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LETTER TO THE EDITOR

Difficulties in immunofixation analysis: a concordance study on the IFM 2007-02 trial

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Multiple myeloma response is evaluated according to the International Myeloma Working Group Uniform Criteria.[1] Among these criteria, serum electrophoresis has a pivotal role as it represents the first step to detect the persistence of the monoclonal protein identified at diagnosis, while performing immunofixation tests in case of normalized electrophoresis. Interpretation of immunofixation according to diagnosis profile is sometimes difficult and requires a careful examination, especially when very thin bands are observed. Indeed, atypical serum immunofixation patterns,[2] also named oligoclonal bands[3] or small abnormal protein bands,[4] have been often reported following not only allogeneic transplantation but also autologous transplantation and even following intensive chemotherapy for leukemia. In fact, if no monoclonal component is detected by serum protein electrophoresis, immunofixation interpretation with bone marrow evaluation determines the type of response, stratifying patients between complete response (CR) and a very good partial response (VGPR).[1] Because immunofixation interpretation is based on human evaluation, it presents a certain degree of subjectivity that conditions its performances. The purpose of this work was to estimate the inter-operator variability and intra- and inter-laboratory performances.

Therefore, we considered serum evaluations conducted within the framework of the IFM 2007-02 trial, in which the objective was to compare bortezomib − dexamethasone (VD) as an induction before a high-dose therapy and autologous stem cell transplantation (ASCT) with a combination comprising reduced doses of bortezomib and thalidomide plus dexamethasone (vTD) in patients with multiple myeloma.[5] Blood samples were analyzed at baseline, after cycle 2, after cycle 4 (post induction) and after ASCT. We first selected immunofixation tests performed during the three last assessments when serum electrophoresis profile was normalized. A total of 119 immunofixation tests were selected as difficult to read and were revised by five biologists of our department of biochemistry. The samples tested corresponded to 70 multiple myeloma with complete monoclonal immunoglobulin and one light-chain myeloma. In 61% of the cases, patients presented monoclonal immunoglobulins of more anodic migration (on β_1- or β_2-globulin zone) than the γ-globulin zone. In addition, immunofixation tests were realized in 54% of the assessments after autograft. These two circumstances represent the situations in which interpretation is the trickiest.

Two questions were asked to the five biologists:

- Do you consider that the monoclonal abnormality characterized at diagnosis is still present?
- Does the immunofixation result suggest an oligoclonal profile?

Statistical analysis of results was performed by the calculation of Kappa − Fleiss coefficient (software STATA version 11MP), which is used to evaluate the degree of concordance between several qualitative variables.[6]

Concerning the first question, the results obtained showed a good global inter-operator concordance ($K = 0.75$). In detail, among the 119 immunofixation tests, we noted 26 cases of discordancy (21.8%): this proportion seemed to be important but corresponded to two principal situations. For some of these 26 samples, the monoclonal protein was present at a concentration close to the limit of detection of immunofixation. For others, the immunofixation presented an oligoclonal profile, and in this situation it is difficult to determine whether one of the bands corresponds to the monoclonal abnormality identified at diagnosis or to a different one with a similar electrophoretic mobility.

Concerning the second question, results showed an average concordance with a Kappa coefficient of 0.63: 22 discordances were noted highlighting the major problem of 'oligoclonal' definition. Should we consider monoclonal protein among several bands identified or should we count only additional bands to define an oligoclonal profile?

In the second part of our work, we performed an inter-laboratory evaluation. We sent 26 serum samples to the two other centers (MayoClinic, Rochester, USA, and Hospital of Barcelona, Spain) where the immunofixation test is performed using the same technology (Sebia, Hydragel 4IF, Evry, France). These samples represented at least VGPR assessments with monoclonal component not detectable by electrophoresis. Interpretation had to be considered with respect to the screening profile, and the results were compared across the two centers and our laboratory. We observed an agreement for 24 immunofixation tests out of 26: one case of discordancy concerned a myeloma case with IgD Kappa monoclonal protein associated with monoclonal free light Kappa chains at diagnosis not retrieved at post-cycle 2 immunofixation by one center. The other discordancy was an IgA Kappa monoclonal component not detectable at post-ASCT immunofixation for one center.

This short study confirms a correct homogeneity of the practices and highlights important points. First, we all do not have the same way to interpret immunofixation profiles presenting several thin monoclonal bands: some biologists describe precisely monoclonal proteins composing immunofixation results, whereas others use the concept of oligoclonal profile consisting in the identification of at least three monoclonal bands at immunofixation. This situation is likely to be due to transient dysregulation of the regenerating B-cell compartment during recovery post transplantation,[7,8] which has been associated with a good prognosis and reveals a more durable immune reconstitution. Specifically, for myeloma patients, oligoclonal profiles may potentially represent either a change in the antibody production of the original plasma cell clone or the emergence of a new malignant clone. Oligoclonal profiles raise the difficult assessment of monoclonal protein persistence potentially hidden by several monoclonal bands when electrophoretic mobility is similar to the one seen at diagnosis. It specially occurs after ASCT and requires laboratory knowledge for characterization of the monoclonal immunoglobulin in terms of isotype, light chain and electrophoretic mobility, which should be documented in paper or in electronic data systems for comparison purposes.[4] There is a risk for a wrong negative

immunofixation result considering thin bands in the oligoclonal profile, consequently defining a CR. In these uncertain conditions, we consider that it is preferable to answer the immunofixation test as doubtful, that is, with a positive test result until proved otherwise, and thus classify patients into very good partial response. Conversely, when possible, identification of a different band from the original one has to be mentioned on the report, although its clinical significance is uncertain.[3]

Finally, our study confirms a correct homogeneity of both intra- and inter-laboratory practices. They allow identifying the degree of variability in the immunofixation response and highlight the necessity of precise immunofixation comment: indeed, beyond a simple positive or negative immunofixation test result, we should precisely comment the presence or absence of monoclonal abnormalities. This evaluation is very important as it conditions the stratification of the result type, particularly for CR and VGPR. Thus, it is well known that, in the context of ASCT, achieving CR or at least VGPR is associated with longer progression-free survival and in most studies longer survival.[9] Follow-up in the same center is the minimum requirement we should have to certify an optimum serum evaluation according to previous evaluations. Identification of oligoclonal profiles and a persistent assessment of the monoclonal protein identified at diagnosis requires careful reporting and monitoring as these bands may occasionally represent true isotype switching leading to disease relapse: we really need a precise and consensual definition of an oligoclonal profile. In conclusion, we have to emphasize on regular reviews between biologists to harmonize our practices and interpretations.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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