Concentrations of D-lactate can in-
crease during diabetes mellitus and DKA, methylglyoxal resulting in neurological symptoms and severe metabolic acidosis, respectively. Measurement of plasma D-lactate concentrations helps to account for the increasing anion gap. Reduction of plasma D-lactate concentrations correlated well with improvement of bicarbonate concentrations and anion gap following treatment.

In conclusion, our findings confirm the hypothesis that D-lactate levels correlate with the severity of DKA and high anion gap and monitoring DKA progression.

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Ninety-Minute vs 3-h Performance of High-Sensitivity Cardiac Troponin Assays for Predicting Hospitalization for Acute Coronary Syndrome

To the Editor:

In patients undergoing transcoronary ablation for septal hypertrophy, concentrations of high-sensitivity cardiac troponin T (hs-cTnT)1 increase within 15 min

1 Nonstandard abbreviations: hs-cTnT; AMI, acute myocardial infarction; ACS, acute coronary syn-

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of the procedure (1). If this situation translates to patients who present with chest discomfort who are suffering an acute myocardial infarction (AMI), hs-cTnT probed at time zero and at least 3 h later should be adequate to diagnose or exclude AMI (2, 3). Moreover, the kinetics shown in those with septal ablation suggest that an even earlier evaluation might be possible for clinical decision-making (1, 2). To assess the usefulness of early troponin measurements, we assessed the performance of hs-cTnT and hs-cTnI at 90 min and 3 h for predicting hospitalization for acute coronary syndrome (ACS) in patients presenting to the emergency department (ED) within 6 h of chest pain onset.

This study included patients enrolled in the RING study (Reducing the time Interval for identifying New Guideline defined MI in patients with suspected ACS in the ED; research ethics board approved) (4) who had EDTA plasma frozen (−80 °C) at presentation (baseline), 90 min, and 3 h (n = 130 patients). Inclusion criteria were: ≥18 years old with onset of ACS symptoms in the past 6 h, blood sample collection ordered by the ED physician for cardiac troponin measurement, informed consent obtained, and availability for telephone follow-up. We excluded patients presenting with ST-elevation myocardial infarction, those referred directly to surgery, trauma patients, and previously enrolled individuals. We followed all patients for 72 h to determine if an ACS hospitalization occurred (combined outcome of AMI or a hospitalization for unstable angina or refractory ischemic cardiac pain), defined as recurrent ischemic symptoms lasting 15 min or more, along with documented electrocardiographic changes indicative of ischemia or leading to a referral for an additional intervention such as thrombolytic therapy or cardiac catheterization or revascularization. An emergency physician and internal medicine specialist independently adjudicated all outcomes and were blinded to the hs-cTnT (lot #157120) and hs-cTnI results (Abbott ARCHITECT precommercial prototype assay measurement after second thaw). We used StatsDirect software for descriptive and nonparametric testing (Mann–Whitney, Friedman, and Conover testing) and Analyse-it software for ROC analyses. A P value of <0.05 was considered significant.

The median [interquartile range (IQR)] age of the study cohort was 60 years (50–71 years) with 64% (n = 83) males. Of the 130 patients enrolled, there were 14 patients hospitalized for ACS (n = 8 AMI diagnosed with cTnT fourth generation, n = 6 unstable angina or refractory ischemic cardiac pain). There was no difference in age between the ACS and non-ACS groups of patients [59 years (52–69 years) vs 60 years (49–71 years); P = 0.92], and the peak high-sensitivity cardiac troponin concentrations (obtained at baseline, 90 min, and 3 h) were higher in the ACS group [hs-cTnT ACS median (IQR) = 75 ng/L (11–262 ng/L) vs non-ACS median (IQR) = 7 ng/L (3–18 ng/L), P < 0.01] and hs-cTnI ACS median (IQR) = 260 ng/L (6–744 ng/L) vs non-ACS median (IQR) = 3 ng/L (1–9 ng/L), P < 0.01]. In the ACS group the 3-h concentration of hs-cTnT [median (IQR) = 74 ng/L (10–262 ng/L)] and hs-cTnI [median (IQR) = 260 ng/L (6–744 ng/L)] was significantly higher than baseline [median (IQR) hs-cTnT = 30 ng/L (11–100 ng/L), P < 0.01; median (IQR) hs-cTnI = 33 ng/L (6–316 ng/L), P < 0.01] and 90-min postbaseline [median (IQR) hs-cTnT = 38 ng/L (8–156 ng/L), P < 0.01; median (IQR) hs-cTnI = 39 ng/L (6–438 ng/L), P = 0.02]. However, there was no significant difference in concentrations between baseline and 90 min for hs-cTnT (P = 0.11) and hs-cTnI (P = 0.05).

Using ROC curve analysis to assess the discrimination of ACS patients (n = 14) and non-ACS patients (n = 116), we observed that 3-h measurements were superior to 90-min measurements (Fig. 1.) [hs-cTnT 3-h area under the ROC curve (AUC) 0.81 (95% CI, 0.65–0.97) vs 90-min AUC 0.80 (95% CI, 0.64–0.95); P < 0.01 and hs-cTnI 3-h AUC 0.83 (95% CI, 0.68–0.97) vs 90-min AUC 0.81 (95% CI, 0.66–0.96); P < 0.01, DeLong method]. The absolute change in concentration from baseline to 3 h (i.e., ∆) was also assessed via ROC analyses. In this analysis, the 3-h hs-cTnT concentration AUC was significantly higher than the absolute 3-h ∆ hs-cTnT AUC [0.72 (95% CI, 0.53–0.91); P = 0.04]; however, there was no difference in the 3-h hs-cTnI concentration and the absolute 3-h ∆ hs-cTnI AUC [0.84 (95% CI, 0.73–0.95); P = 0.81]. The absolute ∆ at 3 h which gave a positive likelihood ratio of 10 (to rule in) for hs-cTnT was ≥9 ng/L and for hs-cTnI was ≥28 ng/L.

These data suggest that the performance of measurements at 3 h for both hs-cTnT and hs-cTnI is superior to measurements at 90 min. The use of earlier decision points such as 90 min may not be prudent, because a 3-h measurement for the high-sensitivity cardiac troponin assays will still be necessary in most patients. The 3-h time-point for hs-cTnT has recently been reported as equivalent to the 6-h timeframe (3). Whether this will also apply for hs-cTnI remains to be determined, because that assay is more sensitive than the hs-cTnT assay (5). Larger prospective studies will be required to validate the 0–3-h evaluation period.
Fig. 1. ROC curve analyses for predicting hospitalization for ACS within 72 h after ED presentation with hs-cTnT (A) and hs-cTnl (B).

References


Identification of Complete Hydatidiform Mole Pregnancy–Associated MicroRNAs in Plasma

To the Editor:

The presence of pregnancy-associated, placenta-specific microRNAs (miRNAs)1 in the plasma of pregnant women has recently been reported (1). Such miRNAs have potential as molecular markers for complete hydatidiform mole (CHM), which is usually diploid and androgenetic in origin. We aimed to identify CHM pregnancy–associated miRNAs in plasma. All participants gave written informed consent, and the Research Ethics Committee of Nagasaki University approved the study.

First, we obtained a set of CHM tissues and blood samples from a CHM pregnant woman at 10 weeks' gestation and normal villous tissue from a woman with an uncomplicated singleton pregnancy who underwent artificial abortion at 10 weeks' gestation. Both the androgenetic origin of the CHM (46,XX) and the biparental origin of normal villous tissue (46,XX) were confirmed by DNA genotyping analysis.

Isolation of total RNA (including small RNAs), assessment of their quality, concentration measurements, construction of a small-RNA library, next-generation sequencing (NGS), miRNA mapping, and NGS analysis of the differential expression of miRNA-encoding genes were performed as described previously (2). NGS analysis of the sample set yielded 16 920 412 reads from CHM tissue, 17 462 519 reads from the patient’s blood cells, and 30 447 835 reads from normal villous tissue. All of these sequence data were deposited in the DNA Data Bank of Japan (DDBJ) Sequence Read Archive (http://www.ddbj.nig.ac.jp/index-e.html). The accession ID is DRA001009 (http://trace.ddbj.nig.ac.jp/DRASearch/).

To compare miRNA concentrations across the data set, we corrected the sequencing bias of GC content for each miRNA (3) and normalized the sequencing read count as reads per kilobase of exon model per million mapped reads (RPKM) (4). NGS analysis of the sample set detected 503 miRNAs in CHM tissue, 497 miRNAs in the patient’s blood cells, and 715 miRNAs in normal villous tissue. Homo sapiens miRNA 520b (hsa-miR-520b), -520f, and -520c-3p were detected in CHM tissue at concentrations >50 times higher than in normal villous tissue. Conversely, hsa-miR-136-3p, -127-5p, -370, -127-3p, -493-3p, -184, and -493-5p were detected in CHM tissue at concentrations >50 times lower than in normal villous tissue. We selected hsa-miR-520b, -520f, and -520c-3p (which were undetectable in blood cells) as candidate plasma CHM pregnancy–associated miRNAs. These candidate miRNAs were located on chromosome 19q13.42, which is commonly referred to as the chromosome 19 miRNA cluster (C19MC). The fold changes (i.e., the RPKM of CHM miRNA divided by the RPKM of normal villous miRNA) for hsa-miR-520b, -520f, and -520c-3p were 62.38 (i.e., 13 760.74 ÷ 220.59), 78.71 (6711.02 ÷ 85.26), and 115.41 (310 662.75 ÷ 2691.72), respectively. Compared with normal villous tissue, the expression of miRNA-encoding genes on C19MC were upregulated, whereas those on C14MC were downregulated in CHM tissue (raw data deposited in the DDBJ).

Subsequently, we analyzed the 3 candidate plasma CHM pregnancy–associated miRNAs by absolute reverse-transcription quantitative real-time PCR (RT-qPCR) with the TaqMan® MicroRNA Assay (Applied Biosystems) in villous tissues and plasma samples from 14 CHM pregnancies (CHM group) and 20 uncomplicated pregnancies (control group). There were no significant differences between the CHM and control groups in maternal age or gestational age (data not shown). Preparation and extraction of total RNA containing small RNA molecules from tissue samples or from 1.2-mL samples of cell-free plasma, as well as absolute RT-qPCR analysis, were performed as described previously (1, 2). The intraassay CVs for hsa-miR-520b, -miR-520f, and -520c-3p in the absolute RT-qPCR assays were 8.1%, 6.4%, and 6.8%, respectively. Absolute RT-qPCR results for villous tissues and plasma samples are shown in Fig. 1. The concentrations of candidate CHM pregnancy–associated miRNAs were significantly higher in the CHM group than in the control group, for both villous tissues and plasma samples. These CHM-associated miRNAs were identified in both CHM tissues and plasma samples. Placenta-specific C19MC is imprinted in the placenta, with expression from the paternally inherited chromosome (5). Therefore, the increased expression of genes encoding CHM pregnancy–