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One-year in vitro stability of cardiac troponins and galectin-3 in different sample types

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ABSTRACT

Background: We assessed the long-term in vitro stability of cardiac troponins and galectin-3 under different storage conditions.

Methods: We used high-sensitivity cardiac troponin I (hs-cTnI) and galectin-3 assays from Abbott and a high-sensitivity cardiac troponin T (hs-cTnT) assay from Roche. The analyte concentrations of 30 patients were measured in heparin-treated plasma, EDTA-treated plasma and serum samples after the following storage conditions: 1) samples used immediately after blood collection for baseline measurements; 2) samples stored for 0.5 year at − 80 °C after one freeze-thaw cycle; 3) samples stored for 1.0 year at − 80 °C after two freeze-thaw cycles; and 4) samples stored for 1.0 year at − 80 °C after one freeze-thaw cycle. According to the concept of acceptable change limits (ACL) the analytes were considered stable as long as the median concentration at a particular time point was > 80%.

Results: Baseline hs-cTnI, hs-cTnT and galectin-3 concentrations ranged from 2.3 to 5436 ng/L, from 5.3 to 850 ng/L, and from 8.3 to 79.3 ng/mL, respectively. After applying the default criterion for analyte stability, the three analytes were stable for at least 1.0 year even after two freeze-thaw cycles for each sample type. We observed the following variation in analyte concentrations after storage relative to the baseline values: the interquartile range (IQR) of cardiac troponin results extended from approx. 80 to 115%, and the IQR of galectin-3 results extended from approx. 90 to 110%.

Conclusion: hs-cTnI, hs-cTnT and galectin-3 were stable under the reported storage conditions irrespective of the sample type used. However, we observed a considerable variation in analyte concentrations after sample storage, which might affect the diagnostic/prognostic value of these analytes in individual patients using frozen blood samples.

1. Introduction

Blood-based biomarkers, such as cardiac troponin (cTn) and galectin-3, are widely used in clinical trials and/or in clinical routines as an aid for the diagnosis and risk stratification of patients with heart disease [1–7]. Currently, a high-sensitivity cardiac troponin I (hs-cTnI) assay from Abbott Diagnostics, a high-sensitivity cardiac troponin T (hs-cTnT) assay from Roche Diagnostics and a recently developed galectin-3 assay from Abbott Diagnostics are of special interest because all three assays can be run on high-throughput analysers in routine clinical practice, and two of the three assays were recently approved by the U.S. Food and Drug Administration (FDA) [8–15].

When blood samples are used for biomarker measurements in clinical routines, efforts should be made to process these samples immediately after blood collection to 1) provide immediate results for timely diagnosis and therapy and 2) avoid any pre-analytical errors [16,17]. In clinical studies, however, this theoretical goal must give way to a more pragmatic approach to sample handling [16,17]. In many clinical studies, for example, the hs-cTnI, hs-cTnT and galectin-3 analytes are measured from frozen aliquots to (retrospectively) evaluate the diagnostic and prognostic value of these three biomarkers for several diseases. However, data in the literature are rare reporting on long-term in vitro stability of hs-cTnI, hs-cTnT or galectin-3 as measured with the above mentioned assays, and the published observations are mainly restricted to hs-cTnT [12,18–21].

In our present study, we therefore systematically assessed the long-term in vitro stability of hs-cTnI, hs-cTnT and galectin-3 under different storage conditions and revealed possible implications for

Abbreviations: ACL, acceptable change limit; CV, coefficient of variation; EDTA, ethylenediaminetetraacetic acid; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; IQR, interquartile range; RCV, reference change value

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epidemiological and large-scale clinical studies that utilize frozen blood samples.

2. Materials and methods

2.1. Study design

To test the hypothesis that the analytes hs-cTnI, hs-cTnT and galectin-3 are stable in vitro at a storage temperature of − 80 °C for at least one year, we used blood samples from clinical routines in our present study. Specifically, we used residual blood samples from 30 patients with various diseases who were admitted to the St. John of God Hospital in Linz, Austria. All patients provided informed signed consent that the residual material from blood samples collected in routine clinical tests would be made available for scientific purposes. Because there was no study-specific blood collection or any other study-specific intervention, approval of this study by the local ethics committee was not deemed necessary.

2.2. Assays

Circulating concentrations of hs-cTnI were measured with the "STAT High Sensitive Troponin-I" assay (Abbott Diagnostics, Vienna, Austria/Europe) on an ARCHITECT $i2000_{SR}$ analyser [9,10]; this assay has been used for routine clinical tests in our hospital since 2013. As described in the European package insert, this assay has a blank limit 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. 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). The gender-neutral 99th percentile hs-cTnI cut-off value of a healthy population is 26.0 ng/L according to the European Abbott package insert.

We measured hs-cTnT concentrations with the "Troponin T hs" assay (Roche Diagnostics, Vienna, Austria/Europe) on a cobas e 411 [8,9]. As stated in the European package insert, this 5th generation cTnT assay has a measurement range of 3.0–10,000 ng/L (defined as a blank limit to the maximum value of the master curve) and a limit of detection of 5.0 ng/L. The manufacturer reports a 10% coefficient of variation at an hs-cTnT concentration of 13.0 ng/L (i.e., limit of quantification). The gender-neutral 99th percentile hs-cTnT cut-off value of a healthy population is 14.0 ng/L according to the European Roche package insert.

Furthermore, we measured galectin-3 with the "routine Galectin-3" assay (Abbott Diagnostics) on an ARCHITECT $i2000_{SR}$ analyser [11,12]. According to the package insert, this assay has a measurement range of 4.0–114 ng/mL, and the limit of detection is claimed to be < 1.0 ng/mL by the manufacturer. The manufacturer describes a 10% coefficient of variation at galectin-3 concentrations of < 4.0 ng/mL (i.e., limit of quantification). Reference values of the galectin-3 assay have been reported previously [22].

2.3. Imprecision data of the assays

We evaluated the precision of the Abbott hs-cTnI assay and the Roche hs-cTnT assay in 2017, applying a protocol according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) guideline EP5-A [23]. For each of the two assays, we used the same three pooled patient heparin-treated plasma samples (pools 1–3) which we had aliquoted into twenty plastic tubes for each concentration level and frozen at − 80 °C. We analysed these samples in duplicate in one run per day for 20 days with the two assays using a single calibration for each assay. Within-run and total analytical imprecision (CV) were calculated with the CLSI single-run precision evaluation test [23] for each assay. The Abbott hs-cTnI assay had a within-run CV of 5.7% and a total CV of 6.8% at a mean concentration of 8.0 ng/L (pool 1), a within-run CV of 3.2% and a total CV of 3.3% at a mean concentration of 104 ng/L (pool 2), and a within-run CV of 2.5% and a total CV of 3.1% at a mean concentration of 490 ng/L (pool 3). The Roche hs-cTnT assay had a within-run CV of 4.4% and a total CV of 6.1% at a mean concentration of 14.6 ng/L (pool 1), a within-run CV of 1.6% and a total CV of 2.1% at a mean concentration of 81.6 ng/L (pool 2), and a within-run CV of 1.4% and a total CV of 2.1% at a mean concentration of 128 ng/L (pool 3).

We evaluated the precision of the Abbott galectin-3 assay, also applying a protocol according to the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) guideline EP5-A [23]. For this evaluation, we used three pooled patient EDTA-treated plasma samples (pools 1–3) which we had aliquoted into twenty plastic tubes for each concentration level and frozen at − 80 °C [24]. We analysed these samples in duplicate in one run per day for 20 days with the assay using a single calibration. Within-run and total analytical imprecision (CV) were calculated with the CLSI single-run precision evaluation test [23] for this assay. The Abbott galectin-3 assay had a within-run CV of 3.6% and a total CV of 4.9% at a mean concentration of 16 ng/mL (pool 1), a within-run CV of 1.8% and a total CV of 2.6% at a mean concentration of 22 ng/mL (pool 2), and a within-run CV of 2.8% and a total CV of 4.5% at a mean concentration of 32 ng/mL (pool 3).

2.4. Study protocol

In our study, we used blood samples from patients in which a hs-cTnI measurement was ordered by the attending physicians as part of a routine clinical test and the attending physicians simultaneously collected venous blood in a Vacuette lithium heparin tube (Greiner Bio One, Kremsmuenster, Austria; product reference number, 454029), a Vacuette K3 EDTA tube (Greiner Bio One, Kremsmuenster, Austria; product reference number, 454217), and a Vacuette serum tube (Greiner Bio One, Kremsmuenster, Austria; product reference number, 454067) for the determination of additional analytes.

A doctor in the Department of Laboratory Medicine (Margot Egger) identified appropriate patients for this study according to hs-cTnI concentrations obtained from heparin plasma in routine clinical tests. We obtained blood samples from 15 patients with hs-cTnI plasma concentrations above the detection limit (i.e., 1.9 ng/L) and below the 99th percentile hs-cTnI cut-off value of a healthy population (i.e., 26.0 ng/L). Furthermore, we obtained blood samples from 15 patients with hs-cTnI plasma concentrations above the 99th percentile cut-off value of a healthy population.

All blood collection tubes were centrifuged at 2250 × *g* during 10 min with a centrifuge temperature of 18–21 °C.

The baseline concentrations for hs-cTnI, hs-cTnT and galectin-3 reported in this study were then determined between 6 and 7 h after blood collection in all three sample types (i.e., heparin-treated plasma, EDTA-treated plasma and serum). For this study, we used reagent lot number 62027UI0 for hs-cTnI, reagent lot number 138684 for hs-cTnT and reagent lot number 50487M50 for galectin-3 measurements. The baseline measurements from the 30 study participants were performed between March 29, 2016, and April 20, 2016. During this time interval, we used a single calibration for each analyte.

Directly after the measurement of the baseline values for each patient, we separated the remaining heparin-treated plasma, EDTA-treated plasma and serum into two aliquots for each sample type (i.e., aliquots A and B). These six aliquots were then frozen at − 80 °C within 15 min after the baseline measurements. At the end of 0.5 year, the aliquot A samples of heparin-treated plasma, EDTA-treated plasma and serum were thawed, vortexed, centrifuged and immediately analysed on the same day (i.e., on October 10, 2016). We used reagent lot number 62027UI0 for hs-cTnI, reagent lot number 138696 for hs-cTnT and reagent lot number 62021M80 for galectin-3 measurements. A single calibration was used for the measurements after 0.5 year for each analyte (i.e., on October 10, 2016). Within 30 to 60 min after thawing, these three aliquots were again frozen at − 80 °C.