Original Study

Evaluation of the Impact of Renal Failure on Correlation and Concordance Between 2 Free Light Chain Assays

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Abstract

Measurement of serum free light chains (FLCs) is recommended for diagnosis of monoclonal gammopathies. FLC measurements with Freelite (Binding Site) and N Latex FLC (Siemens) assays were performed on 1215 fresh sera samples from patients with or without monoclonal gammopathy and renal failure. A good correlation was demonstrated between both assays, but it remained 7.6% to 20.8% discordances between the methods related to the FLC ratio interpretation. In patient follow-up, few discrepancies were observed. Neither of the assays performed better than the other: they provide comparable but not equivalent results, and discrepancies are not linked to renal failure stage. Interpretation must take into account clinical data and the same assay must be used for patient follow-up.

Background: Free light chain (FLC) assays are essential for diagnosis and follow-up of plasma cell dyscrasia. Two assays are available: Freelite (Binding Site) and N Latex FLC (Siemens). The aim of our study was to evaluate the impact of renal failure on concordance and correlation between the 2 FLC assays. **Methods:** FLC measurements using both assays were performed on 1215 fresh serum samples from patients with or without monoclonal gammopathy and renal failure. Concordance and correlation were evaluated using Passing-Bablock regression, Pearson correlation coefficient, and the Cohen kappa coefficient, taking into account the renal failure stage (evaluated with Chronic Kidney Disease–Epidemiology Collaboration formulae) and evaluation of treatment response in patients' follow-up. **Results:** A good correlation was demonstrated between both assays, irrespective of the renal failure stage (Pearson correlation coefficient > 0.90). For FLC ratio interpretation, there remained 7.6% to 20.8% discordances between the 2 methods throughout the whole range of renal impairment. To evaluate FLC evolution in patient follow-up, 41 patients were selected with at least 6 consecutive serum samples being collected during the study period: we observed a concordant evolution of FLC concentrations between both assays. However, few discrepancies were observed with 4 patients. **Conclusions:** Despite adjusted reference ranges for Freelite FLC ratio, there are approximately 12.5% discrepancies in interpretation of FLC ratio between the 2 available assays. They are not linked to renal failure stage and neither of the assays performed better than the other: results must be interpreted taking into account clinical data and the same assay must be used for patient follow-up.

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Introduction

Monoclonal gammopathy (MG) constitutes a heterogeneous family of pathologies, including MG of undetermined significance (MGUS), multiple myeloma (MM), smoldering MM (SMM), and Waldenström macroglobulinemia. MGUS and SMM are asymptomatic, premalignant stages of MM; however, SMM is at an intermediary clinical stage between MGUS and MM, with a higher risk of progression to malignant disease.[1,2] To diagnose and monitor

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patients with MG, protein electrophoresis, immunofixation, and kappa (κ) and lambda (λ) free light chains (FLCs) measurement in serum are the gold standard. The FLC ratio (ratio of the concentration of κ-FLC to λ-FLC) reflects the FLC type distribution. Since 2006, normalization of this ratio has been considered to be a marker for treatment response in recommendations.[3] Since 2011, the FLC ratio has become the definition of complete response for light chain MMs and nonsecreting myelomas. Furthermore, the FLC ratio can be applied for prognostication.[4] At diagnosis, it represents an independent risk factor for progression in MGUS, SMM, and MM, as well as in solitary plasmacytoma.[5,6] MM diagnosis criteria were updated in 2014 by a consensus of the International Myeloma Working Group (IMWG) to integrate biological markers of malignancy.[7] In particular, these new criteria include a serum FLC ratio of involved/uninvolved FLCs of 100 or greater, provided the absolute range of the involved FLC is at least 100 mg/L. In this context, developing a method for FLC quantification is of special interest because it represents the tumor marker of choice directly connected to physiopathology. The first available method for FLC measurement was developed in 2001 by The Binding Site Company (Birmingham, UK).[8] Freelite assay is an immunonephelometric or immunoturbidimetric method using polyclonal antibodies directed against the hidden epitopes of the FLC, and it recognizes only FLCs that are untied to heavy chain. In 2011, another N Latex FLC method using monoclonal antibodies was developed by Siemens (Marburg, Germany).[9] These 2 assays are not entirely equivalent and seem to have limited clinical utility in detecting MG in some clinical situations, and care should be taken for interpretation if assays are switched.[10-17]

Plasma cells normally produce light chains in excess that do not bind to heavy chains to form complete immunoglobulin molecules and instead enter the bloodstream as FLCs. The excess of polyclonal FLCs is rapidly eliminated via glomerular filtration, followed by tubular reabsorption and degradation. In patients with chronic kidney disease (CKD) having a reduced glomerular filtration rate (GFR), the renal clearance of polyclonal FLCs decreases and serum concentrations rise. In 2008, Hutchison et al[18] described an increase in the FLC κ/λ ratio using the Freelite method in patients with CKD (estimated GFR [eGFR] < 60 mL/min/1.73 m^2) and established an extended FLC ratio reference range for these patients. Similar work on the N Latex FLC method was performed by Jacobs et al[19] in 2014, who reported that reference values in the FLC κ/λ ratio did not differ in patients with CKD compared with healthy subjects. They concluded that N Latex FLC ratio in patients with CKD without MG was eGFR independent, with parallel increase of κ and λ FLCs with decreasing eGFR. They also demonstrated that N Latex κ-FLC and Freelite κ-FLC are similar in patients with CKD, whereas N Latex λ-FLCs are higher than Freelite λ-FLCs in patients with CKD.[19] As 20% to 40% of patients with newly diagnosed MM present renal impairment, and as renal impairment is a common complication of MM,[20] it is important to understand the impact of renal function on the existing diagnostic methods. Do these 2 assays allow patients with monoclonal FLC to be similarly identified? To address this issue, the aim of our study was to evaluate the concordance and the correlation between the 2 FLC assays in 1215 serum samples from patients with or without gammopathy.

Materials and Methods

Samples

This was a monocentric prospective study performed in the biochemistry laboratory of Rennes University Hospital. Consecutive sera of patients screened or followed-up for MG were collected between July 2012 and December 2012.

FLC Quantification

FLC measurements were performed on fresh serum, within 5 days after blood sample, on a BN Prospec (immunonephelemeter; Siemens) using the Freelite commercial kit (The Binding Site Ltd) based on polyclonal antibodies from sheep recognizing only FLCs that are not bound to heavy chains,[8] and the N Latex FLC commercial kit (Siemens) using a cocktail of monoclonal antibodies of murine origin.[9] Both kits were used according to the manufacturer's instructions.

FLC Interpretation

We chose to use the reference ranges proposed in the literature[8,9,18]: Freelite κ-FLC 3.30 to 19.40 mg/L, Freelite λ-FLC 5.71 to 26.30 mg/L, Freelite FLC κ/λ ratio 0.26 to 1.65 or 0.37 to 3.10 if renal insufficiency (eGFR < 60 mL/min/1.73 m^2); N Latex κ-FLC 6.7 to 22.4 mg/L, N Latex λ-FLC 8.3 to 27.0 mg/L and N Latex FLC κ/λ ratio 0.31 to 1.56 without specific range required for patients with CKD.

Renal function was assessed on the basis of serum creatinine levels and GFR was estimated using the Chronic Kidney Disease—Epidemiology Collaboration formula.[21] The GFR was defined as normal or slightly decreased when eGFR is ≥ 60 mL/min/1.73 m^2 (designated $CKD_{\geq 60}$). We consider a moderate renal insufficiency as eGFR of 31 to 60 mL/min/1.73 m^2 (designated $CKD_{31\text{-}60}$), severe renal insufficiency as eGFR 15 to 30 mL/min/1.73 m^2 (designated $CKD_{15\text{-}30}$), and renal failure as eGFR < 15 mL/min/1.73 m^2 (designated $CKD_{<15}$).

The purpose of FLC serum assay is to detect monoclonal components in the distribution of FLCs. The presence of a monoclonal component leads to an increase in the FLC κ/λ ratio with a monoclonal κ-FLC and to a decrease in the FLC ratio with a monoclonal λ-FLC. We considered concordant results to be those with the same clinical interpretation of FLC ratio (normal or suspected monoclonality) and discordant to be those with a divergent clinical interpretation of FLC ratio with each assay. In patients with renal impairment, the Freelite FLC ratio reference ranges were adjusted to avoid monoclonality misinterpretations.[18]

To evaluate the correlation of FLC evolution during follow-up with both assays, patients with at least 6 consecutive samples were selected. We focused on a patient's response to therapy. FLC response was examined even if the patients had measurable disease in serum and/or urine protein electrophoresis. Partial response (PR) and very good PR were defined as ≥ 50% and ≥ 90% decrease, respectively, in the difference between involved and uninvolved FLC levels.[3]

Statistical Analysis

To evaluate the correlation between the 2 assays, analysis of the quantitative results of κ-FLC, λ-FLC, and FLC κ/λ ratio was