Clinical Biochemistry 49 (2016) 1109–1112

Contents lists available at ScienceDirect

Clinical Biochemistry

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

Highlight Article

The applied statistical approach highly influences the 99th percentile of cardiac troponin I

Kai M. Eggers [a,*], Fred S. Apple [b,d], Lars Lind [a], Bertil Lindahl [a,c]

[a] Department of Medical Sciences, Uppsala University, SE-751 85 Uppsala, Sweden
[b] Department of Laboratory Medicine and Pathology, Hennepin County Medical Center, Minneapolis, MN, USA
[c] Uppsala Clinical Research Center, Uppsala University, SE-751 85 Uppsala, Sweden
[d] Minneapolis Heart Institute, Abbott-Northwestern Hospital, Minneapolis, MN, USA

ARTICLE INFO

Article history:
Received 31 July 2016
Accepted 8 August 2016
Available online 21 August 2016

Keywords:
Cardiac troponin
Upper reference level
99th percentile
Statistics

ABSTRACT

Background: Cardiac troponin (cTn) is the biomarker of choice for assessment of patients with acute coronary syndromes. Guidelines recommend the cTn 99th percentile derived from a cardiovascular healthy reference population as decision threshold. The importance of standardized criteria for the composition of such a reference population is well acknowledged. In this analysis, we investigated to which extent different statistical methods might have bearing on the calculated cTnI 99th percentile.

Methods: cTnI (Abbott) 99th percentiles were determined in 521 cardiovascular healthy community-dwelling subjects using the nonparametric method, the Harrell-Davis bootstrap method and the robust method together with different tests to identify potential outliers (Dixon, Tukey, Reed) and different statistical softwares.

Results: The cTnI 99th percentiles (nonparametric method) were 37 ng/L (total population), 42 ng/L (men) and 25 ng/L (women). These estimates differed by −7.4% to +5.7% using the Harrell-Davis bootstrap method and were up to 64.1% lower using the robust method. For the robust method, cTnI 99th percentiles varied by 44.2% depending on the applied software. The method of Tukey classified nine subjects as outliers while no outlier was detected using the other methods. Excluding these nine subjects resulted in up to 60.2% lower cTnI 99th percentiles.

Conclusions: Our results emphasize the need of a standardized statistical approach to calculate cTnI 99th percentiles. Our findings support the use of the nonparametric method and a conservative approach to detect outliers. This requires that the assessed population is sufficiently large and well selected on the basis of stringently applied clinical criteria.

1. Introduction

Cardiac troponin (cTn) is the biomarker of choice for assessment of patients with acute coronary syndromes. The cTn 99th percentile upper reference limit determined from a cardiovascular (CV) healthy reference population is acknowledged as cutpoint to indicate cardiomyocyte necrosis consistent with myocardial infarction [1]. With the implementation of high sensitivity (hs) cTn assays, it has become clearer that the composition of such a reference population is crucial for the calculation of the 99th percentile. For this reason, recent position papers outlined specifying criteria: a suitable reference population should be well balanced with respect to sex, age and ethnicity and sufficiently large, i.e. >300 subjects per sex- and age-stratum. Moreover, subjects with a history of previous CV disease, CV risk conditions (e.g. diabetes, renal failure) or biochemical, electrocardiographic and echocardiographic substrates of prevalent CV disease should be excluded [2,3].

Further, even the statistical approach to calculate cTn cutpoints matters. While nonparametric methods are recommended, the current IFCC-CLSI document optionally suggests the robust percentile method in case of smaller cohorts, i.e. n = 40–120 [4]. Such data have been reported occasionally [5]. A third alternative might be the use of the Harrell-Davis bootstrap method [6]. Potentially even more important for calculating far cutpoints such as the cTn 99th percentile is the identification of subjects with aberrant, i.e. outlying values. The IFCC-CLSI document favors the method of Dixon [4,7] but data using other methods [8,9] have been reported as well [5,10].

Considering these issues, our goal was to investigate the effect of different statistical methods used to calculate the 99th percentiles from a population of elderly subjects using a single cTnI assay.

Abbreviations: cTnI, cardiac troponin I; CV, cardiovascular; Hs, high-sensitivity; PIVUS, Prospective Investigation of the Vasculature in Uppsala Seniors; LoD, level of detection.

* Corresponding author at: Department of Medical Sciences, Cardiology, Uppsala University, S-751 85 Uppsala, Sweden.
E-mail address: kai.eggers@ucr.uu.se (K.M. Eggers).

http://dx.doi.org/10.1016/j.clinbiochem.2016.08.012
0009-9120/

2. Material and methods

2.1. Study population

All subjects aged 70 years living in Uppsala, Sweden were eligible for participation in the Prospective Investigation of the Vasculature in Uppsala Seniors (PIVUS) study. Potential study participants were randomly chosen from the registry of community inhabitants. Of the 2025 subjects invited, 1016 participated in the study and reported their medical history, underwent 12-lead ECG recording and blood sampling at baseline [11]. Echocardiography was performed in 924 subjects, as described previously [12]. All subjects gave written informed consent, and the ethics committee at the faculty of Medicine of Uppsala University approved the study.

The present analysis is based on a subset of PIVUS participants regarded as being CV healthy. CV health was defined as the absence of previous CV disease (stroke, myocardial infarction, coronary revascularization), self-reported heart failure, ischemic ECG findings, NT-proBNP > 210 ng/L for men and >250 ng/L for women [13,14], left-ventricular ejection fraction < 0.60 or left-ventricular hypertrophy on echocardiography. Subjects with an estimated glomerular filtration rate < 60 ml/min/1.73m^2 were excluded.

2.2. cTnI assay

cTnI concentrations had been measured in frozen EDTA plasma samples obtained at baseline and using the ARCHITECT *STAT* hs-cTnI assay (Abbott Laboratories, Abbott Park, IL). The level of detection (LoD) of this assay has been reported to range from 1.1 to 1.9 ng/L and the lowest concentration measurable with a 10% coefficient of variation is 5.6 ng/L [15]. According to the manufacturer, the overall 99th percentile is 26 ng/L, with sex-specific 99th percentiles of 34 ng/L for men and 16 ng/L for women.

2.3. Statistical analysis

cTnI 99th percentiles were calculated in the total population and in men and women separately. The following statistical softwares were used: Analyse-it for Microsoft Excel 4.65 (Analyse-it Software, Ltd., Leeds, U.K.), MedCalc 11.6 (MedCalc Software, Ostend, Belgium) and SPSS 21 (SPSS Inc., Chicago, IL). Both the nonparametric method, the Harrell-Davis bootstrap method and the robust method (with Box-Cox transformation followed by a second transformation to eliminate residual kurtosis) were applied. Whenever possible, bootstrap sampling with at least 1000 replications was performed. Subjects with outlying cTnI values were identified in the total population and in men and women separately using the methods of Dixon, Reed or Tukey [7–9]. Because of their non-Gaussian distribution, cTnI values were ln-transformed for this part of the analysis.

3. Results

Our data set included 521 subjects being CV healthy according to our prespecified criteria (266 men, 255 women). The distribution of hs-cTnI levels is illustrated in Fig. 1. Depending on the applied LoD, cTnI detection rates ranged from 84.3 to 99.0%.

The calculated cTnI 99th percentiles are presented in Table 1. The cTnI 99th percentile using the nonparametric method (SPSS) was 37 (95% confidence interval [CI] 21–45) ng/L for the total population, 42 (95% CI 22–73) ng/L in men and 25 (95% CI 11–31) ng/L in women. The 95% CI differed to those calculated using Analyse-it. MedCalc provides only 90% CI. Notably, Analyse-it did not provide 95% CI when the nonparametric method was used in cohorts of men or women. Compared to the nonparametric method, the cTnI 99th percentiles determined using the Harrell-Davis bootstrap method differed by −4.1% to −7.4% in the total population, by +4.6% to +5.7% in men and by −1.8% to −5.2% in women. The cTnI 99th percentiles calculated using the robust method varied by up to 44.2% depending on the applied statistical software and differed by −12.3% to −64.1% compared to the nonparametric method.

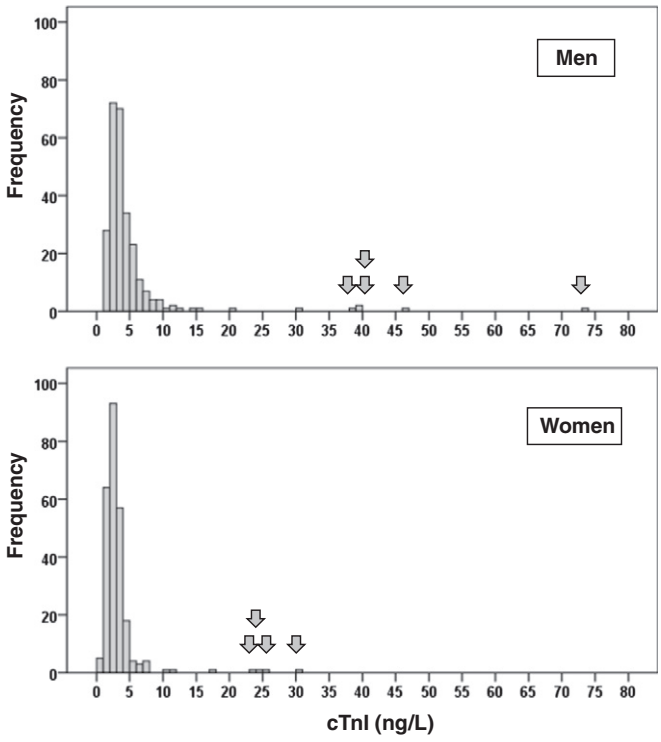

Fig. 1. Distribution of cTnI levels. Arrows indicate subjects with outlying cTnI values according the method of Tukey.

No outliers were detected using the methods of Reed and Dixon. Using the method of Tukey, nine subjects were classified as possible outliers (5 men [cTnI > 38 ng/L]; 4 women [cTnI > 23 ng/L]), see Fig. 1. Excluding these subjects from the calculations lowered the cTnI 99th percentiles by up to −60.2% (nonparametric method), up to −59.0% (Harrell-Davis bootstrap method) and up to −50.9% (robust method).

4. Discussion

Our unique observations demonstrate considerable differences in the cTnI 99th percentiles depending on the applied statistical approach to calculate these estimates.

The current IFCC-CLSI document recommends the use of the nonparametric method as standard [4]. This method, however, requires larger sample sizes and can be highly influenced by outliers. As an alternative, the robust method may be used [4]. This method iteratively weights observed values which minimizes the effect of extreme values and makes it suitable to establish reference intervals from smaller samples. We noted up to 64.1% lower cTnI 99th percentiles when using the robust method compared to the nonparametric method. This corroborates to data reported by Krintus et al. [5]. In addition, the calculated 99th percentiles and their 95% CI varied considerably depending on the applied software. The robust method is computationally demanding and requires several transformation steps for which different options may be chosen, i.e. different tuning constants, numbers of bootstrap replications or modalities of power transformation. Accordingly, the found discrepancies might mirror differences in the algorithms employed by the tested softwares.

While these findings discourage the use of the robust method, the Harrell-Davis bootstrap method might serve as an alternative. This method is a nonparametric estimator using a weighted linear