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Peter Mollee*, Jill Tate and Carel J. Pretorius

Evaluation of the N Latex free light chain assay in the diagnosis and monitoring of AL amyloidosis

Abstract

Background: We compared a novel assay for free light chain (FLC) quantitation based on monoclonal antibodies (N-Latex, Siemens, Germany) to the established polyclonal antibody-based assay (Freelite™, The Binding Site, UK) in AL amyloidosis.

Methods: Sixty-two diagnostic samples were analysed on a BNII nephelometer, 32 of which also had a post-treatment sample.

Results: In the diagnostic samples: for AL of κ type, the median involved FLC (iFLC) was significantly lower by the N-Latex assay (289 vs. 667 mg/L, p=0.0002) whereas for λ AL the values were similar (148 vs. 161 mg/L, p=0.84). Measurable disease, defined as a difference between involved and uninvolved FLC (dFLC) >50 mg/L was present in 82% by the N-Latex assay compared to 89% by the Freelite™ assay. For diagnostic sensitivity, the FLC ratio was normal in 21% (95% CI 12%–33%) and 15% (95% CI 7%–26%) of patients by the N-Latex and Freelite™ assays, respectively. The combination of serum and urine immunofixation electrophoresis with either FLC assay allowed identification of the amyloidogenic clone in 98% producing comparable sensitivity. For the monitoring samples the median reduction in dFLC was 68% for the N-Latex assay and 77% for the Freelite™ assay (p=0.04). This led to some differences in assigning response categories. Partial response as assigned by both assays predicted overall survival (N-Latex p=0.0015, Freelite™ p=0.022).

Conclusions: There are differences between FLC as measured by the N-Latex and Freelite™ assays, but overall the two assays have similar diagnostic sensitivity. Disease response calculated by both assays predicts survival but more clinical validation is required.

Keywords: AL amyloidosis; diagnosis; free light chain assay; monitoring.

***Corresponding author: Peter Mollee,** Associate Professor, Department of Haematology, Pathology Queensland, Princess Alexandra Hospital, Ipswich Road, Woolloongabba, QLD 4102, Australia, Phone: +61 7 31762396, Fax: +61 7 31767042, E-mail: peter_mollee@health.qld.gov.au

Peter Mollee: School of Medicine, University of Queensland, Brisbane, Australia
Jill Tate and Carel J. Pretorius: Department of Chemical Pathology, Pathology Queensland, Brisbane, Australia

Introduction

Since its first description [1], the availability of a commercial serum free light chain (FLC) assay (Freelite™, The Binding Site, UK) has been a major advance in the diagnosis and monitoring of AL amyloidosis. When the FLC assay is used in conjunction with serum and urine immunofixation electrophoresis, more than 95% of plasma cell clones can be detected at diagnosis, making this combination an essential part of the work-up of patients suspected to have AL amyloidosis [1–3]. Changes in the FLC concentration in response to therapy have been shown to predict both organ response and overall survival (OS) [1, 4–6]. The importance of the FLC assay in management of AL amyloidosis is now firmly established in consensus guidelines [7–9]. All of this clinical validation has been done with the Freelite™ assay.

Despite these important advances, the Freelite™ assay suffers from several analytical limitations. The polyclonal anti-FLC antibodies are obtained from sheep immunised against human Bence Jones protein and batch-to-batch variation in the polyclonal antisera used in the assay has been noted [10, 11]. The assay is also prone to non-linearity [10] leading to inaccurate quantitation and antigen excess [11] which can occasionally lead to misdiagnosis. This necessitates additional work and expense for the laboratory when samples need to be retested at further dilutions as recommended by the manufacturer. Rare samples do not react with the Freelite™ assay [10]. There are also reports that the antisera may cross react with κ and λ light chains that are bound to heavy chains leading to overestimation of the involved FLC [12]. These analytical limitations demonstrate the need for assay improvement.

Recently, serum κ and λ FLC immunonephelometric assays based on monoclonal antibodies have become commercially available (N Latex FLC assay, Siemens)

[13]. This new FLC assay appears to have some analytical advantages over the Freelite™ assay [14] but lacks clinical validation, particularly in AL amyloidosis where FLC measurement has such a central and critical role in patient management. We assessed the utility of the N Latex FLC assays in the diagnosis and monitoring of AL amyloidosis and compared it with the polyclonal Freelite™ κ and λ FLC assays.

Materials and methods

Patient samples

Sixty-two patients with newly diagnosed biopsy proven systemic AL amyloidosis who had serum stored at diagnosis were retrospectively identified. Thirty-two of these patients also had a post-treatment serum sample available. These patients had presented to the Princess Alexandra Hospital Haematology Department or its recently established Amyloidosis Clinic or were enrolled on a prospective clinical trial of risk-adapted intravenous melphalan [15]. Amyloid subtype was confirmed as follows: laser capture microdissection and tandem mass spectrometry (n=13); immunohistochemistry or immunofluorescence (n=29); non-diagnostic immunohistochemistry with clonal light chain abnormality and consistent clinical history (n=10); clinical trial patient where subtyping method not known but there was a protocol requirement to exclude non-AL types of amyloid (n=10). In the latter two categories the involved FLC was determined on the basis of the serum and urine protein immunofixation electrophoresis, bone marrow and FLC studies. In no case did these studies yield contradictory light chain restriction. Treatments received included: no therapy (n=9); high-dose melphalan and autologous stem cell transplantation (n=15); melphalan and dexamethasone (n=26); cyclophosphamide, thalidomide and dexamethasone (n=8); bortezomib-based therapy (n=3); and other (n=1). Serum samples had been stored frozen at −80°C for up to 10 years (range 2–121 months) prior to analysis. Ethical approval for the study was obtained from the Metro South Human Research Ethics Committee.

Laboratory assays

Serum FLC were measured with two commercial reagent kits: Freelite™ (Human Kappa and Lambda Free kits; The Binding Site Ltd, Birmingham, UK) and N Latex FLC κ and λ assays (Siemens Healthcare Diagnostics, Marburg, Germany) on a Siemens BNII nephelometer. Freelite™ and N Latex FLC measurements were performed on the same days on the same thawed samples. All initial Kappa and Lambda FLC assays were done at 1 in 100 sample dilution. For the Freelite™ assay the next higher dilution, 1 in 400, was also performed if Kappa FLC was >50 mg/L and below the upper measuring limit, or Lambda FLC was >100 mg/L and below the upper measuring limit, to detect samples exhibiting antigen excess and gross non-linearity. One AL sample exhibited antigen excess by Freelite™ Kappa FLC assay. For the sake of brevity, the respective analytical systems are referred to as Freelite™ and N Latex FLC hereafter. The diagnostic range for the Freelite™ κ/λ ratio was 0.26–1.65 and for the N Latex FLC κ/λ ratio was 0.31–1.56. Serum (and urine) protein immunofixation electrophoresis were not repeated on the stored sera, but the results from the routine clinical analysis at the time of sample collection were used. For the most part, these were performed on Hydrasys gel systems (Sebia, France).

Definitions

Median diagnostic values from the current study were used to dichotomise the continuous variable, difference between the involved and uninvolved FLC (dFLC), in assessment of its prognostic value: 150 mg/L for N Latex assay and 190 mg/L for Freelite assay. Disease considered "measurable" for response was defined as having a dFLC >50 mg/L. Assessment of haematological response was defined according to the recently published response criteria [16]. In brief, the dFLC was calculated for all diagnostic and post-treatment samples. Response criteria were defined as follows: partial response (PR) – >50% reduction in the dFLC; very good partial response (vgPR) – reduction in the dFLC to <40 mg/L; a complete response (CR) – normalisation of the free light chain ratio, negative serum and urine immunofixation electrophoresis.

Statistical analysis

Method comparison was performed using Passing-Bablok regression and Bland-Altman plots with Analyse-It (Version 2.21) and Method Validator software packages. Continuous variables were compared using the Wilcoxon rank sum test. The correlation between survival and FLC response was studied using Kaplan-Meier analysis and the curves were compared using the log-rank test. These latter statistical analyses were performed using the STATA (University of Texas) statistical software package.

Results

Diagnostic sensitivity

Of the diagnostic samples, 18 patients had amyloid of κ type and 44 had amyloid of λ type. The diagnostic sensitivity of the two FLC assays is shown in Table 1. The FLC ratio was normal in 21% (95% CI 12%–33%) and 15% (95% CI 7%–26%) of patients by the N Latex assay and Freelite™ assays, respectively. This difference was not significant (p=0.3). Both FLC assays showed abnormal FLC ratios for all 18 patients with κ light chain amyloidosis. In patients with λ light chain amyloidosis, however, the FLC ratio was abnormal in 31/44 by the N Latex FLC and 35/44 by the Freelite™ assay. In this λ group, both assays showed an abnormal FLC ratio in 29 cases, both ratios were normal in seven cases, in two cases the N Latex FLC ratio was

Table 1 Diagnostic sensitivity of immunofixation electrophoresis and either N Latex FLC or Freelite™ κ/λ ratio in 62 patients with AL amyloidosis.

n	κ light chain amyloid (95% CI)	λ light chain amyloid (95% CI)	All patients (95% CI)
	18	44	62
Serum IFE	56% (31%–78%)	73% (57%–85%)	68% (55%–79%)
Urine IFE	89% (65%–97%)	80% (65%–90%)	82% (70%–91%)
Serum and urine IFE	89% (65%–97%)	89% (75%–96%)	89% (78%–95%)
N Latex FLC			
κ/λ ratio	100% (81%–100%)	70% (55%–83%)	79% (67%–88%)
κ/λ ratio+serum and urine IFE	100% (81%–100%)	98% (88%–100%)	98% (91%–100%)
Freelite™			
κ/λ ratio	100% (81%–100%)	80% (65%–90%)	85% (74%–93%)
κ/λ ratio+serum and urine IFE	100% (81%–100%)	98% (88%–100%)	98% (91%–100%)

CI, confidence interval; IFE, immunofixation electrophoresis; FLC, serum free light chain.

abnormal and the Freelite™ was normal, and in six cases the N Latex FLC ratio was normal and the Freelite™ was abnormal. The combination of serum and urine IFE with either FLC assay, however, allowed identification of the amyloidogenic clone in 98% producing comparable sensitivity for both methods.

Comparison of baseline FLC concentrations

For patients with amyloidosis of κ type the median involved FLC (iFLC) was significantly lower by the N Latex assay (289 vs. 667 mg/L, p=0.0002) whereas in amyloidosis of λ type the values were similar (148 vs. 161 mg/L, p=0.84). According to current consensus criteria 82% of AL amyloidosis would be measurable by the N Latex assay compared to 89% by the Freelite™ assay. As a prognostic factor, a higher dFLC predicted worse OS whether measured by the N latex (2 yr OS 22% vs. 67%, p=0.0041) or Freelite™ assay (2 yr OS 20% vs. 69%, p=0.0005) (Figure 1).

Monitoring utility

For the 32 patients with monitoring samples the post-treatment sample was taken at a median of 4 months (range 1–12 months) post-initiation of chemotherapy. The median follow-up of survivors in this group was 50 months. The median reduction in dFLC was 68% (range, 35% increase to 100% reduction) for the N Latex assay and 77% (range, 7% increase to 100% reduction) for the Freelite™ assay (p=0.04). By current consensus criteria, more patients had measurable disease by the Freelite™ assay (n=29) than by the N Latex assay (n=25). This led to some differences in assigning response categories. The response according to the N Latex assay (n=25) was: CR (16%), vgPR (32%), PR (12%), no response (40%). The response according to the Freelite™ assay (n=29) was: CR (7%), vgPR (38%),

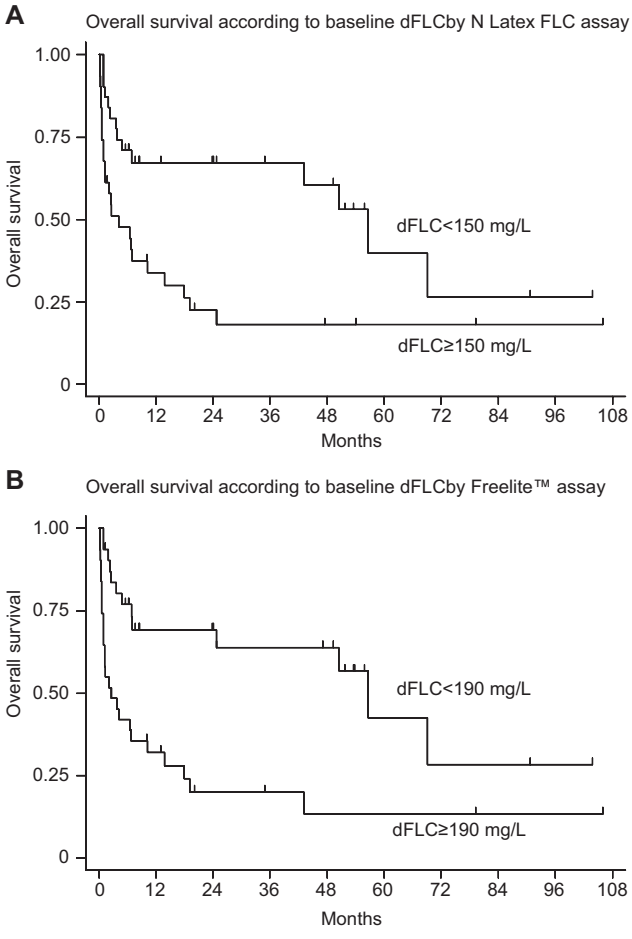

Figure 1 Overall survival according to difference in involved and uninvolved FLC (dFLC) at baseline for: (A) N Latex FLC assay and (B) Freelite™ FLC assay.

PR (17%), no response (38%). Concordance of response categories according to the two assays in patients with measureable disease by both assays (n=24) are detailed in Table 2. In those patients with measurable disease, a partial remission predicted OS by the N Latex (n=25, 2 yr OS 82% vs. 27%, p=0.0015) and Freelite™ assays (n=29, 2 yr OS 75% vs. 36%, p=0.02) (Figure 2).

Analytical comparison

For an analytical method comparison Freelite™ and N Latex FLC concentrations and K/L ratio were compared by Passing-Bablok regression analysis for the 94 sample results (Figure 3) and by Bland-Altman plot for mean difference (Figure 4). While there is good agreement between the Lambda FLC concentrations as measured by both assays, there was bias in the measurement of Kappa FLC concentration. The Freelite™ assay gave lower values at low FLC concentrations (<10 mg/L) and higher values at high FLC concentrations (>75 mg/L) compared to the N Latex FLC assay. This was explored further by examining the Bland-Altman plot according to whether the measurand was the involved versus non-involved FLC. This demonstrates that the Freelite™ assay is giving lower values for the non-involved polyclonal κ light chain and higher values for the monoclonal involved κ light chain (Figure 4). While this tendency was less marked for the Lambda FLC comparison, the uninvolved Lambda FLC concentrations generally were lower by Freelite™ compared to the N Latex FLC assay (Figure 4). Table 3 summarises the Passing-Bablok regression slope for the κ and λ assays in this study of AL amyloidosis compared to a more general hospital population [14]. In AL amyloidosis where the measured FLC corresponded to that involved by the AL disease process the slope is significantly lower compared to the uninvolved FLC as the 95% confidence intervals do not overlap.

An analysis of batch-to-batch variation was also performed. Of the 59 ratios that were available for comparison

Table 2 Response category among 24 patients with AL amyloidosis with measurable disease by both N Latex FLC and Freelite™ assays.

		N Latex FLC response			
		CR	vgPR	PR	NR
Freelite™ response	CR	2	0	0	0
	vgPR	0	5	2	1
	PR	1	2	1	1
	NR	0	1	0	8

CR, complete response; NR, no response; PR, partial response; vgPR, very good partial response.

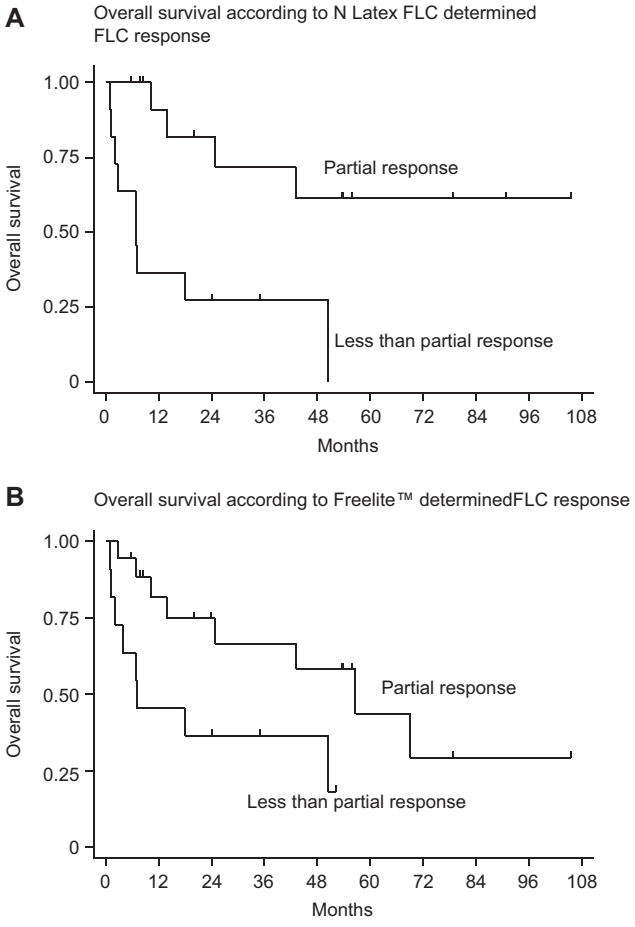

Figure 2 Overall survival according to FLC response for: (A) N Latex FLC assay and (B) Freelite™ FLC assay.

to previous results obtained over different batches of Freelite™ reagent over 10 years, there were eight different assignments by Freelite™ (5 abnormal κ/λ ratios changed to normal ratios; 3 normal ratios changed to abnormal ratios) and a 13.6% difference in κ/λ ratio assignment. The between-reagent variation was greater for Freelite™ Kappa FLC [PB slope: 1.237 (95% CI 0.934–1.468)] whereas Freelite™ Lambda FLC was better correlated [PB slope: 1.044 (95% CI 0.988–1.096] to previous reagent lots.

Discussion

In terms of diagnostic sensitivity, we demonstrated that the FLC κ/λ ratio was abnormal in 79% and 85% of AL amyloid cases when measured by the N Latex FLC and Freelite™ assays, respectively. This non-significant difference was due to fewer cases of λ light chain amyloid having an abnormal κ/λ ratio by the N Latex FLC assay.

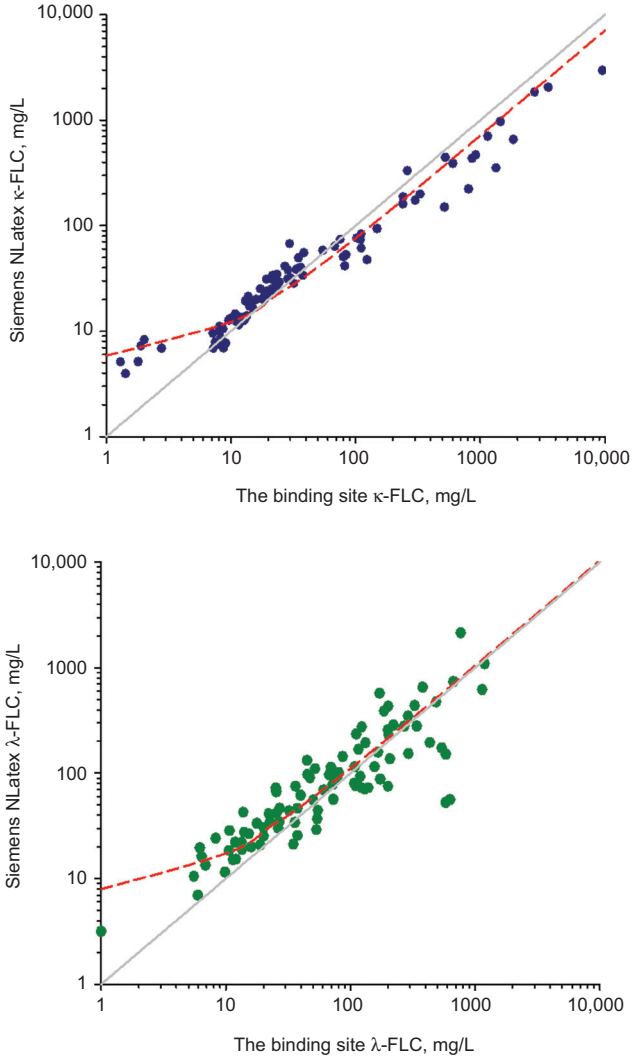

Figure 3 N Latex and Freelite™ FLC assay method comparisons in AL amyloidosis.
Grey line is line of identity; dotted line is Passing-Bablok regression line on a logarithmic scale.

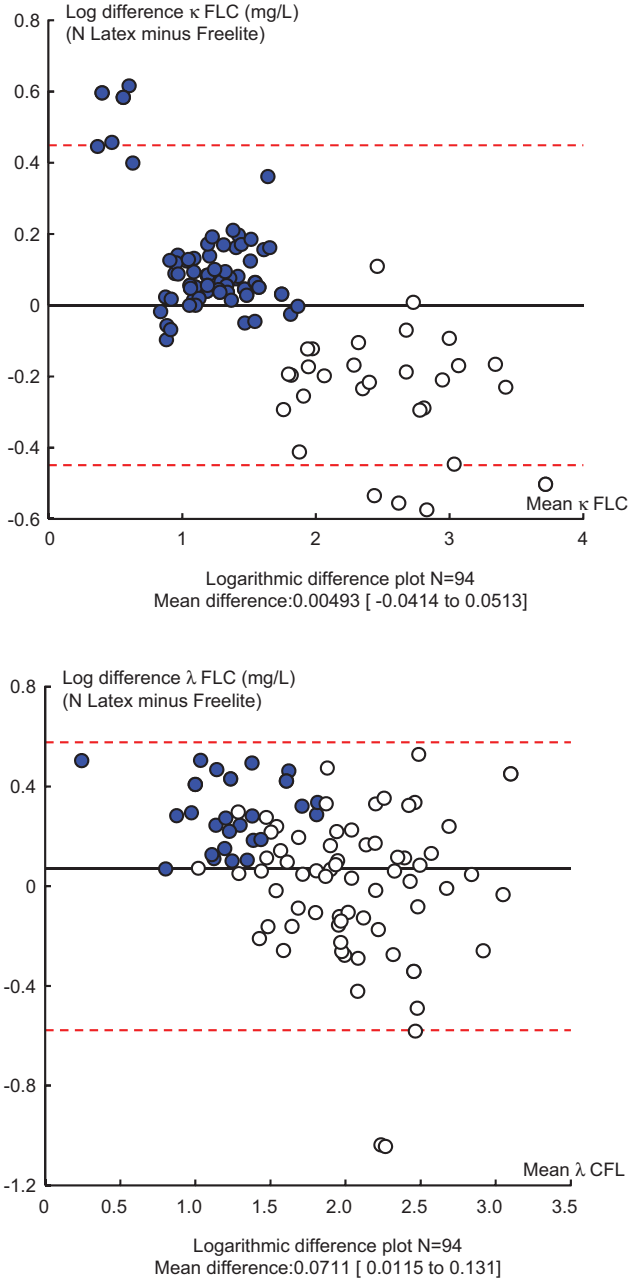

Figure 4 Bland-Altman difference plots in AL amyloidosis.
Solid line is mean difference; dotted lines are 95% confidence intervals at zero mean difference; closed circles represent uninvolved (polyclonal) FLC values; open circles represent involved (monoclonal) FLC values.

Thus, the N Latex and Freelite™ assays "miss" 13 and nine cases of AL amyloidosis, respectively. The routine screening for AL amyloidosis, however, consists of the combination of serum and urine IFE with the FLC assay. This combination, using either FLC assay, allowed identification of the amyloidogenic clone in 98% producing comparable sensitivity for both methods. These results are very similar to those presented by Palladini et al. [17] where 338 patients with newly diagnosed AL amyloidosis were assessed. In this report the κ/λ ratio was abnormal in 84% and 82% of AL cases when measured by the N Latex FLC and Freelite™ assays, respectively. When either FLC assay was combined with serum and urine IFE, 98% of cases were detected providing confirmatory evidence of the diagnostic sensitivity of the N Latex assay for AL amyloidosis.

As reported with the Freelite™ assay [18], we found that the absolute level of the dFLC at diagnosis was predictive of outcome with higher levels associated with worse OS. In comparing the two FLC assays the median dFLC levels in our study were very similar to those from

Table 3 Passing-Bablok regression slope and 95% confidence intervals (CI) for κ and λ free light chains (FLC) assays.

Subject group, n	κ FLC measurand	λ FLC measurand
Total, involved or uninvolved FLC	**Slope (95% CI)**	**Slope (95% CI)**
AL amyloidosis [this study]		
Total (n=94)	0.71 (0.65–0.83)	1.07 (0.92–1.22)
Involved (monoclonal) FLC (26 κ, 68 λ)	0.58 (0.45–0.65)	1.02 (0.82–1.24)
Uninvolved (polyclonal) FLC (68 κ, 26 λ)	1.16 (1.06–1.29)	2.11 (1.70–2.61)
Hospital population [14]		
Total (polyclonal and monoclonal) FLC (n=116)	1.36 (1.22–1.54)	1.37 (1.26–1.50)

the Pavia Amyloidosis Research and Treatment Center [17]: 150 mg/L vs. 165 mg/L for the N Latex FLC assay and 190 mg/L vs. 180 mg/L for the Freelite™ assay.

In the monitoring context, we found that slightly fewer patients had measurable disease (defined as dFLC >50 mg/L) when using the N Latex FLC assay results (82% vs. 89%). In Palladini's study 75% of patients had measurable disease as assessed by either assay although only 64% had baseline dFLC >50 mg/L by both assays. These "measurable disease" criteria are based on studies using the Freelite™ assay. Given the analytical differences between the two assays, it may be that consensus guidelines will need to consider different cut-offs for different assays or a single cut-off that balances both assay's monitoring precision with the need to have as many patients with measurable disease as possible. Our sample size was too small to address these issues. While response categories as calculated using either assay results were broadly similar there were some differences, particularly in the partial remission category. Again, our findings were similar to those of Palladini's larger study. Due to the small sample size, we were only able to examine the impact of partial response or better (includes CR, vgPR and PR) on survival with both assays significantly predicting outcome.

The analytic comparison demonstrated differences between the two assays, particularly in the measurement of the Kappa FLC. In particular, these differences related to whether the FLC being measured was the involved FLC (comprising predominantly the amyloidogenic FLC plus smaller amounts of polyclonal FLC of same isotype) or the uninvolved polyclonal FLC. Thus, the amyloidogenic light chain is reacting differently in one or both assays to the polyclonal light chains of the same isotype. Due to the lack of standards for immunoglobulin light chains, it is difficult to determine which assay is over-estimating (or under-estimating) the true FLC value. These differences in involved FLC values between assays may be due to non-parallel immunoreactivity between the calibrator (polyclonal) and the samples (monoclonal) in one or both assays possibly due to limited antigenicity of the amyloid molecule and a unique amino acid sequence in the variable region. Lot-to-lot variation of polyclonal antibody-based Freelite™ reagent may contribute to some of the difference as demonstrated by the comparison of FLC ratios in the current study compared to that obtained initially with a different reagent lot. Campbell et al. [19] describe the presence of "gaps" at low FLC concentration when Freelite™ was compared with monoclonal FLC antibody assays and that these may contribute to falsely elevated FLC ratio. Figure 3 highlights there is a difference between the two methods at low concentrations below 10 mg/L FLC, in particular for Kappa FLC.

Differences between the two assays may also reflect the subject group evaluated, e.g., AL group versus a more heterogeneous hospital population [14] (Table 3) or the FLC concentration. This demonstrates the importance of performing analytic validation studies in different light chain disease populations rather than just in general hospital or normal donor populations. Further, the study demonstrates that there are differences in absolute values between Freelite™ and N Latex such that values cannot be used interchangeably when monitoring disease response in AL amyloidosis or other monoclonal light chain diseases. Rather, clinicians should monitor FLC using the same manufacturer's assay on the same instrument.

Our study does present some limitations. Our sample size is relatively small, especially for the monitoring group, so conclusions must be made with caution. Nonetheless, the consistency of the findings with the only other study in the field lends support to the conclusions. The study is also retrospective in nature, treatment was not standardised across the patient cohort, and the post-treatment serum sample was only available on a subset of patients and was not taken at a uniform timepoint. All these factors can potentially introduce bias into the study but should not significantly affect the comparison of the two FLC assays.

In summary, we assessed the new N Latex FLC assay in the diagnosis and monitoring of patients with AL amyloidosis. The new assay appears to have similar diagnostic, prognostic and monitoring utility compared to the established Freelite™ assay but further prospective validation in larger cohorts of patients is required to confirm these findings and support the introduction of the N Latex assay into clinical practice for this group of patients. Importantly, in the changeover from one manufacturer's FLC (or any tumour marker) assay to another manufacturer's assay there should be a lengthy overlap period where FLC are measured by both assays when monitoring patients with previously diagnosed disease. This enables the clinician to become familiar with any differences in absolute numbers between assays.

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Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article. Research funding played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.
Research funding: Siemens Healthcare supplied N Latex FLC kits free of charge for this study. JT and PM have also previously performed studies using Freelite™ kits provided free of charge from the Binding Site.
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