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Development of detection methodologies for free light chains and their applications in clinical practice: A review

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Abstract: Free light chains are low-molecular-weight protein byproducts released by plasma cells during the synthesis of immunoglobulin. Under normal physiological conditions, serum-free light chain concentrations are maintained at low levels; however, they increase markedly in diverse pathological states, especially in cases of abnormal proliferation of plasma cells. This review systematically outlines the evolution of methodologies for detecting free light chains, from traditional electrophoresis and immunofixation to modern quantitative techniques such as mass spectrometry, the Freelite test, enzyme-linked immunosorbent assay, and particle-enhanced turbidimetric immunoassay. It critically evaluates the principles, analytical performance, and clinical applicability of each method. Beyond multiple myeloma, the article also explores the growing significance of free light chains in a broad spectrum of conditions, including autoimmune diseases, acute allergy, viral infection, multiple sclerosis, and chronic kidney disease. The expanding clinical utility of free light chains as biomarkers for diagnosis and monitoring underscores their indispensable role in modern laboratory medicine and personalized patient care.

1. Introduction

Immunoglobulin (Ig), a typical component of the adaptive immune system, is a tetramer structure formed by two identical heavy chains and two identical light chains connected by disulfide bonds. Light chains can be divided into two types, κ and λ, with a ratio of approximately 2:1 in the human body. Free light chains (FLCs) are always released as by-products during the plasma cell assembly of Ig due to the slightly higher synthesis rate of light chains compared to heavy chains. In healthy individuals, these low-molecular-weight FLCs are present in serum at very low concentrations, freely filtered by the glomeruli, and subsequently almost completely reabsorbed and metabolized by the proximal tubules of the kidney. However, abnormal proliferation of plasma cells is a hallmark of various hematological diseases, leading to significant overproduction of FLCs. Thus, serum FLC levels have become a key biomarker for tumor burden and disease activity in plasma cell abnormalities, prompting the International Myeloma Working Group (IMWG) to include them in the latest guidelines for the diagnosis of plasma cell tumors.

The scientific journey of understanding FLCs is a rich chapter in the history of medicine, beginning with the seminal observation of what is later known as the Bence Jones protein (BJP) by Henry Bence Jones in 1845[1]. Early investigations have focused on elucidating the physicochemical and structural properties of FLCs, with Gerald M. Edelman made a crucial contribution in defining their molecular identity[2]. The development of detection methods for FLCs began in the early 1960s, evolving from basic semi-quantitative techniques such as immunodiffusion to initial quantitative radioimmunoassays, which, despite their innovation, suffered from poor reproducibility. A significant limitation of these early immunoassays is their inability to distinguish between FLCs and light chains bound within intact Ig. A method for determining plasma FLCs was discovered in 2001, characterized by the use of specific polyclonal antibodies that can selectively bind to free chains[3]. This breakthrough has catalyzed the development of increasingly sophisticated and clinically applicable assays.

This review aims to comprehensively summarize the evolution and current landscape of FLC detection methodologies, critically analyzing the principles, advantages, and limitations of various techniques, including electrophoresis, mass spectrometry, the Freelite test, enzyme-linked immunosorbent assay (ELISA), particle-enhanced turbidimetric immunoassay, the N Latex serum FLC determination test, and the Seralite test. Furthermore, we extend the discussion beyond multiple myeloma to explore the expanding clinical utility of FLCs across a spectrum of conditions, such as autoimmune diseases, acute allergy, viral infection, multiple sclerosis, and chronic kidney disease, thereby underscoring their multifaceted role as biomarkers in modern medicine.

2 Detection methods for FLCs

2.1 Electrophoresis

Electrophoresis was first applied in the study of multiple myeloma in 1939. BJP is a monoclonal Ig light chain found in the urine of patients with plasma cell disorders, and it was first described by Henry Bence Jones in 1845[1]. Dr. Putnam demonstrated in 1967 that BJP has different peptide sequences, leading to specific migration patterns[4]. Thus, using urine protein electrophoresis and immunofixation electrophoresis (IFE) to bind antiserum with specific total κ and λ light chains to form heavy chain-specific antiserum provides a relatively simple and low-cost method for detecting FLCs. The standard electrophoresis analysis methods lack sensitivity for detecting lower levels of monoclonal FLCs, as they only include total light chain reagents. Thus, a small monoclonal FLC peak (measured in mg/L) may often be masked by intact polyclonal Ig and total light chain background (measured in g/L), making it difficult to accurately measure[5]. Although electrophoretic analysis using urine as a substitute for serum overcomes the issue of the background of polyclonal intact Ig, the significance of these tests is limited, as monoclonal FLCs need to be secreted excessively to exceed the reabsorption capacity of the kidneys. Thus, there is an urgent need to develop technologies that can improve the sensitivity of serum IFE, including the use of size exclusion ultrafiltration and FLC-specific antiserum. However, their applicability for large-scale use has not yet been explored, and they only represent analytical methods in the research and development stages.

2.2 Mass spectrometry

Complete light chain analysis based on MALDI-TOF-MS with high throughput capability is more widely explored in clinical studies, and several studies encourage it to replace IFE, which has been approved by the IMWG in 2021[6]. Currently, there are two MALDI-TOF-MS-based detection methods available for clinical use. The MASS-FIX testing method has been developed by the Mayo Clinic, which is the only institution capable of conducting this type of testing. It serves as a qualitative analysis interpreted in a binary manner, which utilizes antiserum against IgG, IgA, IgM, and total antibodies without any FLC-specific reagents, and has been shown to be more sensitive than IFE. EXENT (formerly known as QIP-MS) is the first commercially available complete light chain detection method based on MALDI-TOF-MS, which has been approved for clinical use. At present, it has been approved for use in Europe but is awaiting approval from the US Food and Drug Administration (FDA). Similar to MASS-FIX, standard EXENT testing does not include FLC-specific reagents; however, there are some methodological differences between the two. EXENT utilizes antiserum bound to magnetic particles, while MASS-FIX utilizes camel nanobodies[7]. There is no comparative study on the sensitivity of these two detection methods currently, so it is not yet clear what impact the differences in reagents have on detection performance. Moreover, EXENT detection software automatically interprets mass spectra and provides a complete quantification of monoclonal proteins for Ig, which is opposite to the qualitative results provided by MASS-FIX. However, the use of MALDI-TOF-MS for complete light chains is limited, as only a few centers have relevant instruments, and the detection cost is much higher than electrophoresis.

2.3 Freelite test

A method called Freelite emerged in 2001 for detecting FLCs using polyclonal antibodies. The principle of this method is to prepare sheep immune-tolerant polyclonal anti-κ and λ FLC antibodies to purify FLCs from human urine samples and then coat them with polystyrene latex particles. Latex particles can enhance the progress of the reaction, and antibodies only recognize antigen epitopes that are hidden by binding to Ig heavy chains, which are re-exposed when not bound. Thus, the antibody of this reagent only binds to FLCs rather than the light chains on intact Ig. This method has strong specificity, with detection sensitivities of 1.5 and 3 mg/L for κ and λ FLCs, respectively. The Freelite kit is suitable for various immunoturbidimetric detection systems produced by companies such as Beckman Coulter, Roche, Siemens, and Binding Site. Compared with traditional M protein detection methods, it is a better quantitative detection method with the advantages of high sensitivity and specificity. However, this method still has certain limitations, as different reagent batches can lead to significant differences in results when detecting low concentrations of FLCs, and the results of testing at high dilution may sometimes be higher than those at low dilution[8]. Considering the non-linear relationship between antigen concentration and immune complex precipitation, excessive antigen dilution can lead to the hook effect. Furthermore, the differences in the results obtained by Freelite for detecting the same sample on different analysis platforms limit the possibility of applying the guidelines to all automated instruments in clinical laboratories.

2.4 ELISA

A fully automated ELISA detection method developed by the French company Sybio is based on the principle of adsorbing rabbit anti-κ and λ FLC polyclonal antibodies onto a solid-phase carrier surface and then using horseradish peroxidase-labeled non-specific polyclonal antibodies for double antibody sandwich detection[9]. This method with good linearity is not affected by antigen excess and has excellent repeatability and batch consistency. However, further research is needed to confirm the clinical value of this method, especially in plasma cell diseases.

2.5 Particle-enhanced turbidimetric immunoassay

Recently, Smith and Wu have analyzed the reference range, linearity, precision, and other aspects of this method that utilizes polyclonal antibodies and verified its suitability for the Siemens Advia 1900 analyzer[10]. To monitor patients with myeloma, they have also proposed that if clinical labs need to change techniques, they should consider storing previously tested samples and re-determining the baseline of new reagents to avoid the incidence of outliers that may affect the judgment of test results. This method is not FDA-approved, so it should only be used in research and lab testing.

2.6 N Latex serum FLC determination test

This technique, suited to Siemens BN systems, is a novel methodology developed in 2011 that employs monoclonal antibodies for latex-enhanced scattering turbidity-specific FLC measurement. The method involves purifying BJP from multiple myeloma patients’ urine to create monoclonal antibodies against κ and λ FLCs. Proteins in serum or urine samples may then form immunological complexes with the antibodies. These immunological complexes scatter the light beam that passes through the sample, with the intensity of the scattered light proportional to the concentration of the relevant proteins. To determine the concentration of κ and λ FLCs, we compare them to a known standard value. This approach, when utilized in Siemens BN systems, may avoid the hook effect induced by excessive antigen due to built-in pre-reaction. Velthuis et al. have validated the reference range, precision, interbatch consistency, repeatability, linearity, and other aspects of this method, and the results have indicated that it has excellent precision, sensitivity, and interbatch consistency[11]. Compared with Freelite, this method has the advantage of adequate clinical consistency; however, further research is still needed to demonstrate its clinical value.

2.7 Seralite test

This approach uses chromatography to identify plasma levels of κ and λ FLCs within 10 minutes, making it suitable for emergency screening of tiny clinical samples in hospital labs. This approach uses known pure FLCs to competitively bind gold-labeled anti-FLC monoclonal antibodies to serum FLCs, producing a signal that is inversely proportional to serum FLC concentrations. The signal is determined by the reader (ADxLR5) of the detection system, which provides the ratio of the absorbance value of the FLC test line to that of the control line and then utilizes the calibration curve to obtain the concentration[12]. This method is fast and convenient, and research has indicated that it is suitable for diagnosing and monitoring multiple myeloma in the absence of severe kidney damage. Heaney et al. have evaluated its application as a screening tool in multiple myeloma kidney injury and non-multiple myeloma kidney injury and provided a reference range. They have indicated that this method can be applied to the diagnosis of acute kidney injury caused by myeloma, which is crucial for timely intervention and prognosis improvement in clinical practice. However, this method is not applicable to other plasma diseases that meet the criteria for myeloma.

3. Clinical applications of FLCs

3.1 Autoimmune diseases

Autoimmune diseases, the immune system deficiency of B lymphocytes or T lymphocytes involved in adaptive immune activation, are primarily characterized by chronic inflammation. It can be divided into systemic autoimmune diseases and tissue-specific autoimmune diseases. In this case, FLCs play a related role in activating mast cells and phages, but the mechanism of action is unclear. The earliest relevant research can be traced back to the 1960s, when researchers observed significantly higher concentrations of FLCs in serum and urine compared to healthy controls in rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) [13]. This is the first time that the FLC concentration in the blood is linked to disease progression, and subsequent studies have shown the potential of FLCs as biomarkers. Continuous activation of B cells and disease activity during clinical practice may increase the risk of lymphoma. Elevated concentrations of serum FLCs and β2-microglobulin can be used for monitoring treatment or evaluating recurrence after remission. Few studies have quantified FLCs in urine, which is a non-invasive sampling method for patients with different rheumatic diseases. An increase in urinary FLCs has been proven in SLE patients, and this sudden significant increase can present with severe clinical manifestations at 4–8 weeks. However, these research findings may be partially influenced by proteinuria, which is a typical characteristic of SLE patients. This study has also indicated that when FLCs increase, the κ/λ values do not change, which determines the polyclonal nature of FLCs. It has been confirmed in a recent study, where FLCs in the urine of patients with rheumatism are elevated and the κ/λ values are normal. The correlation between FLCs and inflammatory markers demonstrates their potential in predicting disease activity. However, further research is still needed to determine whether FLCs can serve as relevant biomarkers for response therapy (especially B cell depletion) and risk indicators for lymphoma in autoimmune diseases.

Monoclonal anti-CD20 antibody, rituximab (RTX), is one of the most commonly used drugs for the treatment of RA and SLE, especially in patients who have failed conventional treatment. RTX selectively acts on CD20-positive B lymphocytes (mature and pre-B cells), keeping plasma cells almost intact. Due to the lack of effective biochemical markers, monitoring RTX therapy has become complex, and the correlation between existing biochemical markers and accompanying clinical manifestations is low. For instance, rheumatoid factors usually decrease after RTX treatment; however, it does not always indicate improvement in the patient's clinical condition and cannot determine whether to suspend treatment. This situation explains the lack of consensus on the optimal method for evaluating the patient's response to RTX and the timing of further treatment. The consumption of B cells in peripheral blood reduces the concentration of serum FLCs, indicating that they are more effective biomarkers for single monitored patients receiving RTX therapy. Furthermore, there is a significant correlation between the levels of κ FLCs and the consumption of C3 complement components in serum[14]. This previously unconfirmed correlation has been described in a preliminary study linking FLCs to disease activity. This study has confirmed for the first time that the correlation between FLCs and complement consumption in serum remains even after B cell depletion. The rapid turnover of FLCs, especially the κ light chains, proves that they are ideal markers for RTX reactions.

3.2 Acute allergy

The location of FLCs on nasal mucosal mast cells in patients with allergic and non-allergic chronic rhinitis has confirmed the hypothesis that they can promote the expansion of inflammatory phenomena. In addition, the FLC levels are closely related to granulocyte mediators (i.e., mast cell trypsin and eosinophil cationic protein), further confirming their involvement in the allergic inflammatory cascade. In both allergic and non-allergic rhinitis, an increase in FLCs occurs on the κ and λ chains, which is attributed to an increase in B cell activity. Similarly, with the increase of specific IgE synthesis in allergic syndrome, the synthesis of antigen-specific FLC polyclonal occurs. According to the recommendations of the working group of the European Society of Allergy and Clinical Immunology, idiopathic rhinitis and non-allergic rhinitis syndromes are FLC-mediated and need to be reclassified[15]. However, it is necessary to identify antigens that induce FLC-mediated mucosal reactions and conduct further research to better understand the pathophysiology of non-allergic rhinitis.

3.3 Viral infection

The clinical interest in measuring FLCs in patients with human immunodeficiency virus (HIV) or hepatitis C virus (HCV) stems from the observation that their increase may serve as early biomarkers for tumor development, particularly lymphoma[16]. In chronic HIV infection, in addition to the gradual loss of CD4 lymphocytes, B cells also undergo a series of functional abnormalities, characterized by low levels of antibodies against specific antigens and poor response to vaccines. Paradoxically, the high circulating levels of Ig lead to non-specific polyclonal activation of B cells. The result of this B-cell disease is a progressive immunodeficiency, which in turn constitutes some risk factors for tumor development, such as non-Hodgkin lymphoma (NHL). In a recent study on HIV-infected patients, it has been shown that an increase in plasma FLC concentration can highly predict NHL risk. The determination of FLCs and κ/λ values in HCV patients may be useful in clinical practice. High concentrations of κ FLCs have been observed in HCV-positive patients, and the κ/λ values are positively correlated with the degree of HCV infection.

3.4 Multiple sclerosis

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease characterized by the intrathecal synthesis of oligoclonal Ig bands (OB). The detection of OB in cerebrospinal fluid has been the sole commonly utilized biochemical analytical approach since the late 1960s. Moreover, assessing any damage to the blood-brain barrier by measuring the ratio index of albumin to Ig in serum and cerebrospinal fluid has also become a part of laboratory diagnosis for MS. Recent studies have exhibited that the absolute concentration of κ FLCs has high reliability and specificity for the diagnosis of isolated syndromes and the recurrence and primary progression of MS in clinical settings[17]. FLCs can passively diffuse into cerebrospinal fluid, and their half-life in cerebrospinal fluid is longer than plasma (2–6 hours), comparable to other proteins.

3.5 Chronic kidney disease

FLCs are closely related to the kidney, and the clear differences between polyclonal and monoclonal FLCs may help us better understand their role in determining renal dysfunction. Under normal circumstances, the synthesis of light chains is slightly imbalanced, resulting in a small excess of FLCs (κ and λ FLCs) in the serum. They are easily destroyed, pass through the glomeruli, and are then almost completely reabsorbed in proximal tubular cells; thus, only a small amount of polyclonal FLCs appear in the urine of healthy subjects. On the other hand, when their production rate significantly increases, excess FLCs after filtration exceed the reabsorption capacity and are excreted with urine. In addition, kidney diseases characterized by reduced glomerular filtration rate or proximal tubular cell damage lead to increased FLC serum levels or FLC proteinuria, respectively. The accumulation of FLCs in the proximal lumen emphasizes their endocytosis mechanism and induces a series of events, including inflammation, activation of redox pathways, transcription of pro-fibrotic cytokines, and apoptosis of tubular cells, ultimately resulting in internalization of epithelial-mesenchymal transition[18]. The progression of renal fiber rupture induces the regeneration of nephron masses, leading to the accumulation of FLCs in the distal tubule lumen and the formation of protein casts. These casts are often identified in renal pathology: they are composed of various proteins, including FLCs. Furthermore, polyclonal FLCs can impair the function of neutrophils, thereby severely impairing the immune response.

There is a correlation observed between high levels of polyclonal FLCs and subsequent mortality risk in the population with early chronic kidney disease (CKD) [19]. Considering that systemic inflammation is a related factor for cardiovascular disease and mortality in CKD patients, it may support the use of polyclonal FLCs as sensitive biomarkers, adding prognostic indicators to traditional acute phase biomarkers (e.g., high-sensitivity C-reactive protein). Therefore, serum polyclonal FLCs may become sensitive biomarkers for adaptive immunity in CKD patients, especially for evaluating subclinical infections. A separate reference range is required in the presence of renal impairment due to the inverse relationship between serum FLC levels and renal function.

3.6 Multiple myeloma

Multiple myeloma (MM) is a hematological malignancy originating from hematopoietic cells in the bone marrow that belongs to B-cell lymphoma. It is a pathological condition induced by aberrant neutrophil plasma cell growth in the bone marrow, which results in inappropriate release of M protein or other fragments. MM is more common in men over 40, especially those over 60. It has become one of the most serious illnesses, endangering the health of the elderly. Currently, the cause of bone destruction in MM patients is not fully understood; however, previous studies have indicated that the expression of Ig plays a crucial role in the diagnosis of MM[20]. The malignant proliferation of plasma cells in MM patients leads to a large amount of Ig in the serum, which in turn increases the levels of κ and λ FLCs correspondingly. Research has indicated that the detection of changes in serum Ig and FLC levels is critical in the prevention, diagnosis, and treatment of this disease.

4. Conclusions

Free light chains (FLCs) have evolved from being regarded merely as by-products of immunoglobulin synthesis to becoming important biomarkers in modern clinical medicine. Over the past decades, remarkable advances in analytical technologies have substantially improved the sensitivity, specificity, and accessibility of FLC detection. Traditional electrophoretic techniques remain valuable for screening and characterization of monoclonal proteins, whereas newer methodologies, including Freelite assays, ELISA-based platforms, particle-enhanced immunoturbidimetric assays, N Latex FLC tests, and mass spectrometry, have enabled increasingly accurate quantification and monitoring of FLCs. Among these approaches, mass spectrometry-based techniques show particular promise due to their high analytical sensitivity and potential to improve the detection of monoclonal gammopathies, although broader clinical implementation is currently limited by cost and instrument availability.

Beyond their established role in multiple myeloma and related plasma cell disorders, FLCs have demonstrated growing clinical relevance in a wide range of pathological conditions, including autoimmune diseases, allergic disorders, viral infections, multiple sclerosis, and chronic kidney disease. Their rapid turnover, close association with B-cell activity, and responsiveness to disease progression make them attractive biomarkers for diagnosis, prognosis assessment, therapeutic monitoring, and risk stratification. Nevertheless, several challenges remain, including the lack of assay standardization across platforms, inter-method variability, and the need for disease-specific reference intervals, particularly in patients with impaired renal function.

Future research should focus on harmonizing detection methodologies, establishing universally accepted reference standards, and conducting large-scale clinical validation studies. With continued technological innovation and deeper understanding of FLC biology, FLC-based testing is expected to play an increasingly important role in precision medicine, facilitating earlier diagnosis, individualized treatment strategies, and improved patient outcomes across diverse clinical settings.

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