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Cardiac Troponin Composition Characterization after Non ST-Elevation Myocardial Infarction: Relation with Culprit Artery, Ischemic Time Window, and Severity of Injury

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BACKGROUND: Troponin composition characterization has been implicated as a next step to differentiate among non-ST elevation myocardial infarction (NSTEMI) patients and improve distinction from other conditions with troponin release. We therefore studied coronary and peripheral troponin compositions in relation to clinical variables of NSTEMI patients.

METHODS: Samples were obtained from the great cardiac vein (GCV), coronary sinus (CS), and peripheral circulation of 45 patients with NSTEMI. We measured total cTnI concentrations, and assessed both complex cTnI (binary cTnIC + all ternary cTnTIC forms), and large-size cTnTIC (full-size and partially truncated cTnTIC). Troponin compositions were studied in relation to culprit vessel localization (left anterior descending artery [LAD] or non-LAD), ischemic time window, and peak CK-MB value.

RESULTS: Sampling occurred at a median of 25 hours after symptom onset. Of total peripheral cTnI, a median of 87[78-100]% consisted of complex cTnI; and 9[6-15]% was large-size cTnTIC. All concentrations (total, complex cTnI, and large-size cTnTIC) were significantly higher in the CS than in peripheral samples ($P < 0.001$). For LAD culprit patients, GCV concentrations were all significantly higher; in non-LAD culprit patients, CS concentrations were higher. Proportionally, more large-size cTnTIC was present in the earliest sampled patients and in those with the highest CK-MB peaks.

CONCLUSIONS: In coronary veins draining the infarct area, concentrations of both full-size and degraded troponin were higher than in the peripheral circulation. This finding, and the observed associations of troponin composition with the ischemic time window and the extent of sustained injury may contribute to future characterization of different disease states among NSTEMI patients.

Introduction

Cardiac troponin (cTn) is uniquely present inside cardiomyocytes consisting of two cardiospecific isoforms (cTnT and cTnI) and one non-cardiospecific form (TnC). After myocardial infarction (MI), circulating cTn primarily consists of complex forms (1–6). Recently, cTn composition was described to arise from a stepwise transformation of full-size ternary cTnT-cTnI-cTnC complex (cTnTIC) into smaller sized complexes and fragments of cTn (5). As a future development in biomarker diagnostics, characterization of patterns of cTn composition may contribute to diagnostic differentiation and could be indicative of underlying disease status (7–9). However, direct evidence that links cTn composition to diseased myocardium in the setting of MI is lacking. Interestingly, a connection between cTn composition and disease status might exist, as incidental observations showed a relation with the ischemic time window (4, 5, 9–12) and injury severity (13–15). The difficulty of these investigations was that laboratory

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analyses were complex, time consuming, and were performed in a limited number of MI patients. Additionally, blood was sampled remote to the injured myocardium in all but one study. In that study, cTnT composition was assessed in the coronary venous system (CVS) and a concentration gradient was observed for all known cTnT fragments towards the peripheral circulation (10) .

In view of this, we extended our analyses on cTn composition in blood obtained from various sites within the CVS. With the use of 2 recently developed precommercial assays, cTn composition could be studied in a larger-sized cohort of non-ST elevation MI (NSTEMI) patients. Using a high-sensitive platform complex cTnI (i.e., full-size, partially truncated, and low molecular weight cTnTIC [LMW cTnTIC], and binary cTnI-cTnC [cTnIC]), and large-size cTnTIC complexes (i.e., full-size or partially truncated cTnTIC) were measured, in addition to total cTnI (16, 17). The size of the cohort allowed comparison of patients with left anterior descending (LAD) or non-LAD culprit arteries, which together with multiple site CVS sampling allowed linkage of cTn form concentrations to the area of injured myocardium. In addition, subgroup comparisons were made of severity of sustained injury and ischemic time interval.

Methods and Materials

PATIENT POPULATION

For the present analysis we studied a selection of NSTEMI patients who participated in the TRAMICI study (TRanscardiac Assessment of Myocardial Injury and Coronary Inflammation). TRAMICI is a biomarker study in NSTEMI patients, including multi-site CVS blood sampling and serial peripheral blood samples. This study aimed to assess and compare markers of myocardial injury and inflammation in samples from areas draining blood from infarcted and non-infarcted regions. For inclusion, patients were eligible in case of a NSTEMI with a rising cTn and significant coronary artery disease at coronary angiography (CAG). Main exclusion criteria were: indication for immediate percutaneous coronary intervention (PCI), prior acute coronary syndrome, coronary artery revascularization in the last 3 months, or hemodynamic instability. Patients with ST-elevation myocardial infarction were excluded, to ensure restoration of coronary flow as soon as possible without delays related to informed consent for and execution of a rather comprehensive study protocol. The full lists of inclusion and exclusion criteria have been described elsewhere (18).

Participants underwent routine catheterization (including left ventricular angiogram) and study procedures at the catheterization laboratory of the Radboud University Medical Center (Nijmegen, The Netherlands). After CAG and upon identification of the culprit coronary artery by the interventional cardiologist, patients were included and study procedures were started. Oral informed consent was obtained prior to CAG, and written informed consent was provided afterwards. The local ethical committee (2004-186) of the Radboud University Medical Center approved the TRAMICI protocol. Study procedures were in accordance with the Declaration of Helsinki.

For the present analysis we selected a subgroup of patients fulfilling the following criteria: 1) a definite culprit coronary artery at CAG; 2) availability of a sample from the coronary sinus (CS) and the great cardiac vein (GCV).

CAG ANALYSIS

Prior to biomarker analyses, a team of cardiologists performed a standardized evaluation of wall motion abnormalities of the left ventricle, lesion characteristics, and coronary flow to identify the culprit lesion. Of the patients in whom the culprit lesion was different compared to what was decided by the interventional cardiologist, a third evaluation was performed by an independent cardiologist naive to all data. Upon agreement with either of the 2 previous evaluations, the culprit artery was defined accordingly. If not, the patient was classified as culprit unknown, and blood was not analyzed. For the present analysis we allocated patients to either having a culprit lesion located in the left anterior descending artery (LAD) or in a non-LAD location (circumflex or right coronary artery).

STUDY PROCEDURES

The CVS anatomy was recorded during CAG by filming the complete washout of contrast dye. CVS access was gained by means of a right-sided catheterization procedure. A Terumo wire (Terumo Europe NV) and CHAMP multipurpose catheter (Medtronic) were used for CVS cannulation and blood sampling.

CVS samples. Supplemental Fig. 1 presents a schematic representation of the sampling protocol. After cannulation of the CVS, the CHAMP catheter was advanced into the GCV. A baseline blood sample at the GCV (GCVT0) represents a selective measure of biomarkers from injured anteroseptal myocardium in patients with an LAD culprit lesion. After sampling the GCV, the CHAMP catheter was pulled back and placed distal in the CVS, preferably as close to the point where the middle cardiac vein merges with the CS, but always distal from the second posterolateral vein for the second baseline sample from the CS (CST0).

The protocol specified the procedures for blood sampling as follows. First, the position of the catheter was confirmed using an injection with contrast dye in

the CVS. Second, the catheter was flushed, and the blood was allowed to run freely from the catheter end for a few seconds to ensure sufficient blood reflux. Third, a first blood sample of 3-4 mL was drawn to perform immediate blood gas analysis to double check the catheter position. Fourth, the blood sample of interest was obtained.

Peripheral venous (PV) blood samples. After removal of the CHAMP catheter, peripheral blood samples were obtained from the femoral venous sheath (PVT0).

Follow-up samples. Finally, at 6 and 12 hours post-procedure, additional venous samples were obtained from a peripheral vein (PVT6 and PVT12).

Collected blood samples were divided over serum tubes, immediately centrifuged, divided in 250 µL aliquots, and stored at -80° C until thawed for further analysis. To prevent potential effects of repeated freeze-thaw cycling a separate aliquot for each analysis was used.

BIOMARKER MEASUREMENTS

Figure 1 depicts the cTnI forms of interest, determined with the assays that are described into more detail below.

Three assays were used to determine the different cTn compositions in our NSTEMI patients: 1) For total cardiac troponin I (a combination of complex and non-complex forms) the high-sensitivity cardiac troponin I Pylon immunoassay (ET Healthcare) was used; it has been previously described (16). This assay uses a capture antibody targeting amino acid residues 27–40, and a detection antibody which recognizes cTnI amino acids residues 41–49; 2) To measure complex cTnI (i.e., complexes of full-size cTnTIC, partially truncated cTnTIC and LMW cTnTIC, and binary cTnIC), the same Pylon assay analyzer platform was used applying a different capture antibody with specificity for complex cTnI: 20C6 (Hytest Ltd). This antibody recognizes cTnIC and cTnTIC complexes, whereas noncomplexed cTnI

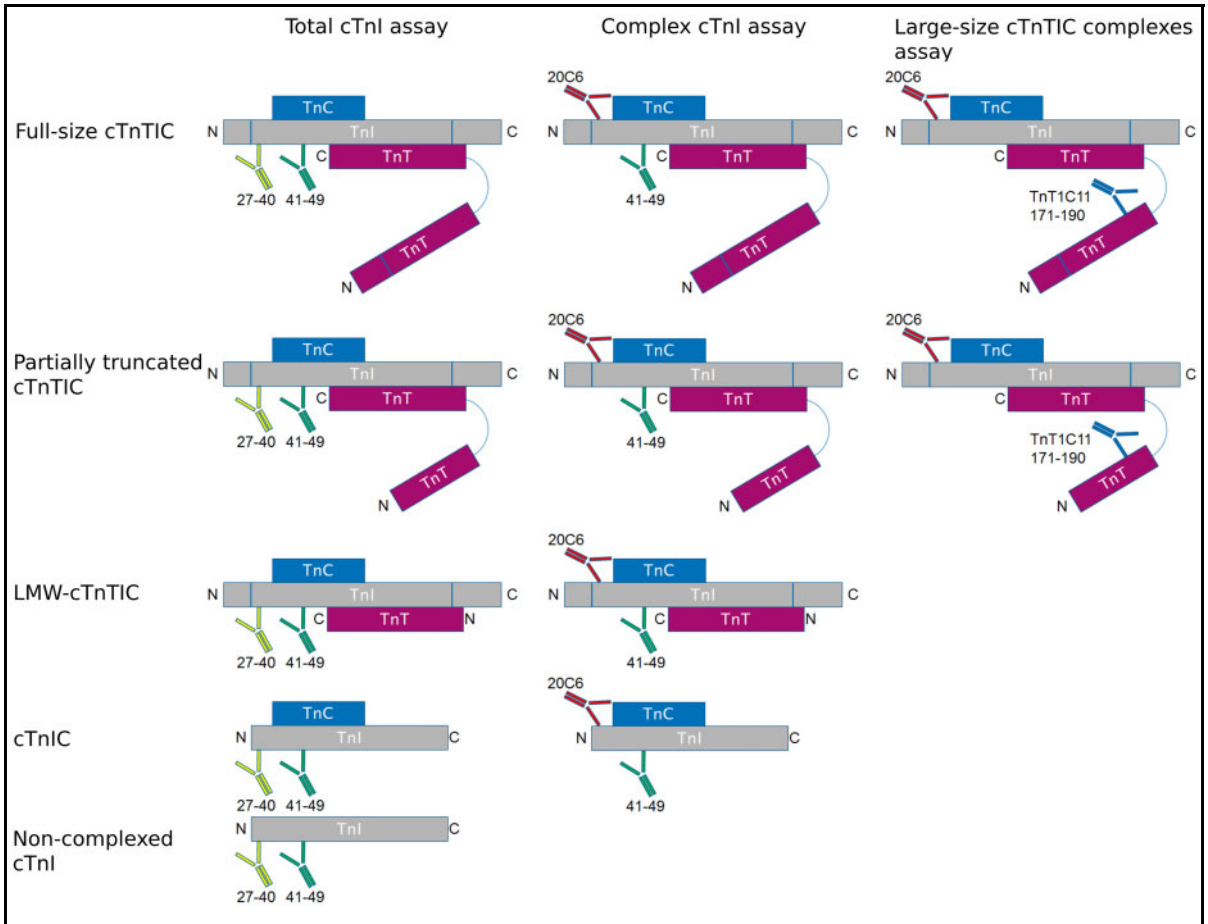

Fig. 1. cTnI forms of interest with the different capture and detection antibodies. Total cTnI: capture cTnI aar 27-40 and detection cTnI aar 41-49. Complex cTnI: capture 20C6 and detection cTnI aar 41-49. Large-size cTnTIC complexes: capture 20C6 and detection TnT1C11 (cTnT aar 171-190). Aar: amino-acid residue; cTn: cardiac troponin; LMW: low molecular weight.

remains undetected. 3) To determine large-size cTnTIC complexes (i.e., full-size cTnTIC and partially truncated cTnTIC) again the Pylon assay analyzer platform was used to combine the capture antibody 20C6 with a detection antibody that binds cTnT amino acid residues 171–190 (i.e., 1C11 from Hytest). The specificity of this cTnT antibody is such that this assay detects cTnTIC complexes with intact cTnT (37 kDa) and its 29 kDa fragment, but not cTnTIC complexes with a smaller-sized 14 kDa cTnT degradation product, previously referred to as LMW cTnTIC (5). Assay performance characteristics have been described elsewhere (16, 17). In addition, assay specificity data are reported in the Supplemental Fig. 2.

CK-MB analysis. Creatinine kinase myocardial brain (CK-MB) was assessed at baseline, and 6 and 12 hours thereafter to assess the severity of myocardial injury. The CK-MB assay was provided to us by Roche Diagnostics, and analyses were performed on a Cobas analyzer (Roche). The CK-MB immunoassay has a lower limit of detection of 0.1 ng/mL. Peak CK-MB was defined as the highest of the three measurements.

STATISTICAL ANALYSIS

Continuous data were analyzed for Gaussian distribution and were expressed as medians with interquartile ranges (IQR). Numerical data were described as a number with a percentage. Paired data were compared using the Wilcoxon signed rank tests or related-samples Friedman's two-way ANOVA tests whenever appropriate. The Mann-Whitney U test was performed for comparisons between unrelated groups. Correlation coefficients between continuous variables were investigated using Spearman's Rho. A *P*-value less than 0.05 was considered statistically significant. All analyses were performed using IBM SPSS Statistics software (version 22.0, IBM Corp).

Results

A total of 45 out of 71 patients that were included in the TRAMICI study qualified for biomarker analyses in the present study (Supplemental Fig. 3). Table 1 shows baseline clinical and angiographic characteristics. The LAD was the culprit lesion in 18 patients, while the other 27 had a non-LAD culprit lesion. The duration between symptom onset and the start of study procedures was 25 [19–36] hours, with a duration between hospitalization and study procedures of 21 [13–27] hours. Peak CK-MB value measured during hospitalization was 10.47 [4.19–19.15] ng/mL.

PERIPHERAL CTNI COMPOSITION

Total cTnI concentration at PVT0 was 612 [203–1666] ng/L, of which 87 [78–100] % was present as complex cTnI and 9 [6–15] % as large-size cTnTIC. Over time, total cTnI concentration showed an increase at PVT6 compared to PVT0 ($P = 0.018$), whereas at PVT12 total cTnI was significantly lower as compared to PVT6 ($P = 0.013$) (Table 2).

As for complex cTnI, in pairwise comparisons absolute median concentrations increased between PVT0 and PVT6 ($P = 0.024$), and were nonsignificantly different between PVT6 and PVT12 ($P = 0.052$) (Table 2). Expressed as a proportion of total cTnI, complex cTnI did not change between the different time points (Fig. 2A).

Absolute median large-size cTnTIC concentrations decreased between PVT6 and PVT12 ($P = 0.001$) (Table 2), as did large-size cTnTIC as a proportion of total cTnI between PVT6 and PVT12 ($P = 0.01$) (Fig. 2B).

CORONARY VENOUS SYSTEM VERSUS PERIPHERAL VENOUS

The absolute median CST0 concentrations of total cTnI, complex cTnI, and large-size cTnTIC were significantly higher as compared to the PVT0 samples

Table 1. Baseline clinical and angiographic characteristics.

	All patients n = 45
Age	65 [54–73]
Male gender	32 (71%)
Smoking	21 (47%)
Hypertension	21 (47%)
Diabetes mellitus	4 (9%)
Hypercholesterolemia	16 (36%)
History of MI	9 (20%)
History of coronary revascularization	5 (11%)
eGFR (mL/min/1.73 m^2)	93 [66–115]
Number of diseased vessels	
1	13 (29%)
2	22 (49%)
3	10 (22%)
Culprit artery	
RCA	11 (24%)
RCX	16 (36%)
LAD	18 (40%)
Severity culprit stenosis	
50-70%	0 (0%)
70-90%	17 (38%)
>90%	28 (62%)

Values are medians (interquartile ranges) or numbers (percentages).

Table 2. Absolute cTnI concentrations for the total population.

N = 45	CST0	PVT0	PVT6	PVT12	*P*-value*	*P*-value†
Total cTnI	1223 [281-2881]	612 [203-1666]	759 [299-2212]	719 [343-1718]	<0.001	0.02
Complex cTnI	950 [238-2065]	631 [166-1337]	653 [276-1649]	674 [325-1386]	<0.001	0.05
Large-size cTnTIC	127 [38-406]	64 [15-144]	67 [20-195]	37 [16-138]	<0.001	0.002

Median concentrations of cTnI and its subforms in ng/L with interquartile ranges.
**P*-value for difference between CST0 and PVT0; †*P*-value for difference between 3 related groups (PVT0, PVT6, and PVT12); comparison between total cTnI at PVT0 and PVT6: $P = 0.018$; between PVT6 and PVT12 $P = 0.013$; comparison of complex cTnI between PVT0 and PVT6 $P = 0.024$, between PVT6 and PVT12 $P = 0.052$; comparison between large-size cTnTIC at PVT0 and PVT6 $P = 0.518$; between PVT6 and PVT12 $P = 0.001$.

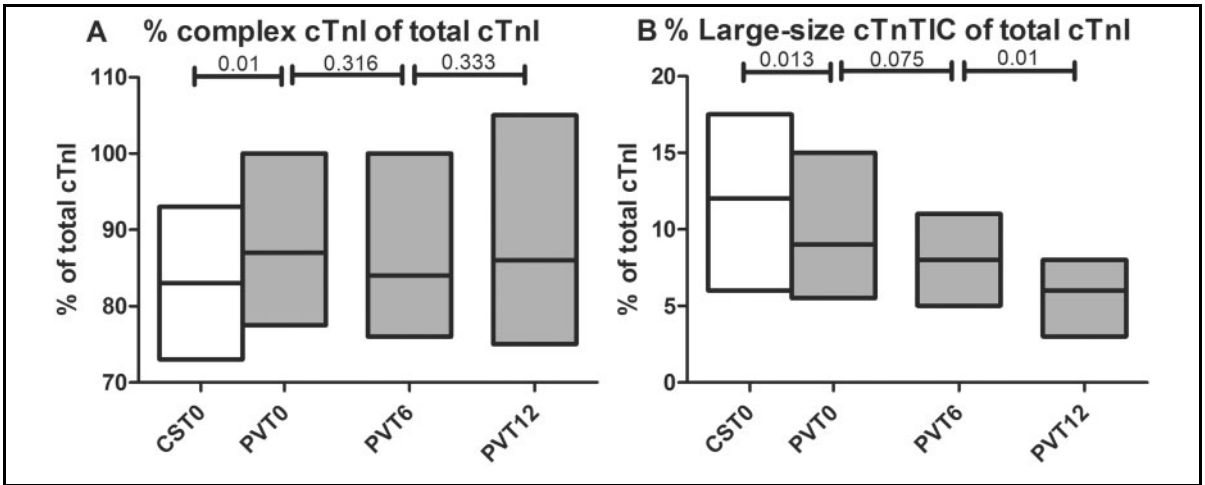

Fig. 2. Percentages of cTnI forms relative to the total cTnI concentration. Median percentages of the different cTnI forms with interquartile ranges. A) Complex cTnI (full-size cTnTIC + partially truncated cTnTIC + low molecular weight cTnTIC + cTnIC) as proportion of total cTnI. B) Large-size cTnTIC (full-size cTnTIC+partially truncated cTnTIC) as proportion of total cTnI.

(all: $P < 0.001$) (Table 2). The relative contribution of complex cTnI as a proportion of total cTnI was higher in PVT0 samples as compared to CST0 samples ($P = 0.01$) (Fig. 2A); for large-size cTnTIC complexes proportions were lower in the PVT0 as compared to CST0 (Fig. 2B).

Figure 3 shows absolute median concentrations in the GCVT0 and CST0 according to localization of the culprit lesion. In patients with an LAD culprit lesion (n = 18, Fig. 3A), absolute concentrations were higher in the GCVT0 for total cTnI, complex cTnI, and large-size cTnTIC complexes as compared to concentrations in the CST0 (all: $P < 0.05$). In patients with non-LAD culprit lesions (n = 27, Fig. 3B), absolute concentrations were higher in the CST0 as compared to concentrations in the GCVT0 (all: $P < 0.01$). As a proportion of total cTnI, proportions of complex cTnI were 81 [74–94]% and 82 [76–96]% ($P = 0.255$) in the GCVT0 and CST0 respectively, in patients with an LAD culprit lesion; for large-size cTnTIC complexes these proportions were 11 [6–14]% and 13 [9–18]% ($P = 0.007$). In patients with non-LAD culprit lesions, the proportions that were observed for complex cTnI were 88 [74–97]% and 84 [72–92]% ($P = 0.06$), and for large-size cTnTIC complexes were 8 [5–18]% and 12 [6–17]% ($P = 0.464$), respectively.

CTNI COMPOSITION IN RELATION TO INJURY SEVERITY AND ISCHEMIC TIME WINDOW

*Injury severity.*Total cTnI concentration was 1666 [886–4004]ng/L in the PVT0 of patients with the highest peak CK-MB levels (upper 50th percentile), and 278

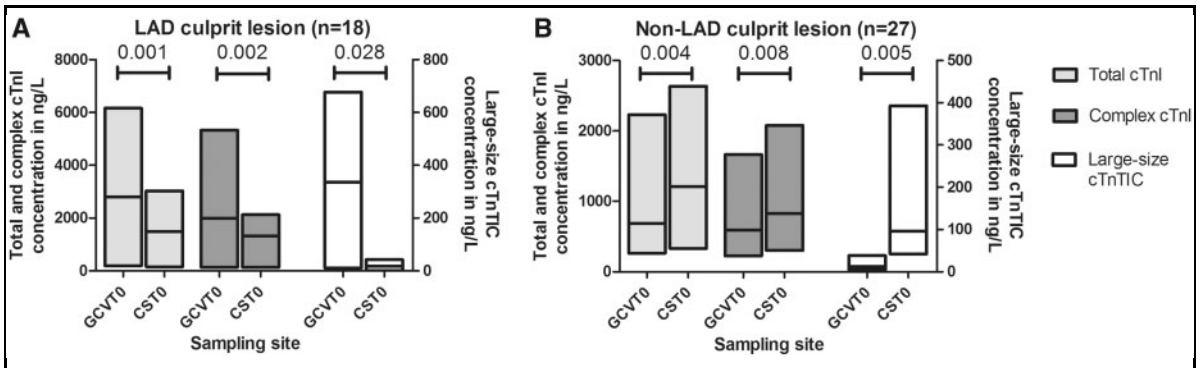

Fig. 3. Absolute cTnI concentrations for patients with a culprit lesion in the LAD or non-LAD. Median concentrations of cTnI and its subforms in ng/L with interquartile ranges. A) LAD culprit lesion. B) non-LAD culprit lesion.

[147–583]ng/L in those with the lowest peak CK-MB levels (lower 50th percentile) ($P < 0.001$). The median proportions of complex cTnI showed no difference between the two groups (Fig. 4A). Proportions of large-size cTnTIC were 14 [9–19]% in the PVT0 of patients with the highest peak CK-MB levels, and 8 [4–10]% in those with the lowest ($P = 0.002$) (Fig. 4B). Differences between these subgroups in proportions of large-size cTnTIC complexes were also observed for GCVT0 and CST0 samples and persisted at PVT6 and PVT12 (Fig. 4B).

Ischemic time window. Of the patients (n = 18) in the lowest 50th percentile of ischemic time window blood sampling occurred at a median of 19 [15–23] hours after symptom onset; for patients (n = 19) in the highest 50th percentile sampling occurred at 35 [29–48] hours after symptom onset. Eight patients were excluded because of uncertainty regarding time of symptom onset. Total cTnI concentration in the PVT0 was 1118 [441–3002]ng/L in patients with the lowest 50th percentile time intervals, and 583 [147–1228]ng/L in those with the highest ($P = 0.210$). The median proportions of complex cTnI showed no difference between patients sampled 'early' or 'late' (Fig. 4C). Proportions of large-size cTnTIC complexes were 14 [10–19]% in the PVT0 of patients with the shortest time intervals, and 8 [5–11]% in those with the longest ($P = 0.002$) (Fig. 4D). Similar differences in proportions of large-size cTnTIC complexes were observed for GCVT0 and CST0 samples (Fig. 4D).

The observed higher proportions of large-size cTnTIC complexes at PVT0 in the group with the shorter ischemic time window was regardless of the severity of injury (15% in smaller MIs, 17% in larger MIs). At PVT6 and PVT12, the difference in proportions of large-size cTnTIC complex in relation to the ischemic time interval was no longer present.

Discussion

The present multi-site coronary venous and peripheral sampling study addresses the cTn composition in a large well-described series of NSTEMI patients. Using a highly sensitive immunoassay platform, enabled determination of total cTnI, complex cTnI and, as a separate measurement, large-size cTnTIC complexes. In coronary veins draining the infarct area, concentrations of the large-size cTnTIC complexes, and also of complex cTnI, were higher than in the peripheral circulation. Together with the marked differences observed within the CVS, this indicates that observed degraded forms in the peripheral circulation are not merely the result of peripheral breakdown. In addition, proportions of large-size cTnTIC were higher in patients with shorter ischemic time windows, and higher CK-MB peaks.

Collectively, these findings may contribute to future work on characterization of disease status in NSTEMI patients according to differences with regard to cTn composition.

Until now it has remained uncertain where cTn is transformed after cell injury occurs. The only evidence of intracellular degradation has been derived from a few small-sized studies (15, 19–22). In line with intracellular degradation, several intracellular proteases have been implicated to selectively proteolyze cTnI and cTnT (23–27). Recently, in a pilot study on two NSTEMI patients, concentrations of intact cTnT, but also of the 29 kDa and smaller 15–18 kDa fragments were higher in the CVS than in the peripheral circulation (10). The current analysis of a larger TRAMICI cohort showed similar results. Higher concentrations of total cTnI, complex cTnI, and large-size cTnTIC complexes were observed in the CVS. Additionally, considering the marked differences within the CVS, the highest

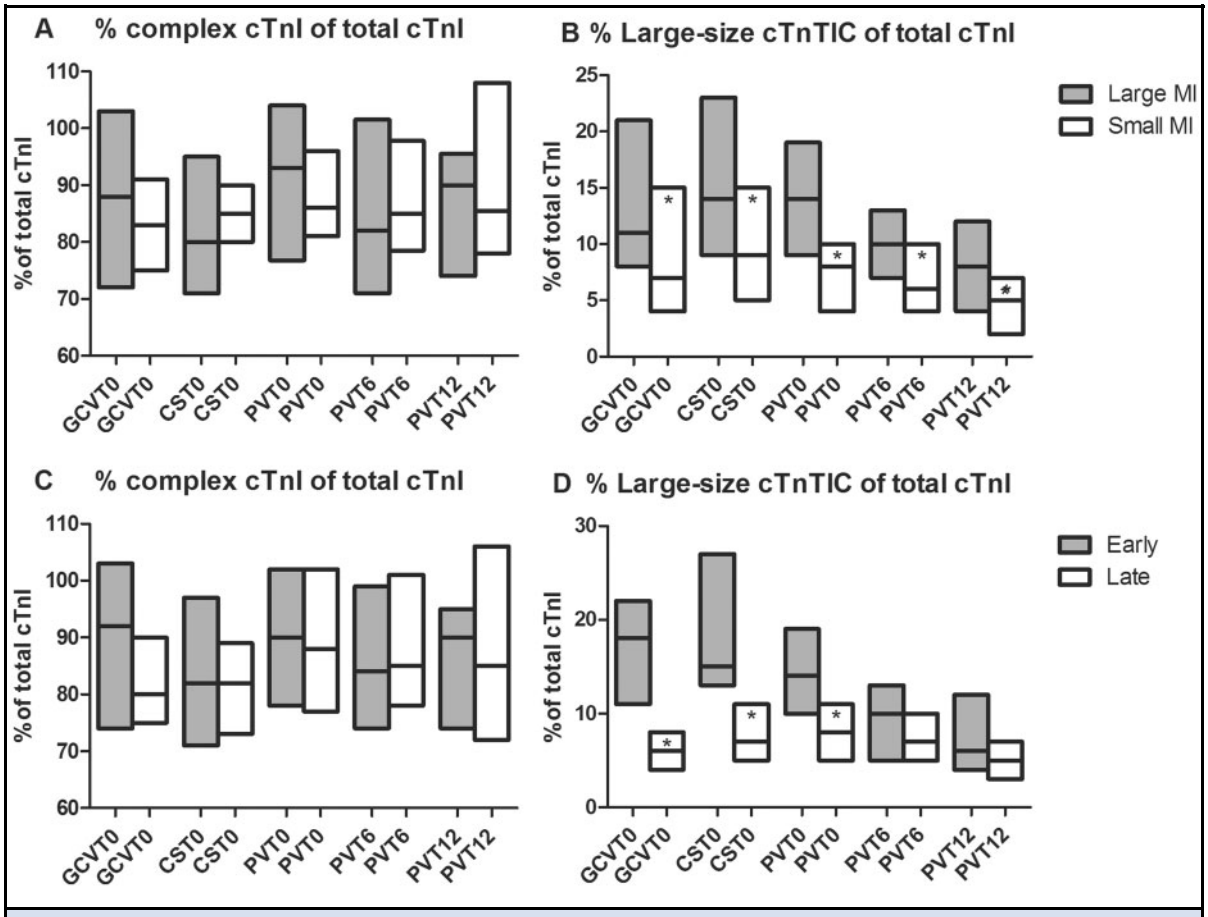

Fig. 4. Percentages of cTnI forms relative to the total cTnI concentration according to severity of injury and ischemic time interval. Median percentage of the different cTnI forms with interquartile ranges. *$P<0.05$ for differences between large and small; and early and late. A-B) Small MI vs. large MI: patients divided based on peak CK-MB levels in small MI (peak CK-MB lower 50th percentile) and large MI (peak CK-MB upper 50th percentile) C-D) Early vs. late blood sampling: based on time interval between symptom onset and blood sampling; early time intervals: lower 50th percentile; late time intervals: upper 50th percentile.

concentrations were observed at the site closest to the injured myocardium. Together with the fact that complex cTnI and large-size cTnTIC complexes as proportions of total cTnI remained relatively constant between the GCVT0 and CST0 samples, our observations are supportive of intracellular breakdown of cTnTIC complexes prior to release. In a study on cTnI degradation in post-MI patients, a similar conclusion was drawn based on ratios of full-size cTnI and its fragments, which remained unchanged over time (28).

Nonetheless, we recognize that part of cTn degradation may also have occurred in the circulation. As previously shown, thrombin acts as a proteolytic enzyme capable of cleaving cTnT at the same amino acid residues (R68/S69) as intracellular μ-calpain (29–31). With increased thrombin activity in NSTEMI patients, the chance of cTn degradation inside the circulation is substantial. Moreover, as observed in the present study, peripheral blood proportionally contained more complex cTnI, and less large-size cTnTIC than CVS samples. This finding may be due to peripheral conversion of large-size cTnTIC into smaller-sized complexes. Finally, as previously discussed, the effect of thrombin may also have occurred preanalytically during serum production (10, 29). Appreciating that this potential effect applies to both CVS and PV samples, within patient differences were not likely affected.

Differential cTn patterns were found previously, which appeared related to the underlying cardiac condition. First, with increasing injury severity cTnI and cTnT degradation appeared to be more extensive (9, 13–15). Second, with longer time-intervals between

onset of ischemia and cTn assessment, larger cTn fragments predominated in early samples, whereas smaller-sized fragments were seen later in the MI aftermath (5, 9–12, 14). In two studies which focused on cTn while in complex with other isoforms, large-size cTnTIC was more prominent in early stages, and upon disease progression more cTnIC complexes were seen (5, 6). As was suggested, disintegration of complexes and degradation of cTnI and cTnT into fragments are regarded as parallel processes. Troponins are bound in a complex while cleaved by proteases. With ongoing ischemia, cTn fragments will dissociate, leaving smaller sized complexes behind. When these steps occur in a time-dependent manner, large-size forms might reflect an earlier stage of disease.

In keeping with abovementioned considerations, we observed higher proportions of large-size cTnTIC in patients with a shorter ischemic time window.

As for the injury severity, we observed higher large-size cTnTIC complex proportions in the patients with the higher peak CK-MB levels. Exploratory analyses on the small subgroups showed that higher CK-MB levels were correlated with shorter ischemic time intervals, which may be related to a higher symptom burden in case of more severe injury.

Despite a median ischemic time window of more than a day, we still observed differential release of cTn according to ischemic time window and sustained injury severity. In case of earlier sampling, especially in large-size infarctions, in which the enzymatic capacity might be inadequate to cause overall degradation, higher large-size cTnTIC proportions might be expected.

Implications

In this study, we have provided arguments that cTn composition can be linked to the area of diseased myocardium. As we also linked cTn forms to clinical variables such as the ischemic time window and injury severity, a first initiative is presented that underscores the potential of cTn form status as a direct image of underlying disease status in NSTEMI patients. Currently, regular cTn measurements are highly sensitive, but cannot differentiate between NSTEMI patients. Development of an immunoassay that allows clinical profiling according to cTn characteristics, could ultimately identify NSTEMI patients with 'early' or 'late' damage with potential impact on timely treatment, and prognosis.

Also, an assay with the capacity to differentiate between cTn forms might improve specificity in another way. As shown in recent studies on patients with end-stage renal disease and healthy marathon runners, cTnT consisted of small-size fragments of cTnT (32–34). In this respect, a newly designed assay with a focus on cTn forms might importantly differentiate between cTn levels due to a type I MI or those seen in other etiologies.

Limitations

We lacked a direct measure of noncomplex cTnI forms (i.e., free noncomplex cTnI forms). Consequently, the relative contribution of free cTnI and fragments to the total composition has not been determined. Given the fact that at PVT0 87% of total cTnI is considered complex, automatically noncomplex will be 13% of total.

In terms of generalizability, this study only relates to NSTEMI patients, as patients with ST-elevation myocardial infarction were excluded, for reasons mentioned before. With cardiac catheterization performed at a median of 25 hours after symptom onset, cTn composition in the earliest phase after symptom onset has not been assessed and could prove different with respect to the ratio of complex and noncomplex forms to total cTn.

With the use of the current assays with improved sensitivity, we were able to study patients with a much smaller infarct-size than in previous reports on this subject (1–4). However, our study did not include patients with cTn concentrations just above the 99th percentile reference limit, and findings can therefore not be inferred to the smallest forms of injury in NSTEMI.

We acknowledge that in addition to our specificity data (Supplemental materials) we should preferably have included analyses on partially truncated- and LMW cTnTIC. However, these cTn complexes are not commercially available. We therefore relied on the recent publication from Vylegzhanina that unmistakably showed the presence of these forms using very similar antibodies to the ones used in the present manuscript (5).

Conclusion

In NSTEMI patients, concentration differences of complex cTnI and large-size cTnTIC within the CVS, and between the CVS and peripheral samples provide evidence of linkage of post-MI composition of cTn forms and the area of diseased myocardium. In addition, the observed differences in cTnI composition with respect to the time passed since symptom onset and extent of sustained injury suggest that, at least partially, cTn composition reflects disease status of NSTEMI patients. This characterization may improve biomarker diagnostics and facilitate differentiation between NSTEMI patients, and those with other etiologies, that may cause release of cTn.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: cTn, cardiac troponin; cTnT, cardiac troponin T; cTnI, cardiac troponin I; TnC, noncardiospecific troponin C; MI, myocardial infarction; cTnTIC: ternary cTnI-cTnT-TnC complex; CVS: coronary venous system; NSTEMI: non-ST elevation myocardial infarction; LMW cTnTIC: low molecular weight ternary cTnTIC complex; cTnIC: binary cTnI-TnC complex; LAD: left anterior descending coronary artery; TRAMICI: TRanscardiac Assessment of Myocardial Injury and Coronary Inflammation; CAG: coronary angiography; PCI: percutaneous coronary intervention; eGFR: estimated glomerular filtration rate; CS: coronary sinus; GCV: great cardiac vein; CST0: coronary sinus at baseline; GCVT0: great cardiac vein at baseline; PV: peripheral vein; PVT0: peripheral vein at baseline; PVT6: peripheral vein 6 hours after baseline; PVT12: peripheral vein12 hours after baseline; CK-MB: creatinine kinases myocardial brain; RCX: circumflex coronary artery; RCA: right coronary artery.

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