Clinica Chimica Acta 444 (2015) 149–153

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Age, sex, and racial influences on the Beckman Coulter AccuTnI+3 99th percentile

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ARTICLE INFO

Article history:
Received 29 October 2014
Received in revised form 30 January 2015
Accepted 4 February 2015
Available online 12 February 2015

Keywords:
Troponin
cTnI
99th percentile
AccuTnI+3
False positive
Beckman Access

ABSTRACT

Background: Beckman Coulter recently released a new cardiac troponin I (cTnI) assay, AccuTnI+3, for the Access 2 and DxI platforms. We validated the stated 99th percentile (20 ng/l) using a large population of healthy adults representative of the Northern California population.
Methods: Within a large sample of healthy adult members receiving care at Kaiser Permanente, cTnI was quantified in residual specimens using the AccuTnI + 3 assay. Patients were selected based on pre-defined criteria extracted from a comprehensive electronic medical record. All specimens with a cTnI concentration >30 ng/l were repeated; specimens that had a reproducible result >30 ng/l were subject to heterophile blocking procedure. 99th percentiles were calculated based on age, sex, race and body mass index categories.
Results: Among 1764 tested subjects, the 99th percentile for all samples was 25 ng/l. Sex differences were observed; the male and female 99th percentiles were 31 and 21 ng/l, respectively ($p = 0.001$). Age (range evaluated 18–89 y, median 47 y) also had a significant influence on the value ($p = 0.003$), but there were no significant differences by race. False positive results were detected in 0.9% of specimens (0.6% "fliers" and 0.3% heterophile antibodies), corresponding to 52% of all results >30 ng/l.
Conclusions: Among a large, representative cohort of healthy adults, we found a 99th percentile value consistent with prior studies based on highly selected small patient samples. Sex and age-specific upper reference limits for cTnI should be considered. In this cohort, about half the findings above the 99th percentile were false positives. Avoiding reporting erroneous results requires implementation of quality indicators.

1. Introduction

International clinical practice guidelines include the troponin 99th percentile as a critical part of the diagnostic criteria supporting the presence or absence of acute myocardial infarction [1]. Therefore, establishment of an accurate 99th percentile, either by manufacturers or individual laboratories, is critical to implementing any troponin assay across the spectrum of patients in clinical practice. Unfortunately, no guidelines exist to harmonize practices or to advise users/manufacturers of the ideal establishment protocol [2]. Furthermore, studies have shown that the method by which the population is selected influences the absolute value of the 99th percentile [3–7], which supports the argument that standard criteria should be utilized.

Studies have indicated that there are sex differences between the 99th percentile troponin cut-offs, which are particularly apparent in the high sensitivity assays [5,8–12]. Ethnic differences are relatively unexamined. A recent study comparing troponin 99th percentiles between American and Vietnamese populations observed no significant difference between values, but is one of the first to address possible ethnic differences in the upper reference limit [13]. Similarly, age-related differences are rarely taken into account, especially because as patients age, the boundary between a pathological and a physiological concentration becomes less apparent [4,5,8].

Beckman Coulter recently modified the troponin assay for the Access2 and DxI platforms, releasing it under the name AccuTnI + 3 [14]. While the manufacturer claims that only subtle changes were made between this assay and the previous generation (AccuTnI), the 99th percentile was re-established. Using a highly selected group of "healthy" individuals, the manufacturer published that the 99th percentile for the AccucTnI + 3 reagent is 50% lower than previously claimed

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http://dx.doi.org/10.1016/j.cca.2015.02.005
0009-8981/

for the AccuTnI (20 vs 40 ng/l). In addition, the package insert includes an "acute myocardial infarction cut-off value" of 30 ng/l. The clinically derived receiver operator characteristic (ROC) curve data supporting the latter cut-off was recently published [15].

A criticism of published studies of troponin 99th percentiles is that they were underpowered and/or unable to evaluate the potential influence of age, sex, and race on these thresholds [2]. A second problem is the apparent need to utilize cardiac imaging to obtain a cardiac healthy population. To address these knowledge gaps and assess the feasibility of using statistically robust numbers in place of cardiac imaging, we evaluated the 99th percentile of the AccucTnI + 3 assay using a large, diverse population of healthy individuals, overall and across age, sex and racial groups.

2. Materials and methods

2.1. Study design, sampling frame and patient characteristics

The study was conducted within Kaiser Permanente Northern California, a large integrated health care delivery system. The Kaiser Permanente membership is highly representative of the local surrounding and statewide population, except for slightly lower representation at the extremes of age and income. This was approved by the Kaiser Foundation Research Institute's Institutional Review Board.

Between January 11, 2014 and March 18, 2014, daily reports were generated based on data from a comprehensive health plan electronic medical record that identified "healthy" adult patients for which there was fresh, residual serum available (submitted for a wide range of routine tests). Each report included 250–400 eligible specimens, of which ~85/day were selected three times per week. Specimens were chosen from the reports at random, but were intended to give equivalent sex and age representation across the adult range.

Specimens were included in the report if fasting glucose and lipoproteins were within normal limits (defined as glucose < 100 mg/dl (<5.55 mmol/l), total cholesterol < 200 mg/dl (<5.18 mmol/l), triglycerides < 150 mg/dl (1.70 mmol/l), LDL < 130 mg/dl (<3.37 mmol/l) and HDL > 40 mg/dl (>1.0 mmol/l) in men or >45 mg/dl (>1.2 mmol/l) in women) on the available residual sample as well as an estimated glomerular filtration rate >60 ml/min/1.73 m^2 within the previous two years based on outpatient serum creatinine results calculated by the chronic kidney disease epidemiology collaboration (CKD-EPI) equation [16]. Patients were excluded if they met any one of the following three criteria: (1) presence of any one of the following comorbid conditions (based on diagnostic codes) arthritis, heart failure, hypertension, hepatic disease, pulmonary disease, peripheral artery disease, stroke, unstable angina, diabetes, or malignancy; (2) no renal function data; and (3) pharmacy dispensings for any cardiovascular or chemotherapeutic medications, including statins, beta blockers, calcium channel blockers, diuretics, anticoagulants, vasodilators, angiotensin converting enzyme inhibitors, angiotensin II receptor blockers and digoxin.

Information was obtained on patient age, sex, self-reported race/ethnicity and body mass index (BMI, kg/m^2) from health plan databases. Race/ethnicity and BMI were unavailable in 65 and 47 of all evaluated specimens, respectively.

2.2. Troponin and NTproBNP assays

Patient specimens were collected into serum separator tubes (SST) and were stored at 4 °C for up to 72 h before cTnI was measured using the Beckman Coulter AccuTnI + 3 assay on the Access 2 platform (Beckman Coulter Inc.). We previously validated that low concentrations (<100 ng/l) of TnI using this assay are unlikely to be affected by these storage conditions [17]. Both cTnT and NTproBNP were measured on each specimen as an independent assessment of cardiac function, but specimens were not excluded based on these result. cTnT and NTproBNP were either measured immediately after cTnI analysis or an aliquot was made and frozen at −80 °C for <4 weeks before analysis.

The CV of Beckman Coulter AccuTnI + 3 assay is <10% at 30 ng/l, the limit of quantitation (LOQ, 10% CV) is 20 ng/l; the limit of detection (LOD) is 8 ng/l; the limit of blank is 5 ng/l and the assay is linear to 1000 ng/l [17]. cTnT (4th generation assay) and NTproBNP were measured on either the e602 or the e411 (Roche Diagnostics). The 4th generation cTnT assay has a 10% CV at 30 ng/l and an analytical measuring range of 0.01–25.0 μg/l. The NTproBNP assay has a CV ≤ 7% across the measuring range (5–35000 pg/ml or 0.6–4130 pmol/l).

Specimens that gave a cTnI result >30 ng/l (the manufacturers claimed optimal AMI cut-off (15)) were repeated. Those that gave the same result were treated with a heterophile blocking reagent (Scantibodies) as specimen quantity allowed. Suspect specimens that repeated positive and were unaffected by heterophile blocking were evaluated for the presence of a high molecular weight interference by incubating the specimen with either 25% polyethylene glycol (PEG) or buffer and testing the supernatant for recovery of cTnI immunoreactivity (effective 1 in 2 dilutions following treatment). Specimens determined to have a false positive result were excluded from the 99th percentile analysis. Specimens that gave a cTnT result >10 ng/l (greater than the LOQ, n = 1) were repeated and treated with heterophile blocking reagent.

2.3. Subpopulations

Results were analyzed as a total population or grouped based on corresponding demographics and/or biochemical data. Subpopulations were defined by sex, age, BMI or ethnic background. Additional subpopulations were grouped based on NTproBNP concentrations using age-specific NTproBNP cut-offs, as reported by Hildebrandt et al. [18].

2.4. Statistical approach

All analyses were conducted using SAS statistical software, ver 9.3. The upper 99th percentile of the cTnI results was calculated for all specimens and stratified categories using a one tailed distribution. The 95% confidence intervals were calculated non-parametrically. Kruskal–Wallis tests were performed between subgroups to assess whether the distributions of cTnI results were the same.

3. Results

3.1. Demographic characteristics of healthy subjects

We examined specimens from 1764 eligible healthy subjects identified during the study period. The distributions of age, sex and race of the cohort are shown in Table 1. With older age, there was a lower frequency of males in the sample. cTnI was detected (>7 ng/l) in 18.9% of selected samples, with a higher prevalence of detectable cTnI in older subjects and those with higher body mass index.

3.2. False positive cTnI results

A total of 29 samples had a cTnI concentration >30 ng/l and were repeated. On second analysis, 34% of these specimens were undetectable (n = 9) or reduced substantially (n = 1; 199 ng/l to 20 ng/l (Table 2)). Of the 19 samples that had reproducible cTnI concentrations >30 ng/l, 21% (n = 4) decreased by ≥50% after incubation with a heterophile blocking reagent. Out of the 15 potentially "true" cTnI >30 ng/l samples remaining, two were highly suspicious (cTnI concentration 83 and 240 ng/l) and were incubated with PEG or buffer. Control specimens showed decreases of similar magnitude when incubated with PEG or buffer (control 1 — original result 100 ng/l; PEG- and buffer-treated 45 and 57 ng/l, respectively; control 2 — original result 160 ng/l; PEG- and buffer-treated 73 and 90 ng/l, respectively). In