SHORT COMMUNICATION

Measuring *Bence Jones* Proteins with Antibodies Against Bound Immunoglobulin Light-Chains: How Reliable Are the Results?

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(Received January 5/March 5, 1993)

Summary: Protein mass concentrations of nine individual urinary *Bence Jones* proteins were determined via protein dry weight and compared (i) to immuno-nephelometric measurements using two different antisera against bound light-chains and (ii) to total protein concentrations determined by trichloroacetic acid nephelometry and the Biuret method. In each case, these four measurements differed significantly from each other and from the expected value (as determined by protein dry weight). The extent and direction of deviation were different for each measurement and varied between cases, but similar patterns were observed in different samples from the same patient. Apparently, these measurements can not be quantitatively compared between different individual *Bence Jones* proteins, but can be interpreted in terms of disease activity and therapeutic success for each patient. Accordingly, the quantitative expression of *Bence Jones* proteins should be restricted to relative terms, e. g. "arbitrary units" or "serum light-chain equivalents". This avoids the (potentially incorrect) statement of protein mass concentrations.

Introduction

There is a strong clinical demand to quantitate urinary excretion of free immunoglubin κ- and λ-light-chains (*Bence Jones* proteins) by methods that can be mechanised, such as immunonephelometry or turbidimetry (1, 2). The "hidden" epitopes, specific for the free form of light-chains, are poor antigens. Moreover, di(oligo)merization of free light-chains in the urine obscures these epitopes (3). Alternatively, the use of antisera directed against bound ligth-chains, cross-reacting with the free form, has been advocated (4, 5). Usually, intact serum immunoglobulins are used for calibration of these assays.

A number of theoretical considerations council against this approach:

(i) The primary structure of the constant parts of free and bound light-chains should be identical, but this does not necessarily apply to the secondary and tertiary structure. Differences in the epitopal architecture may result in differences in the antibody-antigen reaction with respect to the kinetics of precipitate formation and the size of the particles. This, in turn, would compromise the interpretation of the measuring signal in quantitative terms.

(ii) In light-chains the variable and hyper-variable parts constitute a major proportion of the molecule. Antisera, raised against and calibrated with a heterogeneous mixture of these proteins do not necessarily react in the same way with homogenous proteins of monoclonal origin. This may alter the measuring result.

(iii) Due to aberrant expression, fragmentation and di(oligo)merization the molecular weight of *Bence Jones* proteins varies (6, 7). This influences the immune-reaction and may invalidate the calibration.

What should be true in theory, apparently also holds in praxi: Applying the above approach to routine analysis of *Bence Jones* proteins and comparing the results to the total protein concentration as determined by nephelometric detection of trichloroacetic acid precipitation we repeatedly observed large (> 50%) differences between the values of light-chain and total protein concentration. In many of these cases it was confirmed by sodium dodecyl sulphate gelelectrophoretic analysis that these large differences were not accounted for by the presence of proteins other than monoclonal free light-chains. In principle, similar imponderabilities apply to the method of total protein determination as to the immuno-nephelometric measurement of *Bence Jones* proteins. Accordingly, on the basis of our observations it was impossible to decide which of the two discrepant measurements was the correct one.

We selected nine cases and tried to gauge the validity of our measurements. We determined the weight of lyophilised, salt-free *Bence Jones* proteins, purified from a defined aliquot of each urine sample in order to obtain a true measure for *Bence Jones* protein mass-concentration. This we compared to immuno-nephelometric and chemical measurements carried out in the same sample.

Methods

Urine samples were from nine patients, excreting more than 90% pure *Bence Jones* proteins (5 κ-isotype, 4 λ-isotype). The purity of urinary *Bence Jones* proteins was determined by so-

dium dodecylsulfate gelelectrophoretic analysis and standard procedures (8). Ten ml aliquots were desalted by gel-filtration using a Sephadex G25 (Pharmacia, Freiburg) column (65 × 3.6 cm) equilibrated with 100 mmol/l ammonium carbonate buffer, pH 7.0. Desalted urinary proteins were collected in 12 ml polypropylene screw-cap vials (Sarstedt, Heidelberg) and lyophilised. The amount of protein present in the caps was determined after lyophilisation by subtracting the weights of the vials before and after the procedure. The weight of the empty vials did not change during lyophilisation. The recovery of total protein and *Bence Jones* proteins was routinely checked by reconstituting the lyophilised proteins in 10 ml of distilled H_2O, and comparing the values of total and *Bence Jones* protein concentration with the values obtained in parallel in the starting material using the same measuring procedure (see below). Usually more than 90% of total protein and more than 95% of *Bence Jones* proteins were recovered in the lyophilisate. When multiple aliquots of the same sample were processed in parallel, the gravidimetric result varied by less than 15% (CV = 12%, n = 5).

The concentration of κ- or λ-light-chains was determined immuno-nephelometrically (5), using two different sets of antisera (Behringwerke AG, Marburg; Immuno AG, Vienna), directed against the bound form. Total protein concentration was determined by nephelometry of trichloroacetic acid precipitation (9) and by the Biuret method after 10-fold concentration of the protein samples (10). A Behring Nephelometer Analyzer (Behringwerke AG, Marburg) was used for nephelometry.

Results and Discussion

The results obtained in the nine patient urine samples by the different measuring techniques are summarized in table 1. The relative deviation of the various nephelometric and the colorimetric measurements from the gravidimetric reference procedure are shown in figure 1a: It can clearly be seen that in all nine cases the results of the two immuno-chemical measurements and of the two procedures for total protein determination differed significantly from each other and were also different from the value expected on the basis of the weight of the lyophilised proteins. In each sample the extent and direction of deviation from the expected value was different for the two immuno-nephelometric measurements and the two total protein measurements. It also varied considerably from case to case, so that a generalised conclusion as to the validity of either method can not be drawn. Obviously, underestimations are as frequent as overestimations. This applies as well to the chemical determination of total protein concentration as to immuno-chemical measurements. Although here the deviations did not exceed 60%, it can not be excluded that in other cases even greater differences between measured and true values might exist. From these observations we conclude that the assessment

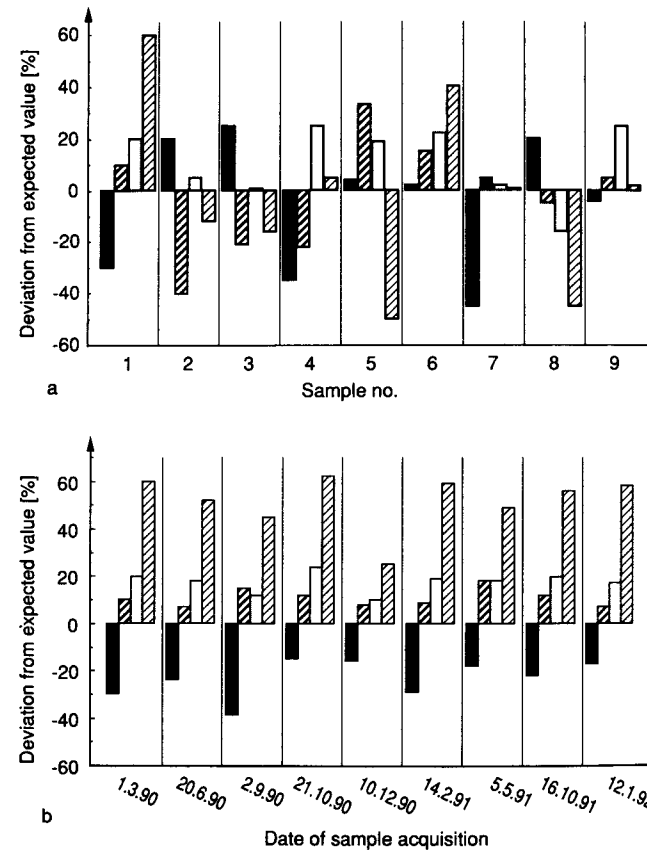

Fig. 1. Differences between values of urinary concentration of *Bence Jones* proteins, measured by different methods:
(i) Urinary *Bence Jones* proteins were quantified via the weight of the salt-free lyophilised proteins contained in a 10 ml urine aliquot.
(ii) In parallel, urinary concentrations were determined by trichloroacetic acid precipitation nephelometry (black bars), the Biuret method (dark diagonally striped bars), and immune-nephelometry using two different antisera (white and light diagonally striped bars, respectively) against bound light-chains and serum immunoglobulins as a standard. The differences between the values derived from procedure (i) and those from (ii) are plotted as relative deviation.
A: Urines samples from nine different patients analysed on the same day.
B: Nine samples obtained from the same patient (donor of sample 1 in A) on different days and analysed in the same way.

Tab. 1. Urinary *Bence Jones* protein concentrations measured[1]) in nine patient samples by different methods.

Sample no.	1	2	3	4	5	6	7	8	9
Acid precipitation[2])	329	1561	1244	3075	943	2032	1766	471	7537
Biuret[3])	531	749	764	3608	1131	2237	3466	358	8321
anti-κ/λ 1[4])	586	1321	995	5902	1020	2403	3401	335	10048
anti-κ/λ 2[4])	759	1092	818	4910	423	2720	3319	230	8086
Weight lyophilised[5])	480	1270	980	4590	850	1930	3270	390	7850

[1]) values in mg/l
[2]) nephelometry of trichloroacetic acid protein precipitation
[3]) measured after 10-fold concentration of the urine samples
[4]) immuno-nephelometry using two different antisera (anti-κ/λ 1, 2) against bound light-chains and serum immunoglobulins as a standard
[5]) gravidimetric determination after gelfiltration and lyophilisation of a 10 ml aliquot

of urinary light-chains either by the nephelometric or the colorimetric method has to be considered semiquantitative. It reflects, rather than measures, the urinary *Bence Jones* protein concentration. However, the factors influencing the measurement are apparently constant for each individual *Bence Jones* protein: similar patterns of deviation were observed when analysing urine samples obtained from the same patient at different times during the disease (fig. 1b). Accordingly, quantitative values of *Bence Jones* proteins obtained for the same patient during the course of the disease can be compared with each other and are interpretable in terms of therapeutical success and disease activity (for an example see fig. 2). Moreover, urinary light-chain concentrations differ between healthy individuals/successfully treated patients and untreated/relapsed patients by several orders of magnitude. It appears that the precision of the methods used to quantitate urinary *Bence Jones* proteins is sufficient to gauge these large differences.

A more precise nephelometric measurement of urinary *Bence Jones* proteins probably could be obtained by using polyclonal anti-peptide antibodies exclusively directed against the constant epitopes of κ- and λ-light-chains, but it remains doubtful if such antisera will provide a precipitation signal sufficiently strong enough for sensitive detection. From the clinical point of view, a fast quantitative measure of *Bence Jones* proteinuria is highly desirable, but it must be doubted that *Bence Jones* proteins can be precisely measured by chemical or immunochemical methods. Our data seem to confirm that such measurements can not be compared in quantitative terms between different individual *Bence Jones* proteins. However, these values can be interpreted in terms of disease activity and therapeutic success within each patient, which gives them a pronounced advantage over entirely qualitative, electrophoretic techniques. Obviously, the mass-concentration of *Bence Jones* proteins derived from the calibration with serum proteins is not true and should not be explicitly given. However, in relation to the calibration with serum proteins, this quantitative value appears

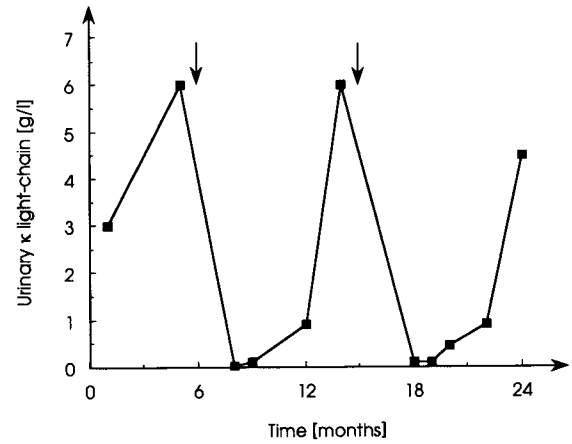

Fig. 2. Quantitative analysis of urinary *Bence Jones* proteinuria (κ-isotype) over a period of 2 years. The arrows indicate cycles of therapy. Free κ-light chain concentrations were determined immuno-nephelometrically using antisera directed against the bound form.

to be a constant feature of a given individual *Bence Jones* protein, which can be reproducibly measured. This raises the question of the unit in which these measurements should be expressed. We would like to propose an expression in relative terms such as, e. g., "arbitrary units" or "serum light-chain equivalents". This avoids the expression of protein mass concentration, which is potentially incorrect, but still provides a clinically desirable term for the quantity of urinary *Bence Jones* proteins.

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