Serum-free light chains in HIV-associated lymphoma: no correlation with histology or prognosis

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Objectives: Serum-free light chains (sFLCs) are a biomarker of B-cell proliferation. Two case-control studies found elevated levels of polyclonal sFLCs predict the development of HIV-associated lymphomas (HALs) in people living with HIV. This effect appears greater for non-Hodgkin's lymphomas than Hodgkin's lymphoma. In this study, we measured sFLCs at diagnosis of HALs, and correlated levels with histology and survival.

Methods: The clinic database of the National Centre for HIV Malignancy was used to identify HAL patients, in the antiretroviral treatment era. Levels of sFLCs were measured using stored sera (cases from 1996 to 2008) and prospectively from 2008 to 2014. Serum immunoglobulins were available for 201 patients. We assessed correlations between sFLCs, serum immunoglobulins, and histological subtypes and overall survival.

Results: Two hundred and sixty-four patients were identified and 70% had polyclonal sFLC, 8% monoclonal sFLC (90% kappa sFLC), and 22% normal sFLC levels. No significant difference in sFLCs was observed between the three major histological subtypes of HAL (Hodgkin's lymphoma, diffuse large B-cell lymphoma, and Burkitt lymphoma). Elevated sFLCs did not influence overall survival in HAL or for the three subtypes individually.

Discussion: Whilst these data confirm the finding of elevated sFLC in HAL, there was no significant difference in sFLC measurements between histological subtypes despite differences in pathogenesis. sFLC did not predict survival in HAL overall or by histological subtype. Elevated sFLCs may predict HAL, but measurement of sFLCs has limited utility in the classification and prognostication of these cases.

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Introduction

Both high-grade non-Hodgkin's lymphoma (NHL) and Hodgkin's lymphoma occur at an increased frequency amongst people living with HIV (PLWH). Although there has been a fall in the incidence of NHL since the introduction of combined antiretroviral therapy (cART), HIV-associated lymphoma (HAL) remains a significant

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cause of morbidity and mortality [1]. Whilst the pathogenesis of HAL is incompletely understood, HIV-induced B-lymphocyte proliferation and reactivation of Epstein–Barr virus (EBV) in latently infected lymphocytes are both implicated. Biomarkers of B-cell proliferation, EBV reactivation and immune activation have all been evaluated as risk factors for the development of HAL in cohort studies [2–5]. A recently published case-controlled study of serum biomarkers in PLWH identified a set of 17, including chemokines, cytokines, acute-phase proteins, tissue remodelling agents and bone metabolic mediators, which were significantly altered in those with NHL compared to matched controls [6].

Immunoglobulin light chains are typically bound to one of the five heavy-chain immunoglobulins (IgA, IgD, IgE, IgG and IgM) as an antibody. However, unbound light chains may be detected in the serum, known as serum-free light chains (sFLCs). There are two types of sFLCs: kappa (κ) and lambda (λ). In HIV, there is a paradoxical B-cell activation that leads to increased levels of immunoglobulins, including sFLCs.

Two case-control studies have found that elevated levels of polyclonal sFLCs predict the development of HAL [2,4], and one of these suggested that the effect was greater for NHL than for Hodgkin's lymphoma [4]. As sFLCs are biomarkers for B-cell proliferation, differences in pathogenesis of the histological subtypes of HAL may account for these findings. Elevated sFLCs at diagnosis have also been shown to be associated with poor outcomes in HIV-seronegative patients with diffuse large B-cell lymphoma (DLBCL) [7] and Hodgkin's lymphoma [8]. The National Centre for HIV Malignancy at Chelsea and Westminster Hospital is one of the largest centres in Europe, and we prospectively collected routine data on PLWH who attended since 1986. In this study, we aimed to measure sFLCs at diagnosis of HAL, and correlate levels with histology and survival.

Methods

We used the clinic database to identify patients with systemic HAL diagnosed in the post-cART era (1996–2008) and retrospectively retrieved serum samples stored at HAL diagnosis prior to chemotherapy (186 samples). These samples were measured for sFLCs using the Freelite assay at The Binding Site Group Ltd. Birmingham, UK. We prospectively measured sFLCs in a further 78 PLWH at HAL diagnosis prior to chemotherapy between 2008 and 2014. Monoclonal elevation of FLC is defined as an elevated FLC, with the corresponding FLC κ-to-λ ratio outside the reference range (0.26–1.65). Polyclonal elevation of FLC is defined as an elevation of either κ or λ outside the laboratory normal range, but with a normal κ-to-λ ratio [9]. Serum immunoglobulin levels prospectively measured prior to chemotherapy were available for 201 of the 264 patients.

Comparison of variables between groups was done by chi-square test for nominal variables and by Kruskall–Wallis test for non-parametric variables. Survival was calculated from the day of HAL diagnosis until death or the date of last follow-up. Univariate Cox's proportional-hazards regression analysis was used to determine the prognostic significance of serum FLCs and immunoglobulin variables at presentation of HAL [10].

The study received funding from the Joint Research Committee of Chelsea and Westminster Hospital NHS Foundation Trust. Freelite assays for retrospective samples were supplied by The Binding Site Group Ltd. Birmingham, UK. A favourable ethical opinion to conduct the research was obtained from the UK National Research Ethics Service.

Results

In total, 264 PLWH with systemic HAL in the post-cART era had sFLCs measured at HAL diagnosis (129 DLBCL, 59 Hodgkin's lymphoma, 40 Burkitt lymphoma, 14 plasmablastic lymphoma, seven T-cell lymphoma, nine primary effusion lymphoma and six other low-grade lymphomas). The mean age was 45 years (range 19–78 years); 231 (88%) were men and the median $CD4^+$ cell count was 178/μl (range 0–1160). At HAL diagnosis, 137 (52%) were on cART, of whom 87 (64%) had an undetectable HIV viral load.

Overall, 192 (73%) had an elevated κ sFLC (median 28 mg/l, range 2–932) including 21 with monoclonal κ sFLC. Similarly, 162 (61%) had an elevated λ sFLC (median 33 mg/l, range 5–7802) including two with monoclonal λ sFLC. Overall, 205 (78%) had an elevated κ and/or λ sFLC, although only 21 (8%) had a monoclonal sFLC.

For 201 patients, heavy-chain immunoglobulin levels were also measured. Ninety (45%) had an elevated IgG (median 15 g/l, range 2.5–43), 27 (14%) elevated IgM (median 0.93 g/l, range 0.006–16) and 108 (54%) elevated IgA (median 3 g/l, range 0.2–30). However, there were reduced levels of heavy-chain immunoglobulin in 47 (24%) patients [5 (3%) low IgG, 7 (4%) low IgA and 42 (21%) low IgM].

Analysis of the sFLC and immunoglobulin levels according to histology of HAL has been performed for the three major histological subtypes: DLBCL, Burkitt lymphoma and Hodgkin's lymphoma (see Table 1). There are no significant differences in the levels of κ or λ sFLCs, in the proportion of patients with elevated levels of κ and/

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Table 1. Results of serum immunoglobulin and free light chain measurements in HIV associated lymphomas according to histological subtypes.

	Diffuse large B-cell lymphoma ($n=129$)	Burkitt lymphoma ($n=40$)	Hodgkin's lymphoma ($n=59$)	P
Serum-free light chain measurements				
Median kappa (mg/l) (range) laboratory reference range 3.3–19.4	29 (5.8–62)	28 (2.0–571)	29 (4.9–932)	KW $P=0.99$
Median lambda (mg/l) (range) laboratory reference range 5.7–26.3	32 (7.7–262)	31 (5.5–389)	37 (5.1–160)	KW $P=0.86$
Elevated kappa (%)	97/129 (75%)	27/40 (67%)	45/59 (76%)	χ^2 $P=0.57$
Elevated lambda (%)	81/129 (63%)	22/40 (55%)	37/59 (63%)	χ^2 $P=0.84$
Elevated either (%)	103/129 (80%)	30/40 (75%)	46/59 (78%)	χ^2 $P=0.80$
Monoclonal sFLC (%)	8/129 (6%)	6/40 (15%)	4/59 (7%)	χ^2 $P=0.37$
Serum immunoglobulin measurements				
Median IgA (g/l) (range) laboratory reference range 0.8–4.0	3.1 (0.27–31)	2.3 (0.62–6.4)	3.3 (0.73–9.4)	KW $P=0.043$
Median IgG (g/l) (range) laboratory reference range 5.3–16.5	16 (2.6–43)	16 (5.5–43)	15 (6.4–39)	KW $P=0.63$
Median IgM (g/l) (range) laboratory reference range 0.5–2.0	1.1 (0.08–17)	1.1 (0.13–9.9)	0.70 (0.17–2.1)	KW $P=0.011$
Elevated IgA (%)	51/87 (58%)	13/36 (36%)	30/59 (51%)	χ^2 $P=0.06$
Elevated IgG (%)	43/88 (49%)	18/36 (50%)	22/52 (42%)	χ^2 $P=0.70$
Elevated IgM (%)	15/88 (17%)	5/36 (14%)	2/52 (4%)	χ^2 $P=0.07$
Elevated any immunoglobulin (%)	67/88 (76%)	22/36 (61%)	35/52 (67%)	χ^2 $P=0.21$

KW, Kruskal-Wallis; sFLC, serum-free light chain.

or λ sFLCs, or in the proportion with monoclonal sFLCs between the different histological subtypes of HAL. Patients with Hodgkin's lymphoma have significantly higher levels of IgA and lower levels of IgM than patients with DLBCL or Burkitt lymphoma.

The median follow-up for the entire cohort was 6 years and the 5-year overall survival from HAL diagnosis was 62% [95% confidence interval (CI) 56–68]. In Cox proportionate-hazards model, elevated κ sFLC ($P=0.50$), λ sFLC ($P=0.69$) or monoclonal elevation of sFLC ($P=0.57$) did not correlate with overall survival. Adjusted Cox models were performed for the three common histological subtypes. For DLBCL, the 5-year overall survival was 55% (95% CI 45–65), but again the sFLCs did not correlate with survival: κ sFLC ($P=0.83$), λ sFLC ($P=0.92$) or monoclonal elevation of sFLC ($P=0.97$). For Burkitt lymphoma, the 5-year overall survival was 54% (95% CI 40–68), but the sFLC did not correlate with survival: κ sFLC ($P=0.43$), λ sFLC ($P=0.96$) or monoclonal elevation of sFLC ($P=0.29$). Finally, for Hodgkin's lymphoma, the 5-year overall survival was 87% (95% CI 78–96) and the sFLC did not correlate with survival: κ sFLC ($P=0.84$), λ sFLC ($P=0.21$).

Discussion

Published case–control studies demonstrate that elevated sFLCs in PLWH increase the risk of subsequent HAL [2,4]. These two studies include a total of 96 patients with NHL and 16 with Hodgkin's lymphoma. Here, we show that over 70% of 264 PLWH have elevated levels of sFLC at the time of HAL diagnosis, including 8% with monoclonal elevations. The pathogenesis of the three major histological subtypes of HAL is thought to differ: Hodgkin's lymphoma is associated with higher $CD4^+$ cell counts and latency type II EBV infection, Burkitt lymphoma occurs at higher $CD4^+$ cell counts with chromosomal translocations between immunoglobulin gene and c-myc oncogene, whilst DLBCL occur at lower $CD4^+$ cell counts and often harbour the most permissive latency III pattern of EBV expression. Nevertheless, sFLCs – biomarkers of B-cell proliferation are over-produced in 75–80% of all three histological subtypes of HALs, with no significant differences in sFLC levels between the subgroups, suggesting that B-cell proliferation may play a role in the pathogenesis of all three.

The relationship between sFLC and prognosis has been evaluated in HIV-seronegative patients with DLBCL and Hodgkin's lymphoma. In 295 DLBCL patients, 32% had elevated sFLC at diagnosis, and elevated levels correlated with a poor overall survival after correction for other known risk factors [7]. Similarly, a recent study of 175 patients found that monoclonal sFLCs were present in 19% and conferred a worse prognosis, particularly in patients with non-germinal centre B-cell (non-GCB) type DLBCL. Conversely, polyclonal FLC, present in 36%, was associated with a poor prognosis in germinal centre B-cell (GCB)-type DLBCL [11]. It has been postulated that monoclonal sFLCs occur more frequently in the aggressive non-GCB subtypes of DLBCL [12]. Sub-classification of DLBCL into GCB and non-GCB subtypes has not been prospectively performed on this series of tumours. In an analysis of 100 HIV-seronegative patients with Hodgkin's lymphoma, 30% had elevated sFLC at diagnosis and again this conferred a worse prognosis [8].

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Our findings in this series of 264 people with HAL differ from reports in the HIV-seronegative population. We found a greater proportion of PLWH had elevated sFLCs at lymphoma diagnosis, but there was no significant correlation with overall survival in patients with DLBCL, Hodgkin's lymphoma or Burkitt lymphoma. The B-cell proliferation and polyclonal hypergamma globulinaemia that is a feature of HIV infection may account for these differences. PLWH and Hodgkin's lymphoma have significantly higher levels of IgA and lower levels of IgM than patients with DLBCL or Burkitt lymphoma, a finding that has been recognised in the HIV-negative population for decades [13,14].

In conclusion, elevated sFLCs are found in over 70% PLWH at HAL diagnosis, including 8% monoclonal sFLCs, but elevated sFLCs do not correlate with histological subtypes or overall survival. Thus, they have limited value as a prognostic biomarker, although there is some evidence that elevated levels predict lymphoma development in PLWH.

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Conflicts of interest

There are no conflicts of interest.

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