Immunoturbidimetry of Apolipoprotein B In Lyophilized Human Serum Pools Using Primary Standard, Alexander B. Sigalou and Olga V. Alexandrovich (Dept. of Biochem. Standardization, Center for Preventive Med., 10 Petroverigsky St., Moscow 101953, Russia)

Measurements of apolipoproteins (apo) A-I and B have not realized their full potential as predictors of risk for coronary artery disease because of inadequate standardization and problems in methodology (1). A key step in standardization is the assignment of accurate values to the reference materials and secondary standards (currently, lyophilized serum pools) through the use of primary standards for apo A-I and apo B. We have examined the influence of various additives (bovine or human serum albumin (BSA and HSA, respectively), immunoglobulin fractions from human serum, and lipoprotein-free human serum) to the primary standard on the results of apo B measurements in three lyophilized human serum pools by immunoturbidimetry, using a manual version of the known methods (2).

Pools 1–3, received from the WHO Lipid Reference Center (Institute for Clinical and Experimental Medicine, Prague, Czechoslovakia), had apo B concentration values assigned by the “consensus values” method, i.e., by using the results of different immunochromatography measurements performed in 84 laboratories in 34 various countries. To analyze each pool, we used as the primary standard the narrow fraction of low-density lipoproteins (LDL, d = 1.03–1.05) after quantification by sodium dodecyl sulfate-Lowry’s procedure. Lipoprotein-free serum (48 g/L), BSA or HSA (40 g/L), and immunoglobulin fraction (26 g/L) were added to the LDL standard, in a proportion similar to that of the native serum samples (based on a normal apo B value of 0.90 g/L). The results are given in Table 1.

As shown, the application of different additives to model the matrix of native serum allowed us to measure apo B values in the control pools that did not significantly differ from the consensus values. Variation in the concentration of the additives (e.g., BSA) within the range 10–90 g/L, as well as the substitution of BSA for HSA, did not influence the analytical results. In contrast, the use of LDL without additives yielded data that were 37–43% higher than the consensus values, within the apo B measured range (0.46–1.04 g/L). These are in full accord with the data obtained by immunonephelometry for native serum pools, which was similarly biased by +34% by utilization of LDL as the primary standard instead of LDL + lipoprotein-free serum (3).

Thus we recommend the use of LDL with added BSA (30–40 g/L) as the primary standard for assigning accurate apo B values to the reference materials and secondary standards (currently, lyophilized serum pools) by immunoturbidimetric assay.

References


Serum triglyceride (TG) can be determined enzymatically after elimination of endogenous free glycerol (FG) (1). An erroneously high result for TG may be obtained when the endogenous FG is not eliminated completely or a falsely low value is obtained when the reagents are consumed by excess FG. Lately, fructose-added glycerol is frequently used to relieve intracranial pressure to prevent the development of brain edema (2). Patients so treated will have extremely high concentrations of circulating FG. In conventional enzymatic assays it is impossible to measure endogenous FG.

To improve this point, we introduced an error signal for the TBA-80S automated discrete analyzer (Toshiba Co., Ltd., Tokyo, Japan) to indicate samples with high concentrations of endogenous FG. For the manual method, we used the ultraviolet-visible recording spectrophotometer UV-265 (Shimadzu Co., Kyoto, Japan). To determine serum TG we used Reagents 1 and 2 from a commercial kit, Clinitek TG-2 (Daiichi Pure Chemicals Co., Ltd., Tokyo, Japan). Reagent 1 consists of 90 U of glycerol kinase (EC 2.7.1.30), 375 U of glycerol-3-phosphate oxidase (EC

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Table 1. Influence of the Nature of Apo B Primary Standard on Accuracy of Apo B Immunoturbidimetric Assay

<table>
<thead>
<tr>
<th>Standard</th>
<th>Pool 1 (0.48)</th>
<th>Pool 2 (0.84)</th>
<th>Pool 3 (1.04)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LDL</td>
<td>0.66 ± 0.07</td>
<td>1.18 ± 0.05</td>
<td>1.45 ± 0.12</td>
</tr>
<tr>
<td>LDL + LP-free serum</td>
<td>0.45 ± 0.02</td>
<td>0.85 ± 0.06</td>
<td>1.02 ± 0.06</td>
</tr>
<tr>
<td>LDL + BSA</td>
<td>0.43 ± 0.04</td>
<td>0.86 ± 0.02</td>
<td>1.04 ± 0.02</td>
</tr>
<tr>
<td>LDL + Ig-fraction</td>
<td>0.48 ± 0.03</td>
<td>0.87 ± 0.05</td>
<td>1.05 ± 0.04</td>
</tr>
</tbody>
</table>

*Consensus values given in parentheses.

LD, lipoprotein.
Fig. 1. Reaction time courses for detection of glycerol in the manual modified enzymatic method for serum triglyceride. Sample A is 1.61 g/L glycerol solution, B is human serum. 25 μL of sample and 2.0 mL of Reagent 1 (R-1) were mixed. After 3 min, 1.2 mL of Reagent 2 (R-2) was added to the mixture, and the absorbance change at 600 nm was recorded at 37°C.

1.1.3.21), 195 U of peroxidase (EC 1.11.1.7), 150 U of ascorbate oxidase (EC 1.10.3.3), 0.21 mmol of ATP, and 60 mg of sodium 3,5-dimethoxy-N-ethyl-N-(2-hydroxy-3-sulfopropyl)aniline (DAOS) dissolved in 100 mL of 200 mmol/L 2-(N-morpholino)ethanesulfonic acid buffer (pH 6.5). In our modified method, we additionally dissolved 11.8 mg of 4-aminoantipyrine in the 100 mL of Reagent 1. In Reagent 2, 30 mg of 4-aminoantipyrine and 270 kU of lipoprotein lipase (EC 3.1.1.34) are dissolved in 100 mL of 200 mmol/L 2-(N-morpholino)ethanesulfonic acid buffer (pH 6.5).

Our modified enzymatic method is performed in two steps. The first step is the color development to monitor the high concentration of FG. Endogenous FG is reacted with glyceral kinase and glyceral-3-phosphate oxidase to form hydrogen peroxide and then with peroxidase to form quinonimine dye. The second step is as in the routine enzymatic assay: lipoprotein lipase acts on TG to form glycerol, and the glycerol is measured as in the first step of the procedure.

The first-step reaction reaches maximum at an FG concentration of 1.50 g/L. Thus, samples containing FG >1.50 g/L show increased absorbance at 600 nm at the initiation of the second reaction. This value is fixed as a baseline value in TBA-80S analyzer; any further increase in absorbance in the second step is read as TG (Figure 1).

Within-assay CVs were 0.89–1.83% and between-assay CVs were 2.69–3.47%. Linearity was observed for TG concentrations up to at least 32.0 g/L. Values obtained by this modified method correlated well (r = 0.998) with those obtained by conventional enzymatic assay. An error signal indicating the need to dilute samples was observed when we used serum obtained from patients receiving glycerol treatment. We obtained similar results when we used another commercial kit, Triglyceride Color (Boehringer Mannheim Yamanouchi Co. Ltd., Tokyo, Japan), and applied this method to a Cobas-FARA centrifugal analyzer (F.

Hoffmann-La Roche & Co. Ltd., Basel, Switzerland). Thus, our modified method for TG is applicable to other automated analyzers and should be used for samples from patients receiving medication containing glycerol.

References

Impact of Inhibition of Angiotensin-Converting Enzyme on Urinary Excretion of Proteins in Chronic Heart Failure, G. Ellekilde, J. Holm, H. Hemmingsen, B. Koczula, and F. E. von Eyben (Depts. of Intern. Med. and Clin. Chem., Central Hospital Nykøbing Falster, Nykøbing Falster 4800, Denmark)

Chronic heart failure (CHF) is often associated with a reduction of renal blood flow (1). Hence, concentrations of serum creatinine are commonly increased in CHF patients (1). Abnormalities in the renal handling of proteins, i.e., increased urinary excretion rates of albumin (a marker of glomerulopathy) and retinol-binding protein (RBP; a marker of proximal tubular dysfunction reflecting impaired reabsorption of low-M₆ proteins), are also seen in CHF (2). Angiotensin-converting enzyme (ACE) inhibitors, which increase renal blood flow, are commonly used as supplementary medication to treat CHF (1, 3). Here, we report a study to monitor the impact of ACE inhibitor treatment on the urinary excretion pattern of proteins in CHF.

We studied 19 patients, 16 men (median age 75 years, range 68–87) and 3 women (ages 56, 67, and 78 years), consecutively admitted to our hospital with a diagnosis of CHF and dyspnea during physical exercise (New York Heart Association groups II and III). Exclusion criteria for the study were diabetes mellitus, arterial hypertension, myocardial infarction within six months, valvular disorder, acute pulmonary edema, primary renal disorder, unstable angina, and treatment with ACE inhibitors. All 19 patients received conventional treatment for CHF, i.e., diuretics alone or in combination with digoxin, before and during hospitalization. Serum creatinine concentrations were normal in 13 patients (3 women ≤115 μmol/L; 10 men ≤133 μmol/L) but above normal in 6 men (134–200 μmol/L). All urine specimens were AlbuSintX negative.

Treatment with the ACE inhibitor (Captopril, Bristol-Myers Squibb, Jægersborg) was begun during hospitalization (25–75 mg/day). Albumin and RBP concentrations were determined by immunochromatographic assays (4, 5) in overnight urine specimens collected before and after the start of treatment. Creatinine in serum and urine was determined by a routine method (kinetic Jaffé, with a Hitachi 717 analyzer).

As shown in Table 1, the urinary excretion of albumin decreased significantly from days 0 (before ACE inhibitor treatment) to 7 after initiation of therapy. The decrease seemed to continue on days 14 and 30, although the between-day changes were not statistically significant. The proportion of patients with above-normal urinary albumin

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