ABSTRACT

Introduction: Release kinetics of high-sensitivity cardiac troponin (hs-cTn) T and I in patients with acute myocardial infarction (AMI) are incompletely understood.

Objective: We aim to assess whether hs-cTnT/I release in early AMI is near-linear.

Methods and results: In a prospective diagnostic multicenter study the acute release of hs-cTnT and hs-cTnI within 1h and 2h from presentation to the emergency department was quantified using three hs-cTnT/I assays in patients with suspected AMI. Primary endpoint was correlation between hs-cTn changes from presentation to 1h versus changes from presentation to 2h, among all AMI patients and different pre-specified subgroups. Final diagnosis was adjudicated by two independent cardiologists, based on serial hs-cTnT from the serial study blood samples and additionally locally measured (hs)-cTn values.

Among 2437 patients with complete hs-cTnT data, AMI was the adjudicated diagnosis in 376 patients (15%). The correlation coefficient between 0/1h-change and 0/2h-change was 0.931 (95%CI 0.916-0.944), p<0.001. Similar findings were obtained with hs-cTnI (Architect) with correlation coefficients between 0/1h-change and 0/2h-change of 0.969 and hs-cTnI (Centaur) of 0.934 (p<0.001 for both). Findings were consistent among Type 1 and type 2 AMI and in the subgroup of patients presenting very early after chest pain onset.

Conclusions: Patients presenting with early AMI show a near-linear release of hs-cTnT and hs-cTnI. This near-linearity provides the pathophysiological basis for rapid diagnostic algorithms using 0/1h-changes as surrogates for 0/2h or 0/3h-changes.

Key words: cardiac troponin release, Acute Myocardial Infarction, linear release kinetics.

INTRODUCTION

Patients with symptoms suggestive of acute myocardial infarction (AMI) account for about 10% of all emergency department (ED) consultations.[1] ECG and cardiac troponin (cTn) T and I form the diagnostic cornerstones and complement clinical assessment.[2, 3]

Recently developed high-sensitivity (hs) cTn assays provide a new non-invasive window to the heart and enable precise measurements of cTnT and cTnI blood concentrations around the 99th percentile of healthy individuals and even within the normal range, which was not possible with prior generations of tests.[4-8] Their analytical superiority translated into clinical superiority in the early diagnosis of AMI.[4-10] However, their analytical kinetics are not completely understood yet.

Pilot data generated in an experimental AMI-model (alcohol septal ablation) and a registry provided first hints that cTnT may be released in a near-linear fashion in early AMI.[11] Obtaining insights into the release kinetics of cTnT and cTnI from cardiomyocytes into the circulation within the first hours of AMI would contribute to advancing our understanding of AMI pathophysiology including the temporal relationship between symptom onset and the development of cardiomyocyte necrosis and possible also help in the clinical implementation and adoption of recently developed rapid hs-cTn-algorithms.[12-20] We therefore aimed to test the hypothesis that cTn release in early AMI is near-linear in a large multicenter study.

METHODS

Study design and patient population

XXX2 is an ongoing prospective international diagnostic multicenter study enrolling patients in 12 centres in 5 European countries (Switzerland, Spain, Italy, Poland, Czech Republic).[5, 21, 22] The aim of XXX2 is to contribute to advancing the early diagnosis of AMI. Adult patients presenting to the ED with symptoms suggestive of AMI with an onset or peak of those within the last 12 hours were enrolled after written informed consent was obtained.

Enrollment was independent from renal function, while patients with terminal kidney failure on chronic dialysis were excluded. For this analysis, patients were also excluded if A) measurements at presentation, after 1h, or after 2h were not available for the respective hs-cTn assay investigated, or B) the final diagnosis remained unclear even after adjudication and at least one hs-cTnT concentrations was elevated (possibly indicating the presence of AMI). C) the final diagnosis was other than AMI. Because some patients had missing data for some of the three investigational hs-cTn assays, three assay-specific sub-cohorts with a large overlap but numerically not identical sizes were derived from the main cohort.

The study was carried out according to the principles of the Declaration of Helsinki and approved by the local ethics committees. The authors designed the study, gathered, and analysed the data according to the STARD guidelines[23, 24] for studies of diagnostic accuracy (only-online supplement), vouch for the data and analysis, wrote the paper, and decided to publish.

Routine clinical assessment

All patients underwent a clinical assessment that included medical history, physical examination, 12-lead ECG, continuous rhythm monitoring, pulse oximetry, standard blood test, and chest radiography, if indicated. Concentrations of cTn were measured at presentation and serially thereafter as long as clinically indicated. Treatment of patients including drug therapy was left to the discretion of the attending physician.

Adjudication of the final diagnosis

Adjudication of the final diagnosis was performed centrally in a core lab (University Hospital Basel) for all patients. Two cardiologists reviewed all available medical records - patient history, physical examination, results of laboratory testing, radiologic testing, ECG, echocardiography, cardiac exercise test, lesion severity and morphology in coronary angiography, if available - pertaining to the patient from the time of ED presentation to 90-day follow up. In order to take advantage of the higher sensitivity and higher overall diagnostic accuracy offered by hs-cTn [12, 22, 25], the adjudication was mainly based on serial hs-cTnT measured in a central laboratory from the serial study blood samples, additionally the adjudicating cardiologists had access to serial concentrations of cTn/hs-cTn measured locally as part of routine clinical care. In situations of disagreement about the diagnosis, cases were reviewed and adjudicated in conjunction with a third cardiologist (approximately in 10% of all patients).

AMI was defined and cTn concentrations interpreted using a uniform cut-off value (the 99th percentile) as recommended in current guidelines and as done in the vast majority of contemporary large diagnostic studies [2, 3,26]. In brief, AMI was diagnosed when there was evidence of myocardial necrosis in association with a clinical setting

consistent with myocardial ischemia. Myocardial necrosis was diagnosed by at least one hs-cTnT value above the uniform 99th percentile, 14ng/L (for women and men) together with a significant rising and/or falling.[3] Absolute changes in hs-cTnT were used to determine significant changes based on the diagnostic superiority of absolute over relative changes.[21, 27] Based on studies of the biological variation of cTn[28] as well as on data from previous chest pain cohort studies [29, 30], a significant absolute change was defined as a rise or fall of at least 10ng/L within six hours or 6ng/L within three hours. .[5, 21, 22]

Patients with AMI were further subdivided into type 1 AMI (primary coronary events) and type 2 AMI (ischemia due to increased demand or decreased supply, for example tachyarrhythmia or hypertensive crisis).[2, 30]

Measurement of hs-cTn

Blood samples for determination of the three hs-cTnT/I assays (hs-cTnT (Elecsys, Roche), hs-cTnI (Architect, Abbott), hs-cTnI (Centaur, Siemens)) were collected at presentation to the ED, after 1h, after 2h and after 3h in serum or plasma tubes during recruitment period (from April 2006 to August 2015) in all centers. Serial sampling was discontinued when the diagnosis was clear and required transfer e.g. to the catheter laboratory or coronary care unit. In addition, serial sampling had to be interrupted at the time of other diagnostic procedures, which required patient transfer to a different unit within the hospital, e.g. for CT-scans. After centrifugation, samples were frozen at -80°C until assayed in a blinded fashion in a dedicated core laboratory. Analytical details of the three hs-cTnT/I assays are described in the online supplement.

Primary and secondary endpoints

The primary objective was to evaluate the possible near-linearity of the acute release of hs-cTnT and hs-cTnI from cardiomyocytes into the circulation as quantified by acute changes of hs-cTnT and hs-cTnI concentrations in patients with early AMI. Changes from presentation to 1h (delta 0h-1h) were compared to the changes from presentation to 2h (delta 0h-2h) with three hs-cTnT/I assays.

The secondary objective was to compare the deltas 0h-1h versus 0h-3h to extend the observation to the first 3h in the ED. Predefined subgroups included patients with NSTEMI, as the clinical implications of the linearity might be most profound in this population, type 1 AMI, type 2 AMI, patients presenting very early (within the first 2h) after chest pain onset, as the release of hs-cTnT and hs-cTnI may be delayed for some time. Another subgroup included patients with total or subtotal coronary occlusion (culprit lesion severity 95% to 100%), as severely impaired (or even absent) coronary artery blood flow to the necrotic cardiomyocytes may impact on release kinetics. The identification of the culprit lesion was left to the discretion of the attending physician.

Statistical analysis

To assess linearity, we performed linear regressions evaluating the correlation between both deltas (delta 0h-1h: changes from presentation to 1h; vs. delta 0h-2h: changes from presentation to 2h) with the correlation coefficients and slopes. Alternative analyses excluding outliers were also performed, showing no significant difference. A supplementary slope analysis was conducted to confirm near-linearity: Values at time points 1 and 2h were normed to the values at time point 0h, and the linear regression slopes between 0-1h and 1-2h were plotted together and graphically compared with the linear regression slope between 0-2h. No formal sample

size calculation was performed. A minimum a sample size within all assays was not predetermined because the main goal was to show real-world data for the implementation and monitoring of hs-cTn in a dynamic cohort and to provide a consistent internal validity within different assays.

Continuous variables are presented as median with inter quartile ranges (IQR), categorical variables as numbers and percentages. All hypothesis testing was two-tailed and p-value of less than 0.05 was considered statistically significant. All statistical analyses were performed using SPSS for Windows 25.0 (SPSS Inc, Chicago, IL) and MedCalc 9.6.4.0 (MedCalc software, Mariakerke Belgium)

RESULTS

Characteristics of the patients

Patient flow is shown in Figure 1S. Overall, 2437 patients had hs-cTnT concentrations available at presentation, after 1h and after 2h, 376 had a final diagnosis of AMI. Baseline characteristics were similar among the cohorts underlying the analyses of the three different hs-cTn assays (Table 1): median age was 62 years, about one-third of patients were women and about one-third had known coronary artery disease.

Acute hs-cTn-changes in patients with AMI

The adjudicated final diagnosis was AMI in 15% of patients in the analysis of hs-cTnT, hs-cTnI Architect and hs-cTnI Centaur. As shown in Figure 1, among all three assays changes between 0h-1h correlated strongly with changes between 0h-2h with correlation coefficients ranging from 0.931 to 0.969 (p<0.001 for all assays). Additionally, Figure 2 shows supplementary slope analysis. For each assay, the slopes 0-1h and 1-2h were comparable with the slopes 0-2h, as given by overlapping confidence intervals of the linear regressions.

Acute hs-cTn-changes among different AMI subgroups

Findings in patients with **NSTEMI** were comparable to the overall AMI cohort **(Figure 3)** with correlation coefficients ranging from 0.862 to 0.966 (p<0.001 for all assays). Similarly, findings in patients with **type 1 AMI** were comparable to **type 2** (Online Supplement, Figures 2S and 3S). Results were comparable among all patients irrespective of the time since **chest pain onset** (Figure 4, Online Supplement Figure 4S) Correlations between 0h-1h changes and 0h-3h changes were similar to the

correlations observed between 0h-1h changes and 0h-2h changes (Online Supplement, Figure 5S).

Acute hs-cTn-changes according to the severity of the culprit lesion

In the hs-cTnT dataset, coronary angiography was performed in 281 AMI patients. Among these patients, 27% had total coronary occlusion (100% diameter stenosis), 61% had a severe coronary culprit lesion stenosis (75-99% diameter stenosis) and 12% had a less severe coronary culprit stenosis (<75% diameter stenosis). The correlation between changes between 0h-1h and changes between 0h-2h was similar among all patients independently of the stenosis severity (Figure 1D). Similar results were observed for the two hs-cTnI assays (Online Supplement, Figure 6S).

DISCUSSION

This study was performed to contribute to a better understanding of acute release kinetics of hs-cTnT and hs-cTnI from cardiomyocytes into the circulation within the first hours of spontaneous AMI. It was our hypothesis that hs-cTnT and hs-cTnI release is near-linear in these patients. We report two major findings.

First, in patients with AMI (including NSTEMI and STEMI) acute 0/1h-changes correlated very closely with 0/2h-changes and with 0/3h-changes. Additional slope analysis, where slopes 0-1h and 1-2h were comparable with the slopes 0-2 h, confirmed the close correlations. These findings were highly consistent with all three hs-cTn assays and confirmed the hypothesis of a near-linear release of hs-cTnT and hs-cTnI within the first hours of AMI. Second, release of hs-cTnT and hs-cTnI was near-linear also in all pre-defined subgroups including NSTEMI, type 1 and type 2 AMI, patients presenting very early after symptom onset, patients with total/subtotal coronary occlusion.

These findings extend and corroborate previous observations, particularly those made from several experimental models that at least partly reflect the pathophysiology of spontaneous AMI in humans.[11, 31, 32] After transcoronary alcohol ablation of septal hypertrophy, a combination of toxic and ischemic damage induced by temporary septal branch occlusion for selective therapeutic injection of 96% ethanol in 21 patients with hypertrophic obstructive cardiomyopathy, hs-cTnT plasma concentrations showed a near-linear increase.[11] Assessing graded duration of acute coronary ischemia with ST depression versus release of cTnI using a conventional assay in 15 ischemic pigs, cTnI increased from 0.05 ug/L to 0.52 ug/L and 0.76 ug/L (P<0.05) with 10 and 20 min of ischemia, and to 30.77 ug/L (P<0.05) with 30 min of ischemia.[32] In 452 patients with NSTEMI, hs-cTnT plasma concentrations showed a near-linear increase with

increasing time from symptom onset within the first hours after arrival to the ED and then they plateaued.[10]

Recently, several distinct cellular mechanisms other than necrosis have been described to possibly result in hs-cTnT and hs-cTnI release from injured cardiomyocytes.[33] These include apoptosis, transient increases in cell permeability due to cell wounds, formation and the release from membranous blebs or microparticles.[33] In addition, experimental evidence suggests that first, cellular homeostasis may require an active transport of hs-cTnT and hs-cTnI back into the cardiomyocytes, and second, that clearance mechanisms might differ between hs-cTnT and hs-cTnI.[34] Putting these observations into perspective with our findings of consistent release kinetics among different AMI phenotypes, different time intervals, different coronary lesion morphology, and among three different hs-cTn assays, it seems appropriated to conclude the dominant mechanism seems to be identical for hs-cTnT and hs-cTnI in early AMI. These insights into AMI pathophysiology may contribute to advancing our understanding of the temporal relationship between symptom onset and the development of cardiomyocyte necrosis. They seem to support the extension of the "time is muscle" concept from the treatment of patients with STEMI to also patients with NSTEMI. This has fundamental consequences and implies the need for very early and accurate diagnosis. Recently developed rapid hs-cTn-algorithms[12-17] for sure will have a major role in this. In addition, these novel insights into AMI pathophysiology might also help in the clinical implementation and adoption of the rapid hs-cTn-algorithms, which use hs-cTn changes occurring within the first hour after ED presentation as surrogates for hs-cTn changes occurring within 3 hours or 6 hours.[12-15, 17]

It is important to highlight that our findings apply to early AMI, but not to the late phase of AMI, e.g. 12h to 48h after symptom onset, as blood concentrations of hs-cTnT and hs-cTnI plateau in the late phase of AMI and then fall again.(10,11) While the vast majority of patients with AMI present within the first 12h after chest pain onset, the inclusion criteria of this study, clinicians must appreciate the fundamental differences in release kinetics in late presenters. In these, concentrations at presentation usually are already markedly elevated, but do not show a relevant short-term change during serial sampling in the ED.

LIMITATIONS

Some limitations merit consideration when interpreting our findings. First, this study analyzed the near-linearity of the acute release of hs-cTn using three hs-cTn assays. As findings were consistent among the 3 different assays, we assume that it can be generalized to hs-cTnT/I in general. Of course, this assumption needs to be confirmed in additional studies. Second, we cannot comment on release kinetics in patients with terminal kidney failure on chronic dialysis, since these patients were excluded from our study. Third, as a cohort of patients presenting with suspected AMI to the ED, our dataset has an underrepresentation of patients with STEMI, as these patients often are identified by the 12-lead ECG in the ambulance and brought directly to the catheter laboratory, bypassing the ED. Therefore, the number of STEMIs was low in this cohort. Forth, we could not asses release kinetics in patients in whom serial sampling was discontinued, because their diagnosis was clear and/or they required early transfer e.g. to the catheter laboratory or coronary care unit. As the diagnosis was clear at an even earlier time point in these patients, it is unlikely that release kinetics would have been different than observed in this study. Fifth, these analyses are specific to patients presenting within 12h after symptom onset or peak. While the majority of patients with AMI present within this time window, late

presenters represent an important minority. Usually, these patients have significant elevations in hs-cTn already at the first blood draw, helping their identification as AMI. Sixth, the documented near-linearity of hs-cTnT and hs-cTnI release in early AMI does not allow to delineate necrosis as the exclusive mechanisms involved. Seventh, since the results are based on an observational design, they only provide empirical evidence of the near-linear release of hs-cTn the first hours after myocardial ischemia, but they do not allow to demonstrate the causal nature of this fact. Eighth, contrarily to the adjudication of final diagnoses, the interpretation of coronary angiograms and hence of the stenosis grade was performed by the treating physician and not centrally.

CONCLUSION

In conclusion, patients presenting with suspected AMI to the ED show a near-linear release of hs-cTnT and hs-cTnI. Given the consistency of these findings among different AMI phenotypes, different time intervals, different coronary lesion morphology, and among three different hs-cTn assays, these results provide empirical evidence that the near-linearity of release in early AMI applies to hs-cTnT and hs-cTnI in general. This near-linearity provides the pathophysiological basis for rapid diagnostic algorithms and support for extending the concept of "time is muscle" to NSTEMI.

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FIGURE LEGENDS

Figure 1	Correlation between 0h-1h changes and 0h-2h changes in AMI patients
Scatter plots showing the association between Delta 0h-1h and 0h-2h of hs-cTn	

Figure 2	Linearity between 0h-1h and 2h values of hs-cTn in AMI patients
Slopes analyses showing the near-linearity between 0h-1h and 2h of hs-cTn	

Figure 3	Correlation between 0h-1h changes and 0h-2h changes in NSTEMI patients
Scatter plots showing the association between Delta 0h-1h and 0h-2h of hs-cTn	

Figure 4	Correlation between 0h-1h changes and 0h-2h changes in AMI patients according to chest pain onset
Scatter plots showing the association between Delta 0h-1h and 0h-2h of hs-cTnT among AMI patients according to time since chest pain onset in AMI patients	

Figure 5	Correlation between 0h-1h changes and 0h-2h changes in AMI patients according to stenosis grade
Scatter plots showing the association between Delta 0h-1h and 0h-2h of hs-cTnT among AMI patients according to stenosis grade of the culprit lesion	

TABLES

Table 1 Baseline Characteristics	hs-cTnT (n=376)	hs-cTnI Architect (n=338)	hs-cTnI Centaur (n=243)
Male no. (%)			
	279 (74)	249 (74)	177 (73)
Age, y			
Median	71	71	70
IQR	(58-81)	(59-81)	(59-80)
Risk Factors, no. (%)			
Hypertension	283 (75)	261 (77)	187 (77)
Hypercholesterolemia	249 (66)	226 (67)	166 (68)
Diabetes	103 (27)	98 (29)	64 (26)
Smoking	258 (69)	231 (68)	166 (69)
Family history	152 (40)	141 (42)	107 (44)
History, no. (%)			
Coronary artery disease	169 (45)	156 (46)	118 (49)
Previous AMI	127 (34)	118 (35)	87 (36)
Previous revascularization	130 (35)	118 (35)	90 (37)
Peripheral artery disease	46 (12)	42 (12)	32 (13)
Previous stroke	38 (10)	35 (10)	18 (7)
ECG findings, no. (%)			
New left bundle branch block	23 (6)	18 (5)	14 (6)
ST-segment elevation	19 (5)	15 (4)	8 (3)
ST-segment depression	75 (20)	79 (23)	55 (23)
T-wave inversion	41 (11)	39 (12)	30 (12)
No significant changes	218 (58)	194 (57)	136 (56)
Body-mass index (kg/m2)			
Median	26	26	26
IQR	(24-29)	(24-29)	(24-29)
eGFR			
Median	76	76	74
IQR	(60-100)	(60-96)	(60-93)
Medication at presentation			
ASA	181 (48)	172 (51)	118 (49)
Vitamin K antagonists	43 (11)	36 (11)	29 (12)
B-blockers	155 (41)	144 (43)	104 (43)
Statins	166 (44)	150 (44)	108 (44)
ACEIs/ARBs	196 (52)	180 (53)	132 (54)
Calcium Antagonists	85 (23)	77 (23)	55 (23)
Nitrates	78 (21)	73 (22)	108 (44)

hs- cTn indicates high sensitivity cardiac troponin; AMI: Acute Myocardial Infarction; ECG, Electrocardiogram; eGFR, estimated Glomerular Filtration Rate; ASA: Acetyl Salicylic Acid; ACEI, Angiotensin Converting Enzyme Inhibitor; ARB, Angiotensin Receptor Blocker. Values are expressed in percentage or medians ± Inter Quartile Ranges (IQR) * experimental assay

1 **FIGURES**

2 <mark>**Figure 1**</mark>
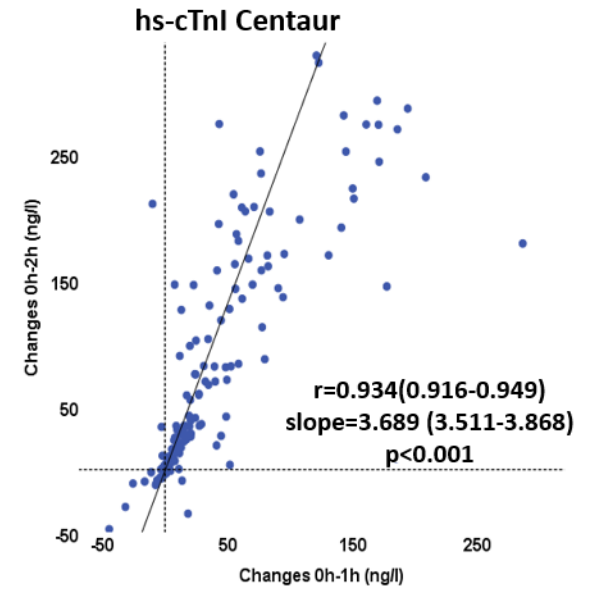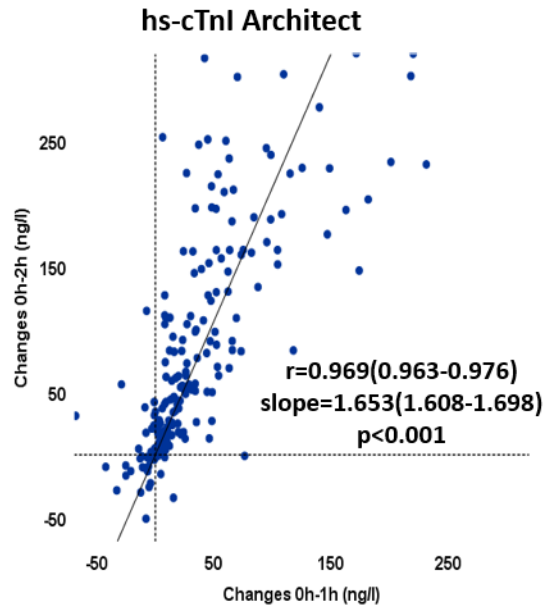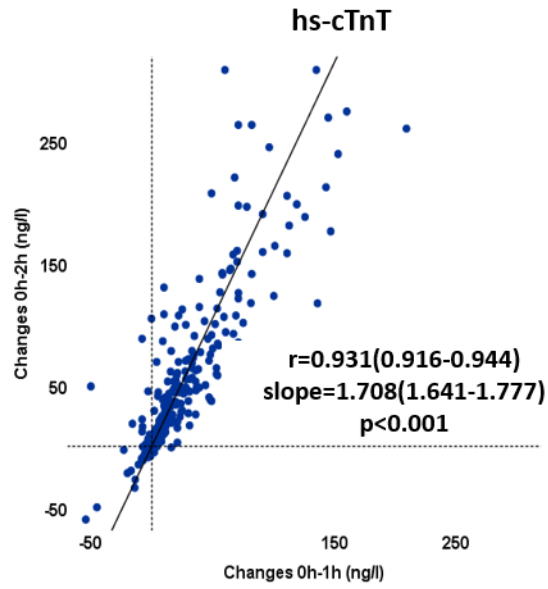
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4 **N=376**   **N=338**   **N=243**

5
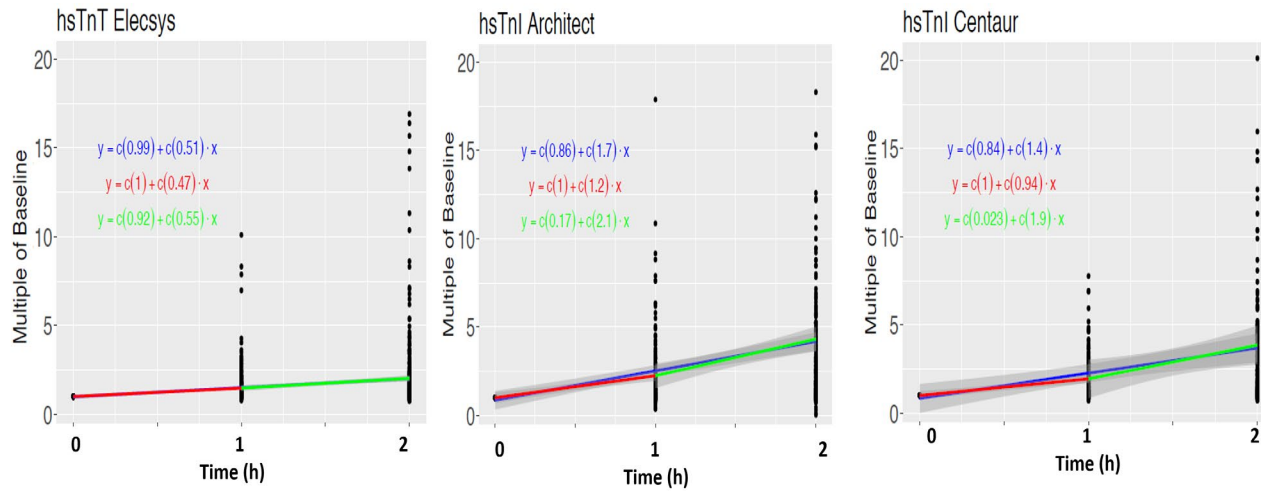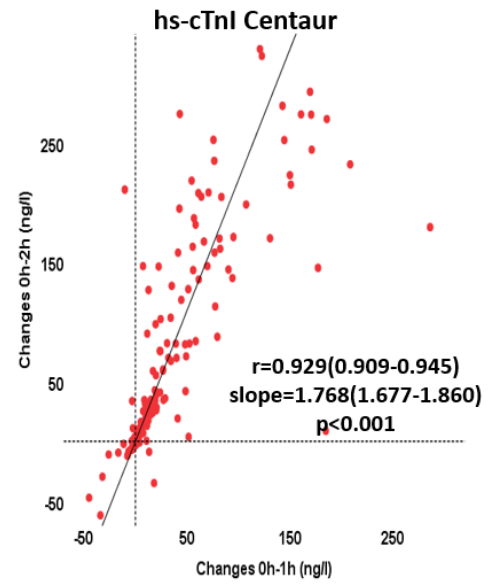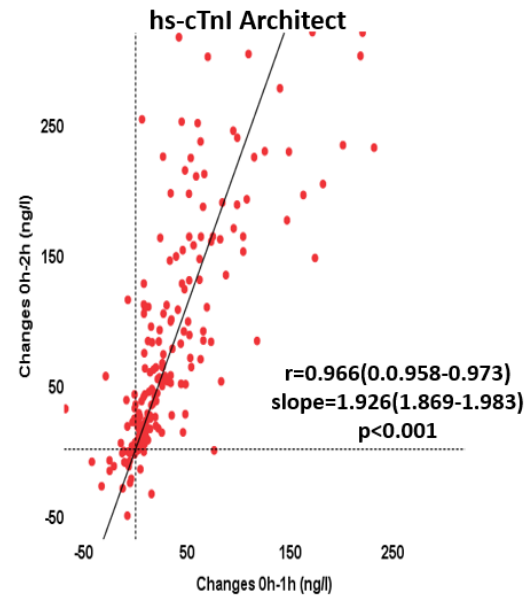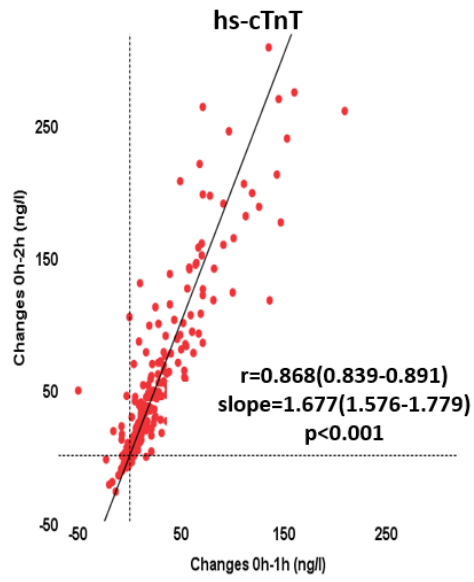
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1   **Figure 2**

2

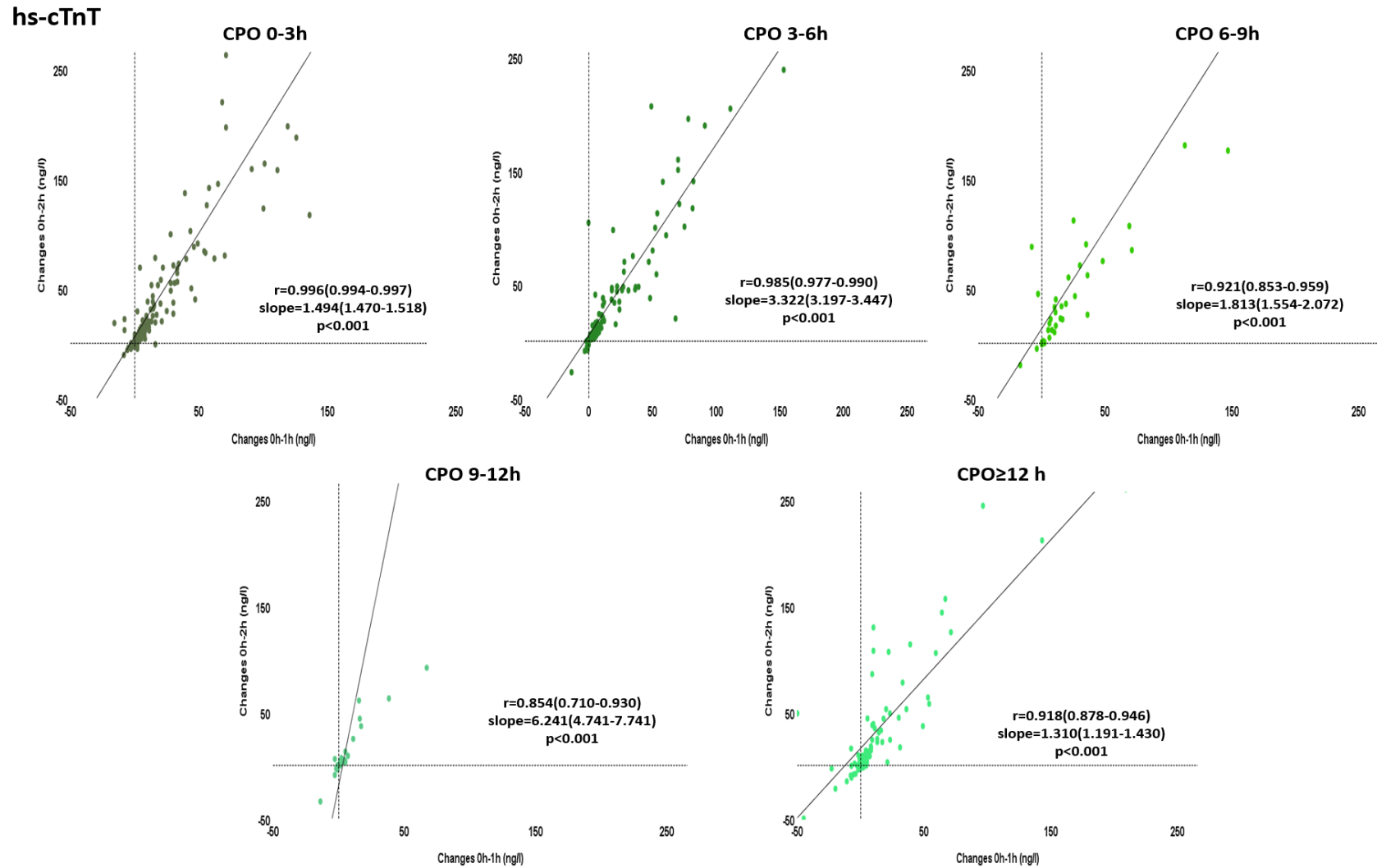3   **N=376**   **N=338**   **N=243**

1 <mark>Figure 3</mark>

hs-cTnT

r=0.868(0.839-0.891)
slope=1.677(1.576-1.779)
p<0.001

hs-cTnI Architect

r=0.966(0.0.958-0.973)
slope=1.926(1.869-1.983)
p<0.001

hs-cTnI Centaur

r=0.929(0.909-0.945)
slope=1.768(1.677-1.860)
p<0.001

2

3   **N=349**   **N=318**   **N=**
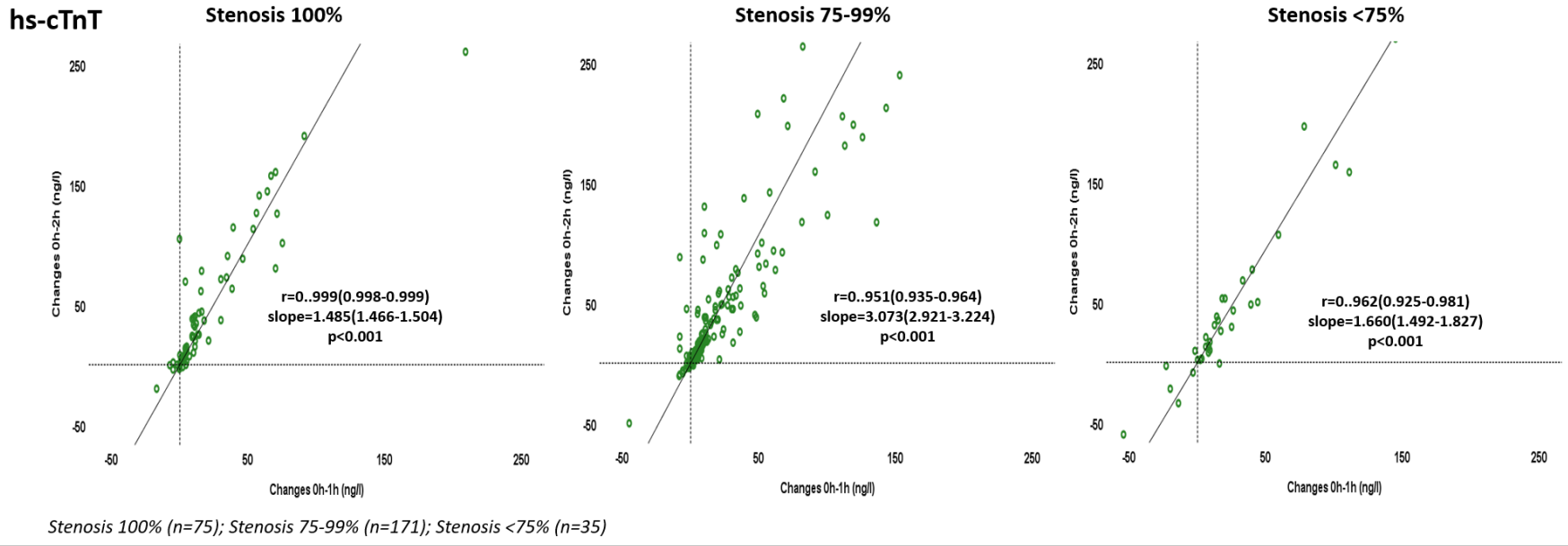
1 <mark>Figure 4</mark>

hs-cTnT

CPO 0-<3h (n=128); CPO 3-<6h (n=90); CPO 6-<9h (n=38); CPO 9-<12h (n=29); CPO≥12h (n=90); missing data CPO (n=1)

2

Figure 5

hs-cTnT

Stenosis 100%

r=0..999(0.998-0.999)
slope=1.485(1.466-1.504)
p<0.001

Stenosis 75-99%

r=0..951(0.935-0.964)
slope=3.073(2.921-3.224)
p<0.001

Stenosis <75%

r=0..962(0.925-0.981)
slope=1.660(1.492-1.827)
p<0.001

Stenosis 100% (n=75); Stenosis 75-99% (n=171); Stenosis <75% (n=35)