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ORIGINAL ARTICLE

Serum-free light chains adjusted for renal function are a potential biomarker for post-transplant lymphoproliferative disorders

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Abstract

Post-transplant lymphoproliferative disease (PTLD) is a serious complication of solid organ transplantation. As early diagnosis remains challenging, we investigated the utility of serum-free light chain (FLC) and heavy chain/light chain pairs (HLC) as diagnostic biomarkers. Pre-treatment serum FLC and HLC levels were measured in 20 patients at their first diagnosis of B cell PTLD and in 14/20 patients during follow-up. Results were compared to serum FLC/HLC levels of 90 matched PTLD-free transplanted controls. Renal dysfunction was common in both cohorts, and combined FLC levels were often elevated above the conventional upper limit of normal (45.7 mg/L). Combined FLC levels were higher in patients with PTLD than in transplant controls ($p = 0.013$), and levels above the conventional ULN were associated with PTLD (OR 3.2, $p = 0.05$). Following adjustment to cystatin C as a marker of renal function an even stronger association was found for a (dimensionless) threshold value of 37.8 (OR 8.9, $p < 0.001$). In addition, monoclonal proliferation (abnormal FLC ratio, using an established renal range cutoff) was more common in PTLD than in controls (3/20 vs. 2/90, $p = 0.04$). Following therapy, at the time of protocolised restaging, patients experiencing subsequent sustained complete remission displayed lower FLC levels than those not experiencing such remission ($p = 0.053$). No relationship with HLC results was seen. Elevated polyclonal FLC levels (especially when adjusted for renal function) and monoclonal proliferation are a potential biomarker for PTLD diagnosis and disease surveillance. However, prospective validation is necessary before FLC measurement should be incorporated in follow-up of transplant recipients and PTLD management.

Keywords PTLD · Serum-free light chain · Serum-free heavy chain · Biomarker

Abbreviations

CHOP	Chemotherapy with cyclophosphamide, doxorubicin, vincristine and prednisone
cFLC	Combined free light chain
CR	Complete remission
CRP	C-reactive protein
DLCBL	Diffuse large B cell lymphoma
EBV	Epstein–Barr virus
ECOG	Eastern Cooperative Oncology Group
FLC	Free light chain

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FLC_{adj}	Adjusted free light chain
HLC	Serum heavy/light chain
MALT	Mucosal-associated lymphoid tumour
OR	Odds ratio
PTLD	Post-transplant lymphoproliferative disorder
ROC	Receiver operating characteristic
ULN	Upper limit of normal

Introduction

The development of post-transplant lymphoproliferative disease (PTLD) is a serious complication of solid organ transplantation [1]. The clinical presentation is varied, with potential for delayed diagnosis. As a result, there is interest in rapid, available and standardised non-invasive markers for early identification of PTLD. One common consequence of lymphoproliferative disease is abnormal immunoglobulin light chain synthesis, including (i) production of excess clonal immunoglobulin light chain and/or (ii) disturbances in polyclonal light chain production. The recent development of assays to quantify serum levels of immunoglobulin light chain (serum-free light chain FLC) has allowed the quantitative assessment of monoclonal and polyclonal changes in light chain levels in disease states. This has been best characterised for multiple myeloma, but there is also increasing evidence for a diagnostic role in other haematological malignancies, infections and chronic disease [2, 3].

There have been two studies of serum FLC levels in PTLD to date [4, 5]. Engels et al. analysed serum from 29 patients with PTLD and compared to case controls. They showed an increased risk of the development of PTLD in patients who had a monoclonal elevation in FLC before the diagnosis of PTLD [4]. Furthermore, polyclonal elevation of FLC was common and also associated with the development of PTLD. In contrast, a smaller study of 10 patients was unable to confirm these associations [5].

The purpose of the current study was to further define and refine the utility of serum FLC levels in PTLD. We carefully evaluated renal function in this context, as measured by cystatin C. This was an important omission from previous studies as serum FLC are freely cleared by the kidneys; therefore, renal impairment is a major confounder in studies of FLC levels [6]. Furthermore, we quantified heavy chain/light chain for assessment of clonal expansion of intact immunoglobulin production that has been observed in a variety of settings including non-Hodgkin lymphoma [7, 8]. Finally, we explored whether changes in these measurements were associated with early and medium-term remission from PTLD.

Material and methods

Cases and controls

Subjects comprised 20 patients with a confirmed diagnosis of B cell PTLD included in the PTLD-1 trial or the prospective German PTLD registry. CD20-positive subtypes such as polymorphic, DLBCL-, MALT- or Burkitt-PTLD were treated in (or in analogy to) the PTLD-1 trial; until 2007, patients received sequential treatment with four courses of rituximab monotherapy followed by four cycles of CHOP chemotherapy [9]. Starting in 2006, protocol treatment was amended to risk-stratified sequential treatment: patients in a complete response after rituximab induction received rituximab consolidation and all others received R-CHOP immunochemotherapy consolidation [10]. The treatment of rare histological PTLD-subtypes was recently summarised [11]. All patients had available serum samples at the time of diagnosis of PTLD. The samples were taken between 1998 and 2011 and had been stored at −20 °C in batches of 1 mL to avoid multiple freeze/thaw cycles. Fourteen of the 20 cases also had after therapy samples available that were taken at restaging 4 weeks after completing therapy and in follow-up intervals of 3 months during the first 2 years.

Serum samples were collected within 7 days of lymphoma diagnosis in all patients and none of them underwent immunosuppression reduction, immunotherapy or chemotherapy prior to sample collection. To control for FLC production caused by inflammation, CRP levels at the time of sample collection were retrieved from patient files. Control cases were selected from liver and kidney transplant patients at the University Hospital of Kiel (Germany) and the Queen Elizabeth Hospital Birmingham (UK). Control patients had no sign of PTLD, infection or any other inflammatory disease at time of inclusion and were individually matched by sex, age at time of transplantation and time from transplantation.

Ethical approval for this study was provided by the local committees of both institutions. The study was conducted according to the guidelines of the Declaration of Helsinki.

Laboratory assay

Serum FLC concentration was determined in all samples by using a quantitative FLC assay (Freelite; the Binding Site, Birmingham, UK) performed on an SPA Plus Analyser. To detect free kappa (κ) and free lambda (λ) light chains assays were undertaken for each isotype. Results are reported as levels of free kappa, free lambda or combined free light chain levels (kappa + lambda). Polyclonal FLC levels are predominantly reported as combined FLC (cFLC) levels, reflecting the hypothesis and aims of the study; results for individual isotypes are also reported. Monoclonal FLC elevations were defined as an abnormal kappa/lambda FLC ratio in association

with elevation of the involved isotype. A normal ratio with elevated kappa and/or lambda levels was considered as polyclonal elevation. For adjustment of FLC levels to renal function, cystatin C concentration was measured in the same available samples by the SPA Plus Analyser.

The established 'conventional' upper limit of normal (ULN) for combined free light chains is 45.70 mg/L, with a 'normal' free light chain ratio considered as values between 0.26 and 1.65 [12]. But the presence of renal dysfunction is known to alter both these parameters. Specifically, polyclonal FLC may be elevated many fold in patients with renal impairment, although an upper limit of normal in this context is not established. Similarly, normal ranges for FLC adjusted for cystatin C levels, which represents the best established renal function marker for such adjustment, are also not established [6, 12]. The normal range for FLC ratio in patients with renal impairment ('renal range' FLC ratio) has been suggested as 0.37–3.10 [12].

Serum heavy/light chain (HLC) quantification were carried out using a serum HLC assay (Heavy light, The Binding Site, Birmingham, UK) performed on the SPA Plus analyser. The HLC assay consisted of separated assays to detect the respective heavy chain and light chain pairs, and therefore provides quantifiable values that identify a clonal intact immunoglobulin. The assay is available for IgGκ and IgGλ, IgAκ and IgAλ and IgMκ and IgMλ. The ratio between κ and λ chains for each Ig can be calculated and has a recognised reference range. Similar to the interpretation of FLC ratios, an abnormal HLC ratio (for any of the Ig isotype heavy and light chain pairs) is suggestive of monoclonal proliferation.

EBV

EBV association of PTLD was detected histologically by staining for EBNA-1, LMP-1 and ZEBRA, or by EBER in situ hybridization in cases of negative EBV-protein expression. The lymphoma sample was considered EBV associated if at least one of the assays gave a positive result.

Statistical analysis

Data are presented as mean ± standard deviation or median (range) where appropriate. Most FLC and HLC data was non-normally distributed and non-parametric analysis was undertaken, specifically Mann–Whitney test for independent data and Wilcoxon signed-rank test for paired data. Categorical data was analysed using Fisher's exact test. Receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive performance of the studied assays. The 'optimal cutoff' was defined as the value for which the sum of sensitivity and specificity was maximal. With PTLD as the outcome measure, logistic regression analysis was undertaken to evaluate the effect of FLC and HLC parameters as independent predictor variables. These analyses were adjusted for other baseline demographic measures as described in the results section. For all analyses, a type 1 error rate ≤ 5% ($p \leq 0.05$) was considered statistically significant. SPSS version 22.0 was used for analysis.

Results

Patients and controls

Of the 20 patients diagnosed with PTLD (Table 1), 25% were female. The recipients had a median age of 43 years at time of solid organ transplantation and developed PTLD after a median of 3.9 years. PTLD was associated with Epstein–Barr virus in 11 of 20 cases (55%, negative in 40%, no data available in 5%) and 16 of 20 patients each had either received a liver (40%) or kidney (40%) transplant. PTLD was monomorphic in 19/20 patients. In these, the most frequent histological subtype was diffuse large B cell lymphoma (DLBCL, $n = 11$) with 2 cases for each of Burkitt-, Hodgkin-, MALT- and plasmacytoma-like PTLD. None of the patients with plasmacytoma-like PTLD displayed bone marrow involvement or osteolytic lesions; one of these patients had been included in the series of plasmacytoma-like PTLD published recently by our group showing that this form of PTLD is different from multiple myeloma in the non-immunosuppressed host and that excessive paraprotein levels are rare in plasmacytoma-like PTLD [13]. Only 3 patients (15%) had been diagnosed with stage I disease, with 85% undiagnosed until advanced stages of disease (Ann Arbor stage III/IV). Localised treatment strategies, specifically irradiation to involved sites, had been applied in a single patient with Hodgkin-like PTLD, with all others receiving systemic treatment strategies. There were no significant differences between EBV-associated and non-EBV-associated cases for any of the FLC or HLC measures studied. Data on EBV DNAemia were not available.

Control patients were either liver or kidney transplant recipients representing the two major groups of patients with PTLD and had sample collection at a median of 4.1 years after transplantation ($n = 90$, range 0.1 to 17.9 years). There were no significant differences between patient and control baseline data.

Presence of abnormal FLC ratios at the time of first diagnosis of PTLD

Monoclonal elevations in free light chains were detected as an abnormal FLC ratio. Using the established 'conventional' range for FLC ratio of 0.26–1.65, abnormal values were found in 4/20 patients with PTLD and in 11/90 controls (20% vs. 12%, $p = 0.4$ by Fisher's exact test). Using the established 'renal' range of 0.37–3.1 [6], abnormal FLC ratios were significantly more

Table 1 Characteristics of solid organ transplant recipients

Characteristic	Subjects with PTLD (cases) $n = 20$	Subjects without PTLD (transplant controls) $n = 90$	p value
Female, *N* (%)	5 (25)	26 (29)	
Age at transplant in years, median (range)	43 (14–67)	48 (14–71)	0.727
Time post-transplant in years, median (range)	3.9 (0.2–18.2)	4.1 (0.1–17.9)	0.904
Organ type, *N* (%)			
Kidney	8 (40)	45 (50)	
Liver	8 (40)	45 (50)	
Heart	4 (20)	0 (0)	
PTLD pathology, *N* (%)			
Polymorphic	1 (5)	–	
Monomorphic	19 (95)	–	
PTLD nodal status, *N* (%)			
Nodal	3 (15)	–	
Extranodal	7 (35)	–	
Nodal + extranodal	10 (50)	–	
PTLD EBV status, *N* (%)			
Positive	11 (55)	–	
Negative	8 (40)	–	
n.d.	1 (5)	–	
PTLD type, *N* (%)			
DLBCL	11 (55)	–	
Hodgkin/Hodgkin-like Lymphoma	2 (10)	–	
Burkitt Lymphoma	2 (10)	–	
Polymorphic	1 (5)	–	
Plasmacytoma-like	2 (10)	–	
MALT	2 (10)	–	
Ann Arbor, *N* (%)			
I	3 (15)	–	
II	0 (0)	–	
III	4 (20)	–	
IV	13 (65)	–	

n, number; *PTLD*, post-transplant lymphoproliferative disorder; *EBV*, Epstein–Bar virus; *DLBCL*, diffuse large B cell lymphoma; *MALT*, mucosal-associated lymphoid tumour

frequent in patients with PTLD (3/20) than in controls (2/90) (15% vs. 2%; $p = 0.04$ by Fisher's exact test). One patient with PTLD and abnormal FLC had been classified as a 'plasmacytoma-like' PTLD. However, exclusion of this patient still resulted in a trend toward an association between abnormal FLC ratios and PTLD (10% vs. 2%, $p = 0.15$).

Elevated polyclonal free light chain levels at the time of first diagnosis of PTLD

Levels of free light chains demonstrated a non-parametric distribution with positive skewing and levels of kappa, lambda and combined FLC were frequently elevated above the predefined upper limit of normal (ULN) in both groups. However, median combined FLC levels were significantly higher in patients with first diagnosis of PTLD (69.94 mg/L [range 4.60 mg/L – 2774.44 mg/L]) than in controls (43.65 mg/L [range 10.65–272.97 mg/L], $p = 0.013$) and levels of cFLC above the 'conventional' ULN (45.70 mg/L) were significantly more frequent in patients with first diagnosis of PTLD than in controls (15/20 vs. 40/90, $p = 0.024$, Table 2). Logistic regression using the conventional ULN as 'cutoff' and adjusted for age, sex and time post-transplantation demonstrated an independent association of elevated cFLC levels and PTLD diagnosis with an odds ratio (OR) of 3.2 (95% CI 1.3–9.7, $p = 0.05$).

Table 2 Associations between circulating free light chain, heavy chain/light chain pairs and PTLD among solid organ transplant recipients

	PTLD (cases)	Transplant controls	*p* value	Normal range
Serum-free light chain level				
	n = 20, mg/L	*n* = 90, mg/L		mg/L
Kappa, median (range)	41.69 (2.47–2053.31)	22.05 (5.45–161.44)	0.015	3.30–19.40
Kappa above 19.4 mg/l (ULN) (%)	15 (75)	50 (56)	0.140	
Lambda, median (range)	32.06 (2.14–2304.00)	20.78 (5.20–128.00)	0.107	5.71–26.30
Lambda above 26.3 mg/l (ULN) (%)	12 (60)	30 (33)	0.040	
Ratio kappa/lambda, median (range)	1.20 (0.20–277.94)	1.00 (0.27–4.85)	0.218	0.37–3.10
Combined FLC, median (range)	69.94 (4.60–2774.44)	43.65 (10.65–272.97)	*0.013*	9.01–45.70
Combined FLC above 45.7 mg/L (ULN) (%)	15 (75)	40 (44)	*0.024*	
Cystatin C-adjusted serum–free light chain level				
	N = 20, mg/L	*N* = 90, mg/L		
Cystatin C, median (range)	1.91 (0.83–11.19)	1.74 (0.62–5.74)	0.086	0.56–0.99
Combined FLC adjusted, median (range)	35.74 (2.88–693.37)	24.80 (8.85–100.91)	0.020	
Combined FLC Adjusted above 37.76 (%)	10 (50)	10 (11)	< 0.001	
Serum heavy chain/light chain pair level				
	N = 20, g/L	*N* = 64, g/L		g/L
IgA kappa, median (range)	1.64 (0.13–6.12)	1.29 (0.25–6.30)	0.369	0.57–2.08
IgA lambda, median (range)	1.18 (0.18–4.61)	1.03 (0.03–4.09)	0.488	0.44–2.04
IgA ratio, median (range)	1.27 (0.70–1.75)	1.30 (0.77–7.00)	0.850	0.78–1.94
IgG kappa, median (range)	7.68 (0.89–15.38)	6.56 (2.87–20.15)	0.455	3.84–12.07
IgG lambda, median (range)	4.21 (0.74–9.80)	3.98 (1.46–10.00)	0.785	1.91–6.74
IgG ratio, median (range)	1.68 (0.71–18.03)	1.65 (0.69–13.36)	0.897	1.12–3.21
IgM kappa, median (range)	0.77 (0.05–5.61)	0.62 (0.13–3.21)	0.644	0.19–1.63
IgM lambda, median (range)	0.36 (0.04–4.54)	0.32 (0.07–1.86)	0.896	0.12–1.01
IgM ratio, median (range)	1.81 (0.49–2.39)	1.72 (0.79–2.96)	0.686	1.18–2.74

n, number; *ULN*, upper limit of normal

CRP level at time of sample collection was available in 16/20 patients and was determined 1.8 days prior to sample collection in mean (± 2.8 days). Median CRP levels were 1.9 mg/dL (range 0.1–20.9 mg/dL). Only 6 patients had CRP level higher than 5 mg/dL and only 3 patients (19%) had CRP level higher than 10 mg/dL. There was no significant correlation between CRP levels and any of the FLC or HLC measures studied and 2 of 3 patients with CRP levels above 10 mg/dL had cFLC levels close to median cFLC levels (66.9 mg/dL, 83.5 mg/L and 193.0 mg/dL).

Adjusting FLC levels to renal function by normalisation to serum cystatin C (both values reported as mg/L and thus dimensionless) again showed significantly higher combined FLC levels ($cFLC_{adj}$) in patients with first diagnosis of PTLD than in controls (35.74 [range 2.88–693.37] vs. 24.80 [range 8.85–100.91], Table 2, $p = 0.020$). Results were essentially similar following exclusion of the 3 patients with abnormal FLC ratios (data not shown). ROC curve analysis excluding the 3 patients with abnormal FLC ratios demonstrated an adjusted combined FLC level of 37.76 as the optimal diagnostic cutoff. Eight out of seventeen cases (47%) and 8/90 controls (9%) demonstrated $cFLC_{adj}$ levels above this cutoff resulting in a sensitivity of 57%, a specificity of 91%, a positive predictive value of 50% and a negative predictive value of 90%. Logistic regression analysis showed an OR of 8.9 (95% CI 2.7–29.5, $p < 0.001$). Logistic regression analysis addressing the combined role of either polyclonal FLC elevation identified by $cFLC_{adj}$ levels above 37.76 or monoclonal FLC production identified by an abnormal FLC ratio using the renal range showed an OR of 9.8 (95% CI 3.3–29.4, $p < 0.001$).

Elevated heavy chain/light chain pair levels at the time of first diagnosis of PTLD

No differences in median levels of any of the immunoglobulin heavy chain subclass ratio parameters were identified between cases and controls (Table 2). There were also no differences suggesting a more frequent monoclonal production of heavy chain/light chain pairs in PTLD. In fact, only one patient showed an abnormal HLC ratio. This was one of the two patients diagnosed with plasmacytoma-like PTLD.

Normalisation of FLC and HLC disturbances with response to PTLD treatment

Post-treatment serum samples were available in 14/20 patients at the time of final staging. Eight out of fourteen patients (57%) had reached complete response (CR) at final staging and remained in CR during a median follow-up of 1.5 years (range 0.8–8.5 years). Six out of fourteen patients did not reach CR. At the time of initial diagnosis and prior to the institution of therapy, there were no differences in any of the FLC or HLC levels between patients reaching CR and those showing stable disease or disease progression (non-responder, $n = 6$). However, at the time of protocol-driven restaging, patients in CR demonstrated significantly lower adjusted cFLC levels than non-responders: 22.63 (range 9.09–59.56) vs. 36.78 (range 25.68–98.69; Mann–Whitney $p = 0.053$) and a notable fall in median-adjusted cFLC levels was seen when pre- and post-treatment samples of responders were compared (Table 3, $p = 0.130$). No significant difference in any of the HLC ratio levels was identified between patients reaching CR and those who did not, although a numerical difference was found for IgG lambda: 3.4 (range 1.2–5.4) vs. 4.9 (range 2.3–10.1, $p = 0.07$). However, there was a significant fall of IgG lambda as well as IgG kappa and IgM kappa levels in paired pre- and post-treatment samples of patients reaching CR ($p \leq 0.05$ for all), but total numbers of patients with abnormal HLC ratios were too small to allow meaningful interpretation.

Discussion

In this study, we show that abnormalities in FLC detected by a sensitive, reproducible and available assay are associated with PTLD after solid organ transplantation. Our exploratory analyses also suggest that normalisation of FLC levels may be associated with clinical remission from disease.

The levels of FLC seen in the current study strikingly resemble those seen in the study by Engels, together suggesting that the results are generalizable to other populations [4]. Moreover, our results are in agreement with the findings of that study whereby polyclonal and monoclonal FLC elevations (separately and in combination) are associated with PTLD diagnosis. Whilst this was not found in another study by Fernando [5], the smaller size of that study was a limitation in robustly excluding an effect. Whilst FLC levels are a generic marker of inflammation, it is important to notice that in our adult patient series with a first diagnosis of PTLD after solid organ transplantation, most patients did not show clinically relevant CRP elevations. The few patients that did probably did not influence the result as their FLC levels were close to the median.

The results of the current study also extend previous work. Specifically, we have investigated the role of renal (dys)function in interpreting FLC levels in patients with kidney transplants (who by definition display chronic kidney disease) and in other forms of non-renal solid organ transplantation where kidney disease is commonly found [14]. There were two specific aspects of relevance in the current work. First, we used the 'renal' diagnostic range for FLC ratio, with values falling outside this range suggestive of monoclonality [6]. At least in the current series, this resulted in detecting a marked difference between PTLD cases and controls, which was not seen using the 'conventional' diagnostic range for FLC ratio. We therefore suggest that future work in patients with a significant burden of chronic kidney disease should consider investigating the utility of this 'renal' range.

Second, and on the basis that polyclonal FLCs are removed from the circulation by glomerular filtration and so are dependent on renal function, we adjusted the raw FLC levels to account for this confounder. Previous work has suggested the closest correlation between FLC levels and cystatin C as a renal excretory marker, and it has been suggested this adjustment be undertaken in renal cohorts [6, 15]. We hypothesised that this adjustment would improve the predictive utility of the FLC measurements, particularly as so many cases and controls displayed levels above the conventional upper limit of normal. Certainly, our data shows increasing odds ratios for PTLD diagnosis and associated increases in the level of significance as analyses sequentially examined: (i) 'conventional' upper limits of normal FLC levels and (ii) the optimal cutoff value in the analysis adjusted for renal function (cystatin C). Furthermore, the ROC curve analyses

Table 3 Pre- and post-therapeutic FLC level in the 8/14 patients reaching sustained complete response at final staging

	Prior to therapy	Final staging	*p* value	Normal range
Cystatin C-adjusted serum–free light chain level				
	n = 8, mg/;	*n* = 8, mg/L		mg/L
Cystatin C, median (range)	1.90 (1.32–11.19)	1.45 (1.19–2.04)	0.189	0.56–0.99
Kappa adjusted, median (range)	22.76 (8.00–42.05)	10.93 (4.07–36.6)	0.195	–
Lambda adjusted, median (range)	16.12 (7.39–205.91)	10.57 (5.02–22.93)	0.105	–
Combined FLC adjusted, median (range)	34.94 (16.54–247.95)	22.63 (9.09–59.56)	0.130	–

n, number

suggest potential predictive utility, which has not been previously examined in this context; the specificity of 91% for the diagnosis of PTLD seen with adjusted FLC levels represents a potential tool to 'rule in' disease. We acknowledge the limited sensitivity at this proposed 'cutoff" value and so limited utility to 'rule out' disease. We also acknowledge the importance of not over-interpreting such data, drawn from a single cohort and with margins of error around these point estimates. But adjusting FLC levels to renal function seems intuitive, and future prospective investigations in transplant cohorts should seek to define whether there is clear advantage in this approach, and whether the 'diagnostic' cutoff values we have identified here are robust and generalizable.

Engels proposed three (not necessarily mutually exclusive and potentially complementary) hypotheses to explain the link between elevated FLCs and PTLD [4]. First, polyclonal B cell activation may be a core component of lymphoproliferative disease, with polyclonal FLC production by activated (albeit not clonal) B cells [3, 16, 17]. Second, monoclonal FLCs are released by the clonally proliferating lymphoma cells themselves [18]. Third, FLC elevations reflect EBV proliferation and (by implication) EBV-associated lymphoma [19]. The current study supports the first two of these hypotheses, as we also found associations between polyclonal and monoclonal FLC elevations and PTLD. We cannot confirm or refute the third hypothesis, as no data regarding EBV viraemia was available in the dataset. PTLD specimens were stained for EBV-related proteins; no differences in FLCs between those expressing and not expressing such proteins were evident, but the sample size does not allow robust interpretation. Larger studies are required to investigate this issue more completely.

We also examined the relationship between FLC levels and clinical course. At the time of surveillance tumour restaging, levels of FLC_{adj} were lower in patients achieving sustained complete remission than in those with either partial remission or disease progression. This data potentially suggests a role for serial FLC measurements for disease surveillance.

In contrast, HLC levels seem to offer less utility in this setting. We were particularly interested to see if an abnormal HLC ratio, which is indicative of clonal proliferation and which has diagnostic value in the setting of non-transplant lymphoma, was associated with PTLD [7]. We failed to detect such an association. Although patients in complete remission experienced greater falls in (some) HLC levels, these exploratory data should be interpreted with caution. It should also be recognised that the fall in IgGκ and IgGλ seen with complete remission can be detected with more conventional measured of Ig levels, rather than specifically requiring this assay of both heavy and light chain pairs.

In summary, we believe the current study points to a role for FLC measurements in the diagnosis and possibly surveillance of PTLD in solid organ transplant recipients. We propose adjusting for renal function improves the diagnostic value of polyclonal FLC levels, and propose novel thresholds for this purpose. However, associations from case-control studies with a limited number of patients can be considerably influenced by non-controlled confounding variables. Prospective clinical trials therefore are necessary to confirm our findings and to determine the additional value of FLC levels in the management of PTLD.

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Author contributions RB and RUT are the principal investigators, coordinated the research and take primary responsibility for the paper. HZ, HR and RUT recruited the patients and collected clinical data. FB, RB and PC recruited the controls. AS did the laboratory work. AS, RB, PC and RUT analysed and interpreted the data. IA served as reference pathologists. AS, RB, PC, HZ and RUT wrote the paper. All authors had full access to the final version of the manuscript and agreed to publication.

Compliance with ethical standards

Ethical approval for this study was provided by the local committees of both institutions. The study was conducted according to the guidelines of the Declaration of Helsinki.

Disclosures The Binding Site provided FLC, HLC and cystatin C assays free of charge. The Binding Site played no role in the analysis of the clinical data, its interpretation or the manuscript preparation. The content of the manuscript remains the responsibility of the listed authors.

Conflicts of interest The authors declare that they have no conflict of interest.

Informed consent Informed consent was obtained from all individual participants included in the study.

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