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ImmunoNephelometric Determination of Free Light Chains of Immunoglobulins in Urine for the Detection of Bence Jones Proteins, and Comparison with Immunofixation

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Introduction

Urinary ImmunoFixation (IFE), the classical and reference method (1, 2) of detecting Bence Jones Protein (BJP) (monoclonal Free Light Chains – FLC – in urine), is laborious, expensive and difficult to interpret, and **above all, impossible to standardize as intra as inter laboratories**.

Leaving aside the polyclonal increase of Ig in lupus, rheumatoid arthritis, etc., and dwelling, instead, on the so-called Monoclonal Components (MC) it should be said that these on the one hand characterise multiple clinical situations and on the other a sub-clinical situation whose current definition, Monoclonal Gammopathy of Undetermined Significance (MGUS), demonstrates the uncertainty in the matter. BJP are no exception, as they can be an indication of MGUS and myeloma, or AL amyloidosis or Light Chain Deposition Disease (LCDD).

Introduction (2)

In the evaluation of these anomalies the separative techniques and the immunological techniques used individually have their limits whereas the combined use of both types of techniques guarantees the best results.

The detection of BJP is rendered even more uncertain and complex by the particular metabolism of the FLC which constitute it and which involves renal function and, consequently, by being their evaluation as well as “sample-dependent”.

The idea is to use a first level screening test to detect the real presence of FLC in the sample (immuno-specific technique for FLC) followed by, on the positive samples in the first test, a second test to verify the electrophoretic mobility of FLC: monoclonal or polyclonal.

As regards the quantitative evaluation, that is the concentration of the BJP, this would seem important above all in the follow up of MGUS, myeloma and other gammopathies.

Objectives – Methodology - Samples

Kits for the direct immunonephelometric determination on unconcentrated urine of kappa and lambda free light chains by automatic nephelometer have been available commercially for several years (see below “Materials”).

The general objective of the work is to evaluate the potentiality of including nephelometric determination of the FLC (IN-FLC) with the kit in question in the routine protocol for the detection of BJP as a first level test able to identify, with sufficient reliability, the definitely negative samples and those in which the presence of FLC should be followed by IFE in order to determine mono or polyclonality.

To be more precise, the objective was to verify whether the use of this protocol could offer concrete advantages in terms of objectivity and uniformity in the final result compared to the use of IFE notoriously affected by :

- a certain lack of reproducibility both intra-laboratory and inter-laboratory, even for the same commercial product utilised (3);
- the semi-automatic operation, at least in our set-up, and therefore the possibility of error in identification of the sample;
- and lastly the subjectivity of result interpretation.

In other words, if the IN-FLC method had proved itself analytically reliable in detecting the presence of FLC in the sample, this information would have served as an objective and reproducible basis for identifying those samples to be considered BJP-negative compared to those potentially BJP-positive and subject to quality IFE so as to highlight the FLC polyclonal or monoclonal (BJP) detected by IN-FLC.

In this way, the IN-FLC would have served as quality control of IFE; avoiding sample exchanges and inversion of antisera, providing a guide on sample concentration, etc.

From this viewpoint, in the first place we examined the analytical performances of the IN-FLC method.

We also verified the qualitative value of the method as first level test in the sense indicated above and therefore made a parallel comparison between IN-FLC and IFE on all the urine samples coming to our Laboratory with the request for the detection of Bence Jones Proteins in three different periods of three months, each between 1998 and 1999, for a total of 572 samples from 526 patients.

It must be stated that the vast majority of requests for the detection of BJP which come to the ImmunoHematology Laboratory are “aimed at” and supported by precise clinical motives, diagnostic suspect or follow-up.

The results were compared and analysed only subsequently; for this reason the verification of dubious situations was carried out only on a few samples.

Materials and Methods

A. ***ImmunoNephelometric Determination of Free Light Chains (IN-FLC)***
We used the "New Scientific Company" kit, code K.BNA.FRK.FRL, (New Scientific Company, Cormano, Milan, Italy) on the "Dade Behring" BNII Nephelometer, (Dade Behring, Milan, Italy).

B. ***Standard Urine Immunofixation (u-IFE)***
The "Sebia" Hydrasys equipment and kits were used.
The anti Free Light Chain antisera are those of Sebia and of the New Scientific Company.
u-IFE was carried out on all the samples concentrated 25 times, using the manufacturer's method.

C. ***Protur Urine Immunofixation (u-IFE-Protur)***
In few cases (15 in all) Beckman Coulter's Protur Plus kit was utilised.

Analytical Performances Evaluation

We primarily assessed the following analytical performances:

- Reproducibility of the calibration curves, linearity and parallelism, imprecision: very good
- Sensitivity: analytical limit: FRK = 0.8 – FRL = 0.5 mg/l;
  biological limit: FRK and FRL = 3 mg/l
- Cross-reaction with intact Ig: not demonstrable
- Antigen excess: not demonstrable until over 40,000 mg/l

IN-FLC / IFE comparison – Samples Evaluation

We carried out parallel IN-FLC and IFE with anti FLC antisera on all our laboratory urine samples requesting the detection of Bence Jones Protein in three different periods of three months, each between 1998 and 1999 for a total of 572 samples from 526 patients. Patient recurrence was: 31 patients present twice, 6 patients three times and 1 patient four times.

It must be emphasised that the vast majority of the requests for the detection of BJP in our ImmunoHematology Laboratory are “aimed at” and supported by precise clinical motives, diagnostic suspect or follow-up. In fact, in our Hospital a Centralised Laboratory works with a protidology section.

Positivity Marker

Standard IFE was interpreted by the usual reader and, in case of doubt, by a second person. The cases remaining dubious by both were classified thus, that is "dubious".

IN-FLC was considered positive if one of the two FLC had concentration > 5 mg/l.

Result Analyses and Verifications

The results of standard IFE and of IN-FLC were compared and analysed as a whole only subsequently, and consequently verification of dubious situations was carried out on few samples - 15 in all - of the most recent. IFE on Protur Plus with anti LC(b&f) and anti FLC antisera were used for this, and the samples were opportunely concentrated or diluted in such a way as to obtain as accurate a result as possible.

IN-FLC / IFE comparison – Samples Evaluation (3)

Table 11

Samples 572			Comparison results - IFE and IN-FLC						
Standard Urine IFE concentrated 25 times			Nephelometry Free Light Chains			**Urine IFE** Protur Verification			
Type	No.	% of totale	type resulted	no. of samples	% of group	run	BJ-	BJ+	BJ-?
BJ-K	42	7,34%	K<,L<	**2**	4,8%	0			
			suspect	40	95%				
BJ-K suspect	3	0,5%	K<,L<	0	0%	2			
			suspect	3	100%			**2**	
BJ-K+BJ-L	2	0,3%	K<,L<	0	0%	0			
			suspect	2	100%				
BJ-L	40	7,0%	K<,L<	0	0%	0			
			suspect	40	100%				
BJ-L suspect	4	0,7%	K<,L<	0	0%	0			
			suspect	4	100%				
poly and ladder κ & λ	51	8,9%	K<,L<	0	0%	1			
			suspect	51	100%				1
negative	430	75%	L<,K<	284	66%				
			suspect	146	34%	12	5	**3**	4

Total BJP & BJP suspect	91	16%	K<,L<	**2**	2,2%
			suspect	89	97,8%

The results are shown on Table 1.
In order to simplify the comparison layout between standard IFE and IN-FLC, the results have been grouped based on the result of the standard IFE and are shown under the following points.

1. Standard IFE – BJP-kappa positive
   42 samples resulted positive on IFE (7.3% of the total) and 2 of these resulted negative on the IN-FLC test.
   Verifying of such discrepancy between standard IFE and IN-FLC, which is in fact the only check, was not possible.

2. Standard IFE – BJP-kappa suspect

There were 3 samples all positive on IN-FLC.

It was possible to carry out IFE on Protur Plus as verification on 2 of these samples and both resulted BJP positive.

One of these samples was of a current patient with a case history of two recurrences:

- September 1998 IN-FLC FRK = 62 mg/l (positive)
  standard IFE = BJP-kappa positive
- May 1999 IN-FLC FRK = 27 mg/l (positive)
  standard IFE = BJP-kappa dubious

3. Standard IFE – double BJP: kappa + lambda

Two samples, both positive on IN-FLC.

Both samples, from two different patients, had double IgM-K + IgM-L MC in the serum, which are also the only MC of this type on record.

4. Standard IFE – BJP-lambda positive

   40 samples (7% of the total) all positive on IN-FLC.

5. Standard IFE – BJP-lambda suspect

   4 samples, all positive on IN-FLC.

   It was not possible to do further checks on any of these.

6. Standard IFE – polyclonal and ladder kappa and lambda

   51 samples (9% of the total) all positive on IN-FLC.

7. Standard IFE – negative

   430 samples (75% of the total) resulted negative on standard IFE; out of these, 284 (66% of IFE negative) were also negative on IN-FLC, whilst 146 (34% of IFE negative) resulted positive.

   Verification by IFE on Protur Plus of 12 of these samples was carried out, chosen amongst those with IN-FLC values more suspect and the result was that 3 were BJP-kappa positive and 4 positive for polyclonal-FLC.

In conclusion,

including the suspects, standard IFE detected 91 (16%) BJP positive samples and of these 2 resulted negative on IN-FLC (2.2% of the IFE positive).

Furthermore, IN-FLC showed all the samples as BJP suspect by IFE, of which the two checkable cases were confirmed BJP positive.

Also, IN-FLC indicated 146 samples suspect of those negative on standard IFE and, of the 12 checkable ones by IFE on Protur, 3 resulted BJP positive, that is "false BJP-negatives" on standard IFE.

Conclusions

IN-FLC has demonstrated that it can be well placed as a first level test in the routine protocol for the detection of Bence Jones Proteins and could take on a role in uniformity of the quality of response in that area.

IFE will always have to be used as a test to indicate the mono- or polyclonality of the FLC shown up in IN-FLC, but the latter should, as it were, "pilot" the quality and sensitivity of IFE.

In other words, ***if a suspect sample on IN-FLC results "negative" on standard IFE, IFE with greater sensitivity should be reverted to, for example concentrating the sample further.***

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