Patients presenting to the emergency departments (ED) with symptoms of acute coronary syndrome (ACS) and with a nondiagnostic electrocardiogram (ECG) pose a management challenge (1). Cardiac troponins [cTns, troponin I (cTnI) and troponin T (cTnT)], creatine kinase (CK), and CK-MB are frequently used in the assessment of ACS. cTns are superior in their analytical specificity and diagnostic sensitivity and specificity for myocardial injury (2, 3). Findings from both animal and clinical studies show that cTnI is released into the blood in various cardiac conditions, including angina, acute myocardial infarction (1, 4, 5), congestive heart failure (6), and myocarditis (7). Because cTns in serum represent myocardial damage and increased risk of future adverse outcomes (8), improving the detection of serum cTns has implications for better diagnosis of myocardial damage and better risk stratification for patients with ACS.

With current clinical assays, cTns are detectable in the circulation 4–6 h after the onset of pain in acute myocardial infarction, peaking within 12–24 h and remaining increased for a few days (9). However, a recently developed Western blot method, WB-DSA (10), detected minute amounts of cTnI in serum of patients undergoing bypass surgery within 10 min after reperfusion (11), suggesting increased detection of TnI by the WB-DSA method. Although WB-DSA does not permit analysis of troponin’s quaternary structure, it does allow accurate assessment of the chemical status of individual troponin subunits, such as the extent and pattern of cTnI degradation. cTnI is specifically degraded in ischemic/reperfused injured rat myocardium (4, 12), and TnI degradation products are detected in myocardium of patients undergoing coronary artery bypass surgery. Because ACS represents a spectrum of cardiac pathophysiology, unique patterns of cTnI degradation may be present in serum at various points along this spectrum and detectable by the WB-DSA. This study presents a series of cases to evaluate the potential clinical applicability of this novel method for the detection of cTnI and any of its degradation products in serum from patients presenting for emergency medical care with symptoms of ACS.

Serum samples were obtained from patients presenting within 4 h of onset of symptoms of ACS to the Kingston General Hospital ED. We recruited patients between May and August 2000 and selected those with no or mild increases in CK, CK-MB, or cTnI as measured by clinical methods. Patients were excluded if they had renal impairment or skeletal injury. Patient selection was meant to be illustrative.

Patients underwent a history and clinical examination, a 12-lead ECG was recorded, and blood samples were obtained at presentation and at 1, 2, 4, 6, and 16–24 h for routine clinical testing of biochemical cardiac markers and for analysis by the WB-DSA (10). Samples were stored at −80°C until analyzed. This study was approved by the Queen’s University Health Sciences and Affiliated Teaching Hospitals Research Ethics Board. All participants gave written informed consent.

Total CK was assayed on the CX7 (Beckman Instruments); CK-MB and cTnI were assayed on the Immunol (Bayer Corporation). The reference intervals for CK were 55–197 U/L (men) and 35–155 U/L (women), with precision estimates (CV) from daily quality-control samples of 2.9% at 131 U/L and 2.7% at 480 U/L. A 20% increase in CK above the upper limit was considered meaningful. CK-MB was interpreted as positive when >8 µg/L with a relative index [(CK-MB × 100)/CK] >3%. The CVs of the CK-MB assay were 3.2% (4.5 µg/L) and 2.9% (18.2 µg/L). The minimum detectable cTnI concentration reported by the manufacturer for the Immunol assay was 0.1 µg/L. The CV of the cTnI assay was 10% at 0.5 µg/L, 7.0% at 3.0 µg/L, and 5.7% at 27 µg/L.

Detection of serum cTnI by WB-DSA was performed under denaturing and reducing conditions (10). Serum was diluted 12-fold in sample buffer containing, per liter, 3.3 g of sodium dodecyl sulfate, 3.3 g of CHAPS, 3.3 g of Nonidet P-40, 0.1 mol of dithiothreitol, 1 mol of urea, 50 mmol of Tris-HCl (pH 6.8), and 500 mL of glycerol. We used 2 µL of serum per lane. Proteins were resolved by 12% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (14 cm × 14 cm × 0.75 mm) in electrode buffer containing, per liter, 25 mmol of Tris, 192 mmol of glycerine, and 1 g of SDS, at 130 V for 4 h. Gel-resolved proteins were then transferred to nitrocellulose (45 µm; Micton Separation) in the presence of 10 mmol/L CAPS, pH 11, for 1 h at 100 V, using a Trans-Blot Cell apparatus (Bio-Rad). Membranes were blocked overnight at 4°C in 10% blocking reagent (Boehringer Mannheim) and probed separately with monoclonal antibody 8I-7 (epitope amino acids 136–154; Spectral Diagnostics), which detects most forms of cTnI (4, 11), and polyclonal antibody P3 (epitope amino acids 26–58; BiosPacific). Although the exposure times of the Western blots were optimized for better visual interpretation of results, all blots shown are in the linear range of detection. Sera were analyzed three times with each antibody. Results were consistent each time. Multiple exposures were conducted for each Western blot, ensuring that blots were in the linear range of detection.

For comparison, human recombinant intact cTnI1-209 and cTnI1-192 [the primary cTnI degradation products observed in stunned myocardium from isolated rat hearts (4, 12, 13)] in human serum were resolved alongside each
patient’s samples. Serum from each patient was also resolved and probed with only secondary antibody to control for cross-reactivity with the patients’ IgG.

**Patient 1.** A 64-year-old woman with a history of coronary artery disease presented with nausea, retrosternal chest heaviness at rest radiating to both arms, and diaphoresis. The ECG showed first-degree heart block and new ST depression. CK and CK-MB were not increased later. TnI was detectable by the Immunol assay only at 15, 22, and 24 h postpresentation, reaching a peak of 0.18 µg/L (Fig. 1A). WB-DSA detected no cTnI on admission, but cTnI was present at 1 h and thereafter for 24 h postpresentation. The discharge diagnosis was second-degree heart block. The patient returned 3 months later with chest pain.

**Patient 2.** A 73-year-old man presented with chest pain for 1.5 h and no other cardiac symptoms. He had experienced a myocardial infarction within the last 6 months and had a family history of cardiovascular disease. The ECG showed inferior-lateral T-wave inversion with inferior Q

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**Fig. 1.** Serial serum samples, analyzed by WB-DSA, from representative patients diagnosed with heart block (A) or unstable angina (B–D), and two patients diagnosed as chest pain, not yet diagnosed (E and F).

cTnI was detected with the anti-cTnI monoclonal antibody 81-7. The corresponding values for CK (U/L), CK-MB (µg/L), and cTnI (µg/L) are indicated (H, samples that were hemolyzed). Direct comparison of the intensities of bands between patients is inappropriate. Relative positions of molecular markers shown in kDa.
waves. CK, CK-MB, and cTnI were not increased (Fig. 1B). The WB-DASA detected cTnI on admission and thereafter. The discharge diagnosis was unstable angina; he returned 6 months later with chest pain.

**Patient 3.** A 47-year-old man with hypertension and coronary artery disease presented with retrosternal tightness with radiation down his left arm and no other cardiac symptoms. The ECG showed no acute changes from past ECGs. CK and CK-MB were slightly increased at 4 and 6 h, but were considered nondiagnostic. cTnI was 0.2 and 0.6 μg/L at 1 and 4 h postpresentation, respectively. The WB-DASA detected cTnI at admission and at all subsequent times (Fig. 1C). The discharge diagnosis was unstable angina.

**Patient 4.** An 80-year-old woman with hypertension and a history of angina presented with a pattern of chest pain consistent with unstable angina. The ECG showed a new left bundle branch block. CK and CK-MB were increased, but remained constant throughout her hospital admission. The magnitudes of the increases in CK and CK-MB were consistent with previous admissions. cTnI was undetected by the clinical assay, but was detected by the WB-DASA at admission, with the signal tapering off by the 6th h after admission (Fig. 1D). The discharge diagnosis was unstable angina.

**Patient 5.** A 69-year-old woman with no previous history of cardiac illness presented with central chest pain radiating to her left shoulder. The ECG showed T-wave flattening in anterior leads. CK, CK-MB, and cTnI were normal. The WB-DASA detected cTnI at admission, with the signal increasing progressively thereafter (Fig. 1E). Discharge diagnosis was chest pain, not yet diagnosed. She revisited the ED 2 months later with chest pain.

**Patient 6.** A 73-year-old woman with hypertension and congestive heart failure presented with weakness and chest pain radiating to the back. The ECG showed a sinus rhythm with left bundle branch block. CK, CK-MB, and cTnI were nondiagnostic. The WB-DASA detected cTnI from 1 h after admission and onward, although at 1 h the signal was faint (Fig. 1F). The discharge diagnosis was chest pain, not yet diagnosed.

**Patients 7–10.** Serum samples from four additional patients with nondiagnostic CK, CK-MB, and TnI were analyzed by the WB-DASA. Three were discharged with the diagnosis of angina and one with chest pain, not yet diagnosed. The WB-DASA detected no cTnI (data not shown). At the 3-month follow-up, one angina patient had occasional chest pain, but had not revisited the ED, another had had angioplasty with insertion of a stent after revisiting the ED, and the other two had not revisited the ED for cardiac-related problems.

The present findings demonstrate that WB-DASA can detect cTnI in samples that are negative by the Technicon Immulon assay. Because prognosis is related to the magnitude of cTnI increase (14), the increased ability of WB-DASA to detect low quantities of TnI may provide improved risk stratification. Three of the six patients in whom cTnI was detected by only WB-DASA returned to the hospital 2–3 months later with chest pain.

Only intact cTnI was observed in this cohort of patients with nonsignificant increases of biochemical cardiac markers and nondiagnostic ECG. This finding, which may reflect subtle myocardial damage, is in contrast to previous observations of multiple cTnI degradation products in serum of patients diagnosed with acute myocardial infarction (10), as assessed by WB-DASA. cTnI was not detected by the WB-DASA in serum of apparently healthy individuals (10), suggesting that the cTnI detectable in the present study by WB-DASA reflected myocardial injury, but that it was subtle compared with those with acute myocardial infarction. Moreover, the slight increase in CK-MB for patients 1 and 3 (Fig. 1, A and C) appears to parallel the detection of cTnI by the WB-DASA, possibly corroborating this more limited myocardial damage. As more patients are recruited to this study, we will look for cTnI modifications that may offer insight into the pathophysiologic process of ACS. The status of cTnI and its pattern of degradation in serum may reflect the state of the myocardium because cTnI can be modified in the myocardium before release into the circulation.

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References


Serum CrossLaps: Pediatric Reference Intervals from Birth to 19 Years of Age, Patricia M. Crofton,1,2* Nancy Evans,3 Mereyn K.H. Taylor,1 and Celia V. Holland4 (1 Department of Paediatric Biochemistry, Royal Hospital for Sick Children, Edinburgh EH9 1LF, United Kingdom; 2 Section of Child Life and Health, Department of Reproductive and Developmental Sciences, University of Edinburgh, Edinburgh EH9 1UW, United Kingdom; 3 The National Children’s Hospital, Tallaght and Department of Paediatrics, Trinity College, Dublin 4, Ireland; 4 Department of Zoology, Trinity College, Dublin 4, Ireland; * address correspondence to this author at: Department of Paediatric Biochemistry, Royal Hospital for Sick Children, Sciennes Road, Edinburgh EH9 1LF, United Kingdom; fax 44-131-536-0410, e-mail croftonp@aol.com)

During childhood growth, bone undergoes extensive modeling involving separate osteoblastic and osteoclastic processes. Markers of bone formation and resorption circulate at higher concentrations in children than in adults, parallel the childhood growth curve, and correlate with height velocity (1,2). Not only do these markers provide insight into the pathophysiology of bone turnover during growth, but they also give an early surrogate measure of its response to treatment. The markers of bone formation are all measured in plasma, and their use as markers of growth and bone formation in children is well established (1,2). However, most markers of bone resorption have traditionally been measured in urine. In infants and children, the practical difficulties associated with urine collection are compounded by marked circadian variation and high within-individual biological variation in urinary markers. Results are generally expressed in relation to creatinine, itself subject to considerable biological variation and changing with age as muscle mass increases. There is therefore a need for a sensitive and specific marker of bone resorption that can be measured in plasma and directly compared with markers of bone formation measured in the same sample.

Serum CrossLapsTM is a promising new marker for bone resorption (3), but its application in children has been hampered by lack of suitable reference data. Here we report age- and sex-related reference data for serum CrossLaps in children from birth to 19 years of age.

Neonates, infants, and children 0–5 years of age. Surplus plasma remaining after routine biochemical tests had been completed was retrieved from 59 neonates, infants, and children (37 males) who presented with various minor conditions that were considered not to have either a short- or long-term effect on growth. Children with systemic disease or intercurrent infections were specifically excluded. Samples were fully anonymized and stored at −70 °C until analysis.

Children 4–19 years of age. We analyzed stored plasma from 287 children (142 males, 145 females) 4–19 years of age who had participated in an earlier population-based epidemiologic study on the seroprevalence of toxocariasis in Irish schoolchildren (4). Ten samples from each sex and year group were analyzed, except for girls 4 years of age and boys 18 years of age, for whom only five and two samples, respectively, were available. Nonfasting samples were collected between 0900 and 1500. All children were well enough to attend school that day. No formal pubertal staging was undertaken because it would have been ethically inappropriate in this context. Blood samples were taken for the original study with the informed consent of both children and parents and after approval by the local ethics committee. The excess plasma remaining after completion of that study was fully anonymized and stored at between −40 and −70 °C until analysis.

We measured serum CrossLaps using a sandwich ELISA assay (Osteometer Biotech) as described (3). The assay has recently been restandardized on a weight basis by the manufacturer, using a synthetic, cross-linked polypeptide containing two identical residues of the sequence EKAHD-β-GGR originating from the C-telopeptide of type I collagen. The conversion from pmol/L [based on the previous reference material prepared from deasalted urinary antigens (3)] to the new units of ng/L is:

\[ x \text{ (ng/L)} = \frac{y \text{ (pmol/L)}}{138} \times 0.775 \]

In addition to the manufacturer’s control material, we included pooled plasma from prepubertal and pubertal children as further quality-control samples. Within- and between-run CVs in our study were 8.7% and 12% at 223 ng/L, 8.8% and 10% at 463 ng/L, and 8.2% and 14% at 786 ng/L, respectively.

The data were analyzed separately for neonates (younger than 1 month postnatal age), for infants (ages 1 month to 1 year), for each sex and year of age thereafter (e.g., the 1-year-old age band comprised children ≥1.0 to <2.0 years), and also for various combinations of ages. We compared medians, means, geometric means, and indices of skewness and kurtosis for log-transformed and untransformed data and found that log transformation gave