Journal of Leukemia
ISSN: 2329-6917

Matschke et al., J Leuk 2014, 2:1
DOI; 10.4172/2329-6917.1000127

Research Article Open Access

Abnormal Free Light Chain Ratios are Significantly Associated with Clinical Progression in Chronic Lymphocytic Leukemia

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Abstract

Serum Free Light Chains (FLC) have prognostic significance in diverse plasma cell dyscrasias. Although monoclonal protein secretion is a typical feature of these diseases, it can also be detected in other B cell malignancies including chronic lymphocytic leukemia. Recent data suggests a significant correlation between abnormal ratio of FLC and outcome. Therefore, we investigated the role of FLC in a large cohort of 135 patients and the correlation to immunofixation (IF) and flow cytometry. Abnormal FLC ratios were found in 78 patients (58%) whereas the IF was positive in only 32 cases (24%). In 55 cases the FLC ratio was positive while IF was negative and in only 9 cases IF was positive while the FLC ratio was normal. In 52 of 98 patients (53%) light chain restriction determined by flow cytometry was concordant with the monoclonal FLC whereas in 5 patients they did not agree. In 41 of 98 patients (42%) a normal ratio of FLC was observed while the immunophenotype was positive for lambda or kappa.

Patients with an abnormal FLC ratio for lambda had a significantly shorter time to first therapy (TFT) than patients with an abnormal ratio for kappa FLC or with a normal FLC ratio (median TFT: 34 versus 76 versus 88 months, p for trend=0.039). Additionally, monoclonal FLC had a significantly shorter time to first treatment compared to polyclonal normal and abnormal FLC ratios (p for trend=0.0489). As expected, polyclonal sFLC correlated significantly with normal and abnormal serum-creatinine (p<0.0001). Future studies are warranted to elucidate the role of FLC as biomarkers of disease and as a prognostic factor for response.

Keywords: Chronic lymphocytic leukemia; Free light chains; Monoclonal protein; Prognostic marker

Introduction

Chronic Lymphocytic Leukemia (CLL) has a variable clinical course. Some patients have an excellent prognosis never requiring treatment, whereas in others survival is short despite early initiation of therapy. Staging systems devised by [1] and [2] are useful for predicting survival and treatment requirements in patients with CLL. However, these staging systems are of limited prognostic value in early stages of the disease (Binet A or Rai stage 0 to II) which today applies to most of the patients at first diagnosis. During the last few years there has been a continuous effort to identify novel prognostic factors in B-CLL which may help define patient subgroups that may benefit from early therapeutic intervention.

Molecular markers like IGVH mutation status, CD38 or ZAP70 expression are able to provide this information. However, the limitations of these new molecular markers are the cost and complexity of the technical procedures, the limitation in clinical use and the necessity for further standardization. Due to these limitations the focus of research has recently changed to new simple prognostic markers allowing wide-spread clinical practice. Currently, the prognostic influence of the number of smudge cells in CLL has been demonstrated [3,4].

Although secretion of monoclonal immunoglobulins is a typical feature of plasma cell dyscrasias, it can also be detected in other B cell malignancies including CLL [5]. Serum Free Light Chains (FLC) have prognostic significance in monoclonal gammopathy of undetermined significance, solitary plasmocytoma of bone, smouldering myeloma, multiple myeloma, Waldenstroms macroglobulinaemia and AL amyloidosis [5-8]. Thus, we were intrigued by three recent studies showing a significant correlation between abnormal ratio of FLC or sFLC levels and outcome in patients with CLL [9-12]. In this study we investigated the role of abnormal ratio of FLC in a large, well characterized cohort of 135 CLL patients. Moreover, we investigated the correlation of FLC ratio to immunofixation and flow cytometry.

Patients and Methods

Study population and CLL patients

Between 1999 and 2011, 135 patients with B-CLL were enrolled in this retrospective analysis and analyzed with regard to several biological and clinical characteristics: Binet stage, age, gender, CD38 and ZAP-70 status, IGHV status, β2-microglobulin, time from diagnosis to first treatment and overall survival. The diagnosis of B-CLL required a persistent B cell lymphocytosis of more than 5.0×10^9/l and a typical CD19+, CD20+ CD5+, CD23+, Ig light chain (κ or λ light chain) restricted immunophenotype as revealed by flow cytometry of peripheral blood cells. Comprehensive clinical information including treatment histories was available for all patients. Patient selection was based on the availability of cryopreserved plasma samples in our CLL cell bank collected at the time before the initiation of therapy or six months after finishing therapy.

Standard clinical criteria were used for the initiation of chemotherapy [13]. CD38 and ZAP-70 expression were determined by flow cytometry as recently described [14,15]. In Table 1 the clinical and laboratory data are shown.

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Received December 26, 2013; **Accepted** January 20, 2014; **Published** January 25, 2014

Citation: Matschke J, Eisele L, Sellmann L, Kalhori N, Nusch A, et al. (2014) Abnormal Free Light Chain Ratios are Significantly Associated with Clinical Progression in Chronic Lymphocytic Leukemia. J Leuk 2: 127. doi:10.4172/2329-6917.1000127

	All patients no. (%), n=135		Normal FLC ratio no. (%), n=57		Abnormal FLC-ratio (involving kappa) no. (%), n=60		Abnormal FLC-ratio (involving lambda) no. (%), n=18		p-value
Age at diagnosis years (median), range	60	(30-84)	61	(43-84)	61	(36-80)	56	(30-84)	0.027*
Sex (M/F ratio)	135	(100)	57	(42.2)	60	(44.4)	18	(13.3)	
M	94	(69.6)	41	(72.0)	40	(66.7)	13	(72.2)	
F	41	(30.4)	16	(28.0)	20	(33.3)	5	(27.8)	0.8
Binet stage	124	(100)	52	(41.9)	54	(43.6)	18	(14.5)	
A	77	(62.1)	33	(63.5)	36	(66.7)	8	(44.4)	
B	31	(25.0)	14	(26.9)	13	(24.1)	4	(22.2)	
C	16	(12.9)	5	(9.6)	5	(9.3)	6	(33.3)	0.09
IGVH status	63	(100)	24	(38.1)	29	(46.0)	10	(15.9)	
Unmutated	30	(47.6)	10	(41.7)	14	(48.3)	6	(60.0)	
Mutated	33	(52.4)	14	(58.3)	15	(51.7)	4	(40.0)	0.62
ZAP-70	75	(100)	30	(40.0)	33	(44.0)	12	(16.0)	
Positive	39	(52.0)	18	(60,0)	17	(51.5)	4	(33.3)	
Negative	36	(48.0)	12	(40.0)	16	(48.5)	8	(66.6)	0.29
CD38	125	(100)	54	(43.2)	54	(43.2)	17	(13.6)	
Positive	73	(58.4)	33	(61.1)	35	(64.8)	5	(29.4)	
Negative	52	(41.6)	21	(38.9)	19	(35.2)	12	(70.6)	0.03
ß2M	124	(100)	55	(44.4)	55	(44.4)	14	(11.3)	
Normal	27	(21.8)	20	(36.4)	6	(10.9)	1	(7.2)	
Abnormal	97	(78.2)	35	(63.6)	49	(89.1)	13	(92.9)	0.002
Immunofixation	133	(100)	56	(42.1)	60	(45.1)	17	(12.8)	
Positive	32	(24.1)	9	(16.0)	18	(30.0)	5	(29.4)	
Negative	101	(75.9)	47	(84)	42	(70.)	12	(70.6)	0.18
Creatinine	123	(100)	56	(45.5)	54	(43.9)	13	(10.6)	
Normal	60	(48.9)	24	(42.9)	26	(48.2)	10	(77.0)	
Abnormal	63	(51.2)	32	(57.1)	28	(51.8)	3	(23.0)	0.09

*Kruskal Wallis Test

Table 1: Patient characteristics. All three groups have been statistically analyzed and compared with each other

The levels of serum FLC were assessed using commercially available immunoassays (FREELITE, The Binding Site, UK) and quantified nephelometrically with the BNII analyzer (Siemens, Germany). These tests detect κ or λ FLC at a low concentration of 3 to 4 mg/L. An abnormal κ:λ ratio was defined as <0.26 or >1.65 [16]. Moreover, in all cases we evaluated the M band on Immunofixation (IF) using an approach combining standard serum electrophoresis with screening immunofixation (Hydragel 12IF, Sebia, Fulda, Germany). The screening IF uses a pentavalent antiserum that is electrophoresed in parallel to the standard electrophoresis on the same gel. Whenever a monoclonal protein was detected, confirmatory IF with classification of the monoclonal protein followed. A normal serum-creatinine was defined for women 0.6 to 1.1 mg/dl and men 0.6 to 1.3 mg/dl, as well as β2-microglobulin 0.8 to 2.4 mg/l. In Table 1 the characteristics of all treated and untreated patients are shown. The samples of 88 patients were taken before treatment, 42 samples were taken after treatment (at least 6 months interval from last therapy). In 5 samples therapy status was unknown.

Statistical analysis

The primary endpoint of this analysis was time to first treatment (TFT). TFT was calculated from the date of first diagnosis until first therapy. Times of patients who never got treated were censored. We used Kaplan-Meier survival analysis, the log-rank test and the log-rank test for trend to investigate the prognostic importance of FLC. Prognostic factors for TFT were analyzed using Cox's proportional hazards regression. Comparison of clinical or laboratory parameters between patient subgroups was performed using the non-parametric Mann-Whitney-U test for continuous variables, the Kruskal-Wallis test and the χ^2 test for categorical data. An effect was considered as statistically significant if the p-value of its corresponding test statistic was not larger than <0.05. All statistical computations were done using GraphPad Prism 3.0 (**GraphPad Software,** San Diego, CA, USA) or SPSS version 17 (SPSS, Chicago, IL, USA).

Results

Correlation of tumor cell immunophenotype, abnormal FLC ratio and immunofixation

First we correlated the clonal light chain of the CLL cells according to tumor cell immunophenotype with the ratio of the free light chains and IF. In 98 patients all parameters were available. In 52 of 57 patients (91%), excluding those patients with normal FLC ratio (41 patients), we observed the same clonal light chain in tumor cell immunophenotype as well as in the ratio of FLC whereas in 5 patients the clonal light chain did not agree. There was an association between light chain restriction determined by flow cytometry and serum FLC ratio (p<0.0001). However, a significant correlation between FLC ratio and tumor cell immunophenotype was not evident (p=0,18). Only 27 of 98 patients (28%) were positive in IF (Table 2).

Correlation between abnormal FLC ratio and immunofixation

Abnormal FLC ratios were found in 78 of 135 patients (58%) (Figure 1) whereas the IF was positive in only 32 cases (24%). In 54 cases the FLC ratio was abnormal while IF was negative and in 9 cases the IF was positive while the FLC ratio was normal. In total, 23 patients had both a positive IF and an abnormal FLC ratio (Table 3).

Light chain restriction by flow cytometry	Normal FLC-ratio	Abnormal FLC-ratio (involving kappa)	Abnormal FLC-ratio (involving lambda)	IF positive
Kappa	19	42	2	20
Lambda	22	3	10	7

Table 2: Association between light-chain restriction by flow cytometry and serum FLC ratio Correlation of the light chain restriction determined by flow cytometry with the ratio of the free light chains and immunofixation. In 98 patients the results of the clonal light chain in CLL patients were evaluable. There was an association between light chain restriction determined by flow cytometry and serum FLC-ratio ($p<0.0001$).

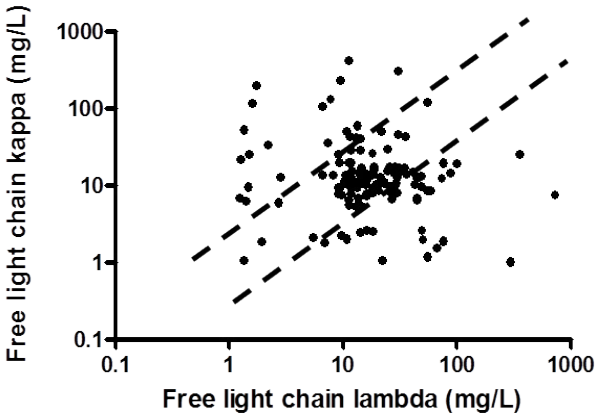

Figure 1: A scatterplot showing serum free kappa and lambda levels for 135 CLL patients In 42% of cases a normal FLC ratio (between the dashed lines) was demonstrated.

	All patients no. (%)		Normal FLC-ratio, no. (%)		Abnormal FLC-ratio, no. (%)	
Immunofixation	133	(100)	56	(42.1)	77	(57.9)
Positive	32	(24.1)	9	(28.2)	23	(71.9)
Negative	101	(75.1)	47	(46.5)	54	(53.5)

Table 3: Association between immunofixation and FLC ratio.

Association of abnormal FLC ratio and treatment free survival

Patients with an abnormal FLC ratio involving λ FLC had a significantly shorter Time To First Treatment (TFT) than patients with an abnormal ratio for κ and those with a normal FLC ratio (median TFT: 34 versus 76 versus 88 months, p for trend=0.039) (Figure 2a). Additionally, monoclonal FLC ratios had a significantly shorter time to first treatment compared to polyclonal normal and abnormal FLC ratios (p for trend=0.0489) (Figure 2b). Additionally, the trend in untreated (88 patients) and treated patients (42 patients) was nearly identical, but the p value was not significant due the small patient number. Therefore, we analyzed both patient groups together.

Multivariate analysis

Using multivariate Cox regression analysis, we compared the abnormal ratio of FLC with other prognostic factors - namely, age, gender, early and advanced clinical stage (Binet stage A vs. B/C), CD38 (cut off 30%) and ZAP-70 expression (cut off 20%) and genomic aberrations (deletion 17p and 11q). High risk cytogenetics (Hazard ratio (HR) 3.2; 95% Confidence interval (CI) 1.8-5.6; p=0.0001), positive CD38 status (HR 3.5; CI 1.7-7.3; p=0.001) and advanced stage according to Binet (HR 3.1; CI 1.7-5.5; p=0.0001) had independent power predicting an unfavorable prognosis.

Association of abnormal FLC ratio and established prognostic markers

As shown in Table 1 we could find an association of abnormal sFLC ratios and the established markers CD38 (p=0.03) and β2-microglobulin (p=0.002). However, there was no association between ZAP-70 (p=0.29) and IGHV (p=0.62).

Moreover, we analyzed the correlation between sFLC and serum-creatinine. In this CLL-cohort, in 13 of 123 patients (11%) we found both an abnormal FLC-ratio and renal dysfunction. A significant correlation between abnormal sFLC and renal damage was not evident (p=0.58) (data not shown). However, for polyclonal FLC´s a significant higher serum-creatinine was demonstrated (p<0.0001) (data not shown).

Combined analysis of abnormal FLC ratio with CD38 and ZAP-70

By summing the number of risk factors defined as abnormal FLC ratio, ZAP-70 and CD38 we separated the cohort into four subgroups (Figure 3). For those patients with no risk factor the TFT was not reached, for patients with one of the three markers the median TFT was 119 months, with two prognostic factors markers 43 months, and with all three markers 26 months (p<0.0001, Figure 3).

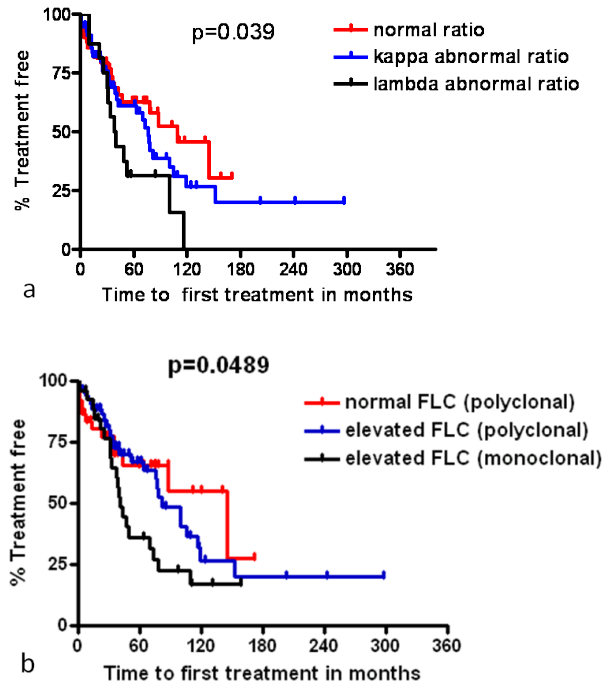

Figure 2: Association of Time to first treatment (TFT) and sFLC ratio Patients with an abnormal FLC ratio involving l FLC had a significantly shorter TFT than patients with an abnormal ratio for k and those with a normal FLC ratio (median TFT: 34 versus 76 versus 88 months, p for trend=0.039) (a). Monoclonal FLC had a significantly shorter time to first treatment compared to polyclonal elevated FLC and normal polyclonal FLC (p for trend=0.0489) (b). Differences in survival times were compared with the log-rank test and the log-rank test for trend.

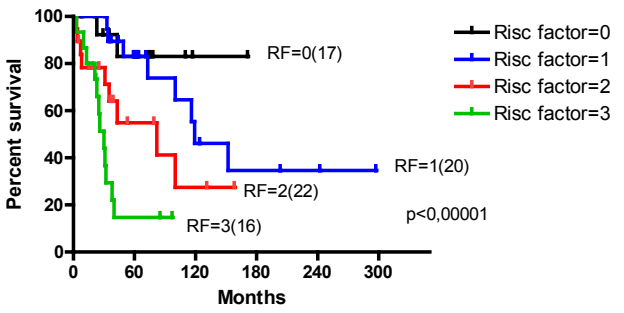

Figure 3: Time to first Treatment (TFT) survival curves in relation to combined analysis of abnormal FLC ratio with CD38 and ZAP-70
Kaplan Meier curves for TFT survival: median time to first treatment not reached (RF=0), 119 months (RF=1), 43 months (RF=2), 26 months (RF=3). Statistical analysis was performed using the log-rank test. RF=risk factor.

Discussion

So far, analyses of abnormal free light chain ratios were explored in multiple myeloma, monoclonal gammopathy of undetermined significance, solitary plasmocytoma, Waldenstrom´s macroglobulinemia and AL amyloidosis [5-8,17]. However, sFLC can be also detected in a substantial fraction of patients with CLL in about 30-40%. Recently, the first evaluation of non-plasma cell B-cell lymphoid malignancies using sFLC assays was reported from the Mayo Clinic. They demonstrated an abnormal sFLC ratio in 44% of a low number of 18 CLL patients [18]. However, [9] could show an abnormal sFLC ratio in 100 out of 259 CLL patients (39%). Moreover, they demonstrated a poor overall survival for patients with an abnormal sFLC ratio. Furthermore, Perdigao presented a correlation between an abnormal sFLC ratio, time to first treatment, IGHV status and overall survival in 84 patients with CLL [19,20]. Studied 34 CLL patients at various disease stages and found an abnormal sFLC ratio in 18 out of 34 patients (53%).

Recently, [10] also showed an abnormal ratio in 30 patients out of 101 CLL patients (30%) with shorter overall survival. Actually, Maurer et al. demonstrated an association of monoclonal FLC and polyclonal FLC with poor overall survival compared to patients with normal FLC [11].

Therefore, we investigated the role of an abnormal ratio of FLC in a large, well characterized cohort of 135 patients and also analyzed the association with immunofixation and light-chain restriction determined by immunophenotyping.

Firstly, we were able to confirm an abnormal FLC ratio in 78 of 135 CLL patients (58%), but interestingly, in only 53% of patients we found both the same clonal B-cell light chain restriction by flow cytometry and the involved FLC of an abnormal ratio. The IF was positive in only 32 of 134 patients and among the 32 patients 23 (17%) patients had both an abnormal FLC-ratio and a positive IF. One reason for the discrepancy can be explained by the fact that some M-proteins could not be detected despite existing FLC`s in the serum. Tsai et al. found a similar result [21]. The other reason is the higher sensitivity of the FLC assay compared to immunofixation. CLL patients with an abnormal FLC-ratio had a detectable M-protein in 15% of cases.

In 5 of 98 patients (5%) we observed a disagreement of the clonal light chain in tumor cell immunophenotype and the abnormal ratio of FLC. A similar result was specified by [23]. (8%) and Ruchlemer et al. (15%) [20]. However, monoclonal gammopathy of undetermined significance (MGUS) is expected in 3-5% of the normal population over 50 years [22] suggesting that the differences in tumor cell immunophenotype and abnormal FLC ratio could be explained by an underlying MGUS in these patients in addition to the CLL. Moreover, the differences could be produced by false positive FLC results.

Secondly, in this retrospective study we observed that patients with an abnormal FLC ratio involving lambda FLC are significantly associated with a worse TFT than kappa and normal ratio (median TFT for abnormal lambda: 34 months; for abnormal kappa 76 months and for normal ratio 88 months; p=0.039) confirming the results of [23].

Concerning high FLC serum-concentrations, it has been shown in Multiple Myeloma that a reduced renal function causes a higher serum FLC [24]. It has also been shown that in chronic kidney disease increasing levels of polyclonal κ and λ sFLC correlate with declining kidney function. Further, studies revealed an extended FLC ratio of 0.37 to 3.1 in patients with renal dysfunction [16,25]. We therefore analyzed the correlation between FLC and serum-creatinine. 13 of 123 patients had renal dysfunction and an abnormal FLC-ratio. 4 patients had abnormal FLC ratio for lambda and a renal dysfunction. A significant correlation between high sFLC and renal damage was not found (p=0.332) and polyclonal sFLC correlated significantly with normal and abnormal serum-creatinine (p=<0.0001), as expected (data not shown).

Following the aforementioned analysis we investigated the influence of abnormal sFLC ratio in the different risk groups like ZAP-70 status, CD38, and IGVH status. We could partly find a correlation between FLCs and these CLL prognostic factors. However, there was an association between CD38 and β2-microglobulin.

Furthermore, we could not confirm abnormal sFLC ratio as an independent prognostic factor in multivariate analysis [23]. And also Maurer [11] describe the abnormal sFLC ratio as an independent prognostic factor analyzed by Cox regression analysis. However, the correlation of CD38 and abnormal FLC (Table 1) and the strong impact of CD38 on TFT could explain this fact in our cohort.

Finally, we investigated whether combining information on the expression of CD38, ZAP-70 and abnormal FLC could help refine the prognostic information provided by any of the three factors alone. By summing the number of risk factors, this approach allowed for separation of our patient cohort into four subgroups which differed significantly with regard to their clinical outcome. Summarized, our results confirm the results of the other studies and demonstrate the pivotal role of abnormal FLC in CLL prognosis. Furthermore, we demonstrate for the first time the correlation of FLC and IF in CLL.

Follow-up studies are warranted to elucidate the role of FLC as biomarkers of disease and as a predictive factor for response.

References

1. Rai KR, Sawitsky A, Cronkite EP, Chanana AD, Levy RN, et al. (1975) Clinical staging of chronic lymphocytic leukemia. Blood 46: 219-234.
2. Binet JL, Lepoprier M, Dighiero G, Charron D, D'Athis P, et al. (1977) A clinical staging system for chronic lymphocytic leukemia: prognostic significance. Cancer 40: 855-864.
3. Johansson P, Eisele L, Klein-Hitpass L, Sellmann L, Dührsen U, et al. (2010) Percentage of smudge cells determined on routine blood smears is a novel prognostic factor in chronic lymphocytic leukemia. Leuk Res 34: 892-898.
4. Nowakowski GS, Hoyer JD, Shanafelt TD, Zent CS, Call TG, et al. (2009) Percentage of smudge cells on routine blood smear predicts survival in chronic lymphocytic leukemia. J Clin Oncol 27: 1844-1849.

5. Dingli D, Kyle RA, Rajkumar SV, Nowakowski GS, Larson DR, et al. (2006) Immunoglobulin free light chains and solitary plasmacytoma of bone. Blood 108: 1979-1983.

6. Dispenzieri A, Lacy MQ, Katzmann JA, Rajkumar SV, Abraham RS, et al. (2006) Absolute values of immunoglobulin free light chains are prognostic in patients with primary systemic amyloidosis undergoing peripheral blood stem cell transplantation. Blood 107: 3378-3383.

7. Kyrtsonis MC, Vassilakopoulos TP, Kafasi N, Sachanas S, Tzenou T, et al. (2007) Prognostic value of serum free light chain ratio at diagnosis in multiple myeloma. Br J Haematol 137: 240-243.

8. Rajkumar SV, Kyle RA, Therneau TM, Melton LJ 3rd, Bradwell AR, et al. (2005) Serum free light chain ratio is an independent risk factor for progression in monoclonal gammopathy of undetermined significance. Blood 106: 812-817.

9. Pratt G (2008) The evolving use of serum free light chain assays in haematology. Br J Haematol 141: 413-422.

10. Yegin ZA, Ozkurt ZN, Yagci M (2010) Free light chain: a novel predictor of adverse outcome in chronic lymphocytic leukemia. Eur J Haematol 84: 406-411.

11. Maurer MJ, Cerhan JR, Katzmann JA, Link BK, Allmer C, et al. (2011) Monoclonal and polyclonal serum free light chains and clinical outcome in chronic lymphocytic leukemia. Blood 118: 2821-2826.

12. Morabito F, De Filippi R, Laurenti L, Zirlik K, Recchia AG, et al. (2011) The cumulative amount of serum-free light chain is a strong prognosticator in chronic lymphocytic leukemia. Blood 118: 6353-6361.

13. Hallek M, Cheson BD, Catovsky D, Caligaris-Cappio F, Dighiero G, et al. (2008) Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. Blood 111: 5446-5456.

14. Dürig J, Naschar M, Schmücker U, Renzing-Köhler K, Hölter T, et al. (2002) CD38 expression is an important prognostic marker in chronic lymphocytic leukaemia. Leukemia 16: 30-35.

15. Dürig J, Nückel H, Cremer M, Führer A, Halfmeyer K, et al. (2003) ZAP-70 expression is a prognostic factor in chronic lymphocytic leukemia. Leukemia 17: 2426-2434."

16. Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF, et al. (2002) Serum reference intervals and diagnostic ranges for free kappa and free lambda immunoglobulin light chains: relative sensitivity for detection of monoclonal light chains. Clin Chem 48: 1437-1444.

17. van Rhee F, Bolejack V, Hollmig K, Pineda-Roman M, Anaissie E, et al. (2007) High serum-free light chain levels and their rapid reduction in response to therapy define an aggressive multiple myeloma subtype with poor prognosis. Blood 110: 827-832.

18. Martin W, Abraham R, Shanafelt T, Clark RJ, Bone N, et al. (2007) Serum-free light chain-a new biomarker for patients with B-cell non-Hodgkin lymphoma and chronic lymphocytic leukemia. Transl Res 149: 231-235.

19. Perdigao J, Cabrai MJ, Costa N, Carrao C, Rodrigues T, et al. (2008) Prognostic factors in CLL: Is serum free light chain ratio a new biological marker? Annals of Oncology 19: 204-410.

20. Ruchlemer R, Reinus C, Paz E, Cohen A, Melnikov N, et al. (2007) Free light chains, monoclonal proteins and chronic lymphocytic leukaemia. Blood 110: 4697.

21. Tsai HT, Caporaso NE, Kyle RA, Katzmann JA, Dispenzieri A, et al. (2009) Evidence of serum immunoglobulin abnormalities up to 9.8 years before diagnosis of chronic lymphocytic leukemia: a prospective study. Blood 114: 4928-4932.

22. Eisele L, Dürig J, Hüttmann A, Dührsen U, Assert R, et al. (2012) Prevalence and progression of monoclonal gammopathy of undetermined significance and light-chain MGUS in Germany. Ann Hematol 91: 243-248.

23. Pratt G, Harding S, Holder R, Fegan C, Pepper C, et al. (2009) Abnormal serum free light chain ratios are associated with poor survival and may reflect biological subgroups in patients with chronic lymphocytic leukaemia. Br J Haematol 144: 217-222.

24. Drayson M, Begum G, Basu S, Makkuni S, Dunn J, et al. (2006) Effects of paraprotein heavy and light chain types and free light chain load on survival in myeloma: an analysis of patients receiving conventional-dose chemotherapy in Medical Research Council UK multiple myeloma trials. Blood 108: 2013-2019.

25. Hutchison CA, Plant T, Drayson M, Cockwell P, Kountouri M, et al. (2008) Serum free light chain measurement aids the diagnosis of myeloma in patients with severe renal failure. BMC Nephrol 9: 11."