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 reanalyzed 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.

Fig. 1. Changes in serum concentrations of cardiac marker proteins in a patient after coronary artery bypass grafting.

Ordinate: multiples of URL. Note the missing increase of cTnI above the URL (dotted line).

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

**Immunoturbidimetric Method for Determination of Hemoglobin A₁c**

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