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Guidelines for the Analysis of Bence Jones Protein

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The detection and quantification of monoclonal free light chains in urine (Bence Jones protein, BJP) are thorny issues for the laboratorian. Immunoelectrophoretic techniques (immunofixation) allow the characterization of the two pathognomonic features of light chains: monoclonality and absence of heavy chains. Immunochemical methods such as nephelometry and turbidimetry are widely used in clinical practice to exclude the presence of BJP. However, these methods are limited by several metabolic and analytical problems. The accuracy of quantitative immunochemical methods is hampered by the heterogeneous molecular forms (fragments and polymers) of BJP and by the lack of reference materials, and the precision of the methods in clinically relevant regions of the dynamic range is poorly defined. Immunoelectrophoretic methods, especially immunofixation, are recommended because of their ability to demonstrate monoclonality and the absence of heavy chains. Immunofixation is also considered the best method to document the disappearance of the monoclonal protein (complete remission). The physiology of immunoglobulins and the clinical relevance of BJP are illustrated in the two appendices to this paper. Clin Chem Lab Med 2003; 41(3):338–346

Key words: Monoclonal immunoglobulin light chains; Immunofixation; Nephelometry; Turbidimetry; Monoclonal gammopathies.

Abbreviations: AL, monoclonal light chain-related amyloidosis; BJP, Bence Jones protein; FLC, free light chain; HC, heavy chain; IF, immunofixation; Ig, immunoglobulin; LC, light chain; LCDD, light chain deposition disease; MC, monoclonal component; PAS, periodic acid-Schiff; pI, isoelectric point; SAP, serum amyloid P component.

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Definition

The Bence-Jones protein (BJP) was described in 1962 as "free monoclonal light chains", synthesized by a single clone of B cells (1, 2).

Normal plasma cells appear to produce a slight excess of light chains (LCs), but B cell neoplasms may produce a much greater excess. Once the mechanism of tubular reabsorption becomes saturated, BJPs are excreted in urine. The molecular mass of BJP is quite variable; BJPs appear in urine as monomers (22 kDa), dimers (44 kDa), or low-molecular mass fragments (5–18 kDa), or show a high degree of polymerization.

Associated Diseases

The most frequent associations are with the following:
- multiple myeloma,
- Waldenström's macroglobulinemia,
- monoclonal light chain-related amyloidosis (AL), and
- light chain deposition disease (LCDD).

These are relatively rare conditions. Their incidence in the western countries varies from 4/100000/year for multiple myeloma to 0.9/100000/year for AL.

BJP may also be associated with lymphomas and chronic lymphocytic leukemia. BJP can also be idiopathic (benign or of undetermined significance).

Clinical Utility

BJP determinations are useful in the following (3):
- Subjects with serum monoclonal component (MC): at diagnosis and during follow-up.
- Patients suspected of having a monoclonal gammopathy, clinically or from laboratory findings as follows:
  - bone pain, fatigue, recurrent infections, purpura, edema,
  - unexpected hypogammaglobulinemia in adults, unexplained increased erythrocyte sedimentation rate, anemia, leukopenia, and thrombocytopenia, proteinuria.

Detection

Sample

The College of American Pathologists issued guidelines defining the methods for BJP detection and quantification based on a 24-hour urine specimen (3, 4). However, 24-hour collections are cumbersome and er-

ratic, and BJP is particularly prone to bacterial degradation. The latter can be minimized by adding an antibacterial agent (*e.g.*, 1 g/l sodium azide). Considering these drawbacks, we recommend the use of the second morning void (5) and expressing the concentration of BJP relative to urinary creatinine.

If the method in use is sensitive enough (see below), the urine can be used unconcentrated (as passed); when the greatest sensitivity of BJP detection is clinically needed, urine should be concentrated. In such case, the membrane used in the concentrating devices should have a cut-off preferably of 5 kDa and, in any case, less than 10 kDa.

Method

The chosen method should verify that the two fundamental characteristics of the LCs are present: *i.e.*, that they are free and monoclonal. Immunofixation combines an electrophoretic step to verify the molecular homogeneity of the protein with immunologic typing (3, 4, 6, 7); it is the recommended method for BJP detection.

Antisera to κ and λ LCs together with antiserum to the heavy chain (HC) of the serum monoclonal immunoglobulin should be used. Antisera to free light chains (FLCs) are expensive; in addition, they are often nonspecific and can have low avidity (Figure 1). They can be useful only if it is suspected that a BJP is co-migrating with an intact immunoglobulin. This suspicion is raised when there is a discrepancy between the HC and the LC signal grossly in favor of the latter (8, 9) (Figure 2).

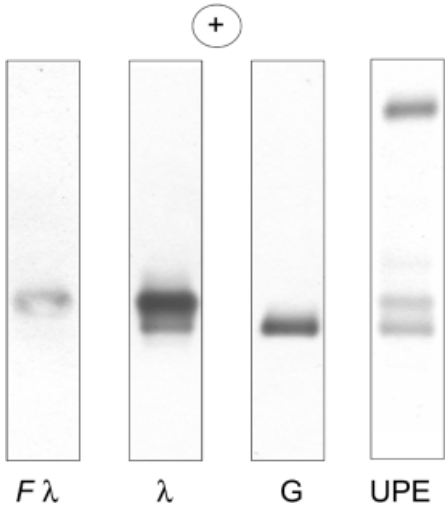

Figure 1 An example of BJP associated with the complete monoclonal immunoglobulin. Each monospecific antiserum used is indicated. Anode at the top. On the right the urine protein electrophoretic pattern (UPE). Two bands are clearly visible in the γ anodal zone in addition to the albumin. Immunofixation shows that the more anodal band is constituted by monoclonal free λ light chains, as confirmed by the antiserum against free λ light chains (*F*) (note the antigen excess due to the low titer of this antiserum), while the cathodal band is constituted by the complete IgGλ, although the λ epitopes were scarcely accessible to the antiserum.

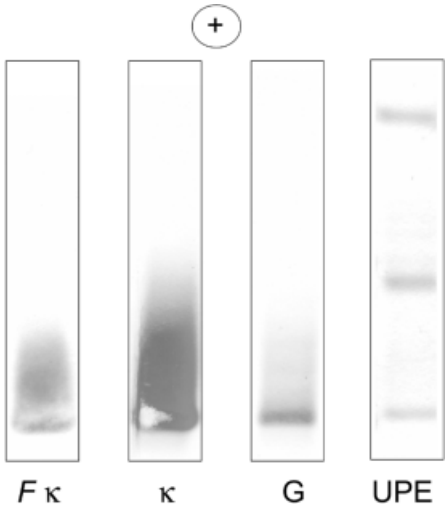

Figure 2 Example of BJP co-migrating with the complete monoclonal immunoglobulin. Each monospecific antiserum used is indicated. Anode at the top. On the right the electrophoretic pattern of urine (UPE). The following proteins are visible: albumin at the anodal end, β_2-microglobulin constituting a band in the β region and a band in the γ cathodal region. The immunofixation shows that the last band is constituted by a monoclonal protein IgGκ with superimposed monoclonal free κ light chains. Note that in comparison with the band immunoprecipitated with anti-IgG antiserum, the anti-κ light chain antiserum produced a more intense band with slight antigen excess. The antigen excess effect is much more evident with the weaker antiserum against free κ light chains (*F*).

Sensitivity

The indication of a detection limit for BJP can only be approximate since there is no way of obtaining an accurate quantification of the protein. However, since the indicated amount for polyclonal LC excretion is approximately 10 mg/l (10, 11), a method with a sensitivity down to this limit is suggested. Among the sensitive stains, colloidal gold provides the highest sensitivity (1–2 mg/l); colloidal Coomassie stain can detect BJP at 10 mg/l or less (12).

Interpretation

Using methods of high resolution and adequate sensitivity, the appearance of so-called LC ladder patterns is common. These multiple, evenly-spaced bands have been well described and are the consequence of the excretion of polyclonal LCs in individuals with impaired tubular reabsorption (13, 14). The pattern is typical, and an experienced eye can distinguish them from BJP; however, it may sometimes be difficult to identify a BJP band co-migrating with one of the multiple bands.

Alternative approaches

Immunochemical methods (nephelometry, turbidimetry) for the quantification of FLCs in urine can be used for BJP detection as an initial screening to exclude BJP, thus reducing the number of samples to process further. However, the amount of BJP can range from a few milligrams to grams per liter, so that the assay can eas-

ily fall into the antigen excess zone. Therefore, it is mandatory to control and avoid antigen excess and to document a detection limit below 10 mg/l.

A positive test should be followed by immunofixation (IF) for the following reasons:

– If the antisera used in the immunochemical method are against LCs (free and bound) it is necessary to document that the LCs are free;
– Since FLC antiserum is incapable of distinguishing monoclonal from polyclonal LCs it is necessary to define the clonality. Although in LC multiple myeloma the synthesis of polyclonal LCs is usually depressed, in several other clinically important instances (AL, LCDDs) the concentration of polyclonal FLCs in urine can be significant and variable in the course of the disease;
– IF is presently recommended to define the response to high-dose chemotherapy in multiple myeloma (15). It has been shown that patients achieving a negative IF have the best prognosis (16); accordingly, therapeutic strategies are presently designed to achieve negative IF.

The cost-benefit ratio of screening samples for BJP using quantitative immunochemical methods should be carefully evaluated depending on the type of population to be examined and the analytical performance of the immunochemical method used.

Tests to be discouraged

1. Methods for measuring total proteins in urine (both precipitating and dye-binding) are insensitive and not accurate for detecting BJP.
2. Dipsticks used to screen for protein in the standard urine examination are impregnated with a buffered dye which is sensitive mainly to albumin and can completely miss the presence of BJP.
3. The unreliable heat test is mentioned only because of its historical value since not all BJP precipitate upon heating.

Quantification

Several staging systems, definitions of indolent disease and treatment guidelines for multiple myeloma and related conditions, are based on decision levels of BJP 24-hour excretion (17–20). However, none of these studies specifies how to identify and measure "something called BJP".

The clinical value of BJP quantification is limited by metabolic and analytical problems. The excretion of BJP is influenced by its degree of polymerization, by renal function, and by the deposition rate of the protein in different tissues, so the amount of BJP in urine is not directly related to the tumor cell mass. Again, an accurate measurement of BJP cannot be easily achieved with present laboratory techniques.

The guidelines of the College of American Pathologists suggest the following procedure (4):

– measurement of total protein in a 24-hour specimen;
– electrophoresis and IF of concentrated urine to detect BJP;
– densitometric scan of the BJP peak; and
– determination of the ratio of the BJP peak percentage to the total protein.

This procedure has some drawbacks:

– inaccuracy of the methods in use to measure total protein in urine: these methods (both precipitating methods and dye binding) are often insensitive to microproteins in general and to BJP in particular. However, if the urine electrophoresis shows that BJP constitutes almost all urinary protein excretion, the determination of total urinary protein performed in the same laboratory by the same method at two points in time may provide useful indications regarding the efficacy of therapy;
– different proteins can have different affinities for the dyes used to stain electrophoretic strips, and thus a lack of linearity of the densitometric response can be observed;
– quite often multiple bands of BJP are present in the urine or the BJP co-migrates with other proteins, so that it is difficult to delimit the BJP peak correctly by densitometry.

It is suggested that follow-up of patients be performed in the same laboratory in order to minimize analytical variability.

Immunochemical methods (nephelometry, turbidimetry) using antisera against FLCs (7, 11, 21, 22) have the drawbacks listed in the "Alternative approach" paragraph. Moreover:

– antisera raised against a polyclonal mixture of LCs do not necessarily react in the same way with the monoclonal LCs of the sample;
– the molecular mass of BJP is quite variable; in urine, they appear as monomers (22 kDa), dimers (44 kDa), low-molecular mass fragments, or can show a high degree of polymerization. The state of aggregation/fragmentation of FLCs in urine is highly variable and unpredictable, depending on many factors (*e.g.*, LC concentration, pH). In addition, the antisera used for the quantification of FLCs are directed against epitopes that are hidden in whole immunoglobulin molecules. In some severe conditions, such as AL, LC fragments of 5-18 kDa, comprising the amino-terminal region, are present in serum and urine and are the main constituents of amyloid fibrils. These pathogenic LC fragments can lack some or all relevant epitopes and be poorly recognized, or missed, by the antisera. All these factors can influence the immune reaction and may invalidate the calibration making the quantification of urinary monoclonal LC unreliable;
– the precision of the quantitative methods at the extremes of the dynamic range (clinically very relevant: at low level to assess remission, and at high level to document progression) is poorly defined;
– there is no reference material for monoclonal LCs, and accuracy therefore remains an open problem;
– there is no standardization of the several methods available for the quantification of urine FLCs. Results

could differ significantly between methods; this represents a serious problem in consideration of the present extreme mobility of patients.

Despite all these drawbacks, the immunochemical estimation of BJP may be of clinical value to monitor the clone during treatment, but it is necessary to utilize the same antisera and calibrators throughout the follow-up and to keep in mind all the caveats listed above.

Recently, it has been reported that in LC myeloma the quantification of FLCs in serum by nephelometry correlates with changes in urinary FLC excretion (23). The authors suggest that serum measurements may be an alternative to the cumbersome 24-hour urine collections in monitoring patients with LC myeloma. However, more data are needed before considering this alternative.

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References

1. Edelman GM, Gally JA. The nature of Bence Jones protein: chemical similarities to polypeptide chains of myeloma globulins and normal γ globulins. J Exp Med 1962; 116: 202–27.
2. Solomon A. Light chains immunoglobulin. Structural-genetic correlates. Blood 1986; 68:603–7.
3. Kyle RA. Sequence of testing for monoclonal gammopathies. Serum and urine assays. Arch Pathol Lab Med 1999; 123:114–8.
4. Keren DF, Alexanian R, Goeken JA, Gorevic PD, Kyle RA, Tomar RH. Guidelines for clinical and laboratory evaluation of patients with monoclonal gammopathies. Arch Pathol Lab Med 1999; 123:106–7.
5. Hofmann W, Guder WG. A diagnostic programme for quantitative analysis of proteinuria. J Clin Chem Clin Biochem 1989; 27:589–600.
6. Merlini G, Aguzzi F, Whicher J. Monoclonal gammapathies. J Internat Fed Clin Chem 1997; 9:171–6.
7. Beetham R. Detection of Bence Jones protein in practice. Ann Clin Biochem 2000; 37:563–70.
8. Levinson SS, Keren DF. Free light chains of immunoglobulins: clinical laboratory analysis: critical review. Clin Chem 1994; 40:1869–78.
9. Attaelmannan M, Levinson SS. Understanding and identifying monoclonal gammopathies. Clin Chem 2000; 46:1230–8.
10. Solling K. Free light chains of immunoglobulins in normal urine determined by radioimmunoassay. Scand J Clin Lab Invest 1975; 35:407–12.
11. Bradwell AR, Carr-Smith HD, Mead GP, Tang LX, Showell PJ, Drayson MT, *et al.* Highly sensitive, automated immunoassay for immunoglobulin free light chains in serum and urine. Clin Chem 2001; 47:673–80.
12. Aguzzi F, Bergami MR, Gasparro C, Merlini G. High sensitivity electrophoretic method for the detection of Bence Jones protein and for the study in unconcentrated urine. Ann Clin Biochem 1993; 30:287–92.
13. Harrison HH, The "ladder light chain" or "pseudooligoclonal" pattern in urinary immunofixation electrophoresis (IFE) studies: a distinctive IFE pattern and an explanatory hypothesis relating it to free polyclonal light chains. Clin Chem 1991; 37:1559–64.
14. Mac Namara EM, Aguzzi F, Petrini C, Higginson J, Gasparro C, Bergami MR, *et al.* Restricted electrophoretic heterogeneity of immunoglobulin light chains in urine. A cause of confusion with Bence Jones protein. Clin Chem 1991; 37:1570–4.
15. Blade J, Samson D, Reece D, Apperley J, Bjorkstrand B, Gahrton G, *et al.* Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. Br J Haematol 1998; 102:1115–23.
16. Lahuerta JJ, Martinez-Lopez J, Serna JD, Blade J, Grande C, Alegre A, *et al.* Remission status defined by immunofixation vs. electrophoresis after autologous transplantation has a major impact on the outcome of multiple myeloma patients. Br J Haematol 2000; 109:438–46.
17. Durie BG, Salmon SE. A clinical staging system for multiple myeloma. Correlation of measured myeloma cell mass with presenting clinical features, response to treatment, and survival. Cancer 1975; 36:842–54.
18. Alexanian R. Localized and indolent myeloma. Blood 1980; 56:521–5.
19. Kyle RA, Greipp PR. Smoldering multiple myeloma. N Engl J Med 1980; 302:1347–9.
20. Weber DM, Dimopoulos MA, Moulopoulos LA, Delasalle KB, Smith T, Alexanian R. Prognostic features of asymptomatic multiple myeloma. Br J Haematol 1997; 97:810–4.
21. Tillyer CR. The estimation of free light chains of immunoglobulins in biological fluids. Int J Clin Lab Res 1992; 22:152–8.
22. Boege F. Measuring Bence Jones protein with antibodies against bound immunoglobulin light chains: how reliable are the results? Eur J Clin Chem Clin Biochem 1993; 31: 403–5.
23. Abraham RS, Clark RJ, Bryant SC, Lymp JF, Larson T, Kyle RA, *et al.* Correlation of serum immunoglobulin free light chain quantification with urinary Bence Jones protein in light chain myeloma. Clin Chem 2002; 48:655–7.

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Appendix A: Physiology of Immunoglobulins

Structure

An immunoglobulin molecule comprises two identical polypeptide HCs, molecular weight approximately 50 kDa, and two identical polypeptide LCs, molecular weight approximately 22 kDa, linked to each other by a

variable number of disulfide bonds and noncovalent interactions.

HCs consist of 3 or 4 subunits (domains) called constant regions with high homology, and one variable region localized at the N-terminal end of the chain.

Different structural and antigenic characteristics of the HC constant region result in different immunoglobulin (Ig) classes and subclasses: IgG (IgG 1, 2, 3, 4) IgA (IgA1 and IgA2), IgM, IgD, and IgE, whereas LCs are classified into two types, kappa (κ) and lambda (λ). The complete immunoglobulin molecule contains only one type of LC, either κ or λ.

Based on the homology of the variable region, human V_H genes may be separated in seven families, V_κ into five major families, and V_λ into ten families. The amino acid residues of heavy and light variable portions contribute to creating the antigen-combining site.

Constant regions (FC fragments) carry out effector functions, such as cellular receptor binding, complement cascade activation, etc.

Synthesis

Immunoglobulin HC and LC are synthesized on ribosomes, under different genetic control (chromosome 14 for HC, chromosome 2 for κ and chromosome 22 for λ LC).

The assemblage of HC and LC occurs in the endoplasmic reticulum. LC are usually synthesized in slight excess of HC. The excess LCs are secreted in blood and excreted through the kidney.

LC catabolism

LC catabolism occurs in the kidney (1–3). Both polyclonal and monoclonal LCs, as well as other proteins with a molecular weight less than 40 kDa are easily filtered through the glomeruli. Microproteins such as enzymes (*e.g.*, ribonucleases, lysozyme), immunoglobulin LCs, peptide hormones (*e.g.*, insulin, growth hormone, parathormone) and others (β_2-microglobulin, α_1-microglobulin, retinol-binding protein) are reabsorbed by the proximal renal tubular cells.

Glomerular membrane permeability to microproteins is variable and depends on the microproteins' physico-chemical characteristics (molecular weight, isoelectric point (pI), glycosylation, *etc.*). Even a minor reduction of glomerular filtration rate determines a rapid increase in the concentration of the microproteins in the plasma. Microproteins are captured from the ultrafiltrate by the cells of the proximal tubule and then degraded into oligopeptides and amino acid residues inside the lysosomes. Such degradation products are secreted into the circulation and re-used.

In normal conditions the reabsorption process can be schematically summarized as follows:

– microprotein adhesion to the luminal membrane of the proximal tubular cells,
– protein segregation in endocytic vesicles,
– migration of endocytic vesicles from the apical side of the tubular cell to the cytosol,
– fusion with lysosomes and reaction with hydrolytic enzymes,
– enzymatic degradation of protein, a process taking from a few minutes to days depending on the type of protein.

Since there is no evidence of specific receptors for each protein nor for groups of proteins on the luminal cellular membrane, the protein tubular reabsorption is generally considered to be non-selective. Actually there is a binding selectivity depending on the interaction kinetics between positive charges on the protein and the negative charges on the reabsorbing cellular membrane (in this case the cells of the proximal tubules). In addition to the charge, the size and shape of the protein influence the reabsorption process. The observations that albumin is less reabsorbed than insulin and ribonucleases, and that all cationic amino acids enhance microprotein excretion support the existence of this type of selectivity.

It has been proposed that the glycoprotein receptor called cubilin (gp280), scattered along the pathway of the endocytic scavengers, may play a physiologic role as a binding site for LC at the level of the renal cell brush border (4).

References

1. Maack T, Johnson V, Kau ST, Figueiredo J, Sigulem D. Renal filtration, transport, and metabolism of low–molecular weight proteins: a review. Kidney Int 1979; 16:251–70.
2. Sumpio BE, Maack T. Kinetics, competition, and selectivity of tubular absorption of proteins. Am J Physiol 1982; 243: 379–92.
3. Solling K. Free light chains of immunoglobulins. Scand J Clin Lab Invest 1981; 157 (Suppl):1–83.
4. Batuman V, Verroust PJ, Navar GL, Kaysen JH, Goda FO, Campbell WC, *et al.* Myeloma light chains are ligands for cubilin (gp 280). Am J Physiol 1998; 275:246–54.

Appendix B: Clinical Manifestations Caused by Bence Jones Protein (BJP)

As is the case for certain MCs constituted by complete immunoglobulin, so too can BJP cause tissue damage through various not completely elucidated mechanisms. The observation that only certain BJPs are pathogenic and that they tend to determine specific clinical manifestations supports the hypothesis that their pathogenicity is linked to specific structural and functional characteristics of the single BJP. The pathogenic effect of LC can be enhanced by host contributing factors.

1. LC nephrotoxicity

The kidney is the site of polyclonal and monoclonal light chain catabolism and represents the target organ of BJP toxicity. However, several patients have been described with high daily excretion of BJP for long periods without any renal damage (1, 2).

BJP can cause the following patterns of kidney damage:

– myeloma kidney,
– immunoglobulin LCDD,
– amyloidosis, and
– renal tubule dysfunction syndromes.

1.1 Myeloma kidney

Renal failure affects approximately one third of myeloma patients at the time of diagnosis, and reduced creatinine clearance is present in one half (3). It is caused by myeloma kidney in most cases.

The name "light chain cast nephropathy" reveals the pathogenic role of monoclonal LCs in determining the nephropathy, characterized by proteinuria and casts in distal and collecting tubules.

Under a light microscope the casts appear big, dense, and refractive with characteristic multilamellar aspects and fractured borders; they are eosinophilic and periodic acid-Schiff (PAS)-positive (4). The casts are usually surrounded by epithelial cells, lymphocytes, polymorphonuclear cells, and multinucleated giant cells, of monocytic and macrophagic nature, which often incorporate cast fragments. The tubular atrophy associated with these lesions is accompanied by an increase in interstitial fibrous tissue.

Under an electron microscope some casts appear highly dense and homogeneous, while others are coarsely fibrillar (the fibrils are different from amyloid ones). Long crystals of different sizes are present in the lumen and sometimes inside tubular cells (5). The monoclonal LC constituting the BJ proteinuria can be identified in the casts by immunohistochemical techniques in approximately 50% of cases, associated with other proteins: the Tamm-Horsfall protein is always present, whereas albumin, C3, polyclonal LC, HCs, and others are irregularly present (6).

Several hypotheses of the mechanism of cast formation have been proposed. Since there is not an evident correlation between BJ proteinuria and cast formation, it is believed that only some BJPs are particularly predisposed to this phenomenon; this theory is supported by experimental data in animals (7, 8). The pathogenic effect has been attributed to the variable portion of LCs which is responsible for the particular physico-chemical and functional characteristics of each individual BJP. Earlier studies identified the high pI (>5) of the BJP as an important predisposing factor based on possible electrostatic interactions with the acidic Tamm-Horsfall protein, whose pI is 3.5 (9). Subsequent studies, both clinical and experimental, excluded any correlation between protein pI and onset of renal failure (10–13). In addition to the still undetermined physico-chemical characteristics of BJP, there are some host factors which contribute significantly to the onset of myeloma cast nephropathy: dehydration, hypercalcemia, hyperuricemia, urinary tract infections, and nephrotoxic drugs.

The possible role of contrast media now appears remote, considering that the ionic contrast media which are more likely to interact with BJP are no longer used. Most probably, the occasional renal damage following administration of contrast media reported in early studies was caused by dehydration during the procedure.

2. Kidney diseases caused by LCs

While myeloma kidney is caused by the tendency of monoclonal LCs to precipitate inside the lumen of renal tubules, several other conditions involving the kidney and other organs are caused by the extracellular deposition of the monoclonal LCs. Two different kinds of monoclonal LC deposition have been described: fibrillar, typical of amyloidosis, and non-fibrillar, characterizing LCDD.

2.1 LCDD

Kidney damage can be caused by the non-fibrillar deposition of LC only, of LC associated with complete immunoglobulins, or of HC (non-amyloid monoclonal immunoglobulin deposition disease: NAMIDD) (14). Here, we discuss briefly only the most frequent form, LCDD. This condition was identified in 1976 using immunofluorescent techniques (15), although glomerular lesions morphologically similar to those typical of Kimmelstiel-Wilson glomerulosclerosis in diabetic nephropathy had been observed and described in patients suffering from multiple myeloma much earlier. Deposits are found at the level of the basal cell membrane and in the vessel walls, and are characterized by non-fibrillar, non-congophilic, PAS-positive, finely granular, electron dense material when observed under the electron microscope.

Optical microscopy reveals heterogeneous patterns: the glomeruli may show no abnormality or various degrees of mesangial expansion up to nodular glomerulopathy. Deposits are always present at both tubular and vessel levels. Immunohistochemical techniques, preferably immunofluorescence, applied to frozen tissues are required for a correct diagnosis. Although clinical manifestations often correlate with renal damage, deposits have been documented in many other organs (liver, skin, lung, and others) and sometimes produce overt clinical symptoms.

Clinical presentation includes proteinuria, usually mild but sometimes in the nephrotic range, without hematuria or hypertension, associated with rapidly progressing renal failure. In about 25% of patients, no overt lymphoproliferative disease is present, although bone marrow immunofluorescence study in these patients frequently demonstrates a plasma cell clone synthesizing a LC of the same type as the LC present in the deposits. The BJP can be present at very low concentration, and very sensitive techniques are required for its detection.

Considering the pathophysiologic mechanism underlying LC tissue adhesion and deposition, several studies have shown structural LC abnormalities such as abnormal size (longer or shorter LC than usual), tendency to polymeric aggregation, and glycosylation abnormalities. Some studies concerning the structural analysis of the abnormal protein involved in the LCDD

have been published as have genetic and biosynthesis studies. The most interesting results are related to the identification of amino acid residue substitutions increasing the hydrophobicity of the molecule (thus enhancing its interactions with the basement membranes and favoring abnormal protein-protein interactions) and possibly destabilizing the protein molecule (16–22).

2.2 AL (monoclonal light chain-related amyloidosis)

The term amyloidosis refers to different conditions characterized by tissue extracellular deposits of autologous proteins structurally organized in fibrils. The fibrils have the following common properties:

– secondary structure constituted mainly by a β-pleated sheet,
– typical green birefringence under polarized light microscopy after staining with Congo red,
– characteristic quaternary fibrillar structure with peculiar X-ray diffraction.

A high molecular weight glycosylated protein called "serum amyloid P component" (SAP) is always present in the deposits whatever the biochemical composition of the amyloid. Apolipoprotein E is also present in the deposits and may contribute, together with SAP, to fibril formation, or persistence (23).

In AL, fibrils are made mainly of monoclonal LC fragments, sometimes associated with complete LC. The LC fragments, of 5-18 kDa molecular weight, consist of the LC amino-terminal region (variable region and 50 amino acid residues of the constant region) (8).

The amyloidogenic LC are usually λ (with a significant prevalence of the λ *3r* and *6a* germline genes (24)) with acidic pI and are associated with the presence of LC fragments either in serum or in urine (25). At present, no specific amino acid sequence has been associated with amyloidogenicity. Reduced folding stability now appears to be a unifying property of amyloidogenic LCs. The tendency of these proteins to populate a partially unfolded intermediate state is a key event in the self-association that progresses to the formation of oligomers and fibrils (26).

AL may be present in 6–15% of myeloma patients. In primary amyloidosis the bone marrow plasma cell infiltration is modest (< 10%). The bone marrow monoclonal plasma cell population can be easily demonstrated by immunofluorescent techniques. The disease is usually systemic and rarely localized. The clinical expression is protean, depending on the organs involved and on the severity of organ damage. AL can be localized, usually in the upper respiratory tract, but also in the urethra and other districts. The dominant organ involved is the kidney in 52% of patients, followed by the heart in 27%, liver in 8%, gastro-intestinal tract in 4%, peripheral nervous system in 4%, soft tissues in 3%, and other organs in 2% of patients. Although the heart involvement dominates the clinical manifestations in approximately one quarter of the patients (27%), 49% of patients show some sign of heart involvement, 25% having congestive heart failure (CHF) and 24% having only echocardiographic signs of cardiac amyloidosis. The median survival is 2–3 years, and heart disease accounts for most of the deaths.

Using sensitive immunofixation techniques it is possible to detect a monoclonal LC in serum or urine of 96% of the patients (27). λ (76%) predominates over κ (24%) light chains. In 34% of patients, the M-protein is a FLC only.

A few cases characterized by the presence of both fibrillar and non-fibrillar monoclonal LC deposits have been described (amyloidosis associated with LCDD). It has been suggested (28) that there is a spectrum of LC generating tissue deposits: at one extreme are those LCs capable of forming only amyloid, and at the other extreme the LCs causing non-fibrillar deposits and, in between, LCs capable of generating both forms of deposits. An alternative hypothesis suggests that all, or almost all, the proteins forming fibrils pass through a stage in which they are neither non-fibrillar nor congophilic, the duration of such a stage depending on their primary structure. Additional investigations are needed to address this problem.

2.3 Renal tubular dysfunction diseases

BJPs are capable of causing, in addition to structural damage, functional disturbances of the tubular cells in patients without renal failure and with negative renal biopsy (29). Clinical manifestations caused by proximal tubule dysfunction are typical of adult Fanconi syndrome (57 described cases according to Messiaen) (30). The abnormalities include normoglycemic glycosuria, aminoaciduria, phosphaturia, lysozymuria, and proximal tubular acidosis. This syndrome is more frequently associated with κ BJP and may precede the onset of multiple myeloma by many years.

This condition has been considered a peculiar form of plasma cell dyscrasia characterized by a smoldering plasma cell clone, BJP, and the tendency of the protein to crystallize in the proximal tubule cells, without cast formation in the distal tubule. Distal tubular dysfunction is characterized by distal tubular acidosis and rarely by nephrogenic diabetes (31). Cases with both proximal and distal tubule dysfunction have been reported (32).

Reduction of metabolic processes and inhibition of ATPase Na, K-dependent activity has been documented using renal tissue slices incubated with BJP (33). Clinical studies have reported the toxic effect of monoclonal LC on tubular reabsorption of microproteins (34) and on other tubular functions, including excretion of uric acid and phosphates, osmolality, acid excretion, *etc.* (35). The functional alterations may be reversed if specific therapy eliminates the production of BJP, thus confirming the latter's toxic effect on the tubule.

Although uncommon, tubular dysfunction can cause osteomalacia and chronic metabolic acidosis. Reduced concentrating capability and decreased Na reabsorption can lead to dehydration, thus enhancing the toxic effect of BJP on the kidney (myeloma cast nephropathy).

Structural studies performed on a monoclonal κ LC involved in Fanconi syndrome showed that the LC variable domain (V_L), obtained from the enzymatic digestion of native fragment, not only might produce crystals in both plasma cells and tubular cells but also (at variance with other monoclonal κ LC) was resistant to additional enzymatic proteolysis (36).

A study performed on 11 patients with Fanconi syndrome (30), with heterogeneous clinical expression, excreting monoclonal LC resistant to a lysosome enzyme, cathepsin B, revealed a surprising homogeneity. All BJPs were κ, eight of them belonged to the V_{kI} subgroup, probably encoded for by only two germline genes (LC02/12 and LC08/018), and one hydrophobic or non-polar amino acid residue was found at position 30 in 5 sequences encoded by LC02/012. The authors postulated that this peculiar primary structure could be responsible for the molecules' resistance to proteolysis and subsequent LC deposition in the endocytic compartment of proximal tubular cells and the correlated functional deficit.

3. Other immunoglobulin deposition nephropathies

Although not correlated with BJP, two recently described conditions which represent 1% of glomerulopathies are worth mentioning (37).

Fibrillar non-amyloidogenic glomerulopathy is characterized by fibrillar deposits at the level of mesangium and glomerular capillary vessels. The fibrils are thicker than the amyloid counterpart, are not stained by Congo red, do not have any ultrastructural organization and are made of polyclonal immunoglobulin.

In *immunotactoid glomerulopathies* the deposits are constituted in 50–80% of cases by monoclonal IgGκ or IgGλ. The deposits are localized to the kidney and have a crystalline or tactoid structure. In addition, glomerulonephritis with organized microtubular monoclonal immunoglobulin deposits (GOMMID) has been observed in association with chronic lymphocytic leukemia and non-Hodgkin lymphoma. Renal biopsy of immunotactoid variants shows membranous glomerulonephritis, frequently associated with segmental mesangial proliferation or lobular membrano-proliferative glomerulonephritis with monotypic Ig deposits. A circulating MC has been observed rarely. Proteinuria, frequently in the nephrotic range, microhematuria, and hypertension are typical clinical manifestations. The evolution to renal failure is more frequent in fibrillar glomerulopathy.

References

1. Kyle RA, Greipp PR. Idiopathic Bence Jones proteinuria. N Engl J Med 1998; 306:564–7.
2. Woodruff R, Sweet B. Multiple myeloma with massive Bence Jones proteinuria and preservation of renal function. Aust N Z J Med 1977; 7:60–2.
3. Knudsen LM, Hippe E, Hjorth M, Holmberg E, Westin J. Renal function in newly diagnosed multiple myeloma – a demographic study of 1353 patients. The Nordic Myeloma Study Group. Eur J Haematol 1994; 53:207–12.
4. Pirani CL, Silva FG, Appel GB. Interstitial disease in multiple myeloma and other non renal neoplasias. In: Contran RS, Brenner BM, Stein JH, editors. Tubulo-interstitial nephropathies. London: Churchill Livingstone, 1983:208–21.
5. Pirani CL, Silva F, D'Agati V, Chander P, Striker LM. Renal lesions in plasma cells dyscrasia: ultrastructural observation. Am J Kidney Dis 1987; 10:208–21.
6. Picken MM, Shen S. Immunoglobulin light chains and the kidney: an overview. Ultrastructural Pathol 1994; 18:105–12.
7. Koss MN, Pirani CL, Osserman EP. Experimental Bence Jones cast nephropathy. Lab Invest 1976; 34:579–91.
8. Solomon A, Weiss DT, Kattine AA. Nephrotoxic potential of Bence Jones proteins. N Engl J Med. 1991; 324:1845–51.
9. Clyne DH, Pesce AJ, Thompson RE. Nephrotoxicity of Bence Jones proteins in the rat: importance of protein isoelectric point. Kidney Int 1979; 16:645–52.
10. Coward RA, Delamore IW, Mallick MP, Robinson EL. The importance of urinary immunoglobulin light chain isoelectric point in nephrotoxicity in multiple myeloma. Clin Sci 1984; 66:229–32.
11. Norden AGW, Fynn FV, Fulcher LM, Richards JDM. Renal impairment in myeloma: negative association with isoelectric point of excreted Bence Jones protein. J Clin Pathol 1989; 42:59–62.
12. Melcion C, Mougenot B, Baudouin B, Ronco P, Moulonguet-Doleris L, Vanhille P. Renal failure in myeloma; relationship with isoelectric point of immunoglobulin light-chains. Clin Nephrol 1984; 22:138–43.
13. Smolens P, Barnes JL, Stein JH. Effect of chronic administration of different Bence Jones proteins on rat kidney. Kidney Int 1986; 30:874–82.
14. Gallo G, Picken M, Buxbaum J, Frangione B. The spectrum of monoclonal immunoglobulin deposition disease associated with immunocytic dyscrasias. Semin Hematol 1989; 26:234–45.
15. Randall RE, Williamson WC, Mullinax F, Tung MY, Still WJ. Manifestations of systemic light chain deposition. Am J Med 1976; 60:293–9.
16. Decourt C, Cogne M, Rocca A. Structural peculiarities of a truncated V kappa III immunoglobulin light chain in myeloma with light chain deposition disease. Clin Exp Immunol 1996; 106:357–61.
17. Vidal R, Goni F, Stevens F, Aucouturier P, Kumar A, Frangione B, *et al.* Somatic mutations of the L 12a gene in V-kappa(1) light chain deposition disease; potential effects on aberrant protein conformation and deposition. Am J Pathol 1999; 155:2009–17.
18. Ling NR. Immunoglobulin production by cultured human lymphocytes. Protein Sci 1999; 8:509–17.
19. Deret S, Chomilier J, Huang DB, Preud'homme JL, Stevens FJ, Aucouturier P. Molecular modeling of immunoglobulin light chains implicates hydrophobic residues in non amyloid light chain deposition diseases. Protein Eng 1997; 10:1191–7.
20. Gallo G, Goni F, Boctor F, Vidal R, Kumar A, Stevens FJ, *et al.* Light chain cardiomyopathy. Structural analysis of the light chain tissue deposits. Am J Pathol 1996; 148: 1397–406.
21. Bellotti V, Stoppini M, Merlini G, Zapponi MC, Meloni ML, Banfi G, *et al.* Amino acid sequence of k Sci, the Bence Jones protein isolated from a patient with light chain deposition disease. Biochim Biophys Acta. 1991; 1097:177–82.
22. Stevens FJ, Argon Y. Pathogenic light chains and the B-cell repertoire. Immunol Today 1999; 20:451–7.

23. Gallo G, Wisniewsky T, Choi-Miura NH, Ghiso J, Frangione B. Potential role of apolipoprotein E in fibrillogenesis. Am J Pathol 1994; 145:526–30.
24. Perfetti V, Casarini S, Palladini G, Vignarelli MC, Klersy C, Diegoli M, *et al.* Analysis of V(lambda)-J(lambda) expression in plasma cells from primary (AL) amyloidosis and normal bone marrow identifies 3r (lambdaIII) as a new amyloid-associated germline gene segment. Blood 2002; 100:948–53.
25. Bellotti V, Merlini G, Bucciarelli E, Perfetti V, Ascari E. Relevance of class, molecular weight and isoelectric point in predicting human light chain amyloidogenicity. Br J Haematol 1990; 74:65–9.
26. Bellotti V, Mangione P, Merlini G. Immunoglobulin light chain amyloidosis. The archetype of structural and pathogenic variability. J Struct Biol 2000; 130:280–9.
27. Palladini G, Obici L, Merlini G. Hereditary amyloidosis. N Engl J Med 2002; 347:1206–7.
28. Buxbaum J, Gallo G. Non-amyloidotic monoclonal immunoglobulin deposition disease. Light-chain, heavy-chain, and light- and heavy-chain deposition disease. Hematol Oncol Clin North Am 1999; 13:1235–48.
29. Fang LST. Light chain nephropathy. Kidney Int 1985; 27: 582–92.
30. Messiaen T, Deret S, Mougenot B, Bridoux F, Dequiedt P, Dion JJ. Adult Fanconi syndrome secondary to light chain gammopathy. Clinicopathologic heterogeneity and unusual features in 11 patients. Medicine (Baltimore) 2000; 79:135–54.
31. Maldonado JE, Velosa JA, Kyle RA, Wagoner RD, Holley KE, Salassa RM. Fanconi syndrome in adults. A manifestation of latent myeloma. Am J Med 1975; 58:35– 64.
32. Smithline N, Kassirer JP, Cohen JJ. Light chain nephropathy. Renal tubular dysfunction associated with light chain proteinuria. N Engl J Med 1972; 94:71–4.
33. Mc Geoch J, Smith JF, Ledingham J, Ross B. Inhibition of active transport sodium-potassium ATP-ase by myeloma protein. Lancet 1978; 2:17–8.
34. Cooper EH, Forbes MA, Crockson RA, Mac Lennan ICM. Proximal renal tubular function in myelomatosis: observations in the fourth MRC trial. J Clin Pathol 1984; 37:848–52.
35. Colussi G, Barbarano L, Airaghi C. Clinical spectrum of tubular disorders from light chains. In: Minetti L, D'Amico G, Ponticelli C, editors. The kidney in plasma cell dyscrasia. Dordrecht: Kluwer Acad Publ, 1988:191–209.
36. Aucouturier P, Bauwens M, Khamlichi AA, Denoroy L, Spinelli S, Touchard G, *et al.* Monoclonal Ig L chain and L chain V domain fragment crystallization in myeloma-associated Fanconi's syndrome. J Immunol 1993; 150:3561–8.
37. King JA, Culpepper RM, Corey GR, Tucker JA, Lajoie G, Howell DN. Glomerulopathies with fibrillary deposits. Ultrastruct Pathol 2000; 24:15–21.