False-Negative Immunoassay Results for Cardiac Troponin I Probably Due to Circulating Troponin I Autoantibodies

To the Editor:
Cardiac troponin I (cTnI), a regulatory protein unique to heart muscle, recently has been proposed as a highly sensitive and specific marker for myocardial damage [1-3]. We evaluated the diagnostic value of serum cTnI measurements in comparison with other biochemical markers for quantifying myocardial injury in 32 patients undergoing elective coronary artery bypass grafts (CABG) who did not have perioperative myocardial infarction as classified by electrocardiography and myocardial scintigraphy [4]. Serum concentrations of cTnI (Stratus™) Cardiac Troponin I Fluorometric Enzyme Immunoassay; Baxter Diagnostics, Deerfield, IL), troponin T (cTnT; Enzymun-Test™ Troponin T; Boehringer Mannheim Diagnostics, Mannheim, Germany), and creatine kinase isoenzyme MB mass (CK-MB; IMX™ CK-MB; Abbott, Abbott Park, IL) were measured before surgery and after release of the aortic clamp every 2-4 h during the first day, and daily thereafter until day 6.

In all preoperative samples, the marker protein concentrations were below the upper reference limits (URL) for cTnI (0.7 μg/L), cTnT (0.2 μg/L), and CK-MB (6.0 μg/L). After surgery, the three marker proteins increased in 31 of the 32 CABG patients, reaching peak values of 12.9 (median; range 2.9-56.0) μg/L for cTnI, 3.3 (0.4-12.3) μg/L for cTnT, and 35.6 (13.7-136.2) μg/L for CK-MB at 8 (median; range 4-20), 8 (4-24), and 4 (4-20) h, respectively, after aortic unclamping.

Surprisingly, in one CABG patient, a 69-year-old man with diffuse three-vessel disease, no cTnI was detectable postoperatively even though his cTnT and CK-MB showed the expected concentration time courses, increasing at 8 h after aortic unclamping to peak values of 3.6 and 36.2 μg/L, respectively, corresponding to 18 and 6 times above URL (Fig. 1). The obviously false-negative cTnI results suggested the presence of an interfering factor in the serum of this patient.

We investigated this possibility by adding increasing amounts of cTnI (up to 38.5 μg/L) to preoperative serum samples from this patient and incubating for 3 h at 37 °C; no added cTnI was detected. When we repeated the experiment, however, with the patient's serum depleted of IgG by treatment with Protein A or specific anti-human IgG-antiserum, ~97% of added cTnI was recovered. This suggested that the interfering factor is an IgG.

The Stratus cTnI two-site immunoassay uses two mouse monoclonal antibodies, making it susceptible in principle to interference from heterophile (i.e., human anti-mouse) antibodies [5]. However, interference from heterophile antibodies appears to be excluded by the following findings: (a) No interference occurred when postoperative samples of the patient's serum were analyzed for CK-MB with the Stratus CK-MB immunoassay, which is based on the same assay procedure as the Stratus cTnI assay; and (b) the interference was confirmed when these serum samples were reassayed for cTnI with another immunoassay procedure (Opus™ Troponin-I Assay; Behring Diagnostics, Westwood, MA), which uses two polyclonal antibodies from goat to recognize cTnI.

Accordingly, we conclude that the interfering IgG does not belong to the category of heterophile antibodies but works as an analyte-binding antibody and prevents the recognition of cTnI by the two-site immunoassays we used. To the best of our knowledge, this is the first documentation of a false-negative immunoassay result for cTnI that probably results from the interference of circulating IgG-class autoantibodies showing high affinity for cTnI.

References

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Immunoturbidimetric Method for Determination of Hemoglobin A1c

To the Editor:
We present here our evaluation of a new immunoturbidimetric hemoglobin A1c method (Unimate HbA1c; Roche Diagnostics, Mississauga, Canada) in a pediatric population (ages 2-18 years). Using this new method, we analyzed for HbA1c 180 whole-blood samples, venous and
capillary, from insulin-dependent diabetic patients attending the Diabetic Clinic, and we compared the results with the current ion-exchange method used in our laboratory. Controls provided by Roche Diagnostics and our own in-house controls were assayed with each run. The immunoturbidimetric reagents were donated by Roche.

The immunoassay uses latex-bound monoclonal antibodies developed against the glycated N-terminus of the β-chain; total hemoglobin was quantified colorimetrically. The assay was performed on a Cobas Mira as recommended by the manufacturer. Pretreatment of the sample is done on the analyzer. The erythrocytes are lysed by low osmotic pressure, and the released hemoglobin is degraded proteolytically to make the β-N-terminal structures more accessible for the immunoassay. In addition, the heme groups are oxidized before the colorimetric determination of hemoglobin. The final result is computed from the HbA1c and the total hemoglobin concentrations. The method is calibrated against an HPLC method that gives values that are, on average, 1.28 times those of the cation-exchange fast protein liquid chromatography (FPLC) method (Pharmacia, Uppsala, Sweden) used in our laboratory with a MonoS HR5/2 column [1]. For FPLC, the erythrocytes are lysed and the labile fraction is eliminated before chromatography.

Analysis of the 180 whole-blood samples by both methods yielded \( y = 0.82x - 0.37 \), \( S_{xy} = 0.39 \) (Fig. 1). Similarly, when only the capillary samples were compared, \( y = 0.80x - 0.27 \), \( S_{xy} = 0.27 \). Nine samples had increased (1.2% to 1.9%) fetal hemoglobin (Hbf), but this did not influence the final result. [Hbf] was quantified by the Pharmacia FPLC method. Because Hbf does not contain the β-chain, Hbf, is not quantified in the immunoturbidimetric assay. Hbf's contribution to the estimation of total hemoglobin is minimal unless the Hbf concentration is >10%. One sample had an abnormal hemoglobin (HbE of 27.3%), and the HbA1c quantification by chromatography was lower than by immunoturbidimetry. This was not unexpected because the point mutation (B26 Glu → Lys) presumably affected the mobility of the hemoglobin on FPLC but would not affect the antigenic epitope, the N-terminal glycoprotein of the β-chain, relevant to the immunoassay. The within-day CV (n = 4) was <2% and the interassay CV (n = 4) was <3% at 5–12% HbA1c.

The immunoturbidimetric method has the advantage, in theory, of specifically detecting all glycated N-terminal forms of HbA unless a point mutation exists in the N-terminal amino glycopeptide region of the β chain. Hbf (<2%) did not affect the final result. The ease with which this assay can be performed in both capillary and venous samples makes it an attractive alternative method for determination of HbA1c.

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References


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Antibodies Against [125I]Testosterone in Patient’s Serum: A Problem for the Laboratory and the Patient

To the Editor:

Determination of steroids directly in diluted serum samples is widely accepted all over the world, and forms the basis for nearly all commercial steroid immunoassay kits. Provided the reagents include inhibitors against steroid-binding proteins, the kits are reliable and easy to perform. Unless the samples are subjected to extraction before assay, the serum may contain interfering substances that may cause falsely high hormone concentrations. We have recently encountered two such cases with the Spectra Testosterone 125I kit from Orion Diagnostica, Espoo, Finland. The two sera were routine samples sent to the laboratory for diagnostic purposes, and the investigation of the samples, as reported here, is in accordance with the Helsinki Declaration of 1975, as revised in 1983.

Patient 1: A woman, 22 years old, normal ovaries, oligomenorrhea, question of hirsutism, thrombosis, and phospholipid antibodies. Normal female hormone concentrations except for testosterone at 25 nmol/L. The serum was reassayed for testosterone after diethyl ether extraction, giving <0.5 nmol/L.