REVIEW ARTICLE

A Brief Review on Free Light Chain Assays: From Conventional to Current

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ABSTRACT

Free light chains (FLCs) are tumour markers of monoclonal gammopathies. Detection of urinary FLC or also known as Bence-Jones protein through urinary protein and its immunofixation electrophoreses (UPE and uIFE, respectively) have been considered the gold standard for its biochemical diagnosis. This is mainly due to their superior detection limits compared to their counterpart investigations in serum. However, urinalysis is limited in many ways. The emergence of serum FLC assay with markedly improved detection limit circumvents many of these problems and has gained much importance in biochemical investigations of monoclonal gammopathies. Nevertheless, they are not without limitations. This review discusses the advantages and limitations of serum and urinary FLC assays.

Keywords: Free light chain, Monoclonal gammopathies, Urine protein electrophoresis, Serum free light chain assay.

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INTRODUCTION

Free light chains (FLCs) are tumour markers of monoclonal gammopathies, particularly of those secreting light chains. Its biochemical detection in serum and urine is of paramount importance in diagnosing and monitoring these disorders. Traditionally, screening of monoclonal gammopathies relies on serum and urine protein electrophoreses, with the latter long being considered as its 'gold standard' test (1). However, these methods have limitations in detecting FLC, many of which have been overcome by a newer assay of serum FLC. This brief review aims to critically discuss FLC assays, from conventional to current.

LIGHT CHAIN IMMUNOGLOBULINS

Immunoglobulins are products of plasma cells comprising of heavy and light chains. The latter may either be bound to a heavy chain in an intact immunoglobulin, or exists as free light chains. The two types of light chains, kappa (κ) and lambda (λ) are structurally heterogenous, with κ being predominantly monomeric and λ dimeric (2). In addition, higher polymeric forms as well as fragments of both types of FLCs may also exist (2). Serum FLC concentrations depend upon the balance between production and renal clearance (2). They are rapidly cleared through the glomeruli before being reabsorbed and catabolised in the proximal tubules (2). Normally little protein is present in the urine due to the huge tubular reabsorptive capacity (2).

Urinary FLC, also known as Bence-Jones protein (BJP), thus represents an overflow proteinuria that occurs when an overproduction of FLCs overwhelms renal tubular reabsorptive capacity (2). First discovered by an English physician, Dr. MacIntyre in 1845 as a urinary protein in an oedematous patient, it was sent to Dr. Bence Jones, a chemical pathologist, for identification (2). Dr. Bence Jones subsequently published the findings of the peculiar protein, which he described as 'hydrated deutoxide of albumen' in 1847 and hence the eponym (2,3). However, it was not until 1962 that the identity of BJP was established as monoclonal FLC (2,4).

MONOCLONAL GAMMOPATHIES

Monoclonal gammopathies are characterised by proliferation of one or more neoplastic clones of plasma cells, which usually secrete monoclonal immunoglobulins or M-proteins (5). Clinically, they encompass a broad spectrum of diseases ranging from premalignant disorders of monoclonal gammopathy of undetermined significance (MGUS) and smoldering multiple myeloma, to malignant disorders of multiple

myeloma (MM) (6). The M-protein may consist of an intact immunoglobulin, the light chain only, or the heavy chain only (5). Not only M-proteins vary in structure, their concentrations also widely vary. Up to 3% of patients with MM are designated as non-secretory MM with no detectable M-protein in serum or urine (7). On the other hand, oligosecretory MM secrete small amounts of M-protein of less than 10 g/L and 200 mg/24h, in the serum and urine, respectively (8,9). Such diseases include light chain multiple myeloma (LCMM), and related low tumour-burden disorders. The latter is defined as a small clonal proliferation of plasma cells producing toxic M-protein leading to end-organ damage, as seen in amyloid light chain (AL) amyloidosis and light chain deposition disease (LCDD) (10,11).

SERUM PROTEIN ELECTROPHORESIS AND IMMUNOFIXATION

Biochemically, serum protein electrophoresis (SPE) is the standard screening test for monoclonal gammopathies (2). M-proteins migrate as discrete bands on an electrophoretic gel, such as agarose gel, whilst a densitometric peak provides a semi-quantitative value for its concentration (1). Subsequent serum protein immunofixation electrophoresis (sIFE) confirms its clonality. In the absence of reactivity for immunoglobulin (Ig) G, A or M, presence of IgD and IgE monoclonals must be excluded before a diagnosis of monoclonal light chain is made (5,8). The detection limit of SPE ranges from 500-2000 mg/L, whilst that of sIFE is 10-fold lower (50-200 mg/L) (2,12). However, in non- or oligosecretory disorders, serum FLC levels may be missed on SPE and sIFE as their concentrations are well below the detection limits of these assays (1).

URINE PROTEIN ELECTROPHORESIS AND IMMUNOFIXATION

Since it can be concentrated many times, urine protein electrophoresis (UPE) and its immunofixation (uIFE) are much more sensitive than those of serum, with detection limits of 10-40 mg/L and has been traditionally recognised as the gold standard for detection of urinary FLCs (1,5). However, technical issues include cumbersome and inaccurate 24-hour urine collection (1). In recent years, it has been recommended to validate the accuracy of urine collection against creatinine clearance (13). Kaplan & Horowitz 2011 (13) also suggested the possibility of using the BJP to creatinine ratio from random urine samples to determine 24-hour BJP excretion.

Measurement of urinary total protein is part of BJP quantitation, which is important in disease definitions and staging (14). However, methods of both precipitating and dye-binding, are often insensitive to microproteins in general and to FLC fragments in particular (14). Also, proteins differ in their affinities for the dyes used, leading to non-linear densitometric response, giving rise to over- or underestimation of BJP concentration (14). Furthermore, urine concentration can be technically demanding with the existence of different practices (4). Overconcentration of urine, however, may form 'ladder banding' pattern, giving a false impression of monoclonal bands (4). Meanwhile, increased urinary polyclonal FLCs, as seen in systemic lupus erythematosus, increases non-specific background staining, thus obscuring any monoclonal light chain, giving rise to false negative results (5). In addition, batch analysis of urine samples may lead to delayed results (1). Although uIFE offers higher analytical sensitivity than UPE, it is a non-quantitative assay and is prone to antigen excess due to a wide range of urinary FLC concentrations, which may cause falsely negative findings (1,4).

Nevertheless, the underlying renal pathology may be inferred to a certain extent from the degree and pattern of proteinuria seen on densitometry and UPE, respectively. For instance, glomerular proteinuria, indicative of an underlying glomerular damage such as glomerulonephritis typically presents as gross proteinuria of >3.5 g/day and an albuminuric pattern on UPE (4,9). Tubular damage, such as Fanconi syndrome, is suggestive by proteinuria of 1-3 g/day and a more generalised pattern of proteinuria on UPE (4,9).

SERUM FREE LIGHT CHAIN ASSAY

More than a decade ago, an interest in measuring FLC in the serum had emerged. The assay has since been internationally recommended as part of the screening panel for monoclonal gammopathies, with some exception, replacing urinary FLC analysis (7). This assay has been a major shift from the previous serum total light chain assays, which measure both bound and free light chain (1). Serum FLC assays are immunoassays, utilising antibody recognition of epitopes on the FLC constant region, which are otherwise hidden in an intact immunoglobulin (1,2,15). Being a serum assay, it negates urinalysis. With analytical sensitivity of 1.5 and 3.0 mg/L for kappa (κ) and lambda (λ) FLC, respectively, the assay is particularly useful in non- and oligosecretory disorders, which are often undetected by SPE or IFE (1,15). Calculation of κ: λ ratio confirms clonality, whereby monoclonal expansion of a single type of FLC gives an abnormal ratio (1,2). In contrast, a polyclonal production of FLCs, as seen in infection, increases both types of FLCs and thus a normal ratio. Although κ is produced twice as much as λ, being monomeric, the former is cleared much faster, resulting in the normal serum κ: λ ratio of around 0.55 (0.26- 1.65) and both having short half-lives of 2-3 h and 5-6 h for κ and λ, respectively (2,12).

IS SERUM FLC A BETTER TEST?

The arguments in favour of serum FLC measurement are as follow. Physiologically, FLCs are freely filtered at the glomerulus and reabsorbed before being catabolised in the proximal tubules. However, tubular reabsorptive capacity ranges widely, reaching up to 10–30g of FLC daily, which is way beyond the normal plasma cell production of 0.5–1 g per day (6). The median serum FLCs required to produce overflow proteinuria have been measured at 113mg/L and 278 mg/L for κ and λ, respectively (6). Thus, the amount of urinary FLCs is therefore heavily dependent upon renal function. Not only measurement of urinary FLCs may miss oligosecretory but more importantly, it is not reflective of FLC production rate and thus potentially becoming unreliable for diagnosis in both early stages and advanced light-chain myeloma (12). Secondly, the short half-lives of serum FLC allow "real-time" assessment of response to therapy, which is also advantageous in patients with intact immunoglobulin MM (15). Monitoring of the latter by SPE would be less accurate due to longer half-lives of intact immunoglobulins (1). Similarly, assessment of response by UPE may miss low levels of BJP in oligosecretory disorders. Thirdly, as FLCs are produced in excess of heavy chains to ensure proper conformation of intact immunoglobulins, therefore, an increase in serum FLC concentrations may be detected the earliest during relapse (12,15). Patients with intact immunoglobulin MM have been shown to relapse with monoclonal FLCs only, a phenomenon termed 'light chain escape', which may otherwise be missed on SPE or UPE (1).

Definition of myeloma kidney has traditionally been based on merely a fixed level of serum creatinine of 173 μmol/L and later by serum creatinine-based estimated glomerular filtration rate equations (7). Recently, the international guideline has incorporated serum FLC in redefining myeloma kidney as part of its clinical features (7). Patients with myeloma kidney typically have baseline serum FLC concentrations exceeding 500 mg/L (1). The guideline now regards serum FLC of >1500 mg/L, which is presumptive of light chain-cast nephropathy, as myeloma-defining event (10). The invasive but definitive renal biopsy is recommended to clarify the underlying cast nephropathy particularly if serum FLC is less than 500 mg/L (7).

LIMITATIONS OF SERUM FLC ASSAY

The first generation assay, the Freelite assay (Binding Site Ltd., UK) utilises polyclonal antibodies that recognise a wide range of epitopes on the FLCs multimers (1). Much work, including the international guideline has been based on this assay (1,12). However, each FLC is unique with its own set of epitopes, and therefore may not react appropriately with the polyclonal antibodies in the calibrator. Thus, equimolar reactivity of the polyclonal antibodies against FLC multimers, ranging from monomeric κ to dimeric λ as well as higher polymers, is questionable. In addition, the epitope recognised by the antibody varies only slightly from that on an intact immunoglobulin, giving rise to a potential cross-reactivity (11). The second generation, the N Latex assay (Siemens Healthcare Diagnostics, Germany) uses monoclonal antibodies recognising a specific epitope on a specific form of FLC, therefore having an increased specificity (1). However, due to its monoclonal nature, the calibrator in this assay has a limited coverage of the FLC multimers (1). Furthermore, being recently introduced, this assay is less widely validated compared to the first generation and only available on a single platform (12). Recently, a third generation assay based on monoclonal antibody is emerging, which, unlike the previous assays, offers simultaneous measurement of both κ and λ FLCs (16). Likewise, this assay has yet to be widely validated.

In addition, there are several other analytical issues impacting the sFLC assays. Currently, neither assay (Freelite or N Latex assay) is able to fulfill the desirable imprecision targets (17). Both assays are also subject to non-linearity of FLC during immunoassay reaction, resulting in underestimation of FLC concentration and non-specific interferences, causing both falsely high and low FLC concentrations (17). Variation seen in different batches of antisera affects both types of assays, but being monoclonal, it is less of a problem for the N Latex assay, giving rise to inaccuracy problem (17). Furthermore, both are also affected by antigen excess as FLC levels widely range from 1-100,000 mg/L, leading to falsely low results (17).

The major analytical issue is accuracy (12,17). Due to its heterogeneity, the true definition of serum FLC, the measurand, remains elusive and hampers reliable recognition of all its molecular forms equivocally, as discussed earlier (9). Thus, until today, there is no international reference material or method for serum FLC (9). Hence, a uniform calibration traceability is yet to be achieved. Clinically, lack of harmonisation and standardisation raises important issues such as whether a patient meeting certain criteria is very much assay-dependent (9,13). It hinders assay interchangeability (9,12). Therefore, in following up and monitoring of patients, one should use the same serum FLC assay, platform and laboratory, as both within and between-assay variations have been observed (9,12,17).

CONCLUSIONS

In conclusion, although serum FLC assay, with its improved analytical sensitivity, has been integrated into monoclonal gammopathies work up, one should bear in mind its limitations. Alternatively, limited yet sufficient

clinical information is still inferable from the older conventional tests, such as UPE.

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