Editorial

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New insights on the analytical performances for detecting and quantifying monoclonal proteins

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Plasma cell dyscrasias include a broad spectrum of conditions from asymptomatic monoclonal gammopathy of undetermined significance to more serious and life-threatening diseases like multiple myeloma and light chain amyloidosis, all grouped under the unifying term of monoclonal gammopathies.

The clinical laboratory plays a fundamental role in the patient's management as screening, diagnosis, risk stratification and therapy monitoring of monoclonal gammopathies require the identification, characterization, and quantification of the monoclonal protein (M-protein) secreted by the clonal plasma cells in the blood [1]. These diagnostics are conducted using serum electrophoresis and immunotyping, performed either on agarose gel or using capillary technology; in the last years, these techniques are greatly improved and are now fully automated. However, the M-protein quantification remains highly subjective and is affected by a large variability: it is the operator's choice to decide the limits of the monoclonal peak and the method to determine the protein quantification, using a perpendicular drop (PD) or tangent skimming (TS) [2]. This becomes harder when the M-protein is small and/or the polyclonal background is evident; in these circumstances it is rather difficult to proceed with the quantification. The decision whether to quantify the "small" M-proteins or to report them qualitatively, is subjective and adds further variability to the measure and the reporting [2].

Actually, a number of clinical guidelines are available to guide hematologists and laboratorians in the screening, diagnosis, monitoring and treatment of monoclonal gammopathies [3], but they include very few (if any) indications about the analytical characteristics of the laboratory methods, thus leaving a huge space to the individual judgement on how to proceed within the laboratory. The result of this situations is a lack of harmonization among different laboratories procedures and reporting; this could jeopardize the patient's management as the M-protein concentration is used to classify the response to therapy and is one of the parameters to assigning patients to clinical trials. In line with this the current recommendations indicate monitoring patients in the same laboratory using the same methods, in spite of the increased mobility of patients (and samples) among clinical centers and countries [4].

In this issue of *Clinical Chemistry and Laboratory Medicine* a multicenter study reports important results on the topic, examining the factors that impact on the accuracy and precision of the serum electrophoretic methods in detecting, typing and quantifying M-proteins, focusing in particular to small M-proteins [5, 6].

The study has several merits: first of all a large number of centers were involved (14 clinical laboratories and two IVD companies across three continents) thus allowing to include results from the vast majority of technologies and methods available in the market; second, it has a sound basis of accuracy using samples spiked with a determined amount of monoclonal therapeutic antibodies with different migration patterns (cathodal, center gamma and beta) mimicking the presence of M-proteins in the serum; third, the tested samples show a wide range of M-protein concentrations (from 10 to 0.1 g/L) covering the range of concentrations encountered in the clinical laboratory routine and including a high number of samples with low concentrations of the M-protein; fourth, the amount of sera in the samples was enough to allow duplicated measurements (1203 in total) so that it was possible to determine the within-laboratory precision.

The study results have been split into two articles: Part I is related to the factors impacting on limit of quantitation (LoQ) [5], Part II is related to the limit of detection (LoD) and the precision of the methods used to quantify the M-proteins verifying the possibility to follow-up the patients with a precision suitable for the clinical needs [6].

The obtained results are relevant for the laboratory practice. The main factors influencing the LoQ have been identified. The most relevant determinant of the accuracy and of the within- and inter-laboratories precision is the M-protein concentration and this is true across all the methods tested; however, the extent of the deviation from the true value depends on the polyclonal background (as it increases, it is more difficult to gate the small M-proteins),

the migration pattern (the cathodal M-proteins are quantified with better accuracy than the mid-gamma and the beta migrating ones) and the gating technique (PD causes overestimation, while TS produces an underestimation of the small M-proteins). The combined influence of these factors makes it difficult (even impossible) to recommend a single method to quantify the M-proteins; however, the awareness of the limitations of the technology and of the gating method used in the laboratory is of great importance. The most important consideration derived from the study is that quantifying and reporting M-proteins below 1 g/L is affected by an unacceptable loss of accuracy: these should be reported qualitatively. However, the laboratory professionals should be aware that this value could be much higher as the polyclonal background increases and the M-proteins migrate in the mid-gamma zone or overlap other proteins normally present in the electrophoretic patterns.

Regarding the LoD, (examined in Part II) the results of the study show small but significant differences between different methods. All the tested methods are able to detect M-proteins till a concentration of 1 g/L; this value further decreases (around 0.5 g/L) if capillary technology was used and if the M-protein is included in a hypogamma background. As expected, the typing methods (immunofixation and immunosubtraction) can detect M-proteins at lower concentrations, with immunofixation showing the best sensitivity. The factor that influences the LoD most has been identified in the amount of gammaglobulin in the sample; in hypergamma samples, the percentage of laboratories able to detect the M-proteins at concentrations below 1 g/L decreases significantly. Importantly, the study highlights some variability in the LoDs even between laboratories using the same methods. This has potentially serious clinical consequences as the complete remission criteria in patients with multiple myeloma, requires the absence of the M-protein at the immunofixation; thus, a single patient may or may not meet the criteria depending on the analytical performances of the laboratory [7]. This emphasizes once more the attention and the care the laboratory professionals should apply in this field of the laboratory medicine and focuses on the need that dedicated sections should be reserved to the protein diagnostics within the clinical laboratory. The laboratory personnel working there can thus operate in close collaboration (and vicinity) with hematologists, rather than being incorporated in large core laboratories far from the clinics, as it is likely to happen considering the merging and consolidation processes of the clinical laboratory services that are presently carrying on in Western countries [8, 9].

The other aspect investigated in Part II is the within-laboratory variation of M-protein quantification. A total of 1203 duplicate measurements in a range of 1–10 g/L, allowed calculating an overall mean CV of 5.0% (95% confidence intervals 4.7–5.4). The factors that impact on the CV are (once again) the M-protein concentration and the polyclonal background; actually, the CVs range from a mean of 2.6% for an M-protein of 10 g/L in a hypogamma background to a CV of 12.7% for an M-protein of 1 g/L in a hypergamma background. Other precious information for the clinical laboratory is that the CVs do not differ significantly between agarose gel and capillary methods; at the opposite, the gating methods show statistically significant differences, with the PD method showing a lower CV. Due to these low CVs and considering that the spiked samples contain a single M-protein in a stable polyclonal background, the study could evaluate the laboratory performances during the follow-up of a patient with monoclonal gammopathy. The results are satisfactory as all the laboratories, regardless of the method utilized to measure the M-protein, were able to identify an increase or a decrease of the electrophoretic spike in more than 99% of the samples.

There are two crucial conclusions of this study: the electrophoretic quantification of the small M-proteins is affected by a such poor accuracy that it is not advisable to report M-proteins below 1 g/L; at the same time, the CVs within the laboratory are very low and allow a monitoring of the single patient in line with the clinical needs.

The main limitation of the study is that the laboratory tests have been carried out in the participating centers in a short period of time, probably performed by the same operator who was aware that the examined samples did have qualitative abnormalities. In spite of this limitation, the obtained results are so sound that a detailed description of the methods used to detect and quantity the M-proteins should necessarily be included in future papers on the topic, to allow an evidence-based evaluation of the research.

The authors dedicate the work to Jillian Tate; it should be not forgotten that the study is based on a project conceived by Jill Tate who sadly passed away almost 1 year ago. She was able to bring together experts from all over the world in the study; they contributed with their expertise to the success of the research.

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