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Measurement of β_2-Microglobulin in Serum by a Particle-Enhanced Nephelometric Immunoassay

By *M. M. Lievens*[1], *S. Woestyn*[1], *P. De Nayer*[1] and *D. Collet-Cassart*[2]

[1] *Laboratoire de Biochimie Médicale, Cliniques Saint Luc, Université Catholique Louvain, Bruxelles, Belgium*
[2] *Unit of Experimental Medicine, International Institute of Cellular and Molecular Pathology, Brussels, Belgium*

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Summary: A particle-enhanced immunoassay of β_2-microglobulin in serum is described. It is based on the agglutination of complexes formed between the serum β_2-microglobulin and latex particles coated with $F(ab')_2$ fragments of polyclonal anti-β_2-microglobulin antibodies. The analytical range of the method is 0.50 to 16 mg/l; it can be extended by appropriate dilution to 0.12 to 80 mg/l with good precision (CV less than 5% over the whole range). The accuracy and the precision are confirmed by a good correlation with radioimmunoassay ($n = 123$, $r = 0.993$). No error due to antigen excess was observed, even up to 292 mg/l.

The main advantages of the method are its simplicity, its low cost per test and its high sensitivity (final dilution of the sample at 1/1200) with no known interference. The calibration curve is stable for at least 2 weeks.

Introduction

β_2-Microglobulin is a low relative molecular mass (11 800) protein present on the membrane of almost all nucleated cells, including lymphocytes, where it is associated to the class I major histocompatibility antigens (1). It is produced by the cells at a relatively constant rate in healthy subjects, and it is released into body fluids as a result of cell turnover. Serum β_2-microglobulin is freely filtered through the glomerular basement membrane of the kidney and reabsorbed by the proximal tubular cells where it is catabolized. Serum β_2-microglobulin determination is useful in various circumstances. Kidney rejection in transplanted patients is correlated to the level of β_2-microglobulin (2). In patients with multiple myeloma, the serum β_2-microglobulin level is negatively correlated with survival time. It is the best tumour marker in pure *Bence-Jones* myeloma and in some of the non-secretory myelomas (3). More recently, serum β_2-microglobulin has been described as the most powerful predictor of progression of HIV seropositive men to AIDS over 3 years (4).

Different methods for β_2-microglobulin measurement have already been described. They include radioimmunoassay (5), radial immunodiffusion (6), direct immunoturbidimetry (7) or particle-enhanced turbidimetric immunoassay (PETIA) (8) and enzyme immunoassay (9). Most of these methods have one or more drawbacks, such as low sensitivity, high cost or potential interferences.

We describe here a particle-enhanced nephelometric immunoassay for β_2-microglobulin which offers the advantages of being sensitive, automated and inexpensive.

Materials and Methods

Apparatus

We used a Behring nephelometer analyser (BNA) (Behringwerke, Marburg, FRG) for all the determinations.

Reagents and standards

The buffer contains 0.1 mol/l glycine, 0.17 mol/l NaCl, 1 g/l bovine serum albumin (Cohn Fraction V, United States Bio-

chemical Corporation, Cleveland, USA) and 0.6 mmol/l sodium azide adjusted to pH 9.2 with NaOH 8 mol/l. $F(ab')_2$ fragments of anti-β_2-microglobulin IgG (reference A072, Dakopatts, Glostrup, Denmark) were prepared as previously reported for the preparation of anti-C-reactive protein $F(ab')_2$ (10). Polystyrene particles with a diameter of 0.24 µm (Estapor K58, Rhone-Poulenc, Courbevoie, France) were coated as already described (11). In the present case, a protein : latex weight ratio of 1 : 5 was used. A working suspension of 1 g/l of particles in the buffer was used in the test. The particles can be frozen or freeze-dried for long term conservation.

The Phadebas β_2-microglobulin RIA kits were obtained from Pharmacia (Uppsala, Sweden).

A pool of serum was used as standard. Its β_2-microglobulin concentration (3.24 mg/l) was determined by the RIA method using the standard of the Phadebas kit.

Methods

The latex reagent was vortexed before use. A six point calibration curve was obtained by automatic dilutions of the standard (1 : 80, 1 : 160, 1 : 320, 1 : 640, 1 : 1280 and 1 : 2560), giving a concentration range from 0.5 to 16 mg/l.

The method settings are summarized in table 1. The sample is automatically prediluted to 1/400 with 0.15 mol/l NaCl. Diluted sample (70 µl) is mixed with 10 µl latex reagent and 140 µl 0.15 mol/l NaCl. The intensity of scattered light at 840 nm is measured after 10 s and 12 min later. The difference between the two signals is converted into concentration by reference to a calibration curve.

The RIA procedure was performed as recommended by the manufacturer.

Statistics

Regression parameters were calculated using a biometric regression procedure according to *Passing & Bablok* (12). The calibration plot method (3 $s_{y/x}$/slope) was used to determine the lower limit of detection of the proposed method (13).

Results

Range and sensitivity

The calibration curve covers the range 0.50 to 16 mg/l of β_2-microglobulin. If the concentration is higher than 16 mg/l, the apparatus automatically dilutes the sample to 1/2000, raising the upper limit of the range to 80 mg/l. If the concentration is lower than 0.5 mg/l, the sample may be reprocessed at a lower dilution (1/100 vs 1/400), decreasing the lower limit of the range to 0.12 mg/l. The accuracy in the low range remains good at the lowest tested concentration of 0.2 mg/l (fig. 1). The detection limit calculated from the results of the five experiments reported in figure 1 ranged from 0.04 to 0.12 mg/l.

No error due to antigen excess was observed up to 292 mg/l, an unexpectedly high pathological concentration of β_2-microglobulin attained by forcing the instrument to perform the measurement on a serum with a high β_2-microglobulin concentration (73 mg/l) at a 1/100 dilution instead of 1/400 (fig. 2).

Accuracy and parallelism

β_2-Microglobulin was determined in 123 serum samples by RIA (x) and by the present method (y). Correlation was excellent ($r = 0.993$) and the regression equation was $y = 1.03x + 0.09$ with means of $x = 2.73$ and $y = 2.90$ mg/l (fig. 3). At the decision level of 3 mg/l (2), the random error ($p = 0.95$) is 0.17 mg/l (14). Even without correction for a systematic error (0.16 mg/l), the total error is lower than 0.4 mg/l, which is accepted as an allowable error.

Tab. 1. Settings of the immunoephelometric method for β_2-microglobulin measurement on the Behring Nephelometer Analyser.

Test name	β_2-Microglobulin		
Sample vol. [µl]	70	Sample dilution Minimal-dilution	1:400.0 1:100.0
Reagent 1 vol. [µl]	10	B2M Latex	
Reagent 2 vol. [µl]	0		
React. buffer vol. (1)	70	N Reaction Buffer	
React. buffer vol. (2)	70		
Measuring time [min]	12	Fixed Time	
Standard		B2M-15	
No. of standard points	6		
First dilution	1:80.0		
Deviation allowed [%]	5.0		
Validity [days]	14		
Conc. unit	mg/l		
Measuring range [mg/l]			
Lower level	0.47		
Upper level	15.00		

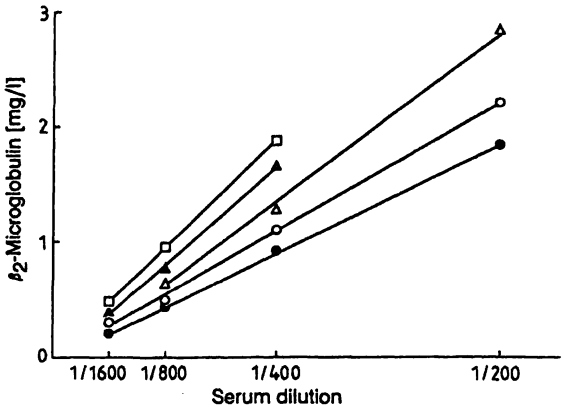

Fig. 1. Linearity of the method in the low concentration range (serial dilutions of 5 different sera).

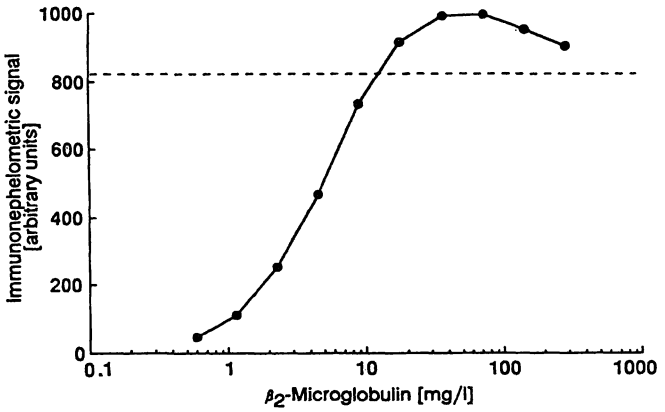

Fig. 2. Effect of high concentrations of β_2-microglobulin on the signal of the nephelometer in the described method. Dotted line: signal of the highest calibration point beyond which the nephelometer asks for a rerun of the sample at a higher dilution.

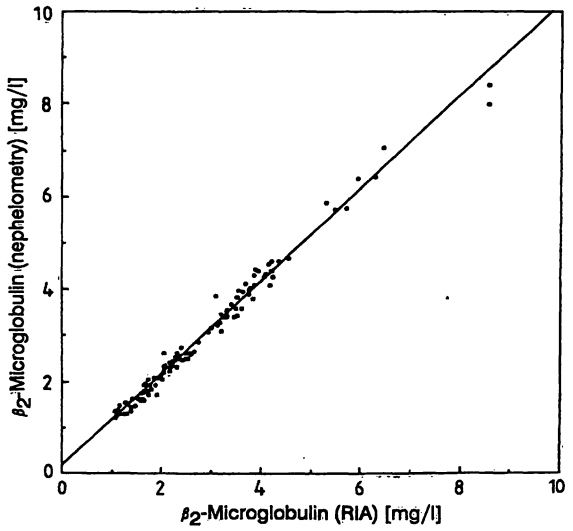

Fig. 3. Comparison of β_2-microglobulin concentrations in 123 patient samples measured by RIA (x) and the immunonephelometric method (y). The linear regression line was $y = 1.03x + 0.09$, $r = 0.993$.

To exclude any matrix effect or interference, we performed serial dilutions of serum samples containing a high concentration of β_2-microglobulin. All measured concentrations were within 0.75 mg/l of the expected values (ranging from 1.4 to 34.0 mg/l), confirming the parallelism of the precipitation curves obtained with the samples and with the serum pool used as standard. No systematic bias was observed for lipaemic or haemolysed samples.

Precision and stability of the calibration curve

Precision was evaluated on serum pools at 3 concentration levels of β_2-microglobulin. The within-run precision was measured for 20 consecutive determinations. The between-run precision was calculated from the mean of 2 consecutive determinations per day performed on 9 days over a period of 21 days. The concentrations were calculated either from the calibration obtained on the first day, or from the curves obtained on the day of the measurement. The results are presented in table 2. The between-run precision is better when the concentrations are calculated from the same calibration curve.

Tab. 2. Within-run and between-run reproducibility of the proposed immunonephelometric method.

		N	Mean mg/l	CV %
Within-run		20	1.53	1.8
		20	4.42	3.0
		20	8.58	1.6
Between-run	first day calibration	9	2.73	3.4
		9	5.96	1.5
		9	9.71	1.7
	every day calibration	9	1.90	4.0
		9	4.70	3.6
		9	9.29	4.1

Three different frozen latex preparations were tested for stability. For each preparation, either refrozen or kept at 4 °C, the calibration curve was stable for at least 15 days.

Effect of polyethyleneglycol

Polyethyleneglycol 6000 (6.7 mmol/l or 4%) was added to the reaction mixture in final concentrations ranging from 0.2% to 1.15%. The sensitivity and the precision of the calibration curve were not improved. Therefore, polyethyleneglycol need not be added to the reaction mixture.

Discussion

The lower limit of detection of proteins by immunonephelometry is in the range of 1 to 10 mg/l, depending on the affinity of the antibodies. By using these antibodies to coat latex particles, the nephelometric signal induced by the antigen-antibody reaction is considerably enhanced. This is exemplified by the present method, in which the sample is assayed at a final dilution of 1/1200 and the final β_2-microglobulin concentration is around 1 µg/l. At such dilutions, turbidity of the serum or haemolysis are no longer a problem. This is an advantage over PETIA (8), which is much less sensitive (final dilution of 1/100). Moreover, rheumatoid factor does not interfere, because latex is coated with the $F(ab')_2$ fraction of the specific antibodies. The antibody used may originate from any animal species. The only potential interference that may occur comes from anti-$F(ab')_2$ antibodies present in exceptional cases. The stability of the calibration curve is satisfactory for at least two weeks, although it does not display the prolonged stability of PETIA reagent.

The cost of the reagent is incomparably lower than any other method, only 1 µl antiserum being consumed per test.

In conclusion, the present method for serum β_2-microglobulin determination is reliable, being sensitive and free from interference. It is very suitable for routine use, because it is inexpensive and can be automated on a commonly used instrument.

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Dr. Michel M. Lievens
Laboratoire de Biochimie Médicale
Cliniques Universitaires Saint Luc
10, avenue Hippocrate (1748)
B-1200 Bruxelles