

APPARENT DISCREPANCIES IN THE QUANTITATION OF FREE LIGHT CHAINS IN SERUM OF PATIENTS PRESENTING WITH A MONOCLONAL GAMMOPATHY

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INTRODUCTION

With the development of a new sensitive technique, measurement of κ and λ free light chains (κ and λ FLC) has become an interesting option. However, while using FLC quantitation to follow up patients with monoclonal gammopathy of undetermined significance (MGUS) or plasma cells diseases, sometimes conflicting results emerged between the immunodetection step showing the lack of free monoclonal light chains (FMLC) in the serum and the quantitation step showing a high level of one type of FLC. In a previous study (poster PO. 408) we showed that less than 50% of the samples with an elevated concentration of κ or λ FLC were positive for FMLC with immunofixation-electrophoresis (IFE). We report here the results of a study undertaken on the sera of 112 consecutive patients followed up for a monoclonal gammopathy associated with various diseases in order to determine the frequency of these discrepancies and in an attempt to explain them.

METHODS

In the serum of 112 consecutive patients followed up for a monoclonal gammopathy we measured serum FLC using a sensitive and automated immunoassay (The Binding Site) on a Dade Behring nephelometer (BN II®). The FLC quantitation was associated with a capillary electrophoresis (Paragon CZE 2000®, Beckman) and to IFE (Helena). Moreover, no matter what the concentration of FLC was, every sample was submitted to an ultra sensitive immunoelectrophoresis (us-IEP) as previously described (Drouet et al. 1999, Clin. Exp. Immunol. ; 118:465-472). Briefly, IEP was performed using home-made ultrathin (500 μ m) layers of agarose (Seakem HEE0, FMC) gels (125x260 mm) in barbital buffer pH 8.6 with precut troughs. According to FLC quantitation, various volumes of serum (1, 5 or 10 μ l) were applied. After completion of the run at 25 V/cm and at 4°C the troughs were filled with antisera directed against total κ or λ (Silenus), κ or λ FLC (The Binding Site and Helena) and alpha-1-antitrypsin (Dade Behring). After an immunodiffusion step of 48 hours, the gel was washed, dried, stained and ready for interpretation.

RESULTS

Figure 1 : Distribution of clinical diagnoses in 112 consecutive patients followed up for a monoclonal gammopathy.

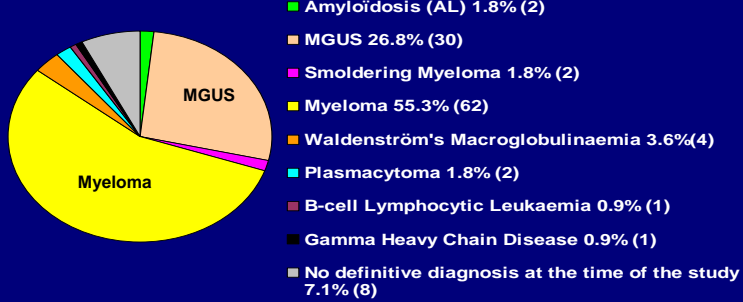


Figure 2 : Distribution of M. components in 112 consecutive patients followed up for a monoclonal gammopathy associated with various diseases.

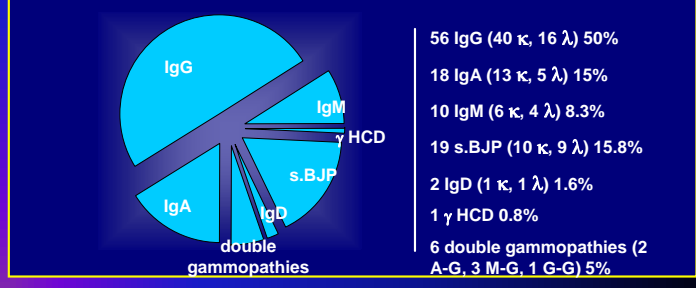


Figure 3 : Serum light chain concentrations and positivity of us-IEP.



Concerning us-IEP, it appeared necessary to fill in twice the troughs with anti-FLC and the best results were obtained with 5 μ l of samples and the antiserum from Helena.

a) In one sample (•), FMLC were detected while both λ_2 concentration and κ_2 / λ_2 ratio were within the normal range. In 4 samples (◊), FMLC were detected while only λ_2 concentration was outside the reference range. The highest λ_2 concentration associated with undetectable FMLC, even with us-IEP, was 66.8 mg/l (•). The lowest λ_2 concentration associated with detectable FMLC was 23.8 mg/l (•). Using us-IEP prevent missing 5 samples, while using FLC quantitation prevent missing 3 samples. Of 24 samples (•) positive for immunodetection of FMLC and/or both λ_2 concentration and κ_2 / λ_2 outside the reference range, 21 (87.5%) were positive using IEP and 21 (87.5%) were positive using FLC quantitation.

b) Of 48 samples with both κ_2 and λ_2 / λ_2 outside the normal interval, 35 (73%) (•) showed FMLC using us-IEP compared to less than 50% with conventional IFE (fig.4). The highest κ_2 concentration not detected by us-IEP was 164 mg/l (ratio 56.5) (•) and the lowest one detected 31.8 mg/l (ratio 3.28) (•). Eight sera out of 13 (61.5%) with undetectable FMLC contained less than 40 mg/l of κ_2 . In 4 patients κ_2 concentration was over 3 g/l (3140, 4200, 5370 and 16600 mg/l) and thus, obviously overestimated. In the four cases, us-IEP showed 2 or 3 arches with different mobilities complexing with A1AT and 2 arches with the same mobility (fig.5). Two arches with the same mobility appeared in some samples with κ_2 concentration over 500 mg/l (3 cases of 4) (fig.6).

Both FLC quantitation and a sensitive immunodetection technique should be used to prevent missing positive samples for FMLC. Besides, both concentration and ratio should be considered.

Using FLC quantitation prevent missing 27% samples (13) with no detectable FMLC even with a sensitive technique.

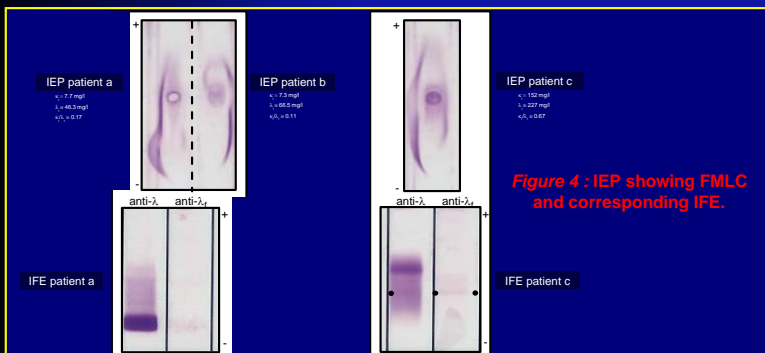


Figure 4 : IEP showing FMLC and corresponding IFE.

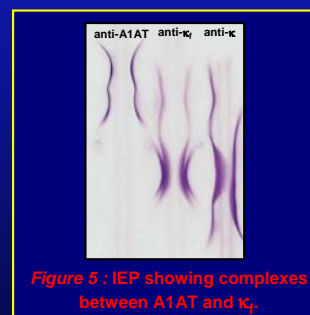


Figure 5 : IEP showing complexes between A1AT and κ_2 .

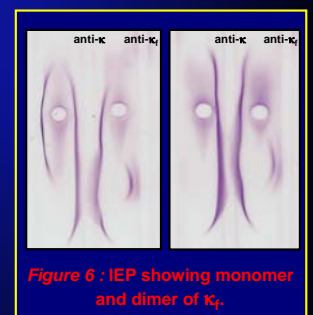


Figure 6 : IEP showing monomer and dimer of κ_2 .

CONCLUSION

Discrepancies between measured FLC and FMLC detected by IFE are partly due to the mobility of FMLC which happen to often migrate in the vicinity of the intact monoclonal immunoglobulin. They are also explained by the fact that antisera directed against FLC have a very low titer. As to the overestimation of κ FLC it is the result of a mixture of different populations of FLC with various molar masses : κ monomers, κ dimers and complexes between κ FLC and A1AT.