"Forlì" Inter-Regional Study Group
on "Bence Jones Proteins and Free Light Chains"

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Recommendations for the Evaluation of Bence Jones Proteinuria

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Introduction

The detection of Bence Jones Proteins (BJP) should be retained important in the diagnosis, prognosis and follow-up of "plasmacellular dyscrasia", as more clearly indicated in the section "Instructions for the detection of BJP" and Figure 2.

Definitions and Synonyms

Synonym: Bence Jones Proteins = Monoclonal Free Light Chains in urine.

Definition: Monoclonal Free Light chains in urine; in electrophoresis the BJP migrate in narrow bands made up of Free Light Chains. There can be several bands of BJP.
(BJP can occasionally be seen in a wide band which, however, reacts as mentioned below.)
In either case, such a band reacts exclusively with one type only of anti Free Light Chain antiserum, kappa or lambda, or with one type only of anti Bound & Free Light Chain antiserum (anti Total Light Chain Antiserum – TLC) and does not react with any of the anti Heavy Chain antisera.

Other situations and definitions which regard Free Light Chains in urine are as follows, and depend on the electrophoretic technique used:

a) Polyclonal Free Light Chains (PFLC)
Wide electrophoretic bands which react with both types of anti FLC antisera, both kappa and lambda. PFLC can co-exist with BJP.

b) Polyclonal Free Light Chains in multiple bands or ladders
Multiple narrow electrophoretic bands which have a characteristic pattern and which react with both types of anti FLC antiserum. They have a similar significance to Polyclonal FLC. They can co-exist with BJP.

Instructions for the Detection of BJP

The detection of Bence Jones Protein should be requested and carried out as below in all situations:

- Operative Protocol in diagnostic of:
  - clinically suspected immunoproliferative diseases such as:
    Waldenström's Macroglobulinaemia, Multiple Myeloma, Chronic Lymphocytic Leukaemia, Primitive Amyloidosis, etc.,
  - serum electrophoresis which highlights a new monoclonal band,
  - laboratory data suspecting micromolecular myeloma.
- Operative Protocol for follow-up in cases of:
  - immunoproliferative diseases,
  - patients with serum monoclonal band but without diagnosis of immunoproliferative disease,
  - patients with BJP (in urine) but without diagnosis of disease.
- Protocol in excluding existence of Multiple Myeloma
  In order to exclude the existence of "multiple myeloma" in the patient, electrophoresis of serum protein is not sufficient; detection of Bence Jones Proteins is necessary.
  Frequently, in the case of "micromolecular myeloma", electrophoresis of the serum does not in fact show significant and specific changes.

See also Fig. 2.

Instructions for the Quantitative Evaluation of BJP

Quantitative evaluation of BJP should be carried out with a view to highlighting their quantitative variations (in level), both in cases of clear disease and its treatment and also in the case of BJP and/or MC in serum not associated with disease (MGUS).
When establishing the frequency of checks and the significance of the quantitative variations of BJP, the very short half-life of Free Light Chains should be taken into consideration.

Sample Type

Detection of BJP should be effected on a 24-hour sample of urine, adding SodioAzide preservative, or alternatively a random sample of which the creatinine should also be measured.
The sample type should be specified in the report.

Methods for the Detection of BJP

The method or the protocol, i.e. the various methods as a whole, for the detection of BJP should be able to evaluate the two elements which characterise it: a) electrophoretically homogeneous band, b) made up of one type only of Free Light Chains. In other words, the methods or protocol should be able to determine the two characteristics, that is:

- Antigenic specificity: Free Light Chains - should be evaluated by the immunochemical method.
- Electrophoretic mobility: homogeneous band - should be evaluated by the electrophoretic method.

As with any analyte, when choosing the method for BJP attention should be paid to the specificity, sensitivity, sample-dependence on sensitivity, intra- and inter-laboratory inaccuracy, sample-dependence on inaccuracy. Procedure and sample controls should be provided for the aforementioned parameters.
In general, the routine methods available for BJP detection are:

1. **ImmunoFixation (IFE)**
   Despite generally being considered a single method, IFE is actually a protocol consisting of two stages, the first of which is electrophoresis and the second immunoprecipitation.
   IFE is a qualitative method and does not provide quantitative information.
   For BJP detection IFE should be carried out both with anti Free & Bound Light Chain antisera (anti Total Light Chain - TLC - antisera) and also with anti Free Light Chain (FLC) antisera: However, to avoid false negatives the anti Free Light Chain antisera should always be utilised in

case the sample results negative for BJP, using anti Heavy and anti Free Light Chain antisera. See Fig. 1.

IFE should, with all antisera, be sensitive enough to at least highlight a band of 1 mg/dl of BJP. Should this not be so, the sample should be suitably concentrated in order to achieve such sensitivity.

In evaluating the result and the sensitivity, attention should be paid to the presence of several bands of BJP, of polyclonal FLC and of polyclonal immunoglobulin.

2. **Electrophoresis (EF)**

   EF should be considered for the detection of BJP:

   - Screening Method in initial verification

     All samples which show by EF a homogeneous band – Transferrin type, or a wide band – Ig type, which may not definitely be attributable to albumin, should be re-examined with IFE.

     In any case, electrophoresis should have the sensitivity to show 1 mg/dl of BJP. If not, the sample should be appropriately concentrated.

   - Quantitative Method in initial verification and subsequent controls

     The Total Proteins (TP) of the sample and densitometry of the electrophoretic pattern are then measured. Here, the following should be noted:

     - Sensitivity of the quantitative determination of the TP should be able to detect a quantity of 1 mg/dl of BJP. If not, the sample should be appropriately concentrated and the volume of the concentration should be such that it enables determination. The imprecision and inaccuracy of the system of concentration should be evaluated.
     - EF should be carried out with dyes compatible with the densitometric scanning, and the dyes should react proportionately well with the BJP and the other proteins.
     - Sensitivity should be such that it detects a 1 mg/dl band of BJP. If it does not do so, the sample would need to be concentrated and the imprecision of the system of concentration evaluated.

3. **Direct Determination of Free Light Chains** with Immunoprecipitation in liquid phase, nephelometric and turbidimetric, with specific anti FLC reagents.

   For the detection of BJP, the following should be considered:

   - Screening Method in initial verification

     All samples with one of the two Free Light Chains > 0.5 or 1 mg/dl should be re-tested with IFE or EF.

   - Quantitative Method in initial verification and in subsequent checks

     For this, the following points should be taken into consideration:

     - Possible contemporaneous presence of polyclonal FLC
     - Possible lack of parallelism between calibrator and monoclonal sample. In order to minimise this phenomenon, the sample should be diluted to a concentration between 1 and 3 mg/dl and then multiplied by that dilution.

Methods for evaluating the quantitative variations of BJP

Diuresis or urinary creatinine should be looked at in quantitative evaluation of BJP.

Other Determinations

In both initial verification and subsequent checks it would be opportune to determine the Immunoglobulin and the serum Total Light Chains. The dosage of alfa-1 microglobulin would be advisable.

Reporting

All useful information should be given in the reports, in order to provide a better comparison of the BJP detection results. Hereunder, the proposal for such a report:

Report

The Liguria Study Group has proposed the following draft report:

Name of Test: Detection of Bence Jones Protein

Screening Test:

Sample: random urine (or other, as stated)

Method …… sensitivity ……. mg/dl)

Result [_] Negative

[_] Confirmation Test required

Confirmation Test:

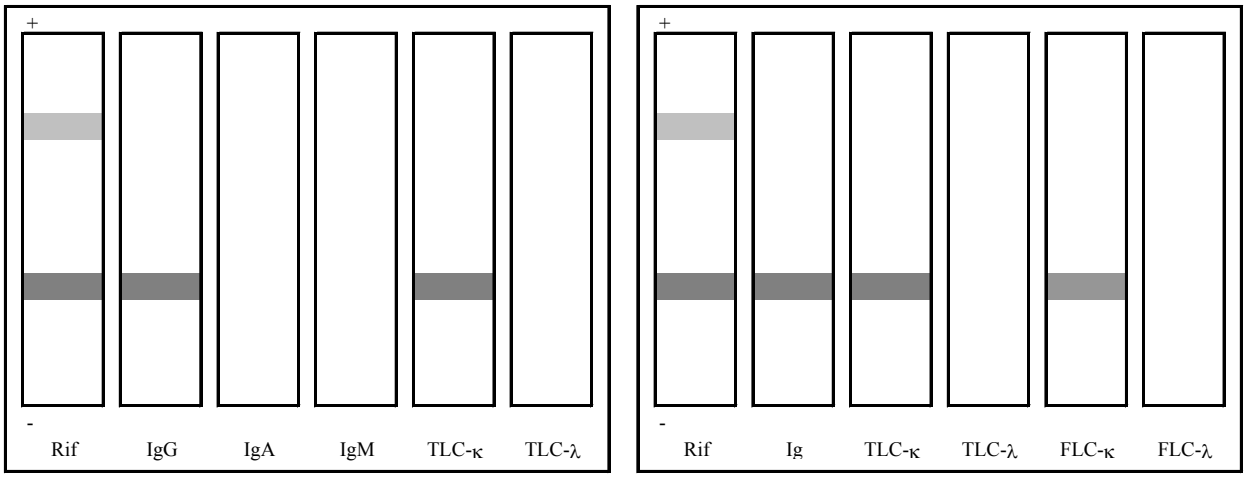
Sample: random urine (or other, as stated)

Method …… sensitivity ……. mg/dl)

Time Lapse for Final Result: ……

Result: …….

Figure: 1 IFE urine sample with MC IgG-k + MC kappa free (BJP kappa)



IFE "serum type" with Antisiera anti Light Chains bound&free (Total Light Chans), without Antisiera anti Free Light Chains

Conclusion MC IgG-kappa - **BJP absent**

IFE with Antisierum anti Ig (polivalente Heavy Chain)and antisiera anti TLC and FLC.

Conclusion MC IgG-k + **BJP-k**

The BJP presents in the sample co-migrates with the intact Immunoglobuin and so we can highlight it only using the Free Ligth Chains Antisera.

Figure: 2 Free Light Chains and Associated Pathology (from the literature)

Pathology

Bence Jones Proteins (BJP)

- Multiple Myeloma (15%-20% being Micromolecular Myeloma)
- Waldenström's Macroglobulinemia
- Chronic Lymphocytic Leukaemia
- Heavy Chain μ Disease
- Other Lymphoproliferative Neoplasia

- AL Amyloidosis
- Light Chain Deposition Disease (LCDD)

- Monoclonal Gammopathy of Undetermined Significance (MGUS)
- Idiopathic Bence Jones Proteinuria

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The BJP do not only indicate a malignancy process but are they themselves a "malignant entity" (micromolecular myeloma is not in itself "more malignant" than other myeloma) which produces pathological effects, above all on the kidneys.

Polyclonal Free Light Chains

- Increase in the Polyclonal Production
  Sarcoidosis, Pulmonary Tuberculosis, Lupus Erythematosus, Rheumatoid Arthritis
- Alteration in Tubular Function
  Fanconi Syndrome, Diabetic Nephropathy, amino acids load such as lysine or arginine, assumption of medication