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Reference values of galectin-3 and cardiac troponins derived from a single cohort of healthy blood donors

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ABSTRACT

Background: Here we describe the determination of upper reference limits (URL) for galectin-3, high-sensitivity cardiac troponin I (hs-cTnI) and high-sensitivity cardiac troponin T (hs-cTnT) in a single cohort of healthy blood donors using routine assays.
Methods: For this reference value study, we used a cohort of 402 consecutive blood donors (64% were male and 36% were female). The median individuals' age was 35.0 years (range, 18.0–64.4). Individuals of this reference population were free of cardiovascular disease, diabetes mellitus, renal disease, cancer, current infection and chronic inflammatory disease. Plasma concentrations of galectin-3 were measured with the "routine Galectin-3" assay (Abbott Diagnostics), of hs-cTnI with the "STAT High Sensitive Troponin-I" assay (Abbott Diagnostics), and of hs-cTnT with the "Troponin T hs" assay (Roche Diagnostics). URLs were calculated by using a non-parametric percentile method.
Results: The 97.5th percentile URL for galectin-3 was 16 ng/mL in males and 17 ng/mL in females; the 99th percentile URL for hs-cTnI was 39 ng/L in males and 24 ng/L in females; and the 99th percentile URL for hs-cTnT was 14 ng/L in males and 11 ng/L in females. Those individuals with hs-cTnI values ≥15 ng/L (n = 8) were different from those individuals with hs-cTnT values ≥10 ng/L (n = 7). Of the 402 individuals, none had galectin-3 values below the limit of detection (LOD, <1.0 ng/mL), 290 (72%) had hs-cTnI values below the LOD (i.e., 1.9 ng/L), and 359 (89%) had hs-cTnT values below the LOD (i.e., 5.0 ng/L).
Conclusion: Plasma concentrations of galectin-3, hs-cTnI and hs-cTnT and corresponding 99th percentile URLs were rather low in our cohort of healthy blood donors compared with previously published data. In our reference population, analyte plasma concentrations above the LOD were detectable in 100% of the individuals with the Abbott galectin-3 assay, but only in less than 50% for both the Abbott hs-cTnI assay and the Roche hs-cTnT assay.

1. Introduction

Galectin-3 is a unique member of chimera type galectins and is involved in a large number of disease processes [1]. Galectin-3 participates in cell adhesion, activation, proliferation, apoptosis as well as cell migration [1,2]. It plays an important role in cancer [2] and inflammation [1,3]. Even in the pathophysiology of cardiac disease, galectin-3 is thought to play a biological role through inflammation and fibrosis [4,5]. As a consequence, the biomarker galectin-3 has been included in the 2013 ACCF/AHA guideline for additive risk stratification of patients with acute and chronic heart failure [6].

To date, only few and conflicting data have been published on upper reference limits (URL) of circulating galectin-3. Thus, the aim of this study was to establish reference values for galectin-3. As reference values for cardiac troponins are still under debate [7–14], we additionally aimed at evaluating URLs of high-sensitivity cardiac troponin I (hs-cTnI) and high-sensitivity cardiac troponin T (hs-cTnT) in a single cohort of healthy blood donors.

2. Materials and methods

2.1. Study protocol

For the present reference value study, we used a cohort of 559 consecutive Caucasian blood donors as described previously [15]. This cohort was recruited at the Red Cross organization in Linz, Austria, in 2008. All study participants completed questionnaires detailing their medical histories and medications in order to confirm their good health before blood collection according to Austrian laws. This healthy reference population was, thus, defined as individuals who were free of

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cardiovascular disease (no arterial hypertension, no history of any cardiac disease, cardiac treatment or cardiac intervention, no history of stroke or transient ischemic attack, no history of peripheral artery disease, no history venous thromboembolism). Additional exclusion criteria included, e.g., pregnancy, age ≥ 65.0 years, history of diabetes mellitus, renal disease and cancer, or current infection and any chronic inflammatory disease. The study protocol was approved by the local ethics committee in accordance with the Declaration of Helsinki, and all study participants gave informed consent.

Blood was obtained by conventional venipuncture avoiding venous stasis. Using VACUETTE® polyethylene terephthalate glycol (PET) blood collection tubes (Greiner Bio-One, Kremsmuenster, Austria), EDTA anticoagulated blood and serum were collected. C-reactive protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6), creatinine, and B-type natriuretic peptide (BNP) were quantified within 4 h of blood collection in all study participants from fresh samples. In addition, serum and plasma samples were frozen in several aliquots at −80 °C within 4 h. One of these frozen aliquots from each study participant was thawed to determine hs-cTnT, another aliquot to determine hs-cTnI, and a third aliquot to determine galectin-3. Of note, each of the aliquots was subjected to one freeze–thaw cycle only.

Criteria for the 559 blood donors to be included into the present reference value study were CRP serum concentrations ≤1.0 mg/dL, PCT serum concentrations ≤0.5 ng/mL, IL-6 serum concentrations ≤15.0 pg/mL, and BNP plasma concentrations ≤100 pg/mL (which are reference values used in our laboratory and are also generally accepted URLs for those same analytes as described in the literature).

Data were statistically analyzed with the SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) and the MedCalc 13.0.0.0 package (MedCalc Software, Mariakerke, Belgium). Comparisons of continuous variables (i.e., galectin-3, hs-cTnI and hs-cTnT) between different reference value groups were performed with the non-parametric Mann–Whitney U test or the non-parametric Kruskal–Wallis test, as appropriate. Obtained p values were not adjusted for multiple comparisons and are therefore descriptive only. URLs were calculated by using a right-sided non-parametric percentile method according to the Clinical and Laboratory Standards Institute (CLSI) guideline EP28-A3c [16]. Ninety percent confidence intervals (CI) were provided for the URLs, if possible.

2.2. Galectin-3, hs-cTnI and hs-cTnT measurements

In December 2009, we measured hs-cTnT plasma concentrations of the blood donors in one batch with the "Troponin T hs" assay (Roche Diagnostics, Mannheim, Germany, reagent lot number 156 593-01) [17,18] on a Modular platform because we started to use this assay at this time in clinical routine in our hospital. As stated in the package insert, this assay has a measurement range of 3.0–10,000 ng/L (defined as limit of blank to the maximum value of the master curve) and a limit of detection of 5.0 ng/L. Accordingly, hs-cTnT concentrations ≤5.0 ng/L were reported as 5.0 ng/L in the present work. The manufacturer reports a 10% coefficient of variation at a hs-cTnT concentration of 13.0 ng/L (i.e., limit of quantification). We confirmed all results with plasma concentrations >15 ng/L by a second measurement; no testing for heterophilic antibodies was performed in these samples.

In February 2013, hs-cTnI plasma concentrations of the blood donors were measured in one batch with the "STAT High Sensitive Troponin-I" assay (Abbott Diagnostics, Vienna, Austria, reagent lot number 23911JN00) [18,19] on an ARCHITECT $I2000_{SR}$ analyzer because in 2013 we switched to this assay for clinical routine use in our hospital. As described in the package insert, this assay has a limit of blank of 0.7–1.3 ng/L, a limit of detection of 1.1–1.9 ng/L, and a measurement range up to 50,000 ng/L. Thus, we decided to report observed hs-cTnI concentrations ≤1.9 ng/L as 1.9 ng/L in this work. The manufacturer claims a 10% coefficient of variation at an hs-cTnI concentration of 4.0–10.0 ng/L (i.e., limit of quantification). We confirmed all results with plasma concentrations >25 ng/L by a second measurement; no testing for heterophilic antibodies was performed in these samples.

In November 2015, we measured galectin-3 plasma concentrations of the blood donors in one batch with the "routine Galectin-3" assay (Abbott Diagnostics, Vienna, Austria, reagent lot number 48441M500) [20,21] on an ARCHITECT $I2000_{SR}$ analyzer. According to the package insert this assay has a measurement range of 4–114 ng/mL, and the limit of detection is claimed to be <1.0 ng/mL by the manufacturer. As previously reported [22], the Abbott galectin-3 assay has a total coefficient of variation of <5.0% for analyte concentrations >16 ng/mL, and the manufacturer describes a 10% coefficient of variation at galectin-3 concentrations of <4.0 ng/mL (i.e., limit of quantification). We confirmed all results with plasma concentrations >20 ng/mL by a second measurement.

2.3. Further biochemical analyses

Serum concentrations of CRP were measured with the standard laboratory method Tina-quant CRP (Latex) (Roche Diagnostics, Mannheim, Germany), a particle-enhanced immunoturbidimetric assay, processed on a Modular platform. PCT serum concentrations were determined with an immunofluorescent assay by using the B.R.A.H.M.S. PCT assay (B.R.A.H.M.S. AG, Hennigsdorf, Germany) on the KRYPTOR analyzer. Serum concentrations of IL-6 were measured with a solid-phase, enzyme-labeled, chemiluminescent sequential immunometric assay on an IMMULITE 2000 instrument (Siemens Medical Solutions Diagnostics, Vienna, Austria). Creatinine serum concentrations were measured by a kinetic colorimetric assay according to the Jaffé method (calibrators IDMS traceable; Roche Diagnostics, Mannheim, Germany) on a Modular platform. Estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation [23]. Plasma BNP concentrations were measured by using the ARCHITECT BNP assay (Abbott Diagnostics, Vienna, Austria) which is a chemiluminescent microparticle immunoassay on an ARCHITECT $I2000_{SR}$ analyzer.

3. Results

Of the 559 blood donors recruited for establishing reference values, 31 did not fulfill the inclusion criteria and, thus, 528 were finally enrolled into this reference value study for determining upper reference limits of galectin-3, hs-cTnI and hs-cTnT. Of the 528 individuals, 338 (64%) were male and 190 (36%) were female. Biomarker concentrations of all three analytes differed significantly between male and female individuals (Mann–Whitney-U tests for each analyte, p ≤ 0.001). In

Table 1
Analyte concentrations of the entire reference value cohort (n = 528) according to renal function.

Analyte	Individuals with eGFR >90 mL/min/1.73 m^2 (n = 402)	Individuals with eGFR <90 mL/min/1.73 m^2 (n = 126)	p value
Galectin-3 [ng/mL]	10.7 (range, 5.0–21.9; 25th–75th percentiles, 9.3–12.4)	12.1 (range, 6.0–24.4; 25th–75th percentiles, 10.2–14.1)	<0.001
hs-cTnI [ng/L]	1.9 (range, 1.9–101.1; 25th–75th percentiles, 1.9–2.0)	1.9 (range, 1.9–48.7; 25th–75th percentiles, 1.9–2.5)	0.005
hs-cTnT [ng/L]	5.0 (range, 5.0–20.1; 25th–75th percentiles, 5.0–5.0)	5.0 (range, 5.0–25.3; 25th–75th percentiles, 5.0–5.2)	<0.001

Abbreviations: hs-cTnI, high-sensitivity cardiac troponin I (Abbott); hs-cTnT, high-sensitivity cardiac troponin T (Roche).
Data are presented as median (range; 25th–75th percentiles). Difference of the analyte concentrations of both groups was calculated with Mann–Whitney-U tests (two-sided p values were not corrected for multiple comparisons).