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Article

Exploring the Role of Cardiac Troponin-Specific Autoantibodies: Prolonged Cardiac Troponin Elimination, Reduced Clearance, and Variable Interference across 5 Commercial Assays

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BACKGROUND: High-sensitivity cardiac troponin (hs-cTn) assays are prone to negative and positive interferences caused by endogenous cardiac troponin-specific autoantibodies (cTnAAbs). Large macrotroponin complexes formed of cardiac troponin (cTn) and cTnAAbs may result in falsely elevated hs-cTn results. This is potentially due to reduced clearance of macrotroponin, but direct evidence is still lacking. In this study, we investigated the possible effects of cTnAAbs on the elimination of cTn.

METHODS: Twenty patients with ST-elevation myocardial infarction (MI) underwent plasmapheresis within 24 h after revascularization to harvest plasma with a high cTn concentration. After clinical recovery, patients returned to the hospital for autologous plasma re-transfusion. Following re-transfusion, blood samples were collected at fixed time points and analyzed with 5 commercial hs-cTn assays. The presence of cTnAAbs in the samples and the epitope specificity of cTnAAbs were investigated with in-house immunoassays.

RESULTS: Altogether, 2 out of 20 patients (10%) were cTnAAb-positive. With 4 commercial hs-cTn assays, cTnAAb-positive patients mainly showed longer elimination half-lives and slower cTn clearances than most cTnAAb-negative patients. One hs-cTn assay was prone to negative cTnAAb interference but correspondingly less prone to positive macrotroponin interference. The central part of cardiac troponin I (cTnI) was predominantly affected by cTnAAbs.

CONCLUSIONS: Endogenous cTnAAbs were for the first time shown to prolong the elimination half-life and reduce the clearance of cTn in the circulation. Additionally, the extent of analytical interference from cTnAAbs and their reactivity to macrotroponin varies among commercial hs-cTn assays, an important consideration for laboratories to ensure accurate diagnosis of MI.

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Introduction

Cardiac troponins (cTns) are the preferred biomarkers to detect myocardial injury, and their measurement with modern high-sensitivity cTn (hs-cTn) assays plays a central role in the diagnosis of acute myocardial infarction (AMI) (1). However, hs-cTn assays, like all immunoassays, are prone to antibody-mediated interferences that may lead to erroneous and clinically misleading cTn results. Endogenous cTn-specific autoantibodies (cTnAAbs) are known to interfere with hs-cTn assays by causing either falsely increased or decreased cTn results that may be inconsistent with the clinical presentation and potentially lead to erroneous clinical decisions (2).

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Circulating cTnAAbs have been found in 2% to 20% of individuals with or without cardiac disease, and thus, they are a relatively common interfering factor and cause of discrepancy between hs-cTn assays (3–10). However, several issues, including the development and clinical significance of cTnAAbs, remain incompletely understood (11). In the circulation, cTnAAbs can bind to cTn and form large macrotroponin complexes, which may have a longer elimination half-life than free cTn. Thus, the presence of cTnAAbs has been speculated to lead to reduced clearance and persistently elevated levels of cTn (8–10, 12, 13). However, direct evidence of the longer elimination half-life of macrotroponin is still lacking. On the other hand, cTnAAbs are also known to cause negative interference by masking critical epitopes and preventing assay antibodies from recognizing cTn (14–20).

Recently, the elimination kinetics of cardiac troponin I (cTnI) and cardiac troponin T (cTnT) were studied by our group in humans using autologous re-transfusion of plasma collected by plasmapheresis during an ST-elevation myocardial infarction (STEMI) (21). The novel method removed the effect of ongoing release of cTn and provided more precise information on the elimination kinetics of cTn in the human bloodstream. However, some deviations, which might be attributable to macrotroponin, were detected in the kinetic profiles. In this study, we intended to thoroughly investigate the potential effects of cTnAAbs on the elimination of cTn.

Materials and Methods

PATIENTS

The study cohort comprised 20 patients who were treated for STEMI (21). Patients underwent plasmapheresis within a median of 25 h [25th–75th percentile 21–29] of symptom onset and within 24 h of revascularization. The membrane-based plasmapheresis filtered molecules according to size (up to 3×10^6 Da). The collected plasma was stored at −20°C. After at least 3 weeks of recovery (median 5.8 [5.0–6.9] weeks) at home, the patients returned to the hospital for an autologous plasma re-transfusion (cTnI dose 1664 to 64 143 ng, cTnT dose 352 to 2921 ng) followed by repeated blood sampling at fixed time points for 8 h. Detailed information on the experimental design, patient eligibility criteria, blood sampling, and ethical considerations are provided in the publication by Kristensen et al. (21).

COMMERCIAL HS-CTN ASSAYS

The collected heparin plasma samples were analyzed with the Atellica IM hs-cTnI assay (Siemens) directly after blood sampling. Aliquots of plasma stored at −80°C were later analyzed with the Dimension Vista hs-cTnI assay (Siemens), the Vitros hs-cTnI assay (Ortho Clinical Diagnostics), the Alinity i STAT hs-cTnI assay (Abbott Laboratories), and the Elecsys hs-cTnT assay (Roche Diagnostics). The characteristics of the hs-cTn assays are described in Table 1.

Table 1. Characteristics of 5 commercial hs-cTn assays used in the study.

Assay	LOD, ng/L	cTnI epitopes recognized by capture and detection antibodies (aar)
Atellica hs-cTnI (Siemens)	1.6	C: 41–50, 171–190 D: 29–34
Dimension Vista hs-cTnI (Siemens)	2.0	C: 29–34 D: 41–50, 171–190
Vitros hs-cTnI (Ortho Clinical Diagnostics)	0.43	C: 87–91 D: 24–40, 41–49
Alinity I STAT hs-cTnI (Abbott)	1.6	ND
Elecsys hs-cTnT (Roche Diagnostics)	3	NR

Abbreviations: LOD, limit of detection; aar, amino acid residue; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; ND, not disclosed; NR, not relevant as epitopes located on cTnT.

ANTIBODIES AND HUMAN CARDIAC TROPONIN COMPLEX

A cTnI-specific 8I7 monoclonal antibody (mAb) was obtained from International Point of Care Inc. All other mAbs and native human cardiac ternary cTnI-cTnT-TnC complex (ITC) were obtained from HyTest (Finland). All used mAbs; their antigens and epitopes as reported by the manufacturer are listed in Table 2. The corrected epitope of 8I7 mAb was reported by Vylegzhanina et al. (23).

CONJUGATION OF ANTIBODIES WITH BIOTIN OR EUROPIUM CHELATE

The capture antibodies were conjugated with biotin isothiocyanate (Biotechnology Unit, University of Turku, Finland), and tracer antibodies were conjugated with intrinsically fluorescent europium chelate (Biotechnology Unit, University of Turku) as described previously (15).

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Table 2. Monoclonal antibodies used in the study.

mAb	Antigen	Epitope
916	cTnI	13–22
801		18–35
4C2		23–29
M155		26–35
19C7		41–49
247		65–74
560		83–93
8E10		86–90
84		117–126
M46		130–145
441		148–158
8I7		169–178[a]
625		169–178
MF4		190–196
7B9	TnC	
3D3	Human IgG	

Abbreviations: mAb, monoclonal antibody; cTnI, cardiac troponin I; TnC, troponin C.
[a]Corrected epitope by Vylegzhanina et al. (23). The epitope reported by the manufacturer is aar 137–148.

IMMUNOASSAY FOR DETECTION OF HUMAN CTNAABS

Plasma samples collected from the participants before and after re-transfusion were analyzed for the presence of cTnAAbs using a previously published cTnAAb immunoassay (principle shown in online Supplemental Fig. 1) (7). First, 150 ng of both biotinylated capture antibodies (M155 and 8I7) were immobilized to the wells of a yellow streptavidin plate (Uniogen Oy) in 25 µL of red assay buffer (Uniogen) with at least 1-hour incubation at room temperature followed by washings with wash buffer (Uniogen). Meanwhile, plasma samples were diluted 5-fold with insulating layer II buffer (Radiometer) and divided into 2 aliquots. One aliquot was spiked with 30 µg/L human ITC complex while the other aliquot remained unspiked. After a 1-hour incubation at +4°C, 30 µL of sample and 200 µL of red assay buffer supplemented with 27 g/L NaCl were added to the wells in triplicates and incubated for 1 hour at +36°C with 900 revolutions per minute (rpm) shaking (iEMS incubator/shaker, Thermo Fisher Scientific). The wells were washed twice and 40 ng of europium chelate-labeled 3D3 tracer antibody diluted in 200 µL of red assay buffer was added to the wells. After another 1-hour incubation at +36°C with 900 rpm shaking, the wells were washed 6 times and dried under a stream of hot air for 5 min. After cooling, the time-resolved fluorescence was measured from the dry surface with a Victor X4 Multilabel Counter (Revvity). Samples were considered cTnAAb-positive when the signal difference between ITC-spiked aliquot and unspiked aliquot was ≥100 counts and the *t*-test gave a P value <0.05.

IMMUNOASSAYS FOR ANALYZING EPITOPE SPECIFICITY OF CTNAABS

Epitope specificity studies were performed on cTnAAb-positive participant samples to determine the target epitopes of cTnAAbs on the cTnI molecule. The analytical recoveries of human ITC complex in cTnAAb-positive samples were compared to the recovery in a cTnAAb-negative lithium-heparin plasma control pool collected from 5 healthy individuals. The sandwich-type immunoassays using different cTnI-specific capture antibodies and the same troponin C-specific tracer antibody to measure the fluorescence signal from unspiked and ITC-spiked plasma samples were performed as described previously (principle shown in online Supplemental Fig. 2) (19). Briefly, 300 ng of biotinylated capture antibody was immobilized to the wells of a yellow streptavidin plate (Uniogen) in 25 µL of red assay buffer (Uniogen) with at least 1-hour incubation at room temperature. Meanwhile, aliquots of plasma samples were spiked with 30 µg/L ITC complex and incubated for 1 hour at +4 °C. After washing the wells twice with wash buffer (Uniogen), 20 µL of sample (unspiked and ITC-spiked aliquots) and 100 ng of europium chelate-labeled 7B9 tracer antibody in 20 µL of insulation layer II buffer (Radiometer) were added to the wells in duplicates and incubated for 1 hour at +36 °C with 900 rpm shaking (iEMS incubator/shaker, Thermo Fisher Scientific). The wells were washed 6 times and dried under a stream of hot air for 5 min. After cooling, the time-resolved fluorescence was measured from the dry surface with a Victor X4 Multilabel Counter (Revvity). Finally, the ITC-specific signal of each cTnAAb-positive sample was compared to the ITC-specific signal of the cTnAAb-negative control pool to obtain the sample-specific recoveries for different capture antibodies.

STATISTICAL ANALYSIS

Elimination and distribution half-lives as well as clearance were estimated for each participant using an exponential 2-phase model and GraphPad Prism software (version 9.4.1). The plateau in the bloodstream was constrained to the assay-specific concentration prior to plasma re-transfusion. Detailed descriptions of analyses regarding the determination of the elimination kinetics of cTn are described in the publication by Kristensen et al (21).

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Comparisons of hs-cTn concentrations and elimination kinetic parameters between cTnAAb-negative and cTnAAb-positive participants were performed with the exact Wilcoxon–Mann–Whitney test using IBM SPSS statistics software (version 29.0). The *P* values were adjusted for multiple testing using the method of Benjamini and Hochberg (22), which controls the risk of false discoveries. An adjusted *P* value <0.10 implies that less than 10% of the reported differences are expected to be false positives, while an adjusted *P* value <0.20 implies that less than 20% of the reported differences are expected to be false positives.

Results

PRESENCE OF CTNAABS AND ELIMINATION KINETICS OF CTN

A total of 2 out of 20 participants (10%) were identified as cTnAAb-positive (participants 8 and 15) based on the analyses of samples collected before and after plasma re-transfusion (online Supplemental Table 1). The cTnAAb-positive participants had similar or longer times from symptom onset or revascularization to plasmapheresis than cTnAAb-negative participants (online Supplemental Table 2).

The hs-cTn concentrations before and after plasma re-transfusion, as well as the elimination kinetic parameters for cTnAAb-positive and cTnAAb-negative participants are presented in Table 3. The baseline cTnI and cTnT concentrations measured with the Atellica, Alinity, Vista, and Elecsys hs-cTn assays prior to plasma re-transfusion were higher in cTnAAb-positive participants (1.6× to 15.3× cTnAAb-negative median) than in most cTnAAb-negative participants (Table 3). After re-transfusion, cTn concentrations seemed to decline more slowly in cTnAAb-positive than in cTnAAb-negative participants (Fig. 1, Supplemental Fig. 3). Particularly, cTnAAb-positive participant 15 exhibited a highly distinctive kinetic profile (Fig. 1). Concentrations measured at final time points were also considerably higher in both cTnAAb-positive participants than in most cTnAAb-negative participants (Table 3).

The Vitros hs-cTnI assay measured the lowest baseline cTnI concentrations specifically in cTnAAb-positive participants (0.3× cTnAAb-negative median) (Table 3). Compared to cTnAAb-negative participants, the cTnI levels of cTnAAb-positive participants measured with the Vitros hs-cTnI assay after plasma re-transfusion were also rather low from the beginning of blood sampling. When an approximation of 5 L was used for the human blood volume, the estimated recovery of the cTnI dose in the circulation after plasma re-transfusion was only 19% for both cTnAAb-positive participants, while the respective value for cTnAAb-negative participants was median 96% [25th–75th percentile 91%–116%] (online Supplemental Fig. 4). When the other four assays were used, the recoveries after re-transfusion for cTnAAb-positive and cTnAAb-negative participants were median 80% [66%–109%] and 97% [86%–110%], respectively. Pairwise comparisons of hs-cTn assays also showed clear differences between the Vitros assay and other hs-cTn assays for cTnAAb-positive samples (online Supplemental Fig. 5).

The cTnAAb-positive participant, 15, showed a notably prolonged elimination half-life and slower cTn clearance than the vast majority of cTnAAb-negative participants when measured by the Atellica, Alinity, Vista, and Elecsys hs-cTn assays. Likewise, the other cTnAAb-positive participant, 8, also showed a longer elimination half-life and slower cTn clearance compared to most cTnAAb-negative participants when the Atellica and Vista hs-cTnI assays were used. However, participant 8 results did not differ from cTnAAb-negative participants when measured by the Alinity and Elecsys hs-cTn assays. Interestingly, cTnAAbs did not seem to have any clear effect on the estimated elimination half-lives of cTn, when the Vitros hs-cTnI assay was used. The clearance values measured by the Vitros assay, however, were exceptionally high for cTnAAb-positive participants.

EPITOPE SPECIFICITY OF CTNAABS

Further analyses were conducted on the cTnAAb-positive samples to determine the binding sites of cTnAAbs on the cTnI molecule. Binding of the sample cTnAAbs to the same epitope as the capture antibody used in the assay resulted in lower recovery. The lowest analytical recoveries of spiked human troponin ITC complex were obtained for the epitopes of mAbs 247 [amino acid residue (aar) 65 to 74], 560 (aar 83 to 93), 8E10 (aar 86 to 90), 84 (aar 117 to 126), and M46 (aar 130 to 145) located at the central part of cTnI (Fig. 2, ITC-specific signals are presented in online Supplemental Table 3). The recoveries for these five epitopes were less than 40% (median 8%, 25th–75th percentile 3%–12%) in the samples of both cTnAAb-positive participants. Furthermore, the ITC recovery for the epitope of mAb 19C7 (aar 41 to 49) was 42% for participant 8 but 110% for participant 15. The recovery values for other epitopes located on the N-terminal and C-terminal parts of cTnI ranged from 73% to 243% (Fig. 2).

Discussion

This is the first study to examine the effect of circulating cTnAAbs on the clearance of cTn. We were able to show that the presence of cTnAAbs in the circulation slowed

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Table 3. hs-cTn concentrations of importance and elimination kinetic parameters for cTnAAb-negative and cTnAAb-positive participants.

	cTnAAb-negative participants[a]	cTnAAb-positive participants		*P* value[b]	Adjusted *P* value[c]
		Participant 8	Participant 15		
Atellica hs-cTnI					
Baseline, ng/L	16 (7–50)	34	169	0.018	0.063
1st sample after re-transfusion, ng/L	3075 (521–6830)	4330	7670	0.126	0.180
Last sample after re-transfusion, ng/L	149 (46–295)	729	4750	0.011	0.055
Distribution half-life, min	23 (5–35)	18	218	0.758	0.842
Elimination half-life, min	125 (62–221)	248	1×10^{31}	0.011	0.055
Clearance, mL/min	54 (32–93)	32	7×10^{-28}	0.029	0.067
Alinity hs-cTnI					
Baseline, ng/L	12 (6–326)	62	60	0.105	0.166
1st sample after re-transfusion, ng/L	2596 (412–6937)	5090	5206	0.059	0.118
Last sample after re-transfusion, ng/L	155 (43–492)	972	2285	0.011	0.055
Distribution half-life, min	26 (3–52)	4	NA	0.211	0.275
Elimination half-life, min	213 (76–957)	176	434	0.758	0.842
Clearance, mL/min	49 (35–94)	30	NA	0.143	0.195
Vista hs-cTnI					
Baseline, ng/L	18 (10–67)	66	275	0.026	0.065
1st sample after re-transfusion, ng/L	2746 (547–8018)	4997	10 695	0.026	0.065
Last sample after re-transfusion, ng/L	191 (66–514)	1219	6832	0.011	0.055
Distribution half-life, min	27 (20–45)	12	283	1.000	1.000
Elimination half-life, min	192 (94–259)	309	1×10^{31}	0.011	0.055
Clearance, mL/min	41 (25–80)	15	7×10^{-28}	0.019	0.063
Vitros hs-cTnI					
Baseline, ng/L	7 (2–24)	2	2	0.011	0.055
1st sample after re-transfusion, ng/L	1759 (320–3903)	457	646	0.095	0.166
Last sample after re-transfusion, ng/L	82 (25–206)	48	109	0.958	1.000
Distribution half-life, min	28 (17–51)	8	23	0.063	0.118
Elimination half-life, min	240 (94–649)	146	306	1.000	1.000
Clearance, mL/min	47 (31–106)	204	118	0.015	0.063
Elecsys hs-cTnT					
Baseline, ng/L	12 (8–19)	19	19	0.033	0.071
1st sample after re-transfusion, ng/L	237 (80–379)	377	164	0.643	0.772
Last sample after re-transfusion, ng/L	19 (13–73)	40	81	0.021	0.063
Distribution half-life, min	22 (17–39)	21	26	0.589	0.736
Elimination half-life, min	130 (88–622)	168	486	0.126	0.180
Clearance, mL/min	78 (7–127)	45	16	0.100	0.166

Abbreviations: cTnAAb, cardiac troponin-specific autoantibody; hs-cTnI, high-sensitivity cardiac troponin I; hs-cTnT, high-sensitivity cardiac troponin T; NA, not available.

[a]Data presented as median (minimum–maximum).

[b]Comparisons of hs-cTn concentrations and elimination kinetic parameters between cTnAAb-negative and cTnAAb-positive participants were performed using the exact Wilcoxon–Mann–Whitney test.

[c]*P* values were adjusted for multiple testing using the method of Benjamini and Hochberg, which controls the risk of false discoveries. An adjusted *P* value <0.10 implies that less than 10% of the reported differences are expected to be false positives, while an adjusted *P* value <0.20 implies that less than 20% of the reported differences are expected to be false positives.

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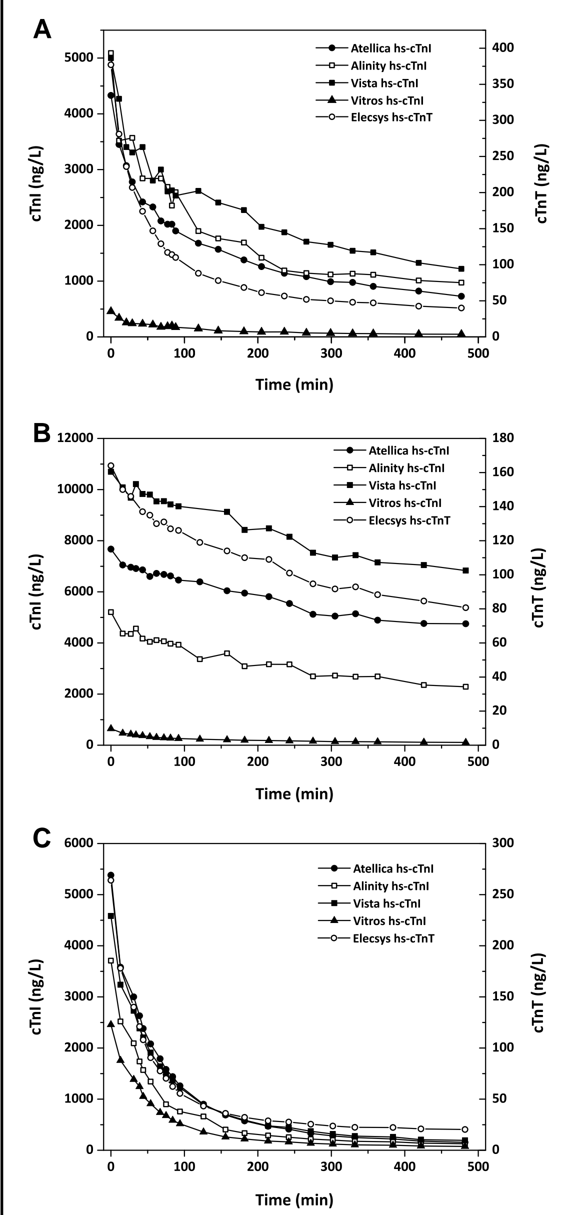

Fig. 1. Elimination of cTnI and cTnT in cTnAAb-positive and cTnAAb-negative participants after plasma re-transfusion. (A), cTnAAb-positive participant 8; (B), cTnAAb-positive participant 15; (C), Representative cTnAAb-negative participant 16. Concentrations of cTnI and cTnT were measured with Atellica hs-cTnI (black circle), Alinity hs-cTnI (open square), Vista hs-cTnI (black square), Vitros hs-cTnI (triangle), and Elecsys hs-cTnT (open circle) assays. Abbreviations: cTnI, cardiac troponin I; cTnT, cardiac troponin T; cTnAAb, cardiac troponin-specific autoantibody.

down the elimination of cTn due to the formation of macrotroponin complexes. This was studied with 5 widely used commercial hs-cTn assays.

Previously, cTnAAbs have been observed to be a common cause of discrepancies between hs-cTn assays, and they are known to interfere with hs-cTn assays by causing either falsely elevated or reduced hs-cTn results (8, 16, 18, 19, 24). In this study, 4 out of 5 assays (Atellica hs-cTnI, Alinity hs-cTnI, Vista hs-cTnI, and Elecsys hs-cTnT) measured clearly elevated baseline cTn concentrations still several weeks after AMI in both cTnAAb-positive participants. Additionally, at least one cTnAAb-positive participant showed prolonged cTn elimination following autologous plasma re-transfusion. This can be explained by the formation of high-molecular-weight macrotroponin complexes consisting of endogenous cTnAAbs and circulating cTn. Due to the prolonged elimination half-life of larger molecules, macrotroponin is cleared more slowly from the circulation than free cTn resulting in persistently increased cTn levels (2). Delayed clearance has been previously reported also for other macroanalyte complexes, such as macroprolactin (25).

Affinities, specificities, and concentrations of cTnAAbs have been observed to be highly variable between cTnAAb-positive individuals (8, 19, 20). In this study, the presence of cTnAAbs was also found to affect the elimination of cTn to varying extent, although similar kinetic trends could be seen for both cTnAAb-positive participants. Participant 15 exhibited a highly distinctive elimination kinetic profile for the Atellica, Alinity, Vista, and Elecsys hs-cTn assays, while participant 8 could not be distinguished from cTnAAb-negative participants as clearly, highlighting the interindividual variability in the characteristics and/or concentrations of cTnAAbs. In the epitope specificity studies, the cTnAAbs in the two patients were mainly found to bind to the central part of cTnI. However, some differences were also observed in the specific cTnI regions targeted by cTnAAbs between the two cTnAAb-positive participants, further highlighting the interindividual variability.

The degree of analytical interference caused by cTnAAbs and reactivity to macrotroponin are known to vary between different hs-cTn assays due to different assay characteristics. For instance, differences in specificities and affinities of assay antibodies as well as in assay conditions are likely to explain why cTnAAbs interfere differently with different hs-cTn assays (9, 20). In this study, macrotroponin in participant 15 seemed to affect the Atellica and Vista hs-cTnI assays most prominently, as the calculated clearance values were remarkably slow. The Atellica and Vista hs-cTnI assays are both manufactured by Siemens and the target epitopes are the same, which likely accounts for the similarity of the results.

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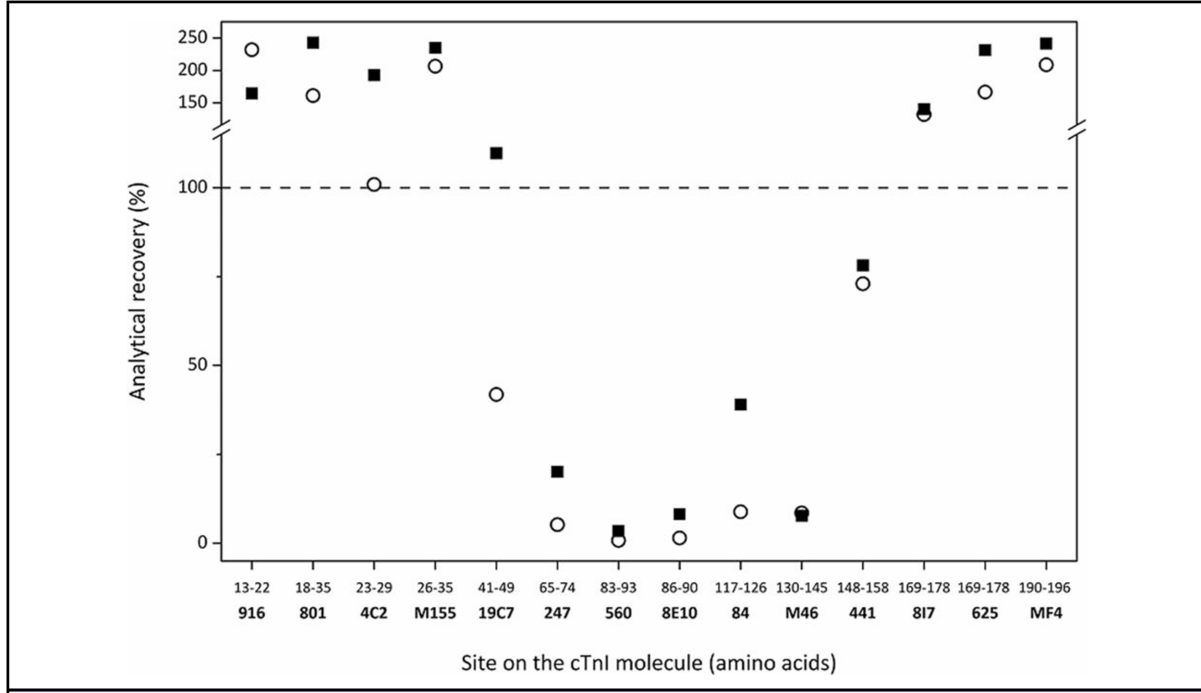

Fig. 2. Analytical recovery of human troponin ITC complex for different cTnI epitopes in cTnAAb-positive samples. Open circle and square indicate participants 8 and 15, respectively. Abbreviations: cTnI, cardiac troponin I; cTnAAb, cardiac troponin-specific autoantibody.

Interestingly, the results obtained with the Vitros hs-cTnI assay were very different from the other 3 hs-cTnI assays. Due to a lack of standardization and differences in calibration, the 4 hs-cTnI assays do not generally give equal results. However, the Vitros hs-cTnI assay measured discordantly low cTnI levels after plasma re-transfusion for both cTnAAb-positive participants. The estimated recovery of the injected cTnI dose in the circulation after plasma re-transfusion was remarkably lower in the 2 cTnAAb-positive than in the cTnAAb-negative participants suggesting that the dose was, to a large extent, not detected by the Vitros hs-cTnI assay in cTnAAb-positive participants. When the other assays were used, the estimated recoveries of the cTn dose in the circulation did not notably differ between cTnAAb-positive and cTnAAb-negative participants.

In our epitope specificity studies, the cTnAAbs in both cTnAAb-positive participants were found to target the epitopes located on the central part of cTnI. Importantly, the only capture antibody used in the Vitros hs-cTnI assay binds to aar 87 to 91, a region corresponding to the epitope of mAb 8E10, which seemed to be almost completely blocked by cTnAAbs in the recovery studies. Thus, the discordantly low cTnI concentrations observed with the Vitros hs-cTnI assay in the two cTnAAb-positive participants can be explained by the inability of the capture antibody to bind to its epitope, which is blocked by cTnAAbs. The negative interference of cTnAAbs may delay the increase of cTnI levels to detectable concentrations in cTnAAb-positive AMI patients and, moreover, cause concerns for the applicability of early rule-out algorithms in cTnAAb-positive patients, noting that approximately 10% of healthy individuals have been reported to be cTnAAb-positive (5, 6). However, the negative interference found in the 2 cTnAAb-positive participants may not apply to other individuals with cTnAAbs targeting other epitopes.

While cTnAAbs interfered negatively with the Vitros hs-cTnI assay, which has not been shown previously with this assay, the other 3 hs-cTnI assays did not seem to be prone to negative interference of the cTnAAbs in the 2 patients in this study. The capture and tracer antibodies used in the Atellica and Vista hs-cTnI assays target the C-terminal and N-terminal parts of cTnI (aar 29 to 34, 41 to 50, 171 to 190) that were not found to be major targets of cTnAAbs in the recovery studies. These findings are consistent with the previous knowledge and recommendations to avoid targeting the central part of cTnI due to its susceptibility to cTnAAb-related interferences (18, 19). The target epitopes of the Alinity hs-cTnI assay are not

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provided by the manufacturer, but evidently, the assay was able to detect macrotroponin indicating that the critical target epitopes were not masked by cTnAAbs. Furthermore, the Elecsys hs-cTnT assay was also found to detect macrotroponin to some extent. Thus, these 4 hs-cTn assays are likely to be susceptible to clinically false positive interference as baseline levels in cTnAAb-positive individuals may be higher than in others.

Hs-cTnI assays utilizing 3 antibodies that bind to different parts of cTnI, i.e., "three-site assays," have been previously suggested to be more susceptible to positive macrotroponin interference than two-site assays (9). However, previous case findings have shown that the three-site Vitros hs-cTnI assay has lower immunoreactivity for macrocomplexes than some other three-site and two-site assays (26). Of the assays investigated in our study, the Atellica, Vista, and Vitros hs-cTnI assays are confirmed three-site assays. However, while the Atellica and Vista assays showed clear positive macrotroponin interference, the Vitros assay seemed to have significant negative interference. Therefore, it is not the number of antibodies used in the assay that will determine the susceptibility to positive or negative interferences, but rather the epitopes that the assay antibodies are targeting. Moreover, cTnAAb may target different cTn epitopes in different individuals making it possible for one assay to have positive macrotroponin interference with certain cTnAAb-positive individuals and negative interference with other cTnAAb-positive individuals.

The central part of cTnI was found to be most affected by cTnAAbs confirming previous findings (19). Some of the investigated cTnI epitopes have been claimed to be masked in the ITC complex by complexation with cTnT (for example the epitope of mAb 247, as indicated in the product data by Hytest). However, we were able to show clear detection of spiked ITC in a pool of cTnAAb-negative healthy plasma with all investigated epitopes and see significant reduction of ITC detection with cTnAAb-positive samples with affected epitopes (Supplemental Table 3). We used native ITC complex for spiking the samples. Possible disintegration of cTnT from the ITC complex during storage or assay might explain the results.

The timing greatly affects the composition of circulating cTn in AMI. In the early hours after symptom onset, cTnI is known to be mostly present as a part of the ternary ITC complex, whereas in the later stages of AMI (>30 h after symptom onset), binary cTnI-TnC (IC) complexes prevail (27). In addition, in the later stages of AMI the central part of cTnT detected by the Elecsys hs-cTnT assay is mostly cleaved apart from the ITC complex (27). Interestingly, one study has shown that cTnAAbs are specific to conformational epitopes in the ITC complex and not to the IC complex (28). The plasmaphereses for cTnAAb-positive participants 8 and 15 were performed 66.5 and 30.0 h after symptom onset, respectively. The timing of plasmapheresis may also affect the findings of this study, as different cTn compositions may explain some of the differences observed between the 2 cTnAAb-positive participants.

One of the cTnAAb-negative participants with chronic kidney disease also exhibited a distinctive elimination kinetic profile for hs-cTnT. Patients with chronic kidney disease often have persistently elevated hs-cTnT levels, and renal clearance has been shown to contribute to cTnT clearance at low cTnT levels (29). Thus, impaired kidney function is likely to account for the prolonged cTnT elimination in this cTnAAb-negative participant.

A key strength of this study is its novelty as it is the first study to demonstrate the effect of circulating cTnAAbs on cTn clearance and to reveal their impact across 5 different commercial assays. Despite the small cohort comprising only 2 cTnAAb-positive and 18 cTnAAb-negative participants, clear evidence was shown of cTnAAbs and macrotroponin formation influencing cTn clearance. These findings build on and provide valuable insights into the results of the previous study published by Kristensen et al. (21).

In conclusion, this study provided first-time evidence that endogenous cTnAAbs and the macrotroponin formation result in prolonged cTn elimination and reduced clearance supporting the hypothesis that macrotroponin causes persistent cTn elevations. While some hs-cTn assays (Atellica hs-cTnI, Alinity hs-cTnI, Vista hs-cTnI, and Elecsys hs-cTnT) detected macrotroponin and showed reduced cTn clearance leading to falsely elevated cTn levels in cTnAAb-positive individuals, the Vitros hs-cTnI assay was affected by negative interference resulting in falsely low cTn levels. The findings highlight the complex effects of cTnAAbs on hs-cTn assays that should be considered in clinical practice and development of new generations of hs-cTn assays. Further research is needed to understand the clinical implication of cTnAAbs and minimize assay interferences.

Supplemental Material

Supplemental material is available at *Clinical Chemistry* online.

Nonstandard Abbreviations: hs-cTn, high-sensitivity cardiac troponin; cTnAAb, cardiac troponin-specific autoantibody; cTn, cardiac troponin; AMI, acute myocardial infarction; cTnI, cardiac troponin I; cTnT, cardiac troponin T; mAb, monoclonal antibody; ITC, native human cardiac ternary cTnI-cTnT-TnC complex; aar, amino acid residue.

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