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The calculation of the cardiac troponin T 99th percentile of the reference population is affected by age, gender, and population selection: A multicenter study in Italy

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ABSTRACT

Background: The aim of this study is to determine the 99th upper-reference limit (URL) for cardiac troponin T (cTnT) in Italian apparently healthy subjects.
Methods: The reference population was selected from 5 cities: Bolzano (n = 290), Milano (CAMELIA-Study, n = 287); Montignoso (MEHLP-Study, n = 306); Pisa (n = 182); and Reggio Calabria (MAREA-Study, n = 535). Subjects having cardiac/systemic acute/chronic diseases were excluded. Participants to MEHLP project underwent cardiac imaging investigation. High-sensitive cTnT was measured with Cobas-e411 (Roche Diagnostics).
Results: We enrolled 1600 healthy subjects [54.6% males; age range 10–90 years; mean (SD): 36.4 (21.2) years], including 34.6% aged <20 years, 54.5% between 20 and 64 years, and 10.9% over 65 years. In the youngest the 99th URL was 10.9 ng/L in males and 6.8 ng/L in females; in adults 23.2 ng/L and 10.2 ng/L; and in elderly 36.8 ng/L and 28.6 ng/L. After the exclusion of outliers the 99th URL values were significantly decreased ($P < 0.05$) in particular those of the oldest (13.8 ng/L and 14 ng/L). MEHLP participants were divided in healthy and asymptomatic, according to known cardiovascular risk factors (HDL, LDL, glucose, C-reactive protein): the 99th URL of cTnT values of these subgroups was significantly different (19.5 vs. 22.7, $P < 0.05$).
Conclusions: 99th URL of cTnT values was strongly affected by age, gender, selection of subjects and the statistical evaluation of outliers.

1. Introduction

Cardiac troponins (cTns) I and T are the marker of choice for the detection of myocardial injury and the diagnosis of myocardial infarction, as recommended by the most recent guidelines [1,2]. In fact, the recommended criteria for the diagnosis of AMI is the evidence of a rise and/or fall of cardiac cTnI or cTnT with one or more values above the 99th percentile upper reference limit (URL), found in a clinical setting suggestive of myocardial ischemia [3,4]. As a result, the correct and precise (10% CV) estimation of the 99th URL represents the cornerstone for the differential diagnosis of the acute coronary syndromes. Over the past 10 years cTn assays have been improved in analytical sensitivity and precision thereby allowing the measurement of cTn in healthy subjects. According to Apple's scorecard for the classification of cTn assays, a high-sensitive assay must measure the 99th URL with 10% CV and more than 50% of healthy subjects must have detectable cTn levels [5].

The increasing analytical sensitivity of cTn assays greatly influences the 99th URL estimation. However the main factor that influences the 99th URL estimation is the selection of the reference population. ESC

Abbreviations: BMI, body mass index; BNP, brain natriuretic peptide; cTn, cardiac troponin; ECG, electrocardiogram; IQR, interquartile range; LVM, left ventricular mass; Q1, 25th percentile; Q3, 75th percentile; URL, upper reference limit.

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guidelines (2010) suggest selecting a sex- and age-matched healthy reference population, which should have a normal cardiac function as assessed by imaging.

Age- and gender-dependent effects have been observed both for cTnI [6–10] and cTnT [10–14] high-sensitive assays: cTn levels are lower in women and increase with age in both genders, in particular a sharp increment is observed after 65 years of age [6,12,15]. The age-dependent increase in cTn might be related to a progressive increase of heart failure due to the aging of myocardial tissue [16]. This suggests that the selection of elderly people for the 99th URL estimation must be performed very carefully.

Patient selection criteria have been shown to greatly affect both the 99th URL value and the cTn distribution in genders [17–20]. While, no clear definition has been proposed for the selection of a reference population for cTn 99th URL estimation, several Authors agree that inclusion criteria should be based on data obtained from a health questionnaire, on screening for renal function through the estimation of glomerular filtration rate measurement, and evaluation of hemodynamic stress and ventricular dysfunction through brain natriuretic peptide (BNP) or NT-proBNP measurement. In addition the reference population should be split equally by sex, include both young and elderly people, and be representative of the ethnicity present in the region [17,18]. Finally, the mathematical approach, used to identify and exclude the outliers, may also play a significant role in the calculation of cTn 99th URL [21,22].

The principal aim of this study was to determine the 99th URL for high-sensitive cTnT (hs-cTnT) assay according to age and gender in healthy subjects representative of the Italian population. Another aim of this work is to evaluate the effect of different statistical methods to exclude outliers from the 99th URL estimation for hs-cTnT assay.

2. Materials and methods

2.1. Study population

Samples from apparently healthy subjects were obtained from the *G. Monasterio Tuscany Foundation* (Pisa, Italy), and in collaboration with the clinical biochemical laboratory of the *San Maurizio Regional Hospital* (Bolzano; Italy) and three Italian population studies (MEHLP, CAMELIA and MAREA studies).

Briefly, the MEHLP project is a screening study aimed at evaluating the amount of cardiovascular subclinical pathology in an asymptomatic general population. To this aim, the population >40 years from the community of Montignoso (Massa, Italy) was enrolled (1474 people, mean ± SD 61 ± 14 years, males 48%, left ventricular ejection fraction 58 ± 5%, cardiac mass index 118 ± 42 mg/m2). CAMELIA and MAREA [23,24] studies are coordinated by the University of Milan (San Paolo Hospital, Milano, Italy), the "*Associazione Calabrese di Epatologia*" (Hepatology Association of Calabria, Reggio Calabria, Italy) and the "*Istituto Superiore di Sanità*" (Institute of Health, Roma, Italy). The CAMELIA (CArdiovascular risks, MEtabolic syndrome, Liver, and Autoimmune) study is aimed to investigate interactions among liver disease and cardiovascular risk and atherosclerosis. To this aim, 3550 individuals, aged 18–75, participated to the study. Subjects were randomly enrolled by censoring lists in two towns that are representative of the socio-economic and lifestyle characteristics of Northern Italy (Abbiategrasso, Milan) and Southern Italy (Cittanova, Reggio Calabria). According to the order of randomization, carotid artery echography was performed on 1 out of 3 individuals in order to measure the carotid intima–media thickness (IMT). Troponin T was measured in 484 out of 1180 participants who underwent IMT measurement and for which a lithium heparin plasma aliquot was available.

With similar aims, the MAREA (Metabolic Alterations in Reggio Calabria Adolescents) study has been carried in a population of adolescents. A sample of 843 adolescents aged 10–14 years was randomly selected from an updated school census list; troponin T was measured in 537 participants for which a lithium heparin plasma aliquots was available.

Subjects enrolled at the *San Maurizio Regional Hospital* (Bolzano; Italy) answered to questionnaire about an on-going therapy, past or present of cardiovascular diseases – including hypertension – cardiovascular surgeries, endocrine dysfunction, and kidney failure. Plasma C-reactive protein and creatinine values were also registered.

Subjects enrolled in CAMELIA and MAREA studies underwent a health investigation on lifestyle habits and medical history by questionnaires, clinical examination, carotid ultrasonography, and laboratory tests (serum creatinine, glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, total bilirubin, aspartate-aminotransferase, alanine-aminotransferase, gamma-glutamyltransferase, alkaline phosphatase, C-reactive protein, ferritin, iron, transferrin, homocysteine, TSH, complete blood count). NT-proBNP was measured only in MAREA participants.

Subjects recruited at the *G. Monasterio Tuscany Foundation* (Pisa, Italy) and participants to the MEHLP study answered a detailed questionnaire about lifestyle habits and medical history, and underwent clinical examination and laboratory tests (creatinine, glucose, insulin, total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, bilirubin, aspartate-aminotransferase, alanine-aminotransferase, gamma-glutamyltransferase, alkaline phosphatase, C-reactive protein, ferritin, iron, transferrin, homocysteine, TSH, NT-proBNP, complete blood count). Participants to the MEHLP Study were subjected to electrocardiogram (ECG) and cardiac imaging analysis (computed tomography scan, carotid echography, echocardiography).

This study was carried out in compliance with the principles set forth in the Declaration of Helsinki. The informed consent was obtained from all subjects enrolled in the study; the respective local ethical committee approved all population studies.

2.2. Exclusion criteria and definitions

On the basis of all collected data, we excluded all subjects presenting cardiac or systemic acute or chronic diseases, such as myocardial infarction, heart failure, coronary heart disease, hypertension, diabetes, kidney disease, obesity, tumor, hepatitis, and chronic obstructive pulmonary disease. Subjects using drugs, except for substitutive hormonal therapy, were also excluded.

For the present investigations, hypertension was defined as a systolic blood pressure of 140 mm Hg or higher, a diastolic blood pressure of 90 mm Hg or higher, or the use of antihypertensive medications. Body mass index (BMI) was calculated as the weight in kilograms divided by the square of the height in meters (kg/m^2); obesity was defined as BMI ≥ 30 kg/m^2. Diabetes was defined as a fasting blood glucose level of 126 mg/dL (7 mmol/L) or greater, or the use of any hypoglycemic agent. Glomerular filtration rate (GFR) was calculated according to the "modification of diet in renal disease" (MDRD) formula for serum creatinine assays not standardized to the IDMS reference method: $GFR = 186 \times \text{serum creatinine}^{-1.154} \times \text{age}^{-0.203} \times 0.742$ (if female) [25]. All considered subjects were Caucasian. Renal disease was defined as GFR < 60 ml/min/1.73 m^2.

2.3. Blood sampling and laboratory analysis

Blood samples were obtained from fasting participants between 8 and 9 AM; analyses were performed using standard clinical laboratory procedures with automated analyzers. cTnT levels were evaluated in aliquot of lithium heparin plasma stored at −80 °C. Plasma concentrations of cTnT were measured at the Fondazione Toscana G. Monasterio using the hs-cTnT method (Ref. 05092744) with the automated Cobas e411 platform by Roche Diagnostics; the assay was performed according to the recommendations made by the manufacturer (Roche Diagnostics, Germany) using the recalibrated control materials (lot 167345 and subsequent) [26]. The limit of blank (LoB) and the limit of

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