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Combined light chain immunofixation to detect monoclonal gammopathy: a comparison to standard electrophoresis in serum and urine

Abstract

Background: The purpose of this study was to evaluate a combined κ and λ light chain immunofixation (CLIF) as a screening tool to detect monoclonal immunoglobulins in serum and urine. A secondary aim was to investigate the impact on workflow and reagent utilisation of a systematic implementation of CLIF in addition to routine protein electrophoresis (PE) on all samples.

Methods: Light chain antisera (κ and λ) were mixed in a 1:1 ratio and loaded in the same sequence as the PE to create a superimposable image.

Results: The CLIF procedure agreed significantly better with standard immunofixation procedures in the serum and urine. In 33 (22%) new patients and in 114 (15%) follow-up patients CLIF detected a band missed by PE in serum. In 34 (4.5%) of previously categorised cases the monoclonal band was below the detection limit of CLIF in serum, but still detectable by conventional immunofixation electrophoresis. In one case (0.7%) a band in a urine specimen was missed by CLIF compared to 70 (49%) missed by PE. After the systematic introduction of CLIF turn-around-times (TATs) and utilisation of laboratory consumables decreased significantly (p<0.001).

Conclusions: A systematic implementation of CLIF led to the detection of monoclonal bands missed by PE with an improvement in TATs and a decrease in cost.

Keywords: amyloidosis; immunofixation electrophoresis; monoclonal gammopathy of uncertain significance (MGUS); monoclonal immunoglobulin; myeloma; plasma cell proliferative disorder; protein electrophoresis.

Introduction

The laboratory contribution to the diagnosis and monitoring of plasma cell proliferative disorders largely depends on the electrophoretic demonstration of a monoclonal immunoglobulin in serum and/or urine [1, 2]. Protein electrophoresis (PE) separates the constituent proteins according to charge with most immunoglobulins migrating to the anodal or γ-region on agarose gel or capillary zone electrophoresis. The detection limit for a monoclonal immunoglobulin is approximately 1–2 g/L and this depends on the background polyclonal immunoglobulins [2]. To demonstrate the monoclonal nature of a band seen on PE an immunological technique such as immunofixation electrophoresis (IFE) is required. The latter procedures characterise monoclonal immunoglobulins according to the heavy (γ, α, μ, ε or δ) and light (κ or λ) chain composition and have detection limits of approximately 100–200 mg/L, again depending on the background polyclonal immunoglobulins [2]. To demonstrate the monoclonal nature of a band seen on PE an immunological technique such as immunofixation electrophoresis (IFE) is required. The latter procedures characterise monoclonal immunoglobulins according to the heavy (γ, α, μ, ε or δ) and light (κ or λ) chain composition and have detection limits of approximately 100–200 mg/L, again depending on the background polyclonal immunoglobulin staining [2]. Additional information regarding the monoclonal nature of the disease process can be obtained from the relative abundance of free light chains in serum [3, 4].

The disease spectrum of the monoclonal gammopathies ranges from the frankly malignant multiple myeloma (MM) with gross over production of a monoclonal immunoglobulin to a cryptic presentation such as in non-secretory MM or some cases with primary amyloidosis (AL) where no detectable monoclonal immunoglobulin is demonstrable in serum or urine. The mere presence of a monoclonal immunoglobulin is also not diagnostic of malignancy as implied by the aptly named monoclonal gammopathy of uncertain significance (MGUS) which constitutes the majority of newly identified cases with a
monoclonal immunoglobulin in serum [5, 6]. Thus the detection of small bands below the detection limit of PE with an analytically more sensitive technique such as IFE is not routinely performed in new patients unless there is a specific clinical suspicion of multiple myeloma or a related disorder [1, 2, 7].

The response criteria for MM include a quantitative assessment of the monoclonal immunoglobulin band size in the follow-up assessment and in selected patients IFE is mandated when the band is no longer visible on PE to categorise complete-, near complete remission and other categories [1, 2]. To provide a clinically relevant diagnostic service and to comply with the reporting guidelines can result in a complicated laboratory workflow with a sequential and reflexive addition of IFE and other tests based on the supplied clinical information, findings on the initial PE, results of other diagnostic tests and by comparison with previous results [2]. To complicate matters further screening algorithms based on the supplied clinical information is fraught with danger, given the protean manifestations of plasma cell proliferative disorders [3].

The purpose of this study was to evaluate the diagnostic performance of a combined κ and λ light chain immunofixation procedure (CLIF) as a screening tool to detect monoclonal immunoglobulins in serum and urine. In addition the impact on workflow and reagent utilisation was assessed after a systematic implementation in parallel to routine electrophoresis.

Materials and methods

The study was performed in a tertiary referral laboratory that provides a service to a range of clinicians ranging from primary to tertiary care, including bone marrow transplantation. All samples with a PE request on serum or urine during an 8-week period were included in the assessment of diagnostic performance and underwent a CLIF investigation. Our routine protocol for investigating patients complied with national guidelines [2]. In patients that were followed up with known disease the absence of a previously identified monoclonal immunoglobulin on PE was confirmed with IFE. In new patients IFE was performed to characterise newly identified bands and in selected cases where the investigation was specifically requested on the basis of a high clinical index of suspicion. The protocol complied with local ethical standards and was performed according to the World Medical Association Declaration of Helsinki regarding ethical conduct.

Protein electrophoresis (PE) and immunofixation electrophoresis (IFE)

All PE, IFE and CLIF reagents, equipment and quality control materials were obtained from SEBIA (Cedex, France). Urine samples were concentrated up to 80-fold with Minicon CS15 concentrators (Millipore Ireland Ltd, Cork, Ireland). For the PE gel undiluted serum or concentrated urine samples were applied and stained with Amido Black. For IFE serum samples were diluted with the Sebia diluent according to the manufacturer’s recommendations relative to the total globulin result (>30 g/L a 1:11, 20–30 g/L a 1:6 and <20 g/L a 1:3 dilution). IFE was performed by applying antisera (γ, α, μ, κ, λ, free-κ, free-λ) and when indicated δ and ε followed by an acid violet stain. All procedures were performed with fresh samples under routine examination conditions.

Combined light chain immunofixation (CLIF)

The CLIF antisera reagent was prepared in-house by mixing the Sebia κ (SE4736) and λ (SE4737) light chain antisera in a 1:1 ratio and stored at 2–8 °C. Serum and urine samples were loaded as described for IFE and the κ+λ-antisera was applied to all tracks of the gel (20 μL) after which the gels were processed as for IFE.

The samples were loaded on an IFE gel in the same sequence as for the PE to create a superimposable image in order to facilitate interpretation (Figure 1).

The adjudication of the PE and CLIF gels were performed by two staff members and controlled by a third person when the results are entered into the laboratory information system. A retrospective audit was systematically conducted after the completion period to resolve any discrepancies (WJ, SK, CP). An adjudicated final reference diagnosis was based on a review of the clinical case history with incorporation of the standard IFE and iso-electric focussing results where it was performed.

The detection limits of PE, IFE and CLIF were determined by serially diluting samples with a characterised monoclonal immunoglobulin band in Sebia diluent and separately with a pooled normal serum to imitate both an immunoparetic and polyclonal background.

Turn-around-time (TAT) and test utilisation

The effect on workflow was assessed by comparing the TAT before and after implementation of the CLIF and the reorganised laboratory process over two equivalent periods of three calendar months. The TAT was obtained from the laboratory information system and based on the elapsed time between the initial capture of the request and the validation of the electrophoresis report. The PE and IFE consumable utilisation was estimated from the electronic stores ordering information system. To accommodate for the variability in the number of antisera used per individual patient, the utilisation of IFE gels were standardised relative to the number of PE gels.

Statistics

Agreements in classification relative to the adjudicated reference diagnosis are presented as the 95% confidence interval of the Cohen’s κ statistic (κ_{adjus}) [8]. The distributions of skewed data were described as medians and interquartile ranges and differences between medians were assessed with the Mann-Whitney procedure. Significance testing was performed at the 5% level (p<0.05).
Results

A total of 3597 samples were received during the study period (male=1953, female=1644) and no sample was excluded from this analysis. There were 1884 serum and 469 urine samples collected from test-naïve patients with no record of a previous PE, IFE or FLC request. We received 983 serum and 264 urine samples from patients known to have a monoclonal immunoglobulin or plasma cell proliferative disorder from previous investigations for monitoring purposes.
Limit of detection

The detection limit for all methods was a function of the background immunoglobulin staining. PE detected a band of approximately 0.25 g/L with no background staining and approximately 0.95 g/L with a polyclonal background. Both IFE and CLIF were able to detect very faint bands of approximately 0.05–0.10 g/L with a clear background, but with a polyclonal background this decreased to approximately 0.25 g/L for IFE and 0.50 g/L for CLIF.

Diagnostic performance

The CLIF procedure detected monoclonal protein bands in serum and urine for both naïve and known patient groups that were not detected by standard PE. The retrospective audit with the benefit of clinical history, IFE and iso-electric focussing results allowed us to further assess the accuracy of both procedures and in all categories the agreement was significantly improved for CLIF over PE (Table 1).

Naïve patient serums

The agreement between the adjudicated reference diagnosis and CLIF ($\kappa_{Cohen} 0.85$) was significantly improved over that of PE ($\kappa_{Cohen} 0.73$). Both PE and CLIF were equally prone to identifying faint bands as potentially monoclonal ($p=0.27$) and in the vast majority (38 PE; 42 CLIF) of these cases multiple small oligoclonal bands could be demonstrated with the definitive methods of IFE and IEF (Figure 2, example 1). Haemolysis (two cases) and fibrinogen (one case, Figure 2, example 2) resulted in small bands detected on PE, but not with CLIF. In six CLIF cases we concluded that an application artefact was misinterpreted as a possible band.

Of the 33 patients missed by PE but detected by CLIF, 17 had bands in the $\beta$-region which typed as follows: seven IgA$\kappa$, six IgA$\lambda$, two IgM$\kappa$ and two with $\lambda$ Bence Jones Protein. The remainder (16 patients) had small monoclonal immunoglobulin bands in the $\gamma$-region <1 g/L. Examples of these trace monoclonal immunoglobulin bands are shown in Figure 1, lane 18 and Figure 2, examples 3 and 4. In one case a small band in the $\gamma$-region was not detected by both procedures.

Naïve patient urines

The agreement with the adjudicated reference diagnosis of CLIF ($\kappa_{Cohen} 0.99$) was significantly improved over that of PE ($\kappa_{Cohen} 0.49$). In one case a trace monoclonal $\lambda$-band was not detected on CLIF compared to 26 cases where PE did not detect a monoclonal immunoglobulin in the urine. In one patient with marked proteinuria a candidate band detected with PE was not confirmed as monoclonal after further investigation.

Known patient serums

CLIF ($\kappa_{Cohen} 0.90$) detected 114 monoclonal immunoglobulin bands that were missed with PE ($\kappa_{Cohen} 0.66$). In 34 (4.5%) samples a band was detected with IFE, but not with CLIF and in the majority (32 cases) we concluded that a trace monoclonal immunoglobulin below the CLIF limit was missed.

**Table 1** Diagnostic performance of patient classified with PE or CLIF compared to the adjudicated diagnosis (incorporating clinical history, immunofixation electrophoresis and iso-electric focussing).

<table>
<thead>
<tr>
<th></th>
<th>TP</th>
<th>TN</th>
<th>FP</th>
<th>FN</th>
<th>$\kappa$ (95% CI)</th>
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<tr>
<td>Naïve patients</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Serum</td>
<td>CLIF</td>
<td>149</td>
<td>1686</td>
<td>48</td>
<td>1</td>
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<tr>
<td></td>
<td>PE</td>
<td>116</td>
<td>1693</td>
<td>41</td>
<td>34</td>
</tr>
<tr>
<td>Urine</td>
<td>CLIF</td>
<td>40</td>
<td>428</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>PE</td>
<td>14</td>
<td>428</td>
<td>1</td>
<td>26</td>
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<td>Known patients</td>
<td></td>
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<tr>
<td>Serum</td>
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<td>4</td>
<td>34</td>
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<tr>
<td></td>
<td>PE</td>
<td>608</td>
<td>227</td>
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<td>148</td>
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<tr>
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<td>CLIF</td>
<td>101</td>
<td>154</td>
<td>5</td>
<td>1</td>
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<tr>
<td></td>
<td>PE</td>
<td>56</td>
<td>158</td>
<td>1</td>
<td>46</td>
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</table>

CLIF, combined light chain immunofixation; FN, false negative; FP, false positive; naïve, no record of previous monoclonal immunoglobulin; known, previously detected monoclonal immunoglobulin or known to have a plasma cell proliferative disorder; PE, protein electrophoresis; TN, true negative; TP, true positive. *IFE not performed in all cases (only when specifically requested based on clinical suspicion); †IFE was not performed if a band visible on PE corresponded to a previously characterised band.
of detection was present. One patient had a monoclonal IgAκ immunoglobulin that reacted poorly with the κ light chain sera and in one case the band was detected after repeat analysis at a different dilution. In four instances oligoclonal banding against a polyclonal background was misinterpreted as monoclonal with CLIF.

Known patient urines

PE (κCohen 0.59) missed 46 of the 102 urine monoclonal bands while in only one case a small band was missed by CLIF (κCohen 0.95). Five cases with a false-positive CLIF interpretation were ascribed to a misinterpretation of an application artefact.

Turn-around-time and test utilisation outcomes

The systematic incorporation of CLIF as part of the initial laboratory investigation for all samples resulted in a significant TAT reduction of approximately 28 h in all categories (Table 2). This occurred despite the increase in patient workload of 32% which was consistent with the general increase of test utilisation between the respective periods. The improvement in TAT were due to a number of factors the most significant of which was the earlier finalisation of reports in known patients where a monoclonal band corresponding to one previously typed was present – approximately 45% of urine and 16% of serum sequential IFE procedures were eliminated in this category of patients. Second in new patients the confident identification of bands, especially in urine and the serum non-γ-region, allowed quicker finalisation of negative results. Third IFE results on newly identified bands and on follow-up cases were available earlier due to a reduction in total IFE workload.

The utilisation of IFE consumables, which are required for both the IFE and CLIF procedures, declined by 17% compared to PE consumables. This occurred despite the fact that every sample received a CLIF procedure. We ascribed this to the reduction in IFE procedures performed, especially in known patients, accompanied by increased efficiency of CLIF with 54 patients per gel.

Discussion

A novel finding of our study was that the systematic application of CLIF with a superimposable image complementary to routine electrophoresis improved the workflow, reduced TATs and decreased the use of total IFE consumables in our environment. The reduction of IFE procedures to confirm the presence of a previously characterised monoclonal immunoglobulin band and the avoidance of further investigations in new patients without a strong clinical indication were the major contributors to this finding. In the group of 983 patients followed up with a known monoclonal immunoglobulin in serum, CLIF detected 114 patients that were missed by PE. According to standard protocols this group of patients would have

Table 2  The effect of CLIF implementation on the turn-around-time (TAT) and the utilisation of immunofixation electrophoresis (IFE) consumables.

<table>
<thead>
<tr>
<th></th>
<th>Pre implementation</th>
<th>Post implementation</th>
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<tr>
<td>Serum samples, n</td>
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<td>4548</td>
</tr>
<tr>
<td>Urine samples, n</td>
<td>641</td>
<td>860</td>
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<tr>
<td>Serum TAT, h&lt;sub&gt;a&lt;/sub&gt;</td>
<td>94.6 (50.7–118.5)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>62.3 (29.6–95.6)</td>
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<tr>
<td>Urine TAT, h&lt;sub&gt;b&lt;/sub&gt;</td>
<td>118.5 (90.7–141.3)&lt;sup&gt;b&lt;/sup&gt;</td>
<td>92.5 (57.8–120.3)</td>
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<tr>
<td>IFE utilisation&lt;sup&gt;c&lt;/sup&gt;</td>
<td>2.64&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.19</td>
</tr>
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</table>

<sup>a</sup>Median (interquartile range); <sup>b</sup>p<0.001 compared to the post implementation period; <sup>c</sup>Utilisation expressed as the number of IFE gels relative to the PE gels.
required at least a subsequent IFE to assist in classifying the therapeutic response [1, 2]. The reduction in IFE procedures in this patient group after implementation of CLIF partially explained the improved TATs and decreased usage of IFE consumables.

The ability to detect serum monoclonal bands migrating in the α- and β-regions and the knowledge that bands below the usual PE detection limit can be confidently detected, resulted in decreased sequential and reflexive IFE procedures that are time consuming. This had the effect of fewer IFE procedures in new patients without a strong clinical suspicion of a plasma cell proliferative disorder. The systematic implementation of CLIF in parallel with PE therefore led to improved workflow in our laboratory as evidenced by the improvement in TAT by approximately 1 day because the CLIF result was available at the time of PE interpretation, which allowed finalisation of more reports. Even though all samples underwent an abbreviated immunofixation, the use of a single lane on the IFE gel per sample as opposed to the multiple lanes used for routine IFE contributed to the relative reduction in IFE consumables.

Second our results demonstrated that CLIF significantly improved the detection of monoclonal immunoglobulin bands over that of conventional PE. This was consistent in both serum and urine specimens and in both new patients investigated de novo and in known patients whose therapeutic responses were monitored. This was expected as CLIF is a modified immunofixation procedure which has a superior detection limit for monoclonal immunoglobulins [1, 2, 7]. Our finding that 22.1%±6.7% (95% CI) of monoclonal immunoglobulins were not detected with a standard PE in the serum of newly investigated patients were similar to the report by Eisele et al. (27.3%±6.8%) who used a screening immunofixation procedure with antisera directed against a mixture of heavy and light chains [7]. Our performance of CLIF in serums of known patients and in all the urine samples contributed additional information on the analytical performance of a screening immunofixation procedure.

The detection limit experiment and the results in urine samples demonstrated that in the absence of background immunoglobulin staining the CLIF procedure performs similarly to IFE. With a polyclonal background the detection limits of both CLIF and IFE deteriorate and the mixture of both κ and λ signals masks the identification of monoclonal bands with CLIF to a greater extent. The differential decrease in the detection limits was mostly confined to the γ-region and no monoclonal immunoglobulins detected by IFE in the α- or β-regions were missed by CLIF. Thus although the performance is an improvement over that of PE, IFE still has a role in defining complete response in known patients as well as the initial investigation of selected new patients with a high index of clinical suspicion. Standard IFE will still be required to further characterise the heavy and light chain composition of new patients identified with CLIF.

The importance of not relying on PE alone when investigating for Bence Jones proteinuria was reinforced by our findings as approximately 49% of monoclonal immunoglobulin bands in urine would be missed unless some form of immunofixation was performed (Figure 1 lanes 30–53). In our opinion the only utility of PE in urine is to assist in the quantification of the monoclonal fraction excreted in timed urine specimens when a band is visible. The comparable performance of CLIF and IFE in urine, with only one case in 142 (0.7%) missed by CLIF, can be ascribed to the low background immunoglobulin staining which optimises the analytical sensitivity in this matrix.

The detection of 33 additional small monoclonal immunoglobulin bands with CLIF in the serums of naïve patients was expected, especially when the monoclonal proteins were migrating in the β-region [7]. Although satisfying from a laboratory point of view, the clinical benefit of detecting small monoclonal bands especially in the γ-region in these patients is not a foregone conclusion as the majority of these patients may end with a MGUS diagnosis and we may inadvertently have contributed to the Ulysses syndrome in some [9]. Due to the short duration of this study we did not investigate the clinical outcomes of patients in whom small monoclonal immunoglobulins were detected as these may only be settled after a number of years and will require a lengthy prospective study [7]. If we accept the premise that the detection of small monoclonal immunoglobulin bands are of clinical benefit, 22.1% of monoclonal immunoglobulins would have been missed by PE alone in naïve patients (p<0.001) with approximately one additional patient detected per 57 tests performed in this patient group.

We considered performing PE in a sequential manner and only for the quantitation of bands detected with CLIF and IFE, since the contribution of PE in the redesigned process was largely limited to this purpose. We have not pursued this avenue further because modelling suggested that a major portion of the improved TAT of the new process would be sacrificed by a sequential process. Second the potential reduction in PE consumables will be offset by an increase in manual labour due to sample retrieval and setup. Although not a major consideration a potential risk of relying only on CLIF in new patients is that cases with heavy chain disease may be missed.
Limitations

A limitation of this study is the lack of IFE investigations in all naïve patients in whom no band was detected with a resultant overestimation of the diagnostic performance of CLIF. In known patients 4.5% of monoclonal bands were below the detection limit of CLIF and we therefore estimate that approximately eight small bands may have been missed by CLIF of which we detected one which had an IFE performed due to clinical suspicion. It would, however, be impractical to perform IFE on every sample received for PE and this is also not the current standard of care [2, 7]. In addition we did not perform IFE on follow-up samples where the monoclonal immunoglobulin detected on PE corresponded to a previously characterised band; this is, however, not expected to have a significant influence on our findings. Lastly one should be careful to extrapolate our findings to other settings with a different clinical case mixture. The impact on workflow and consumable utilisation will depend on local laboratory practice and the ratio of new and follow-up patients.

In summary, a systematic implementation of CLIF in parallel to PE allowed improved detection of monoclonal immunoglobulins and improved our workflow with a reduction in TAT and usage of IFE consumables. CLIF can potentially reduce the number of routine IFE procedures in all urines and in sera of monitored patients with plasma cell proliferative disorders.

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Conflict of interest statement

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References


