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Biomarkers

Circulating Cardiac Troponin T Exhibits a Diurnal Rhythm

Lieke J. J. Klinkenberg, MSc,* Jan-Willem van Dijk, MSc,† Frans E. S. Tan, PhD,‡ Luc J. C. van Loon, PhD,† Marja P. van Dieijen-Visser, PhD,* Steven J. R. Meex, PhD*

Maastricht, the Netherlands

Objectives The goal of this study was to test the unverified assumption that chronically elevated cardiac troponin T (cTnT) levels fluctuate randomly around a homeostatic set point.

Background The introduction of high-sensitivity cardiac troponin (cTn) assays has improved sensitivity for acute myocardial infarction (AMI). However, many patients with a single positive cTn test result do not have AMI. Therefore, the diagnosis of AMI relies strongly on serial testing and interpretation of cTn kinetics. Essential in this regard is a profound understanding of the biological variation of cTn.

Methods Two studies were conducted to assess biological cTnT variation and to investigate the presence of a diurnal rhythm of cTnT. Study 1 comprised 23 male subjects with type 2 diabetes, with no acute cardiovascular disease. Serial venous blood samples were drawn over an 11-h period (8:30 AM to 7:30 PM). In study 2, the presence of a diurnal cTnT rhythm was investigated by hourly sampling of 7 subjects from study 1 over 25 h.

Results In study 1, we observed a gradual decrease in cTnT concentrations during the day (24 ± 2%). This decrease was present in all participants and was most prominent in subjects with the highest baseline cTnT values (Pearson's R 0.93). Diurnal variation of cTnT, as assessed in study 2, was characterized by peak concentrations during morning hours (8:30 AM, 17.1 ± 2.9 ng/l), gradually decreasing values during daytime (8:30 PM, 11.9 ± 1.6 ng/l), and rising concentrations during nighttime (8:30 AM the next day, 16.9 ± 2.8 ng/l).

Conclusions A diurnal cTnT rhythm substantiates the recommendation that all dynamic changes in cTnT should be interpreted in relation to the clinical presentation. Epidemiological studies and risk-stratification protocols with the use of cTnT may benefit from standardized sampling times. (Exercise and Glycemic Control in Type 2 Diabetes; NCT00945165) (J Am Coll Cardiol 2014;63:1788–95) © 2014 by the American College of Cardiology Foundation

Cardiac troponin (cTn) is a sensitive marker of cardiac injury and is the preferred biomarker for the diagnosis or exclusion of acute myocardial infarction (AMI). Since the introduction of the high-sensitivity cTn assays, detectable cTn levels are no longer restricted to cardiac patients but can also be reliably assessed in apparently healthy subjects. Persistently elevated cTn concentrations near or above the 99th percentile of a healthy reference population are frequently observed in patients with stable coronary artery disease, elderly subjects, and patients with various chronic diseases such as type 2 diabetes (1–4). Some have suggested that a higher decision limit (3-fold the 99th percentile limit) might be advisable for diagnosing AMI in these patient groups (3,5).

Regardless of the cutoff value used, the critical tool for discriminating between AMI-induced cTn release and relatively stable cTn elevations in chronic diseases is serial testing and the assessment of kinetic changes in cTn. Guidelines advocate a rise and/or fall of cTn in patients with evidence of myocardial ischemia, with at least 1 cTn value exceeding the 99th percentile of the reference population (6). It should be recognized, however, that there is no perfect diagnostic threshold, and the application of any change criteria thus far is a trade-off, directed by the need for a high sensitivity or a high specificity. Therefore, a profound understanding of the naturally occurring biological variation of cTn is essential when interpreting dynamic changes in serial testing. By

From the *Department of Clinical Chemistry, Cardiovascular Research Institute Maastricht (CARIM), Maastricht University Medical Center (MUMC), Maastricht, the Netherlands; †Department of Human Movement Sciences, School for Nutrition, Toxicology and Metabolism (NUTRIM), Maastricht University Medical Center (MUMC), Maastricht, the Netherlands; and the ‡Department of Methodology and Statistics, Maastricht University, Maastricht, the Netherlands. This study was supported by a research grant from Stichting De Weijerhorst (Maastricht, the Netherlands) to Dr. van Dieijen-Visser. The authors have reported that they have no relationships relevant to the contents of this paper to disclose. The first 2 authors contributed equally to this work, and the Departments of Clinical Chemistry and Human Movement Sciences, Maastricht University Medical Center, contributed equally as well.

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definition, biological variation is measured by serially sampling healthy subjects at regular intervals (7). Thus far, studies in healthy subjects have reported short-term (hourly) biological variation of cTn ranging from 3% to 48%, which translates into reference change values (RCVs) of 45% to 85% (8–11). This finding means that a difference in serial cTn measurements which exceeds this threshold is unlikely due to analytical and biological variation only and is therefore considered a "true" change at a p value <0.05. However, it should be noted that healthy subjects may not be truly representative of the typical patient who requires clinical evaluation for chest pain.

The present study comprised a highly standardized assessment of within-day, diurnal, and between-week biological variation of cardiac troponin T (cTnT) levels in subjects with increased odds of requiring hospital examination for chest pain.

Methods

To examine within-day, diurnal, and between-week variation in cTnT levels, 2 distinct studies were conducted. Both studies complied with the principles of the Declaration of Helsinki and were approved by the institutional review board and the ethics committee at Maastricht University Medical Center. All participants provided written informed consent.

Study 1: within-day and between-week variation. The study group consisted of 23 male subjects (body mass index [BMI] 30 ± 4 kg/m^2; mean age 63 ± 7 years) who were diagnosed with type 2 diabetes for a mean duration of 7 ± 5 years (Online Table 1). According to their BMI, 16 subjects were classified as overweight (BMI 25 to 30 kg/m^2) and 7 subjects as obese (>30 kg/m^2). Subjects were recruited by using advertisements in the local newspaper. Exclusion criteria were self-reported renal failure, liver disease, morbid obesity (BMI >40 kg/m^2), history of severe cardiovascular problems (AMI or stroke in the past year), hypertension (>160 mm Hg systolic blood pressure and/or >100 mm Hg diastolic blood pressure), and exogenous insulin therapy. This study is part of a more extensive project investigating glycemic control and lifestyle interventions (12).

Participants visited the laboratory by car or public transportation on 3 occasions (experiments A to C) in randomized order (Fig. 1). Subjects were asked to refrain from exhaustive physical labor and exercise training for 2 days before each test day. Experiment A estimated within-day cTnT variation under sedentary conditions. From 8:30 AM until 7:30 PM, subjects were restricted to a sedentary laboratory environment and spent the day seated in a chair or couch, while reading, talking, watching television, or working on a laptop. Participants received standardized breakfast, lunch, and dinner at 8:30 AM, 12:30 PM, and 5:00 PM. Blood samples (8 ml) were collected from an antecubital venous catheter 5 min before each meal, as well as 90 and 150 min after each meal, resulting in a total of 9 blood samples collected within 11 h.

Experiment B was conducted to investigate whether within-day cTnT variation was affected by light physical activity. The test day was identical to experiment A, except for the addition of 15 min of slow-paced walking (total distance 800 to 1,000 m, including 2 staircases) after each meal (at 9:15 AM, 1:15 PM, and 5:45 PM) to mimic a day with low-to-moderate levels of physical activity. Experiment C was conducted to examine between-week variation of cTnT. Before breakfast at 8:30 AM, a blood sample was collected. Between-week variation was calculated by using the morning samples (8:30 AM) of experiments A through C. For all subjects, experiments A through C were performed on the same day of the week, within a total study period of 15 to 29 days.

Abbreviations and Acronyms

AMI = acute myocardial infarction
BMI = body mass index
CI = confidence interval
CK = creatine kinase
cTn = cardiac troponin
cTnT = cardiac troponin T
CV_i = within-person biological coefficient of variation
eGFR = estimated glomerular filtration rate
IQR = interquartile range
RCV = reference change value

Study 2: diurnal variation. After finalizing study 1, additional experiments were conducted to investigate the hypothesis that circulating cTnT exhibits a diurnal rhythm. Seven subjects who also participated in study 1 were sampled every hour (8 ml) by using an antecubital venous catheter over a time span of 25 h (Fig. 1). Participants were restricted to a sedentary laboratory environment from 8:30 AM till 9:30 AM the next day with standardized meals consumed at 8:30 AM, 12:30 PM, and 5:00 PM (breakfast, lunch, and dinner, respectively). Subjects went to bed at 11:30 PM, and the lights were off at 11:35 PM until 7:00 AM. During the night, polyethylene coiled extension lines (Vygon, Ecouen, France) were used for blood sampling to prevent disturbance of participants' sleep.

Laboratory measurements. Blood samples were collected in ethylenediaminetetraacetic acid–containing tubes. Immediately upon collection, the blood samples were centrifuged, and plasma was stored at −80°C until analysis. Hematology parameters were analyzed immediately in the diurnal samples on a Sysmex XE-5000 analyzer (Sysmex Corporation, Kobe, Japan). cTnT was measured in duplicate by using a high-sensitivity cTnT assay (Roche Diagnostics, Indianapolis, Indiana) on the cobas 6000 analyzer (lot number 167650). The limits of blank and detection of the assay were 3 and 5 ng/l, respectively; the 99th percentile among healthy subjects is 14 ng/l (13). Creatinine, creatine kinase (CK), and albumin were measured on the cobas 6000 analyzer. The estimated glomerular filtration rate (eGFR) was calculated according to the Chronic Kidney Disease Epidemiology Collaboration formula (14).

Statistical analysis. Between-person, within-person biological (CV_i), and analytical coefficients of variation were calculated by using a balanced analysis of variance with a nested random design in 2 levels; 95% confidence intervals