

Elevated Cardiac Troponin T in Patients With Skeletal Myopathies



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ABSTRACT

BACKGROUND Cardiac troponins are often elevated in patients with skeletal muscle disease who have no evidence of cardiac disease.

OBJECTIVES The goal of this study was to characterize cardiac troponin concentrations in patients with myopathies and derive insights regarding the source of elevated troponin T measurements.

METHODS Cardiac troponin T (cTnT) and cardiac troponin I (cTnI) concentrations were determined by using high sensitivity assays in 74 patients with hereditary and acquired skeletal myopathies. Patients underwent comprehensive cardiac evaluation, including 12-lead electrocardiogram, 24-h electrocardiogram, cardiac magnetic resonance imaging, and coronary artery computed tomography. cTnT and cTnI protein expression was determined in skeletal muscle samples of 9 patients and in control tissues derived from autopsy using antibodies that are used in commercial assays. Relevant Western blot bands were subjected to liquid chromatography tandem mass spectrometry for protein identification.

RESULTS Levels of cTnT (median: 24 ng/l; interquartile range: 11 to 54 ng/l) were elevated (>14 ng/l) in 68.9% of patients; cTnI was elevated (>26 ng/l) in 4.1% of patients. Serum cTnT levels significantly correlated with creatine kinase and myoglobin ($r = 0.679$ and 0.786 , respectively; both $p < 0.001$). Based on cTnT serial testing, 30.1% would have fulfilled current rule-in criteria for myocardial infarction. Noncoronary cardiac disease was present in 23%. Using cTnT antibodies, positive bands were found in both diseased and healthy skeletal muscle at molecular weights approximately 5 kDa below cTnT. Liquid chromatography tandem mass spectrometry identified the presence of skeletal troponin T isoforms in these bands.

CONCLUSIONS Measured cTnT concentrations were chronically elevated in the majority of patients with skeletal myopathies, whereas cTnI elevation was rare. Our data indicate that cross-reaction of the cTnT immunoassay with skeletal muscle troponin isoforms was the likely cause. (J Am Coll Cardiol 2018;71:1540-9) © 2018 The Authors. Published by Elsevier on behalf of the American College of Cardiology Foundation. This is an open access article under the CC BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/>).



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The troponin complex critically regulates actin and myosin cross-bridge cycling in striated muscle. It is bound to tropomyosin on the actin filament and consists of 3 subunits: troponin T (TnT), troponin I (TnI), and troponin C. While TnI inhibits actin-myosin cross-bridge cycling, TnT is responsible for binding tropomyosin, and troponin C interacts with cytosolic Ca²⁺ to trigger conformational changes of the complex allowing actin-myosin interaction (1). Distinct cardiac and skeletal isoforms of TnT and TnI ensure that the respective troponin complex is best suited for different physiological roles. As a consequence, cardiac-specific isoform expression enables the use of TnT and TnI as tissue-specific biomarkers, which has profoundly impacted the diagnosis and definition of acute coronary syndromes.

SEE PAGE 1550

Clinical measurement of cardiac troponin (cTn) levels began in the 1990s. Since then, they have evolved into the biochemical gold standard for the diagnosis of myocardial necrosis. From the first definition in 2000 until the third universal definition of myocardial infarction (MI) in 2012 (2), cTn assays increasingly form the cornerstone for the diagnosis of MI. Therefore, high sensitivity and specificity of these assays are essential. However, conditions other than MI may lead to (often chronically) elevated cTn levels. Patients experiencing chronic kidney disease, for example, may have persistently elevated cTn concentrations, which has been interpreted as kidney disease-related subclinical cardiac damage (3) and/or reduced renal cTn clearance (4). In contrast, cardiac troponin T (cTnT) elevation in skeletal muscle disease has been less understood, and although specificity problems of early-generation cTnT assays seemed to be resolved in newer assay generations (5), concerns about the specificity of newer generation assays recently re-surfaced in patients with skeletal myopathies (6,7). Increased cTnT levels in these patients often pose a diagnostic dilemma and frequently lead to unnecessary and sometimes invasive diagnostic evaluations.

There are several explanations for the high prevalence of elevated cTnT levels in patients with skeletal muscle disease. Assays may detect subtle cardiac involvement in these patients that is unapparent clinically and on imaging. Another explanation is re-expression of cardiac isoforms in diseased skeletal muscle. Lastly, cTn assays may cross-react with other troponin isoforms and thus yield false-positive cTn concentrations.

In the present paper, we comprehensively investigated cardiac involvement in a range of skeletal

myopathies and analyzed cTnT, cardiac troponin I (cTnI), and other biomarker levels. In *in vitro* experiments, we discovered cTnT antibody reactivity with diseased and healthy skeletal muscle specimens and skeletal muscle-spiked healthy sera, whereas mass spectrometry only revealed skeletal troponin isoform expression in these samples.

METHODS

Patients with hereditary or acquired skeletal muscle disease were included in this cohort study. At baseline, patients underwent comprehensive cardiac examinations, including blood sampling, electrocardiogram (ECG), 24-h ECG, and cardiac magnetic resonance (CMR) imaging with late gadolinium enhancement (LGE). The study was approved by the ethics committee of the Medical University of Graz (26-282 ex 13/14) and conformed with all pertaining regulations and the principles of the Declaration of Helsinki (8). Patients provided written informed consent to participate in the study.

PATIENT POPULATION. Ninety-one patients >18 years of age with the diagnosis of a genetic or acquired neuromuscular disease who presented at the neuromuscular outpatient clinic of the Department of Neurology, Medical University of Graz, and 1 patient from the Department of Internal Medicine, Medical University of Innsbruck, were recruited between June 2014 and June 2016. Diagnoses included myotonic dystrophy types 1 and 2, nondystrophic myotonias, Duchenne muscular dystrophy (DMD), Becker muscular dystrophy (BMD), facioscapulo-humeral muscular dystrophy, limb girdle muscular dystrophies, X-linked myopathy with postural muscle atrophy (XMPMA), inclusion body myopathy, and inflammatory myopathies. A subgroup of patients had primarily neuronal muscular diseases such as amyotrophic lateral sclerosis or spinobulbar muscular atrophy.

LABORATORY EXAMINATIONS. High sensitive cTnT, creatine kinase (CK), creatine kinase-myocardial band, N-terminal pro-B-type natriuretic peptide, aspartate aminotransferase, and myoglobin were determined on a Cobas automated analyzer (Roche Diagnostics, Mannheim, Germany). From each sampling, 2 serum aliquots were snap-frozen and stored for later analysis. cTnI was determined by using the Abbott Architect STAT Troponin-I high sensitive assay (Abbott Laboratories, Chicago, Illinois). A second blood sample was taken after 3 h at the first visit

ABBREVIATIONS AND ACRONYMS

BMD	= Becker muscular dystrophy
CK	= creatine kinase
CMR	= cardiac magnetic resonance imaging
CTA	= computed tomography angiography
cTn	= cardiac troponin
cTnI	= cardiac troponin I
cTnT	= cardiac troponin T
DMD	= Duchenne muscular dystrophy
ECG	= electrocardiogram
IQR	= interquartile range
LC-MS/MS	= liquid chromatography tandem mass spectrometry
LGE	= late gadolinium enhancement
MI	= myocardial infarction
TnI	= troponin I
TnT	= troponin T
XMPMA	= X-linked myopathy with postural muscle atrophy

TABLE 1 Patient Characteristics and Laboratory Results Classified According to Myopathy Group

	Dystrophic Myopathy (n = 22)	Myotonic Dystrophy (n = 31)	Nondystrophic Myotonia (n = 7)	Inflammatory (n = 9)	Primarily Neurogenic (n = 3)	Other Myopathies (n = 2)
Female	36.4 (8)	58.1 (18)	71.4 (5)	44.4 (4)	0.0 (0)	50.0 (1)
Age, yrs	44.1 ± 15.5	46.7 ± 13.4	46.6 ± 10.9	54.7 ± 14.2	29.0 ± 7.2	51.0 ± 0.0
BMI, kg/m ²	23.7 ± 4.1	26.8 ± 7.1	27.7 ± 5.3	28.4 ± 4.0	23.3 ± 2.7	29.7 ± 0.4
cTnT, ng/l	52 (18-145)	24 (11-36)	3 (3-6)	26 (18-247)	65 (19-67)	19 (12-26)
cTnT >14 ng/l	81.8 (18)	71.0 (22)	0.0 (0)	77.8 (7)	100.0 (3)	50.0 (1)
cTnI, ng/l	5 (2-9)	5 (3-7)	3 (2-4)	4 (3-6)	2 (2-2)	3 (2-3)
cTnI >26 ng/l	9.1 (2)	3.2 (1)	0.0 (0)	0.0 (0)	0.0 (0)	0.0 (0)
NT-proBNP, ng/l	70 (37-201)	61 (28-155)	66 (41-215)	57 (37-83)	9 (7-24)	74 (23-124)
CK, U/l	621 (234-1,040)	309 (208-432)	60 (40-298)	405 (186-1,105)	1,190 (232-3,730)	1,812 (541-3,083)
CK-MB, U/l	37 (20-60)	15 (13-19)	12 (10-15)	23 (20-44)	45 (19-75)	48 (26-70)
Myoglobin, µg/l	156.5 (64.4-227.5)	81.4 (55.7-120.7)	24.1 (21.0-60.8)	149.0 (49.9-252.2)	196.6 (40.8-483.3)	187.4 (83.6-291.2)
eGFR, ml/min/1.73 m ²	125.8 ± 39.6	105.6 ± 15.7	91.2 ± 15.7	100.6 ± 17.1	126.8 ± 13.5	116.4 ± 7.2

Values are % (n), mean ± SD, or median (interquartile range).

BMI = body mass index; CK = creatine kinase; CK-MB = creatine kinase-myocardial band; cTnI = cardiac troponin I; cTnT = cardiac troponin T; eGFR = estimated glomerular filtration rate; NT-proBNP = N-terminal pro-B-type natriuretic peptide.

to evaluate if cTnT and cTnI levels were stable and thus reflected chronically elevated cTn. Laboratory measurements were repeated after 1 year.

IMAGING. All patients without contraindications for magnetic resonance imaging underwent 1.5-T ECG-gated CMR with LGE according to current guidelines (9). Patients with elevated serum cTnT levels who had no contraindications and were ≥40 years of age or had cardiovascular risk factors underwent coronary computed tomography angiography (CTA) (Somatom 64 Multislice, Siemens, Erlangen, Germany). Details on the imaging protocols are given in the [Online Methods](#).

MUSCLE SPECIMENS. In total, skeletal muscle samples from 9 patients who had given informed consent were available. Control tissue samples from patients with no known skeletal muscle disease were obtained from autopsy. [Online Table 1](#) presents a list of tissue samples used.

PROTEOMICS. Western blot analyses of tissue samples were performed with cTnT- and cTnI-specific antibodies (provided by Roche Diagnostics) directed against the same epitopes as the capture and detection antibodies used in the respective commercial assays. Positive bands obtained in Western blot analysis were subjected to on-blot digest and liquid chromatography tandem mass spectrometry (LC-MS/MS). The [Online Methods](#) present details on the Western blot and LC-MS/MS methods.

cTn MEASUREMENTS IN MUSCLE SAMPLES. Plasma of a healthy subject was spiked with increasing concentrations of homogenized healthy skeletal muscle, myocardium, or liver tissue, and cTnT, cTnI, and myoglobin concentrations were analyzed.

The [Online Methods](#) present details regarding these measurements.

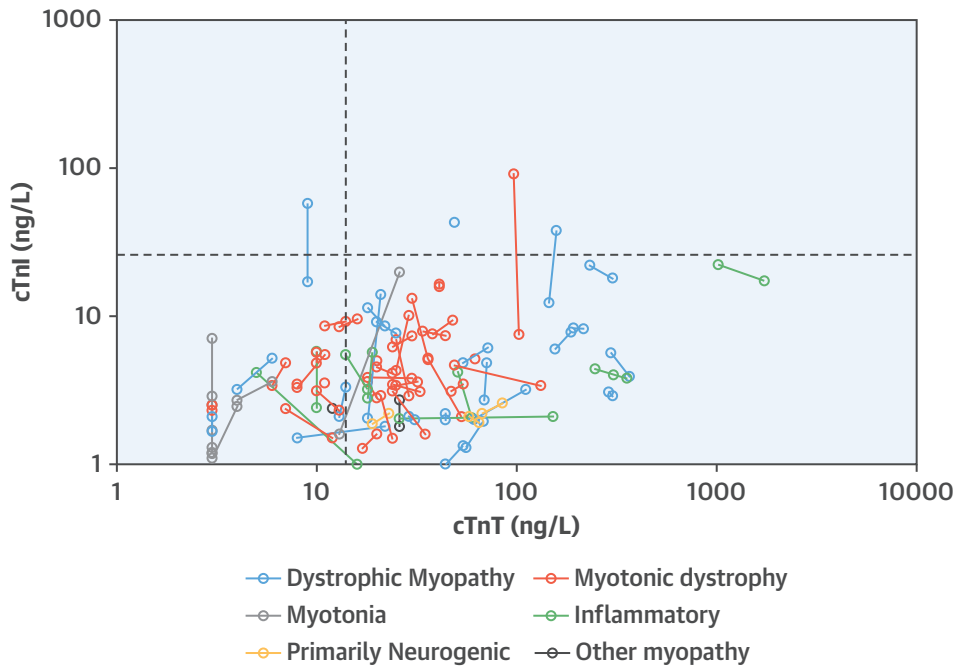
STATISTICAL ANALYSIS. Patients with previous MI, significant coronary artery disease, or other known causes of cardiac impairment other than skeletal muscle disease were excluded from further analyses. Patients were grouped into 6 categories: myotonic dystrophies, dystrophic myopathies, nondystrophic myotonias, inflammatory muscle diseases, primarily neurogenic disease, and other myopathies. Patients were further stratified according to cardiac involvement, which was defined as any of the following: presence of LGE, reduced ejection fraction <50% (10), increased left ventricular mass index, increased end-diastolic volume index (11), atrial fibrillation, or relevant conduction disorder (left bundle branch block or atrioventricular block ≥IIb).

Values are presented as mean ± SD, median (interquartile range [IQR]), or relative and absolute frequencies. Skewed laboratory variables were log-transformed to achieve normal distribution if necessary. The Pearson correlation coefficient was used to measure correlations between cTnT, cTnI, and markers of skeletal muscle damage. The Student's *t*-test, Mann-Whitney *U* test, or the Fisher exact test was used for group comparisons as appropriate. Left ventricular mass and end-diastolic volume was indexed to body surface area. A *p* value <0.05 was considered statistically significant.

RESULTS

PATIENT CHARACTERISTICS. Ninety-two patients were screened and underwent baseline examinations. CMR imaging was performed in 83 patients, and

CENTRAL ILLUSTRATION Cardiac Troponin T and Troponin I Levels in Patients With Skeletal Muscle Disease at Baseline and 1 Year



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Cardiac troponin T (cTnT) levels were elevated in a large proportion of patients with skeletal muscle disease, whereas cardiac troponin I (cTnI) levels were normal in the majority of patients. A scatterplot (logarithmic scale) of cTnT and cTnI levels at the first visit and after 1 year is presented. The 2 cardiac troponin measurements in individual patients are connected by **solid lines**; assay cutoffs are shown as **dashed lines** (26 ng/l for cTnI and 14 ng/l for cTnT). At the first visit, 68.9% had raised cTnT levels, and 4.1% had raised cTnI levels (see also contingency table in [Online Table 3](#)).

38 patients underwent coronary CTA imaging. Nine patients were excluded because an initially suspected myopathy could not be confirmed or remained questionable. Another 9 patients had other causes of cardiac impairment (6 had previously experienced MI, 2 had significant coronary artery disease, and 1 had dilated cardiomyopathy after myocarditis) and were also excluded, leaving 74 patients for analysis. Their mean age was 46 ± 14 years, 49% (36 of 74) were female, median cTnT was 24 ng/l (IQR: 11 to 54 ng/l), median cTnI was 4 ng/l (IQR: 2 to 7 ng/l), and the mean estimated glomerular filtration rate (per the Chronic Kidney Disease Epidemiology Collaboration equation) was 111 ± 27.4 ml/min/1.73 m². No patient had an estimated glomerular filtration rate <60 ml/min/1.73 m². The different myopathy categories, including demographic parameters and laboratory results, are shown in [Table 1](#); [Online Table 2](#) presents details on a patient level.

PLASMA cTnT MEASUREMENTS ARE ELEVATED IN PATIENTS WITH SKELETAL MYOPATHY. Although 69% (51 of 74) of patients had cTnT concentrations above the established assay cutoff (14 ng/l), only 4% (3 of 74) had raised cTnI levels (>26 ng/l) ([Central Illustration](#), [Online Table 3](#)). The vast majority of patients either had cTn levels below the respective cutoff in both assays (30%) or raised cTnT and normal cTnI levels (66%). cTnT elevation was most pronounced in patients with XMPMA (233 ng/l; IQR: 216 to 289 ng/l; n = 5) within the category of dystrophic myopathies (52 ng/l; IQR: 18 to 145 ng/l; n = 22). cTnT levels were chronically elevated with a delta of 0.1 ± 9.4 ng/l after 3 h and -6.7 ± 27.6 ng/l at 1 year in the entire cohort. In 72 patients, 15.3% (n = 11) had a 3-h delta >10 ng/l, and 30.1% (22 of 73) would have fulfilled rule-in criteria for MI using both a proposed 2-h algorithm (12) or the 3-h European Society of Cardiology algorithm (3).

TABLE 2 Cardiac Involvement in the Cohort			
All Patients			
LGE	16.2 (11/68)		
EF <50%	7.2 (5/69)		
Increased LVMI	1.5 (1/68)		
Increased EDVi	0.0 (0/68)		
AF, LBBB, or AV block \geq IIb	6.8 (5/74)		
Any of the Variables Above	Cardiac Involvement 22.9 (16/70)	No Cardiac Involvement 77.1 (54/70)	p Value
cTnT, ng/l	37 (15-181)	23 (10-49)	0.025
cTnT >14 ng/l	75.0 (12/16)	66.7 (36/54)	0.760
cTnI, ng/l	9 (6-22)	3 (2-5)	<0.001
cTnI >26 ng/l	18.8 (3/16)	0.0 (0/54)	0.010
NT-proBNP, ng/l	128 (49-328)	56 (24-108)	0.003
CK, U/l	619 (277-1,026)	309 (160-602)	0.169
CK-MB, U/l	25 (16-50)	18 (13-38)	0.160
Myoglobin, μ g/l	102.1 (79.6-206.1)	81.3 (41.6-157.4)	0.205
eGFR, ml/min/1.73 m ²	117.4 \pm 40.0	109.0 \pm 23.6	0.769
Framingham risk, %	10.0 (4.1-14.9)	4.6 (0.9-9.2)	0.043

Values are % (n/N), median (interquartile range), or mean \pm SD. Cardiac involvement in the cohort was defined as any of the following: presence of late gadolinium enhancement (LGE), ejection fraction (EF) <50%, increased left ventricular mass index (LVMI), increased end-diastolic volume index (EDVi) (11), atrial fibrillation (AF), or relevant conduction disorder (left bundle branch block [LBBB] or atrioventricular [AV] block \geq IIb). Cardiac and skeletal muscle markers are presented according to cardiac involvement. p values from Student's t-test, Mann-Whitney U test, or the Fisher exact test.

Abbreviations as in [Table 1](#).

CK levels in the blood reflect myocyte damage and are clinically used as an indicator of disease activity in myopathies. CK levels were elevated in the majority of patients (78% [58 of 74]). CK, myoglobin, and aspartate aminotransferase levels strongly and significantly correlated with cTnT ($r = 0.679$, $p < 0.001$; $r = 0.786$, $p < 0.001$; and $r = 0.576$, $p < 0.001$, respectively) but not with cTnI ($r = 0.124$, $p = 0.293$; $r = 0.182$, $p = 0.121$; and $r = 0.177$, $p = 0.131$) ([Online Figure 1](#)). The correlation between cTnT and cTnI concentrations was weaker, albeit still significant ($r = 0.316$; $p = 0.006$). In patients with XMPMA, a longer observation period (6 to 8 years) was available. Their CK and cTnT levels were chronically elevated with largely parallel curves over time ([Online Figure 2](#)).

EVIDENCE OF CARDIAC INVOLVEMENT IN PATIENTS WITH MYOPATHIES. We characterized cardiac involvement or underlying cardiac disease in the study cohort using CMR, coronary CTA, and ECG measurements and excluded patients with evidence of previous MI, significant coronary artery disease, or other known underlying cardiac disease ($n = 9$). Cardiac involvement was defined as any of the following: presence of LGE, ejection fraction <50%, increased

left ventricular mass or end-diastolic volume index, atrial fibrillation, or relevant conduction disorder (left bundle branch block or atrioventricular block \geq IIb).

Signs of cardiac disease were present in 23% (16 of 70) of the cohort ([Table 2](#)). In certain patient groups, cardiac abnormalities were more pronounced ([Online Table 4](#)), particularly in the category of dystrophic myopathies that includes entities with known cardiac involvement, such as XMPMA (13), DMD, BMD (14), and in myotonic dystrophies (15).

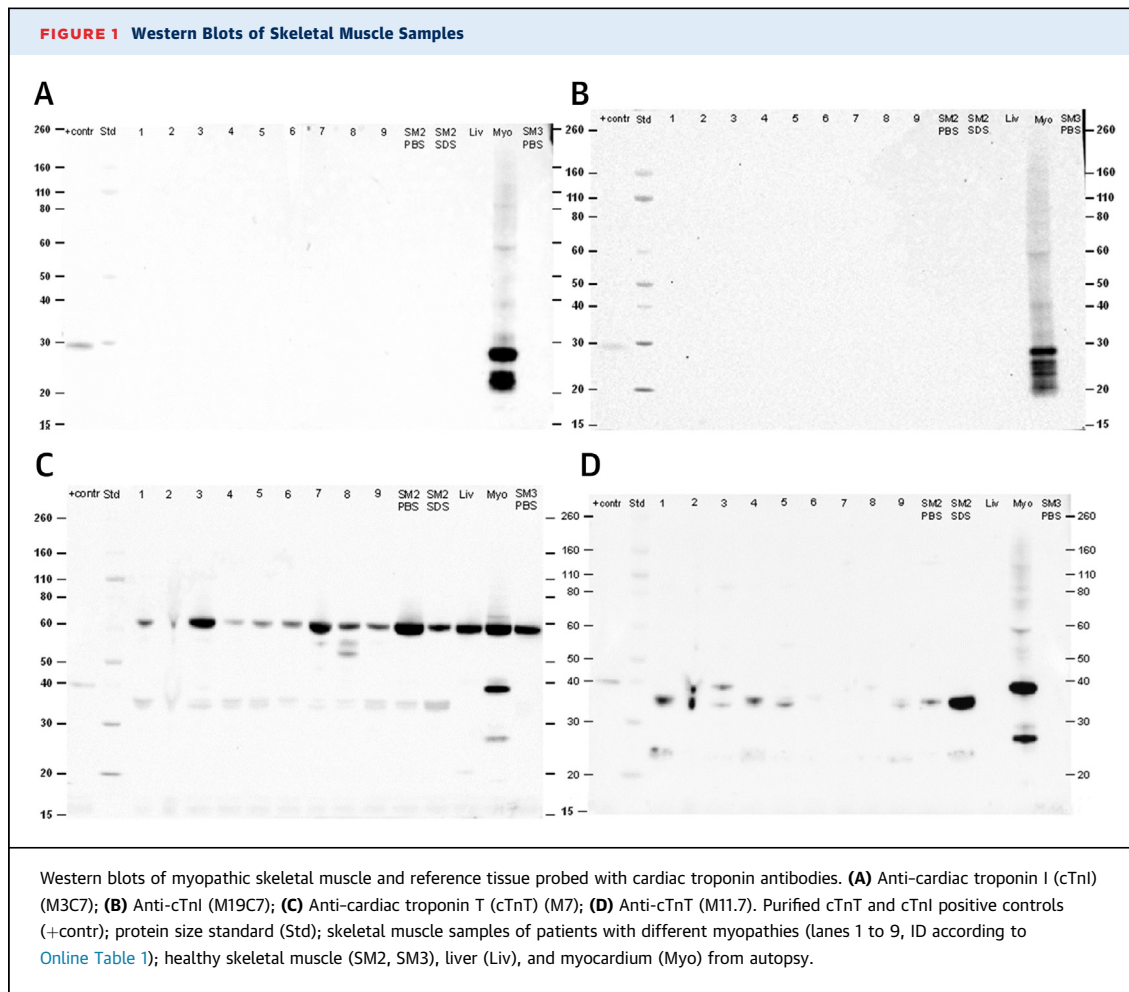
Levels of cTnI, N-terminal pro-B-type natriuretic peptide, and, to a lesser extent, cTnT were significantly higher in patients with cardiac involvement ($p < 0.001$, $p = 0.003$, and $p = 0.025$, respectively) ([Table 2](#)), whereas CK and myoglobin were not ($p = 0.169$ and $p = 0.205$). Importantly, in patients with no signs of cardiac disease, cTnT remained elevated in the majority of patients (67% [36 of 54]), and the proportion of patients above the diagnostic cutoff did not differ between groups.

cTnT BUT NOT cTnI ANTIBODIES DETECT BANDS IN SKELETAL MUSCLE SAMPLES.

We next assessed the reactivity of cTn antibodies in skeletal muscle samples of patients with myopathy and healthy reference tissue ([Online Table 1](#)). Using cTnI antibodies, only myocardium showed positive bands ([Figures 1A and 1B](#)). In blots probed with cTnT antibodies ([Figures 1C and 1D](#)), we detected 1 to 3 bands per lane in skeletal muscle samples with either cTnT antibody at molecular weights below cTnT ([Online Figures 3 and 4](#)). These bands were present in diseased skeletal muscle but also in healthy skeletal muscle reference tissues. The M7 cTnT antibody additionally detected a strong positive band in all samples, including liver tissue, at approximately 60 kDa. This band was not detected with the M11.7 antibody and is therefore not recognized in the clinically used assay, which requires positivity with both antibodies. As expected, myocardium showed a typical band with both antibodies at the molecular weight of cTnT, whereas liver tissue did not show positive bands at a molecular weight consistent with troponin ([Online Table 5](#) presents details on molecular masses of troponin isoforms).

MASS SPECTROMETRY DETECTS SKELETAL TnT IN cTnT-ANTIBODY POSITIVE SKELETAL MUSCLE WESTERN BLOT BANDS.

The positive bands detected with both antibodies were subjected to LC-MS/MS. Slow skeletal TnT was present in all samples in the recognized bands. Fast skeletal TnT was present in 13 of 14 samples. There was no evidence of cTnT in any of the analyzed skeletal muscle samples ([Online Tables 6 and 7](#)), whereas purified cTnT was clearly



identified by using this method. The 60-kDa band detected by the M7 cTnT antibody did not contain troponin isoforms ([Online Table 7](#)).

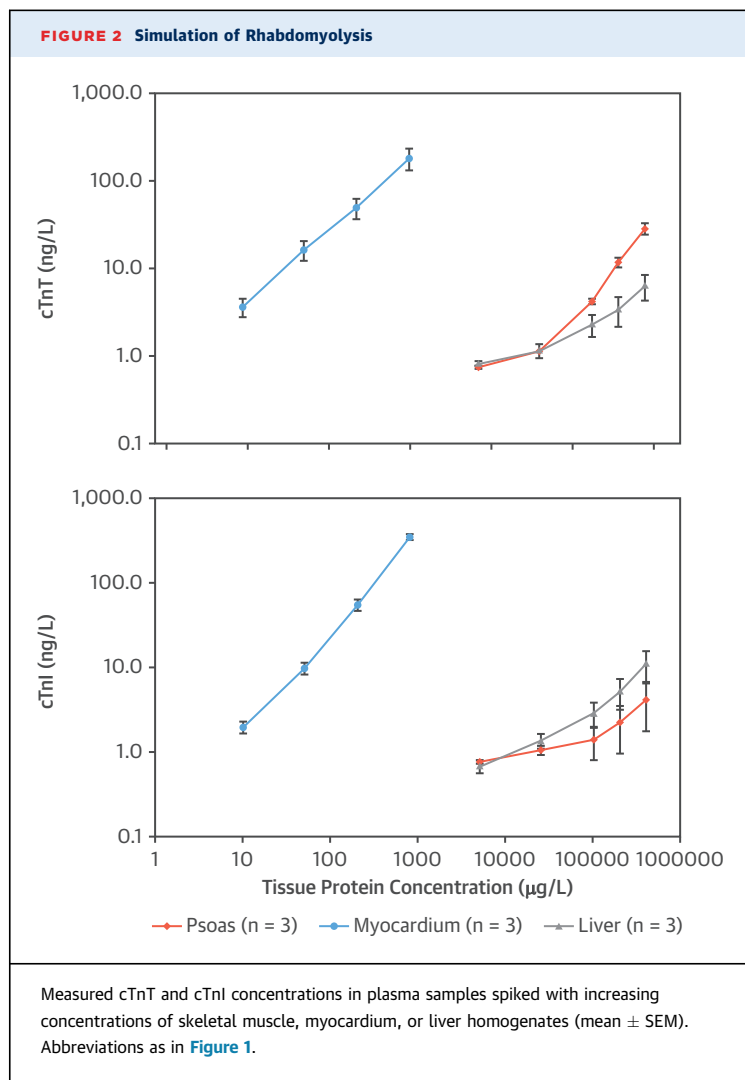
cTnT CONCENTRATIONS ARE ELEVATED IN PLASMA SPIKED WITH HOMOGENIZED HEALTHY SKELETAL MUSCLE SAMPLES. To investigate if skeletal muscle proteins would cause positive test results not only in the Western blot setting but also in the clinically used assay, we spiked healthy human plasma with healthy cardiac or skeletal muscle homogenates and then measured cTnT, cTnI, and myoglobin concentrations ([Figure 2](#), [Online Figure 5](#)).

Measured cTnT and cTnI linearly increased with rising concentrations of myocardial muscle protein spiked in plasma samples. For cTnT, there was a comparable linear increase at higher protein input concentrations when spiking skeletal muscle into plasma resulting in a rightward shift of the cTnT concentration curve. cTnI measurements yielded much lower values in the same approach and

remained below the established assay cutoff and nonmuscle control tissue (liver) ([Figure 2](#)).

DISCUSSION

In the present study, cTnT serum concentrations were substantially elevated in patients with neuromuscular disorders, whereas the cTnI serum concentrations were not. Although only 23% of patients had (mostly subtle) signs of cardiac involvement as assessed by a thorough cardiac characterization, cTnT concentrations were well above the established cutoff (99th percentile of healthy subjects) in 69% of patients. When considering cTnT concentration changes in serial measurements, 1 in 3 patients would have fulfilled rule-in criteria for acute MI. Skeletal muscle diseases are rare; however, in these patients, the diagnostic utility of cTnT in discovering acute myocardial injury seems limited, at least if stratification is based on established cutoffs. In contrast,



cTnI serum concentrations were below the upper limit of normal in the vast majority of patients.

SKELTAL MYOPATHIES AND CARDIAC INVOLVEMENT.

Our study cohort comprised several different myopathies, which we categorized into 6 groups to reduce heterogeneity (Online Table 2). In contrast to the other groups, nondystrophic myotonias rarely feature significant muscle degeneration (16). Accordingly, they were the only group in which cTnT and cTnI concentrations were normal in all patients. In primarily neurogenic diseases with muscle wasting (cTnT elevated in all 3 patients, cTnI normal), a myocardial involvement is highly improbable, whereas in the remaining groups, cardiac involvement has been variably reported (13,17). In our own examinations, cardiac abnormalities were more prevalent in categories that are known to have cardiac involvement, especially in the group of muscular dystrophies in which development of cardiomyopathy

is particularly frequent in XMPMA, DMD, BMD (13,14) and, to a lesser extent, in patients with myotonic dystrophies (15). Although cTnT concentrations were higher in myopathy patients with cardiac disease, they were also increased in the absence of cardiac disease, and the proportion of subjects with values above the established cutoff was comparable. In contrast, the proportion of cTnI values above the established cutoff was significantly higher in skeletal myopathy patients with evidence of cardiac disease compared to patients without such evidence.

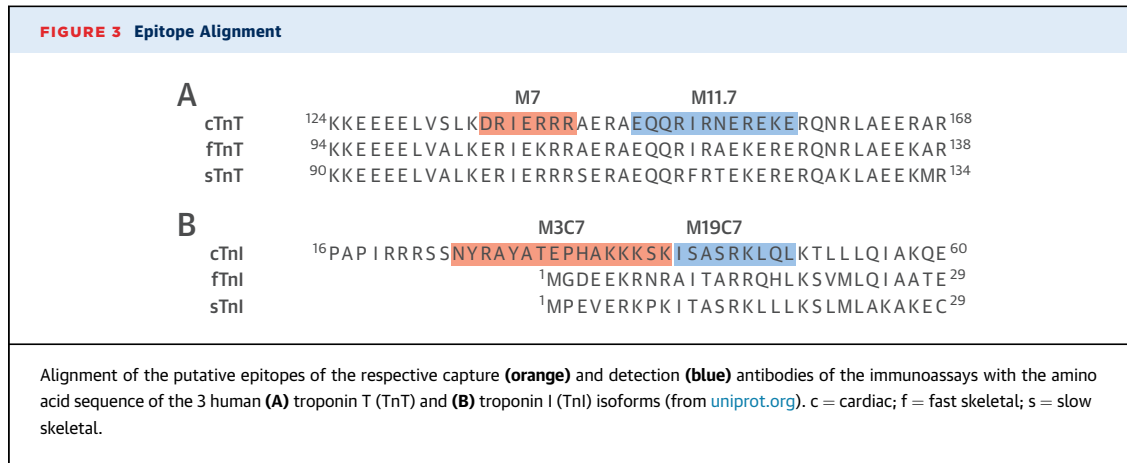
BIOMARKERS OF SKELETAL AND CARDIAC MUSCLE DAMAGE.

CK or myoglobin levels are used to monitor disease activity in myopathies with myocyte damage, and their concentrations were high in a large proportion of the study patients. Similarly, cTnT levels were elevated in the majority of patients and were strongly correlated with CK and myoglobin levels. If cardiomyocyte necrosis was the source of elevated cTnT values, cTnI would be expected to be positive, too. However, cTnI was only elevated in a few patients and, unlike cTnT, cTnI did not correlate with CK and myoglobin. Indeed, cTnT correlated better with the unspecific markers of muscle damage CK and myoglobin (which are quantitatively mostly derived from skeletal muscle in patients with myopathy) than with cardiac-specific cTnI. These data imply that skeletal muscle is the predominant source of elevated measured cTnT concentrations in these patients.

Several studies have described an elevation of cTnT in patients with skeletal myopathies. Although some of these studies maintain that cTnT reflects the extent of cardiac involvement (18,19), others found evidence on a skeletal muscle origin and questioned the cardiac specificity of cTnT assays (6,7,20). Indeed, unspecific reactivity had been a problem with early-generation cTnT and cTnI assays in patients with skeletal muscle disease (21-23). Further development and better antibody specificity improved the assays (5); however, a number of ensuing publications reported on elevated cTnT levels in patients with skeletal muscle disease (24-27), including recent assay generations (7).

SKELTAL MUSCLE ORIGIN OF MEASURED PLASMA cTnT: CROSS-REACTIVITY OR RE-EXPRESSION OF CARDIAC ISOFORMS?

Although several groups proposed a skeletal muscle origin of measured cTnT in myopathies, the exact mechanisms are still under debate. The antibodies used in the cTnT immunoassays have been tested in Western blots on diseased skeletal muscle samples before to address this question. Ricchiuti et al. (28) found positive bands in patients with renal disease-induced myopathy; however, the bands differed in molecular weight



between the 2 antibodies. In contrast, Jaffe et al. (6) reported positive bands of a similar molecular weight with both antibodies in tissue of patients with skeletal myopathy.

In our Western blot experiments, the 2 cTnT antibodies detected corresponding positive bands in skeletal muscle of patients but also healthy control samples at a molecular weight consistent with skeletal TnT. Using LC-MS/MS analysis, we identified skeletal TnT isoforms in these bands but did not find cTnT. These data support that cross-reaction of the assay and not re-expression of cTn isoforms in diseased skeletal muscle caused the elevated measurements in our patients.

In an additional experiment, we simulated skeletal muscle necrosis by adding healthy skeletal muscle homogenate into healthy plasma. This test resulted in cTnT measurements that were a linear function of skeletal muscle input protein concentration at concentrations that elicited an increase in measured myoglobin comparable to clinically observed values. Thus, skeletal muscle protein is not only detected by cTnT antibodies in Western blots but also the clinically used cTnT immunoassay in this setting. In contrast, cTnI remained below the assay cutoff and below values of nonmuscle control tissue.

Even if assay cross-reaction with skeletal troponin is small, it is apparently relevant considering that there is approximately 100 times more skeletal than myocardial muscle mass and that skeletal myopathies often lead to extensive muscle injury with elevation of skeletal troponin levels by several orders of magnitude (29). In line with this interpretation, the amino acid sequence of the 3 TnT isoforms shows strong homologies in the putative epitope regions, particularly regarding the M7 antibody (Figure 3).

A different explanation for the increased measured cTnT concentration in skeletal myopathies

is re-expression of cardiac or fetal TnT isoforms in diseased skeletal muscle. Wens et al. (30) recently reported the results of a large cohort of patients with Pompe disease. The authors detected both messenger ribonucleic acid of cTnT and a tryptic peptide specific for cTnT by mass spectrometry in skeletal muscle. In patients with DMD, studies found cTnT and/or cTnI messenger ribonucleic acid expression in skeletal muscle (20,31). Although the latter 2 of these studies did not assess protein expression, they suggest that re-expression of cTnT in diseased skeletal muscle is possible and may be the source for some of the measured cTnT in such patients. However, it remains questionable how much of cTnT messenger ribonucleic acid is translated to protein and if re-expression may quantitatively account for the observed cTnT concentrations in patients. In our data, antibody reactivity in Western blot experiments did not differ between diseased and healthy skeletal muscle samples, and homogenized healthy skeletal muscle spiked in healthy plasma caused a clear increase in measured cTnT concentrations in the immunoassay. This outcome supports the hypothesis that cross-reactivity rather than re-expression is the main driver for elevated measured cTnT values in our patient cohort. Importantly, irrespective of the exact nature of measured cTnT, our data and research by other groups strongly suggest that it originates from skeletal rather than cardiac muscle.

STUDY STRENGTHS AND LIMITATIONS. In contrast to previous studies that found elevated cTnT levels in patients with skeletal myopathies (7,25-27), our patients underwent comprehensive cardiac evaluation, including CMR, coronary CTA, 12-lead ECG, and ambulatory ECG, thus allowing the thorough assessment of potential cardiac disease causing elevated

cTn levels. In addition, we were able to acquire a relatively large number of 9 human myopathic skeletal muscle specimens and healthy control tissues. Our Western blot experiments were complemented by LC-MS/MS, which allowed us to identify the proteins present in unspecific Western blot bands. Lastly, our spiking experiments with homogenized healthy skeletal muscle clearly showed assay reactivity with simulated skeletal muscle injury.

A limitation is the study cohort's limited sample size in some subcategories that is owed to the rareness of certain myopathies. This limitation necessitated pooled analysis and interpretation. An important precaution is that our data relate to specific antibody and test manufacturers and as such to individual sensitivities and antibody epitopes.

CONCLUSIONS

Patients with skeletal muscle diseases have a high prevalence of elevated measured cTnT levels, whereas cTnI concentrations are largely normal. Thorough cardiac evaluation and good associations of cTnT with markers of skeletal muscle damage suggest a skeletal muscle origin of measured cTnT in these patients. cTnT antibodies detect protein in diseased and healthy skeletal muscle. In patients with extensive skeletal muscle damage, such cross-reactivity may cause positive cTnT test results. cTnT measurements should therefore be interpreted with caution in skeletal muscle disease. Changes of cTnT in serial measurements, as well as clinical, ECG, and imaging characteristics, should be accounted for in these patients in accordance with current guidelines.

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PERSPECTIVES

COMPETENCY IN MEDICAL KNOWLEDGE: Even when myocardial disease is absent, patients with skeletal muscle disease may have elevated levels of cTnT.

COMPETENCY IN PATIENT CARE AND PROCEDURAL SKILLS: In patients with skeletal myopathy, serial assessment of dynamic troponin changes in addition to other clinical evidence is important for establishing a diagnosis of myocardial infarction. In these patients, cTnI appears more specific for the detection of myocardial injury than cTnT.

TRANSLATIONAL OUTLOOK: Further research is required to clarify the relative contributions of myocardial damage, assay cross-reactivity, and expression of cardiac isoforms by diseased skeletal muscle to elevated serum cTnT in patients with skeletal myopathy.

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KEY WORDS cardiac troponin, myopathy, skeletal muscle

APPENDIX For an expanded Methods section, as well as supplemental figures and tables, please see the online version of this paper.