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The Detection of Bence Jones Proteins
A Review from the Records of the Ospedali Galliera, Genoa

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Abbreviations

BJP	Bence Jones Proteins
EF	Electrophoresis
IFE	Immunofixation
LC(b&f)	Total Light Chains (bound & free)
FLC	Free Light Chains
MC	Monoclonal Component

Introduction

The SIBioC Protein Study Group's recent publication of "Guidelines for the Determination of Bence Jones Proteins" (1) led me to examine our records of monoclonal gammopathies and, in particular, cases of urine with BJP co-migrant with intact monoclonal Ig.

I must thank Professor Romano, Director of the Galliera Analysis Laboratory, for his co-operation and Dr. Campanella and Dr. Pittaluga for their active help.

The file in question is computerised and was opened in 1992, closing mid-1999 (the year in which the programme and recording system were changed) and contains 10,146 entries of protidological serum tests in patients with monoclonal gammopathy of which 2,355 are also complete with data relative to the determination of BJP in the urine.

Study Objective

The contents of the file were not examined purely for statistical purposes but rather to focus attention on the comparison of positivity for BJP in relation to the antisera utilised in IFE to highlight them.

Data and methods

The review concerns the recording of the protidological testing of urine showing positive for the presence of MC during the period from 1992 to the whole of 1997. This delimitation was necessary in order to have uniformity in the criteria for determination of BJP and in the recording of the data. In fact in this period the urine – a fresh sample of the second urine of the morning was, as a rule, requested – of patients with serum monoclonal gammopathy, and/or requesting the determination of clinically-suspected BJP, was analysed as follows:

- electrophoresis (EF) on acetate (Gelman-Dasit) colloidal gold stain (Dasit)
- immunofixation (IFE) on such urine and concentrated 100 times (Minicon system) with anti Total Light Chains (bound & free) (LC(b&f) kappa and lambda antisera and also anti Free Light Chains (FLC) kappa and lambda, as well as the anti heavy chain antisera of the monoclonal Ig(s) present in the serum.

  Immmunofixation was carried out either with the standard Beckman IFE Paragon systems or Sebia manual Hydragel with relative antisera kit, whilst the anti FLC antisera were alternately either Dako or NSC.

  A first reading was always taken after staining with Coomassie blue and a subsequent reading after re-staining with violet.

Results

The results are shown in Tables 1 and 2, and Figures 1 to 4. It should be pointed out that:

a. in the various groups the patients are only counted once if the analytical data is recurrently identical; if the analytical data is different, the same patient is included in more than one group.
b. The groups in Table 1 and the relative Figures are "schematic and summarising", although homogeneous from the logical-interpretative point of view in the composition of the MC(s), including EF and IFE diversified images.

There are 1,043 cases in total, sub-divided as follows:

- 635 patients with urine completely negative for both BJP and intact monoclonal Ig.
- 101 patients with urine positive for intact monoclonal Ig but negative for BJP (Table 1 - Column D, Fig. 4); of these, 95 showed one MC only and six showed two MCs, all of which naturally consisted of intact Ig.
- 307 patients with urine positive for BJP (Table 1 – Columns A+B+C) divided as follows:
  - 202 patients with positivity for BJP only (Table 1 - Column A; Fig. 1); in reality BJP can be seen with one, two or more bands and rarely with ladder**.**
  - 85 patients with BJP positivity and for monoclonal intact Ig with distinct bands (Table 1 - Column B; Fig. 2); also here the BJP can be seen in various bands and the intact Ig can be seen in two different classes (for example, IgG-kappa+IgM-kappa), or also combinations of the aforementioned situations.
  - 20 patients with BJP positivity and monoclonal intact Ig with co-migration (single band). In these latter cases, the monoclonal intact Igs were 12 of kappa type (6 IgG and 6 IgM) and 8 lambda (7 IgG and 1 IgM) (Table 1 - Column C; Fig. 3, 3/A, 3/B).

    The group is actually made up of:
    - 15 samples showing a single band with monoclonal intact Ig perfectly overlapping the BJP (Fig. 3)
    - 3 samples showing one band of intact Ig overlapping the BJP and another band of solely intact Ig, which in one case corresponds to the first and in two cases is different (Fig. 3/A)
    - 2 samples which show a band of intact Ig co-migrant with BJP and another band of BJP only (Fig. 3/B).

Discussion

Thus we can see that the phenomenon of co-migration was verified in 6.5% of the patients with BJP positive urine and in 4.9% of the patients with MC in the urine.

It is still more interesting nevertheless to analyse these data in the light of that which emerges from the SIBioC guidelines and in particular where it is stated:
"The antisera to be used are anti κ and anti λ total with the addition of the anti heavy chain antiserum of the immunoglobulin present in the serum as per Fig 1". (Fig. 6)
In our case records, 121 are as per the example shown in the figure 6 on the left (Table 1 – Columns C+D); that is, MC reacting positively with the anti LC(b&f) antiserum and with that of the anti heavy chain of the serum Ig.
However, in 20 of these, reaction can also be seen with anti FLC antiserum; we can thus verify 16.5% cases of BJP co-migration and intact Ig which would not have been detected without the use of anti FLC antiserum (Table 1 – Column C; Fig. 3).
The percentage does not seem negligible to me.

The relative guidelines state:
"Anti free light chain antisera are not recommended in that they often have low titre, little avidity, are expensive and can produce cross-reactivity with bound light chains. They can be useful in particular cases such as, for example, the identification of a BJ protein which co-migrates with intact immunoglobulin".
As regards the first two statements, it seems to me that they do not exclude the possibility of procuring anti FLC antisera with satisfactory avidity and titre, and it would therefore perhaps have been more appropriate to suggest the use of antisera of this kind and/or of criteria to evaluate such requirements.
I do not wish to go into the question of costs.
I again note that nothing is mentioned about the criteria to distinguish the cases of co-migrant BJP, for which the use of the anti FLC antisera is actually recommended.
I would, however, like to look for a moment at the question of possible Cross-Reactivity. Reading the file carefully, amongst the 20 co-migration patients I did, in fact, find 10 in which this eventuality can be reasonably excluded for the following reasons:

- in six patients, a positive co-migrant BJP sample with intact Ig is recorded and, at different times, a sample which in the same position shows solely the intact Ig, with no reaction with anti FLC antisera (Fig. 5).
- in two patients, positive reaction with anti FLC antisera only in the concentrated urine sample was observed, whilst the un-concentrated one showed solely intact Ig. However, for the same patients on different dates BJP with anti FLC antisera was also seen in the un-concentrated sample, and in exactly the same position.
  Furthermore, in group A clear positivity was frequently seen in similar urine with anti LC(b&f) antisera and, in the same position a less evident positivity or negativity with anti FLC antisera. The re-staining of the strip or the concentration of the sample sometimes also made reaction with the anti FLC antisera evident. Urine samples like this on different dates showed good positivity with both types of antisera (presumably they contained a higher quantity of BJP).
- in three patients 2 MCs, both with intact Ig, were observed in the urine, but co-migrant BJP was seen in only one of these (Fig. 3/A).

Besides, if we consider the 105 patients in our records in whose urine BJP together with intact monoclonal Ig (Table 1 – Columns B+C) was found, in 85 of these (81%) the two bands were distinct and the anti FLC antisera reacted positively with only one of the two; that is, with the one which did not react with the anti heavy chain antisera of the serum Ig. Neither could any form of a-specific reaction towards the other MC (that made up of intact Ig) be seen. (Figure 2).
Also, no suspected a-specific reaction was observed using anti FLC antisera on the MC of the 101 patients with urine positive for intact Ig only (Table 1 - Column D; Fig. 4).
Regarding the sensitivity (avidity, titre) of the anti FLC antisera compared to the anti LC(b&f) ones, the records can say no more - where the MCs were recorded, their composition, their mobility and their presence in the urine sample as such or only in the concentrated urine. Yet my experience, which, however, is based on what I recall, is that the first are, in effect, often a little less sensitive than the second. I recall, in fact, a weak positivity reaction to the visible BJP with the anti LC(b&f) on un-concentrated urine and with the anti FLC on concentrated urine and/or re-stained.
However, the evaluation of the varied sensitivity shows up well in our Multi-centres' work (2) (3).

Again on the subject of Cross-Reactivity, I note that C. R. Tillyer's (4) work in the guidelines says that this is not usually a problem with urine samples unless faced with serious cases of non-selective proteinuria. A potential problem of Cross-reaction for serum and CSF is spoken about and studies with Dako antisera are referred to, in which percentages of Cross-Reactivity measuring from 0.02% to 0.03% for kappa and between 0.03% and 0.06% for lambda were found, against purified human IgG.
I would again add that the Tillyer (4) and Boege (5) articles in the guidelines, advising against using the FLC quantitative methods of dosage, were really interesting and I would propose their distribution, once translated.

In the first, the numerous defects of all the measurement systems of MCs in general and of BJP are examined in detail, concluding in favour of a prudent use of immunometry with antisera from a good commercial source, together with the periodic use of EF to verify monoclonality.
The second measures the BJP of nine patients at various therapeutic points with gravimetric determination after gel-filtration and lyophilization; this data is used as a reference and comparison with four other measurement systems, two of which are immunometric.
This thus shows that, apart from the gravimetric system, measurement of the BJP is not possible; that is, a quantitative comparison between BJP of different individuals. On the other hand, for the same patient these measurements intended as arbitrary units would seem able to be utilised and to correspond to the therapy.
In both works, the necessity for international standard reference emerges.
Their conclusions are encouraging along with the work set up by the Multi-centres.

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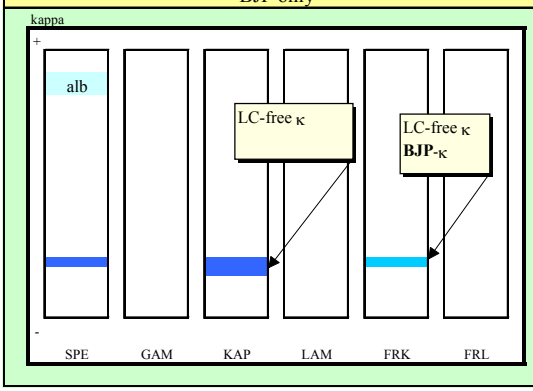
Tables and Figures

Table 1	Laboratory Records, Ospedale Galliera, Genoa - Period: 1992 - 1997 Urine - Detection of Monoclonal Components of Ig and/or Fragments - ***Patients***									
Explanatory comparison of Galliera procedure and SIBioC 2001 Document proposal										
	Partials (Groups)					Totals (sums of Groups)				
Galliera Schema **Antisera:** **- Heavy Chains** **- Total Light Chains (b&f)** **- Free Light Chains**	A BJP only	B BJP + mono-Ig (two separate bands)	C **BJP + co-migrant mono-Ig**	D mono-Ig (no BJP)	E Negative (no MC)	A+B+C BJP	B+C BJP + mono-Ig	B+C+D mono-Ig (with and without BJP)	A+B+C+D Total MC	Total Patients
	202	85	20	101	635	307	105	206	408	1043
% of total C+D			17%	83%						
% of total BJP	66%	28%	**7%**			100%	34%			
% of total MC	50%	21%	5%	25%		75%	26%	50%	100%	
% of total patients	19%	8%	2%	10%	61%	29%	10%	20%	39%	
SIBioC Schema **no Free Light Chains Antisera**	"Resolved" Patients Columns A + B		"Unresolved" Patients Columns C + D		In 30% of the non-MC cases it would be necessary ***to repeat IFE*** with anti Free Light Chain antisera					
No. of Patients	287		121							
% of total MC	70%		30%							
The table shows the "patients" on record, since recurrences of the patient expressed by the number of samples can be influenced by the first result, as well as being very variable for numerous factors.										

Table 2	Laboratory Records, Ospedale Galliera, Genoa - Period: 1992 - 1997 Urine - Detection of Monoclonal components of Ig and/or Fragments - **Samples**									
Explanatory comparison of Galliera procedures and SIBioC 2001 Document proposal										
	Partials (groups)					Totals (sums of Groups)				
Galliera Schema **Antisera:** **- Heavy Chains** **- Total Light Chains (b&f)** **- Free Light Chains**	A BJP only	B BJP + mono-Ig (two separate bands)	C **BJP + co-migrant mono-Ig**	D mono-Ig (no BJP)	E Negative (no MC)	A+B+C BJP	B+C BJP + mono-Ig	B+C+D mono-Ig	A+B+C+D Total MC	Total Samples
No. of Samples	464	152	24	149	1021	640	176	325	789	1810
% of total C+D			14%	86%						
% of total BJP	73%	24%	**3,8%**			100%	28%	51%		
% of total MC	59%	19%	3,0%	19%		81%	22%	41%	100%	
% of total samples	26%	8%	1,3%	8%	56%	35%	9,7%	18%	44%	
SIBioC Schema **No Free Light Chains Antisera**	"Resolved" MC Samples Columns A + B		"Unresolved" MC Samples Columns C + D		In ***22%*** of the cases with MC, it would be necessary ***to repeat IFE*** with anti Free Light Chain Antisera					
No. of Samples	616		173							
% of total MC	78%		22%							

Fig. 1 **Urine IFE Chart - Table 1, column A**

BJP only | 202 patients (66% of total BJP)

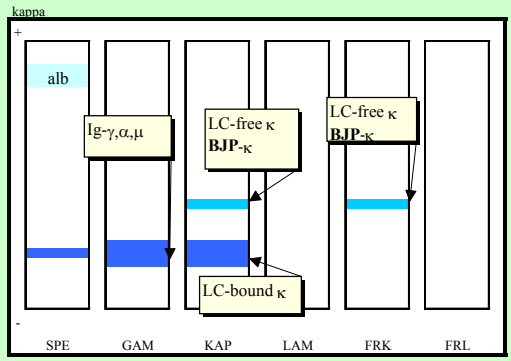


Key
SPE = Electrophoresis (reference)
GAM = Polyvalent IgG,IgA,IgM Antiserum - Heavy chain
KAP/LAM = Total Light Chain (bound & free) Antisera
FRK/FRL = Free Light Chain Antisera: kappa/lambda
LC = Light Chains

Note
The BJP appears in one, two or more bands and is rarely with ladder.

Fig. 2 — **Urine IFE Chart - Table 1, column B**

Intact mono-Ig (like MC serum) + non co-migrant BJP



85 patients (28% of total BJP)

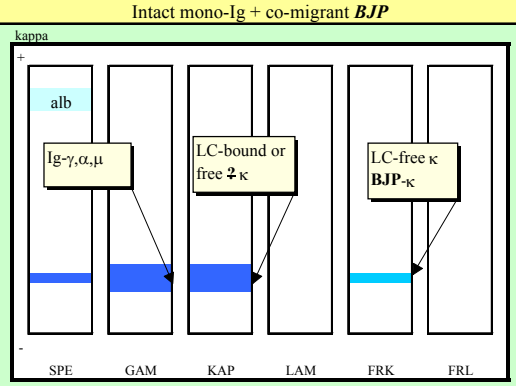
Key
SPE = Electrophoresis (reference)
GAM = Polyvalent IgG,IgA,IgM antiserum - Heavy chain
KAP/LAM = Total Light Chain (bound & free) Antisera
FRK/FRL = Free Light Chain Antisera: kappa/lambda
LC = Light Chains

Note
The BJP appears with one, two or more bands and sometimes with ladder. The band(s) of intact mono-Ig can be of different types (e.g. *IgG-kappa+IgM-lambda*) with all possible combinations.

The anti Free Light Chain antisera do not highlight cross-reaction with intact mono-Ig.

Fig. 3 — **Urine IFE Chart - Table 1, column C**

Intact mono-Ig + co-migrant ***BJP***



20 patients (6.5% of the BJP total) - Base (a) Type

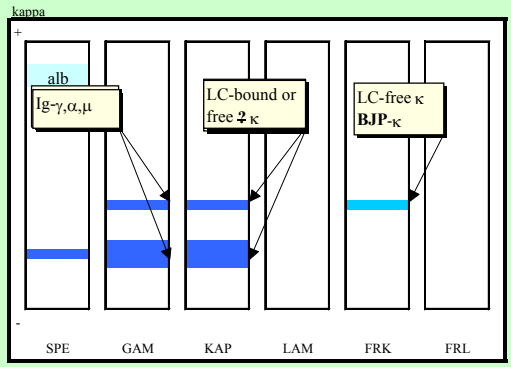
Key
SPE = Electrophoresis (reference)
GAM = Polyvalent IgG,IgA,IgM Antiserum - Heavy Chain
KAP/LAM = Total Light Chain (bound & free) Antisera
FRK/FRL = Free Light Chain Antisera: kappa/lambda
LC = Light Chains

Note
a) **15 samples with a single band of intact mono-Ig perfectly overlapping the BJP.**
b) 3 samples as in a) but with the addition of another band of intact mono-Ig. (Fig. 3/A).
c) 2 samples as in a) with the addition of another BJP band.

The anti Free Light Chain antisera highlight cross-reaction with intact mono-Ig???

Fig. 3/A — **IFE Urine Chart - Table 1, column C**

Intact mono-Ig + co-migrant ***BJP***



20 patients (6.5 % of the BJP total) - type (b)

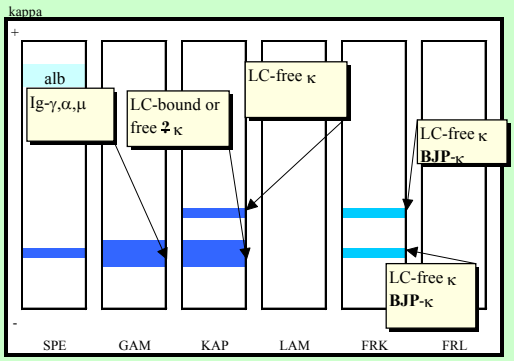
Key
SPE = Electrophoresis (reference)
GAM = Polyvalent IgG,IgA,IgM Antiserum - Heavy Chain
KAP/LAM = Total Light Chains (bound & free) Antisera
FRK/FRL = Free Light Chain Antisera: kappa/lambda
LC = Light Chains

Note
a) 15 samples with a single band of intact mono-Ig perfectly overlapping the BJP.
b) 3 samples as in a) but with the addition of another band of intact mono-Ig.
c) 2 samples as in a) with the addition of another BJP band.

The anti Free Light Chain antiserum does not highlight cross-reaction with cathode intact mono-Ig.

Fig. 3/B — **Urine IFE Chart - Table 1, column C**

Intact mono-Ig + co-migrant ***BJP***



20 patients (6.5 % of the BJP total) - case (c)

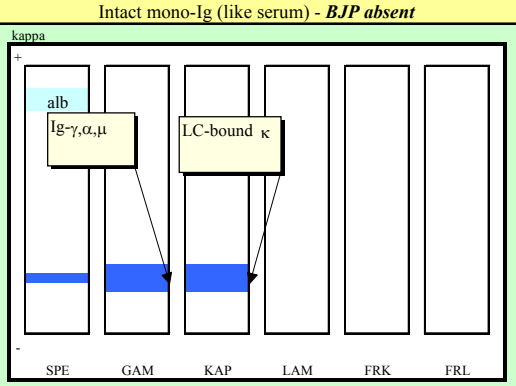
Key
SPE = Electrophoresis (reference)
GAM = Polyvalent IgG,IgA,IgM Antiserum - Heavy Chain
KAP/LAM = Total Light Chain (bound & free) Antisera
FRK/FRL = Free Light Chain Antisera: kappa/lambda
LC = Light Chains

Note
a) 15 samples with a single band of intact mono-Ig perfectly overlapping the BJP.
b) 3 samples as in a) but with the additiion of another band of intact mono-Ig. (Fig. 3/A)
c) 2 samples as in a) with the addition of another BJP band.

The anti Free Light Chain antisera highlight cross-reaction with the intact mono-Ig~~???~~

Fig. 4 — **Urine IFE Chart - Table 1, column D**

Intact mono-Ig (like serum) - ***BJP absent***



101 patients (33% of BJP total)

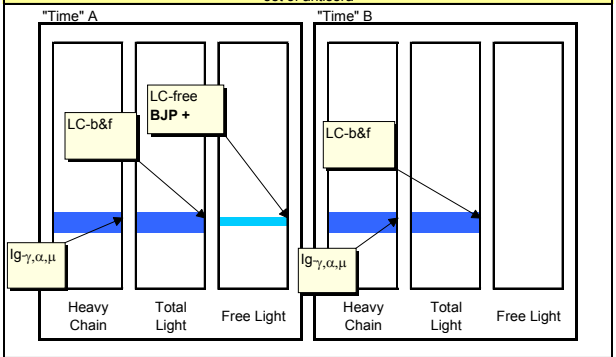
Key
SPE = Electrophoresis (reference)
GAM = Polyvalent IgG,IgA,IgM Antiserum - Heavy chain
KAP/LAM = Total Light Chain (bound & free) Antisera
FRK/FRL = Free Light Chain Antisera: kappa/lambda
LC = Light Chains

Note
a) 95 samples with a single band of intact mono-Ig.
b) 6 samples as in a) but with the addition of another band of intact mono-Ig.

The anti Free Light Chain antisera do not highlight cross-reaction with the intact mono-Ig.

Fig. 5

Urine IFE Schema of 6 patients examined at different times in their case history with the same set of antisera

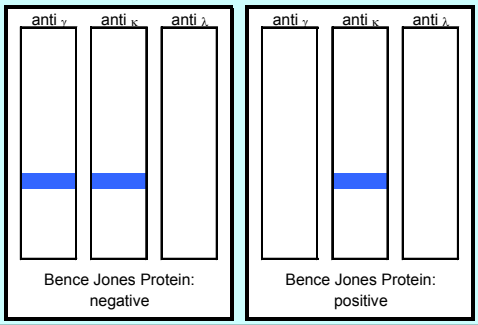


If the "Time A" result was due to cross-reaction of the anti FLC antisera with the intact Ig, the "Time B" result would never have been obtained.

Fig. 6

Fig.1 of SIBioC 2001 document (modified graph)

Patient with kappa serum IgG



Note: anti κ – λ means anti Total Light Chain (bound & free) antisera (see text)