sample processing 92

4.6 2.6 Histology and Immunohistology methods 101

4.6.1 2.6.1 Patients 101

4.6.2 2.6.2 Histology sample processing 101

4.6.3 2.6.3 Histological staining 102

4.6.4 2.6.4 Histological assessment 102

4.6.5 2.6.5 Immunohistochemistry sample processing 103

4.6.6 2.6.6 Two dimensional stereology 104

4.7 2.7 Immunology methods 107

4.7.1 2.7.1 Patients 107

4.7.2 2.7.2 FACS sample processing 107

4.7.3 2.7.3 Multiplex sample processing 110

4.7.4 2.7.4 Statistical methods 111

4.8 2.8 Results 112

4.8.1 2.8.1 Patient demographics 112

4.8.2 2.8.2 Reduced bacteria in anal fistula tracts 114
4.8.3 Reduced rectal bifidobacteria and *Bacteroides* in Crohn’s patients 117

4.8.4 Histopathological features of anal fistula tracts 118

4.8.5 Similar levels of acute and chronic inflammation in Crohn’s and idiopathic fistulae 118

4.8.6 Inflammation is greatest near the luminal surface in Crohn’s and idiopathic fistula tracts 119

4.8.7 Higher T lymphocytes in Crohn’s than idiopathic anal fistula tracts 121

4.8.8 Dendritic cells lack homing markers in Crohn’s anal fistula tracts 123

4.8.9 Crohn’s and idiopathic anal fistula contain similar levels of TNF but IL-17a is reduced in Crohn’s compared to idiopathic anal fistulae 123

4.8.10 Associations between bacteria and immunity 123

4.9 Discussion 124

4.10 Summary 128

4.11 Limitations 128

5 An audit of the surgical management of anal fistulae: Fistulotomy in the tertiary setting can achieve high rates of fistula cure with an acceptable risk of deterioration in continence 130

5.1 Abstract 130

5.2 Introduction 131

5.3 Hypothesis and Aims 132

5.3.1 Hypotheses 132

5.3.2 Aims 132

5.4 Methods 132

5.4.1 Patient identification 132

5.4.2 Review of records 132

5.4.3 Database and statistics 133

5.4.4 Surgical procedures 133

5.5 Results 134

5.5.1 Demographic and baseline disease data 134
Long-term MRI-guided combined anti-TNF$\alpha$ and thiopurine therapy for Crohn’s perianal fistulae

Abstract

Introduction

Hypothesis and Aims

Hypotheses

Aims

Methods

Patients

Study design

Radiology

Fistula complexity

Treatment

Clinical follow-up

Radiological follow-up

Statistical Analysis

Ethical considerations

Results

Baseline data

Clinical and radiological outcome up to three years

Delay between clinical remission and radiological healing

Factors influencing clinical and radiological outcome

Effect of early response to treatment on disease course

Surgical procedures during treatment

Stopping treatment

Maintaining radiological healing after treatment cessation

Adverse events
Table of figures

Figure 1 - Intersphincteric and low transsphincteric perianal fistulae (a), high transsphincteric fistula and transsphincteric perianal fistula with multiple external openings and associated abscess (b), in coronal section.................. 19

Figure 2 - Sagittal section showing the proportions of fistulae in Crohn’s patients (63). SB = small bowel, B = bladder, U = uterus, R = rectum........ 20

Figure 3 - T2 weighted pelvic MRI images with fat suppression showing improvement of anal fistula from baseline (a) at six months (b) and 21 months (c)........................................................................................................ 47

Figure 4 - a. A bulky silastic seton and b. a low profile ethibond seton with just three throws and the ‘whiskers’ tied back with silk. ........................................ 49

Figure 5 - Parks’ classification of anal fistulae, (169) by kind permission of John Wiley and Sons................................................................. 58

Figure 6 - a. trans-sphincteric fistulae with extensions into the ischioanal fossae and pararectal space and b. the three planes in which horseshoeing can occur (intersphincteric, ischioanal fossa and pararectal) (169) by kind permission of John Wiley and Sons. ...................................................... 58

Figure 7 - Graphs of published healing rates over time in studies of fibrin glue in anal fistulae. # = only high transsphincteric fistulae studied. * = enhanced glue. ........................................................................................................ 65

Figure 8 - Graphs of published healing rates over time in studies of fistula plug in anal fistulae.................................................................................. 66

Figure 9 - Fistula healing rates with the anal fistula plug as reported in articles found in PubMed over the past 4 years. Each triangle represents a publication. The horizontal bars represent median and range of values (191). By kind permission of Wolters Kluwer Health......................................................... 67

Figure 10 - Intersphincteric sphincter conserving approach in high anal fistula (197). .................................................................................................. 69

Figure 11 - The protective outer sheath from an 18G cannula is inserted into the EO to prevent contamination of the biopsy forceps....................... 93

Figure 12 - The biopsy forceps are passed down the sheath....................... 93

Figure 13 - The biopsy forceps are passed until resistance is met when a biopsy is taken................................................................. 94
Figure 14 - The biopsy is retrieved using a sterile needle, before washing in sterile PBS and placement in a sterile cryovial for snap freezing. .......................... 94

Figure 15 - An internal opening biopsy taken in theatre. The silastic seton marks the IO in the anal canal. ........................................................................................................ 95

Figure 16 - An example 4 well slide .............................................................. 96

Figure 17 - Full thickness fistula tract wall sample retrieved after fistulotomy using scalpel and forceps. ........................................................................................................... 101

Figure 18 - Example tessellation .................................................................. 105

Figure 19 - Separate tessellations across zones ........................................... 105

Figure 20 - Perfect tessellation ..................................................................... 106

Figure 21 - Separate tessellations across two zones in full cross section specimen ........................................................................................................... 106

Figure 22 - Separate tessellations across a cluster and the remaining tissue ........................................................................................................... 106

Figure 23 - Increased macrophages and T lymphocytes at luminal surface of fistula tract compared to deeper layers .......................................................... 120

Figure 24 - H&E x 20 of fistula tract with lumen in lower left corner and full thickness of tract wall up to fat. Inflammatory zone seen near lumen of tract. ........................................................................................................... 120

Figure 25 - Close up of figure 24 (x100) showing epithelium of tract lumen in lower left corner with peri-luminal T lymphocytes ........................................... 121

Figure 26 - Increased T lymphocytes in luminal layer of Crohn's compared to idiopathic fistula tracts .......................................................... 122

Figure 27 - Increased T lymphocytes in deep layer of Crohn's compared to idiopathic fistula tracts .......................................................... 122

Figure 28 - Fluorescent in situ hybridisation using eubacterial probe showing mucus devoid of bacteria alongside epithelium (EUB x630) ......................... 114

Figure 29 - Fluorescent in situ hybridisation using eubacterial probe showing bacteria within mucus layer alongside epithelium (EUB x630) ......................... 115

Figure 30 - Scanning electron microscopy image of tract surface showing a small cluster of cocci (x10000) ........................................................................ 116

Figure 31 - Scanning electron microscopy image of fistula tract surface devoid of bacteria (x10000) ........................................................................ 117
Figure 32 - Position of internal openings ............................................................. 135
Figure 33 - Height of fistula tracts in fistulotomy patients .............................. 135
Figure 34 - Deterioration in continence seen post fistulotomy ..................... 136
Figure 35 - Flowchart for selection of surgical procedure ............................ 150
Figure 36 - Referral pattern of rectovaginal fistula study patients .................. 152
Figure 37 - Aetiology of rectovaginal fistula study patients ........................... 152
Figure 38 - Initial study algorithm for treatment of Crohn’s perianal fistulae used in this cohort of 41 patients started in 2006 ........................................... 170
Figure 39 - Kaplan Meier plot showing delay between clinical remission and radiological healing .................................................................................. 177
Figure 40 - Number of patients undergoing n procedures during study period .................................................................................................................. 179
Figure 41 - Number of patients undergoing n fistula related procedures during study period .......................................................... 179
Figure 42 - Fistula related procedures during study period ......................... 180
Figure 43 - T2 weighted axial MRI with fat suppression demonstrating a. perianal fistula, b. improvement on combination thiopurine and anti-TNF therapy, c. radiological healing of fistula tract and d. recurrence after cessation of anti-TNF therapy ................................................. 183
Table of Tables

Table 1 - A summary of differences and similarities in the suggested aetiological factors in Crohn’s-related and idiopathic perianal fistulae ........... 24

Table 2 - Randomised Controlled Trials (RCT) or meta-analyses of medical treatments of Crohn’s-related perianal fistulae ........................................ 43

Table 3 - Studies of fistula glue in anal fistula including Crohn’s patients ..... 52

Table 4 - Studies of the fistula plug in anal fistula including Crohn’s patients 53

Table 5 - Studies of fistula glue, fistula plugs and bioprosthetic meshes including Crohn’s and non-Crohn’s rectovaginal fistulae ......................... 81

Table 6 - Oligonucleotide probe sequences ................................................. 97

Table 7 - Hybridisation temperatures ......................................................... 98

Table 8 - Post-hybridisation washes .......................................................... 99

Table 9 - Antibody data ............................................................................. 104

Table 10 - An example labelling grid with antibodies and isotype controls .. 108

Table 11 - Antibody labelling of compensation tubes ............................... 109

Table 12 - Summary of aetiological findings ............................................... 112

Table 13 - Patient demographics and baseline disease data ........................ 113

Table 14 - Studies of sphincter saving infill materials in RVF repair ............. 146

Table 15 - Baseline characteristics ............................................................. 174

Table 16 - Clinical and radiological outcomes for combination treatment with infliximab and thiopurine over three years ....................................... 175

Table 17 - Clinical and radiological outcomes for combination treatment with adalimumab and thiopurine (previous infliximab failure) over three years ... 176

Table 18 - Clinical and radiological outcomes for all patients treated with combination thiopurine and anti-TNF therapy over three years .................. 176

Table 19 - Number of procedures performed during study period .............. 178

Table 20 - Procedures performed during study period ............................... 180

Table 21 - Protocol for tissue processing .................................................... 196
Table 22 - Tract acute inflammation results ........................................ 203
Table 23 - Tract acute inflammation results ........................................ 203
Table 24 - Tract chronic inflammation results ...................................... 204
Table 25 - Tract Macrophages and T-lymphocytes by zone.................. 204
Table 26 - Tract Macrophages and T-lymphocytes by aetiology .......... 205
Table 27 - Rectal bacteria results: EUB ............................................. 205
Table 28 - Rectal bacteria results: BAC ............................................. 205
Table 29 - Rectal bacteria results: BAC ............................................. 206
Table 30 - Rectal bacteria results: BIF ............................................. 206
Table 31 - Rectal bacteria results: EREC .......................................... 206
Table 32 - Rectal bacteria results: E COLI ....................................... 207
Table 33 - Rectal bacteria results: F PRAU ....................................... 207
Table 34 - Tract flow cytometry results ............................................ 208
Table 35 - Tract cytokine results ..................................................... 209
Table 36 - Tract IL-17a results after adjustment ............................... 209
Acknowledgements

I owe a debt to the following individuals and groups, without whom I would not have been able to complete this thesis:

Dr Kevin Whelan, Dr Neil Rayment and Mr Barry Hudspith who supported, informed and guided the microbiological studies;

Dr Hafid Omar, Professor Stella Knight, Ms Alison Scoggins and the members of the APRG who supervised the immunology studies;

Dr Tahera Ansari, Dr Paul Sibbons, Dr Anna Nowocin, Dr Kate Widdows and the members of NPIMR who helped with the Histopathological studies;

Professor Thomas Guenther who reviewed histopathological specimens;

Dr Damian Balmforth and Dr Babar Kayani who collected data on rectovaginal fistulae;

Dr Stefano Sala, Dr Valentina Cianci, Dr Kate Kalmar, Mr Gary Atkin and Dr Pravin Ranchod for their help with the clinical anal fistula database;

Dr Siew Ng, Ms Sherrill Tripoli and other members of the MRI group;

Dr David Burling and Dr Arun Gupta who endured early morning meetings to review MRI scans fuelled by coffee, croissants and a love for research;

Dr Eddie Edwards and his team of undergraduate students at the Centre for Computing, Imperial College London, who created the prototype computer software, SIMI, for measurement of fistula volume;

Dr David Burling for his help, advice and support, in particular with regard to imaging and manuscript preparation;

The consultant staff of St Mark’s Hospital, in particular Miss Sue Clark, Dr Simon Gabe and Miss Carolyne Vaizey, for their support and help;

My fellow research fellows Mr James Hollingshead, Miss Ruchi Tandon, Dr Dominic Bullas, Mr Anil George, Mr Najib Daulatzai, Mr Goher Rhabour, Dr Aravinth Murugananthan, Dr Cheng Tee, Dr Simon Peake, Dr John Landy and Dr Ana Ignjatovic, who provided both specific help and a wonderful atmosphere in which to develop academic skills and firm friendships;

Dr Ignjatovic for her role as adviser, sounding board, colleague and friend throughout our time at St Mark’s and beyond;

Mrs Marilyn Tozer and Dr Himali Patel, for proof reading services and advice offered in spite of the subject matter situated, as it is, some 2-3 feet from the latter’s area of expertise and further still from the former’s comfort zone;
Mr Daniel Singer for advice and encouragement, often over noodles, and for offering to proof read despite his already very heavy workload;

My parents, Paul and Marilyn, who provided IT skill and moral support whenever they were needed, and enabled me to enter this world of higher education and attainment;

The charities Core, the Ileostomy Association, for Crohn’s and its founders Tasha and Lisa, and all the staff but especially Maxine, Riyah and Sarah at the St Mark’s Foundation, which provided the funds vital to this work.

I must also acknowledge the sacrifice of our patients, who entered my studies without reluctance or question despite their often difficult situations, and who demanded precision and perseverance of me by virtue of their own fortitude in the face of adversity.

Dr Ailsa Hart supervised me with great skill and compassion, recognising my weaknesses and driving from me a work of which I am proud – I am very grateful to her for the many hours spent examining every detail of my studies and for supporting me throughout.

Professor Robin Phillips inspired, directed and supported this thesis with his inimitable style, work ethic and thoughtfulness, guiding me through the highs and lows of research and clinical life before, during and after my time at St Mark’s.

To these two I owe this thesis (which is as much theirs as mine) and a renewed enthusiasm for academic surgery.

Finally, I thank my wife Himali, and our wonderful daughters Anaiya and Maya, who endured my absence and fatigue during the years of this research, and supported me unconditionally throughout it.
1 Introduction

The problems associated with fistula-in-ano have vexed sufferers and surgeons for thousands of years. Civilisations from East and West have documented symptoms and medical and surgical treatments associated with perianal fistulation; authors on the subject include Hippocrates and John of Arderne. The questions raised by these authors often mirror the ongoing dilemmas of fistula surgery: what is the optimal treatment method? How does one prevent recurrence? How does one avoid incontinence?

Famous sufferers have included Louis XIV and Charles Dickens, the latter treated by Frederick Salmon at his Hospital for Fistula &c. founded in 1835 in the City of London (now relocated to Harrow) where patients with complex anal fistulae are still seen today, often having failed treatment elsewhere and seeking that elusive outcome desired by so many over the centuries: a cure without risk of incontinence.

In searching for this Holy Grail of fistula surgery, one must understand what drives the creation of anal fistulae and also what allows them to persist, where the current treatments risk failure or incontinence and what makes this failure more likely. One must also consider Crohn’s disease very carefully. Although not a common disease, it is becoming more so, and the risk of perianal fistulation in this group is far higher than in the general population. This group of patients is important not only because it is at such high risk of developing perianal fistulae, but also because it may help us understand the aetiology of anal fistulae, particularly when studied alongside patients without inflammatory bowel disease who also develop fistulae.

Although rarely life threatening, perianal sepsis and in particular perianal fistulae are the source of enormous suffering to those afflicted and account for personal, work and social disruption which may be devastating. In this thesis the aetiology and current management of perianal fistulae will be explored in order to help transform the outcome of patients.
1.1 A background to Crohn’s-related perianal fistulae

1.1.1 Historical context

Crohn’s disease is a chronic inflammatory bowel disease characterised by transmural bowel involvement, anywhere from mouth to anus, which can lead to fistula development, particularly in the perianal region. A perianal fistula is a pathological connection between the anorectal mucosal surface and the perianal skin causing pain and purulent discharge. Perianal fistulae can be idiopathic or secondary to various diseases, including Crohn’s disease.

Figure 1 - Intersphincteric and low transssphincteric perianal fistulae (a), high transsphincteric fistula and transssphincteric perianal fistula with multiple external openings and associated abscess (b), in coronal section

Penner and Crohn described the first case of perianal fistula in a patient with regional enteritis in 1938 (1) and subsequently noted perianal lesions in 14% of a cohort of patients. It is now recognised that perianal fistulae are a common complication of Crohn’s disease, occurring in around a third of all patients with Crohn’s disease (2).
In a recent community based study there was a cumulative risk of developing a fistula of 50%, including a perianal fistula in 26% at 20 years (3).

![Image of a sagittal section showing the proportions of fistulae in Crohn's patients. SB = small bowel, B = bladder, U = uterus, R = rectum.]

**Figure 2 - Sagittal section showing the proportions of fistulae in Crohn's patients (63).** SB = small bowel, B = bladder, U = uterus, R = rectum.

It is now known that perianal fistulating Crohn’s disease represents a distinct disease phenotype (4), which appears to be separate from luminal fistulating disease with differing disease behaviour and which often requires different therapeutic strategies. The Vienna Classification for Crohn's disease was devised in 1998 to categorise diverse Crohn's disease phenotypes (5) recognising the heterogeneity of the disease, but did not distinguish between perianal and luminal fistulae. The recent Montreal modification to the Vienna Classification provides a separate modifier for perianal disease (6) and this underlines the now recognised importance of this phenotype with regard to impact on disease prognosis and treatment options.
The impact of perianal disease on Crohn's

While Crohn's disease is classically described as progressing from inflammatory to penetrating and/or stricturing disease (7), this does not necessarily apply to perianal disease. Many patients are diagnosed with perianal fistulae before or at the time of diagnosis of Crohn's disease. In one cohort of Crohn's disease patients, 25% of patients with fistulizing disease had perianal fistulae which predated their diagnosis of Crohn's disease (8). Another 46% developed fistulae soon after.

Perianal disease generally denotes a more aggressive Crohn's disease phenotype. If perianal disease is present at initial diagnosis, the disease is more likely to follow a severe course, progress more quickly from inflammatory to stricturing or penetrating complications and require several medical therapies and surgical interventions (9-11). Beaugerie and colleagues in 2006 reported that perianal lesions at diagnosis were independently associated with a more disabling course, even when there were other factors such as age below 40 years at onset or need for steroids for the first flare of disease (9). Lakatos and colleagues in 2009 reported that perianal disease (in addition to other factors such as small bowel disease, smoking, prior steroid or early azathioprine/anti-TNF therapy) is a predictor of disease behaviour change (from inflammatory to stricturing or penetrating) over time in patients with Crohn's disease (12).

The natural history of perianal fistulae in Crohn’s patients at St Mark’s Hospital, London before the introduction of anti-TNF drugs was examined by Bell and colleagues in 2003 (13). On long term follow-up 76% of perianal fistulae had healed, 16% were persistent and 7% had a seton in situ. Relapse occurred with 32% of simple fistulae (defined as low, single external opening, no evidence of abscess, rectovaginal fistula or anorectal stricture [figure 1a]) and 23% of complex fistulae (defined as high, multiple external openings, perhaps associated with perianal abscesses and/or rectovaginal fistulae, anorectal strictures or active rectal inflammation [figure 1b]). Median time to healing of perianal fistulae was 44 months with a median of 3 (simple) or 6 (complex) treatments per patient. Complex fistulae required more complex surgery; 50% ultimately underwent proctectomy, stoma or resection, while only 6% of those with simple fistulae ever required a major operation.
1.1.3 Factors associated with perianal Crohn’s disease

There are several factors that influence development of perianal disease, including gender, age, race and disease location. The data regarding gender are conflicting, with some reports of an increased incidence of perianal fistula in males (8), while other patient cohorts demonstrated no difference between males and females (4). Age at disease onset appears to influence development of perianal disease, with younger patients at the greatest risk. One study found age less than 40 years to be a significant risk factor for penetrating complications, including perianal disease, and another suggested that patients diagnosed after age 50 were less likely to present with perianal complications (7), although duration of disease and age at diagnosis may represent the same risk factor for perianal disease.

Non-Caucasian race appears to be associated with perianal disease (4;7). Sephardic Jews have a higher risk for perianal disease than Ashkenazi Jews (14). Fistulae are most common when colonic disease, in particular distal colonic disease, is present (8). For example, patients with Crohn’s colitis were more than three times as likely to develop perianal fistulae as those with ileitis (4). Anti-Saccharomyces cerevisiae antibodies (ASCA) are associated with Crohn’s disease and also predict a higher likelihood of (oral and) perianal Crohn’s (odds ratio 1.89) (15). Whether these antibodies to saccharomyces cerevisiae are part of a genetic predisposition to Crohn’s or an adaptive immune response to an unknown microbe or environmental factor is not known.

1.1.4 Hidradenitis Suppurativa

Hidradenitis Suppurativa (HS) is associated with perianal Crohn’s disease, can coexist with it, and causes diagnostic difficulty in some patients. The clinical and histological features of the two diseases can be similar and it is possible to confuse HS with Crohn’s perianal sepsis or with the cutaneous manifestations of Crohn’s. Early series highlighted the diagnostic difficulty (16;17); the presence of granulomas in the submucosal layer of cutaneous biopsies led to a diagnosis of Crohn’s disease but the appearance of abscesses in the axillae suggested HS and subsequent intestinal inflammation confirmed the dual diagnoses. Epithelioid granulomas, known to occur in systemic granulomatous disease such as Crohn’s and sarcoid, were subsequently shown also to exist in patients with HS alone (18).
Further series not only reinforced the association between HS and perianal Crohn’s disease and the potential for missing one or other diagnosis but also described their outcome - in one series 23 of 24 had had a laparotomy, 22 had a stoma and 17 had undergone proctectomy (19;20). Further evidence of a link between the diseases comes from two case reports in which concurrent luminal Crohn’s and HS responded to treatment with infliximab with improvement at all HS sites (21;22).

Clinical and radiological features may be valuable in distinguishing the two diseases. The presence of abscesses at other sites including the groin and axillae are clearly suggestive of HS. Case reports in the literature suggest that MRI may be valuable; skin thickening and subcutaneous induration away from the perianal area, subcutaneous abscesses remote from the rectum or anus, enlarged inguinal lymph nodes, and no communication with pelvic organs are all consistent with HS rather than Crohn’s anal fistulae (23).

1.2 Aetiological factors

The aetiology of perianal fistulating Crohn’s disease is unclear. Historically, these lesions have been considered infective entities, as in the Parks cryptoglandular theory (discussed later). Professor L Hughes, in 1978, divided anorectal lesions in Crohn’s disease into primary and secondary lesions. Primary lesions included ulcers and fissures which, under mechanical influences, would lead to secondary lesions such as strictures and epithelialised fistula tracts. His contention was that a deep fissure, caused by a flare of proximal disease, created a pocket into which faeces would pass. As the flare of proximal disease resolved and under the pressure of defecation, this pocket would erode into the subcutaneous tissues leading to abscess formation (24).

It is now believed that, as with luminal Crohn’s disease, the aetiology of perianal Crohn’s disease involves interaction between microbiological, immunological and genetic factors (Table 1).
<table>
<thead>
<tr>
<th>Table 1 - A summary of differences and similarities in the suggested aetiological factors in Crohn’s-related and idiopathic perianal fistulae</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Genetics</strong></td>
</tr>
<tr>
<td>IBD5</td>
</tr>
<tr>
<td>IRGM rs4958847</td>
</tr>
<tr>
<td>NOD2/CARD15</td>
</tr>
<tr>
<td><strong>Microbiology</strong></td>
</tr>
<tr>
<td>Microbiota found in fistula tracts</td>
</tr>
<tr>
<td><strong>Efficacy of antibiotics</strong></td>
</tr>
<tr>
<td><strong>Efficacy of faecal diversion</strong></td>
</tr>
<tr>
<td><strong>Efficacy of immunomodulators</strong></td>
</tr>
<tr>
<td><strong>Immunology</strong></td>
</tr>
<tr>
<td>T cells</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Macrophages</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>B cells</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Monocytes</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Epithelial to mesenchymal transition (EMT)</td>
</tr>
<tr>
<td>Myofibroblasts</td>
</tr>
<tr>
<td>Defensins</td>
</tr>
<tr>
<td>Matrix Metalloproteinases</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>TNFα</td>
</tr>
<tr>
<td>Epithelialisation</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
1.2.1 Genetic factors

An association of perianal Crohn’s disease with a susceptibility locus on chromosome 5 has been described (25;26). On 5q31 (IBD5), the carnitine/organic cation transporter (OCTN) is associated with Crohn’s disease and particular OCTN variants are associated with perianal disease (25;27;28). It is not clear how these variants may relate to disease, but impaired OCTN activity or expression may reduce carnitine transport. This can lead to defects in oxygen burst mediated pathogen killing which may partially explain how the previously implicated altered handling of bacteria is relevant in the pathogenesis of Crohn’s disease. Reduced carnitine transport may also lead to impaired fatty acid β-oxidation in intestinal epithelium, as carnitine is an essential co-factor for β-oxidation of long chain fatty acids in mitochondria. Impaired fatty acid β-oxidation is exacerbated by bacterial metabolites and causes colitis in experimental models (26). It has also been shown that impaired OCTN activity may diminish uptake of physiologic compounds while increasing uptake of potential toxins derived from bacterial catabolism. Overall, a role for OCTNs in handling enteric bacteria or their products and interacting with the epithelial barrier/mucosal immune system appears to be important and it is hypothesised that aberrant stimulation through OCTN transporters may lead to inflammation characteristic of Crohn’s disease.

More recently, another susceptibility locus has been described on chromosome 5q33.1. IRGM rs4958847 is part of the autophagy pathway which eliminates intracellular organisms and polymorphism is associated with an increased risk of penetrating complications (odds ratio 1.48) and perianal fistulae (odds ratio 1.61) (29).

Very few studies have investigated the association between NOD2/CARD15 genotype and perianal CD. The one study that has investigated this association was unable to show correlation between this genotype and perianal fistulating disease in a cross-sectional study of 275 patients (30), but there may be an influence of NOD2/CARD15 gene status with regards to the response of perianal fistulae to antibiotic therapy, greater response being noted in patients with NOD2/CARD15 wild-type (31).

Several studies have examined genetic associations with penetrating Crohn’s disease (including perianal fistulae) but they do not differentiate within their
penetrating disease cohorts so the implications for perianal disease specifically are not clear (32;33). There are no genome wide association scans for perianal Crohn’s disease and it is possible that other genetic associations exist that are currently unknown.

1.2.2 Microbiological factors

One theory for the aetiology of perianal fistulating Crohn’s disease is that fistulae occur following cryptoglandular infection, as with idiopathic anal fistulae. This initially leads to abscess and finally to fistula formation caused by surgical or spontaneous rupture of the abscess through the perianal skin. Surgical diversion of the faecal stream has been shown to lead to long term improvement in perianal fistulae in 26% of patients (34) implying that the luminal stream, and likely the microbiota within it, in some way contributes to or drives the pathogenic process. Surgical diversion also improves luminal Crohn’s disease so the improvement seen in patients with perianal Crohn’s disease may reflect a decrease in Crohn’s activity rather than a direct effect on the perianal fistulae. Changes in the microbiota within perianal fistulae in the context of defunctioning do not seem to have been explored.

In luminal Crohn’s disease, there is evidence that the antigens that drive the tissue-damaging response in intestinal inflammation are derived from the normal bacterial microbiota. Proof of this concept has come from studies of at least 11 models of inflammatory bowel disease. In these models, inflammation is dependent on the presence of a normal microbiota; in its absence there is no disease. The phenomenon is seen in different species (mice, rats and guinea pigs) and occurs in manipulated systems, such as knockout or transgenic animals, as well as in induced models, such as indomethacin- or carrageenan-induced colitis.

In addition, in humans diversion of the faecal stream after surgery prevents recurrence of Crohn's disease and re-anastomosis results in inflammation (35;36). Furthermore, this effect has been demonstrated directly by infusion of luminal contents into excluded ileum (37). The inflammatory lesions tend to occur in areas of bowel with the highest bacterial counts - the terminal ileum and colon.

The search for a single organism as the causative agent has yielded inconsistent results. Entero-adherent strains of Escherichia coli in ileal mucosa have been found in patients with Crohn's disease (38;39). Other single organisms that have been associated with intestinal inflammation include Chlamydia, Listeria, and
Mycobacteria, (40-43), but whether these changes are primary or secondary to the disease process is not clear and as yet an infectious origin for Crohn's disease has not been confirmed. Other evidence points to an alteration in the balance of microbes in inflammatory bowel disease, known as dysbiosis. Microbial studies have shown a persistent reduction in biodiversity of mucosa-associated flora in patients with active inflammatory bowel disease compared with the microbiota in controls (reviewed in (44)). A loss of normal anaerobic components (Bacteroides, Eubacterium, and Lactobacillus spp.) has contributed to this difference (45;46). A shift to an increased representation of Gram-negative bacteria appears to accompany a reduced bacterial diversity in Crohn's disease patients during remission, implying that it may represent a primary modification rather than a response to disease or inflammation (47). Decreased bifidobacteria are found in Crohn's disease (48) as well as an overall increase in the number of mucosal adherent bacteria (49). At relapse in a group of patients with ulcerative colitis, a decreased richness and diversity of the flora was found (50).

However, studies assessing microbiological changes in Crohn's fistulae are sparse. One study in which 13 Crohn's patients with perianal disease had pus aspirated from their fistula tracts which was analysed by standard bacterial culture techniques reported that Gram-positive organisms, in particular staphylococci, streptococci and corynebacter, predominated over Gram-negative enteric organisms (51) which is different to the findings in idiopathic fistulae (52;53), where microbes found on excised granulation tissue were found to be largely of gastrointestinal origin. These reports are limited by differences in the compartments analysed (exudates versus mucosa) and the use of bacterial culture as opposed to molecular techniques to quantify the microbes. Bacterial culture techniques are inadequately selective and are unable to detect all the different types of microbes present within the GI tract (54). Furthermore, there has been no comparison between microbes in Crohn's perianal fistulae compared with idiopathic.

Antibiotics temporarily work quite well when used in fistulating Crohn's disease, supporting a role for the gut flora in the aetio-pathogenesis of Crohn's fistulae. Case series and one placebo-controlled pilot trial supply the evidence for antibiotics in treating patients with fistulating CD. Metronidazole, with activity against anaerobic bacteria, was used in 21 consecutive patients with perianal CD and, of the 18 patients who remained on therapy, 10 were considered to have complete healing of their fistulae at 10 weeks (55). However after stopping metronidazole, 78% had
symptomatic recurrence (56), so it would seem that the fistulae were suppressed rather than cured. Ciprofloxacin, with broad-spectrum activity including Gram negative aerobic organisms, has been used in a small uncontrolled study. Eight patients with perianal Crohn’s disease were treated with ciprofloxacin for 3-12 months and all eight patients improved. However, half continued to have persistent drainage (57). A three month course of metronidazole and ciprofloxacin was assessed in 14 patients with perianal Crohn’s disease. Nine out of 14 patients improved and 3 patients had complete clinical healing of their fistulae (58).

Even with attempts to provide broader spectrum antibiotic cover there is limited efficacy in true healing of Crohn’s fistulae, suggesting that optimal selection of antimicrobials may be lacking or that infection may not be the whole story. In routine clinical practice, antibiotic sensitivity of microbes from Crohn’s fistulae is not assessed or used to tailor appropriate antibiotic choice. It may be that an environmental factor exists (e.g. excess TNFα) that must be treated concurrently for antibiotics to be effective in treating Crohn’s fistulae, in a similar way to that in which eradication of gastric colonisation with Helicobacter Pylori requires reduction of acid secretion in addition to antimicrobial treatment. Another explanation for the partial efficacy of antibiotics is that the underlying genotype of the patient may dictate antibiotic responsiveness versus non-responsiveness. For example, as described above, NOD2 status appears to predict responsiveness to ciprofloxacin in Crohn’s perianal fistulae (31).

The microbiological environment of the anal fistula tract remains unknown and any influence of bacteria on the immunological status of the tract is also therefore entirely unclear. Whether fistula tracts associated with Crohn’s disease contain different bacteria to idiopathic tracts, and whether the bacteria themselves are key to the creation or persistence of the tracts is based on an understanding of these facts. The microbiological status of the rectum in patients with anal fistulae is also poorly understood. Chapter 2 explores the microbiology of Crohn’s and idiopathic fistula tracts and the rectal microbiota present in these patients.

1.2.3 Immunological factors

Histologically, Crohn’s fistulae seem to arise when a fissure penetrates the lamina propria and muscularis mucosae into the deeper layers of the underlying tissue.
They tend to be surrounded by granulation tissue with a cellular infiltrate and capillaries.

There are sparse data exploring quantitative and qualitative changes in immune cells in perianal Crohn’s fistulae. Bataille et al. histologically examined the nature of fistula tracts from patients with Crohn’s disease and controls with fistulae of non-IBD origin (59). They examined a broad range of internal and external fistulae, including 14 perianal fistulae (8 in the Crohn’s group and 6 in the non-IBD control group). Using immunohistochemical techniques on surgical specimens they were able to demonstrate differences in the position and number of T and B cells and macrophages between the two groups, as summarised in Table 1 (Crohn’s vs. non-IBD control fistulae). In the Crohn’s group there was an infiltrate of T cells in the internal wall of the fistula, then a small band of macrophages, and finally a dense infiltrate of B cells in the outer wall of the fistula. In the non-IBD control group, the fistula wall was intensely infiltrated with macrophages throughout the wall with only a few B cells found. T cells were found in the outer two thirds of the fistula wall. Only a small number of patients with perianal Crohn’s disease were included in this study and it is not clear whether the described changes, which represent the entire cohort of patients studied so far, also apply to those patients with perianal disease specifically.

Myofibroblasts are key cells in tissue injury and repair in the gut (60). They become activated, proliferate and migrate in the early stages of the injury and repair process. They can cause gut damage by secreting matrix metalloproteinases (MMPs) (61), which are involved in extracellular matrix degradation (62). There appears to be an aberrant healing process in Crohn’s fistulae, such that instead of optimal wound healing there is tissue destruction; myofibroblasts play a role in this aberrant process. In fistulae from patients with Crohn’s disease, there is an abnormal arrangement and distribution of myofibroblasts. CD myofibroblasts differ functionally from control myofibroblasts. They have a reduced ability to migrate (63), an enhanced proliferative capacity and they release increased amounts of TGFβ and TNFα (64;65). Increased tissue-degrading MMPs, in particular MMP3 and MMP9, are strongly expressed in patients with fistulae, both of Crohn’s and idiopathic origin (66), suggesting a role in fistulation in general.

With defects in fibroblasts known to be present in Crohn’s fistulae, it is perhaps surprising that a novel approach used human autologous fibroblasts seeded onto
biocompatible scaffolds to treat Crohn’s fistulae. A case report described fistulectomy followed by insertion of autologous fibroblasts. Complete healing was noted in 3 weeks and the patient remained healed throughout a 20 month follow-up period (67). This study will be discussed in greater detail below (page 53).

About one third of Crohn’s fistulae are (at least partially) epithelialised (59). In addition to being a physical barrier to bacterial invasion, the epithelium protects the host by secreting substances with anti-microbial activity, including defensins. Defensins form part of the innate immune system and can protect mucosal surfaces from infections with Gram-negative and -positive bacteria, fungi, enveloped viruses and protozoa. High levels of the defensins hBD2 and hBD3 have been found in idiopathic anal fistulae (68) and in levels similar to those found in the perianal skin. The rectal mucosa in these patients expressed almost no hBD2 or 3 and only low levels of hBD1. The same study also indicated high levels of CK5/6 and low levels of CK8 in the fistulae examined which, again, is similar to the findings in a multi-layered epithelium like skin and quite the opposite to the findings typical in intestinal epithelium. A comparative study in Crohn’s fistulae does not seem to have been performed, but there is evidence in luminal Crohn’s disease of impaired induction of human beta-defensins (HBD2 and 3) in Crohn’s colitis and the alpha-defensins HD5 and 6 in ileal disease (69).

In Crohn’s fistulae that are non-epithelialised (present in more than two-thirds of cases), there is a thin layer of myofibroblast-like “transitional cells” with gap junctions that form a basement membrane-like structure. It has been shown that epithelial cells can change into mesenchymal cells, a process referred to as epithelial-mesenchymal transition (EMT), which has been seen in epithelial cancers and also in normal wound healing, and it appears that this occurs in Crohn’s fistulae (70).

When a defect occurs in the intestinal barrier, epithelial cells migrate to the site of injury. EMT, which is an essential component of tissue remodelling and wound repair, occurs and the epithelial cells, characterised by strong cell–cell junctions and polarity, are replaced by a mesenchymal phenotype, with reduced cell–cell adhesions, and a fibroblast morphology and function making them more mobile and able to migrate more easily to repair mucosal defects. Markers of EMT include decreased E-cadherin and β-catenin (which translocates to the nucleus) expression, and increased expression of β6 integrin. TGF-β is an inducer of EMT. This pattern of markers has been shown in both Crohn’s-related and idiopathic perianal fistulae.
EMT may be a malfunctioning defence mechanism forming part of the aetiology of all perianal fistulae and may provide a target for novel therapies.

Defects in our understanding of the immunology of Crohn’s anal fistula tracts include knowledge of the basic constituents of the immunological response in the tracts themselves, and the location of immune cells within the wall of the tract. Whether immune cells are found in the superficial sections of the wall, perhaps in response to a luminal provocation, what level of inflammation is seen and which cells form this inflammatory response are not known. Nor are any similarities or differences in dendritic cell function between luminal and perianal fistulating Crohn’s disease or between Crohn’s and idiopathic fistula tracts. These issues will be explored in the immunology section of Chapter 2.

1.2.4 Aetiological implications of immunosuppressant therapy

The effect of immunosuppressants, such as azathioprine/6-mercaptopurine and most notably anti-TNFα antibodies, suggests that immune mediated mechanisms play a role in Crohn’s fistula formation. Furthermore, the efficacy of immunosuppressants implies that a purely infective pathogenic process is unlikely in Crohn’s fistulae. Use of immunosuppressants to treat Crohn’s fistulae may be expected to exacerbate an infective origin. Although perianal abscesses have been reported in trials of immunosuppressants and anti-TNFα antibodies, they do not appear to be a major problem.

A meta-analysis of nine randomised controlled trials of azathioprine or 6-mercaptopurine in Crohn’s disease (71) reported a 54% response to treatment in various Crohn’s related fistulae (including perianal fistulae) compared with only 21% in the placebo arms. In another study, azathioprine was added to a 20 week antibiotic course and improved the rate of response from 15% to 48% in 49 patients (72).

Anti-TNFα strategies have revolutionised the medical treatment of Crohn’s fistulae, not only in allowing complete healing of fistulae in about half of treated patients, but also in providing much more rapid resolution of the draining fistulae with patients often responding to infliximab in about 2 weeks. Nearly 10 years ago the first randomised double-blind placebo-controlled trial demonstrated efficacy of infliximab in the treatment of fistulating Crohn’s disease with approximately 62% of patients in
the infliximab group responding compared with 26% in the placebo group, and nearly 50% of all patients in the infliximab group experiencing complete fistula closure (73). Furthermore, in the ACCENT II trial, regular infusions of infliximab, given every 2 months, enabled 46% of infliximab-treated patients to continue responding and 36% of infliximab-treated patients to remain completely healed at 1 year (74). Not only systemic but also local injections of infliximab into the fistula tract itself have been shown to be efficacious (75;76). This may indicate a role for local TNFα production in initiating or perpetuating Crohn’s fistulae.

Serum and rectal mucosal cytokines have been assessed. One study, using ELISA techniques, demonstrated raised rectal mucosal IL-1β and IL-6, and raised serum IL-6 and TNFα in perianal Crohn’s patients compared to healthy controls and Crohn’s patients with only small bowel disease (77). The rectal mucosal levels of all three cytokines correlated with disease activity or severity. The cytokine milieu of idiopathic anal fistula tract tissue has been considered in a single study but local rectal and skin biopsies were used as control tissues (68). The validity of this control tissue is not clear. The study demonstrated elevated levels (vs. perianal skin or rectal mucosa) of IL-8 and IL-1β but normal levels of TNFα, IL-10 and IL-6. The cytokine milieu of Crohn’s anal fistula tracts themselves is not known and cytokines in Crohn’s and idiopathic fistula tracts have never been compared directly.

It is not clear why some patients respond to infliximab and others do not, but the genotype may influence responsiveness. For example, polymorphisms in the Fas ligand -843 C/T may be a genotypic predictor of response to infliximab in fistulating perianal Crohn’s disease (78).

Although infliximab appears to have altered the natural history of Crohn’s fistulae, radiological studies show that the fistula tract and associated disease process may persist (79;80); prolonged treatment appears to be necessary to optimise deep tissue healing. It may be that other cytokines are involved in this stage of the disease process, so other therapeutic options may be needed further to enable healing.

The detailed cytokine milieu of both Crohn’s and idiopathic anal fistula tracts is poorly understood. TNFα levels in the tracts themselves have not been described and cytokine profiles for both types of tract may represent valuable information, whether differences or similarities between the two are present. An examination of the cytokine milieu of Crohn’s and idiopathic anal fistulae is described in Chapter 2.
1.2.5 Comparison with idiopathic fistulae

The aetiology of idiopathic fistulae is also unclear. The cryptoglandular hypothesis suggested by Parks in 1961 implicates infection of anal glands, a theory first proposed in 1878 by Chiari (81). Specifically, it is the intersphincteric anal glands (representing between one and two thirds of the total number (82)) that are initially infected by an abscess which subsides but persists deep to the internal sphincter and leaves behind the now diseased gland which becomes the seat of chronic infection. This, in turn, leads to formation of a granulation tissue lined tract or fistula. The intersphincteric component of the infected system is fed with bacteria from the gastrointestinal tract which leads to persistence of the tract.

Henrichsen et al. showed that of 50 patients with idiopathic perianal, ischiorectal or intersphincteric abscesses, 21 grew coliforms or streptococcus faecalis (gut-derived organisms) from their cultures whereas 17 grew staphylococcus, proteus or streptococcus (skin-derived organisms). None of those with skin-derived organisms had or developed associated fistulae whereas 13 of those with gut-derived organisms did, either at operation or within 6 months follow-up (83).

Seow-Choen et al. studied granulation tissue from the tracts of idiopathic perianal fistulae in 18 patients and processed the samples using standard microbiological techniques. Sixty-nine isolates representing at least 17 species were obtained. The predominant organisms were *Escherichia coli* (22 per cent), enterococcus (16 per cent) and *Bacteroides fragilis* (20 per cent) with streptococci and staphylococci present in only a minority of cases (53).

These findings support the infective process as an aetiological factor in idiopathic fistula formation. However, Lunniss et al. commented on the paucity of organisms which led to enrichment techniques being required to grow organisms at all (52). This suggests that infection does not fully account for the persistence of idiopathic perianal fistulae. Epithelialisation of the tract has been suggested as a possible cause of persistence (84), but this has never been substantiated. There is limited published work investigating the immunological nature of idiopathic fistula tracts.

By comparing idiopathic and Crohn’s-related anal fistulae a causative or at least permissive factor required for fistula formation or persistence may be identified, opening future therapeutic approaches.
### 1.3 Assessing perianal fistulae in Crohn’s disease

#### 1.3.1 Fistula classification

Low intersphincteric fistulae, low transsphincteric fistulae and those with a single tract with no extensions or complicating abscesses are classified as ‘simple’. Complex fistulae have extensions, high tracts (whether intersphincteric, transsphincteric or suprasphincteric) involve the vagina, or have an associated stricture or proctitis (85). The differentiation between simple and complex has prognostic relevance. Complexity worsens the prognosis, reduces the chance of healing and threatens continence (13). As yet no medical trials have randomised specifically based on complexity of fistula. Perhaps contrary to expectation, a prospective cohort of patients with perianal Crohn’s disease treated with anti-TNFα antibodies at St Mark’s Hospital showed no relation between fistula complexity and the likelihood of healing with anti-TNF agents (80).

The Montreal classification recognition that perianal disease requires a separate sub-classification because it is distinct from and is not usually associated with internal fistulating/penetrating disease (6), guides management and may have an aetiological basis as discussed above.

#### 1.3.2 Assessment of perianal Crohn’s disease

There are three ways to assess a patient with perianal Crohn’s disease, besides thorough history and physical examination:

1) Endoscopy, for evaluating proximal luminal disease and in particular the state of the rectum;
2) Local imaging with MRI or anal endosonography;
3) Examination under anaesthesia with surgical drainage of any abscesses and seton placement as required.

These assessment methods are generally complementary and are often undertaken in combination. Endoscopy will assist in diagnosis and map the extent and severity of the known case. Additional small bowel imaging may be used fully to map the disease.
Pelvic MRI has been shown to be the most accurate method for classification of the primary tract and any secondary extensions (86). Anal ultrasound, particularly when enhanced by hydrogen peroxide, is more accurate at determining the site of any internal opening (IO). Anal ultrasound (in combination with anorectal physiology tests) can also assess sphincter integrity and function which has been claimed by some surgeons to help them formulate an optimal surgical strategy. However, ultrasound will not reliably identify ischioanal fossa or supralelevator sepsis because of rapidly declining resolution with distance from the probe. Furthermore, there is considerable variation in operator expertise and insertion of the ultrasound probe may be very uncomfortable in the setting of active sepsis, and impossible with stenosis.

On the other hand, MRI can be performed without a radiologist needing to be present at the time the test is done. Sequential images are easier to compare, particularly when monitoring treatment. Interpretation is more intuitive and the strategic view is better, which can be helpful intra-operatively. However, the test is expensive and the internal opening is less well seen (its position is usually inferred), and some patients feel claustrophobic in the confined space of the MRI scanner (and others with, for example cardiac pacemakers, are precluded).

As well as being diagnostic, examination under anaesthetic (EUA) by an experienced surgeon allows infection to be drained and either definitive surgery (lay open or other curative procedures) or placement of a temporary loose seton for drainage while medical management is optimised. However, injudicious probing may cause iatrogenic tracts, increasing the complexity of the fistula and thereby the risk of recurrence or subsequent incontinence.

In 2001 Schwartz and colleagues reported 34 patients with perianal Crohn’s disease taking part in a prospective study comparing the accuracy of 3 methods: EUS, early generation MRI and EUA. There was good agreement between all three. EUS had 91% accuracy, MRI 87% accuracy and EUA 91% accuracy. A combination of any 2 methods yielded an accuracy of 100% (87). Two years earlier, Orsoni and colleagues had detected more abscesses and fistulae in 22 Crohn’s patients when using EUS compared to MRI (88). However the quality of MRI in this era was relatively poor.
A later blinded study at St Mark’s hospital compared clinical examination, EUS and MRI in 104 patients (89). Classification of fistula, presence of secondary tracts/abscesses and the site of internal openings were all most accurately assessed at MRI. For example, tract accuracy was 90% with MRI, 81% with ultrasound and 61% with clinical examination. The question of which modality is best for discriminating simple and complex fistulae was specifically addressed using established Evidence Based Practice principles by Sahni and colleagues in 2008 (86). They found that MRI is the superior technique with sensitivity and specificity of 97% and 96% compared with 92% and 85% for anal ultrasound and 75% and 64% for clinical examination. MRI remains the bedrock of fistula imaging at St Mark’s Hospital, partly for this reason, partly because the surgeons and gastroenterologists are able to interpret them themselves to some degree in theatre or in clinic, and partly because, as a more objective (less operator-dependent) examination, MRI also facilitates serial assessment and comparison of images over different time points.

This latter benefit is crucial in the medical management of Crohn’s anal fistulae and its utility is examined in Chapter 5. How to monitor the efficacy of the medical treatment of Crohn’s anal fistulae, when to increase the dose, change to a new regime or declare healing and halt treatment, are unknown but vital to optimising the use of anti-TNFα agents in this group.

1.4 Medical treatment of perianal Crohn’s disease

The established principles are to drain infection, use setons as required, aggressively manage active proctitis, give antibiotics, immunosuppressants, and employ anti-TNFα therapy, all of which demands significant co-operation between gastroenterologists and surgeons.

Few patients improve without any therapy (10% in the placebo arms of the early trials) (73;90;91). Also, improving, either spontaneously or with treatment, is not the same as being cured. The majority of recent medical trials (particularly those assessing the anti-TNFα agents) use clinical assessments of healing such as Fistula Drainage Assessment, where response is defined as a reduction of 50% or more from baseline in the number of draining fistulae observed at 2 or more consecutive study visits ≥ 4 weeks apart (55;73;85). A fistula is considered to be closed when it no longer drains despite gentle finger compression at examination. Remission is
defined as the absence of any draining fistulae at two consecutive visits. However, studies utilising MRI findings as a more rigorous end-point of deep tissue healing demonstrate undrained sepsis in these patients who, in turn, are very likely to recur or develop more complex sepsis (80). So fistula closure is not the same thing as fistula healing.

1.4.1 Corticosteroids

There is no demonstrable role for corticosteroids in perianal Crohn’s disease, although corticosteroids are sometimes used to treat concomitant luminal disease.

1.4.2 Metronidazole and ciprofloxacin

Both metronidazole and ciprofloxacin demonstrate slow and incomplete response, early recurrence and unwanted side effects. In an uncontrolled series, Bernstein and colleagues reported 21 patients with perianal Crohn’s disease treated with Metronidazole 20mg per kg daily (55). All had less discomfort and 10 of 18 (56%) had complete clinical healing. Improvement typically occurred after 6 to 8 weeks. Brandt and colleagues followed up 17 of these patients and added another 9 (56). Three quarters relapsed on stopping metronidazole and side effects including peripheral neuropathy and nausea limited long term use. Jakobovtis and colleagues reported clinical healing in 33 to 50% of metronidazole-treated patients (92).

Ciprofloxacin has been used in a small open series. Turunen and colleagues in 1989 reported 8 patients with perianal fistulating Crohn’s disease resistant to metronidazole; all improved but half had persistent drainage (57). Solomon and colleagues reported 14 patients with perianal Crohn’s disease treated with both metronidazole and ciprofloxacin with approximately two thirds of patients responding (58). Metronidazole has been compared with ciprofloxacin or placebo in a small randomised double-blind placebo-controlled pilot study reported in 2008 (93). Twenty-five patients with perianal Crohn’s disease were randomised to ciprofloxacin 500mg twice a day or metronidazole 500mg twice a day or placebo twice a day and were treated for ten weeks. Clinical remission and response rates were 30% and 40% with Ciprofloxacin, 0% and 14% with Metronidazole and 12.5% and 12.5% with placebo, none of these differences being significant. There were no differences seen.
in the scores of PDAI, CDAI, IBDQ, and patient and physician global assessment at any time point during the study.

Overall, antibiotics remain a mainstay of treatment for perianal Crohn’s disease despite the lack of controlled trial evidence. If metronidazole is used, a dose of 750–1500 mg/day is suggested. Adverse events include metallic taste, glossitis, nausea and neuropathy. It should be discontinued if any signs of neuropathy occur, but generally therapy is continued for 3–4 months. If ciprofloxacin is used, a dose of 500–1000 mg/day is adequate and again is usually required for 3-4 months. Adverse events include headache, diarrhoea, nausea and rash.

1.4.3 Immunomodulators

There are no controlled trials using the immunomodulators azathioprine and 6MP with fistula outcome assessed as primary end points. Efficacy is suggested by a meta-analysis of controlled trials in which fistulae were assessed as secondary end points. This meta-analysis reports a clinical fistula response of 54% in the azathioprine/6MP group compared with 21% in the placebo group (71). There are two uncontrolled case series (one in children) that have been reported (94;95). The larger series by Korelitz and Present in 1985 included 18 patients with perianal fistulae. Six patients demonstrated complete fistula closure and 4 patients demonstrated clinical improvement of their fistulae.

The combination of azathioprine and antibiotics has also been assessed (72). Fifty-two patients with predominantly simple Crohn’s perianal fistulae were given metronidazole or ciprofloxacin for 8 weeks. Patients who received azathioprine as well had a better response at week 20 than those who had not, suggesting combination treatment was better.

The slow initial response, side effects and relatively low remission and high recurrence rates with the drugs so far discussed have all left the door open to newer treatments.
1.4.4 Infliximab

With the anti-TNF drugs, comes a change in treatment options for perianal fistulating Crohn’s disease. The potential not only to improve quality of life but also to heal the fistula tracts has been realised and the time to clinical improvement has changed from the order of several months to a few weeks.

The first placebo controlled trial using infliximab was reported by Present and colleagues in 1999 (73). Ninety-four patients were given three infusions of infliximab at 0, 2 and 6 weeks. Sixty-eight percent of patients had a clinical response and approximately 50% of patients closed all fistulae. The median time to achieve response was 2 weeks.

In the maintenance trial of infliximab in patients with perianal fistulating Crohn’s disease (ACCENT II), 282 patients initially received 3 infusions of infliximab at 0, 2 and 6 weeks and responders at week 14 were randomised to receive placebo or maintenance infliximab 8 weekly for 54 weeks (74). There was a significant difference in the primary outcome measure, which was time to loss of response. Among patients who had a response at the time of randomisation, those given infliximab maintenance therapy had a significantly longer time to loss of response (greater than 40 weeks) than those who received placebo maintenance (14 weeks) (p< 0.001). Forty-six percent of patients in the infliximab maintenance group had a fistula response at 54 weeks and 36% of patients had a complete response at 54 weeks. In the placebo maintenance group, 23% of patients still had a response at week 54 and 19% had a complete response.

The impact of infliximab maintenance in the ACCENT II patients on hospital admissions and the need for surgery was examined by Lichtenstein in 2005 (96). The treatment maintenance group had a reduction of >50% in hospital admissions compared with the placebo maintenance group and their length of stay was also significantly reduced (2.5 to 0.5 days). The treatment group also had an approximately 50% reduction in the mean number of all operations and procedures, rising to >80% for major procedures. Not only does infliximab influence the number of hospital admissions and operations that a patient with perianal Crohn’s disease undergoes, but there is evidence that anti-TNF therapy improves health-related quality of life in patients with Crohn’s perianal fistulae (97).
When infliximab is stopped, however, the risk of recurrence is high and it is higher in perianal than luminal disease. Domenech and colleagues followed patients with perianal Crohn’s disease for 1 year after they had stopped a one year course of infliximab (induction plus maintenance). They found 83% of luminal CD patients were free of relapse compared to only 34% of perianal CD patients (98).

More recently, a large open label case series from Hungary of 148 Crohn’s anal fistula patients reported a remission rate of 49% at 12 weeks follow-up but longer term follow-up and confirmed healing on MRI were lacking (99). An Italian multicentre group reported an initial response to induction with infliximab in 76% of 188 patients with perianal Crohn’s disease and a 44% clinical remission rate (100). In a prospective study from St Mark’s Hospital, 34 patients with perianal Crohn’s fistulae were followed up long term. At 6 months, 58% of the infliximab treated patients were in clinical remission, 37% were in clinical response and 5% had had no response (80).

The question of whether infliximab should be used with concomitant immunomodulators such as thiopurines to achieve a better outcome, is difficult to answer in the setting of perianal Crohn’s disease. It is interesting to note that the majority in remission in the Hungarian study were on a concurrent immunomodulator, most frequently Azathioprine. In the Accent II trial, about one third of patients were on concomitant immunomodulators. A post-hoc analysis of the ACCENT II data by Lichtenstein and colleagues published in 2009 identified that approximately one third of patients in both placebo and infliximab arms were on concomitant immunomodulators at baseline and it appeared that there was no major benefit of dual therapy (101). However, in a recent study to assess whether concomitant immunomodulators are useful in patients with Crohn’s disease, it was found that perianal complications were less frequently observed at times when patients were on concomitant immunomodulators (102). The balance of risk and benefit of dual therapy needs to be considered in individual cases. In the recent SONIC trial, which assessed efficacy of infliximab monotherapy, azathioprine monotherapy and the two drugs combined in over 500 adults with moderate to severe Crohn’s disease who had not had previous immunomodulator or anti-TNF therapy, overall about 12% of patients had perianal fistulae - but no separate analysis was performed in this group to determine the relative benefits of single or dual therapy (103).
The presence of proctitis has been shown to be a predictor of poor response to anti-TNFα (80). There are no other clear predictors of failure or success of anti-TNFα in Crohn’s perianal fistulae. However, it is interesting to note that perianal fistulae heal more commonly than other fistulae, particularly internal fistulae, with this treatment (104).

The long term use of anti-TNF agents such as infliximab is costly and although rare, side effects of these drugs can be serious and may include septic complications, autoimmune phenomena such as drug induced lupus, demyelinating disease and malignancy (74). Their efficacy over the longer term, particularly with reference to true healing of fistula tracts, remains unknown with most reports detailing follow-up to several months rather than years. In Chapter 5 the efficacy of anti-TNF agents after 3 years of maintenance treatment will be examined with both clinical and radiological assessment.

1.4.5 Local injection of infliximab

Two open label studies have used infliximab as a local instillation in situations where disease is limited to the anus or there are contraindications to systemic infliximab. Poggioli and colleagues in 2005 reported the results of 3 to 12 infusions of infliximab (15 to 20mg) directly injected into the tissue surrounding both openings and into the wall of the tract (76). Healing of fistulae was noted in 10 of 15 patients.

Asteria and colleagues in 2006 reported 6 of 11 patients treated with local infliximab achieving a clinical response and 4 of the 11 remained healed at a median of 10 months follow-up (75).

1.4.6 Adalimumab

In the CHARM (Crohn's trial of the fully Human Antibody Adalimumab for Remission Maintenance) study, 113 patients with Crohn's fistulae were given adalimumab at week 0 (80mg), week 2 (40mg) and then maintenance with either weekly or fortnightly adalimumab or placebo (105). Fistula response and fistula remission (cessation of drainage from all orifices) were secondary endpoints. At 26 weeks, 30% of patients treated with adalimumab had complete closure and this rose to 33% at 56
weeks compared with 13% in the placebo arm. In CLASSIC-1 (Clinical Assessment of Adalimumab Safety and Efficacy Studied as an Induction Therapy in Crohn's disease) adalimumab at 80/40 mg, 160/80 mg or placebo was administered and short term effects were assessed. 32 of the 299 patients had draining perianal fistulae and no difference was found between placebo and any of the ADA induction doses for fistula response or remission (106).

Other studies have considered adalimumab in patients who have already failed an anti-TNF drug. In GAIN (Gauging Adalimumab efficacy in Infliximab Non-responders), Crohn’s disease patients who had lost response or who were intolerant to infliximab were treated with an induction regime of 160/80 mg adalimumab or placebo (107). Forty-five of the 325 patients had perianal fistulae and no difference was found between placebo and any of the ADA induction doses for fistula response or remission. In an open label trial in which 22 patients with perianal fistulae who had lost response or showed intolerance to infliximab were treated with adalimumab 160/80 mg, remission was noted in 23% at 4 weeks (108). The study of fistulating Crohn’s disease, described above, in which 34 patients with fistulating perianal disease (85% anal fistulae, 15% rectovaginal fistulae) were followed up at St Mark’s hospital, also evaluated the response to adalimumab in patients who lost response to or were intolerant of infliximab (80). At 6 months, 14% of the adalimumab treated patients (who had failed infliximab) were in clinical remission, 43% in clinical response and 43% having had no response. The data suggest that a second anti-TNF agent is effective in patients who have failed one anti-TNF therapy already, but the effect is reduced.

The long term use of adalimumab is shown in the CHARM extension study to 2 years in which 60% of adalimumab-treated patients who completed follow-up throughout both trials maintained fistula healing at 2 years, although the non-responder imputation efficacy at 2 years, which includes those patients who started on Adalimumab but left the trials for any reason (including stopping the drug due to lack of efficacy, adverse events, protocol violations etc.), was 31% (109). A further snapshot of efficacy at 3 years indicated that over 90% of those patients with healed fistulae at the end of CHARM, maintained healing for a further 2 years on either weekly or fortnightly adalimumab (110).
Table 2 - Randomised Controlled Trials (RCT) or meta-analyses of medical treatments of Crohn's-related perianal fistulae

<table>
<thead>
<tr>
<th>Drug</th>
<th>Study</th>
<th>Type of study</th>
<th>n</th>
<th>FU at end point</th>
<th>Fistula remission</th>
</tr>
</thead>
<tbody>
<tr>
<td>Metronidazole</td>
<td>Thia 2009 (93)</td>
<td>RCT</td>
<td>7</td>
<td>10 weeks</td>
<td>Rx 0% Placebo 12.5%</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>Thia 2009 (93)</td>
<td>RCT</td>
<td>10</td>
<td>10 weeks</td>
<td>Rx 30% Placebo 12.5%</td>
</tr>
<tr>
<td>Aza/6-MP</td>
<td>Pearson 1995 (71)</td>
<td>Metaanalysis</td>
<td>41</td>
<td>-</td>
<td>Rx *54% Placebo *21%</td>
</tr>
<tr>
<td>Tacrolimus</td>
<td>Sandborn 2003 (91)</td>
<td>RCT</td>
<td>42</td>
<td>10 weeks</td>
<td>Rx 10% Placebo 8%</td>
</tr>
<tr>
<td>Infliximab</td>
<td>Present 1999 (73)</td>
<td>RCT induction</td>
<td>94</td>
<td>14 weeks</td>
<td>Rx 55% Placebo 13%</td>
</tr>
<tr>
<td>Infliximab</td>
<td>Accent II (74)</td>
<td>RCT maintenance</td>
<td>282</td>
<td>54 weeks</td>
<td>Rx 36% Placebo 19%</td>
</tr>
<tr>
<td>Adalimumab</td>
<td>CHARMM # (105)</td>
<td>RCT maintenance</td>
<td>113</td>
<td>56 weeks</td>
<td>Rx 33% Placebo 13%</td>
</tr>
</tbody>
</table>

* = remission and response included together  
# = In CHARM patients with luminal and perianal Crohn’s disease were assessed

1.4.7 Other medical options

Other immunomodulators including Tacrolimus, Thalidomide, Methotrexate and Ciclosporin have been considered. Alternative options include GMCSF, absorptive carbon and hyperbaric oxygen.

1.4.7.1 Tacrolimus, Thalidomide, Methotrexate, Ciclosporin

1.4.7.1.1 Tacrolimus

Tacrolimus was assessed in a placebo-controlled trial in which it was given orally for 10 weeks in 42 Crohn’s anal fistula patients. There was significant clinical improvement in the tacrolimus group (45%) compared with placebo (9%) but no significant improvement in fistula remission rates was seen (91). Gonzalez Lama and colleagues in 2005 showed that longer term therapy may be required to achieve healing (111). Topical tacrolimus has been used in a placebo controlled trial in which 19 patients were stratified according to whether they had ulcerating or fistulating
disease (112). There was a benefit in the ulcerating but not the fistulating group. There was no systemic detection of tacrolimus and side effects were not encountered. This lack of systemic absorption was also noted in a case series of children with oral or perianal (6/8) Crohn’s and in whom the topical preparation was effective in improving fistulae after failure of numerous other therapies (113).

1.4.7.1.2 Thalidomide

Thalidomide has been used in the context of perianal Crohn’s disease. Vasiliauskas and colleagues in 1999 reported an open series of 12 male patients with Crohn’s disease of whom six had fistulae and 5 out of 6 demonstrated significant improvement (114). Ehrenpreis and colleagues in 1999 reported an open label study of 13 patients with fistulating Crohn’s disease (115). Six out of 13 achieved complete remission of their fistulae at 12 weeks. Plamondon and colleagues in 2007 reported an open label study of 11 patients with fistulating Crohn’s disease treated with Thalidomide (116). Nine out of the 11 responded to the Thalidomide and 3 out of 9 had a complete response. The data suggest that Thalidomide can heal fistulae in patients naïve or refractory to anti-TNF drugs.

1.4.7.1.3 Methotrexate

There is little evidence to support the use of methotrexate as monotherapy to treat perianal Crohn’s disease. There are no randomised data, only small case series. In one case series, 56% of patients with Crohn’s anal fistulae on methotrexate showed a complete or partial response to therapy (117). Schroder and colleagues used infliximab induction as a bridge to methotrexate (20 mg/week) in 12 consecutive patients of whom four (33%) had complete closure and three (25%) had partial response (118).

1.4.7.1.4 Ciclosporin

There have been small case series using intravenous ciclosporin, usually at a dose of 4mg/kg, to treat perianal Crohn’s disease. Although about three quarters of patients initially responded, the treatment was limited in that following oral transition or discontinuance of ciclosporin, a majority of patients relapsed (119).
1.4.7.2 GM-CSF

Human granulocyte-macrophage colony-stimulating factor (GM-CSF) has been examined in luminal Crohn’s disease in a small number of studies, including a double blind RCT where it was used as a steroid-sparing agent in steroid dependent Crohn’s disease. The primary end point was steroid free remission, which was achieved in 18.6% of treatment patients vs. 4.9% of those taking placebo (p = 0.03) (120). Evidence of improvement in perianal fistulae is limited and a small case series of heterogeneous and complex patients published in 2010 failed to find any improvement in fistulae in Crohn’s disease after GM-CSF treatment (121).

1.4.7.3 Hyperbaric Oxygen Therapy (HBOT)

The mechanism of action of HBOT is thought to be multifactorial, including raising the oxygen tension in damaged tissue, improving oxygen-burst mediated phagocytosis, decreasing circulating LPS-stimulated pro-inflammatory cytokines such as IL-1, IL-6 and TNFα levels, and inhibiting neutrophil-associated inflammation (122;123). Several studies during the 1980s and 1990s described cases or small case series of patients with severe perianal Crohn’s disease who partially or completely responded to HBOT (124-126).

In these trials, side effects of HBOT included tympanic membrane perforation and blurred vision (which resolved after treatment finished). Patients spent between 30 and 130 hours in the HBOT chamber in 1.5-2 hour sessions. It is difficult to draw firm conclusions from these heterogeneous studies on efficacy or side effects in the absence of randomised controlled data.

1.4.7.4 Oral absorptive carbon

Fukuda and colleagues reported a randomised controlled trial of oral absorptive carbon in which 57 patients received either activated absorptive carbon or a non-absorptive carbon molecule as placebo (127). The treatment group achieved 37% improvement and 30% remission of anal fistulae after 8 weeks treatment compared to 10% and 7%, in the placebo group. The authors suggested that the mechanism of action might be a combination of firming of stool, absorption of toxins or intestinal...
stimulation factors (such as serotonin and histamine) and restoration of the normal luminal environment and flora.

**1.4.8 Monitoring response to therapy**

The improved medical treatment for Crohn's perianal fistulae has prompted new questions: how to monitor patients, how long to treat them with drugs which have serious side effects and are expensive, and when or perhaps if to stop treatment.

Studies have shown that although clinical healing (in the form of a closed external opening or cessation of drainage) has occurred in the short term, imaging modalities including MRI indicate that the fistula tract may remain for some time with deep and true healing of the fistula lagging behind clinical remission by months or with reactivation after cessation of treatment or during maintenance treatment (79;80). In those in whom healing does take place, determining the moment of tissue healing using MRI and stopping treatment at this point, rather than based on clinical remission, ought to mean a lower risk of later recurrence.

Sequential MRI scanning may also select those patients who are non-responders at an earlier stage, permitting an early change to another anti-TNF therapy or the decision to stop altogether or continue on maintenance treatment. This early diagnostic impact on decision making should improve treatment efficiency and patient safety.

The prospective study of 34 patients with fistulating Crohn's disease at St Mark's Hospital, discussed above, also evaluated the impact of MRI on clinical decision making (80). Patients were treated with infliximab and, if there was a lack or loss of response or if the patient was intolerant to infliximab, they were treated with an alternative agent (adalimumab or thalidomide). Patients were assessed clinically and by MRI at 6, 12, 18 and 24 months, and yearly thereafter with a median follow-up of 83 weeks (range 52 to 131 weeks).

At 6 months, 58% of the infliximab treated patients and 14% of adalimumab treated patients were in clinical remission. They found that deep tissue healing as assessed by MRI was slower to occur than clinical healing and the rate of deep tissue healing
varied between patients. The presence of proctitis, but neither the complexity nor the duration of fistula, was associated with a lack of response.

Figure 3 - T2 weighted pelvic MRI images with fat suppression showing improvement of anal fistula from baseline (a) at six months (b) and 21 months (c).

Imaging may also be useful in guiding surgical intervention during medical treatment and thereby improve outcome. A small study by Spradlin and colleagues (2008) showed that serial EUS during maintenance infliximab treatment, which was used to time seton removal and repeat abscess drainage and seton insertion, reduced fistula recurrence and treatment failure (128).
Long term treatment with expensive drugs that pose potentially serious risks to health is increasingly common, so an understanding of whether treatment is working and what the long term future holds for an individual on anti-TNF agents is crucial, but currently conspicuous by its absence. This is partly because of the blunt clinical assessments used to monitor patients on these drugs. The natural history of patients on anti-TNF agents including radiological monitoring, as well as factors predicting outcome, are examined in Chapter 5.

1.5 Surgery for perianal Crohn’s disease

The surgeon’s role has changed, management being in combination with the physician. Some fistulae can be definitively cured, usually by lay open or advancement flap. Other patients need a proctectomy. Others need palliation of their symptoms, usually employing drainage of any trapped infection and thereafter with a long term, comfortable loose seton (Figure 4).

Examination under anaesthetic, drainage of sepsis and insertion of comfortable setons now forms the first stage in the truly combined surgical-medical eradication of anal fistulae using infliximab as described above. If pre-treatment surgical drainage is inadequate, abscess formation during the infliximab course may be more likely and could cause the treatment to fail. In the ACCENT II trial of maintenance infliximab in patients with fistulating Crohn’s disease, there was no signal that infliximab-treated patients were more likely to develop new perianal sepsis (129). Twenty-one (15%) patients in the infliximab maintenance group had at least one newly developed fistula-related abscess compared with 27 (19%) in the placebo maintenance group (P = 0.526). The number of fistula-related abscesses diagnosed over time did not differ between groups (Sands and colleagues 2006). Loose seton insertion seeks to palliate, reducing pain and discharge and in particular preventing recurrent abscess formation. In addition to the described role with infliximab treatment, it remains a key tool in the surgeon’s arsenal for the long term management of Crohn’s perianal fistulae, stubbornly resistant to all attempts at eradication, and may prevent deterioration requiring proctectomy in some patients (130-133).
Figure 4 - a. A bulky silastic seton and b. a low profile ethibond seton with just three throws and the ‘whiskers’ tied back with silk.

1.5.1 Surgery and infliximab

The combination of infliximab and surgery has been examined in a number of small trials in three ways; the application of surgery before infliximab treatment, combined maintenance with infliximab and seton insertion, and the application of infliximab before surgery.

The use of pre-infusion examination under anaesthetic, drainage of trapped sepsis and insertion of setons improved outcome by increasing initial response rate by almost a fifth, lowering recurrence rate by a third and increasing time to recurrence by 10 months (134).

Several small uncontrolled case series have reported the successful use of combined seton insertion and maintenance infliximab with remission rates of between 47% and 78% (135-137).

A series of 226 patients undergoing various surgical procedures for Crohn’s anal fistulae included 79 who received preoperative infliximab (138). The groups in this study were not randomised and were heterogeneous. For example the combined treatment group had a higher proportion of transsphincteric (as opposed to intersphincteric) fistulae, a higher proportion of seton insertions (as opposed to fistulotomies) and a higher proportion of patients with proctitis. Both groups achieved similar healing rates of approximately 60%. However, there were signals in this report which suggest there was a benefit to combined treatment. Time to healing was reduced from 12.1 to 6.5 months and two subgroups, patients with transsphincteric fistulae or those treated with setons as their only surgical intervention, healed more frequently if treated with infliximab too. Proctectomy rates
were similar in both groups (8-10%) and, interestingly, although proctitis was reduced in the combination group, this did not correlate with healing. The above mentioned heterogeneity makes these results more challenging to interpret but they none-the-less hint at the benefits which might be observed in a well-designed randomised trial of combined infliximab and surgical treatment.

A smaller series of 17 patients in whom 7 underwent either fistulotomy (for low fistulae) or advancement flap (for higher fistulae) alone and 10 had preoperative infliximab found 100% initial healing in both arms with 2 recurrences (29%) in the surgery alone group and 1 (10%) in the infliximab group (139).

1.5.2 Lay open

Some Crohn’s fistulae can be laid open and cured. As a general rule, surgeons are more cautious in Crohn’s disease and will not lay open fistulae that in the idiopathic setting they would be happy to treat. Not only are there concerns regarding future fistula formation and problems with healing, but the increased bowel frequency and the tendency for this to worsen with disease activity or future resection makes incontinence a greater threat.

1.5.3 Advancement flaps

Advancement flaps can be used as a sphincter preserving technique for some higher fistulae in Crohn’s disease with a success rate of around 50% (140;141). Patients are chosen who do not have active proctitis or extensive cavitating ulceration in the anal canal. A defunctioning stoma is usually used in an attempt to improve the chance of success, although there is little evidence suggesting an improved success rate. An adjunctive Martius flap may be helpful. An advancement flap is easier when there is perineal descent or internal intussusception.

Two studies from the Cleveland Clinics of Florida and Ohio, published in 2002, demonstrated a worse outcome for advancement flap surgery in Crohn’s anal and rectovaginal fistulae than those of cryptoglandular origin in more than 100 patients each (140;141). Mizrahi and colleagues showed an increased recurrence rate after surgery from 33% in non-Crohn’s fistulae to 57% in the Crohn’s patients which was
similar to the findings of Sonoda and colleagues with 30% recurrence rising to 50% in Crohn’s disease. This latter study also demonstrated a lower success rate in rectovaginal than anal fistulae and found that early surgery on a well-drained fistula complex increased the chance of success. Smoking has been shown to increase the risk of failure in cryptoglandular fistulae, although in this as well as most studies of advancement flap surgery, patients with Crohn’s disease were excluded (142).

1.5.4 Glues and plugs

Fistula infill materials, such as fibrin or collagen glues and collagen plugs, have not maintained the same initial enthusiasm and excellent results that their first published trials enjoyed. None-the-less they remain a low risk (albeit expensive) option in the management of idiopathic anal fistulae. Many of the trials of both glue (table 3) and plugs (table 4) have included small numbers of Crohn’s patients, although independent analysis of efficacy in the Crohn’s cohorts is often not possible due to these small numbers and the absence of MRI validation of healing.

Vitton and colleagues studied 14 Crohn’s fistulae (superficial, inter-trans and suprasphincteric and anovaginal) and observed complete clinical healing in 8 of them at a median of 23 months follow-up. The fistulae were described as refractory, although the complexity of the fistulae and extent of Crohn’s disease was not described (143).

A recently published study of fibrin glue in Crohn’s perianal fistulae, an open label, randomised controlled trial, demonstrated initial success (clinical remission at week 8) in 38% (13 of 34) of the fibrin glue group compared to 16% in the observation only group (144). Twenty of the 37 patients in the observation only group crossed over to fibrin glue treatment at week 8 and 9 of these patients went into remission at week 16. Unfortunately, of the 54 who underwent fibrin glue treatment at some point in the trial, only 11 (20%) were in remission at long term follow-up (median 37 months for initial fibrin glue group and 17 months for cross over group). No imaging was used to assess deep tissue healing in this study.
### Table 3 - Studies of fistula glue in anal fistula including Crohn’s patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>FU (months)</th>
<th>Routine preop seton</th>
<th>Total n</th>
<th>Crohn’s n</th>
<th>Overall success rate</th>
<th>Crohn’s success rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abel+ (145)</td>
<td>1993</td>
<td>3-12</td>
<td>No</td>
<td>10</td>
<td>3</td>
<td>6/10 (60%)</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td>Venkatesh# (146)</td>
<td>1999</td>
<td>12</td>
<td>No</td>
<td>30</td>
<td>6</td>
<td>18/30 (60%)</td>
<td>0/6 (0%)</td>
</tr>
<tr>
<td>Cintron# (147)</td>
<td>2000</td>
<td>12</td>
<td>No</td>
<td>79</td>
<td>6</td>
<td>48/79 (61%)</td>
<td>2/6 (33%)</td>
</tr>
<tr>
<td>Lindsey (148)</td>
<td>2002</td>
<td>17.1</td>
<td>No</td>
<td>19</td>
<td>2</td>
<td>12/19 (63%)</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>Zmora (149)</td>
<td>2003</td>
<td>12.1</td>
<td>No</td>
<td>24</td>
<td>5</td>
<td>8/24 (33%)</td>
<td>2/5 (40%)</td>
</tr>
<tr>
<td>Sentovich (150)</td>
<td>2003</td>
<td>22</td>
<td>Yes</td>
<td>48</td>
<td>5</td>
<td>33/48 (69%)</td>
<td>4/5 (80%)</td>
</tr>
<tr>
<td>Loungnarath (151)</td>
<td>2004</td>
<td>26</td>
<td>No</td>
<td>39</td>
<td>13</td>
<td>12/39 (31%)</td>
<td>4/13 (31%)</td>
</tr>
<tr>
<td>Singer# (152)</td>
<td>2005</td>
<td>27</td>
<td>No</td>
<td>75</td>
<td>3</td>
<td>25-44%</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td>Vitton# (153)</td>
<td>2005</td>
<td>23.4</td>
<td>No</td>
<td>14</td>
<td>14</td>
<td>8/14 (57%)</td>
<td>8/14 (57%)</td>
</tr>
<tr>
<td>Chung$ (153)</td>
<td>2010</td>
<td>3</td>
<td>No</td>
<td>(81)</td>
<td>2</td>
<td>23/79 (29%)</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td>de Parades# (154)</td>
<td>2010</td>
<td>11.7</td>
<td>Yes</td>
<td>30</td>
<td>11</td>
<td>15/30 (50%)</td>
<td>7/11 (63.6%)</td>
</tr>
<tr>
<td>Grimaud# (144)</td>
<td>2010</td>
<td>2</td>
<td>No</td>
<td>34</td>
<td>34</td>
<td>13/34 (38%)</td>
<td>13/34 (38%)</td>
</tr>
</tbody>
</table>

#Includes some rectovaginal fistulae
+The one success in the CD group had a rectovaginal fistula (RVF) which healed after 2 treatments
$Idiopathic results from contemporary but separately published results by same authors

The anal fistula plug has been used in trials of Crohn’s perianal disease with success rates between 25% and 100% (table 4) including the two largest studies (of 14 and 20 patients) which were at opposite ends of this spectrum of success (26.6% and 80%). Failure occurred due to extrusion of the plug, abscess formation or ongoing discharge. Long term follow-up or radiological proof of fistula healing in an adequately powered randomised controlled trial is required to establish efficacy of this expensive treatment.
Table 4 - Studies of the fistula plug in anal fistula including Crohn’s patients

<table>
<thead>
<tr>
<th>Study</th>
<th>Year</th>
<th>FU (months)</th>
<th>Routine preop seton</th>
<th>Total n</th>
<th>Crohn's n</th>
<th>Success rate</th>
<th>Crohn's success rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>O’Connor* (155)</td>
<td>2006</td>
<td>10</td>
<td>depends</td>
<td>20</td>
<td>20</td>
<td>16/20 (80%)</td>
<td>16/20 (80%)</td>
</tr>
<tr>
<td>van Koperen (156)</td>
<td>2007</td>
<td>7</td>
<td>No</td>
<td>17</td>
<td>1</td>
<td>7/17 (41%)</td>
<td>1/1 (100%)</td>
</tr>
<tr>
<td>Ky (157)</td>
<td>2008</td>
<td>6.5</td>
<td>No</td>
<td>44</td>
<td>14</td>
<td>24/44 (54.6%)</td>
<td>4/14 (26.6%)</td>
</tr>
<tr>
<td>Schwandner (131)</td>
<td>2008</td>
<td>9</td>
<td>No</td>
<td>18</td>
<td>7</td>
<td>12/18 (61%)</td>
<td>6/7 (85.7%)</td>
</tr>
<tr>
<td>Christofidis (158)</td>
<td>2008</td>
<td>6.5</td>
<td>No</td>
<td>47</td>
<td>3</td>
<td>20/47 (43%)</td>
<td>NR</td>
</tr>
<tr>
<td>Schwandner† (159)</td>
<td>2009</td>
<td>9</td>
<td>No</td>
<td>9</td>
<td>9</td>
<td>7/9 (77%)</td>
<td>7/9 (77%)</td>
</tr>
<tr>
<td>Safar (160)</td>
<td>2009</td>
<td>4</td>
<td>No</td>
<td>36</td>
<td>4</td>
<td>5/36 (13.9)</td>
<td>1/4 (25%)</td>
</tr>
<tr>
<td>Zubaidi (161)</td>
<td>2009</td>
<td>12</td>
<td>No</td>
<td>22</td>
<td>2</td>
<td>19/22 (86%)</td>
<td>1/2 (50%)</td>
</tr>
<tr>
<td>Chung$ (153)</td>
<td>2010</td>
<td>3</td>
<td>No</td>
<td>50</td>
<td>4</td>
<td>27/46 (59%)</td>
<td>3/4 (75%)</td>
</tr>
<tr>
<td>Owen (162)</td>
<td>2010</td>
<td>15</td>
<td></td>
<td>35</td>
<td>3</td>
<td>13/35 (37%)</td>
<td>1/3 (33%)</td>
</tr>
</tbody>
</table>

*The O’Connor study included one pouch patient, 2 anovaginal fistulae and 4 patients on concomitant infliximab. Single rather than multiple tracts were significantly associated with successful closure. Preoperative setons were used if the tract was too wide for the plug to sit snugly.

NR – not reported or cannot be concluded form the data presented

†In this study, 2 of the 10 patients enrolled were defunctioned and one was lost to follow-up.

$Idiopathic results from contemporary but separately published results by same authors

1.5.5 Modified infill materials

The waning published efficacy of glues and plugs over time has led to the addition of agents designed to improve healing rates. Preclinical work at St Mark’s has demonstrated that the addition of autologous fibroblasts to a collagen glue improved the histological appearance of the healed tract compared to glue alone and clinical studies are in progress (163). A single case report of autologous fibroblasts on a biologic scaffold (HYAFF) has been described in a Crohn’s patient with a transsphincteric fistula (67). The patient had a seton inserted and three doses of intravenous infliximab administered before fistulectomy and then insertion of the scaffold with autologous fibroblasts into the tract until it was completely filled. The
patient was free of recurrence throughout a 20 month follow-up period, but MRI validation of healing was not performed.

In 2008 Garcia-Olmo and colleagues reported the administration of adipose derived stem cells (ASCs) in fibrin glue vs. glue alone in a randomised controlled trial of 49 patients (14 with Crohn's disease) and found a significant increase in healing from 16% without ASCs to 71% with them (164). ASCs are living adult stem cells of mesenchymal origin which are extracted from subdermal adipose tissue obtained through liposuction. The study's authors suggest that the ASCs may have both an initial anti-inflammatory effect and also aid tissue repair through differentiation into epithelial cells.

This approach of adding constituents to correct specific aspects of the pathology in persisting anal fistula tracts is extremely attractive and could in the future lead to personally tailored treatments in both idiopathic and Crohn's related anal fistulae, incorporating aspects of immunology and microbiology specific to an individual.

1.5.6 Defunctioning and proctectomy

Some patients have either failed medical therapy and undergone several operations finding nothing that helps to alleviate their symptoms and gain the quality of life they want, or are subdued by the systemic effects of their colorectal disease to such an extent that a stoma becomes a realistic option. Proctectomy (+/- colectomy depending on the extent of disease) in the patient with severe rectal and/or perianal disease can leave patients with an improved quality of life in spite of a recurrence (of luminal disease) rate of around 20% and a similar risk of reoperation. The principle risks of this operation include damage to the pelvic nerves and the delayed or unhealed perineal wound, which latter affects around 30% of patients up to 6 months and 10% beyond that time. A small group of these will require further surgical procedures to aid healing of the wound, including curettage or (usually myocutaneous) flap reconstruction.

Defunctioning with an ileostomy provides temporary relief of symptoms but disease often recurs and most of these stomas will never be reversed. Yamamoto and colleagues reported their series of 31 patients who underwent defunctioning alone in Crohn's disease for perianal or anovaginal disease (34). Only 8 (26%) went into long
standing remission and only 3 patients (10%) had their stoma reversed. Hong and colleagues reported a series of 21 patients who were defunctioned for perianal Crohn’s disease and found the chance of having continuity restored was not influenced by infliximab. At median follow-up of 22 months (4-121), 4 patients (19%) had undergone stoma closure, 11 had had a proctocolectomy, and 6 had both a stoma and their rectum in situ. The effect of the procedure on severity of perianal disease was no effect in 4 (19%), temporary improvement in 6 (29%), initial improvement with a sustained benefit in 7 (33%), and healing in 4 (19%) (165). As a general rule, if a patient needs their large bowel Crohn’s disease excised, defunctioning alone will leave on-going ill-health with little or no chance of reversal in the longer term and should be avoided.

1.6 Fistula-associated carcinoma in Crohn’s disease

It is important to note that malignancies can arise in Crohn’s related perianal disease. Although it is uncommon, a recent literature review revealed 61 cases of carcinomas arising in perineal fistulae in Crohn’s disease. Sixty-one percent of the patients were females, who were also significantly younger than males at the time of diagnosis of cancer, and had a shorter duration of fistulating perianal disease prior to cancer transformation. Adenocarcinoma was the most common histology followed by squamous cell carcinoma (166).

1.7 Summarising the current management of Crohn’s anal fistulae

Anal fistulae are a common, unpleasant and treatment-resistant manifestation of Crohn’s disease.

There is evidence for genetic, immunological and bacteriological factors in the aetiology of Crohn’s perianal fistulae. A combination of tackling the environmental milieu in the tract and the microbes may hold the key to more effective treatment of Crohn’s perianal fistulae. A specific focus on factors which lead to persistence of the tracts, including further microbiological characterisation, study of the host immune cells and responses to the micro-organisms present, and genetic predisposing factors is needed to elucidate this and guide the next steps in research and
management. The pathogenesis of idiopathic fistulae is also poorly understood and advances in this area would improve our general understanding of perianal fistulae in both the idiopathic and Crohn’s disease settings.

Medical treatments with antibiotics and immunomodulators have had limited success, but the advent of anti-TNF drugs has had a dramatic effect on the prognosis of these fistulae, offering the patient a realistic chance of response and indeed remission. However, loss of response and side effects are all too common. Local administration, combinations of drugs and new anti-TNFα agents all offer potential improvements.

Surgery likewise can have a high rate of recurrence for most procedures and the risks of stomas, impairment of continence, unhealed wounds and repeated operations are ever present.

The primary goal of treating Crohn’s anal fistulae should be eradication without recurrence. This may sometimes be achieved by surgery in the form of laying open in low fistulae, or alternatives such as advancement flaps or fistula plugs (although the evidence for these latter is scant and contradictory). Healing is more likely to occur using anti-TNF treatments. Surgery and imaging remain absolutely crucial to this mode of management with EUAs with drainage of trapped sepsis and insertion of setons before and sometimes during treatment, and high quality radiological monitoring of response so that appropriate decisions on interventions such as surgery, cessation of medical treatment or dose or drug change can be made.

1.8 Idiopathic anal fistulae

1.8.1 Background

Idiopathic anal fistulae most often occur in patients aged between 30 and 60 with an incidence of around 1-2 per 10,000 people (167), far less commonly than in Crohn’s disease, however they pose a very significant problem both to the sufferers and to colorectal surgeons. The symptoms of pain or discomfort, recurrent abscess formation, persistent discharge and limitations to sexual and other activities of daily living can be very debilitating.
1.8.2 Aetiology

As discussed above, idiopathic fistulae are also known as cryptoglandular based on the aetiological theory proposed by Parks in which he described an intersphincteric anal gland becoming infected and this infection becoming chronic with a fistula tract forming as a result (81). Parks noted dilated anal glands in 8 of 30 patients described in his aetiological paper and suggested that this might represent a congenital abnormality, making some people more susceptible to the formation of a granulation lined tract fed and kept open by the abscess around this gland in the intersphincteric space.

However, deeper examination of the cryptoglandular hypothesis has taken place only rarely since Parks’ original work. Goligher and colleagues found evidence of intersphincteric sepsis in less than half the anal fistulae they examined for his 1967 paper (168). Also, this theory implies a bacteriological aetiology with organisms feeding the infection from the internal opening and it is for this reason that surgical treatments focus upon draining sepsis, obliterating the internal opening or disconnecting it from the rest of the fistula tract. However, a consistent culprit organism has never been found (1.2.5) and indeed Lunniss and colleagues surprisingly found an enrichment medium was necessary to culture organisms from what one might expect to be a floridly infected wound, given this aetiological explanation (52).

As mentioned above, epithelialisation has been suggested as a potential factor in the persistence of anal fistulae and epithelialisation has been found to occur from one end of the tract or the other more commonly than evidence of an infected anal gland (84).

In fact, as with Crohn’s disease, the histological, immunological and microbiological nature of idiopathic anal fistula tracts remains obscure and an examination of these factors is reported in Chapter 2.

1.8.3 Classification

The surgical basis for management of idiopathic anal fistulae demands a more rigorous classification system than that employed for the majority of Crohn’s anal fistulae. Parks described anal fistula according to their primary tract and secondary
extensions with the external sphincter as the ‘keystone’ (169). The primary tract is either intersphincteric (comprising 45% of his initial cohort), trans-sphincteric (30%), suprasphincteric (20%) or extrasphincteric (5%) (Figure 5). This latter group is aetiolgically distinct from true anal fistulae and will not be discussed further here.

Figure 5 - Parks’ classification of anal fistulae, (169) by kind permission of John Wiley and Sons.

In addition to the primary tract, secondary extensions can occur in the intersphincteric plane, the ischioanal fossae or the pararectal space and may form linear extensions leading to abscesses or horseshoe extensions which curl around the anorectum on one or both sides.

Figure 6 - a. trans-sphincteric fistulae with extensions into the ischioanal fossae and pararectal space and b. the three planes in which horseshoeing can occur (intersphincteric, ischioanal fossa and pararectal) (169) by kind permission of John Wiley and Sons.
1.8.4 Clinical assessment

The assessment of a patient with an anal fistula is based on a sound history and a focused clinical examination. Aetiological factors, like the presence of diagnosed or occult inflammatory bowel disease, nicorandil use, and anal digitation will potentially change the planned management. The consistency of stool and any likelihood of sphincter deficiency, such as obstetric injury or previous sphincterotomy for fissure, are relevant when considering surgical options.

Some patients may have cultural or religious difficulties with treatment-induced minor discharge or soiling which will inform their decision, and a long suffering patient may well have a higher tolerance of risk than a younger patient with a short history.

Although there is no standard or evidence base to the techniques employed for clinical assessment of anal fistulae, one approach, previously described, is outlined below (170). Examining the patient in the left lateral position, the surgeon performs rigid sigmoidoscopy to identify proctitis after a detailed digital rectal examination. Any external opening (open or healed) should be described and palpated for tenderness and induration. The area between the external opening (EO) and the anal verge should then be palpated with a lubricated finger and any tract felt will of necessity be superficial. The absence of induration implies a deeper course.

Within the anal canal, the internal opening may be palpable and is likely to be lower than assumed, even in higher fistulae. It may be felt as a grain of sand, more tender than surrounding tissues. Induration, a feeling like bone rather than fillet steak, should then be sought within the anal canal, at the anorectal junction and within the rectum itself (170). This gives a clue to the passage of the primary tract or to the presence of any secondary extensions.

The length of good quality contracting external sphincter above the internal opening must be assessed as incontinence of greater magnitude than flatus incontinence and minor anal seepage depends not on how much muscle is cut, but on how much muscle is left behind. Minor incontinence (flatus incontinence and < 1 teaspoon of mucus leakage in a 24 hour period) is probably related to internal sphincter division alone (171-174).
1.8.5 Investigations

The majority of patients can be fully assessed clinically and investigations are not usually necessary. In the case of complex disease, however, or fistulae which cannot be characterised clinically, it is sometimes valuable to undertake pelvic imaging to elucidate the fistula tract’s course. Endoanal ultrasound and pelvic MRI are the most commonly used and their various merits have been discussed above (page 34).

Briefly, although EUS is useful for assessing the sphincter complex and gives an excellent description of the internal opening and any tract close to the anal canal, it cannot assess the ischioanal fossae well due to rapidly declining resolution with distance from the probe, it is operator dependent and it can be uncomfortable in the presence of acute sepsis. Generally speaking, clinicians find the images difficult to interpret and rely on the report from their radiology colleague.

MRI, using a T2 weighted fat suppressed sequence (e.g. STIR), gives excellent images without the need for a radiologist to be present at the time of the scan. The images are more easily interpreted by clinicians who can often identify the important anatomical structures more clearly and can use the images in the clinic or theatre setting. Although the position of the internal opening is often inferred rather than actually seen, any tract can be visualised regardless of how far from the anus it travels.

1.8.6 Surgical treatment

Based on the aetiological and anatomical findings from clinical assessment and any investigation, patient preference and surgical expertise, the most appropriate form of treatment can be determined.

1.8.6.1 Addressing the compromise

All currently available operations for anal fistula represent a compromise. The patient needs to decide whether he or she prefers a higher risk of recurrence or a higher risk of (usually minor) impairment of continence. The word incontinence is both emotive and ambiguous, embracing as it does all situations between full faecal incontinence to formed stool alongside the slightest occasional inadvertent escape of flatus or slight staining of the underwear. Some patients have recurrent problems
with abscess formation and copious discharge, repeated operations and significant interference with their daily lives over many years and are willing to risk a high rate of minor inadvertent escape of gas or slight marking of the underwear to gain a good chance of cure. Each individual’s approach to this compromise is a crucial element of the management plan.

Importantly, not all patients who develop incontinence after fistula surgery are dissatisfied by their outcome (175) and a study published in 2000 by Garcia-Aguilar and colleagues found that the risk of dissatisfaction was greater in patients suffering recurrence (61%) than those who developed impairment of continence (24%) after fistulotomy (176).

1.8.6.2 Fistulotomy

Laying open a fistula and allowing healing to take place by secondary intention remains the treatment of choice for many surgeons encountering low intersphincteric or trans-sphincteric fistulae and has been shown in large series to have a high success rate (177). High transsphincteric fistulae, too, may be laid open in some circumstances. The risk of impairment of continence is the principle concern regarding this procedure and, although the rate of incontinence following this procedure in expert hands is between 1/4 and 1/3, the impairment produced is usually minor with only the inadvertent loss of flatus or mild mucus leakage (171). This is often acceptable to patients if a reasonable chance of cure offsets the risk.

It is reasonable to postulate, given that the impairment is similar in intersphincteric and trans-sphincteric fistulae (171), that internal sphincter division is responsible for this incontinence, which can also be seen after lateral anal sphincterotomy for anal fissure (178;179). Moreover, a policy of leaving behind usually 2cm but at least 1cm of contracting muscle cephalad to the fistulotomy resulted in minimal incontinence in a recently published cohort (171).

Nevertheless, when deciding to offer lay open of a high fistula, factors such as those discussed above including the patient’s usual bowel habit, tendency towards IBS, the presence of IBD, a history of sphincter injury or traumatic vaginal delivery, the quality of the residual sphincter and the presence of extensions which will be hard to drain
adequately, need all to be considered in order to offer a reasonable assessment of the risk of recurrence and ‘incontinence’.

Marsupialisation can be used to reduce the size of the wound (which usually heals well despite florid bacterial contamination) and may decrease time to healing (180). The addition of immediate reconstruction of the divided sphincter has been described in low fistulae and is successful in terms of rapid healing and postoperative pain as well as avoiding recurrence and incontinence (181) (see below). Open excision of the fistula tract delays healing (182), and core out fistulectomy, whilst less destructive and advocated by some (183), remains a less popular technique than fistulotomy.

1.8.6.3 Fistulotomy or core out fistulectomy and primary sphincter repair (FSR)

Fistulotomy or core out fistulectomy and immediate sphincter repair offers the chance to cure fistulae deemed too high to lay open without risking major incontinence. Suitable patients include those with recurrent fistulae, contraindications to advancement flap, or a pre-existing sphincter deficit amenable to surgical repair.

Advocates suggest that post-operative healing is faster than in fistulotomy alone but with comparable recurrence rates (181), and that continence is improved in patients with a preoperative deficit with only a minor risk to those who are fully continent (184).

Large series were reported in 1968 and 1985 with over 100 patients with low fistulae. Parkash and colleagues found that 88% of wounds healed within 2 weeks of surgery and recurrence occurred in 4% of patients with general satisfaction with functional outcome (181).

More recently, 4 case series have been published with cohorts of 14 to 75 patients with high fistulae. Recurrence and post-operative (mostly minor) incontinence rates ranged from 0 to 14% and 0 and 21% with the worst outcomes seen in a report of recurrent fistulae (185;186). High quality studies are lacking and this technique does not enjoy the support of many colorectal surgeons. Randomised controlled data are required to prove its worth.
1.8.6.4 Advancement flaps

Through disconnection of the tract from the bowel and eradication of the internal opening, advancement flaps have been a valuable tool in the fistula surgeon’s armoury for a century. Different techniques of flap repair have been proposed, including full and partial thickness flaps from the rectum cephalad of the internal opening to rhomboid flaps and anodermal flaps from below. The internal sphincter is often taken, at least in part, supposedly to preserve the flap’s blood supply.

Causes of failure are thought to be either technical through flap ischaemia or anastomotic breakdown, or due to underlying disease. In a large (albeit heterogeneous) consecutive series of 96 patients treated with endorectal advancement flap, Mizrahi and colleagues identified the presence of Crohn’s disease but not gender, number of previous repairs, transspincteric vs. rectovaginal fistulae or the presence of a diverting stoma to be associated with failure (140). In a similar sized series of idiopathic anal fistulae, smoking was found to predict failure of transanal flap repair, reducing success from 79% to 60% with a greater impact the more cigarettes the patient smoked per day (142). The overall success rates in these two studies, in line with larger studies in the literature, were 60% and 69% respectively.

Randomised controlled trial data of fistulotomy (with immediate sphincter repair in the Perez trial) vs. two different advancement flap techniques (island flap anoplasty [from below upwards] and transanal endorectal advancement flap [from above downwards]) suggest parity in terms of incontinence and recurrence (187;188). Those fistulae too high to contemplate fistulotomy obviously cannot be included in a randomised trial, so success in the treatment of these very high anal fistulae managed by advancement flap should not be directly compared with fistulotomy outcomes.

1.8.6.5 Infill materials

The ongoing search for fistula treatments that avoid the compromise between recurrence and incontinence led to the advent of first fistula glues and subsequently fistula plugs. These infill materials also disconnect the tract from the bowel along with filling the lumen of the tract. Most trials of either glue or plug have utilised preoperative seton insertion, antibiotic cover, bowel preparation or a combination of
these to try to reduce the septic load and acute inflammation in the perioperative period.

Fibrin glues use fibrinogen and thrombin in separate barrels of a double-barrelled syringe which come together at the point of instillation in the tract. The resulting fibrin plugs the void and allows collagen to be laid down across the scaffold. Unfortunately, the fibrin is absorbed within 7-10 days which may not be long enough for tissue repair to take place (189). The external opening often heals over, however, with epithelialisation occurring rapidly but ultimately breaking down when sepsis recurs within the tract itself, which explains the early healing and later recurrence pattern of failure seen with fibrin glues.

Initial success rates of around 80% have gradually fallen away to around 30-40% seen in some of the more recent trials (Figure 7) with the exception of studies which have examined modifications to the fibrin glue, including the addition of antibiotics (152), using a more concentrated glue (149), and the addition of porcine collagen fibres (190) which are postulated to reduce the bacterial load within the fistula and improve the action of the glue as a scaffold respectively.

Failure adequately to drain sepsis and remove granulation tissue and epithelialisation from the primary tract and secondary extensions will inevitably lead to failure. Early absorption or leakage of glue from the tract will also promote recurrence. However, the great advantage of fibrin glue instillation is that it is repeatable and the sphincter is not damaged, so it may be attempted a number of times before an operation, which latter may offer a higher chance of success but also has a risk of incontinence.
Figure 7 - Graphs of published healing rates over time in studies of fibrin glue in anal fistulae. # = only high transssphincteric fistulae studied. * = enhanced glue.
The collagen plug appears to be following in the footsteps of fibrin glue with initial success rates over 80% decreasing over time (Figure 8).

**Figure 8** - Graphs of published healing rates over time in studies of fistula plug in anal fistulae.
Rather than gradually decreasing in published efficacy, it is probably fairer to say that the efficacy of the fistula plug is extremely variable with the large variation in reported success rates maintained each year from 2006 to 2009 (Figure 9) (191).

Perhaps this variability is due to poor patient selection and/or poor placement technique, including failure adequately to prepare the tract or secure the plug. Poor technique often results in extrusion of the plug and later reports contain fewer extrusions as the cause for failure. The variable nature of the research methodologies and follow-up used may also play a part and randomised controlled trials of the fistula plug are required to demonstrate its efficacy. One RCT of fistula plug vs. advancement flap in high transsphincteric idiopathic fistulae was stopped early due to an unacceptably high failure rate in the fistula plug group of 80% compared to 12.5% in the advancement flap group (192).

![Figure 9 - Fistula healing rates with the anal fistula plug as reported in articles found in PubMed over the past 4 years. Each triangle represents a publication. The horizontal bars represent median and range of values (191). By kind permission of Wolters Kluwer Health.](image-url)

Interestingly, a recent report indicated that tracts >4cm in length were almost 3 times as likely to heal as their shorter counterparts which the authors suggested was due to the presence of a greater surface area of tissue into which the plug could be
incorporated (193). Importantly, one of the authors of this report was an experienced fistula plug surgeon and it must be assumed that placement and technique were optimal in this series – the overall success rate of 43% is therefore particularly disappointing.

In order to reduce the variability in technique and outcome, consensus statements describing optimal patient selection, surgical technique and follow-up were published in 2008 (194). Failure often occurs due to plug extrusion, which is related to surgical technique or tract diameter and should occur less frequently in more experienced hands, where a success rate of 50-60% is expected.

On long term follow-up or through the use of MRI scanning a true healing rate lower than that quoted in some studies of AFP may be found. One study found the success rate fell from 84% at 2 months to 54% at 12 months (157). Ellis and colleagues found a quarter of patients clinically healed for a year had evidence of residual fistula tract at MRI scan (195).

As with fibrin glue, even a modest success rate (if true), given its repeatability and the lack of sphincter damage, would be valuable. The plug is expensive compared to fistulotomy, yet one cost effectiveness study reported AFP (at $2100) to be cheaper per success than endoanal advancement flap for high fistula (196).

1.8.6.6 Intersphincteric approaches and the LIFT procedure

Another method of disconnecting the fistula from the bowel and encouraging healing via an intersphincteric approach was first described at St Mark’s in 1993 by Matos and colleagues (197). The technique involved an intersphincteric approach to the area of intersphincteric sepsis which was eradicated, closure of the internal opening and IAS defect, excision of the external element of the tract, repair of the EAS defect and primary wound repair Figure 10.

The primary outcome in this study was the preservation of both sphincters which was achieved in 7 of 13 patients with a further 2 having preservation of the external sphincter only. Five of the patients experienced primary healing following the procedure.
In 2009 the LIFT (ligation of inter sphincteric fistula tract) procedure was described and is different in two regards: ligation and division of the intersphincteric tract rather than closure of the internal sphincteric defect and IO is performed and curettage rather than excision and primary repair of the external component (198). The authors suggest that ligation is more secure and curettage faster and suggest that their improved results (recurrence of 5.6%) are due to the IS ligation, although the cohort consisted of mainly low fistulae by comparison with the suprasphincteric, rectovaginal and IBD-related fistulae in the St Mark’s cohort.

Further series have been published. A Malaysian group has reported a study of 45 patients with predominantly low trans-sphincteric fistulae (3/4) with primary healing in 82.2% of patients with no deterioration in continence (199). Another study of 39 patients with mostly recurrent fistulae has reported a success rate of 57% and also found no subjective deterioration with regards incontinence (200).
1.8.6.7 Setons

Setons are surgical sutures or wires placed through the lumen of the fistula tract and tied externally with the loose ends external to the external opening and the anus. The role of the seton can be for anatomical assessment, for curative treatment as part of a staged approach or through recurrent tightening, or palliative.

The decision about whether or not it is safe to lay open an anal fistula should be based on the length of good quality contracting sphincter above the internal opening. Clearly, this assessment can only be made when the IO can be felt. At EUA, the placement of a seton in the patient who has not undergone spinal or epidural anaesthesia (which relaxes the sphincter to such an extent that accurate estimation may be impossible) may permit determination of this distance before lay open. Alternatively, further clinical assessment can be carried out in a subsequent clinic appointment and further counselling on the risks of fistulotomy can be undertaken.

Tight or cutting setons are methods of gradual fistulotomy with fibrosis of the sphincter taking place behind, theoretically lowering the risk of impairment of continence. A seton is placed through the fistula and, when the acute sepsis is drained, it is tightened either once (tight) or on a regular basis until it has cut through (cutting). Some patients find the tightening painful (201). Low fistulae as well as higher tracts, including horseshoe fistulae, have been treated with this approach (202;203); the more complex fistulae suffer from similar continence disturbance and recurrence rates as those treated with fistulotomy. Lunniss has favoured what he terms a ‘snug’ seton which he claims cuts through more slowly (204).

A recent systematic review comparing tight or cutting seton placement either with (SIAS) or without (PIAS) surgical division of the internal anal sphincter up to the level of the fistula, found that dividing the IAS at operation reduced recurrence from 5% to 3% but increased incontinence from 5.6% to 25.2% (205). The overall rates of recurrence and faecal incontinence in the 18 studies of tight or cutting seton reported ranged from 0 to 16% and 0 to 75% respectively.

These rates of incontinence are not dissimilar to those seen in single stage fistulotomy which has prompted some to question the value of the tight or cutting seton. Unfortunately this cannot be tested experimentally; in a fistula low enough to undergo fistulotomy, there is no advantage to the cutting seton, whereas in higher
fistulae one cannot randomise patients to a fistulotomy arm in the knowledge of a high rate of faecal incontinence, even to prove that the rate in tight setons is similar.

Loose setons can be placed as part of a staged surgical approach, for example with an advancement flap (206), or as a permanent palliative treatment. Setons initially provide consistent drainage which reduces acute sepsis and encourages healing of accessory tracts and extensions. A well-drained system can then have the seton drain removed to allow healing to take place with or without further surgery. This is a long process and it is not clear that the nature of the final continence outcome is different to that which would have been offered by fistulotomy.

A comfortable, soft, low profile seton can be placed to provide palliation of symptoms of pain and recurrent abscess when the risk of incontinence is too great for the patient to countenance. Sharp, stiff or bulky setons, especially those with multiple knots, can be uncomfortable and may not provide the respite from symptoms which patients desire in order to obtain an adequate quality of life. Even the most comfortable setons may break from time to time and require replacement.

1.8.7 Optimal treatment of high anal fistulae

The presence of so many options for the treatment of high anal fistulae implies the lack of a definitive solution. In Chapter 3 the outcomes of a cohort of mainly high, tertiary referred anal fistula patients treated with fistulotomy is explored with the specific aim of identifying the level of compromise found in this group of patients with experience of prior failure and often a degree of impairment of continence from earlier attempts at cure. The risk of recurrence and the additional risk of incontinence from further surgery are key to deciding whether to offer further attempts at radical treatment or whether palliation may be preferable. Associations between aspects of anatomy and natural history with recurrence or incontinence are also explored in Chapter 3.
1.9 Rectovaginal fistulae

1.9.1 Background

Rectovaginal fistulae are not well tolerated and patient pressure to resolve even the more minor ones is frequently intense. They usually occur following obstetric injury, perianal sepsis or in the presence of Crohn’s disease, although radiation, malignancy and iatrogenic injury are other known causes.

Symptoms include the passage of air, discharge and faeces from the vagina, recurrent urinary tract or genital infections, perianal and vaginal pain and discomfort, including dyspareunia.

The commonest cause of rectovaginal fistula is obstetric injury. The fistula usually occurs following a third or fourth degree tear which is sustained during approximately 5% of labours. One to two percent of these will go on to suffer a rectovaginal fistula (207). A large study of women undergoing vaginal delivery found 0.1% developed a rectovaginal fistula (208).

In a population based survey of Crohn’s disease patients, 35% were found to have fistulae and of these 9% had a rectovaginal fistula (3) which appeared at a mean age of 34 years (209). A larger study of 886 patients revealed that rectovaginal fistulae may occur in up to 10% of those with Crohn’s disease (209). RVF is more common in association with colonic (23%) than small bowel (3.5%) Crohn’s disease (210,211).

1.9.2 Clinical assessment

A detailed history considering symptoms of inflammatory bowel disease or irritable bowel syndrome, stool consistency and frequency and continence is required. The obstetric history is also important, along with a description of any perianal procedures, including previous repairs.

The severity of symptoms is important since surgery may not cure and could involve multiple procedures and possibly a stoma, so a balance of the risks and benefits in each case, and particularly recurrent cases, must be made.
Clinical examination must consider the presence of proctitis, the position of the anorectal opening and the position of the vaginal opening. These may be assessed digitally or with a proctoscope or sigmoidoscope in the vagina. The presence of extensions is clearly relevant, although many tracts will be straight and unbranching.

Internal intussusception within the rectum will make an advancement flap easier and can be assessed with the patient awake by asking them to bear down with a proctoscope in place.

The quality of the anal sphincters and rectovaginal septum can be assessed digitally although anal ultrasound may be required to fully identify any defects.

### 1.9.3 Imaging

Clinical assessment is sometimes augmented with imaging to determine the relations and extent of the fistula disease. Secondary extensions, abscess and the relationship of the fistula tract to the anal sphincter are of particular importance. Transperineal and endoluminal ultrasound, magnetic resonance imaging and fistulography have all been used to identify and delineate fistula tracts.

#### 1.9.3.1 Fistulography

Fistulography relies on the ability of the clinician to identify and cannulate the vaginal opening of the tract, which is rarely possible. It also gives no information on the relation of the fistula to the anal sphincters and is noted to have a low accuracy in delineating the tracts. False positives can follow iatrogenic injury through falsely directed probing (212). Despite these shortcomings, fistulography does have its advocates (213).

#### 1.9.3.2 Ultrasonography

Endoanal ultrasound can be used to identify anal and anovaginal fistulae. Some reports suggest a high sensitivity, others disagree (214-216). This is perhaps explained by differences in the study methodology or the operator dependent nature of the technique. Most reports do agree, however, that ultrasonography offers an
excellent interpretation of the anal sphincters, including revealing any occult sphincter defect before surgery. Trans perineal ultrasound (TPUS) has been shown to have a similar accuracy to endoluminal ultrasound with the added advantages of being a quicker, cheaper, simpler technique requiring less specific training, although some subtle tracts can be missed using this technique (214). Endoluminal ultrasonography is more widespread and is considered, along with MRI, to represent the gold standard in perianal fistula imaging, and is sometimes enhanced with hydrogen peroxide (217).

1.9.3.3 MR techniques

Body coil and surface coil MR imaging have a spatial resolution too low accurately to detect small diameter tracts between the anorectum and the vagina consistently in contrast with the gold standard use of body coil imaging in anorectal fistulating disease (218;219). In addition, fistulae to the vagina are frequently epithelialised, which means they will not show an enhanced signal on MRI. Endoluminal MRI coils improve the spatial resolution at the potential risk of not appreciating more complex tracts extending beyond the field of view of the scan, however a supplementary phased array or body coil scan can be used to provide this information (220;221).

1.9.3.4 Agreement in MRI

Endoluminal MRI is an effective imaging method for anovaginal fistulae, but availability has become more or less non-existent. A study of 20 consecutive patients with proven anovaginal fistulae was undertaken by Dwarkasing et al in 2003 (221). Two radiologists independently reviewed the MRI scans of each patient looking for the presence, course and relation of the tract, secondary extension, abscess or oedema, the openings of the tract and the state of the anal sphincters. T2 weighted images using an endoanal coil had been taken and were retrospectively reviewed. The radiologists had a minimum of 1 year of experience in MR imaging of the abdomen and pelvic floor. The anal opening was identified in all cases and the vaginal opening in 19 (95%) and there was 100% agreement between the two radiologists on all factors, except for the presence of secondary extensions (90%). Anal fistulae, abscesses and oedema of the septum were found in 6 of 10 patients
with an underlying diagnosis of Crohn’s disease or obstetric or iatrogenic injury compared to 1 of 10 patients without a known underlying condition.

1.9.3.5 Endoluminal MRI vs. EUS

The debate regarding the optimal imaging method for rectovaginal fistulae continues. Hussain et al. in 1996 found that classification of fistulae was possible in 17 of 28 patients (61%) with EUS, 25 (89%) with endoanal MR imaging and 26 (93%) with surgery (222). Maier et al. examined Crohn’s related and recurrent fistulae, of which 5 were anovaginal, comparing EUS and MRI with surgery (223). In this small group there was no difference between the two techniques. Stoker and colleagues also found similar accuracy in the two techniques in a slightly larger group of patients (13) with positive predictive values for EUS and endoluminal MRI of 100% and 92%, respectively (215).

1.9.4 Treatment

RVF are notoriously difficult to treat, with surgical success of around 50% rising to 80% with repeated procedures (224-228). Defunctioning stomas and, in the case of Crohn’s disease, bowel resection are sometimes required for healing, with proctectomy rates between 20% and 30% - which is probably lower than in the past, when between a third to a half of patients with RVF underwent proctectomy, sometimes for rectal rather than the fistulous disease itself (131;133;229;230). This reduced rate is probably due to improved control with anti-TNF drugs.

1.9.5 Medical treatment of Crohn’s rectovaginal fistulae

Medical treatments for Crohn’s RVFs have, until recently, centred on improving luminal disease control. Antibiotics, thiopurines, parenteral nutrition and immunosuppressants, such as cyclosporine and methotrexate, have all been used. As with anal fistulae, the anti-TNF drugs have altered the potential of medical treatment, offering the possibility of true healing of the fistula itself. The majority of studies are of Crohn’s disease or perianal Crohn’s disease more generally and contain only a few rectovaginal fistulae, the outcome of which is not always clear.
Antibiotics remain a mainstay of treatment of perianal sepsis in Crohn’s disease as discussed above (1.4.2) and ciprofloxacin and metronidazole are most commonly selected. Short term relief is sometimes achieved but remission is rare. Thiopurines are used for disease control and two small studies have reported a benefit of 6-mercaptopurine over placebo, one finding improvement or remission in 3 of 6 patients (90;94). Present and Lichtiger found that intravenous cyclosporine healed 1 and improved 1 (of 2) rectovaginal fistulae but unfortunately they relapsed on oral maintenance (231). Methotrexate in three patients led to remission in one and improvement in two but unfortunately two of the patients relapsed between 2 and 4 years later and suffered side effects such as alopecia and respiratory infections in the meantime (117).

1.9.5.1 Infliximab

Several studies of infliximab in Crohn’s disease have included RVF patients. In most of these studies, partial response is not the reduction in baseline draining fistulae of at least 50% used for anal fistulae, since RVF are often single in number, so a reduction in drainage, perceived by the patient, is used instead.

A small cohort of RVF patients was assessed by Parsi and colleagues who reported a low remission rate (compared to anal fistulae) of 2 of 12 patients (14%), although 9 of the patients achieved a partial response (104). Luna-Chadid published slightly better results, finding 1/3 improved and 1/2 in remission in their small cohort. Multivariate analysis found no factors predicting success (232). The RVF data from ACCENT II were analysed post hoc by Sands in 2004 (233). Of 28 RVFs, 60% were closed after the 3 dose induction. By week 54, 46% of those on maintenance therapy and 43% on placebo remained in response.

Gaertner and colleagues published an interesting report in 2010 in which they considered combined medical and surgical therapy (234); 51 patients undergoing staged or definitive surgery for Crohn’s rectovaginal fistulae were analysed. All were on some form of medical therapy (ranging from 73% on metronidazole to 5% on methotrexate) and 26 of them had also received infliximab induction. The study considered the impact of infliximab on surgical outcome.
They found that preoperative infliximab decreased proctitis at the time of surgery but that proctitis did not correlate with fistula healing. Eleven of twenty-five (44%) surgery only patients healed compared to 16 of 26 (62%) who had infliximab before surgery, although the difference was not statistically significant. Time to healing was significantly reduced in the infliximab group, however, from 8.1 to 2.9 months, from which it is reasonable to infer that perhaps a larger group might have demonstrated improved healing with infliximab. Interestingly, all patients who underwent seton insertion before definitive surgery healed. Faecal diversion did not improve remission rates. Proctectomy was undertaken in 36% of the surgery only group and 19% of the infliximab plus surgery group, although again the difference was not significant (p=0.25). A larger study is needed to answer the questions raised by this study; whether reducing proctitis preoperatively improves surgical outcome and whether doing so using infliximab, setons, faecal diversion or a combination improves the long term remission rate.

The value of medical treatment of Crohn’s rectovaginal fistulae in the era of anti-TNF agents is unclear. Whether a significant number are healed by medication alone, whether surgery represents the main chance of success, and whether the risk of permanent diversion or proctectomy has changed in the last decade will be examined in Chapter 4.

1.9.6 Surgery

Surgery for rectovaginal fistula is difficult and success rates are lower than with other proctological procedures, particularly in Crohn’s disease. An assessment of faecal incontinence and the state of the sphincters must be made to determine whether sphincter repair is indicated at the same time as fistula repair. The patient and surgeon must also decide whether to defunction and which approach to take to offer the patient the best chance of the best result.

Recurrence is the major complication but stomas, sexual dysfunction and impairment of continence are also all too common and distressing for patients.

Advancement flap repair is generally accepted to be the current gold standard operation but multiple approaches and adjuncts are possible as part of this operation. Several other approaches, conservative through to radical, are also used. Surgery is
generally avoided in the presence of proctitis and medical treatment, seton drainage or defunctioning are often used before definitive surgery.

### 1.9.6.1 Mixed surgical series

Several series of both Crohn’s and mixed aetiology cohorts have been reported. Some earlier reports suggest success rates of around 50% at primary repair rising to 80% or more after multiple attempts (224-228;235). Defunctioning stomas were often used and transanal rectal advancement flaps, perineoproctotomy, transvaginal and transperineal approaches were used.

More recent series report similar results. In 2007 Athanasiadis and colleagues published data on 37 women with Crohn’s disease and RVFs in whom they performed 56 procedures (226). Their overall recurrence rate was 30% with failure being significantly more common in rectal advancement flaps than direct closure of the fistula and anocutaneous advancement, which latter also protected continence more completely. Proctectomy was performed in 13.5% of women. In 2009 Loefler and colleagues performed an average of 2.1 operations on 45 Crohn’s disease patients with RVF with long term success in 53% of cases and 10 proctectomies (228).

El Gazzaz and colleagues reported a series of non-CD RVF patients in 2010 (227). Sixty had obstetric injuries and 40 had a cryptoglandular aetiology. The final success rate was 68% with both a high BMI and repeated repairs increasing the risk of failure on multivariate analysis. They also considered quality of life and found no differences between the healed and non-healed groups. Only 47 were sexually active at follow-up and of these 25% suffered from dyspareunia.

### 1.9.6.2 Advancement flaps

Several different flap techniques and adjuncts are currently used for rectovaginal fistula repair. Depending on the pliability of the perineal, vaginal and rectal tissues, some approaches can be impossible and others made easier. The choice of approach is based partly on the particular expertise of the operating surgeon and the majority of series of flap repairs are of one or two types.
The commonest advancement flap is the transanal rectal advancement flap which was used in several series with success rates ranging from 43% to 92% (141;225;228;236;237). The technique involves raising a flap with a broad base and the rectal opening in the apex. The opening is excised and the flap pulled down to fill the defect and sutured in place. The vaginal opening is left to allow drainage. The lack of a perineal wound means less pain and fewer problems with healing. Concomitant sphincter repair is possible and the risk of sphincter injury is minimal (238).

In a study of transanal advancement flap repair of anal fistulae (in which RVFs and Crohn’s patients were excluded), smoking was found to increase the risk of failure and the number of cigarettes smoked per day was also found to correlate with the healing rate (239).

Rectal sleeve advancement requires a circumferential flap to be raised cephalad from the dentate line. The flap is full thickness and after it has been raised a core out fistulectomy is performed. The diseased cylinder of the flap is then excised and the remaining tissue brought down and anastomosed back onto the dentate line. Success rates in two small series suggest success in 60-75% after multiple operations (240;241).

The anocutaneous flap was first reported in 10 Crohn’s RVF patients in 1993 by Hesterberg and colleagues (242). They reported excising the tract, repairing the sphincteric defects from anal (IAS) and vaginal (EAS) ends and then raising and securing a cutaneous flap up inside the anus to cover the anal opening. Primary success was achieved in 7 of 10 cases. More recent series also describe successful use of this technique (226). Pliable and healthy perianal skin is not always present in Crohn’s disease or radiation, which may preclude this technique. The house advancement flap is another technique of anocutaneous advancement which raises a robust flap based on a dissection down to ischiorectal fat, thereby maintaining vascularity from the perforating vessels that pass through the fat layer (243).

The modified Martius graft uses the fat pad of the labia majora as an interposition to bolster the rectovaginal septum. Small series in the literature have reported success rates between 50% and 94% with impairment of continence and dyspareunia as significant complications (244-247).
Most advancement flaps are placed on the high pressure side of any fistula repair but some surgeons opt for a transvaginal approach. This may be because there is more lax and pliable tissue to raise flaps with or because of the lower likelihood of inflammation, particularly in Crohn’s disease. A systematic review has found that there is no difference in healing rates between rectal and vaginal advancement flaps (248).

1.9.6.3 Glue and plugs

As with anal fistulae, a surgical technique offering success without anal sphincter damage is extremely attractive. The use of fibrin glues, collagen plugs and the interposition of bioprosthetic sheets to repair the rectovaginal septum have all been employed.

The evidence for these techniques, however, comprises several small, uncontrolled trials with mixed fistula types, aetiologies and surgical techniques. For example, some studies included only defunctioned patients whereas others did not, and there are varying proportions of patients with Crohn’s disease from 0-100%. Most of the studies are of various perianal fistulae and include a few rectovaginal fistulae within them; few were specific to anovaginal or rectovaginal fistulae.

Studies of fistula glue, fistula plugs and bioprosthetic meshes including Crohn’s and non-Crohn’s rectovaginal fistulae are listed in Table 5 below.
### Table 5 - Studies of fistula glue, fistula plugs and bioprosthetic meshes including Crohn’s and non-Crohn’s rectovaginal fistulae

<table>
<thead>
<tr>
<th>Technique</th>
<th>Study</th>
<th>Year</th>
<th>Success rate in RVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fistula glue</td>
<td>Venkatesh (146)</td>
<td>1999</td>
<td>6/8</td>
</tr>
<tr>
<td></td>
<td>Cintron (147)</td>
<td>2000</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td>Loungnarath (151)</td>
<td>2004</td>
<td>1/3</td>
</tr>
<tr>
<td></td>
<td>Singer (152)</td>
<td>2005</td>
<td>0/1</td>
</tr>
<tr>
<td></td>
<td>Vitton (143)</td>
<td>2005</td>
<td>3/4</td>
</tr>
<tr>
<td></td>
<td>Grimaud (144)</td>
<td>2010</td>
<td>0/3</td>
</tr>
<tr>
<td></td>
<td>de Parades (154)</td>
<td>2010</td>
<td>0/5</td>
</tr>
<tr>
<td>Fistula plug</td>
<td>Ellis (249)</td>
<td>2008</td>
<td>6/7</td>
</tr>
<tr>
<td></td>
<td>Thekkankattil (250)</td>
<td>2008</td>
<td>1/7</td>
</tr>
<tr>
<td></td>
<td>Gonsalves (251)</td>
<td>2009</td>
<td>3/5</td>
</tr>
<tr>
<td>Bioprosthetic interposition</td>
<td>Ellis (249)</td>
<td>2008</td>
<td>22/27</td>
</tr>
<tr>
<td></td>
<td>Schwandner (252)</td>
<td>2009</td>
<td>19/21</td>
</tr>
</tbody>
</table>

It is difficult to determine the efficacy of any of these techniques based on these studies.

**1.9.6.4 Gracilis muscle interposition**

The Gracilis muscle has two distinct advantages over other thigh and buttock muscles for use in perineal transposition, filling defects in the perineum with the potential to improve continence. Firstly, it has only a vestigial function, unlike gluteus maximus, for example, and secondly its single pedicle lies proximally, meaning that it is both versatile and robust. After mobilisation of the muscle from distal to proximal it is passed into the perineum and can fill a large perineal defect. Technical considerations, such as too short a muscle, inadequate vascularity and rotation or malposition of the muscle, are key intra-operatively. Complications include infection, wound breakdown and venous thromboembolism. Good results have been reported.
using this technique which is used in rectovaginal, rectourethral and rectovesical fistulae (253).

Wexner and colleagues reported success in 5 of 6 non-Crohn’s RVFs treated with gracilis muscle interposition which fell to just 3 of 9 in Crohn’s disease. Complications included pain and infections at both the thigh and perineal wounds (254). Further small series in the last 2 years have reported similar findings, often in recurrent fistulae (255-257), although Furst and colleagues reported a high success rate in their series of Crohn’s patients. Larger series are required to determine the efficacy of this technique.

1.9.6.5 Setons

Permanent loose setons are used in rectovaginal fistulae to palliate, reducing symptoms and the occurrence of new fistulae. Several studies have been shown to improve symptom control and thereby avoid the need for permanent diversion or proctectomy (131-133). This is not a successful strategy in all patients and it may be that sufficient time to achieve symptom control is necessary, but even then some patients will not do so (131). Thornton and colleagues found that age, reduction in anal wall thickness after seton insertion and length of follow-up were significant predictors of long term symptom control. It has been previously demonstrated that anal wall thickness correlates with disease activity (258) and a combination of medical disease control with seton drainage may avoid radical surgery in patients who have failed other treatment options.

1.9.6.6 Diversion

The difficult course of the recurrent rectovaginal fistula will sometimes lead to permanent diversion or proctectomy. The presence of proctitis increases the chance of a patient with perianal Crohn’s disease requiring proctectomy, as does anal stenosis, and diversion rates in rectovaginal fistula vary from 20% to 60% (209;229;230;234;259), although in recent years the rate of proctectomy has decreased, perhaps in response to improved medical management.
As well as higher rates of permanent diversion, a temporary defunctioning stoma is often used in the surgical treatment of rectovaginal fistula, with the attendant risk of the stoma becoming permanent following failed treatments (230). In one study, univariate analysis identified rectovaginal fistula, as well as temporary diversion, as a factor increasing the need for permanent diversion, and although in multivariate analysis rectovaginal fistula was not significant on its own, complex fistula (including RVF and anal fistulae with multiple openings) was a significant factor (230).

In the majority of cases, temporary diversion is used to increase the chance of healing from other medical or surgical interventions. Indeed diversion alone is of limited value in perianal Crohn’s disease, including rectovaginal fistulae. A small study discussed above by Yamamoto and colleagues reported 6 cases of RVF in which early improvement in 4 led to relapse within three years and proctectomy was undertaken in all 6 cases (34).

1.9.6.7 Predicting failure

Attempts to ascertain risk factors for failure have produced varied results. One centre has produced two reports on this subject, in 2001 and 2009, the former incorporating a review of the literature as well as an analysis of 32 of their own patients, comprising a previously published group with 24 consecutive additions (259;260). They found a healing rate of 56% after the first procedure rising to 81% overall with no differences between the various techniques (rectal, cutaneous or vaginal advancement flaps or perineoproctotomy). The initial report suggested that failure was associated with more sites of Crohn’s disease, the presence of extraintestinal disease and proctitis, although only the number of sites was significant on multivariate analysis. In the larger series, duration of disease and a previous extensive colonic resection were associated with failure.

El-Gazzaz and colleagues published a series of 65 patients with a median follow-up of 44 months and healing in 46% (261). Twenty-eight of these women (43%) were sexually active at follow-up, of whom 9 (all with unhealed fistulae) had ongoing dyspareunia. Multivariate analysis revealed that immunomodulators were associated with healing and smoking and steroids with failure.
Different RVF injury complexes require different surgical approaches and the level of success seen with the different injuries and approaches is important. There is also value in identifying factors which increase or reduce the chance of successful healing, particularly in redo repairs or in tertiary patients in whom options may be limited and more radical procedures contemplated. These issues will be further examined in Chapter 4.

1.10 Summary

Despite much research in the aetiology of Crohn’s disease, anal fistulae remain mysterious in their creation and persistence. Many basic aspects of fistula pathology in both Crohn’s and idiopathic tracts remain obscure. Similarly, the optimal medical management of Crohn’s perianal fistulae, and surgical options for any fistula tract but particularly high tracts or those associated with Crohn’s disease, are the subject of debate rather than consensus. Rectovaginal fistulae are also difficult to treat and their management has changed in the last decade following the introduction of the anti-TNF drugs. The use of MRI to monitor and assess the efficacy of these drugs in the various perianal fistulae associated with Crohn’s disease has revealed a flaw in clinical assessment of these tracts which is often used alone to justify ongoing use or cessation of treatment. The studies which follow aim to answer some of the following questions:

1. How are idiopathic and anal fistula tracts similar and how do they differ at a microbiological and immunological level?
2. Do bacteria populate the luminal surface of fistula tracts and if so which bacteria are present?
3. Is there an inflammatory infiltrate in the wall of the fistula tract and if so what are the constituent immune cells?
4. What is the additional burden of impairment of continence in the complex, tertiary anal fistula tract managed by fistulotomy?
5. Which patients are likely to recur or lose continence?
6. What are the current options for medical management and the efficacy of drugs in Crohn’s rectovaginal fistulae in the anti-TNF era?
7. What factors influence success in the surgical management of rectovaginal fistula, which operations are currently employed for the various injury complexes seen and what is the risk of permanent diversion or proctectomy?
8. When treating Crohn’s perianal fistulae with anti-TNF agents, does radiological monitoring describe a different natural history to clinical monitoring alone?

9. Which factors influence clinical and radiological outcome on anti-TNF agents?

10. What happens to patients who stop maintenance treatment after healing has occurred?
2 The aetiology of anal fistulae

2.1 Abstract

Introduction

The aetiology of Crohn’s anal fistula remains obscure. Microbiological, genetic and immunological factors are thought to play a role but are not well understood. We aimed to characterise bacterial and immunological components of Crohn’s and idiopathic anal fistula tracts.

Methods

Crohn’s and idiopathic anal fistula tracts were sampled and analysed using Fluorescent in situ hybridisation, flow cytometry and immunohistochemistry to identify and compare luminal bacteria and immune cells present.

Results

Bacteria were not found in association with the luminal surface of the anal fistula tracts although inflammation was found to be on the peri-luminal surface of the fistula tract. Crohn’s tracts contained more CD3 positive cells in the luminal and deep layers than idiopathic tracts, but fewer CD65 positive cells. Crohn’s anal fistula tracts had lower numbers of immune cells expressing gut and skin homing molecules. TNFα levels were similar in both Crohn’s and non-Crohn’s tracts but IL-17a levels were lower in Crohn’s anal fistula tracts.

Conclusions

Crohn’s anal fistula tracts lack bacteria but inflammation is more pronounced in the peri-luminal zone of the tract wall implying an alternative luminal factor driving the inflammation. Specific differences in cytokine milieu and immune cell composition between Crohn’s and idiopathic fistulae represent potential targets for diagnostic tests, therapy and future research.
2.2 Introduction

Anal fistulae are a common (2) and unpleasant complication of Crohn’s disease (CD) and also occur, less commonly, in patients without Crohn’s or other predisposing disease when they are called idiopathic or cryptoglandular fistulae. Cryptoglandular fistulae are thought to follow infection of an intersphincteric anal gland and are most commonly low, simple fistulae although higher, more complex tracts can occur. Crohn’s anal fistulae, by contrast, are more commonly complex (262) and multiple (13) and are thought to arise from deep penetrating ulcers or fissures in the anal canal (24).

Aetiological factors leading to the formation of fistulae or their persistence remain obscure. As with luminal Crohn’s disease, a combination of genetic, microbiological and immunological factors is thought to play a role. In genetically susceptible individuals, the interaction of normal commensal bacteria with host immunity leads to an aberrant inflammatory response causing persistent inflammation and sometimes ulceration, stricturing and fistulation. The most common site of fistulation is the anus (3).

2.2.1 Microbiology

The temporary improvement seen in perianal disease after faecal diversion (34) and the partial efficacy of antibiotics (56) (57) support the concept of a microbiological element in the aetiology of anal fistulae. Several animal models of inflammatory bowel disease support this concept, since inflammation is dependent on the presence of microbiota and in its absence there is no disease. Single causative organisms and dysbiosis have been suggested as mechanisms by which bacteria might cause inflammation. Adherent invasive strains of *Escherichia coli* have been found in patients with CD (38;39) and are known to persist inside macrophages and epithelial cells producing large amounts of TNF, selectively colonising the terminal ileum of Crohn’s patients (263). A reduction in biodiversity in mucosa-associated microbiota and a loss of normal anaerobic components, a decrease in bifidobacteria and *Faecalibacterium prausnitzii*, and an increase in the number of mucosal adherent bacteria have all been noted in Crohn’s disease (44) (264;265) (48) (49).

Different bacteria exert different immune effects on the host tissue. Some groups (such as Bacteroides and E Coli) are known to be pro-inflammatory within the gut,
whereas others are known to be anti-inflammatory (bifidobacteria, *Faecalibacterium prausnitzii* (266)). Dysbiosis is a change in the proportions of bacteria normally present in the gut and several studies have examined the flora in CD and found a reduced diversity with a change in the ratio of pro- and anti-inflammatory bacteria in active disease (reviewed in (263)).

As discussed in chapter 1, the microbial content of fistula tracts in CD is less well understood. Studies examining cultured pus or curettings from Crohn’s and cryptoglandular fistula tracts have demonstrated different organisms in cryptoglandular than Crohn’s fistulæ (51) (52;53) and enrichment media required due to a paucity of organisms. However, bacterial culture techniques are unable to detect the majority of gastrointestinal organisms (54). Molecular techniques, which can identify not only the presence but in some cases the number and location of bacteria, allow closer examination of the microbiota. Moreover, *in situ* techniques allow an assessment of the clinical relevance of bacteria found by contrast with both culture and qPCR techniques.

### 2.2.2 Immunology

Histologically, Crohn’s anal fistulæ have a central fissure which penetrates the lamina propria and muscularis mucosae to reach the underlying tissues. Previous studies have indicated that most fistula tracts contain some epithelium but it often occurs in small islands, is rarely circumferential and most often occurs near the internal opening (59;84;267). Bataille and colleagues examined 97 fistula tracts from various sites in Crohn’s and non-Crohn’s patients, including 8 Crohn’s and 6 non-Crohn’s anal fistula tracts (59). The remaining fistula tracts were non-anal. Clear differences were reported across the 97 patients, including T cells lying closer to the luminal surface of the Crohn’s fistula tracts and in deeper layers in non-Crohn’s tracts, and macrophages intensely infiltrating the whole wall of non-Crohn’s tracts but lying in only a small band in Crohn’s tracts. Only results describing the entire cohort were presented and it is not clear whether these findings were representative of anal fistula tracts.

Dendritic cells (DC) are antigen presenting cells which sample mucosa-associated bacteria and migrate to lymph nodes to stimulate immune response via T cells. They imprint homing of T cells to organs e.g. skin (cutaneous lymphocyte-associated
antigen, CLA) and gut (α4β7 integrin). DC have been shown to play a key role in the aberrant immune response in Crohn’s disease (268), where they express more Toll-like receptors which recognise microbes and increase inflammatory cytokine levels. The presence, phenotype, homing characteristics and function of DC have not previously been examined in Crohn’s and cryptoglandular anal fistulae.

Serum and rectal mucosal cytokines have been analysed in Crohn’s anal fistula patients in whom higher levels of rectal mucosal IL-1β and IL-6, and raised serum IL-6 and TNFα were found compared with both healthy controls and Crohn’s patients with disease limited to the small bowel (77). In this study correlations were also found between some cytokines and the activity or severity of disease and no influence on cytokines was found from infliximab, azathioprine or steroids. Cytokines from the wall of the fistula tract were not considered. Mucosal cytokines from idiopathic anal fistulae were examined by Kiehne in 2007 who found elevated levels of IL-8 and IL-1β but normal levels of TNFα, IL-10 and IL-6 (68).

Infliximab and other anti-TNFα agents have been found rapidly and, in some cases completely, to heal anal fistula tracts in Crohn’s disease, underlining the importance of cytokines in fistula tract pathology. However, the cytokine milieu of Crohn’s anal fistula tracts themselves is not known.

### 2.3 Hypothesis and Aims

#### 2.3.1 Hypothesis

We hypothesised that Crohn’s and idiopathic fistula tracts differ with regard to microbiological and immunological factors which drive their formation and persistence.

#### 2.3.2 Aims

We aimed:
- to identify the specific bacteria or dysbiosis present in Crohn’s and idiopathic anal fistula tracts themselves and additionally to identify the rectal microbiota in Crohn’s patients with and without anal fistulae; and
- to characterise the inflammatory cell population and cytokine profile in Crohn’s and idiopathic anal fistula tracts.
2.4 Methods

2.4.1 Patient selection

Four groups of patients were approached to take part in this study:

- patients with cryptoglandular, or idiopathic, anal fistulae (IPD),
- patients with Crohn’s anal fistulae (CPD),
- patients with Crohn's disease but no past or present anal fistulae (CD),
- patients without inflammatory or malignant bowel disease.

Demographic and disease related data were noted including duration and extent of disease, smoking status and previous medical and surgical therapy.

Baseline characteristics of all patients are shown in Table 13.

2.4.2 Sample collection

All patients were recruited prior to clinically indicated surgery or colonoscopy. Surgical procedures included fistulotomy, seton insertion and proctectomy. The specific technique for sample collection for each of the three modalities studied, microbiology, immunology and immunohistochemistry, is described in detail in the relevant section below. Some factors were specific to the type of procedure undertaken and these are addressed here.

2.4.3 Surgery

2.4.3.1 Lay open

Patients undergoing lay open of a perianal fistula were placed in the lithotomy position. After washing the perineum with antibacterial skin prep but before any manipulation of the fistula, the fistula tract and anorectal biopsies (taken at the internal opening in the anus) for microbiological assessment were obtained using colonoscopy biopsy forceps (see below). The operation then proceeded and when the fistula tract had been laid open, forceps and scalpel were used to retrieve the tract biopsies for immunology and immunohistochemistry.
2.4.3.2 Proctectomy

Patients undergoing proctectomy also had the perianal portion of their operation carried out in the lithotomy position with antiseptic preparation of the perineum. After the anorectum had been excised with the surrounding perineal tissues, the specimen was quickly dissected on a sterile surface. The fistula tract was identified and laid open using a scalpel or excised intact. All biopsies were then taken from this exposed bed.

2.4.3.3 Seton

Patients undergoing seton insertion or change sometimes underwent a small lay open at the outer end of the fistula tract in which case they were treated as in lay open above. If not, the same process of antiseptic preparation of the perineum followed by fistula tract and anorectal biopsies was undertaken. Tru-cut biopsies were then taken of the wall of the fistula tract, gaining tissue for immunology and immunohistochemistry, although if only very small scraps of tissue were obtained, histological specimens were not processed.

2.4.4 Colonoscopy

Since no internal opening was present in the patients undergoing colonoscopy and anal biopsy would have been difficult to obtain and would have caused discomfort to the patient, rectal biopsies were taken in these patients from low in the rectum. Biopsies for microbiological analysis were obtained in Crohn’s patients without perianal disease. Tissue for cytokine analysis was taken from patients without inflammatory or malignant bowel disease.

2.4.5 Proctitis

At surgery, along with biopsies for microbiological analysis, a further anorectal biopsy was taken and sent for histological assessment in the hospital histopathology department as per normal care. This biopsy was used to assess whether proctitis was present.
2.5 Microbiology methods - Quantification of Fistula Tract and Anal/Rectal Mucosa-Associated Microbiota using Fluorescent In Situ Hybridisation

2.5.1 Patients
Patients with idiopathic or Crohn’s related anal fistulae, or Crohn’s disease but no perianal disease were recruited as described above. Fistula patients underwent a planned operation, usually examination under anaesthetic (EUA) with seton insertion or laying open of fistula although a small number underwent proctectomy. At surgery biopsies were taken and snap frozen in liquid nitrogen as described below. Non-fistula patients underwent a planned colonoscopy and had biopsies taken in the same way.

2.5.2 Anal/rectal and fistula tract sample processing

2.5.2.1 Sample processing and storage

Rectal and fistula tract biopsies were taken using Radial Jaw 3 (Boston Scientific, France) biopsy forceps whether at colonoscopy or surgery. Samples taken at colonoscopy were obtained during the procedure from as low in the rectum as possible. The samples were retrieved on the biopsy forceps, dipped briefly in sterile phosphate buffered saline (PBS) and then placed into dry, sterile, irradiated, 1.2 ml cryovials (VWR international Ltd, Lutterworth, UK) using a sterile hypodermic needle. The cryovials were snap frozen in liquid nitrogen and stored at -80°C.

At surgery, both gut and fistula tract samples were obtained. To obtain fistula tract samples, careful precautions were undertaken. The patient was placed in the lithotomy position for the purposes of the operation and the entire buttock and perianal region was cleaned with antibacterial skin prep. The protective outer sheath from an 18G cannula was inserted into the EO (Figure 11) to prevent the biopsy forceps from picking up bacteria form the surrounding skin (Figure 12).
Figure 11 - The protective outer sheath from an 18G cannula is inserted into the EO to prevent contamination of the biopsy forceps

Figure 12 - The biopsy forceps are passed down the sheath

If the EO was too small to admit the cannula sheath then the forceps were inserted into the internal opening without it. The biopsy forceps were then fed into the tract as
far as possible until resistance was met (Figure 13) at which point the biopsies were taken in the normal way and removed using a sterile needle (Figure 14).

Figure 13 - The biopsy forceps are passed until resistance is met when a biopsy is taken.

Figure 14 - The biopsy is retrieved using a sterile needle, before washing in sterile PBS and placement in a sterile cryovial for snap freezing.
If visible, biopsies were taken from the anal canal at the internal opening (Figure 15). If this was not possible because the IO could not be found, a random anal or low rectal biopsy was taken.

![Figure 15 - An internal opening biopsy taken in theatre. The silastic seton marks the IO in the anal canal.](image)

Both anal/rectal and tract biopsies were then washed in sterile PBS, placed into a cryovial using sterile forceps and snap frozen in liquid nitrogen before transfer to a freezer where they were stored at -80°C.

The samples were transferred from the freezer in containers of dry-ice, orientated so that the luminal surface of the anal/rectal or fistula tract biopsy was facing up, mounted in OCP (Bright Instrument Company, UK) and placed back into the dry-ice. The frozen, mounted tissue was transferred into a cryostat (Bright Instrument Company, UK) and secured. Sections were cut at 6μm thickness and mounted onto plain 4 well slides (Hendley, UK).

![Figure 16](image). Each slide was marked with the patient identifier, the site of the sample (R = rectum or T = tract) and the level of the section (each time the sample was sectioned the slices were taken off in order and labelled so we could identify a
deeper or more superficial section if appropriate) (figure 2.1). The sections were then frozen and stored at -20°C until the hybridisation step.

The orientation of the sample was confirmed by performing a haematoxylin and eosin stain (although in the case of fistula tract samples, if no epithelium was present, orientation could not be confirmed microscopically). Briefly, haematoxylin is added for two minutes and then washed in tap water for five minutes, then slides are counterstained in eosin for a further five minutes before being briefly dipped in tap water and then dehydrated through 70%, 95% and 100% ethanol/distilled water washes for a minute each and placed in xylene before mounting with DPX (Surgipath, UK) and a 22x64mm cover slip.

![Figure 16 - An example 4 well slide](image)

**2.5.2.2 Fixation**

Slides were taken from the freezer and allowed to thaw at room temperature for 10 minutes. They were fixed using filtered 4% paraformaldehyde solution (appendix 1) for 10 minutes. Excess paraformaldehyde was removed and the slides washed in Diethyl pyrocarbonate (DePC) Water (Sigma, USA) (appendix 1), twice, for five minutes each.

**2.5.2.3 Pre-hybridisation**
Each slide was then pre-incubated at the hybridisation temperature (HT) with 20µl of hybridisation buffer (appendix 1) per well for 30 minutes in sealed hybridisation trays. The optimal hybridisation temperatures for each probe are described below (see Table 7). In order to prevent the slides drying out tissue paper saturated in de-ionised water was placed in the tray. The Pre-incubation step reduces non-specific binding and, by raising the temperature of the slides, improves specific binding of the probe in the hybridisation step.

### 2.5.2.4 Oligonucleotide probes

Each probe used was manufactured and supplied by Microsynth, Switzerland. They were supplied as a lyophilised powder of 0.2µmol per probe at room temperature. The probes we used had previously been successfully used in published research. Table 6 lists each probe and its sequence.

The probes were made up to a stock concentration of 50ng/µl or 1000ng/µl in sterile 10mMol Tris-HCl (Trizma, Sigma, UK) buffered to pH 6.5. Once diluted the probe was stored at -20°C in 5 and 50µl aliquots. All the probes were conjugated with Cy3 fluorophore which has an absorption spectrum of 550nm and an emission spectrum of 570nm.

**Table 6 - Oligonucleotide probe sequences**

<table>
<thead>
<tr>
<th>Target bacterial group</th>
<th>Clone</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Panbacteria (269)</td>
<td>EUB 338</td>
<td>5’-GCT GCC TCC CGT AGG AGT-3’</td>
</tr>
<tr>
<td>Bifidobacteria (270)</td>
<td>Bif 164</td>
<td>5’-CAT CCG GCA TTA CCA CCC-3’</td>
</tr>
<tr>
<td><em>Clostridium coccoides</em> – Eubacterium rectale cluster (271)</td>
<td>EREC 482</td>
<td>5’-GCT TCT TAG TCA RGT ACC G-3’</td>
</tr>
<tr>
<td>Bacteroides – Prevotella cluster (272)</td>
<td>Bac 303</td>
<td>5’-CCA ATG TGG GGG ACC TT-3’</td>
</tr>
<tr>
<td><em>Faecalibacterium prausnitzii</em> (273)</td>
<td>Fprau 645</td>
<td>5’-CCT CTG CAC TAC TCA AGA AAA AC-3’</td>
</tr>
<tr>
<td><em>Escherichia coli</em> (274)</td>
<td>Ecoli 1531</td>
<td>5’-CAC CGT AGT GCC TCG TCA TCA-3’</td>
</tr>
</tbody>
</table>
2.5.2.5 Hybridisation

During pre-hybridisation, the appropriate amount of each probe was diluted to a concentration of 5ng/μl with hybridisation buffer warmed to the hybridisation temperature. A final volume of 18μl of probe was placed on each well under a 13mm diameter cover slip used to protect the wells during hybridisation, in order to prevent evaporation of hybridisation fluid and drying out of the sections. The trays were then resealed with cling film with the paper wick still in place, and placed back into the blacked out hybridisation oven set at the appropriate HT for the probe concerned overnight.

As the hybridisation temperatures for some probes were within 2 °C of each other, a compromise temperature in between the two was used so that multiple probes could be hybridised at the same time (Table 7).

A positive control sample, known to contain the bacteria for which we were probing, was used in each experiment.

Table 7 - Hybridisation temperatures

<table>
<thead>
<tr>
<th>Oligonucleotide Probe/Clone</th>
<th>Optimal Hybridisation Temperature (±2°C)</th>
<th>Hybridisation Temperature used</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB 338</td>
<td>48</td>
<td>49</td>
</tr>
<tr>
<td>Bif 164</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>ERE 482</td>
<td>50</td>
<td>49</td>
</tr>
<tr>
<td>Bac 303</td>
<td>45</td>
<td>45</td>
</tr>
<tr>
<td>Fpra 645</td>
<td>35</td>
<td>36</td>
</tr>
<tr>
<td>E. coli 1531</td>
<td>37</td>
<td>36</td>
</tr>
</tbody>
</table>

2.5.2.6 Stringent washes

After overnight incubation, the slides were removed from the oven and the cover slips gently tapped off. The slides were then placed into either DePC water for 5 minutes or washed using Hybridisation wash for 20 minutes on a gentle agitator and then placed in De PC water for 5 minutes (appendix 1, 7.2) (Table 8). During the washing
phase the slides were protected from exposure to light using aluminium foil wrapped around the Coplin jars.

The slides were removed from the wash, excess water tapped off and a fluorescent mountant (Vectashield, Vector Laboratories, USA) was added and then covered with a 22x64mm coverslip.

Table 8 - Post-hybridisation washes

<table>
<thead>
<tr>
<th>Oligonucleotide Probe/Clone</th>
<th>20 minutes hybridisation wash and agitation</th>
<th>5 minutes DePC water</th>
</tr>
</thead>
<tbody>
<tr>
<td>EUB 338</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Bif 164</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>EREC 482</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Bac 303</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Fprau 645</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>E. coli 1531</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

2.5.2.7 Microscopy

All slides were reviewed using a Zeiss Axioskop 2 epifluorescent microscope with a mercury vapour lamp. Each anal/rectal section was reviewed to identify the epithelial border, mucus lying next to it and fluorescing bacteria within it. The best field containing the most bacteria in any of the wells was used and all bacteria lying within mucus associated with the epithelium in this high power field were counted by two researchers and their scores were averaged.

If the positive control samples within an experiment had detected bacteria and at least two sections of a sample appeared morphologically intact with a clear run of epithelium and mucus lying off it but no bacteria were seen, we counted the sample as having no bacteria of the type probed for present.

If there were no positive samples within an experiment then no sample was determined as a zero and the experiment was repeated with new slides from each sample.
If the positive control or any other sample did detect bacteria but a given sample did not have at least two sections containing a run of epithelium with mucus lying off it, it was considered inadequate and the experiment was repeated with new slides from the sample.

Fistula tract samples were viewed in the same way but since there is no consistent mucosal or epithelial surface, all aspects of each sample were considered possible luminal surfaces of the fistula tract and examined for the presence of bacteria.

Good examples of bacteria or samples with good morphology, epithelium and mucus but no bacteria were photographed using a fluorescent Leica DMI2® confocal microscope or the Zeiss Axioskop described above.

2.5.2.8 Gram stain

Slides were allowed to air dry and thaw at room temperature for 10 minutes. After warming for 10 minutes at 37°C they were allowed to cool before flooding with Crystal Violet (Pro-Lab diagnostics, Richmond) for 70 seconds and rinsing with water. The slides were then flooded with Gram’s iodine for 60 seconds before rinsing with water and differentiating with Gram’s Differentiator and immediately rinsing. Safranin counterstain was applied for 20 seconds before rinsing and mounting with DPX.

2.5.2.9 Scanning electron microscopy

On three occasions specimens were taken for scanning electron microscopic (SEM) analysis. Full thickness fistula tract specimens were dissected and placed in cell culture wells. Cotton wool soaked in 2.5% phosphate buffered glutaraldehyde was placed in adjacent wells in order that the specimens were exposed to glutaraldehyde vapour during transport.

Vapour fixation with 1% osmium tetroxide in phosphate buffer was performed overnight. The osmium tetroxide was then removed and the specimens allowed to air dry over 36 hours.
The specimens were removed from the wells and mounted on SEM stubs using silver
dag, and gold coated using a Polaron E5100 SEM coating unit (Quorum
Technologies). The specimens were viewed in a “Quanta” field emission gun SEM
(_FEI UK Ltd) at 5 Kv accelerating voltage in secondary electron mode.

### 2.6 Histology and Immunohistology methods

#### 2.6.1 Patients

Patients with idiopathic or Crohn’s related anal fistulae were recruited as described
above and underwent a planned operation, usually examination under anaesthetic
(EUA) with seton insertion or laying open of fistula although a small number
underwent proctectomy.

#### 2.6.2 Histology sample processing

Samples were taken from the fistula tract using a scalpel and forceps (Figure 17)
after the tract had been examined with a fistula probe and, in the case of fistulotomy,
after the laying open had taken place.
In the case of seton operations, a Tru-cut biopsy needle was used to obtain a sample of the wall of the fistula tract. This was performed by placing the needle as far into the tract as possible and then firing it with the sampling surface pressed up against the wall of the tract. Where a proctectomy was performed, the tract was not disturbed until after resection of the anorectum whereupon the tract was excised intact from the surrounding tissues.

Samples were orientated, if possible, at the time of retrieval. They were placed in sterile saline and on ice for transfer to the laboratory which took place soon afterwards.

Samples were then cut into smaller pieces, if large enough, demonstrating the full thickness of the fistula tract wall across the cut surface and placed into cassettes with this face uppermost. The cassettes were placed into 10% neutral buffered formalin solution for at least 48 hours to fix the tissues. The tissue was then processed to wax using an automated processor (7.1.2.1) and embedded into wax blocks with the full thickness face of the fistula tract wall at the cutting surface. A rotary microtome was used to cut 5µm sections which were then mounted onto APTS (3-aminopropyltriethoxysilane) (Sigma Aldrich, UK) coated (to improve adhesion) glass
microscope slides (Surgipath, UK) using a warm water bath and left to dry overnight at 60°C, cooled and stored at room temperature.

2.6.3 Histological staining

Haematoxylin and Eosin (H&E) staining, according to a standard protocol (appendix 1, 7.1.2.2), was performed to assess basic tissue and cellular morphology. Picro-Sirius Red and Miller’s Elastin (PM) staining (appendix 1, 7.1.2.3) was also used to identify and assess collagen and elastin within the samples.

2.6.4 Histological assessment

An expert histologist (Prof Paul Sibbons) performed a blinded assessment of the histology and morphology of fistula tract specimens using an Olympus BX40 microscope equipped with an Olympus DP70 digital camera.

2.6.4.1 Orientation and morphology

Confirmation (and in some cases primary assessment since orientation at operation had not been possible) of the orientation of the section was sought microscopically with the presence of a non-skin epithelial border taken to indicate the luminal surface of the fistula tract. If orientation was possible, the luminal aspect of the section was marked.

If distinct layers of tissue throughout the section were visible, they were named according to their predominant feature and their size relative to the section was estimated.

2.6.4.2 Inflammation

Inflammation was described as chronic or acute and assigned a severity score on a scale from 0-5 where 0 = no inflammation and 5 = severe inflammation based on the presence of immune cells and tissue responses.

2.6.4.3 Epithelialisation

Epithelialisation was sought in each section.

2.6.4.4 Collagen
The presence, quantity and quality (healthy or denatured) of collagen were assessed using PM stain with polarised light.

2.6.5 Immunohistochemistry sample processing

Immunohistochemistry was performed on all samples. CD3 primary antibodies, staining for T lymphocytes, and Ab-1 primary antibodies, staining for macrophages, were used (Table 9). Protocols (appendix 1, 7.1.2.4 & 7.1.2.5) were developed specifically for the antibodies used on fistula tract tissue in order to provide adequate antibody staining without excessive non-specific staining. Antigen retrieval with Target Retrieval Solution (DAKO, UK) at 98°C for 30 minutes was necessary for the CD3 protocol but not for Ab-1.

<table>
<thead>
<tr>
<th>Antibody name</th>
<th>Clone</th>
<th>Company</th>
<th>Type</th>
<th>Isotype</th>
<th>Cells stained</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calprotectin-Monocyte/Macrophage/L1 Protein Ab-1</td>
<td>MAC 387</td>
<td>Thermo Scientific</td>
<td>Mouse monoclonal antibody</td>
<td>IgG1</td>
<td>Neutrophils, Monocytes, macrophages, squamous epithelia</td>
</tr>
<tr>
<td>CD3</td>
<td>F7.2.38</td>
<td>Dako Cytomation</td>
<td>Mouse monoclonal antibody</td>
<td>IgG1, Kappa</td>
<td>T cells</td>
</tr>
</tbody>
</table>

Briefly, sections were rinsed in distilled water and treated with 3% hydrogen peroxide in methanol to block endogenous peroxidase activity and then washed with PBS alone (CD3) or both distilled water and PBS (Ab-1) before treatment with normal horse serum (Impress Kit, Vector Laboratories, UK) to prevent non-specific staining before being incubated with the primary antibody either overnight at 5°C (Ab-1) or for
1 hour at room temperature (CD3). Sections were then washed in PBS again and anti-mouse secondary antibody (ImmPRESS Reagent, Vector Laboratories, UK) was applied at room temperature. After further PBS washing, diaminobenzidine (ImmPACT DAB) peroxidase substrate (Vector Laboratories, UK) was added to allow visualisation of the target cells with a light microscope. Finally, sections were counterstained with Harris haematoxylin for 15 (Ab-1) or 30 (CD3) seconds, dehydrated, cleared in xylene and mounted using DPX mountant (Surgipath, UK) and cover slips.

The samples were viewed on a light microscope (Olympus BH2) to confirm appropriate staining and a negative control sample was used with each experiment to ensure the absence of non-specific binding.

2.6.6 Two dimensional stereology

Each sample then underwent a 2 dimensional stereological count using a microscope with accurate stage movement measurement and computer software with unbiased sampling frames. The dimensions of the tissue were established using the stage movement measurement device and then the distance between fields of view to achieve approximately 20 different fields was noted. A count for these fields of view was obtained and the coefficient of error (CE) was noted (Figure 18). If it was noted to be above 10%, the number of fields of view was increased to try to reduce the CE by counting a field of view between each of the fields of view already counted and then the CE was noted once again. This process continued until the CE reached the acceptable level of 10% or it plateaued and would not reduce any further. The magnification was kept constant between samples.
Some samples could be orientated according to their appearance on H&E staining and showed zones with different densities of the examined cell types closer to and further from the luminal aspect of the tract (Figure 19, Figure 21). These samples underwent separate counts in the two zones. If the zones were small, a perfect tessellation was performed to try to produce as accurate a result as possible (Figure 20).
Figure 21 - Separate tessellations across two zones in full cross section specimen

Some samples which could not be orientated showed clustering of the examined cells. A note was made of these clusters and counting was performed within them if they were large enough (Figure 22).

Figure 22 - Separate tessellations across a cluster and the remaining tissue

2.7 Immunology methods

2.7.1 Patients

Patients with idiopathic or Crohn’s related anal fistulae were recruited as described above and underwent a planned operation, usually examination under anaesthetic
(EUA) with seton insertion or laying open of fistula although a small number underwent proctectomy. Control patients in the cytokine experiments had no history or evidence of malignancy or colitis on colonoscopy and had been referred with symptoms of rectal bleeding or a change in bowel habit, or for colonic polyp follow-up or family history screening colonoscopy.

2.7.2 FACS sample processing

2.7.2.1 Tissue collection and transport

One to two biopsies were obtained from the luminal surface of the fistula tract at operation. Tissues were placed in complete medium (appendix 1), placed on ice and transported immediately for processing.

2.7.2.2 ‘Walk out’

Samples were removed from the transport medium using sterile forceps and processed within a level 1 fume hood. The samples were washed in complete medium to remove blood and cut into pieces approximately 2mm³ using a scalpel blade. One or two of these pieces were then placed in a test tube for subsequent freezing and multiplex analysis (see below). The other samples were placed in a single well of a 24 well plate (VWR international Ltd, Lutterworth, UK) and incubated overnight at 37°C with 5% CO₂ in air in 1ml of complete medium. After incubation, the samples for cell analysis were removed from the incubator and the supernatant removed from the 24 well plate into a test tube. The well was then washed with 1ml of FACS buffer (appendix 1, 7.3.1) three times and the wash placed into the test tube to obtain as many cells as possible. This test tube was then centrifuged at 1500 rpm for 5 minutes and the fluid was discarded. Walk out cells remain at the bottom of the test tube.

2.7.2.3 Cell surface labelling

The cells were agitated by flicking the bottom of the tube and the resulting volume measured. This volume was made up to 150μl per tube plus 50μl with FACS buffer and antibodies and their isotype controls were added (Table 10). The tubes were then placed in the refrigerator at 4°C for 20 minutes. The cells were washed by
centrifugation in FACS buffer (1500 rpm, 5 minutes), and re-suspended in 200µ/L of paraformaldehyde (appendix 3). The samples were stored at 4°C until acquisition on the flow cytometer within 72 hours.

**Table 10 - An example labelling grid with antibodies and isotype controls**

<table>
<thead>
<tr>
<th>Tube number</th>
<th>FitC</th>
<th>PE</th>
<th>PC5</th>
<th>APC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>CLA</td>
<td>β7</td>
<td>-</td>
<td>CD3</td>
</tr>
<tr>
<td>2</td>
<td>CLA</td>
<td>β7</td>
<td>Lineage cocktail</td>
<td>HLA-DR</td>
</tr>
<tr>
<td>3</td>
<td>rIgM</td>
<td>γ2a</td>
<td>Lineage cocktail</td>
<td>HLA-DR</td>
</tr>
<tr>
<td>4</td>
<td>CD65</td>
<td>CD19</td>
<td>-</td>
<td>γ1</td>
</tr>
<tr>
<td>5</td>
<td>mIgM</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**2.7.2.4 Peripheral blood mononuclear cells (PBMCs) for compensation in flow cytometry**

Human whole blood was obtained by venepuncture and placed into sodium-heparin Vacutainers (Beckton-Dickinson). It was diluted with an equal volume of RPMI-1640 Dutch modification. The whole blood was centrifuged over the same volume again of Ficoll-paque density gradient at 2000 rpm for 25 minutes at room temperature. PBMCs were collected from the interface that resulted and washed twice in FACS buffer at 1500 rpm for 5 minutes. They were then re-suspended in 500µl and labelled (Table 11). After 20 minutes at 4°C a further FACS buffer wash was performed and 1% paraformaldehyde was added to 500µl.

**Table 11 - Antibody labelling of compensation tubes**

<table>
<thead>
<tr>
<th>Tube number</th>
<th>FitC</th>
<th>PE</th>
<th>PC5</th>
<th>APC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Compensation 1</td>
<td>CD8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>C2</td>
<td>-</td>
<td>CD8</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
2.7.2.5 Flow cytometry

Multi-colour flow cytometry allows characterisation of individual cells in suspension by measuring size and granularity as well as identifying the wavelength of light given off by a fluorophore (FitC, PE, PC5 or APC) attached to cells as they pass through one or more focused laser beams.

Twenty microlitres of flow-count fluorospheres (Beckman Coulter, High Wycombe, UK) at a known concentration was added to each tube to enable absolute cell number assessment. Samples were acquired for approximately 5 minutes at high flow rate until all cells had been analysed. Compensation samples were acquired until between 20000 and 50000 events had occurred. All data were then saved as list-mode data files, stored and transferred to a computer for subsequent analysis.

2.7.2.6 FACS analysis

Data were acquired using a FACS Calibur flow cytometer (Becton Dickinson, England) and CellQuest software. Further compensation and analysis of list-mode data were carried out subsequently using WinList software (Verity Software House, Maine). To enable compensation, a region was made around lymphocytes, identified by their size and granularity on the side scatter (SSC) versus forward scatter (FSC) plot and, in each channel, the CD8 (or CD3 in the PC5 tube) marker was evaluated and compensation applied with the compensation tool on WinList. Live cells (again, based on size and granularity) were analysed on a plot of HLA-DR+ versus lineage cocktail- staining which identifies DCs. Lineage cocktail comprised specific monoclonal antibody labels for T cells (CD3), monocytes (CD14, CD16), B cells (CD19), and stem cells (CD34). Using multi-colour analysis, CD11c+ and CD11c− populations within the DC population were assessed for proportion and expression of homing markers. T cells, macrophages, B cells and monocytes were also identified by gating on viable cells and then determining the proportion of cells within this region positive for the fluorophore corresponding to the cell marker in that tube. Both
Region Gating and Enhanced Normalised Subtraction (ENS) were used to analyse the flow cytometry data.

### 2.7.3 Multiplex sample processing

#### 2.7.3.1 ‘Walk out’

The one to two samples set aside for cytokine analysis were incubated overnight (37°C in humidified 5% CO2 in air) in complete medium. The supernatant was then removed into a 1.2ml cryovial and stored at -80°C until multiplex analysis was undertaken on all samples examining for the presence of Th1/Th2/Th17 cytokines (IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ, IL-17A).

#### 2.7.3.2 Multiplex methods

Cryovials containing supernatant were thawed at room temperature and vortexed. Fifty microlitres of supernatant was placed into labelled sample tubes containing 50μl of vortexed human Th1/Th2/Th17 cytokine capture beads (BD Biosciences, UK). Fifty microlitres of human Th1/Th2/Th17 PE Detection Reagent (BD Biosciences, UK) was added to each tube and incubation at room temperature occurred for 3 hours, with the tubes protected from light. One millilitre of wash buffer (BD Biosciences, UK) was then added to each tube before 5 minutes of centrifugation at 200g. The supernatant was discarded and 300μl of wash buffer was added to resuspend the precipitate.

Samples were acquired using a FACS Calibur flow cytometer as described above until all events had been captured. The resultant data were analysed using FCAP Array software (BD Biosciences, UK).

### 2.7.4 Statistical methods

The Stata software package - version 9.2 (StataCorp LP, College Station, Texas, USA) was used for performing all appropriate statistical analyses. Continuous variables were summarised by the medians and inter-quartile range (IQR) due to the skewed distribution of some variables. The number and percentage of subjects in each category were used for categorical variables.
The Chi-square test was used to compare categorical outcomes between groups. Additionally, logistic regression was used to jointly examine the effect of several factors upon the binary categorical outcomes. The size of effects of these analyses was given in the form of odds ratios.

Differences in continuous outcomes between independent groups were evaluated using the Mann-Whitney U test to compare between two groups, or the Kruskal-Wallis test to compare between more than two groups. These non-parametric tests were used due to non-normal distribution of most outcomes. For differences between paired groups the Wilcoxon signed rank test was used. A P value of 0.05, or where multiple testing was used 0.01, was considered significant.

### 2.8 Results

A summary of our findings can be found in Table 12.

**Table 12 - Summary of aetiological findings.**

<table>
<thead>
<tr>
<th>Pathological feature</th>
<th>Crohn’s anal fistula</th>
<th>Idiopathic anal fistula</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fistula tract bacteria</td>
<td>None</td>
<td>None*</td>
</tr>
<tr>
<td>------------------------</td>
<td>------</td>
<td>-------</td>
</tr>
<tr>
<td><strong>Rectal bacteria</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bifidobacteria</td>
<td>Fewer (P=0.02)</td>
<td>More</td>
</tr>
<tr>
<td><em>Bacteroides</em></td>
<td>Fewer (P=0.05)</td>
<td>More</td>
</tr>
<tr>
<td>Faecalibacterium Prausnitzii, Clostridia, Escherichia Coli</td>
<td>Similar</td>
<td>Similar</td>
</tr>
<tr>
<td><strong>Immune cells</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute/Chronic inflammation</td>
<td>Similar</td>
<td>Similar</td>
</tr>
<tr>
<td>Macrophage position</td>
<td>Luminal</td>
<td>Luminal</td>
</tr>
<tr>
<td>Macrophage number</td>
<td>Similar</td>
<td>Similar</td>
</tr>
<tr>
<td>T-lymphocyte position</td>
<td>Luminal</td>
<td>Luminal</td>
</tr>
<tr>
<td>T-lymphocyte number</td>
<td>More luminal (P=0.05) and deep (P=0.04)</td>
<td>Fewer</td>
</tr>
<tr>
<td>DC homing</td>
<td>Less gut (α4β7) and skin (CLA) homing</td>
<td>More (P=0.03)</td>
</tr>
<tr>
<td>CD65 +ve cells</td>
<td>Fewer</td>
<td>More (P=0.04)</td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-17a levels</td>
<td>Lower</td>
<td>Higher (P=0.04)</td>
</tr>
<tr>
<td>TNFα</td>
<td>Similar</td>
<td>Similar</td>
</tr>
<tr>
<td>Other Th1/2/17</td>
<td>Similar</td>
<td>Similar</td>
</tr>
</tbody>
</table>

### 2.8.1 Patient demographics

Sixty-one patients were recruited to the study including 18 with cryptoglandular anal fistulae (IPD), 20 with Crohn’s anal fistulae (CPD), 13 with Crohn’s disease but no previous or current anal fistulae (CD) and 10 normal controls. The demographic details of the 4 groups are shown below (Table 13).

**Table 13 - Patient demographics and baseline disease data.**

<table>
<thead>
<tr>
<th></th>
<th>Crohn’s anal fistula (CPD) n= 20</th>
<th>Luminal Crohn’s disease (CD) n= 13</th>
<th>Idiopathic anal fistula (IPD) n=18</th>
<th>Controls (n=10)</th>
</tr>
</thead>
</table>


<table>
<thead>
<tr>
<th>Age (median, years)</th>
<th>31.5</th>
<th>32</th>
<th>46.5</th>
<th>56.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (M:F)</td>
<td>9:11</td>
<td>5:8</td>
<td>12:6</td>
<td>5:5</td>
</tr>
<tr>
<td>Smokers</td>
<td>6 (30%)</td>
<td>2 (15%)</td>
<td>6 (33%)</td>
<td>2 (20%)</td>
</tr>
<tr>
<td>Disease location*</td>
<td>4 L1p (20%) 7 L2p (35%) 6 L3p (30%) 3 p (15%)</td>
<td>5 L1 (38%) 1 L2 (8%) 7 L3 (54%)</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of luminal disease (median, years)</td>
<td>12</td>
<td>11</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Duration of perianal disease (median, years)</td>
<td>4</td>
<td>N/A</td>
<td>3</td>
<td>N/A</td>
</tr>
<tr>
<td>Stoma</td>
<td>3 (15%)</td>
<td>3 (23%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Seton</td>
<td>8 (40%)</td>
<td>N/A</td>
<td>8 (44%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Immunomodulator (Steroid, Thiopurine, Methotrexate)</td>
<td>7</td>
<td>4</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>


The demographics of the patients used for each experiment were compared to determine any differences. In all experiments, the median age of patients with idiopathic anal fistulae was significantly greater than those with Crohn’s fistulae. In the cohorts undergoing FISH and flow cytometry, patients with idiopathic fistulae had undergone more previous operations than those with Crohn’s fistulae. There was no difference in disease duration or fistula duration between the groups in any experiment.

### 2.8.2 Reduced bacteria in anal fistula tracts

Fistula tract specimens from 32 patients (18 IPD, 14 CPD) were examined using fluorescent in situ hybridisation. No bacteria were identified in 31 patient samples including all 14 Crohn’s anal fistula tracts. Only 1 patient’s fistula tract (a patient with an idiopathic fistula) was found to contain any bacteria associated with the wall of the
tract (Figure 24). These initial 32 specimens were processed as described above with a wash stage in theatre straight after collection.

Figure 23 - Fluorescent in situ hybridisation using eubacterial probe showing mucus devoid of bacteria alongside epithelium (EUB x630)
To control for a technical cause to explain this lack of bacteria, i.e. the washing of bacteria from the sample during processing, a further 4 patient samples (all Crohn’s) were retrieved and placed into cryovials without a washing stage. Three of these were paired samples where washed samples were also processed. In all 4 cases no bacteria were seen.

A Gram stain was performed on 14 patient samples including 3 paired samples (washed and unwashed) and also the sample positive for bacteria in the patient with an idiopathic fistula from the FISH experiments; once again the positive sample was strongly positive but the remaining samples showed no bacteria.

Scanning electron microscopy was performed on 3 patient samples all with no wash stage at any point. One sample (IPD) showed a small cluster of cocci in a very small area on its surface. The rest of its surface and FISH examination of this specimen, as well as SEM assessment of both of the other samples (CPD) found no bacteria.
Figure 25 - Scanning electron microscopy image of tract surface showing a small cluster of cocci (x10000)
2.8.3 Reduced rectal bifidobacteria and *Bacteroides* in Crohn's patients

Crohn's anal fistula patients had fewer rectal bifidobacteria (median 0 bacteria per high powered field, IQR 0-8, vs. median 32, IQR 0-88, P=0.02) and Bacteroides (median 0 bacteria per high powered field, IQR 0-0, vs. median 15, IQR 17-72, P=0.05) than patients with IPD. Crohn's patients without anal fistula also had fewer bifidobacteria than IPD patients. The numbers of *Faecalibacterium Prausnitzii*, *Escherichia Coli* and Clostridia examined using the probes were similar across all three groups.

A longer duration of Crohn's disease (with fistula and non-fistula patients grouped) was associated with more *Bacteroides* (correlation coefficient 0.45, P=0.02).
There was no association found between gender, smoking status, site of luminal disease, presence of a stoma, presence of a seton, or the use of antibiotics, immunomodulators or anti TNF agents and rectal bacteria.

Full results are found in appendix 1 (7.4.2).

2.8.4 Histopathological features of anal fistula tracts

Fistula tract specimens analysed in our study varied with regards to the cells present and their location in the wall of the fistula tract. Some of the Crohn’s fistula tracts contained a keratinised squamous epithelium on their luminal surface with granulation tissue also found on this surface and deeper into the wall of the tract. Ulceration was sometimes seen.

Deeper in the fistula tract wall and surrounding the lumen there was fibrosis with a mostly chronic inflammatory infiltrate composed predominantly of lymphocytes and plasma cells with a few eosinophils and occasional neutrophils also seen. Inflammation was often moderate, chronic and perivascular. Granulomas were rarely seen in the Crohn’s fistula tracts.

The inflammation and fibrosis continued into the deeper layers of the wall of the tract and into the adipose tissue that surrounded the tract.

2.8.5 Similar levels of acute and chronic inflammation in Crohn’s and idiopathic fistulae

Acute inflammation in the fistula tract wall was examined histologically. The median acute inflammation score (based on the types and numbers of cells present, measured on a scale of 0-5) was higher in patients with a seton in situ at the time of surgery (1.5 vs. 0, P = 0.03). There was a trend towards smokers having a higher level of acute inflammation than non-smokers, although this result was not statistically significant (P = 0.08).

A strong positive correlation was found between a longer duration of luminal Crohn’s disease and a higher level of acute tract inflammation in the Crohn’s anal fistula group (Correlation Coefficient = 0.91, P = 0.005). The presence of a seton was also
associated with a higher level of acute tract inflammation (median acute inflammation score = 1.5 vs. 0, P = 0.03).

Chronic inflammation was also assessed (0-5). Although not statistically significant, trends were seen suggesting patients with a stoma had lower and smokers had higher levels of chronic inflammation. Patients with a stoma had a mean chronic inflammation score of 1.0, compared to 2.7 for those with no stoma (P = 0.07). Patients who smoked had a higher level of inflammation, with a mean of 3.1 compared to a mean of 2.1 for those who did not smoke (P = 0.07).

There was no difference in either acute or chronic inflammation between the Crohn’s and idiopathic fistula tracts.

Full results are found in appendix 1 (7.4.1.1).

2.8.6 Inflammation is greatest near the luminal surface in Crohn’s and idiopathic fistula tracts

Fistula tract specimens were examined histologically across the full thickness of the fistula tract wall. A clear demarcation between zone 1 (luminal) and zone 2 (deep) was visible microscopically.

In Crohn’s and idiopathic anal fistula tracts, both Ab-1 (macrophage) and CD3 (T lymphocyte) counts showed significant differences in median number per unit area between zone 1 and zone 2 (10.8 x 10^{-5} \mu m^2 vs. 2.5 x 10^{-5} \mu m^2, P=0.02 and 16.6 x 10^{-5} \mu m^2 vs. 5 x 10^{-5} \mu m^2, P=0.002 respectively) indicating that the luminal layer of the fistula tracts contain more macrophages and T lymphocytes than the deep layers (Figure 27).
Figure 27 - Increased macrophages and T lymphocytes at luminal surface of fistula tract compared to deeper layers

Figure 28 - H&E x 20 of fistula tract with lumen in lower left corner and full thickness of tract wall up to fat. Inflammatory zone seen near lumen of tract.
2.8.7 Higher T lymphocytes in Crohn’s than idiopathic anal fistula tracts

When the number of cells seen in Crohn’s and idiopathic fistula tracts was compared, more CD3 +ve T lymphocytes were seen in both the luminal (Figure 30) and deep (Figure 31) layers of Crohn’s fistula tracts compared to idiopathic tracts (21.7x10$^{-5}$ μm$^2$ vs. 14.4x10$^{-5}$ μm$^2$, P=0.05 and 9.1x10$^{-5}$ μm$^2$ vs. 4.5x10$^{-5}$ μm$^2$, P=0.04 respectively).

No difference in Ab-1 positive cells (macrophages) between Crohn’s and idiopathic patients overall, or in zone 1 or zone 2 counts was found.
Figure 30 - Increased T lymphocytes in luminal layer of Crohn’s compared to idiopathic fistula tracts

Figure 31 - Increased T lymphocytes in deep layer of Crohn’s compared to idiopathic fistula tracts

Full results found in appendix 1 (7.4.1.3).
2.8.8 Dendritic cells lack homing markers in Crohn’s anal fistula tracts

DC in Crohn’s fistula tracts had significantly reduced levels of both the gut (α4β7, median 0% cells positive, range 0-0, vs. median 16%, range 0-96%, P=0.03) and skin (CLA, median 0%, range 0-0, vs. median 78%, range 0-99%, P=0.03) homing markers compared with idiopathic fistula tracts. Crohn’s fistula tracts showed lower levels of CD65 than idiopathic fistula tracts (median positive control intensity ratio 6, range 1-93, vs. median 77, range 0-743, P=0.04). There was no significant difference in proportions of either myeloid or plasmacytoid DC, or of monocytes (CD14, CD16), B lymphocytes (CD19) or T lymphocytes (CD3), or HLA DR positivity between Crohn’s anal fistulae and idiopathic anal fistulae found by flow cytometry.

Full results are found in appendix 1 (7.4.3).

2.8.9 Crohn’s and idiopathic anal fistula contain similar levels of TNF but IL-17a is reduced in Crohn’s compared to idiopathic anal fistulae

There was no significant difference in levels of Tumour necrosis Factor (TNFα) between Crohn’s and idiopathic anal fistula tracts (1.61 pg/ml vs. 2.81 pg/ml, P = 0.48).

IL-17a levels were lower in Crohn’s anal fistula tracts (2.63 pg/ml) than normal rectum (3.01 pg/ml) and idiopathic anal fistula (3.38 pg/ml) (P=0.04).

Levels of IL-2, IL-4, IL-6, IL-10 and IFN-γ were also similar in Crohn’s and idiopathic anal fistula tracts.

The full results are found in appendix 1 (7.4.4).

2.8.10 Associations between bacteria and immunity

When examined across all patients, some rectal bacteria were found to be associated with tract immune cells. This analysis utilised multiple testing and as such a lower level of significance (P=0.01) was used.
Higher levels of *Faecalibacterium Prausnitzii* tended towards association with fewer CD3+ve T cells in the deeper layer of the fistula tract (cc=-0.58, P=0.0367).

Higher levels of *Bacteroides* were associated with higher levels of CLA (cc=0.78, P=0.014) and β7 (cc=0.92, P=0.0004) in tract tissue generally and higher levels of CLA on DC (cc=0.90, P=0.0009).

Rectal and tract cytokines also showed some association with some bacteria.

Higher levels of EREC tended towards association with higher levels of IL-2 (cc=0.64, P=0.024) and IFNγ (cc=0.65, P=0.021).
Higher levels of *Bacteroides* tended towards association with lower levels of IL-10 (cc=-0.62, P=0.025).

### 2.9 Discussion

We aimed to characterise and compare the fistula tract microbiota using a molecular technique. Like standard culture, quantitative polymerase chain reaction amplifies the bacteria present so that contamination can confuse the results achieved. Fluorescent in situ hybridisation identifies bacteria where they are fixed so that it is possible to assess the nature and number of mucosal-associated bacteria, those thought to interact with the host immune cells and drive inflammation, whilst discounting those that sit well away from the tissue or on top of it which are likely contaminants or at least unlikely to be clinically relevant.

Our fistula tract findings suggest that very few if any bacteria occupy a clinically relevant position in anal fistula tracts. A reduced number of bacteria in the established fistula tracts seen in our study may suggest that there is limited bacterial colonisation of the luminal surface of anal fistula tracts which would imply limited interaction with host immune cells and a less significant role for bacteria in the persistence of anal fistula than previously thought.

This finding could be due to a sampling error since only two small biopsies were retrieved for each tract although several levels within these biopsies were examined. The biopsies were taken as far cephalad in the tract as possible but rarely next to the internal opening where most bacteria might be postulated to be found.
Another explanation may be that after the initial infective insult with abscess and ultimately fistula formation, fistula tract immune activity is able to prevent colonisation of the luminal surface of the tract by bacteria, and analysis of this ‘stand off’ reveals active innate immunity and few if any live bacteria (as seen here): an effective host defence. The tract may subsequently fail to fully heal due to a separate pathology or abnormality in tissue repair, for example.

Previous assessment of mucosa-associated bacteria in the rectum by Swidsinski and colleagues using molecular techniques (49) suggests that a. bacteria do not invade denuded mucosal areas but colonise the intact mucosal surface; b. are held in position, despite washing, by a thick bacterial film in which the bacteria sit; c. the bacterial film is only present in specimens with concentrations of 10,000cfu/μl; and d. below this concentration few if any scattered bacteria are seen.

Our fistula tract specimens examined contained little or no epithelium and rarely a mucus layer. An absence of mucus leaves cells open to adherence and invasion by bacteria but also denies them an immediate home and it may be that by washing, either in theatre after collection of the specimen, or during the FISH or Gram experiments, any bacteria present in vivo were simply washed away.

Electron microscopy in 3 patients also revealed sterile tracts, in spite of quite large sections of tissue, in 2 of the 3 cases. The small, isolated cluster of cocci seen in the third sample may represent contamination, or possibly a precursor to fistula re-activation.

Our fistula tract findings may be relevant to persistence of established fistula tracts rather than the initial insult which leads to fistula formation, and the role of bacteria within this aetiopathogenesis.

The rectal microbiota may be a more likely element in the initiation of fistulation. We found reduced bifidobacteria in Crohn’s patients both with and without anal fistulae, compared to patients with idiopathic anal fistulae. This finding is consistent with previous reports of reduced bifidobacteria in Crohn’s disease (48) which suggests our experimental technique and patient population are consistent with those published elsewhere, however a dysbiosis or single causative organism responsible for anal fistulation in Crohn’s disease or across aetiologies was not seen.
However, both idiopathic and Crohn’s fistula tracts showed evidence of acute and chronic inflammation and, significantly, both groups of patients had more CD3 positive and Ab-1 positive (monocytes, macrophages, neutrophils) cells in the luminal zone than the deep zone, suggesting inflammation arising from the luminal surface of the tract.

If the inflammation is peri-luminal and therefore driven by luminal factors, but these factors are not live bacteria, what can be causing this inflammatory reaction? Endotoxin, remnants of bacterial cell walls following lysis or other proteins recognised as foreign may be present. Non-bacterial infective agents were not sought in our experiments and nor were degraded bacterial remnants which, in the absence of RNA, would not have been detected by our FISH probes.

Although, since we cannot find them, we cannot differentiate between luminal agents driving the immune response in Crohn’s and idiopathic fistulae, we can examine the responses themselves.

Dendritic cells direct the immune response to invasion by migrating from the site at which they have encountered an antigen to the lymph node where they imprint T cells with a migration marker which sends them to the site of invasion to mount a local defence. We found a reduced tendency for DC to express both gut and skin homing markers in Crohn’s anal fistulae compared to idiopathic fistulae. This implies a less ordered immune response which might lead to ineffective bacterial killing. If this finding were related to maturity of the tract alone then we would expect a similar low level of homing in idiopathic fistulae.

Crohn’s anal fistula tracts expressed less CD65 than idiopathic anal fistula tracts. CD65 is a cell surface antigen found on monocytes in the blood and macrophages in the tissues, as well as other myeloid cells, including neutrophils and cells of the innate immune system.

Although the proportions of CD3 positive T lymphocytes in Crohn’s and idiopathic anal fistula tract tissue were similar when examined with flow cytometry, immunohistochemistry revealed a difference between the two groups. In samples with a clear luminal surface, Crohn’s patients had higher numbers of CD3 positive cells than idiopathic patients in both the luminal and deep zones of the fistula tract wall.
Similarities in the remaining immune cell composition of fistula tract specimens from both idiopathic and Crohn’s fistula patients suggest that similar persistence factors may be in play in both groups regardless of initial aetiology.

Cytokines, including TNF, were also similar across idiopathic and Crohn’s anal fistula specimens with the exception of IL-17a. Crohn’s fistula tracts expressed less IL-17a than both idiopathic fistula tracts and normal rectum. The potential for IL-17a as a diagnostic or therapeutic target should be explored. IL-17a is generally considered a pro-inflammatory cytokine and levels of both IL-17a itself and IL-17a producing cells are higher in the GI mucosa of Crohn’s patients than healthy controls (reviewed in (276)). However, some recent evidence suggests that IL-17a may have a protective role in T cell mediated intestinal inflammation in vitro (277) and that IL-17 expressing cells can be inflammatory or protective depending on co-expression with other cytokines (276;278).

Since there is no normal control for a fistula tract it is not clear whether the similar TNF levels are depressed, normal, or raised to the same degree in both idiopathic and Crohn’s anal fistula. Samples of rectal mucosa from patients undergoing colonoscopy to investigate fresh rectal bleeding or in colorectal cancer family history screening and found to have neither IBD nor CRC, showed similar cytokine levels to fistula tract patients but the validity of this as a normal control is unknown. Therapeutic strategies directed towards TNF in Crohn’s anal fistulae are often very successful (73) and raised TNF levels have been found in the serum of patients with Crohn’s anal fistulae compared to Crohn’s patients with only luminal disease (77). However, given that tract TNF levels are similar, and if local treatment with anti-TNF agents were successful, there may be value in trials of similar treatments in complex, inoperable or refractory idiopathic anal fistulae.

We should interpret our findings on the association between rectal bacteria and tract immunity with caution given the multiple statistical testing used to identify them. Nevertheless, we found higher levels of Bacteroides were associated with higher levels of CLA and β7 in tract tissue generally and higher levels of CLA on DC in particular with great statistical confidence. The impact of rectal bacteria on fistula tract immune cells and cytokines, and of luminal bacteria on luminal immune cells and cytokines in Crohn’s disease and Ulcerative Colitis, may represent a fruitful avenue of future research.
2.10 Summary

Inflammation in anal fistula tracts is more pronounced in the peri-luminal zone of the tract wall, implying a luminal factor is driving the inflammatory process. The absence of live bacteria associated with the luminal surface of the tract suggests that bacteria may not be that factor.

The broad similarities in the immune cell composition of Crohn’s and idiopathic anal fistula tracts suggest that, at least in mature tracts, a similar process permits persistence. Further, medical treatments advantageous in one group may also prove useful in the other, particularly where, as with anti-TNF agents, levels of the target molecule are similar in the two types of tract.

Specific differences between Crohn’s and idiopathic fistulae such as lower levels of IL-17a, greater numbers of peri-luminal and deep CD3 positive T cells and impaired (skin and gut) homing in the tracts themselves, and an altered rectal microbiota, in Crohn’s disease, represent potential targets for diagnostic tests, therapy and future research.

2.11 Limitations

This study was carefully designed with several control groups and as large a study population as possible given the relatively specialist nature of the patient group. Nevertheless, several factors limit its usefulness and findings.

Patient selection was limited to St Mark’s which allowed for a large throughput of fistula and in particular Crohn’s fistula patients, but meant that most were tertiary referrals with a relatively long duration of Crohn’s and/or anal fistulating disease. This meant that we were examining a group of well-established fistulae rather than newly created tracts which might be postulated to express more of the factors which lead to formation of the tract.

The collection of samples was formalised to limit sampling error but on some occasions the tract diameter did not allow passage of the cannula sheath to protect the forceps as they entered the tract and once inside, we were only able to blindly pass the forceps rather than specify the location of the biopsies or their quality. It might be the case that influential bacteria reside in a particular part of the tract which
we did not sample, for example near the internal opening. Orientation of the samples was also difficult.

The microbiology samples demonstrated the rather surprising finding of a lack of clinically relevant ‘mucosa-associated’ bacteria. Several factors have been discussed above which may mean this is an erroneous finding due to technical problems. The washing stages of sample collection and processing may have cleared bacteria which were influential in vivo and although techniques were used to eradicate this as a factor, the most powerful of these, SEM, was only used in three cases and does not rule out washing as the culprit.

It may also be the case that fistula tract tissue is simply not amenable to the FISH technique. Although the tissue stained appropriately, FISH in the gut demonstrates bacteria held fast in a biofilm alongside intact mucosa and the lack of these structures in the tract may make it a less useful technique here; FISH has not been undertaken in fistula tracts before, to my knowledge.

Our immunological findings are also derived from tissue which was heterogeneous in size, shape and depth of penetration through the wall of the tract, all of which may influence the number and proportions of particular cells seen.

The patient groups were consecutive and although some significant differences were seen, the size of the groups (particularly when one considers that not all patients were used in all experiments) means that the study may have been under powered to detect more subtle differences. In addition to this, the very large number of analyses including subgroup analyses performed, means the risk of type 1 error due to multiple testing is significant.

The lack of a true control tissue for fistula also limits the immunology and particularly cytokine data obtained. It is only possible to infer from our results whether one or other group of patients demonstrates a higher or lower level of a given factor than the other. This is adequate in some circumstances, particularly given the multiple control groups used, but care must be taken in others, for example with TNF where, as discussed above, similar levels may mean high in both Crohn’s and idiopathic fistulae, low in both, unremarkable in both or, indeed, that our sample size was too small to detect the true difference.
3 An audit of the surgical management of anal fistulae: Fistulotomy in the tertiary setting can achieve high rates of fistula cure with an acceptable risk of deterioration in continence

3.1 Abstract

Introduction

Surgery is the mainstay of treatment of anal fistulae. Low fistulae are often laid open but higher fistulae present a more difficult problem. Patient choice centres on a compromise between risk of recurrence and risk of impairment of continence. We aimed to determine the efficacy and safety of fistulotomy at a tertiary referral centre, in particular the additional risk of impairment of continence following fistulotomy of the often recurrent, multiply-operated patients seen.

Methods

Patients undergoing surgery under one Consultant Surgeon (Professor Phillips) for an anal fistula during the study period (2005-2006) were identified and a thorough review of the patients’ clinical records was undertaken. Demographic, fistula anatomy, treatment and follow up data were obtained.

Results

Eighty-four patients underwent either fistulotomy (50) or insertion of a permanent loose seton. The mean length of follow up was 11 months (SD 14.22). In the fistulotomy group there was an overall ‘success rate’ of 93%. Secondary extensions were associated with failure to achieve cure (P=0.008). Nine patients (20%) suffered a deterioration in continence after surgery. A longer time to referral was associated with a greater degree of impairment of final continence.
Conclusions

It is safe and reasonable to offer fistulotomy to appropriate patients despite previous surgery and within the tertiary setting. By so doing, a very high rate of healing can be achieved in patients who have previously failed to heal. The additional risk of impairment of continence is around 1 in 5 and in the majority will represent only minor incontinence.

3.2 Introduction

Anal fistulae are common and usually occur following infection of an intersphincteric anal gland (81). They lead to pain and discharge and patients often suffer recurrent abscesses which undergo surgical or spontaneous drainage before a period of quiescence and then further eruption.

In the absence of Crohn’s disease, surgery is the mainstay of treatment and for low fistulae, fistulotomy (also known as laying open) is the standard treatment. When a higher fistula is seen, sphincter-preserving operations such as ligation of the intersphincteric fistula tract (LIFT) (198;199), advancement flap surgery (188;192) or the placement of an infill material such as fibrin glue (279) or collagen plug (191) is often considered but the risk of recurrence is high. Fistulotomy will cure a high fistula but impairment of continence may occur. Cutting setons probably produce a similar level of impairment of continence to fistulotomy (205) and can be uncomfortable for the patient.

Careful anatomical and bowel function assessment can allow fistulotomy in high fistulae with a low rate of recurrence and an acceptable level of impairment of continence – similar to that seen in lower fistulae (171). Given an appropriate length (1-2 cm) of preserved external sphincter, internal sphincter division leads to the majority of continence impairment seen after fistulotomy (171-173;178).

Patient choice centres on a compromise between risk of recurrence and risk of impairment of continence. The approach to this compromise of patients with a new, primary fistula may differ to that of a patient with multiply recurrent fistulae of a longer duration, who may also have suffered complications or impairment of continence at previous operations.
This study seeks to determine the efficacy and safety of fistulotomy at a tertiary referral centre, in particular the additional risk of impairment of continence following fistulotomy of the often recurrent, multiply-operated patients seen.

3.3 Hypothesis and Aims

3.3.1 Hypotheses

- The anatomy, duration and aetiology of anal fistulae as well as age and smoking status influence surgical outcome;
- The presence of a defunctioning stoma influences outcome;
- Incontinence following laying open of fistulae is usually minor and, in the tertiary setting, additional laying open procedures add little impairment of continence or other morbidity.

3.3.2 Aims

The efficacy and safety of fistulotomy at a tertiary referral centre has been determined, in particular the additional risk of impairment of continence following fistulotomy of the often recurrent, multiply-operated patients seen.

3.4 Methods

3.4.1 Patient identification

Patients were identified from the operation booking diary and surgical log. Any patient undergoing surgery for an anal fistula under Professor Phillips during the study period (2005-2006) was noted and their clinical notes retrieved. If an operation note for a procedure carried out on an anal fistula within this period was available, the records were then reviewed.

3.4.2 Review of records

A thorough review of the patients' clinical notes was undertaken with examination of the clinical notes, clinic correspondence, operation records and examination results. Computer records of imaging tests or other investigations were also accessed where relevant. Demographic, fistula anatomy, treatment and follow up data were obtained. A low fistula was defined as involving no more than the most caudad 1cm of sphincter.
**3.4.3 Database and statistics**

Data were entered into a database on Microsoft Excel 2003™. The Stata software package - version 9.2 (StataCorp LP, College Station, Texas, USA) was used for performing all appropriate statistical analyses. Data were compared using Fisher's exact test, the unpaired t-test and the Mann-Whitney test as appropriate.

**3.4.4 Surgical procedures**

The two surgical procedures studied were fistulotomy and seton insertion. All patients deemed suitable for a fistulotomy were counselled regarding the risks of minor incontinence (≤ flatus incontinence and 1 teaspoon of mucus leakage in 24 hours) occurring in a quarter to a third of patients, recurrence occurring in 2-3% and delayed wound healing. Patients for seton insertion were fully informed regarding the permanent nature of the loose seton, the occasional need for replacement and the small risk of ongoing symptoms requiring further drainage and/or seton insertion.

Both procedures take place in the lithotomy position with the hips and knees flexed to around 110°. A monopolar diathermy pad is placed on the patient's thigh if any fistulotomy is to take place. After skin preparation with antibacterial skin prep the patient is draped and a digital rectal examination is undertaken to gain an appreciation of the anatomy of the internal opening(s) and the course of the primary tract and any extensions. A probe is then passed through the fistula from external to internal opening. If the internal opening cannot be found using the probe or felt digitally, dilute hydrogen peroxide is instilled into the external opening through a 23G cannula and then the external opening is occluded. A view of the anorectum is maintained throughout this procedure using an Eisenhammer retractor and bubbles entering the anal canal through the internal opening identify its position.

The probe is placed carefully through the fistula tract without any pressure to avoid iatrogenic injury. If the tract traverses the roof of the ischiorectal fossa it is sometimes necessary to apply gentle traction to the probe once the tip is at the apex of the tract in order for it to follow the inner aspect of the tract through the sphincters. An hourglass deformity of the fistula tract sometimes precludes passage of the Lockhart-Mummery probe and under these circumstances lachrymal probes are used.
Once the probe is successfully placed through the fistula tract, a further assessment of the quantity of external sphincter cephalad of the internal opening is made. The amount of muscle and any distal keyhole deformity can be assessed under general anaesthetic but the contractile quality of the muscle can only be assessed in the awake patient, usually in the outpatient clinic.

If adequate muscle to maintain continence to stool will remain after fistulotomy (taken to be 1cm in the presence of a normal bowel habit by the senior surgeon) and the patient consents to it, a full laying open of the fistula can take place. If there is insufficient muscle or the patient does not consent to fistulotomy, a comfortable, low profile seton is inserted.

The senior surgeon uses a 1-0 Ethibond tied with a single surgeon’s knot over two fingers placed on the buttock. The loose ends of this suture are then tied back using a 2/0 silk suture with a single surgeon’s knot (Figure 4b). This leaves a low, soft seton with small knots which is unlikely to cause problems for the patient.

### 3.5 Results

#### 3.5.1 Demographic and baseline disease data

Eighty-four patients underwent either fistulotomy (n=50) or insertion of permanent loose seton. Results for the fistulotomy group are presented below. Mean (SD) length of follow up was 11 (14) months.

In the fistulotomy group, 41 (82%) were tertiary referrals with a mean 1.3 (SD 1.1) operations prior to referral. Fourteen (28%) had a seton in situ. Median age was 48 years (range 18-79) and 9 were women. Six patients had inflammatory bowel disease (5 Crohn’s disease, 1 indeterminate colitis), 7 had diabetes and 1 hidradenitis suppurativa. Sixteen were smokers. MRI scanning was performed in 27 patients, endoanal ultrasound in 1 patient and both in 3. Nineteen had no imaging investigations performed.
3.5.2 Fistula anatomy

The median number of both internal and external openings was 1 (range 1-2 and 0-5 respectively). The positions of the primary internal openings are shown in Figure 32. The internal opening was palpable in 41 patients and located at the dentate line in 33, below it in 7 and above it in 10. Goodsall’s rule was obeyed in 76% of cases including 80% of external openings behind the transverse anal line. Tract height is shown in Figure 33.

![Position of internal openings](image)

**Figure 32 - Position of internal openings**

![Height of fistula tracts](image)

**Figure 33 - Height of fistula tracts in fistulotomy patients**
3.5.3 Impairment of continence

A continence deficit was found in 13 (26%) patients at referral and included 9 patients with minor incontinence (flatus +/- < 1 teaspoon of mucus leakage in a 24 hour period), 3 with a greater degree of leakage and 1 with urgency.

Six patients did not attend follow up or were referred for local follow up. Following fistulotomy, 34% of patients (15/44) had some degree of continence deficit, of whom 12 had minor incontinence (Figure 34). Nine patients (20%) suffered a deterioration in continence after surgery (7 previously normal patients developed minor incontinence, 1 with minor incontinence at referral developed more significant leakage leading to the use of a pad and 1 developed urgency).

<table>
<thead>
<tr>
<th>Time of assessment</th>
<th>Referral</th>
<th>Discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urgency or greater discharge</td>
<td>8%</td>
<td>7%</td>
</tr>
<tr>
<td>Minor incontinence</td>
<td>18%</td>
<td>27%</td>
</tr>
<tr>
<td>Full continence</td>
<td>74%</td>
<td>66%</td>
</tr>
</tbody>
</table>

Figure 34 - Deterioration in continence seen post fistulotomy

In the fistulotomy group, time to referral was associated with final continence. Only 7% of patients referred within one year suffered a decrease in continence, whilst this increased to 38% for patients who were referred after two years (P = 0.05). When all 84 patients (the fistulotomy and loose seton groups combined) were examined together, continence at referral was the only factor significantly associated with final continence; 84% of those continent at referral were continent at discharge compared to 27% of those incontinent at referral (P<0.001). Fistulotomy rather than seton insertion (P = 0.07) and tertiary rather than secondary referral (P = 0.09) had a
borderline association with incontinence at discharge but after multiple logistic regression only continence at referral remained significant.

3.5.4 Recurrence

Recurrence occurred in 5 patients. Two of these required a second fistulotomy following early bridging of the fistula wound and were cured following the second procedure giving an overall success rate of 93% (41/44). Of the remaining 3 patients one had an underlying osteomyelitis of the pubis, one had Crohn’s disease and developed recurrence more than a year later and one developed an apparently new fistula after 4 years.

Secondary extensions were associated with failure to achieve cure. Ninety-seven percent of patients (31 of 32) without secondary extensions at fistulotomy were cured compared to only 60% (6 of 10) of those with them (P=0.008). There was a trend towards a longer time to referral being associated with failure to heal, although this did not quite reach statistical significance in our cohort (P=0.09). No other anatomical features were associated with healing in the fistulotomy group.

3.5.5 Complications

Six patients experienced complications other than recurrence and impairment of continence. These included anal pain with no evidence of recurrence in 2 patients, post-operative bleeding at 2 weeks in 1, an anal fissure and pruritus ani in 1, overgrowth of granulation requiring cautery in the outpatient department in 1 and a wound infection in another. None of these 6 patients required further surgery.

One patient with a high transsphincteric idiopathic fistula with internal opening at 6 o’clock and external opening at 4 o’clock had a stoma created at the time of fistulotomy and reversed 7 months later. He had incontinence to stool at referral and this persisted after restoration of continuity which took place at his request.

Of 9 women treated with fistulotomy, 3 had anterior internal openings, 1 a subcutaneous tract, 1 patient with Crohn’s disease had a high transsphincteric tract and 1 a low transsphincteric tract. This latter patient had minor incontinence at
referral which worsened after surgery. The other two remained fully continent following fistulotomy. None developed recurrence.

### 3.6 Tertiary referral patients

Of 50 fistulotomy patients, 41 of whom were tertiary referrals, 37 were referred by another colorectal surgeon having undergone a mean of 1.7 previous operations (SD 1.9). After a mean follow up of 10 months (SD 14), 5 patients were lost to follow up and 4 patients suffered recurrence of fistula including 1 with early bridging who underwent a successful second lay open giving an overall success rate in this group of 29 of 32 patients (91%). The remaining three recurrences were those patients with underlying osteomyelitis and delayed recurrence detailed above.

At referral, 12 of 37 (32%) patients had some degree of continence impairment (9 minor, 2 with greater leakage and 1 with urgency). Following fistulotomy, 13 of 32 (40%) patients had some degree of continence impairment (11 minor, 2 with greater leakage). Six patients (19%) suffered deterioration in their continence following surgery, all to minor incontinence only.

### 3.7 Complex fistulae

Twenty seven patients (54%) who underwent fistulotomy had complex fistulae (high tracts, secondary extensions, inflammatory bowel disease). In this group, one third of patients had impairment of continence at referral (6 minor incontinence, 1 with greater leakage and 1 urgency). Following fistulotomy 3 were lost to follow up. Recurrence occurred in 4 patients in this group (16%) and 5 patients (20%) developed new or worse impairment of continence including 4 with minor incontinence and 1 patient with urgency. Overall final continence impairment in this group was 41%, four fifths of which was minor incontinence.

### 3.8 Inflammatory bowel disease

Six patients (3 female) with inflammatory bowel disease underwent fistulotomy (5 Crohn’s disease, 1 indeterminate colitis). The patients had undergone a median of 1 (range 0-4) operation prior to referral. Tracts were intersphincteric in 1, low transspinchteric in 2 and mid or high transspinchteric in 3. Three had minor incontinence at referral and one of these improved following surgery. One patient,
with a low transsphincteric tract, developed recurrence over a year after successful fistulotomy.

3.9 Setons

Permanent loose seton insertion was undertaken in 28 patients deemed unsuitable for fistulotomy (21) or who declined fistulotomy when offered it (7). A low profile, soft seton fashioned from 1 ethibond tied in a single surgeons knot with the ‘whiskers’ tied back with silk was placed in all cases. Ten seton patients were women, 20 were tertiary referrals, 3 had inflammatory bowel disease, 3 had diabetes, 11 had secondary extensions and 10 had setons in situ at referral. Two presented with continence impairment (1 urgency, 1 minor) and after surgery no new continence impairment was noted. Median follow up was 20 months.

3.10 Conclusions

High or complex anal fistulae, including fistulae in patients with inflammatory bowel disease or other co-morbidities, can be successfully treated even after previous failed surgery and in the presence of impairment of continence.

More than 80% of the present cohort had been seen in another hospital and undergone previous surgery which had been unsuccessful. Patients face a decision when offered fistula surgery: do they opt for a modality offering a higher rate of success but with it a higher risk of impairment of continence, or do they opt for a safer but less successful method of treatment? Previous failed surgery and a longer duration of symptoms may influence this decision. In order correctly to inform patients, the risk of deterioration of continence following fistulotomy must be known. An earlier study showed that a quarter to a third of patients will develop impairment of continence following laying open of a tract traversing the anal sphincter and that internal sphincter division is responsible for the greater part of that impairment (171).

In this group of mostly complex, tertiary referred, previously operated patients, the proportion with continence impairment at referral was 26% which rose to 34% at discharge. Although this change is only 12%, some patients improved following surgery and 1 in 5 patients suffered deterioration in their continence following fistulotomy, the majority from full continence to minor incontinence.
As in an earlier study, lay-language has been used to describe impairment of continence. This is because the utility of these data is in advising patients of their likely outcome whilst obtaining informed consent. The Vaizey (280) and Wexner (281) scores, which add different aspects of incontinence to produce an overall score and which value flatus and solid incontinence with an equal weight, may be less useful in the clinical setting.

Although 5 patients developed fistula recurrence, two were cured following a second fistulotomy and of the remaining three, two developed a new fistula more than a year after treatment, one in the presence of Crohn’s disease. The third had an underlying osteomyelitis. The final healing rate was 93%. A previous study demonstrated healing following fistulotomy in 97-98% of patients in a large cohort of cryptoglandular fistulae using the same principles of treatment (171). In the present study, patients with more complex tracts in the form of secondary extensions were more likely to recur.

It is not true to say that all patients should undergo fistulotomy. The decision making process employed in this study has been previously described (171). Briefly, an assessment is made, usually in Outpatients, of the likely residual sphincter following a planned fistulotomy in order to ensure that at least 1-2cm of good quality contractile muscle will remain, along with an assessment of the current and likely future bowel habit (IBD, IBS, chronic diarrhoea). This requires a thorough physical examination, sometimes aided by high quality imaging with MRI or endoanal ultrasound (rarely), to identify the position of internal and external openings as well as assessment of any extensions or collections.

Alternative techniques in those wishing to avoid any impairment of continence including permanent loose seton insertion, tight setons, infill materials such as fistula plugs and glues, the LIFT procedure and advancement flaps offer alternatives to fistulotomy but risk failure and therefore further abscesses, fistula recurrence and future procedures. LIFT has been most extensively reported in low anal fistulae with success rates of around 80% (198) which falls to 60% (200) in recurrent tracts. Infill materials have suffered a waning success rate down to around 30% with fistula glues (279) and 50% with plugs (191). Nevertheless, all three techniques are repeatable and none precludes future surgery in any form.
A longer time to referral seems to be associated with a higher risk of both recurrence and deterioration in continence. This may be because later referral implies more previous operations or selects those patients with more complex fistula anatomy or biology.

### 3.11 Summary

It is safe and reasonable to offer fistulotomy to appropriate patients despite previous surgery and within the tertiary setting. By so doing, a very high rate of healing can be achieved in patients who have previously failed. The additional risk of impairment of continence is around 1 in 5 and in the majority will represent only minor incontinence.

### 3.12 Future work: a prospective database

The aspects of outcome in fistula surgery which are crucial to the patient are long term recurrence, continence impairment and quality of life. There are few data on these subjects beyond a year or so in the literature and care must be taken when comparing the outcome from different procedures since a. there is often significant selection bias; higher fistulae are less likely to be laid open, more complex fistulae are rarely treated with infill materials etc., and b. heterogeneity in technique between surgeons, for example in long term loose seton material choice, may limit the value of results found.

We have begun prospective data collection on all fistula patients seen in the outpatient department. Simple tick box sheets (Appendix 3, page 213) are filled in at the initial outpatient appointment, in theatre immediately after surgery, and at each subsequent follow-up appointment.

The demographics and comorbidities of the patient, anatomy of the fistula, investigations, details of surgery and outcomes in terms of recurrence, continence, wound healing and overall satisfaction are monitored throughout the patient journey.

As well as monitoring recurrence and continence impairment rates, the overall satisfaction gained from early long term seton use, laying open, (often repeated) advancement flap surgery and newer techniques in the management of high fistulae can be determined.
Prognostic factors will be sought that might help determine that seton placement, for example, is likely to fail to produce a satisfactory outcome, perhaps because of the presence of a high extension of the fistula which might act as a ‘sump’, pouring pus down into the drained fistula tract and preventing the palliation hoped for. Another example might be factors that improve the chance of success in advancement flap surgery, or predict failure early to prevent fruitless repeated operations.

3.13 Limitations

This retrospective study represents the practice of a single surgeon and examines only two of the techniques he uses in the management of anal fistulae. The anatomy of the fistulae has been defined by the same surgeon undertaking surgery and deciding on the procedure to be offered to the patient.

Assessment of healing and recurrence may be imperfect since long term follow up may be undertaken back at the secondary care institution in some cases although our median follow up was comparable with the published literature.

Assessment of incontinence did not utilise a standardised tool as discussed above and although the reasoning behind this is, in my view, sound, this limits comparison with other studies in which such tools are used.

The group of patients is small and heterogeneous. Subgroup analysis and conclusions based thereon must be considered carefully and a largely tertiary group of patients is not the same as a group of patients with complex fistulae hence the analysis of these smaller groups.

The exact process for determining which procedure to offer a patient, whilst clearly described, remains to some extent subjective albeit in the hands of the senior surgeon alone, and may not be applicable directly in other contexts. It is likely that patients arrive at St Mark’s with different expectations and tolerance of impairment of continence to those of a patient with a new fistula which informs the surgeon’s discussion with the patient and selection of the appropriate procedure.
4 An audit of the medical and surgical management of Crohn’s and non-Crohn’s rectovaginal fistulae

4.1 Surgical management of rectovaginal fistulae in a tertiary referral centre: many techniques are needed

4.1.1 Abstract

Introduction

The mainstay of treatment for rectovaginal fistulae (RVF) is surgery. Published success rates vary; initial success being around 50% rising to 80% with repeated surgery. Crohn’s fistulae are more likely to recur.

Methods

A retrospective study of rectovaginal fistula repair between 2003 and 2008 was performed in a tertiary referral centre. Patients undergoing surgery for a rectovaginal fistula under the senior author during the study period were identified and their clinical notes reviewed.

Results

Thirty-five patients underwent 50 operations. Their median age was 42 years and 83% were tertiary referrals. Two patients were lost to follow-up and 19 of 33 patients were ultimately healed (58%) after a mean of 1.4 operations. Median time to success was 11 months (range 2.5 – 48). The ‘curative’ group had an overall success of 73% (19 of 26). Seventy-five percent of non-IBD patients and 67% of those with Crohn’s disease had successful treatment of their RVF.

Twenty-four of thirty-five patients (67%) underwent creation of a stoma. Sixteen of twenty-four (67%) were deemed fit for restoration of continuity. No demographic or disease related factors were found to influence healing.
Discussion

Rectovaginal fistulae require a range of surgical approaches, both abdominal and anal. A variety of different anal techniques is necessary, depending on the integrity of the anal sphincter and the presence or absence of perineal descent/internal intussusception. There remains significant scope for improvement.

4.1.2 Introduction

Fistulae to the vagina from the anus or low rectum are variously called ano-vaginal or rectovaginal fistulae (RVF). The latter term has been employed in this article. They are uncommon. RVF may arise in the vaginal midline following obstetric injury, perianal sepsis or in patients with Crohn’s disease, as well as following radiation, malignancy or iatrogenic injury. Low anastomoses after anterior resection or ileoanal pouch formation may fistulate to the vagina.

There are also abdominal sources, such as appendicitis, terminal ileal Crohn’s disease and diverticular disease, where the fistula, rather than being in the posterior midline, usually is to one of the vaginal fornices. These can best be inspected using a rigid sigmoidoscope in the vagina.

The predominant symptom is of passage of stool or flatus vaginally. Whereas it is usually wise to accept the likely presence of a RVF based on the history, women can sometimes be mistaken, particularly when an anterior internal sphincter injury results in flatus blowing across the vaginal introitus causing an abnormal sensation often interpreted as a fistula.

The commonest cause of rectovaginal fistula is obstetric injury. The fistula usually occurs secondary to a third or fourth degree tear which latter is sustained during approximately 5% of labours. One to two percent of these will go on to suffer a rectovaginal fistula (207). A large study of women undergoing vaginal delivery found 0.1% developed a rectovaginal fistula (208). Injury to the rectovaginal septum in its lowest part is synonymous with an anal sphincter injury, in its upper part with a rectocele, and to both parts with perforation of the central portion with a rectovaginal fistula.
Another frequent cause of RVF is Crohn’s disease. In a population based-survey of Crohn’s disease patients, 35% were found to have fistulae, 9% of whom had a rectovaginal fistula (3), which appeared at a mean age of 34 years (209). A larger study of 886 patients revealed that rectovaginal fistulae may occur in up to 10% of patients with Crohn’s disease (209). RVF is more common in association with colonic (23%) than small bowel (3.5%) Crohn’s disease (210;211).

Surgery for rectovaginal fistula is difficult and success rates are modest, particularly in Crohn’s disease. Recurrence, a permanent stoma, dyspareunia, anal stenosis and incontinence may all ensue.

The outcome of repair is uncertain. Some reports have suggested success in 50% after one operation rising to 80% after many (224;225). Others report similar results in Crohn’s disease (226;228) and non-CD patients (227). In one recent Crohn’s disease series advancement flaps fared badly; proctectomy was required in 14-22% of patients. Success rates were slightly higher in non-CD patients. A high body mass index (BMI) and multiple repairs were associated with failure. Perhaps surprisingly, quality of life was no different whether the RVF was successfully repaired or not. Less than half the patients were sexually active at follow-up and of these a quarter suffered from dyspareunia.

Studies of various types of transanal rectal advancement flaps (TRAF) have been published, usually containing few patients with varied aetiology. Success rates range from 43% to 92% for ‘above down’ flaps (141;225;228;236;237), around 70% for ‘from below up’ (sliding skin) flaps (242), and 60% to 75% for sleeve advancement (240;241). Pliable tissues are required, so Crohn’s disease or extensive scarring from sepsis or previous radiation may preclude these techniques. Adjuncts such as levatorplasty or Martius flaps may be used to bolster repairs. One study reported a 75% success rate with Martius flaps in 8 women with RVF or suprasphincteric anal fistulae (246). Similar rates have been reported by others (244;245;247).

Many surgeons advocate closure approached from the high pressure, rectal side, but there are advocates for a transvaginal approach, access being easier. A systematic review has found little difference in healing rate between either approach (260). Several studies have shown no advantage to defunctioning (235;259;261;282).
As with high anal fistulae, glues, plugs and bioprosthetic sheets are intuitively attractive to some, but numbers are few and experience limited (Table 14).

Table 14 - Studies of sphincter saving infill materials in RVF repair

<table>
<thead>
<tr>
<th>Technique</th>
<th>Study</th>
<th>Year</th>
<th>Success rate in RVF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fistula glue</td>
<td>Venkatesh (146)</td>
<td>1999</td>
<td>6/8 (75%)</td>
</tr>
<tr>
<td></td>
<td>Cintron (147)</td>
<td>2000</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td></td>
<td>Loungnarath (151)</td>
<td>2004</td>
<td>1/3 (33%)</td>
</tr>
<tr>
<td></td>
<td>Singer (152)</td>
<td>2005</td>
<td>0/1 (0%)</td>
</tr>
<tr>
<td></td>
<td>Vitton (143)</td>
<td>2005</td>
<td>3/4 (75%)</td>
</tr>
<tr>
<td></td>
<td>Grimaud (144)</td>
<td>2010</td>
<td>0/3 (0%)</td>
</tr>
<tr>
<td></td>
<td>de Parades (154)</td>
<td>2010</td>
<td>0/5 (0%)</td>
</tr>
<tr>
<td>Fistula plug</td>
<td>Ellis (249)</td>
<td>2008</td>
<td>6/7 (86%)</td>
</tr>
<tr>
<td></td>
<td>Thekkankattil (283)</td>
<td>2008</td>
<td>1/7 (14%)</td>
</tr>
<tr>
<td></td>
<td>Gonsalves (251)</td>
<td>2009</td>
<td>3/5 (40%)</td>
</tr>
<tr>
<td>Bioprosthetic interposition</td>
<td>Ellis (249)</td>
<td>2008</td>
<td>22/27 (81%)</td>
</tr>
<tr>
<td></td>
<td>Schwandner (159)</td>
<td>2009</td>
<td>19/21 (90%)</td>
</tr>
</tbody>
</table>

Muscle transposition might seem like using a sledgehammer to crack a walnut, but RVF surgery is difficult and recurrence is frequent. Gracilis, having only a vestigial function and a convenient axial blood supply, has been favoured over other thigh and buttock muscles. Wexner and colleagues (2008) reported success in 5 of 6 non-Crohn’s RVFs treated with gracilis muscle interposition but just 3 of 9 with Crohn’s disease (254). Further small series in the last 2 years have reported similar findings, often in recurrent fistulae (255-257), although Furst and colleagues reported a high success rate in their series of Crohn’s patients.

In Crohn’s patients, diversion and proctectomy are sometimes required. Proctitis increases the chance of a patient with perianal Crohn’s disease requiring proctectomy, as does anal stenosis. Proctectomy rates in patients with rectovaginal
fistula vary from 20% to 60% (131;133;229;230), with some decline in recent years, perhaps in response to improved medical management.

Besides use for permanent diversion, a stoma may be used to defunction the perineal area with the hope that future reversal might become possible. A small study by Yamamoto and colleagues reported 6 cases of RVF where early improvement in 4 was followed by relapse within three years and ultimately proctectomy in all 6 cases (34).

A large study from the Cleveland Clinic published in 2010 retrospectively examined 125 patients treated for RVF of various aetiologies. Over a 10 year period, 184 operations were performed with an initial success rate of 57% that rose to 87% of patients after a mean of 1.5 operations (235). Smoking and the presence of Crohn’s disease were associated with a higher chance of failure. Only 34% underwent temporary faecal diversion and this conferred no benefit. Gracilis interposition was their most successful procedure.

Variable responses to medical therapies for rectovaginal fistulae in patients with Crohn’s disease have been reported. There is only limited evidence of response of rectovaginal fistulae to cyclosporine, azathioprine or 6-mercaptopurine (94;231;284;285) and the response is not maintained in most patients. The anti-TNF therapy, infliximab, allowed closure of 61% of rectovaginal fistulae when infliximab was given at weeks 0, 2 and 6 in the Accent II trial (A Crohn’s Disease Clinical Trial Evaluating Infliximab in New Long-term Treatment Regimen in Patients with Fistulising Crohn’s Disease) and infliximab provided a longer duration of closure than placebo when assessed by history of symptoms typical of draining rectovaginal fistulae or where possible physical examination (233).

One surgeon’s referral practice and outcomes over a 6 year period are reported below.
4.1.3 Hypothesis and Aims

4.1.3.1 Hypotheses

- The anatomy, duration and aetiology of rectovaginal fistulae as well as age and smoking status influence surgical outcome;
- The type of surgery carried out on rectovaginal fistulae influences outcome;
- The presence of a defunctioning stoma influences outcome;

4.1.3.2 Aims

- To describe the surgery on patients with rectovaginal fistulae under a single surgeon at a tertiary referral centre over a five year period;
- To describe the factors influencing surgical outcome in these patients;

4.1.4 Methods

4.1.4.1 Patient identification

Patients were identified from the operation booking diary and surgical logs, clinic letters (available from 2006 onwards), the CIS system and the ‘medsecs’ system drive containing available data from before that point. Any patient undergoing surgery for a rectovaginal fistula by Professor RKS Phillips during the study period (2003-2008) was identified and their clinical notes drawn.

4.1.4.2 Review of records

A thorough review of the patients’ clinical notes was undertaken with examination of the clinical notes, clinic correspondence, operation records and examination results performed. Computer records of imaging tests or other investigations were also accessed where relevant.

4.1.4.3 Database and statistics

Data were entered into a database on Microsoft Excel 2003™. The Stata software package - version 9.2 (StataCorp LP, College Station, Texas, USA) was used for performing all appropriate statistical analyses. Data were compared using Fisher’s exact test, the unpaired t-test and the Mann-Whitney test as appropriate.
4.1.4.4 Surgical approach

In outpatients, the mainstay of diagnosis was clinical. Examination of the vagina with a rigid sigmoidoscope, permitted fistulae to the fornices or the posterior midline to be seen (they are rarely present anywhere else), the latter aided by using a bevelled proctoscope in the vagina, such as a Graham-Anderson. Anal ultrasound was performed in all patients to assess the state of the anal sphincters. MRI was not found to be useful as the tracks, being usually epithelialised, show no enhanced signal. Clinical assessment of perineal descent and internal intussusception completed the examination.

Perineal descent can be checked by placing the thumb on the ischial tuberosity with the patient in the left lateral position and observing descent below the imaginary line joining both tuberosities. Internal intussusception can be determined clinically by observing with a rigid sigmoidoscope a tongue of anterior wall descending on straining at rigid sigmoidoscopy, or on proctography, or in theatre using Babcock forceps.

Patients with Crohn’s disease were assessed for their overall level of sickness (unlikely to be related to any fistula and guiding any discussion towards ablative surgery) and in those otherwise well, the state of the rectum.

Selection of the appropriate surgical approach is summarised in Figure 35.
In essence, almost all patients with an intact anal sphincter on ultrasound and an anastomosis resulting in a potential 2cm+ distal cuff underwent an abdominal procedure with mucosectomy and colon/pouch pull-through. Patients without an anastomosis but with a sufficiently high rectovaginal fistula to make perineal access difficult also underwent some form of pull-through procedure.

The remaining fistulae were treated using an anal, perineal or vaginal approach. The determining questions were:

a) *Lower Injury, sphincter broken.* Did the anal ultrasound show an intact or a broken anal sphincter? If broken, then the approach was by a sphincter repair with the dissection cephalad beyond the fistula to the pouch of Douglas and both pelvic sidewalls. The fistula was divided and the rectal, high pressure part repaired from within the rectovaginal septum. An adjunct of either a levatorplasty (when the adjacent tissues were supple and easily opposable) or a Martius flap (when more rigid) was used.

b) *Lower injury, sphincter intact, perineal descent.* If the anal ultrasound showed an intact anal sphincter, then the key question was whether there was clinical
evidence of internal intussusception or perineal descent. If there was, then a transanal, ‘Delorme’s-style’ advancement flap was used, usually without a stoma being constructed at St Mark’s.

c) **Lower injury, sphincter intact, no perineal descent.** If the anal sphincter was intact and if there was also no perineal descent/internal intussusception, a ‘U’ shaped incision was made at the posterior introitus and the rectovaginal septum was entered above the anal sphincter. The dissection proceeded as for (a) above but without the perineal incision or need for sphincter repair.

The ‘Delorme’s-style’ advancement flap may be circumferential or hemi-circumferential and is akin to a Sarles’ operation for rectocele. The submucosal plane is developed almost to mid-rectal level. The fistula is divided, any epithelium is removed, and the first level, a direct repair, made. The intussuspecting circular smooth muscle wall is then drawn down and sutured caudad as the second level repair, re-enforcing the first. Tension free mucosa is then sutured caudad still, sometimes accepting a small amount of anal ectropion, as the third level of repair.

Consent for a stoma (except for ‘Delorme’s-style’ flap surgery) was usually sought and almost always given.

### 4.1.4.5 Crohn’s patient data collection

Demographic and disease specific data were collected on patients with Crohn’s disease including smoking status, family history of inflammatory bowel disease, site and duration of luminal and perianal disease and drugs used during the study period. Various Crohn’s medications were used including anti-TNF agents, steroids, antibiotics (metronidazole and ciprofloxacin), thiopurines, 5 ASAs and methotrexate.

### 4.1.5 Results

#### 4.1.5.1 Demographic and baseline disease data

Thirty-five patients underwent 50 operations over 5 years, being on average 7 cases/year. Their median age was 42 years and 83% were tertiary referrals (Figure 36) having undergone a median of 2 (range 0-6) operations before referral to St
Mark’s. The aetiology was Crohn’s disease in 10 cases, obstetric in 10, anorectal sepsis in 6, iatrogenic in 4, Bartholin’s sepsis in 3, UC in 2 and pemphigus, radiotherapy and chemotherapy in 1 patient each (Figure 37). Thirteen patients were smokers and one had peripheral vascular disease.

![Referral pattern of rectovaginal fistula study patients](image)

Figure 36 - Referral pattern of rectovaginal fistula study patients

![Aetiology of rectovaginal fistula](image)

Figure 37 - Aetiology of rectovaginal fistula study patients

### 4.1.5.2 Overall success

Two patients were lost to follow up and 19 of 33 patients were ultimately healed (58%) after a mean of 1.4 operations. This represented a success rate of 68% in non-IBD patients and a 40% rate in those with Crohn’s disease. The median time to success was 11 months (range 2.5 – 48). When loose seton patients were excluded
(as with a seton in place they cannot heal), the overall success for ‘curative’ cases was 19 of 26 (73%), being 75% of non-IBD patients and 67% of Crohn’s cases.

4.1.5.3 Abdominal operations

Eight patients including 1 with Crohn’s disease underwent abdominal operations, two with an intended permanent stoma (proctocolectomy and end ileostomy [CD], pouch excision), and 6 restorative (colonic pouch, rectal advancement, pouch advancement, Soave x 3). Two patients developed recurrence following Soave procedures, One of these underwent successful perineal repair, re-utilising a previously placed adjunctive omental flap, while the other underwent a repeat Soave that left a pinhole fistula, for which she declined further treatment. The final success rate in the whole group was 88% after a mean of 1.25 operations. Five of the 6 patients with restorative procedures had reversal of their stomas after a mean of 4 months. Complications included delayed healing in 1 perineal wound, an incisional hernia in 1 laparotomy wound, dyspareunia in one woman (present at referral), and impaired continence in a patient who arrived at St Mark’s already with a stoma.

4.1.5.4 Lower Injury, sphincter broken.

Anal ultrasound showed a significant sphincter defect and a sphincter repair with an adjunct was undertaken.

Six patients underwent nine perineal operations involving an overlapping sphincter repair, direct repair of the fistula, and an adjunct (levatorplasty 2 operations, Martius flap 5). There were 5 tertiary referrals, 3 smokers, 1 Crohn’s patient and all had a degree of tissue loss. Four had obstetric injuries and a stoma was used in all 6.

Only 2 of 6 patients (33%) healed. No redo procedures were successful. Of the 4 failures, 1 went on to successful lay open, 1 had a seton inserted, 1 was satisfied with a colostomy and 1 declined further surgery. Four stomas were later closed. No dyspareunia or anal stenosis was reported.
4.1.5.5 Lower injury, sphincter intact, perineal descent.

Anal ultrasound showed an intact anal sphincter and perineal descent/internal intussusception was present on clinical examination so a ‘Delorme’s-style’ advancement flap was undertaken.

Twelve patients underwent 13 Delorme’s-style advancement flap repairs. Ten were tertiary referrals, 4 had obstetric injuries, 3 had Crohn’s disease and 1 was of Bartholin’s sepsis origin. Six were smokers and one had peripheral vascular disease. The median length of stay was 2 days. Five patients (already) had stomas.

One patient was lost to follow-up and of the remaining 11, the initial procedure was successful in 6 (55%). One patient had a successful redo Delorme’s-style procedure, one went on to successful lay open, one had only a pinhole recurrence and wanted no further intervention, one was offered further surgery but declined a defunctioning stoma and therefore further attempts at repair, and one had a permanent seton inserted following recurrence. Overall ‘success’ in this group was 73% (8 of 11 patients) with 82% of patients satisfied with their outcome. There were three complications in the group. Difficulty in evacuation occurred in 1 patient and two patients required a short period of Hagar dilatation for temporary anal stenosis.

4.1.5.6 Lower injury, sphincter intact, no perineal descent

Anal ultrasound showed an intact anal sphincter and perineal descent/internal intussusception was absent so a ‘U’ shaped incision at the introitus with direct repair with an adjunct was undertaken.

Six patients underwent seven procedures utilising a ‘U’ shaped incision at the posterior introitus. Dissection was in the rectovaginal septum to the pouch of Douglas and both pelvic sidewalls with direct repair of the fistula and an adjunct. All were tertiary referrals; 3 had obstetric injuries, 1 had Crohn’s disease, 2 were smokers, all had stomas. Adjuncts were levatorplasty in 2, Martius flap in 3 and omentoplasty (mobilised during earlier Soave procedure) in 1.

After the initial procedure, 3 of 6 patients healed and 2 recurred with much smaller tracts, one having few symptoms and being satisfied, the other continuing to be
symptomatic but without wishing to undergo further surgery. The final patient had a second attempt via this approach, which failed, a Delorme’s-style advancement flap, that also failed, and finally successful lay open with no deterioration in continence. Five of the six patients in the group were therefore satisfied with their final outcome (83%). The stoma was reversed in all 6 at a median of 4 months (range 3-23 months). Two women later suffered dyspareunia; they had altogether undergone 4 and 6 perineal operations respectively by the end of the study period. There was no anal stenosis.

4.1.5.7 Miscellaneous operations

Seven patients underwent seton insertion. Four were Crohn’s patients on medical therapy and one patient had UC and a seton on arrival at St Mark’s which she opted to have changed to a more comfortable, low profile seton. The other two patients, with obstetric injuries, had undergone previous perineal repairs and one had anal stenosis following a Delorme’s-style advancement flap; both opted to keep a permanent loose seton. No dyspareunia was reported.

One patient underwent lay open following 5 failed attempts at repair (both before and at St Mark’s). Her stoma was reversed 4 months afterwards (23 months after creation) and, surprisingly, continence was acceptable.

4.1.5.8 Stomas

Twenty-four of thirty-five patients (67%) underwent creation of a stoma either before referral (11) or during treatment at St Mark’s (13). Sixteen of these twenty-four (67%) underwent restoration of continuity or were referred back to their local unit for the procedure.

4.1.5.9 Factors influencing outcome

The use of a stoma, smoking, tissue loss, size and duration of fistula, the presence of IBD or obstetric injury, the patient’s age, and concomitant sphincter injury were not associated with healing.

Success rates were similar whether the stoma was formed some time before an attempted repair (10 of 14 patients), or at the time of repair (5 of 7 patients).
4.1.5.10  Complications

The principle complication was recurrence. One third of patients with a defunctioning stoma never had it reversed. Four patients had new or pre-existing dyspareunia, 5 had transient anal stenosis that responded to regular Hagar anal dilatation over two months, and 3 developed wound problems following surgery. There were no deaths.

4.1.5.11  Crohn’s patients undergoing surgery

The median age of the 10 Crohn’s patients who underwent surgery was 44 (range 19-75). Eight were tertiary referrals, one had sustained an obstetric injury, one had Bartholin’s sepsis, three smoked, and one had peripheral vascular disease. Surgery performed included Delorme’s-style procedure in 3, perineal approach and sphincter repair in 1, ‘U’-shaped incision at introitus with direct repair and levatorplasty in 1, seton insertion or replacement in 4 and panproctocolectomy in 1. Of the 3 patients who underwent Delorme’s-style advancement flap surgery, all three healed with no recurrence.

Both of the patients undergoing a perineal approach had defunctioning stomas sited but suffered recurrence. Four patients had setons inserted or replaced, one of whom had a defunctioning stoma at referral to St Mark’s. Two patients were offered panproctocolectomy with end ileostomy formation and one patient underwent this procedure, the other opting to keep her seton in situ. Two patients had their stomas reversed 4 and 6 months after surgery.

Complications other than recurrence included anal stenosis in 4 patients (present in 3 at referral). Wound problems were reported by two patients; one ileostomy wound suffered delayed healing and in one patient who underwent proctocolectomy, the perineal wound required further EUA and marsupialisation.

Eight patients used medication for Crohn’s disease either before or during the study period. Four used anti-TNF agents, seven Azathioprine, six antibiotics (metronidazole and ciprofloxacin), seven steroids, two 5 ASA agents and one patient used methotrexate. None of these medications led to successful closure of the fistula in any patient although all produced some improvement in most patients. Complications of drug use included an allergic reaction to infliximab, neutropenia,
fever and suspected pneumonitis in three patients on azathioprine, nausea and diarrhoea on ciprofloxacin, low mood on steroids and nausea on methotrexate.

**4.1.6 Discussion**

Surgical results are uncertain, which means that women with few symptoms, such as flatus only vaginally and with a pinhole fistula, should be counselled to try to avoid an operation, although few will actually heed such advice, the presence of a fistula being abhorrent to most.

Given the uncertainty, it is wise to consider the possibility of failure from the outset and the woman’s likely response to that. This study, as well as others, has failed to demonstrate a benefit of a stoma. But most multiply failed cases will at some stage or other end up having an attempt at repair covered with a stoma, whatever the evidence. Among other things, a woman confronted by unsuccessful surgery may wonder whether earlier use of a stoma might not have made a difference. The fistula already being abhorrent, many women, being made aware of this possible future conundrum, will choose to have a stoma from the outset. For them, handling any future failure may be easier if they know they have ‘given their all’ when repair attempts have been made.

Many strategies are needed. Abdominal approaches are probably best when anastomotic fistulae to the vagina have a sufficient residual rectal cuff below them, permitting a pull through (or ‘Soave’-type) operation with mucosectomy and endo-anal anastomosis; success rates are also highest.

Much more difficult is when there is an anastomosis and a cuff, but the fistula actually arises below this at the dentate line (as may be the case with an ileo-anal pouch in IBD). A permanent seton may be the least bad option, permitting continual natural defecation with prevention of worsening of sepsis.

In a young and highly motivated individual with a colo-vaginal anastomotic fistula without a distal cuff to permit a standard pull-through, the Turnbull-Cutait operation may be tried (although not used here) (286), with reasonable success in highly selected cases.
There is no ‘one-size fits all’ perineal approach. It does not seem logical to raise an advancement flap while ignoring a significant underlying anal sphincter injury, nor point in damaging an intact anal sphincter by performing some sort of lay open and repair. Anal ultrasound seems key to a rational approach.

Modern technology has supplied a number of new options that were not tested in this group of patients, from glues through fistula plugs to supporting biological membranes. On the whole, fistulae to the vagina are short and frequently epithelialised, a group not faring well with glues. Fistula plugs are simple to use but published experience is limited (Table 14). The rectovaginal septum is very thin in many patients with a rectovaginal fistula, making the idea of additional support with a biological membrane attractive to some surgeons (Table 14) yet abhorrent to other surgeons contemplating an avascular foreign body in a contaminated wound.
4.2 The clinical course of rectovaginal fistula in Crohn’s disease in the infliximab era

4.2.1 Abstract

Introduction
Crohn’s disease related rectovaginal fistulae (RVF) are common and very unpleasant. They frequently recur after both medical and surgical treatment. There are limited data describing the natural history of Crohn’s RVF in the infliximab era.

Methods
A retrospective review of patients with Crohn’s disease and rectovaginal fistula seen at St Mark’s hospital between 2000 and 2008 was carried out. Demographic, clinical, surgical and outcome data were obtained.

Results
Crohn’s rectovaginal fistulae were healed in 35% (13/37) of women, following multiple surgical and medical interventions. Surgery healed half (6/13) of these fistula with others palliated by seton insertion or defunctioning stomas. Only half (6/13) the stomas created were reversed. Most medical interventions induced improvement in patients’ symptoms but robust healing occurred in only 7 women and side effects, although mostly minor, were common.

Discussion
Rectovaginal fistulae still represent a difficult problem for clinicians and are very unpleasant for their sufferers. Further research in techniques already identified may produce improved outcomes in the near future and should be pursued.

4.2.2 Introduction
Rectovaginal fistula (RVF) occur in up to 10% of women with Crohn’s disease and are extremely unpleasant (209). Predominant symptoms include the passage of wind, discharge or stool from the vagina. They are more common in colonic (23%) than small bowel (3.5%) disease (210;241).
Surgical repair is difficult and recent studies report success rates of around 40-60% at first repair rising to 60-80% after multiple attempts (235;260;261). Crohn’s RVF were more likely to recur than those of obstetric origin (235). Immunomodulator use may be associated with success and smoking with failure (261). Diversion and proctectomy are sometimes required. Proctectomy rates have fallen in recent years, perhaps due to improved medical treatment, but rates in excess of 20% have been reported (13;131;133;229;230).

Medical treatment of Crohn’s RVF is also of limited efficacy although infliximab shows promise in producing robust remission in some patients. Thiopurines and cyclosporine produce a limited and brittle response in most patients (94;231;284;285). The Accent II trial (A Crohn’s Disease Clinical Trial Evaluating Infliximab in New Long-term Treatment Regimen in Patients with Fistulising Crohn’s Disease) examined maintenance infliximab use and included a subset of RVF patients who experienced remission in 60% of cases with a longer duration of cessation of symptoms than placebo (233).

Combined infliximab and surgical therapy has also shown promising results (234). A study of 51 patients undergoing staged or definitive surgery for Crohn’s rectovaginal fistula with an arm receiving preoperative infliximab induction and a second without, found that 11 of 25 (44%) surgery only patients healed compared to 16 of 26 (62%) in the infliximab + surgery group although the difference was not statistically significant. Time to healing was significantly reduced in the infliximab group, however, from 8.1 to 2.9 months, from which it is reasonable to infer that perhaps a larger group might have demonstrated improved healing with infliximab. Interestingly, all patients who underwent seton insertion before definitive surgery healed. Faecal diversion did not improve remission rates. Proctectomy was undertaken in 36% of the surgery only group and 19% of the infliximab plus surgery group, although again the difference was not significant (p=0.25).

A previous report on the natural history of RVF in Crohn’s disease treated at St Mark’s Hospital during the pre-infliximab era found that of 27 patients followed for over 5 years, 22 (81%) were healed at final follow up, the majority following surgical intervention, after a median 26 months of treatment. Eight of these healed patients (30%) required proctectomy. Only 3 of 27 were healed by medical treatment alone (13).
4.2.3 Aims

We aimed to examine the natural history of Crohn’s RVF in the era of anti-TNF agents and identify the outcome that these women currently experience.

4.2.4 Methods

Patients with Crohn’s disease and a rectovaginal fistula, seen at St Mark’s hospital between 2000 and 2008, were identified using computerised patient correspondence, operating diaries and the Clinical Information System. A retrospective review of their notes was carried out and demographic, clinical, surgical and outcome data were obtained.

4.2.5 Results

4.2.5.1 Demographic and baseline disease data

Thirty-seven women with a median age of 44 years (range 19-75) were treated for rectovaginal fistula associated with Crohn’s disease at St Mark’s Hospital between 2000 and 2008. Twelve women were smokers and four had peripheral vascular disease but none had diabetes, HIV or other immunosuppressive diseases. The majority of the fistula were small although 3 women had large (>1cm diameter) openings. Thirteen (35%) had a family history of inflammatory bowel disease.

The median duration of luminal and perianal Crohn’s disease were 14.5 and 11 years (range 3-45 and 3-45 years) respectively. Disease was colonic in 58%, ileo-colonic in 30%, terminal ileal in 9% and restricted to the perineum in 3%.

Operations to repair or palliate the RVF or associated anal fistula had been undertaken in 14 women before referral and included advancement flap repair, seton insertion, fistula glue and fistulotomy. Nine patients had undergone segmental colectomy for luminal Crohn’s disease. Five women had a defunctioning stoma in situ on referral to St Mark’s.
4.2.5.2 Overall success

During the study period, 23 women underwent 56 operations (mean 2.4 operations per patient) for their RVF or the sequelae of RVF surgery. Thirty-two were treated with medications including infliximab (14), thiopurines (28), antibiotics (28), steroids (27), 5 ASA agents (13), Methotrexate (8), Thalidomide (2), Mycophenylate (1) and Adalimumab (1).

Success was achieved in 13 (35%) patients. Seven were cured of their fistula during medical treatment and six following surgical intervention. A further 3 patients were satisfied with the palliation achieved through seton insertion. One patient ultimately underwent proctectomy and a further 3 were offered the procedure. Thirteen patients underwent stoma formation during the course of their treatment and of these 6 either had the stoma reversed or were awaiting reversal.

Most patients had multiple medical interventions in addition to surgery, including several different drugs sometimes in combination, so it is difficult to ascribe benefit to any individual agent. However, 7 patients went into clinical remission during drug treatment including 2 patients on infliximab alone, 1 on azathioprine alone, 1 taking azathioprine with metronidazole, 1 on methotrexate alone, 1 on salazopyrin alone and 1 on prednisolone and metronidazole. Of 14 patients treated with infliximab, 4 of 14 (28%) achieved remission, but 2 of these patients suffered a recurrence. Six of fourteen (47%) infliximab treated patients, some on concomitant immunomodulators, responded without achieving full remission, at a median of 4 weeks (range 1-8 weeks). Of 28 patients treated with thiopurines, 3 of 28 (11%) achieved remission, 17 of 28 (60%) responded.

In addition to those patients experiencing remission, improvement in symptoms occurred in between 40% and 60% of patients at some point during their treatment.

4.2.5.3 Complications

Adverse events, although mostly minor, were common, particularly with infliximab (43%), thiopurines (54%) and steroids (26%). Infliximab was responsible for 3 anaphylactoid reactions, headaches in 1 patient, palpitations in another and reactivation of pulmonary TB in a third. Patients on thiopurines experienced
neutropenia in 4 cases, fever symptoms in 5, nausea in 3 and headaches, pneumonitis and septicemia in 1 each. Steroids produced osteoporosis in 4 women, low mood in 1, erratic blood sugar levels in 1 and nausea in 1. Antibiotics led to nausea in 5 patients. 5 ASA agents caused nausea in 2 patients and methotrexate produced a flu-like illness in 1 patient and nausea in another.

4.2.6 Discussion

Women with Crohn’s rectovaginal fistula achieved overall success in around a third of cases, following multiple surgical and medical interventions. Surgery healed half of these fistula and others were palliated by seton insertion or defunctioning stomas. Only half the stomas created were reversed. Most medical interventions induced improvement in the patient’s symptoms but robust healing occurred in only 7 women and side effects, although mostly minor, were common.

In this retrospective study, patients were followed for the duration of their care in the colorectal and gastroenterological departments during the study period and thereafter. Patients were followed, on average, for 5.6 years (range 7-138 months) during which time they may have undergone several medical and/or surgical interventions. It is impossible to determine the exact timings of each medical intervention, whether they overlapped and what period of observation followed them. Rather than demonstrating the efficacy of any individual intervention, the value of this study is in describing the natural history of women with Crohn’s rectovaginal fistulae experiencing standard care at a tertiary centre, and, in particular, their outcome which can assist in informing patients of their likely disease course.

At first glance, the results achieved by Bell and colleagues a decade ago seem better than those presented here; 81% (22 of 27) healed at final follow up vs. 35% (13 of 37). Bell’s successes came from proctectomy in more than a third of cases and medical treatment alone in only 3. Our results point to a different pattern of treatment. Medical treatment alone was responsible for healing in 7 of 37 women and around half gained significant benefit from medical treatment. Only 1 patient underwent proctectomy and several achieved adequate symptom control from reparative surgery or medical treatment such that they declined further surgical intervention. It may be that improved medical treatment and palliative surgery protect
women from ablative surgery and a permanent stoma; lower healing rates in this study do not necessarily correlate with patient dissatisfaction or an unsuccessful outcome.

In fact, proctectomy is considered by many to be a failure rather than a success in the management of perianal Crohn's disease and this is, perhaps, increasingly the case. Whilst there is certainly a place for proctectomy in patients who are blighted by disease and in whom a great improvement in quality of life may follow the procedure, we might hope that a similar improvement may be found without resorting to ablative surgery in most patients. With this in mind, our success rate falls to 32% and Bell's to 52%.

The present study ran during the first decade of increased infliximab use and the use of infliximab has now changed (from episodic to scheduled use). Anti-TNF therapy, surgery and the two combined have the greatest efficacy in the literature at the present time. Combining anti-TNF therapy and surgery shows great promise but high quality evidence is lacking.

Crohn's rectovaginal fistulae still represent a difficult problem for gastroenterologists and coloproctologists as well as their sufferers. Further research examining combined medical + surgical treatments, such as augmenting surgery with preoperative infliximab induction and the benefits of faecal diversion and preoperative seton use, may produce improved outcomes and can be undertaken in the near future based on the early work in this area if adequate interest and funding can be found.

4.3 Limitations

These studies examining rectovaginal fistulae suffer from several factors which limit their applicability and usefulness. The patients studied are a complex group of mainly tertiary patients with a long duration of Crohn's disease and/or rectovaginal fistulation behind them.

We have studied them retrospectively, capturing them through imperfect databases and reviewing their notes which may not describe all events with complete and particularly temporal accuracy. This is especially important when considering which
treatments led to improvement or healing in our patients and so conclusions around these factors were not drawn.

Defining healing in this group is also difficult. The absence of symptoms is taken to be synonymous with healing unless a defect is found on clinical examination. Imaging is rarely of use here. Several different clinicians were involved with the medical patients and exactly how they individually defined healing cannot be known.

The patients in these studies were heterogeneous, particularly with regard to fistula anatomy, and it is this difference which enabled me to discuss the various options for surgery but which also limits the sample size within each group. Comparisons between techniques or with others, cannot be drawn. As a single surgeon’s experience, the applicability of these results outside our unit is hard to define.

Patients were mostly seen as tertiary referrals and may have also seen secondary providers in either gastroenterology or surgery. Any intervention undertaken in this setting can only be gleaned from correspondence which may be deficient. Patients who experience recurrence after referral back to secondary care may not represent to St Mark’s so some failures may be missed.
5 Long-term MRI-guided combined anti-TNFα and thiopurine therapy for Crohn’s perianal fistulae

5.1 Abstract

Background
Anti-tumour necrosis factor therapy heals many Crohn’s anal fistulae clinically but the rate, extent and durability of deep tissue healing, and factors influencing long term outcome, are unknown.

Methods
Consecutive patients with Crohn’s disease-related perianal (anal, rectovaginal, anolabial) fistulae treated with infliximab or adalimumab were monitored prospectively both clinically and radiologically using MRI.

Results
Forty-one consecutive patients with Crohn’s-related perianal fistulae were treated with infliximab (n=32) or adalimumab (n=9; following infliximab failure) in combination with a thiopurine (unless intolerant). Fifty-eight percent of all patients, comprising 66 percent and 43 percent of infliximab and adalimumab treated patients respectively, demonstrated remission or response at 3 years. Thirty-three percent of infliximab treated patients maintained clinical remission at 3 years. Radiological healing lagged behind clinical remission by a median of 12 months. The likelihood of clinical remission at any time was five times greater in patients who had early clinical response within 6 weeks than those without. The number of fistula tracts was associated with reduced clinical remission. All patients who achieved radiological healing maintained healing on infliximab treatment, whilst only 43% maintained healing after cessation of anti-TNF therapy.

Conclusions
Combination anti-TNF and thiopurine therapy provides sustained benefit in patients with perianal Crohn’s fistula. Early clinical response is associated with subsequent clinical remission. Radiological healing is slower than clinical healing. Radiologically healed fistula tracts maintain healing on infliximab but can recur after cessation of therapy.
5.2 Introduction

Anal fistulae occur in approximately one third of patients with Crohn’s disease (2), represent a distinct phenotype of disease (4), cause significant morbidity, and are associated with a severe and disabling disease course requiring multiple medical and surgical interventions (2;9;24).

Antibiotics (metronidazole and ciprofloxacin) and immunosuppressive drugs (thiopurines) temporarily reduce symptoms but do not lead to radiological healing of anal fistulae (14;17;25). The anti-TNFα therapies, infliximab and adalimumab, have been shown to maintain clinical remission in approximately a third of patients after one year of treatment (4;18) with reduced hospitalisations and operations (96) but recurrence of fistulae can occur during treatment and at a rate of 66% 1 year after cessation of therapy (5;18).

Clinical remission (closure of the external opening, sustained for four weeks and resistant to gentle finger pressure, in all tracts open at baseline) does not reflect true deep tissue healing as demonstrated with MRI (13;15;20;28) and EUS (20). Early cessation of treatment, after clinical healing but before complete eradication of the tract, may be responsible for many recurrences.

A cooperative approach between physicians, surgeons and radiologists is needed to manage Crohn’s anal fistulae and algorithms describing such an approach have been published (287). Adequate drainage before treatment with anti-TNF agents improves outcome by increasing initial response rate by almost a fifth (134). A non-randomised study which examined the impact of preoperative infliximab on the outcome of surgical treatment of Crohn’s anal fistulae found that time to healing was reduced by half and in some surgical subgroups healing was more frequent in the infliximab group (138). Radiology-guided surgical intervention before and during infliximab treatment has produced high remission rates (7;22;26) although these studies were small and radiological proof of healing was not always obtained.

St Mark’s Hospital has previously published data on 34 patients with Crohn’s anal fistulae treated with infliximab, adalimumab or thalidomide, assessed both clinically and using serial MRI scanning to determine deep tissue healing of the fistula tract (80). At 1 year, clinical remission was seen in 53% of infliximab treated patients, and 29% of adalimumab treated patients (adalimumab was the second anti-TNF therapy
used). Radiological healing was seen in 28% of all anti-TNF treated patients. In that study, radiologically healed patients remained healed on maintenance treatment. The length of fistula history and fistula complexity did not influence healing. Proctitis reduced the clinical benefit of anti-TNF therapy; response or remission was seen in 60% with proctitis as opposed to 89% of those without proctitis (P = 0.054).

A recent study showed that MRI improvement coincided with clinical and endoscopic improvement in 50% of patients treated with infliximab at a median follow up of 36 weeks (288). Clinical benefit was seen in more than three quarters of patients but at longer follow up (median 94 weeks) only 1 of 13 patients had no fistula tracts seen on MRI.

Another study, using EUS to guide combination therapy (using antibiotics, thiopurines and anti-TNF agents), demonstrated clinical remission in 76% of patients and radiological healing in 52% at a median of 68 weeks, with a lag time of 3 months between clinical and radiological healing (20).

Besides these studies, maintenance treatment with anti-TNF drugs monitored radiologically beyond 1 year has not been adequately described and studies using radiological assessment of follow up as an end point are lacking. Several groups have published such studies using the van Assche scoring system (13;19;28). This considers the number of fistula tracts, their anatomy, extensions, collections, proctitis and hyperintensity on T2-weighted images. One study found that it was insensitive to change in some patients (13) and another noted that T2 hyperintensity was the factor most clearly associated with clinical benefit (289).

The long term follow up to 3 years in the earlier-reported prospective cohort described above (80), expanded to 41 through consecutive recruitment, of patients treated with infliximab or adalimumab for perianal Crohn’s fistulae will next be reported. Patients have been assessed longitudinally both clinically and radiologically.
5.3 Hypothesis and Aims

5.3.1 Hypotheses

- Infliximab and Adalimumab maintenance provide long term remission in the majority of Crohn’s anal and rectovaginal fistula patients;
- Healing as diagnosed on MRI occurs later than healing as diagnosed clinically;
- Radiologically healed patients can safely stop maintenance treatment.

5.3.2 Aims

The aims were to (i) optimise treatment of Crohn’s perianal fistulae using surgical intervention and a combination of anti-TNF and thiopurine therapy under MRI guidance according to a previously published treatment algorithm; (ii) determine the rate and extent of healing both clinically and radiologically; (iii) characterise the time lag between clinical and radiological healing; (iv) determine whether an early clinical response relates to subsequent clinical or radiological healing; and (v) determine the effect of stopping anti-TNF therapy, in clinically and radiologically healed fistulae, on the subsequent disease course.

5.4 Methods

5.4.1 Patients

Consecutive patients seen at St Mark’s Hospital, London and commenced on combined anti-TNF and thiopurine therapy between 2006 and 2008 for Crohn’s disease with perianal (anal, ano or rectovaginal or labial) fistulae according to our initial study protocol (Figure 38) were included. The diagnosis of CD was confirmed previously on endoscopy, radiology or histology.

Patients with symptomatic luminal obstruction and other major comorbidities were excluded. Patients with undrained perianal sepsis were included only after adequate drainage had been achieved.
5.4.2 Study design

This was a prospective observational cohort interventional study, which took place in a specialist centre receiving primary and secondary referrals. Standard clinical care, defined in an algorithm (Figure 38), was followed for all patients.

Baseline data including demographic information, luminal disease location and duration, the anatomy of draining external openings, Crohn’s Disease Activity Index (CDAI), Perianal Disease Activity Index (PDAI) (290) and the presence of proctitis were recorded. Examination under anaesthesia with abscess drainage and seton insertion was performed if fistulae were complex or if there was undrained sepsis on clinical examination or MRI scanning.

Figure 38 - Initial study algorithm for treatment of Crohn’s perianal fistulae used in this cohort of 41 patients started in 2006
5.4.3 Radiology

An MRI scan was performed in all patients at baseline according to a standardised fistula protocol, using a Siemens Avanto 1.5T MR scanner (Siemens, Erlangen, Germany). High resolution sagittal, axial and coronal T2 sequences with fat saturation (axial and coronal perpendicular and parallel to the plane of the anal canal respectively) were obtained, (TR / TE, 8670 / 96; Matrix 256 x 512; field of view, 300; flip angle, 150, slice thickness 4 mm; two excitations with IPAT factor of 2).

Patients were scanned supine using a phased-array surface coil. The long axis of the anal canal, used to orientate the axial and coronal planes, was identified using a midline sagittal localising scan.

On this baseline MRI scan the number, thickness or volume, and complexity of fistula tracts, anatomical classification, secondary extensions and hyper-intensity on T2 weighted images were determined.

5.4.4 Fistula complexity

Simple fistulae were defined as superficial fistulae, intersphincteric or trans-sphincteric fistulae with one primary tract and no additional tracts and / or extensions or abscesses and ano-labial fistulae. Complex fistulae were defined as intersphincteric or trans-sphincteric fistulae with extensions or abscesses, suprasphincteric, extrasphincteric and recto-vaginal fistulae (291).

5.4.5 Treatment

Patients received infliximab treatment (if anti-TNF agent naïve) or adalimumab treatment, if infliximab therapy had failed previously due to lack of efficacy or adverse reactions. Standard induction courses (infliximab: 5mg/kg at weeks 0, 2 and 6; adalimumab: 160mg at week 0, 80mg at week 2) were followed by maintenance treatment (infliximab: 5mg/kg 8 weekly; adalimumab: 40mg every 2 weeks) until week 22 at which point patients were classified as a clinical responder (who continued maintenance treatment) or a clinical non-responder (who were offered a
change to adalimumab if initially on infliximab). Patients were commenced on azathioprine at a dose of 2-2.5mg/kg/day or, if azathioprine intolerant, on 6-mercaptopurine 1-1.5mg/kg/day from week 0. Episodes of acute perianal abscess were treated surgically and often with antibiotics (metronidazole 400mg po tds and/or ciprofloxacin 500mg po bd). However, maintenance antibiotics were not used due to the risk of adverse effects and lack of supportive evidence in long term use.

5.4.6 Clinical follow-up

Clinical follow up took place at weeks 2, 6, 22, 34 and 52, then 6 monthly to two years and annually thereafter. MRI scanning was undertaken at the start of treatment and at the same intervals as clinical follow up from week 34 onwards. Any clinical or symptomatic improvement noted between weeks 2 and 6 (or thereafter) prompted seton removal, with the aim of achieving fistula healing with anti TNF agents.

At clinical follow up visits an assessment of the number of external openings, fistula healing, development of new external openings, degree of fistula drainage and pain was made. CDAI, PDAI and Inflammatory Bowel Disease Questionnaires (
Appendix 2) were completed by the patients during the initial 18 months of treatment.

Clinical remission was defined as cessation of drainage on history, absence of perianal drainage to gentle finger compression of all external openings and surrounding tissues, and absence of spontaneous drainage between two consecutive visits. Clinical response was defined as (i) closure of ≥50% in the number of externally draining fistulae, or (ii) marked reduction in drainage of fistula(s) together with less pain and induration as reported by the patient, sustained for at least two consecutive visits compared with baseline evaluation.

If a patient did not respond clinically, required surgical intervention or discontinued study medication due to lack of efficacy, they were deemed to have had a lack of response.

5.4.7 Radiological follow-up

MRI scanning was undertaken at 6 monthly intervals for the first 2 years and annually thereafter, as part of routine clinical care of these patients. Each MRI was reviewed in consensus by two experienced gastrointestinal radiologists. The radiologists were blinded to both treatment arm and outcome. Each subsequent MRI scan was compared to both the baseline scan and the previous scan and classified as healed, improved, unchanged or worse.

Radiological healing was defined as complete resolution of the previous high signal tract or a subtle, narrow calibre intermediate signal residual tract (frequently with low signal borders resembling a train track appearance). Radiological improvement was defined as reduction in the number and/or volume (with an estimated reduction of 10% or more) in the fistula tracts or abscesses. No radiological change was defined as a similar number of tracts and volume (any reduction less than 10%). Worsening of the radiological appearance was defined as the development of new tracts or collections, or an increase in the volume (of at least 10%) of any previous cavities or fistula tracts.

Assessment of around 3/4 of these patients for the first 18 months was undertaken and published by other members of the group (Ng). All other clinical and radiological assessment (taken out to up to 4.5 years) forms part of this thesis.
5.4.8 Statistical Analysis

The Stata software package - version 9.2 (StataCorp LP, Texas, USA) was used for performing all appropriate statistical analyses. Continuous variables were summarised by the medians and inter-quartile range (IQR) due to the skewed distribution of most variables. The number and percentage of subjects in each category were used for categorical variables. The Chi-square test was used to compare categorical outcomes between groups. Additionally, logistic regression was used to jointly examine the effect of several factors upon the binary categorical outcomes. The size of effects of these analyses were given in the form of odds ratios.

Differences in continuous outcomes between independent groups were evaluated using the Mann-Whitney U test to compare between two groups, or the Kruskal-Wallis test to compare between more than two groups. These non-parametric tests were used due to non-normal distribution of most outcomes. For differences between paired groups the Wilcoxon signed rank test was used. Cox regression or multilevel linear regression were used for the detection of any factors predicting therapy outcome. Linear regression was used for continuous outcomes, whilst Cox regression was used for survival outcomes. A P value of 0.05 was considered significant.

5.4.9 Ethical considerations

All patients were treated on the basis of clinical need according to standard clinical care at our institution, on an open label basis and according to licensed or published regimes. Ethics Committee approval was therefore not sought, but the study was approved by the hospital Drugs and Therapeutic Committee and Medical Director. All patients were treated only after full and informed consent.

5.5 Results

5.5.1 Baseline data

Forty-one Crohn’s patients with draining anal or rectovaginal fistulae were included in the study. Their baseline characteristics are shown in Table 15.
Table 15 - Baseline characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (median, range)</td>
<td>36</td>
</tr>
<tr>
<td>Male:female</td>
<td>16:25</td>
</tr>
<tr>
<td>Disease duration in years (median, range)</td>
<td>10</td>
</tr>
<tr>
<td>Disease location</td>
<td></td>
</tr>
<tr>
<td>L1</td>
<td>9</td>
</tr>
<tr>
<td>L2</td>
<td>21</td>
</tr>
<tr>
<td>L3</td>
<td>8</td>
</tr>
<tr>
<td>L4 only</td>
<td>2</td>
</tr>
<tr>
<td>p only</td>
<td>1</td>
</tr>
<tr>
<td>Fistula duration in years (median, range)</td>
<td>6</td>
</tr>
<tr>
<td>Fistula location</td>
<td></td>
</tr>
<tr>
<td>anal</td>
<td>31</td>
</tr>
<tr>
<td>vaginal</td>
<td>8</td>
</tr>
<tr>
<td>both</td>
<td>2</td>
</tr>
<tr>
<td>Number of fistulae (median, range)</td>
<td>1</td>
</tr>
<tr>
<td>Concurrent immunomodulator</td>
<td></td>
</tr>
<tr>
<td>Azathioprine</td>
<td>21</td>
</tr>
<tr>
<td>6-Mercaptopurine</td>
<td>4</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>11</td>
</tr>
<tr>
<td>Non or ex-smoker</td>
<td>28</td>
</tr>
</tbody>
</table>

Thirty-two patients were commenced on infliximab and nine on adalimumab (after failing infliximab treatment before the study began). Seven of the infliximab patients were changed to adalimumab during the study due to loss of response or an adverse reaction to infliximab.

Median follow up was 2.5 years. At the time of this report, 13 infliximab treated patients and 7 adalimumab treated patients had reached 3 or more years of follow up.

5.5.2 Clinical and radiological outcome up to three years

Fifty-eight percent of all patients derived clinical benefit (remission or response) up to the end of follow-up, including 66% and 43% of infliximab and adalimumab treated patients respectively. Adalimumab treated patients had previously failed infliximab therapy with adalimumab used as second line agent.

For those patients who had reached a minimum of 3 years follow-up, 21% were in remission and 37% had achieved a response. On infliximab, 33% were in remission and 33% had achieved response. On adalimumab, none were in remission and 43% had achieved response.
Tables 2-4 show the clinical and radiological results for infliximab (Table 16), adalimumab (Table 17) and all anti-TNF treated patients (Table 18).

Table 16 - Clinical and radiological outcomes for combination treatment with infliximab and thiopurine over three years

<table>
<thead>
<tr>
<th>Infliximab</th>
<th>6 months n=32</th>
<th>12 months n=32</th>
<th>18 months n=26</th>
<th>24 months n=22</th>
<th>36 months n=12</th>
</tr>
</thead>
<tbody>
<tr>
<td>clinical remission</td>
<td>31%</td>
<td>44%</td>
<td>35%</td>
<td>36%</td>
<td>33%</td>
</tr>
<tr>
<td>radiological healing</td>
<td>25%</td>
<td>25%</td>
<td>23%</td>
<td>27%</td>
<td>42%</td>
</tr>
<tr>
<td>clinical response</td>
<td>38%</td>
<td>38%</td>
<td>38%</td>
<td>32%</td>
<td>33%</td>
</tr>
<tr>
<td>radiological improvement</td>
<td>38%</td>
<td>41%</td>
<td>27%</td>
<td>9%</td>
<td>8%</td>
</tr>
<tr>
<td>clinical loss of response</td>
<td>16%</td>
<td>13%</td>
<td>15%</td>
<td>18%</td>
<td>33%</td>
</tr>
<tr>
<td>radiological loss of response</td>
<td>22%</td>
<td>16%</td>
<td>19%</td>
<td>27%</td>
<td>42%</td>
</tr>
</tbody>
</table>

Table 17 - Clinical and radiological outcomes for combination treatment with adalimumab and thiopurine (previous infliximab failure) over three years

<table>
<thead>
<tr>
<th>Adalimumab</th>
<th>6 months n=11</th>
<th>12 months n=10</th>
<th>18 months n=9</th>
<th>24 months n=9</th>
<th>36 months n=7</th>
</tr>
</thead>
<tbody>
<tr>
<td>clinical remission</td>
<td>9%</td>
<td>40%</td>
<td>22%</td>
<td>11%</td>
<td>0%</td>
</tr>
<tr>
<td>radiological healing</td>
<td>9%</td>
<td>20%</td>
<td>11%</td>
<td>22%</td>
<td>14%</td>
</tr>
<tr>
<td>clinical response</td>
<td>54%</td>
<td>60%</td>
<td>67%</td>
<td>44%</td>
<td>43%</td>
</tr>
<tr>
<td>radiological improvement</td>
<td>54%</td>
<td>60%</td>
<td>55%</td>
<td>44%</td>
<td>43%</td>
</tr>
<tr>
<td>clinical loss of response</td>
<td>27%</td>
<td>0%</td>
<td>11%</td>
<td>44%</td>
<td>43%</td>
</tr>
<tr>
<td>radiological loss of response</td>
<td>18%</td>
<td>0%</td>
<td>22%</td>
<td>11%</td>
<td>43%</td>
</tr>
</tbody>
</table>

Table 18 - Clinical and radiological outcomes for all patients treated with combination thiopurine and anti-TNF therapy over three years
5.5.3 Delay between clinical remission and radiological healing

Twelve patients achieved both clinical remission and radiological healing. Radiological healing lagged behind clinical remission by a median of 12 months (interquartile range 3 to 30 months) (Figure 39).

<table>
<thead>
<tr>
<th>All Anti-TNFα agents</th>
<th>6 months n=43</th>
<th>12 months n=42</th>
<th>18 months n=35</th>
<th>24 months n=31</th>
<th>36 months n=19</th>
</tr>
</thead>
<tbody>
<tr>
<td>clinical remission</td>
<td>26%</td>
<td>43%</td>
<td>31%</td>
<td>29%</td>
<td>21%</td>
</tr>
<tr>
<td>radiological healing</td>
<td>21%</td>
<td>24%</td>
<td>20%</td>
<td>26%</td>
<td>32%</td>
</tr>
<tr>
<td>clinical response</td>
<td>42%</td>
<td>43%</td>
<td>46%</td>
<td>35%</td>
<td>37%</td>
</tr>
<tr>
<td>radiological improvement</td>
<td>42%</td>
<td>45%</td>
<td>34%</td>
<td>19%</td>
<td>21%</td>
</tr>
<tr>
<td>clinical loss of response</td>
<td>19%</td>
<td>10%</td>
<td>14%</td>
<td>26%</td>
<td>37%</td>
</tr>
<tr>
<td>radiological loss of response</td>
<td>21%</td>
<td>17%</td>
<td>20%</td>
<td>23%</td>
<td>42%</td>
</tr>
</tbody>
</table>

Figure 39 - Kaplan Meier plot showing delay between clinical remission and radiological healing
5.5.4 Factors influencing clinical and radiological outcome

On univariate analysis, the number of fistulae at baseline and fistula complexity were related to the clinical outcome. Patients with 3 or more fistulae were less likely to achieve clinical remission than those with fewer fistulae (HR 0.54 [95% CI = 0.33, 0.89] P = 0.02). The number of fistulae did not relate to radiological healing (P=0.09).

Patients with more complex fistulae were less likely to achieve clinical remission than those with simple fistulae (HR 0.38 [95% CI = .14, 0.98], P = 0.04). Fistula complexity did not relate to radiological healing (P = 0.06).

Multivariable analysis showed that only the number of fistulae at baseline was related to clinical remission.

On univariate analysis, the presence of a defunctioning stoma at commencement of treatment was associated with a decreased chance of clinical remission (p=0.04).

The choice of anti-TNF agent, age, gender, duration of Crohn’s disease, duration of fistula, site of luminal disease, smoking status, and number of previous operations did not influence clinical or radiological improvement or healing.

CDAI and PDAI were used to assess symptomatic improvement in response to anti-TNF treatment. However, the initial change over 6 months in either score did not relate to clinical or radiological response or remission.

5.5.5 Effect of early response to treatment on disease course

An early clinical response (within 6 weeks) was significantly associated with future clinical remission. Patients with an early response within 6 weeks had a likelihood of remission at any time that was 5 times greater than those with no early response (P=0.004). Early clinical response was not associated with radiological healing.

5.5.6 Surgical procedures during treatment

Eighteen (44%) of the forty-one patients underwent 25 operations during the study period (Table 19, Figure 40, Figure 41). The remaining 23 patients (56%) underwent
no surgical procedures. Twenty one (84%) of the operations performed were perianal fistula related and are detailed below (Table 20, Figure 42).

Table 19 - Number of procedures performed during study period

<table>
<thead>
<tr>
<th>Number of procedures</th>
<th>Patients having any procedure</th>
<th>Patients having a fistula related procedure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>23</td>
<td>26</td>
</tr>
<tr>
<td>1</td>
<td>12</td>
<td>10</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

Figure 40 - Number of patients undergoing n procedures during study period
Number of patients undergoing n fistula related procedures during study period

![Pie chart showing number of patients undergoing fistula related procedures during the study period.](image.png)

Figure 41 - Number of patients undergoing n fistula related procedures during study period

Table 20 - Procedures performed during study period

<table>
<thead>
<tr>
<th>Fistula related procedures</th>
<th>Crohn's related procedures</th>
</tr>
</thead>
<tbody>
<tr>
<td>I+D abscess</td>
<td>5</td>
</tr>
<tr>
<td>Lay open fistula</td>
<td>1</td>
</tr>
<tr>
<td>Seton +/- I+D</td>
<td>11</td>
</tr>
<tr>
<td>Defunctioning stoma</td>
<td>2</td>
</tr>
<tr>
<td>Proctectomy</td>
<td>2</td>
</tr>
</tbody>
</table>

181
5.5.7 Stopping treatment

Fifteen patients stopped or changed anti-TNF agents during the study period after a median duration of treatment of 17 months. Ten patients stopped anti TNF treatment; 8 were on infliximab and 2 adalimumab. Of these 10 patients, cessation was due to clinical remission in 5, a failure to achieve remission in 2, and side effects, pregnancy and rectal cancer in 1 each.

In addition to the 2 who never achieved remission, a further 5 of the 15 patients developed recurrent anal fistulae after clinical and, in 3 cases, radiological remission after a median of 8 months. Six of the 10 patients were on azathioprine throughout treatment and after cessation, including 4 of the 7 with either failure to respond or recurrence of fistulae; the remainder were intolerant of thiopurines. A dose escalation was attempted in 3 and was partially successful in 2 patients. Surgery was required in 7 patients including all recurrent fistulae, one of the fistulae that never responded and the patient who developed a rectal cancer. This last patient and one other required proctectomy whereas the other patients underwent drainage of sepsis with or without seton insertion.
Five patients changed treatment from infliximab to adalimumab. Two did so due to lack of response of perianal disease and 3 due to the development of active luminal disease. Treatment was for a median of 11 months before switching. All 3 who switched for luminal disease had both clinically and radiologically healed perianal disease before switching and one of these developed fistula recurrence 11 months after switching. The other two have had only limited follow up since the switch. Two patients required proctectomy following treatment failure with two anti-TNF agents.

5.5.8 Maintaining radiological healing after treatment cessation

Fifteen patients achieved radiological healing on either infliximab (n=12) or adalimumab (n=3), of whom 10 were on combination with azathioprine (thiopurines not tolerated in 5). To the end of follow-up, seven of these fifteen had a recurrence of their fistula.

Treatment was maintained in 7 patients who had achieved radiological healing, of whom 5 were on infliximab and 2 adalimumab. All of the infliximab treated patients who achieved radiological healing and continued on treatment maintained healing. Both adalimumab patients lost response despite maintenance treatment.

Eight of the fifteen patients who had achieved radiological healing stopped their anti-TNF treatment or switched to an alternative agent after luminal disease flare or due to side effects, of whom 5 then lost response. Of these five patients, three were on maintenance azathioprine and two were intolerant of thiopurines.

In this subset of 15 patients, predictors of maintaining radiological remission were assessed. None of the following was associated with loss of radiological remission: whether treatment was maintained, duration of disease or fistula, number of fistulae, previous operations, initial change in PDAI, gender, age, smoking status, fistula complexity, presence of a stoma, presence of a seton at induction or immunomodulator use. All 3 patients on adalimumab failed to maintain radiological healing compared to 4 of the 12 infliximab patients although this difference did not reach statistical significance (P=0.08).

In the 12 infliximab treated patients, a trend was seen if patients stopped treatment; all 5 patients who continued infliximab maintenance maintained radiological healing compared to only three of seven (43%) who stopped treatment (P = 0.08).
Figure 43 demonstrates serial MRI-imaging in a patient whose perianal disease improved clinically and radiologically on combination therapy, but who developed clinical and radiological recurrence within 4 months of stopping the anti-TNF therapy.

5.5.9 Adverse events

An abscess occurred in 14 patients. Sixteen procedures for drainage of perianal sepsis were performed, including seton insertion 11 times. One patient stopped infliximab due to side effects and one developed rectal cancer.
Figure 43 - T2 weighted axial MRI with fat suppression demonstrating a. perianal fistula, b. improvement on combination thiopurine and anti-TNF therapy, c. radiological healing of fistula tract and d. recurrence after cessation of anti-TNF therapy.

5.6 Discussion

Combination anti-TNF and thiopurine therapy provides sustained benefit in patients with perianal Crohn's fistula using MRI imaging to monitor outcomes and deep healing. Early clinical response is associated with subsequent clinical remission. Radiological healing is slower than clinical healing with a time lag of up to one year. Radiologically healed fistula tracts maintain healing on infliximab but can recur after cessation of therapy.
In this prospective open interventional study, MRI has been shown to be a useful tool to monitor fistula healing. The study highlights real life practice of treated patients, prospectively recruited and followed carefully based on predefined algorithms. Approximately one third of infliximab treated patients with perianal Crohn’s disease maintained clinical remission at 3 years of follow up. More patients on adalimumab lost response over time but these patients had previously failed infliximab, and adalimumab was the second anti-TNFα agent used.

Clinical remission occurred earlier than radiological healing, the latter occurring on average a year later. This is the first study to have demonstrated the temporal relationship between clinical and radiological response or healing on MRI in patients with perianal Crohn’s fistulae treated in the long term.

Factors associated with failure or maintenance of healing were also examined. An increasing number and complexity of fistulae, and the presence of a stoma at induction, tended to be associated with a decreased chance of long-term healing. These findings contrast with those from the 18 month follow-up data on this cohort published in 2009 but are consistent with surgical and natural history data, which suggest that complex fistulae are associated with a worse prognosis (13). The presence of a stoma at induction suggests a particularly severe disease phenotype, which is likely to be the factor that reduced the chance of clinical fistula healing, rather than the presence of the stoma per se.

Smoking status and pre-anti-TNF therapy induction seton placement were not associated with an altered outcome. A recent study has suggested that baseline MRI characteristics, such as single-branched fistulae and collections, are associated with a worse outcome, with these patients more likely to undergo surgery (288).

Early clinical fistula response (within 6 weeks) is associated with a 5 times increased likelihood of achieving clinical remission. Early radiological changes in response to treatment, or rate of improvement, may also be helpful in predicting outcome, and therefore influencing therapeutic management.

In order to assess radiological parameters, such as extent or rate of improvement, for their suitability as prognostic indicators, it will be necessary in the future to identify parameters of interest and develop standardised, robust methods of assessment of
these parameters. A simple, computer-aided method of serial assessment would be valuable. The previously described scoring system published in 2003 by van Assche et al. was initially used in this cohort (79). However, in the 18 month follow up data, the scoring system was insensitive to change in some patients (80). For example, those who had a 50-80% reduction in tract volume, a reduction in hyperintensity in secondary tracts but not the primary tract, or reduced volume of sepsis across all tracts but maintaining the same number of tracts, could have an unchanged van Assche score despite improvement.

Another study using the van Assche scoring system with long term follow-up suggested that MRI monitoring is only useful in the first year of follow-up and that after this time clinical surveillance alone is valid (288). However, in this study there was no evidence of improved concordance between clinical and radiological assessment in the latter years and clinical remission was often maintained for more than a year before radiological healing occurred. It is possible that the scoring system used is less sensitive after one year of treatment but the assessment of volume and use of radiological monitoring per se do not seem to diminish in utility through long term follow up. On the contrary, after an external orifice has healed, radiological assessment may be the only way of determining when the tract is completely eradicated.

A recent study noted that a change in T2 hyperintensity was the most valuable factor associated with clinical benefit (289). In the present study, an assessment of the change in fistula volume based on T2 signal intensity was used to determine whether a patient had improved, healed or failed to improve. This method was sensitive to change over time and was clinically useful for determining management but lacks the objectivity of a volume measurement. Refinement of assessment of the volume of the fistula complex and changes in the volume or signal intensity as well as the magnitude and rapidity of these changes might predict ultimate outcome. This would be a fruitful avenue for future research.

Twenty-one operations were performed for anal fistula during the study. The need for surgery indicated clinical loss of response. The majority of operations were performed to drain perianal sepsis. Although abscess formation is known to complicate anti-TNF therapy in some patients, this has not been a significant problem in most studies. Our treatment algorithm required drainage of all sepsis, using setons where necessary, and the presence of undrained pus was sought whenever clinical
deterioration occurred. Proctectomy and defunctioning stoma formation for fistula were each required in two patients. All but 3 patients had undergone surgery before enrolment, the majority for drainage of perianal sepsis, mostly with seton insertion, although 4 had failed previous advancement flap surgery, fibrin glue insertion or fistulotomy. Seventeen patients had undergone previous colonic resection.

Further work is required to determine the feasibility of stopping anti-TNF treatment when deep tissue healing is deemed to have taken place. These data suggest that stopping treatment can lead to recurrence even when the fistula appears radiologically healed (Figure 43).

Several factors may account for this. Firstly, the accuracy of a healed fistula tract on MRI is unknown. The radiological definition in this study was either the absence of a visible tract or the reduction in signal intensity and volume of a tract such that it was no longer hyperintense on T2 weighted images and was visible as either a very thin, intermediate or low signal intensity line which was taken to be a scar. The resolution of MRI scanning and the assumptions used may be inadequate for ruling out tiny residual foci of infection which may increase risk of recurrence. This may also account for the slightly higher rate of radiological than clinical remission seen at 3 years. Further analysis of the nature of a healed fistula tract on MRI is required. Secondly, although the fistula is treated and may be fully healed, the underlying disease process persists and new fistulae may be created. If a new fistula does occur, it may do so through the path of least resistance which might often be the transition between scar and normal fat at the site of an old tract. Finally, it may be that full healing of Crohn’s fistula tracts is very unusual on anti-TNF agents and that the majority of patients will require long term maintenance treatment (perhaps with surgical drainage at intervals) in order to sustain clinical benefit either in the form of remission, or at least a response sufficient to provide a reasonable quality of life.

Whatever the reason for recurrence following radiological healing, there was a trend towards association between stopping maintenance infliximab treatment and the loss of radiological healing even in the small subgroup available. Although not statistically significant, further analysis of this finding in larger cohorts to identify or exclude an association is crucial as the questions of long term maintenance treatment and cost-effectiveness are explored.
In the GETAID conducted STORI cohort study, which focussed on patients with luminal CD, there was a 50% relapse rate over 30 months in 115 patients who stopped infliximab after achieving long term remission under combined therapy with a thiopurine (292). Complete endoscopic mucosal healing and normal CRP were predictive of a lower chance of relapse. It was postulated here that a healed fistula tract on MRI may have a similar prognostic significance to luminal mucosal healing, although the results suggest that this may not be the case.

Domenech and colleagues found that 83% of patients with luminal disease were free of relapse compared to 34% of patients with perianal disease one year after cessation of anti-TNF therapy (98). Identifying those patients who will develop recurrence of their fistula after cessation of anti-TNF treatment is important and the prospective analysis of these patients continues. In those patients who did not obtain clinical or radiological remission, substantial clinical benefit was still often achieved with improved quality of life (97).

### 5.7 Summary

The clinical remission rate of combined infliximab and thiopurine treatment of Crohn’s perianal fistulae is one third at 3 years. A larger number of fistula tracts and the presence of a stoma at induction, the latter a marker of more severe disease, are associated with reduced clinical remission. Early clinical response increases the likelihood of developing clinical remission. Radiological healing lags behind clinical remission. Radiologically healed fistula tracts maintain healing on infliximab but are at risk of recurrence after cessation of therapy.

Further work examining clinical and radiological factors (such as changes in fistula volume) which predict success and failure of anti-TNF treatment of Crohn’s perianal fistulae, further characterisation of healed fistula tracts on MRI, and assessment of the safety of stopping anti-TNF treatment after successful healing are required to optimise the management of these patients.
5.8 Limitations

This study is prospective in recruitment but some observations were made retrospectively. The group is small and although follow up is long (and the longest yet published) the cohort which has experienced 3 or 4 year follow up is smaller still.

No control group was used. This is because our aim is to examine the proportion of patients who remain healed throughout their treatment with anti-TNF agents, examining the difference between clinical and radiological healing and the rate at which efficacy falls over time.

The lack of a placebo arm limits the value of our results in terms of an expression of the overall success in the use of these agents as it means that we cannot know how many of these patients might have been healed at the end of follow up without treatment.

Defining clinical healing is difficult as I have explained above, but even radiological healing is problematic; the appearance of a healed fistula tract on MRI scan is also unknown. Conclusions about recurrence following the finding of a healed tract on MRI must therefore be considered carefully and further work to clarify this is required.

Given the relatively small group of patients and the number of factors examined for their influence on clinical and radiological healing a type 1 error due to multiple testing remains a possibility.
6 Discussion

In this thesis, I have investigated several aspects of Crohn’s and idiopathic anal fistulae. The basis of understanding any disease must be its aetiology and pathogenesis. It is not known why anal fistulae occur, why they persist or why they become complex. It is known that all three are more common in Crohn’s than idiopathic anal fistulae, lending logic to the comparative studies conducted in this thesis.

A number of novel discoveries have been made, including an absence of bacteria lying beside the mucosa in anal fistula tracts. In the gut, it is mucosa-associated bacteria which interact with the host immune system via dendritic cells, driving the inflammatory process. It is true that an inability to find the bacteria does not necessarily mean they are not present in vivo, since the lack of a mucosal surface and adjacent mucus layer may mean that (perhaps tenuous) associations in vivo are lost after any manipulation, such as during the washing phase of the experiments. However, were that to be the case, the SEM experiments, conducted as they were without washing might have been expected to detect more bacteria, which they failed to do. A larger SEM study with consideration of other techniques that might avoid the problem of disturbing bacteria in a fragile relationship with the fistula tract wall, is planned.

If it is assumed that the microbiological findings are correct, the presence of inflammatory cells close to the luminal aspect of the fistula tract wall may imply the presence of another luminal factor driving the inflammatory process in the fistula tract. Endotoxin, bacterial proteins adherent to the tract wall or other pathogens are all possible and would not have been detected by the experiments presented here. Investigation along these lines is also planned in tandem with the microbiological study mentioned above.

Alternatively, one might anticipate that an efficient host, lining a disturbed surface with appropriate immune-competent cells, might prevent adherence of potentially invasive bacteria onto that surface, thereby resulting in the findings presented here. If this were indeed a sine qua non of the competent host, then other granulation tissue-lined wounds not connected to fistula disease could be examined to see if they
are similarly sterile on their immediate surface. The problem may be one of a failure to epithelialise an otherwise properly prepared wound.

Epithelialised surfaces swarm with bacteria. An intact epithelium is energy efficient for the host, as a permanently mobilised defensive immune force does not need to be stationed at the frontier. It is also in the interests of the bacteria, which have a place to feed and multiply. It may be that the host and bacteria together work synergistically to epithelialise a surface, and the absence of bacteria from the depths of a fistula wound may actually harm the final phase of epithelialisation.

Next, the similar levels of TNFα in Crohn’s and idiopathic fistula tracts, and the known efficacy of anti-TNFα agents in Crohn’s anal fistulae, suggests that a trial of infliximab in chronic, inoperable, refractory anal fistulae may be warranted.

As the fistula tracts studied here were chronic and well established, it may be that the findings represent the biology of persistence rather than of pathogenesis. Investigation in a group of new, acute anal fistulae in both Crohn’s and idiopathic patients alongside established fistulae may generate interesting differences both between the Crohn’s and idiopathic patients and also between these findings from chronic fistulae compared to the microbiology and immunology of acute fistulae.

The search for aetiological factors is important in order to improve treatment options and limit the compromise faced by patients. If a fistula can be laid open with an acceptable risk of minor incontinence, then fistulotomy offers the best chance, and a very good chance, of cure. If a risk of minor incontinence is not acceptable to the patient, or the impairment suffered following lay open would be greater than this, the patient faces options including palliation with a loose seton, potentially similar incontinence with a tight seton, or with the vagaries of an advancement flap, glue or plug.

LIFT shows promise as a simple, effective and low risk treatment and appears to be the best alternative to fistulotomy at the present time. Larger studies are required to prove its value, particularly in complex, refractory or Crohn’s related fistulae.

In surgery, when many procedures exist for the same condition, none is satisfactory. This is certainly the case for anal fistula surgery. Like Helicobacter Pylori eradication or Enhanced recovery Programs, anal fistulae may well be multi-faceted beasts
requiring treatment of every factor in order to achieve success. Fistulotomy is successful; it eradicates the tract, its lining and contents, the intersphincteric space and internal opening. The wound is exposed to repeated abrasion by dressings and the normal processes of locomotion and altering posture. The ‘distal obstruction’, necessary to promote fistula persistence elsewhere in the body, is disrupted.

The alternative operations perform only one or two of these functions. Infill materials for example, fill the space and disconnect the tract from the bowel, but they do little to disrupt the environment of the tract itself and any residual space; extrusion of glue or epithelium left behind may lead to recurrence.

Without a full understanding of the aetiological aspects of anal fistulae, including cytokine milieu, pathogens, immune dysfunction, space, ramifications, inflammation, epithelium, not to mention so called ‘unknown unknowns’, it will be difficult to create a regimen that treats every aspect of fistula pathology.

However, infill materials such as plugs, glue and microspheres, may offer a scaffold upon which to hang this multifaceted treatment. With adequate removal of epithelium and granulation tissue, such as with a reaming device, closure of the internal opening, followed by instillation of a collagen based infill material which can be impregnated with agents targeted to the pathogenic features of an individual’s fistula tract, such as infliximab, gentamicin and autologous fibroblasts, one can begin to see a treatment which not only treats all aspects of pathology but is tailored to a given patient.

Antibiotic impregnated fibrin glue offered disappointing results. Local injections of infliximab in small studies of Crohn’s anal fistulae had limited success. Microspheres have been shown to hold and release drugs over time. Autologous fibroblasts improve healing in an animal model. Several elements of this treatment have been considered but a combination based on the concept of countering every aetiological factor has not. The next step in this process, a study of autologous fibroblast enhanced collagen paste after reaming, is planned in the fistula research unit at St Mark’s shortly.

At the present time, many complex or refractory anal fistula patients come to St Mark’s following failed treatment elsewhere. Many of them are suitable for fistulotomy and leave cured of their fistula with only a modest risk of worsening their
incontinence. In some patients, earlier fistulotomy has caused a degree of impairment of continence which is not worsened by a small additional sphincter injury whilst 1cm or more of cephalad functioning muscle remains. In others, years of disappointment and recurrence present a more accepting patient to clinic who is willing to risk flatus incontinence for a 95% chance of cure.

Crohn’s patients are less commonly suitable for fistulotomy and for those who cannot undergo surgery, anti-TNFα agents offer the best chance of fistula healing. Infliximab is expensive and has significant side effects in some patients with only around a 1 in 3 chance of lasting remission whilst on maintenance therapy. However, this is significantly better than any other drug currently available, there is no risk of incontinence, and even if remission is not achieved, improvement in fistula symptoms and quality of life is very common.

Some patients will never gain a significant benefit and will certainly never heal. Some will improve for a time and then relapse. Some will heal if given a sufficient course of treatment. It is very difficult to prejudge which of these groups patients will fall into based on demographic or baseline disease data. The data presented in this thesis have shown that a rapid early clinical response increases the chance of a patient achieving clinical remission, but no radiological prognostic features have been identified so far. The prospective study continues but the radiological parameters for assessing improvement are blunt.

The best measure of a physical response in a fistula tract on MRI scanning may well be the change and rate of change of volume of the tract itself. Clinically, a healed external opening may obscure a growing rabbit warren of extensions and tract expansion. On MRI, this would be obvious. More subtle changes in volume are also detectable on modern scanners but quantifying and delineating change over time is a very labour intensive process. A prototype computer program able to read DICOM images from MRI scanners, semi-automatically measure fistula tract volume on serial scans, and plot changes in volume over time is under development.

Such a programme has two immediate potential uses. Firstly, it might help clinicians to decide when it is appropriate to stop treatment, either because response is being lost or because treatment is complete and the chance of recurrence on cessation is very low. Secondly, change in volume and the rate of change in volume may be useful predictive factors. For example, a future infliximab regimen might benefit from
the knowledge that a reduction in tract volume of 50% or more in the first 6 months suggested a 95% chance of radiological healing at 2 years, or that a change in volume of less than 25% in the first 6 months suggested a 95% chance of relapse within 18 months and no prospect of healing; and so on. This kind of prognostic information could prevent exposure to expensive and potentially harmful drugs in patients with little chance of benefit, and encourage perseverance in those patients who may heal if only treated for long enough.

The large group of patients who gain some clinical benefit from anti-TNF agents without obtaining remission may nevertheless warrant ongoing treatment. Further work is needed to delineate the personal, social and economic cost and benefit of these agents as a ‘palliative’ intervention to consider whether such treatment is beneficial and cost effective for these patients who are often keen to continue treatment in spite of the risks and a failure fully to heal because an improved family and working life is gained.

Like anal fistulae in Crohn’s disease, rectovaginal fistulae do not heal consistently in response to drugs or surgery. Even non-Crohn’s RVF fare poorly at surgery. Preoperative placement of setons and stomas are sometimes used in spite of a lack of evidence, and whilst several studies have commented on the latter as a secondary outcome, randomised data on the influence of a stoma on healing are lacking. Without this kind of trial, like will not be compared with like. For example, a woman may be persuaded to accept a stoma to cover her third RVF repair attempt. Whether or not that repair is successful, one is left asking whether at her first, perhaps best chance of successful surgery, a stoma might have made a difference. It may also be the case that in order to glean a benefit from stoma use, a high rate of defunctioning may be necessary. Other factors such as seton insertion to limit inflammation before surgery may also encourage healing.

In Crohn’s disease, whilst stomas and setons may still be of value, preoperative infliximab treatment is probably the next valuable step to investigate. Good pilot data have found that time to healing is improved in patients treated with infliximab before surgery. A large randomised trial investigating the value of preoperative infliximab induction, along with preoperative seton insertion and, in some cases, defunctioning, in improving surgery for anal and rectovaginal fistulae in Crohn’s disease seems attractive.
6.1 The future

With an improved understanding of the aetiological aspects of anal fistulae, a combined, multi-faceted treatment may represent the future of their management. An optimised approach should deal comprehensively with the factors underpinning fistula occurrence and persistence in both Crohn’s and complex idiopathic disease. Whilst improvements in outcome have been achieved in recent decades, a plateau above which success rates will not climb will be reached (if it has not already) without a shift in approach. Identifying and treating the pathological factors in a combined way seems key.

Consider a tract too high to lay open, perhaps in the presence of Crohn’s disease and with secondary ramifications. Diagnostic laboratory tests might determine bacterial colonisation, TNFα levels, T lymphocyte and DC function and other immunological variables. A period of seton treatment to drain the system might be followed by systemic infliximab induction (+/- maintenance) with MRI monitoring to determine improvement in the ramifications and identify the lone tract that remains. After this priming of the tract it might be reamed to remove any epithelium and the luminal surface of the tract itself and a collagen based infill material impregnated with antibiotics, infliximab, autologous fibroblasts and other agents tailored to the individual to counter each defect in the immunological and repair systems might be instilled.

This thesis provides novel microbiological and immunological findings to stimulate and guide further aetiological analysis as well as a description of the benefits and limitations of some of the current aspects of fistula management. A move towards the combined management of these patients by surgeons, gastroenterologists and radiologists is crucial with shared goals and simultaneous intervention, rather than the alternative of simply passing patients between the two specialities when options from one side become exhausted. This move should govern both clinical and research approaches with combined clinics and academic units, such as the Fistula Research Unit at St Mark’s.
7 Appendix 1

7.1 Histology and IHC materials and protocols

7.1.1 Histology and IHC materials

<table>
<thead>
<tr>
<th>Product</th>
<th>Company/Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gill’s Haematoxylin</td>
<td>Surgipath Europe Ltd, Peterborough</td>
</tr>
<tr>
<td>Harris Haematoxylin</td>
<td>Surgipath Europe Ltd, Peterborough</td>
</tr>
<tr>
<td>Eosin</td>
<td>Pioneer, UK</td>
</tr>
<tr>
<td>2.5% ready to use normal horse serum</td>
<td>Vector laboratories, UK</td>
</tr>
<tr>
<td>Anti-mouse Ig ImmPRESS Reagent</td>
<td>Vector laboratories</td>
</tr>
<tr>
<td>ImmPACT DAB peroxidise substrate</td>
<td>Vector laboratories</td>
</tr>
<tr>
<td>Hydrogen Peroxide (30%w/v in water)</td>
<td>Sigma Aldrich, UK</td>
</tr>
<tr>
<td>Phosphate buffered saline</td>
<td>Sigma Aldrich</td>
</tr>
<tr>
<td>Tris Buffered Saline 0.05M, pH 7.4</td>
<td>Trizma base 6.06g, NaCl 8.06g, KCl 0.20g (all Sigma) in 500ml dH2O</td>
</tr>
<tr>
<td>TRS</td>
<td>DAKO, UK</td>
</tr>
<tr>
<td>Methanol</td>
<td>Sigma</td>
</tr>
<tr>
<td>Acid Alcohol, 1% HCl in 70% IMS</td>
<td>Fisher Scientific, UK &amp; Genta Medical, UK</td>
</tr>
<tr>
<td>Sirius Red, F3B, 1g in 1000ml Picric Acid (Sigma)</td>
<td>BDH lab, UK</td>
</tr>
<tr>
<td>Calcium Chloride</td>
<td>VWR, UK</td>
</tr>
<tr>
<td>1M Sodium Hydroxide</td>
<td>Sigma</td>
</tr>
<tr>
<td>Miller’s Elastin</td>
<td>Surgipath</td>
</tr>
<tr>
<td>CD3 antibody (M7254)</td>
<td>DAKO</td>
</tr>
<tr>
<td>Ab-1 antibody (MS 148P1)</td>
<td>Thermo Fisher Scientific</td>
</tr>
<tr>
<td>Citrate buffer 10mM</td>
<td>2.5g in 1000ml dH2O, pH 6.0</td>
</tr>
<tr>
<td>Anti-rabbit Ig ImmPRESS Reagent</td>
<td>Vector laboratories</td>
</tr>
<tr>
<td>Weigert’s Haematoxylin</td>
<td>Pioneer Research Chemicals (PRC), UK</td>
</tr>
<tr>
<td>Acid K permanganate, 0.5% KMnO₄ in 3% H₂SO₄</td>
<td>Sigma</td>
</tr>
<tr>
<td>1% Oxalic Acid</td>
<td>Sigma</td>
</tr>
</tbody>
</table>
7.1.2 Histology and IHC protocols

7.1.2.1 Tissue processing

After gross cut up, tissue is fixed in 10% neutral-buffered formalin and then processed in graded alcohols, xylene and wax (Table 21) before being embedded in paraffin wax prior to sectioning.

Table 21 - Protocol for tissue processing

<table>
<thead>
<tr>
<th>Solution</th>
<th>Time (hours)</th>
<th>Temperature (°C, RT = Room temperature)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10% neutral-buffered formalin</td>
<td>2</td>
<td>RT</td>
</tr>
<tr>
<td>70 % IMS (Alcohol)</td>
<td>1</td>
<td>RT</td>
</tr>
<tr>
<td>90% IMS</td>
<td>1</td>
<td>RT</td>
</tr>
<tr>
<td>100% IMS</td>
<td>1</td>
<td>RT</td>
</tr>
<tr>
<td>100% IMS</td>
<td>1</td>
<td>RT</td>
</tr>
<tr>
<td>100% IMS</td>
<td>1</td>
<td>RT</td>
</tr>
<tr>
<td>50% absolute IMS/Xylene</td>
<td>1</td>
<td>RT</td>
</tr>
<tr>
<td>Xylene</td>
<td>½</td>
<td>RT</td>
</tr>
<tr>
<td>Xylene</td>
<td>1</td>
<td>RT</td>
</tr>
<tr>
<td>Xylene</td>
<td>½</td>
<td>RT</td>
</tr>
<tr>
<td>Paraffin wax</td>
<td>½</td>
<td>60</td>
</tr>
<tr>
<td>Paraffin wax</td>
<td>1 ½</td>
<td>60</td>
</tr>
</tbody>
</table>

7.1.2.2 H&E protocol

Dewax with Xylene 1                | 5m           |
Dewax with Xylene 2                | 2m           |
Absolute IMS (Industrial Methylated Spirit) | 2m           |
95% IMS                            | 2m           |
70% IMS                            | 2m           |
Running tap water                  | 2m           |
Gill’s Haematoxylin                | 90s          |
Running tap water                  | 5m           |
Differentiate in 1% hydrochloric acid in 70% IMS | 3s (dip 3 times) |
Running tap water                  | 4m           |
0.5% aqueous Eosin                 | 5m           |
Running tap water                  | 20s          |
70% IMS                            | 30s          |
95% IMS                            | 30s          |
Absolute IMS                       | 2m           |
Xylene 1                           | 2m           |
Xylene 2                           | Until ready to mount |
7.1.2.3 PM protocol

Dewax with Xylene 1  
Dewax with Xylene 2  
Absolute IMS (Industrial Methylated Spirit)  
95% IMS  
70% IMS  
Running tap water  
Acid potassium permanganate  
Distilled water  
1% oxalic acid  
Distilled water (fresh)  
95% IMS  
Miller’s Stain  
95% IMS  
Tap water  
Weigert’s haematoxylin  
Tap water  
1% hydrochloric acid in 70% IMS  
Tap water  
Distilled water (fresh)  
Picro Sirius red  
70% IMS  
95% IMS  
Absolute IMS  
Xylene 1  
Xylene 2  
Until ready to mount

7.1.2.4 Ab-1 protocol

1. Xylene1 5 mins
2. Xylene2 2 mins
3. IMS 2 mins
4. 95% IMS 2 mins
5. 70% IMS 2 mins
6. Running tap water 2 mins
7. Hydrogen peroxide 3%, 30 minutes
8. 2xdH2O
9. 3xPBS
10. NSB, Horse serum (2 drops) 30 minutes
11. Primary antibody, dilution 1/1000: Thermo Fisher Scientific: O/N
12. 3xPBS
13. Impress kit, anti-mouse (2 drops), 45 minutes
14. 3xPBS
15. DAB, Neo Marker 3 mins
16. Distilled water 5 min
17. Harris Haematoxylin, 30 seconds
18. Running water 7 minutes
19. 1% Acid Alcohol 3x3 seconds
20. Running water 5 minutes
21. 70% IMS 30 secs
22. 95% IMS 30 secs
23. IMS 30 secs
24. Xylene1 2 mins
25. Xylene2 until mounted

7.1.2.5 CD3 protocol

1. Xylene1 5 mins
2. Xylene2 2 mins
3. IMS 2 mins
4. 95% IMS 2 mins
5. 70% IMS 2 mins
6. Running tap water 2 mins
7. AR: 98° in Dako TRS 30 mins
8. Cool in PBS
9. Hydrogen peroxide 3%, 30 minutes
10. 3xPBS
11. NSB, Horse serum (2 drops) 45 minutes
12. Primary antibody, dilution 1/50: Dako: 60 mins
13. 3xPBS
14. Impression kit, anti-mouse (2 drops), 30 minutes
15. 3xPBS
16. DAB, Neo Marker 3 mins
17. Distilled water 5 min
18. Harris Haematoxylin, 15 seconds
19. Running water 7 minutes
20. 1% Acid Alcohol 3x3 seconds
21. Running water 5 minutes
22. 70% IMS 30 secs
23. 95% IMS 30 secs
24. IMS 2 mins
25. Xylene1 2 mins
26. Xylene2 until mounted
7.2 FISH materials and protocols

7.2.1 FISH materials

<table>
<thead>
<tr>
<th>Product</th>
<th>Company/Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>DePC water</td>
<td>1ml DePC (Sigma, UK) per litre of deionised water, shake vigorously, incubate overnight at room temp, autoclave</td>
</tr>
<tr>
<td>Hybridisation buffer</td>
<td>4.78gL Tris-HCL (Sigma, UK), 79.69g/L NaCl (VWR, UK), deionised water, autoclave, 15ml/L 10% SDS, adjust to pH 7.2, filter at 0.2 μm</td>
</tr>
<tr>
<td>Hybridisation wash</td>
<td>3.15gL Tris-HCL (Sigma, UK), 52.60g/L NaCl (VWR, UK), deionised water, adjust to pH 7.2, autoclave, filter at 0.2 μm</td>
</tr>
<tr>
<td>4% paraformaldehyde</td>
<td>500ml PBS, 20g paraformaldehyde (Sigma, UK), heat to 65°C, add NaOH to clear and filter at 0.2 μm</td>
</tr>
<tr>
<td>Tris buffer, 10mM, pH6.5</td>
<td>Sigma, UK</td>
</tr>
</tbody>
</table>

7.2.2 FISH protocols

7.2.2.1 Probe dilution protocol

The desiccated probe is re-suspended with a microlitre volume of Tris Buffer (10mM, pH6.5) equal to the microgram mass supplied (found on datasheet accompanying probe). This produces a 1μg/μl stock solution which is diluted to a 5ng/μl working concentration solution with hybridisation buffer at the time of hybridisation by adding 1μl of stock solution to 199μl of hybridisation buffer.

7.2.2.2 Hybridisation protocol

1. Remove slides from -20°C storage
2. Warm at RT for 10 minutes
3. Fix in 4% paraformaldehyde for 10 minutes
4. Wash in DePC water for 5 mins x2
5. Add 20μl filtered, warmed hybridisation buffer to each well and place in oven in sealed, humidified box, at appropriate hybridisation temperature for 30 mins
6. Add 18μl probe (5ng/μl) in hybridisation buffer to each well
7. Cover with 13mm round cover slip
8. Place back in oven in sealed, humidified box at same temperature overnight
9. Remove slides from box and tap off coverslips
10. Place slides into filtered hybridisation wash and agitate for 20mins at room temperature (in foil wrapped Coplin jars) (Bif and Bac only)
11. Place in DePC water for 5 mins
12. Mount
13. Store in fridge and protect from light

7.3 **FACS materials and protocols**

7.3.1 **FACS materials**

<table>
<thead>
<tr>
<th>Product</th>
<th>Company/Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete medium</td>
<td>RPMI-1640 Dutch Modification (Sigma Aldrich, UK) containing 10% Fetal Calf Serum, 100 µg/ml streptomycin, 100 units/ml penicillin</td>
</tr>
<tr>
<td>FACS Buffer</td>
<td>PBS with 2% FCS, 0.02% sodium azide and 1mM EDTA</td>
</tr>
<tr>
<td>Phosphate Buffered Saline (PBS)</td>
<td>Sigma, UK</td>
</tr>
<tr>
<td>Fetal calf serum (FCS)</td>
<td>Tissue culture systems</td>
</tr>
<tr>
<td>Ficoll-Paque</td>
<td>Amersham Pharmacia Biotech AB, Uppsala, Sweden</td>
</tr>
<tr>
<td>Flow-count™ Fluorospheres</td>
<td>Beckman Coulter, Bucks</td>
</tr>
<tr>
<td>Optilyse C</td>
<td>Immunotech, Marseille</td>
</tr>
<tr>
<td>Paraformaldehyde (PFA) 1%</td>
<td>BDH chemicals, Poole</td>
</tr>
<tr>
<td>Antibody</td>
<td>Colour</td>
</tr>
<tr>
<td>---------------</td>
<td>--------</td>
</tr>
<tr>
<td>CD11c</td>
<td>FitC</td>
</tr>
<tr>
<td>γ1</td>
<td>FitC</td>
</tr>
<tr>
<td>CD65</td>
<td>FitC</td>
</tr>
<tr>
<td>γ2a</td>
<td>FitC</td>
</tr>
<tr>
<td>rIgM</td>
<td>FitC</td>
</tr>
<tr>
<td>mIgM</td>
<td>FitC</td>
</tr>
<tr>
<td>CLA</td>
<td>FitC</td>
</tr>
<tr>
<td>β7</td>
<td>PE</td>
</tr>
<tr>
<td>CD19</td>
<td>PE</td>
</tr>
<tr>
<td>γ1</td>
<td>PE</td>
</tr>
<tr>
<td>CD3</td>
<td>PE</td>
</tr>
<tr>
<td>CD16</td>
<td>PE</td>
</tr>
<tr>
<td>r γ2a</td>
<td>PE</td>
</tr>
<tr>
<td>γ2a</td>
<td>PE</td>
</tr>
<tr>
<td>Lineage cocktail</td>
<td>PC5</td>
</tr>
<tr>
<td>CD14</td>
<td>PC5</td>
</tr>
<tr>
<td>Gamma 1</td>
<td>PC5</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>APC</td>
</tr>
<tr>
<td>β7</td>
<td>APC</td>
</tr>
<tr>
<td>r γ2a</td>
<td>APC</td>
</tr>
<tr>
<td>CD3</td>
<td>APC</td>
</tr>
<tr>
<td>γ1</td>
<td>APC</td>
</tr>
</tbody>
</table>

7.3.2 FACS protocols

7.3.2.1 Walk out protocol

1. Remove tissue from complete medium
2. Cut in Petri dish to 2mm³ pieces
3. Wash with complete medium
4. Remove any blood using scalpel
5. If sufficient pieces of tissue (at least 3), place 1-2 pieces into a test tube, add 1ml complete medium and incubate overnight at 37°C, 5% CO₂
6. Place remaining pieces into single well of 24 well plate
7. Add 1ml of complete medium
8. Incubate overnight at 37°C, 5% CO₂
9. Remove supernatant from test tube and place in cryovial, store at -80°C for multiplex analysis
10. Remove supernatant from 24 well plate and label

7.3.2.2 Labelling protocol

1. Remove supernatant from 24 well plate and place in test tube
2. Wash well with 1ml FACS buffer x3 putting wash fluid with supernatant
3. Centrifuge test tube at 1400 rpm for 5 mins
4. Tip out fluid and flick to disperse cells in remaining fluid
5. Make up to 100μl per tube plus 50μl with FACS buffer
6. Divide equally between FACS tubes
7. Add antibodies to appropriate tubes using 10μl of FicC antibodies, 12μl of lineage cocktail and 5μl of all other antibodies
8. Refrigerate for at least 20 minutes
9. FACS buffer wash: add 1ml of FACS buffer to each tube, centrifuge at 1400 rpm for 5 mins, tip out supernatant and flick to disperse cells
10. Add 200μl of 1% paraformaldehyde
11. Add 20μl of fluorospheres
12. Perform flow cytometry

7.3.2.3 Compensation tube protocol

1. Add an equal volume of RPMI medium to whole blood
2. Slowly pipette mixture onto equal volume of Ficoll
3. Centrifuge at 2000 rpm for 25 mins
4. Extract PBMCs as interface
5. Add FACS buffer to PBMCs up to 35ml
6. Centrifuge at 1500 rpm for 5 mins, tip out supernatant then flick to disperse cells
7. Repeat FACS buffer wash with 10ml at 1500 rpm for 5 mins, tip out supernatant and flick to disperse cells
8. Make up to 500μl with FACS buffer
9. Add antibodies to appropriate tubes using 10μl of FicC antibodies and 5μl of all other antibodies
10. Refrigerate for at least 20 minutes
11. FACS buffer wash: add 1ml of FACS buffer to each tube, centrifuge at 1400 rpm for 5 mins, tip out supernatant and flick to disperse cells
12. Add 500μl of 1% paraformaldehyde
13. Perform flow cytometry
7.4 Results data

7.4.1 Tract immunohistochemistry results

7.4.1.1 Acute and chronic inflammation

7.4.1.1.1 Acute inflammation

Table 22 - Tract acute inflammation results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Acute Inflammation Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>0 (0, 2.5)</td>
<td>0.80</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>0.5 (0, 1)</td>
<td></td>
</tr>
<tr>
<td>Smoker</td>
<td>No</td>
<td>0 (0, 1)</td>
<td>0.08</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1 (0, 3)</td>
<td></td>
</tr>
<tr>
<td>Disease location</td>
<td>L1</td>
<td>1 (0, 2)</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>0 (0, 0)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>2.5 (0, 5)</td>
<td></td>
</tr>
<tr>
<td>Stoma</td>
<td>No</td>
<td>0 (0, 2)</td>
<td>0.39</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>0 (0, 0)</td>
<td></td>
</tr>
<tr>
<td>Seton</td>
<td>No</td>
<td>0 (0, 0)</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.5 (0, 3)</td>
<td></td>
</tr>
<tr>
<td>Immunomodulators</td>
<td>No</td>
<td>0 (0, 1)</td>
<td>0.44</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1 (0, 3.5)</td>
<td></td>
</tr>
</tbody>
</table>

Table 23 - Tract acute inflammation results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.32</td>
<td>0.17</td>
</tr>
<tr>
<td>Duration luminal disease</td>
<td>0.91</td>
<td><strong>0.005</strong></td>
</tr>
<tr>
<td>Duration perianal disease</td>
<td>-0.15</td>
<td>0.53</td>
</tr>
<tr>
<td>Number of previous operations</td>
<td>0.05</td>
<td>0.85</td>
</tr>
</tbody>
</table>
### 7.4.1.1.2 Chronic inflammation

#### Table 24 - Tract chronic inflammation results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Chronic Inflammation Mean (SD)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male</td>
<td>2.6 (1.3)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>2.3 (1.1)</td>
<td>0.57</td>
</tr>
<tr>
<td>Smoker</td>
<td>No</td>
<td>2.1 (1.0)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>3.1 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Disease location</td>
<td>L1</td>
<td>2.7 (0.6)</td>
<td>0.68</td>
</tr>
<tr>
<td></td>
<td>L2</td>
<td>2.0 (2.8)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>L3</td>
<td>3.3 (1.5)</td>
<td></td>
</tr>
<tr>
<td>Stoma</td>
<td>No</td>
<td>2.7 (1.1)</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>1.0 (1.4)</td>
<td></td>
</tr>
<tr>
<td>Seton</td>
<td>No</td>
<td>2.4 (1.2)</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.7 (1.3)</td>
<td></td>
</tr>
<tr>
<td>Immunomodulators</td>
<td>No</td>
<td>3.3 (1.0)</td>
<td>0.43</td>
</tr>
<tr>
<td></td>
<td>Yes</td>
<td>2.4 (1.8)</td>
<td></td>
</tr>
</tbody>
</table>

#### 7.4.1.2 Luminal vs. deep immune cells

#### Table 25 - Tract Macrophages and T-lymphocytes by zone

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Luminal cells (z1) Median (IQR)</th>
<th>Deep cells (z2) Median (IQR)</th>
<th>Difference (z1 – z2) Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab-1 (macrophage)</td>
<td>10.8 (1.7, 38.8)</td>
<td>2.5 (1.5, 7.5)</td>
<td>10.2 (0.0, 35.4)</td>
<td>0.02</td>
</tr>
<tr>
<td>CD3 (T-lymphocyte)</td>
<td>16.6 (13.1, 22.8)</td>
<td>5.0 (2.9, 6.2)</td>
<td>11.6 (7.3, 23.8)</td>
<td>0.002</td>
</tr>
</tbody>
</table>
7.4.1.3 T-lymphocytes

Table 26 - Tract Macrophages and T-lymphocytes by aetiology

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Variable</th>
<th>Idiopathic Median (IQR)</th>
<th>Crohn's Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ab-1 (macrophage)</td>
<td>Naurea</td>
<td>5.5 (0.9, 13.8)</td>
<td>7.3 (1.3, 22.1)</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>Na z1 (lumen)</td>
<td>10.8 (1.7, 38.8)</td>
<td>18.0 (2.9, 42.8)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>Na z2 (deep)</td>
<td>2.5 (1.5, 7.5)</td>
<td>4.5 (1.7, 7.9)</td>
<td>0.88</td>
</tr>
<tr>
<td>CD3 (T-lymphocyte)</td>
<td>Naurea</td>
<td>7.7 (3.0, 17.1)</td>
<td>4.5 (3.5, 15.5)</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>Na z1 (lumen)</td>
<td>14.4 (11.1, 20.0)</td>
<td>21.7 (16.6, 44.9)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>Na z2 (deep)</td>
<td>4.5 (2.9, 5.3)</td>
<td>9.1 (6.2, 9.1)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

7.4.2 Rectal Fluorescent in situ Hybridisation results

7.4.2.1 EUB/Panbacteria

Table 27 - Rectal bacteria results: EUB

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>IPD</td>
<td>54 (17, 72)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td>CPD</td>
<td>26 (14, 46)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>IPD / CPD</td>
<td>37 (14, 61)</td>
<td>0.75</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>34 (9, 83)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>CPD / CD</td>
<td>26 (12, 61)</td>
<td>0.23</td>
</tr>
<tr>
<td></td>
<td>IPD</td>
<td>54 (17, 72)</td>
<td></td>
</tr>
</tbody>
</table>

7.4.2.2 Bacteroides - Prevotella

Table 28 - Rectal bacteria results: BAC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>IPD</td>
<td>15 (17, 72)</td>
<td>0.05</td>
</tr>
<tr>
<td></td>
<td>CPD</td>
<td>0 (0, 0)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>IPD / CPD</td>
<td>0 (0, 61)</td>
<td>0.56</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>5 (0, 83)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>CPD / CD</td>
<td>0 (0, 19)</td>
<td>0.14</td>
</tr>
<tr>
<td></td>
<td>IPD</td>
<td>15 (0, 47)</td>
<td></td>
</tr>
</tbody>
</table>
Table 29 - Rectal bacteria results: BAC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Correlation Coefficient</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Duration of perianal disease</td>
<td>-0.07</td>
<td>0.71</td>
</tr>
<tr>
<td>Duration of luminal disease (*)</td>
<td>0.45</td>
<td>0.02</td>
</tr>
</tbody>
</table>

(*) Crohn’s patients only

7.4.2.3 Bifidobacteria

Table 30 - Rectal bacteria results: BIF

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>IPD</td>
<td>32 (0, 88)</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>CPD</td>
<td>0 (0, 8)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>IPD / CPD</td>
<td>0 (0, 49)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>0 (0, 7)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>CPD / CD</td>
<td>0 (0, 8)</td>
<td>0.005</td>
</tr>
<tr>
<td></td>
<td>IPD</td>
<td>32 (0, 88)</td>
<td></td>
</tr>
</tbody>
</table>

7.4.2.4 Clostridium coccoides – Eubacterium rectale cluster

Table 31 - Rectal bacteria results: EREC

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>IPD</td>
<td>0 (0, 27)</td>
<td>0.76</td>
</tr>
<tr>
<td></td>
<td>CPD</td>
<td>0 (0, 15)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>IPD / CPD</td>
<td>0 (0, 15)</td>
<td>0.53</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>0 (0, 67)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>CPD / CD</td>
<td>0 (0, 15)</td>
<td>0.99</td>
</tr>
<tr>
<td></td>
<td>IPD</td>
<td>0 (0, 27)</td>
<td></td>
</tr>
</tbody>
</table>
### 7.4.2.5 Escherichia coli

Table 32 - Rectal bacteria results: E COLI

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>IPD</td>
<td>0 (0, 0)</td>
<td>0.74</td>
</tr>
<tr>
<td></td>
<td>CPD</td>
<td>0 (0, 9)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>IPD / CPD</td>
<td>0 (0, 8)</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>0 (0, 0)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>CPD / CD</td>
<td>0 (0, 0)</td>
<td>0.98</td>
</tr>
<tr>
<td></td>
<td>IPD</td>
<td>0 (0, 0)</td>
<td></td>
</tr>
</tbody>
</table>

### 7.4.2.6 Faecalibacterium prausnitizii

Table 33 - Rectal bacteria results: F PRAU

<table>
<thead>
<tr>
<th>Variable</th>
<th>Category</th>
<th>Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>IPD</td>
<td>0 (0, 28)</td>
<td>0.29</td>
</tr>
<tr>
<td></td>
<td>CPD</td>
<td>0 (0, 4)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>IPD / CPD</td>
<td>0 (0, 9)</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>CD</td>
<td>0 (0, 17)</td>
<td></td>
</tr>
<tr>
<td>Group (combined)</td>
<td>CPD / CD</td>
<td>0 (0, 8)</td>
<td>0.55</td>
</tr>
<tr>
<td></td>
<td>IPD</td>
<td>0 (0, 28)</td>
<td></td>
</tr>
</tbody>
</table>
### 7.4.3 Flow cytometry results

<table>
<thead>
<tr>
<th>Variable Group</th>
<th>Variable</th>
<th>Crohn's Median (Range)</th>
<th>Idiopathic Median (Range)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3 - T Cells</td>
<td>% Positive PCIR</td>
<td>2 (0, 85)</td>
<td>16 (0, 84)</td>
<td>0.09</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 (0, 248)</td>
<td>2 (0, 103)</td>
<td>0.70</td>
</tr>
<tr>
<td>CD19 - B Cells</td>
<td>% Positive PCIR</td>
<td>0 (0, 56)</td>
<td>21 (0, 71)</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0, 6)</td>
<td>1 (0, 3)</td>
<td>0.92</td>
</tr>
<tr>
<td>CD65 - Neutro</td>
<td>% Positive PCIR</td>
<td>19 (0, 65)</td>
<td>15 (0, 42)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6 (1, 93)</td>
<td>77 (0, 743)</td>
<td><strong>0.04</strong></td>
</tr>
<tr>
<td>CD16 - Granulocytes</td>
<td>% Positive PCIR</td>
<td>6 (3, 9)</td>
<td>0 (0, 31)</td>
<td>0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td>192 (2, 382)</td>
<td>0 (0, 20)</td>
<td>0.09</td>
</tr>
<tr>
<td>CD14 - Monocytes</td>
<td>% Positive PCIR</td>
<td>25 (0, 54)</td>
<td>0 (0, 45)</td>
<td>0.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (0, 20)</td>
<td>0 (0, 2)</td>
<td>0.32</td>
</tr>
<tr>
<td>B7 - Migration Cells All</td>
<td>% Positive PCIR</td>
<td>0 (0, 45)</td>
<td>26 (0, 65)</td>
<td>0.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (0, 532)</td>
<td>1 (0, 230)</td>
<td>0.72</td>
</tr>
<tr>
<td>CLA - Migration Cells All</td>
<td>% Positive PCIR</td>
<td>0 (0, 2)</td>
<td>0 (0, 40)</td>
<td>0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0, 1)</td>
<td>0 (0, 42)</td>
<td>0.24</td>
</tr>
<tr>
<td>DCs</td>
<td>% DCs</td>
<td>14 (1, 57)</td>
<td>15 (0, 36)</td>
<td>0.77</td>
</tr>
<tr>
<td></td>
<td>% Myeloid</td>
<td>36 (5, 62)</td>
<td>53 (10, 93)</td>
<td>0.46</td>
</tr>
<tr>
<td></td>
<td>% Plasmacytoid</td>
<td>64 (37, 94)</td>
<td>47 (7, 90)</td>
<td>0.46</td>
</tr>
<tr>
<td>B7 - Migration DCs</td>
<td>% Positive PCIR</td>
<td>0 (0, 0)</td>
<td>16 (0, 96)</td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0, 0)</td>
<td>1 (0, 24)</td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td>CLA DCs</td>
<td>% Positive PCIR</td>
<td>0 (0, 0)</td>
<td>78 (0, 99)</td>
<td><strong>0.03</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>0 (0, 0)</td>
<td>1 (0, 18)</td>
<td><strong>0.03</strong></td>
</tr>
</tbody>
</table>
7.4.4 Cytokine results

Table 35 – Tract cytokine results

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal Median (IQR)</th>
<th>CPD Median (IQR)</th>
<th>IPD Median (IQR)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>2.25 (2.02, 2.60)</td>
<td>2.02 (1.80, 2.32)</td>
<td>1.93 (1.76, 2.46)</td>
<td>0.20</td>
</tr>
<tr>
<td>IL-4</td>
<td>1.87 (1.50, 2.08)</td>
<td>1.87 (1.77, 1.95)</td>
<td>1.92 (1.87, 2.14)</td>
<td>0.58</td>
</tr>
<tr>
<td>IL-6</td>
<td>350 (283, 1213)</td>
<td>334 (106, 1345)</td>
<td>1201 (250, 4692)</td>
<td>0.64</td>
</tr>
<tr>
<td>IL-10</td>
<td>2.04 (1.46, 2.81)</td>
<td>1.86 (1.61, 4.05)</td>
<td>2.81 (1.35, 6.22)</td>
<td>0.68</td>
</tr>
<tr>
<td>TNF</td>
<td>2.18 (1.65, 2.85)</td>
<td>1.61 (1.24, 2.66)</td>
<td>2.87 (2.09, 6.61)</td>
<td>0.48</td>
</tr>
<tr>
<td>IFN-g</td>
<td>1.48 (1.43, 2.48)</td>
<td>1.43 (1.24, 1.72)</td>
<td>4.07 (1.72, 7.47)</td>
<td>0.11</td>
</tr>
<tr>
<td>IL-17a</td>
<td>3.01 (0.00, 3.86)</td>
<td>2.63 (2.37, 3.86)</td>
<td>3.38 (3.13, 4.59)</td>
<td>0.05</td>
</tr>
</tbody>
</table>

There was some evidence of an overall difference in IL-17a between the three groups, although this result was only of borderline statistical significance (p=0.05).

As a result the Mann-Whitney test was used to perform pairwise comparisons between groups. The p-values from these analyses were given a Bonferroni adjustment to allow for multiple testing, and the adjusted p-values are shown in the next table.

Table 36 – Tract IL-17a results after adjustment

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normal vs. CPD P-value</th>
<th>Normal vs. IPD P-value</th>
<th>CPD vs. IPD P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-17a</td>
<td><strong>0.04</strong></td>
<td>0.29</td>
<td><strong>0.04</strong></td>
</tr>
</tbody>
</table>
## 8 Appendix 2

### 8.1 Fistula Drainage Assessment

<table>
<thead>
<tr>
<th>End Point</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Improvement</strong></td>
<td>Closure of individual fistulae defined as no fistula drainage despite gentle finger compression. Improvement defined as a decrease from baseline in the number of open draining fistulae of ≥50% for at least 2 consecutive visits (i.e., at least 4 weeks).</td>
</tr>
<tr>
<td><strong>Remission</strong></td>
<td>Closure of individual fistulae defined as no fistula drainage despite gentle finger compression. Remission defined as closure of all fistulae that were draining at baseline for at least 2 consecutive visits (i.e., at least 4 weeks).</td>
</tr>
</tbody>
</table>
### 8.2 Perianal Disease Activity Index

<table>
<thead>
<tr>
<th>Categories</th>
<th>Score</th>
</tr>
</thead>
<tbody>
<tr>
<td>Discharge</td>
<td></td>
</tr>
<tr>
<td>No discharge</td>
<td>0</td>
</tr>
<tr>
<td>Minimal mucous discharge</td>
<td>1</td>
</tr>
<tr>
<td>Moderate mucous or purulent discharge</td>
<td>2</td>
</tr>
<tr>
<td>Substantial discharge</td>
<td>3</td>
</tr>
<tr>
<td>Gross faecal soiling</td>
<td>4</td>
</tr>
<tr>
<td>Pain/restriction of activities</td>
<td></td>
</tr>
<tr>
<td>No activity restriction</td>
<td>0</td>
</tr>
<tr>
<td>Mild discomfort, no restriction</td>
<td>1</td>
</tr>
<tr>
<td>Moderate discomfort, some limitation activities</td>
<td>2</td>
</tr>
<tr>
<td>Marked discomfort, marked limitation</td>
<td>3</td>
</tr>
<tr>
<td>Severe pain, severe limitation</td>
<td>4</td>
</tr>
<tr>
<td>Restriction of sexual activity</td>
<td></td>
</tr>
<tr>
<td>No restriction in sexual activity</td>
<td>0</td>
</tr>
<tr>
<td>Slight restriction in sexual activity</td>
<td>1</td>
</tr>
<tr>
<td>Moderate limitation in sexual activity</td>
<td>2</td>
</tr>
<tr>
<td>Marked limitation in sexual activity</td>
<td>3</td>
</tr>
<tr>
<td>Unable to engage in sexual activity</td>
<td>4</td>
</tr>
<tr>
<td>Type of perianal disease</td>
<td></td>
</tr>
<tr>
<td>No perianal disease/skin tags</td>
<td>0</td>
</tr>
<tr>
<td>Anal fissure or mucosal tear</td>
<td>1</td>
</tr>
<tr>
<td>&lt;3 Perianal fistulae</td>
<td>2</td>
</tr>
<tr>
<td>≥3 Perianal fistulae</td>
<td>3</td>
</tr>
<tr>
<td>Anal sphincter ulceration or fistulae with significant undermining of skin</td>
<td>4</td>
</tr>
<tr>
<td>Degree of induration</td>
<td></td>
</tr>
<tr>
<td>No induration</td>
<td>0</td>
</tr>
<tr>
<td>Minimal induration</td>
<td>1</td>
</tr>
<tr>
<td>Moderate induration</td>
<td>2</td>
</tr>
<tr>
<td>Substantial induration</td>
<td>3</td>
</tr>
<tr>
<td>Gross fluctuance/abscess</td>
<td>4</td>
</tr>
</tbody>
</table>
### 8.3 Crohn’s Disease Activity Index

<table>
<thead>
<tr>
<th>Clinical or laboratory variable</th>
<th>Weighting factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of liquid or soft stools each day for seven days</td>
<td>x 2</td>
</tr>
<tr>
<td>Abdominal pain (graded from 0-3 on severity) each day for seven days</td>
<td>x 5</td>
</tr>
<tr>
<td>General well being, subjectively assessed from 0 (well) to 4 (terrible) each day for seven days</td>
<td>x 7</td>
</tr>
<tr>
<td>Presence of complications*</td>
<td>x 20</td>
</tr>
<tr>
<td>Taking Lomitil or opiates for diarrhoea</td>
<td>x 30</td>
</tr>
<tr>
<td>Presence of an abdominal mass (0 as none, 2 as questionable, 5 as definite)</td>
<td>x 10</td>
</tr>
<tr>
<td>Absolute deviation of Hematocrit from 47% in men and 42% in women</td>
<td>x 6</td>
</tr>
<tr>
<td>Percentage deviation from standard weight</td>
<td>x 1</td>
</tr>
</tbody>
</table>
9 Appendix 3

9.1 Prospective database data collection forms
### Fistula Surgery Database Form

**Date of operation:**
- Surgeon's name (if RRO then 3-4)

**Surgery type:** (not all that apply)
- Rising
- Drains
- Drainage of abscess
- Staged
- Granulation
- Inguinal hernia
- Perforated ulcer
- Other abdominal/pelvic procedures

**Adjuvant therapy:**
- Partial thickness
- Full thickness
- Bowel fistula

**Complications:**
- Partial
- Perforated
- Suppurating
- Ulcer
- Other

* 1 = Performed unoccupied
  2 = Performed, Supervisor attended
  3 = Performed, Supervisor unattended
  4 = Not done

**Special notes:**
- Discharge home
- Estimated hospital stay
- Other complications

---

### Fistula Progress Form

**Date of assessment:**
- Date of fistula surgery
- Status pericarditis or other

**Any additional following surgery:**
- Continuities
- Incision

**Complications:**
- Yes
- No
- Others

---

### Operative Assessment:

**Number of external seams (show on sketch):**
- Number of external sutures placed on skin
- Number of external sutures placed on diseased tissue

**Is there evidence of secondary infection?**
- Drainage of abscess
- Other abdominal/pelvic procedures

**Internal opening:**
- Partial
- Intestinal
- Other

**Fistula:**
- Yes
- No

**Urethra:**
- Yes
- No

**Other:**
- Yes
- No

---

**Patient to complete this section only please:**

**Mark's Description Score:**
- Please mark an X in the appropriate box for each question
- The following give guidelines as to the seven to be marked:

**Wound:**
- In the past 4 weeks
- Within 4 weeks
- More than 4 weeks

**Wound:**
- Every 1 or more episodes a week but less than 2 x week
- Every 2 or more episodes a week

**How often:**
- Never
- Rarely
- Occasionally
- Weekly
- Daily

**Are you uncertain of solid bowel movements?**
- Yes
- No

**Are you uncertain of liquid bowel movements?**
- Yes
- No

**Are you unable to control urination?**
- Yes
- No

**Have you ever had a bowel movement that caused you to be troubled?**
- Yes
- No

**Do you ever need to use a pad or two a pad?**
- Yes
- No

**Do you take cabergoline medications?**
- Yes
- No

**Can you hold for at least 15 minutes with upper arm in room to empty your bowel?**
- Yes
- No

**Have you ever had a bowel movement that caused you to be troubled?**
- Yes
- No

**Do you ever need to use a pad or two a pad?**
- Yes
- No

**Have you ever had a bowel movement that caused you to be troubled?**
- Yes
- No

---

### Checklist:
- Fistula surgery database form
- Fistula surgery database form
- Completed progress form
- Please send to the VRO research fellow

---

**Is there evidence of recurrence?**
- Yes
- No

**Is patient cured from fistula?**
- Yes
- No

---

**Overall satisfaction with your care at St Mark's:**
- EXCELLENT
- GOOD
- FAIR
- POOR

**Thank you for completing the form. Your answers remain confidential and will allow us to improve the care provided at this hospital.**

---

**216**
Reference List


