Time-Resolved Fluoroimmunoassay of β2-Microglobulin in Serum and Cerebrospinal Fluid

To the Editor:

Human β2-microglobulin (β2m) is a small polypeptide (Mr, 11,800) that is noncovalently bound to class I molecules of the major histocompatibility complex. An increase in β2m concentration in serum is considered a marker of immune activation in various disorders, such as malignancies and several infectious conditions, including human immunodeficiency virus (HIV) infection (1). Measurement of β2m in cerebrospinal fluid (CSF) has been proposed for detecting malignant metastases in the meninges of patients with leukemias and lymphomas and of neurological involvement in the acquired immunodeficiency syndrome (AIDS) (2, 3). Most of the reported immunochemical methods for human β2m measurement are based on the use of polyclonal antisera to β2m (4–8). A monoclonal antiserum to β2m has been used in both a radioimmunoassay (9) and a sandwich time-resolved immunofluorometric assay (TR-IFMA) (10).

The aim of this study was to evaluate a new commercial time-resolved fluoroimmunoassay (TR-FIA) (Delfia β2 micro kit; Pharmacia, Uppsala, Sweden) for determining β2m concentrations in the serum and CSF of healthy and HIV-1-infected individuals. The TR-FIA is based on the competition between europium-labeled β2m and sample β2m for binding to specific monoclonal mouse IgG antibodies. The monoclonal IgG-β2m immune complexes are detected by antibody to mouse IgG coated on a solid phase. The enhancement solution dissociates the europium ions from the labeled immune complexes, providing highly fluorescent chelates. Thus, the degree of fluorescence is inversely proportional to the β2m concentration in the sample (11).

The linearity range of the fluorescence signal was established by testing several dilutions (0.2–55.3 μg/ml) of a standard serum containing β2m. Linearity was observed between 0.2 and 32 μg/ml. The detection limit was defined as the concentration corresponding to the mean plus 2SD of the zero standard fluorescence signal (0.1 mg/L). The intra-assay precision was determined by 20 measurements of β2m in three standard serum samples with known concentrations (1.3, 5.6, and 26.5 μg/ml); the coefficients of variation were 3%, 1.9%, and 3.3%, respectively. Interassay precision was determined by 10 measurements of β2m in standard sera with two known concentrations (1.3 and 5.6 μg/ml); the coefficients of variation were 7.5% and 4.0%, respectively. Neither the intra-assay nor the interassay variations were significant (<10%).

Mean β2m concentrations in the serum of 65 healthy subjects without malignant, inflammatory, or psychiatric diseases (35 women, 30 men; ages 20–60 years) and in CSF of 45 subjects (23 women, 22 men; ages 18–82 years) with normal CSF cell counts and protein concentrations were 1.46 μg/ml (SD 0.30; range 0.8–2.4) and 0.96 μg/ml (SD 0.83; range 0.4–1.7), respectively. The 95% nonparametric reference intervals for healthy individuals were 1.40–1.60 μg/ml (median 1.50 μg/ml) in serum and 0.90–1.20 mg/ml (median 1.05 μg/ml) in CSF.

For comparison, β2m concentrations in 81 serum and 28 CSF samples from inpatients at our institution were measured with both the TR-FIA technique and an ELISA test (Phadezym; Pharmacia) in which a polyclonal antiserum to β2m is used (Figure 1). In serum and CSF, the reference interval for healthy individuals (measured by means of TR-FIA) was very close to the value obtained with the comparison method.

Finally, β2m was measured in serum and CSF from 54 HIV-1-infected patients. Five had stage II disease according to the Centers for Disease Control (CDC) classification, 5 were at stage III, and 44 were at stage IV (stage IVA, 23; stage IVB, 16; stage IVC, 23, 4 of whom had cerebral toxoplasmosis, stage IVC; 16; stage IVD, 6). The 16 patients at CDC stage IVB of HIV-1 disease had characteristic neurological disorders: 11 had neurological signs and symptoms of dementia (compatible with HIV-1 encephalitis) and 4 had peripheral neuropathy. In the 54 HIV-1-infected patients the mean (±SE) serum β2m was 3.18 ± 0.29 mg/L and the mean CSF concentration was 3.00 ± 0.22 mg/L. These values were significantly higher than those for healthy control subjects (Mann-Whitney test, P <0.001). In addition, the serum and CSF β2m concentrations were significantly higher (P <0.001) in the subgroups of patients with neurological disorders (n = 20, mean 3.74 ± 0.43 mg/L) and those with only HIV-1 encephalitis (n = 11, 3.57 ± 0.53 mg/L), than in the HIV-1-infected patients without neurological involvement (n = 36, mean 250 ± 0.26 mg/L).

The sensitivity and the specificity of this test was 73% and 85%, respectively. By receiver-operating characteristic curve analysis we determined a threshold value of 3.0 mg/L for β2m in CSF for clinical decision-making. These results are similar to those reported by Brew et al. (2), who found a strong correlation between the β2m concentration in CSF and the severity of the AIDS dementia complex.

TR-FIA and TR-IFMA (10) offer similar advantages: they require a very small sample volume and single incubation without chemical pretreatment, they provide improved precision and have a long shelf-life, and they use nonradioactive europium labeling. The use of monoclonal antibodies increases the specificity and has the added advantages of unlimited supply and constant, predictable quality. In contrast to TR-IFMA, TR-FIA does not require a sample predilution step, a significant advantage when infectious samples were analyzed. Moreover, the one-step competition procedure in the TR-FIA method cuts down the time of analysis. The excellent reliability of this method makes it suitable for assay of β2m in serum and CSF.

![Graph](image-url)
Addition of Sucrese Avoids Effect of Lyophilization on Determinations of Lipoprotein(a) in Serum

To the Editor:

There is recent evidence that high concentrations of lipoprotein(a) (Lp(a)) in human serum are significantly correlated with the risk for development of coronary heart disease (1). Lp(a) contains a cholesterol-rich core that is covered by a complex of apolipoprotein B-100 disulfide (apo B) linked to a large glycoprotein called apolipoprotein(a) (apo(a)). Because Lp(a) closely resembles low-density lipoprotein (LDL), we added sucrose in concentrations similar to those used by Wieland and Seidel (2) for the apo B determination to avoid the denaturation effect during lyophilization.

We collected serum samples from 10 subjects with Lp(a) concentrations ranging from 136 to 907 mg/L measured by radioimmunoassay (Pharmacia apo(a) RIA 100; Pharmacia Diagnost AB, Uppsala, Sweden) and divided these into six aliquots. We kept one aliquot at 4°C for ≤5 days. Another aliquot was frozen at −86°C and lyophilized. We added sucrose to the remaining four aliquots to give final sucrose concentrations of 70 (24.0), 200 (68.5), 400 (136.9), and 600 mmol/L (205.4 g/L), respectively, and then lyophilized them.

In all samples studied, we determined the Lp(a) concentrations by three different assays: (a) an ELISA method (Macra™ Lp(a); Terumo Medical Corp., Elkton, MD), based on the use of microtiter wells containing monoclonal antibody to Lp(a), in which bound Lp(a) antigen from serum is revealed with a polyclonal antibody to Lp(a) conjugated with horseradish peroxidase and with addition of substrate; (b) the above-mentioned RIA, in which two different monoclonal antibodies are directed against the apo(a) part of the Lp(a) particle, which is known only during a sample pretreatment step; and (c) an automated nephelometric immunoassay carried out on the Behring nephelometer and based on the measurement of agglutination by sample Lp(a) of latex particles coated with F(ab′)2 fragments (3) of a polyclonal antibody to Lp(a) (Behringwerke AG, Marburg, Germany). Values for the samples lyophilized in the presence of sucrose were corrected for dilution.

We found that lyophilization of serum reduced the assayed value of Lp(a). However, as shown in Figure 1, addition of sucrose in serum had to reach a concentration of 205.4 g/L or 600 mmol/L before lyophilization would totally inhibit the effect of lyophilization on the determination of Lp(a) in serum samples by the Lp(a) Macra ELISA and by our latex immunonephelometric method. Lower concentrations of sucrose would reduce but not prevent the lyophilization effect, which is method-dependent: in the Lp(a) determination by RIA, no lyophilization effect was observed, possibly because this assay measures concentrations of only the apo(a) part of the Lp(a) particle.

Our results do not agree with those recently published by Sgoutas and Tuten (4). The disagreement is possibly due to the low sucrose concentration they used (25 g/L ≈ 73 mmol/L and not 600 mmol/L as they stated). This fact can also explain the decrease in recovery found in another lyophilization study of different methods for apo B measurement (5).

Our results indicate that addition of sucrose to human serum avoids the denaturation effect of the lyophilization process on different Lp(a) immunoassays. Moreover, an additional advantage of lyophilization with sucrose is that serum remains clear upon reconstitution. Therefore, on the basis of our results, this procedure may be useful for preparing Lp(a) standards and quality-control materials.

References

5. Marcovina SM, Adolphsen JL, Parlavec-