Editorial

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Perspectives in developments of mass spectrometry for improving diagnosis and monitoring of multiple myeloma and other plasma cell disorders

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Assessment of circulating monoclonal components (MC) as clone biomarkers is a central step in the management of monoclonal gammopathies, traditionally achieved through the combined use of electrophoresis and immunofixation (EF/IFE), free light chains (FLC) assessment and quantification of immunoglobulin subclasses. This analytical combination grants that three crucial aspects can be addressed: determination of clonality, MC typing and MC quantification. In recent years, mass spectrometry (MS) has become increasingly present in the clinical analysis of monoclonal proteins [1], to the point that it has replaced traditional tools as first-line approach in centers such as Mayo Clinic [2]. With improved performances and accessibility of instrumentation, in fact, MS is now suitable to provide answers to all the above outlined questions; in addition, it possesses peculiar capabilities, which may be substantially advantageous in specific clinical contexts. Three aspects in which MS could outperform traditional methods are specificity for the clonal MC, analytical sensitivity and ability to detect post-translational modifications (PTMs).

Regarding the first point, MS provides a highly specific, individualized tag to monitor the MC over time: its exact mass. Mass is virtually unique for each MC, and therefore allows to follow the pathologic protein with unprecedented specificity over polyclonal immunoglobulins, oligoclonal

bands or therapeutic monoclonal antibodies. This is especially advantageous with the increasing usage of monoclonal antibodies for managing MM and related conditions [1]. The second advantage of MS is the high analytical sensitivity towards MC detection [1, 3–6], either through evaluation of the whole monoclonal light and/or heavy chains (top-down methods) or of clonotypic peptides thereof (bottom-up approaches). The improved sensitivity of MS compared to the traditional combination of methods has been confirmed by recent clinical studies [3–6] and is especially critical in the context of minimal residual disease (MRD) assessment. Reports by Mayo Clinic investigators showed that not only MRD assessment is feasible on peripheral blood, but also that MS might outperform bone marrow analysis by next-generation flow [5, 6]. Considering the prognostic and decisional implications of MRD detection, the availability of an instrument for spotting minimal amounts of the pathogenic MC on peripheral blood is of key practical importance. Matrix-Assisted Laser Desorption/Ionization-Time of Flight (MALDI-TOF) MS coupled with an immuno-enrichment step was significantly better than IFE in detecting serum monoclonal LC in patients with abnormal serum FLC ratio [7].

Another setting in which MS analysis plays a special role is evaluation of PTMs, most importantly glycosylation. Recent studies of serum monoclonal light chains using the MALDI-based Mass-Fix method [2] showed that glycosylation can be detected on routine testing, and that this modification is a risk factor for progression of the monoclonal gammopathy to AL amyloidosis, myeloma, and other plasma cell disorders [8, 9]. This observation may have important implications for earlier diagnoses and prognosis. In addition, MS could confirm the molecular identity between the light chains present in blood and those deposited in tissues in AL amyloidosis, thus supporting the correctness of typing.

The report published in this issue of CCLM by Deighan and colleagues [10] is exemplificative of the unique potentialities offered by MS in the assessment of individual MC. In

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this study, advanced MS techniques were coupled with EF/IFE to obtain an in-depth characterization of primary sequence and quaternary structure of an atypical MC. This work nicely outlines the potential of next generation proteomic solutions for characterizing individual monoclonal components, with important implications in the perspective of personalized medicine. At the same time, however, the report also exemplifies the required skills and challenges in the setting of MS-based MC analysis, which are among the reasons why only some of the potential applications of MS have so far reached the clinical stage. The term “mass spectrometry” indeed encompasses a spectrum of platforms based on different technologies. While some approaches (especially MALDI-TOF-based ones) have already entered the clinical stage due to their robustness, automatization, and easiness of spectra interpretation [2], other methods are less prone to a high analytical throughput and require significantly more specific training. Currently, the majority of liquid chromatography-mass spectrometry (LC-MS)-based approaches are largely confined to a clinical research setting, with a less clear perspectives regarding rapid translation to the clinical routine. A major additional issue relies in the variability of MCs in terms of primary structure; this translates into the fact that the specific amino acid sequence of each individual MC is typically not available, except than in the rare clinical cases in which it can be sequenced from the bone marrow clone. Availability of the MC sequence would allow predicting the expected mass of the MC and of its peptides, thus increasing confidence in assignment of monoclonal peaks at baseline and facilitating selection of clonotypic peptides for targeted MS. Some of the existing challenges in the MS analysis of monoclonal proteins may indeed now benefit from improvements in other steps of the analytical process, rather than mass spectrometry itself. In the short time, the quest for further improvement is likely to be played on grounds such as effectiveness of analyte enrichment in the preanalytical phase, bioinformatic data analysis and high-throughput next generation sequencing of immunoglobulin transcripts.

Overall, we are at the beginning of a new era in the analysis of monoclonal proteins, in which MS will gain a progressively wider space. MS is a privileged instrument for precision medicine in monoclonal gammopathies; in addition, the increasing usage of therapeutic monoclonal antibodies, the new consciousness of MRD importance and the novel acquisitions on the prognostic role of PTMs impose new challenges on the traditional electrophoretic and immunochemical methods. To which extent and at which pace the clinical routine will change on a wide scale

is, however, still unclear and will depend on several factors. Firstly, EF and MS are not interchangeable. The two approaches cannot be alternatively interrogated regarding MC quantitation, with obvious implications in follow up of existing patients. Moreover, moving from EF/IFE to MS requires profound reorganization in the clinical laboratory, both in terms of instrumentation and of personnel competences, which may not be affordable - nor needed - in smaller laboratories. Further studies are now urgently required to assess the actual impact and the cost-benefits ratio of transition to MS on improving patient outcome.

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