Elevated Plasma Cardiac Troponin T Levels Caused by Skeletal Muscle Damage in Pompe Disease

Stephan C.A. Wens, MD; Gerben J. Schaaf, PhD; Michelle Michels, MD, PhD; Michelle E. Kruijshaar, PhD; Tom J.M. van Gestel, BAS; Stijn in’t Groen, BAS; Joon Pijnenburg, BAS; Dick H.W. Dekkers, BAS; Jeroen A.A. Demmers, PhD; Lex B. Verdijk, PhD; Esther Brusse, MD, PhD; Ron H.N. van Schaijk, MD, PhD; Ans T. van der Ploeg, MD, PhD; Pieter A. van Doorn, MD, PhD*; W.W.M. Pim Pijnappel, PhD*

Background—Elevated plasma cardiac troponin T (cTnT) levels in patients with neuromuscular disorders may erroneously lead to the diagnosis of acute myocardial infarction or myocardial injury.

Methods and Results—In 122 patients with Pompe disease, the relationship between cTnT, cardiac troponin I, creatine kinase (CK), CK-myocardial band levels, and skeletal muscle damage was assessed. ECG and echocardiography were used to evaluate possible cardiac disease. Patients were divided into classic infantile, childhood-onset, and adult-onset patients. cTnT levels were elevated in 82% of patients (median 27 ng/L, normal values <14 ng/L). Cardiac troponin I levels were normal in all patients, whereas CK-myocardial band levels were increased in 59% of patients. cTnT levels correlated with CK levels in all 3 subgroups (P<0.001). None of the abnormal ECGs recorded in 21 patients were indicative of acute myocardial infarction, and there were no differences in cTnT levels between patients with and without (n=90) abnormalities on ECG (median 28 ng/L in both groups). The median left ventricular mass index measured with echocardiography was normal in all the 3 subgroups. cTnT mRNA expression in skeletal muscle was not detectable in controls but was strongly induced in patients with Pompe disease. cTnT protein was identified by mass spectrometry in patient-derived skeletal muscle tissue.

Conclusions—Elevated plasma cTnT levels in patients with Pompe disease are associated with skeletal muscle damage, rather than acute myocardial injury. Increased cTnT levels in Pompe disease and likely other neuromuscular disorders should be interpreted with caution to avoid unnecessary cardiac interventions. (Circ Cardiovasc Genet. 2016;9:6-13. DOI: 10.1161/CIRCGENETICS.115.001322.)

Key Words: creatine kinase □ echocardiography □ mass spectrometry □ myocardial infarction □ troponin T

Troponin T (TnT), troponin I (TnI), and troponin C form the troponin complex involved in muscle contraction. After depolarization, TnT binds tropomyosin resulting in interaction between actin and myosin and subsequent muscle contraction.1-3 Skeletal muscle–specific and cardiac muscle–specific forms of TnT and TnI are expressed from closely related genes.2,4 Cardiac troponins (cTn) are important biomarkers in patients with acute myocardial infarction (AMI) because they are released into the circulation after ischemic damage to cardiomyocytes.5,6 However, increased concentrations of cardiac troponin T (cTnT) have been observed in the general population and in several patient groups including those with heart failure and end-stage renal failure but without AMI.2,6-8 Recently, an elevation of plasma cTnT in the absence of cardiac troponin I (cTnI) in several neuromuscular diseases has been shown.2,9 It has been suggested that diseased skeletal muscle tissue is an alternative source for elevated cTnT levels in certain neuromuscular conditions and dialysis patients.5,9-11 However, because of similarities between members of the troponin family, the presence of cTnT in tissue samples has been the subject of debate because of potential cross-reactivity of antibodies used in immunoblot or ELISA assays.

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From the Department of Neurology (S.C.A.W., E.B., P.A.v.d.), Center for Lysosomal and Metabolic Diseases (S.C.A.W., G.J.S., M.E.K., T.J.M.v.G., S.G., J.P., E.B., A.T.v.d.P., P.A.v.d., W.W.M.P.P.), Molecular Stem Cell Biology, Department of Clinical Genetics (G.J.S., T.J.M.v.G., S.G., J.P., W.W.M.P.P.), Department of Cardiology (M.M.), Department of Clinical Chemistry (R.H.N.v.S.), Erasmus MC University Medical Center, Rotterdam, The Netherlands; Division of Metabolic Diseases and Genetics, Department of Pediatrics, Erasmus MC-Sophia, Rotterdam, The Netherlands (G.J.S., T.J.M.v.G., S.G., J.P., A.T.v.d.P., W.W.M.P.P.); Proteomics Center, Erasmus MC University Medical Center, Rotterdam, The Netherlands (D.H.W.D., J.A.A.D.); Netherlands Proteomics Center, Rotterdam, The Netherlands (D.H.W.D., J.A.A.D.); and NUTRIM School for Nutrition, Toxicology and Metabolism, Maastricht University Medical Center, Maastricht, The Netherlands (L.B.V.).

Drs van Doorn and Pijnappel shared senior authorship.

The Data Supplement is available at http://circgenetics.ahajournals.org/lookup/suppl/doi:10.1161/CIRCGENETICS.115.001322/-DC1. Correspondence to W.W.M. Pim Pijnappel, PhD, Molecular Stem Cell Biology, Department of Clinical Genetics, Erasmus MC University Medical Center, Wytemaweg 80, 3015 CN Rotterdam, The Netherlands. E-mail w.pijnappel@erasusmc.nl or Pieter A. van Doorn, MD, PhD, Department of Neurology, Erasmus MC University Medical Center, ’s-Gravendijkwal 230, 3015 CE Rotterdam, The Netherlands. E-mail p.a.vandoorn@erasusmc.nl © 2016 American Heart Association, Inc.

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Pompe disease is a neuromuscular metabolic disorder with an overall disease incidence at 1:40,000 live births. The disease is caused by mutations in the acid α-glucosidase (GAA) gene (MIM 606800), which encodes a lysosomal enzyme responsible for glycogen degradation. Dysfunctional or low levels of GAA result in glycogen accumulation affecting mainly skeletal muscle tissue. Diagnosis of Pompe disease is based on GAA enzymatic activity measurements. Patients with classic infantile Pompe disease have a residual enzyme activity of <1%, leading to a severe form of the disease. They present shortly after birth with muscle weakness and a hypertrophic cardiomyopathy with progressive cardiac failure, which results in death within 1 year of age if left untreated. Enzyme replacement therapy (ERT) can be life-saving for classic infantile patients and the cardiomyopathy responds well to ERT. Patients with nonclassic or late-onset Pompe disease have a residual enzyme activity of ≤20% and show slower disease progression. They present during childhood or adulthood with limb-girdle weakness and reduced pulmonary function. In these patients, ERT positively alters the natural course by increasing muscle strength and stabilizing pulmonary function.

Increased cTnT concentrations were found in one of our adult patients who reported atypical chest pain. However, a comprehensive cardiac evaluation failed to reveal cardiac abnormalities. This discrepancy between elevated cTnT concentrations and absence of AMI prompted us to investigate the presence of increased cTnT levels in more detail in a large cohort of patients with Pompe disease.

Methods

Study Population

In the Netherlands, all patients with Pompe disease are referred to the Center for Lysosomal and Metabolic Diseases of the Erasmus University Medical Center Rotterdam. In total, 122 patients at our center participated in this study. The patients were subdivided into 3 groups. The first group comprised 14 patients with classic infantile Pompe disease who had developed symptoms in the first year of life and had a hypertrophic cardiomyopathy. All these patients started treatment with ERT before the first year of life. Plasma samples analyzed for cTnT in this study were taken after ERT, when the left ventricular hypertrophy (LVH) was resolved in 10 of 14 patients. The other patients had nonclassic or late-onset Pompe disease: group 2 comprised 13 patients with symptom onset before the age of 18 years (childhood onset), and group 3 comprised 95 patients with symptom onset after the age of 18 years (adult onset). Diagnosis was confirmed by a deficiency of acid α-glucosidase GAA (was already abbreviated earlier in text) in leukocytes or fibroblasts and mutation analysis. The Medical Ethics Committee at Erasmus University Medical Center approved the study protocol. All patients provided written informed consent.

Laboratory Testing

cTnT levels were measured in heparin plasma samples using a fifth generation highly sensitive assay of Roche Diagnostics; 99th percentile 14 ng/L, and limit of detection 5 ng/L. In 7 adult patients, cTnT levels were measured before the start of ERT and ≤2 years after treatment. Plasma cTnI levels were measured using the Access AccuTnI assay of Beckmann Coulter with a 99th percentile cutoff of 0.04 ng/L. Plasma cTnI levels were measured using the Roche Diagnostic assays. As reference intervals for CK we used the age of 2–13 years, <230 U/L; male adolescents (13–17 years), <270 U/L; female adolescents (13–17 years), <125 U/L; male adults (>17 years), <200 U/L; and female adults (>17 years), <170 U/L. The sex-specific 99th percentiles for CK-MB levels were 7.6 μg/L in male patients and 4.7 μg/L in female patients.

ECG and Echocardiography

Standard 12-lead ECG recordings were examined for signs of ischemia, LVH, and rhythm or conduction abnormalities. In addition, 13 patients with the classic infantile form underwent echocardiography within 1 month after the plasma samples were taken (Sonos 5500 or 7500 ultrasound system, Philips, Best, The Netherlands), as well as 48 patients with nonclassic Pompe disease with a suspicion of LVH based on the ECG, or who already underwent this procedure during a previous study. The LV mass index (LVMI) was calculated and indexed by body surface area. We used previously published data as reference values for children and young adults. In addition, the LV end diastolic dimension, the LV end systolic dimension, interventricular septum, LV posterior wall, and fractional shortening were measured.

Human Tissue Biopsies

Skeletal muscle biopsies of patients with Pompe disease were taken from the quadriceps muscle before the start of ERT. Human adult heart and liver biopsies were obtained from the Department of Pathology of Erasmus MC. Biopsies from the quadriceps muscle of healthy controls were obtained from Maastricht University Medical Center.

Expression of cTnT

Tissue biopsies were homogenized in RLT buffer (Qiagen) at 4°C using a PRO200 disruptor. After 2x extraction using phenol/chloroform/isoamylalcohol (Sigma P8030), total RNA was isolated using the RNaseasy Mini Kit with DNase I digestion to remove genomic DNA (Qiagen, Cat. No. 74106). cDNA was synthesized using the iScript cDNA synthesis kit (Bio-Rad). cTnT mRNA expression was quantified using quantitative polymerase chain reaction (qPCR) with the iTaq Universeral SYBR Green Supermix (Bio-Rad) and 10 pmol/μL forward and reverse primers as described. Primers annealed to separate exons to ensure that only cDNA was amplified, rather than genomic DNA. Two primer sets were used for cTnT mRNA; the C-terminal primer set amplified the region encoding the C-terminal part of the cTnT protein that is common between different splice forms; the N-terminal cTnT primer set amplified the region encoding the N-terminal part of the cTnT protein that is known to be differentially spliced. The identity of cTnT qPCR products was determined by cloning the PCR fragment into a plasmid vector using topo cloning (Life Technologies) followed by sequence analysis on an AB3130 Genetic Analyzer (Applied Biosystems, Hitachi). Primer sequences are listed in Table I in the Data Supplement.

Mass Spectrometry

Muscle biopsies were homogenized in 100 μL 4x loading buffer (NuPAGE LDS sample Buffer, Life Technologies, NP0007) in a 2-μL Eppendorf tube filled with stainless steel beads (Qiagen, Cat. No. 69989) using a TissueLyser II (Qiagen, Cat. No. 85300) operated for 2.5 minutes at maximum power at 4°C. Beads were removed, homogenates were heated for 10 minutes at 70°C, cleared by centrifugation and separated on a 4% to 12% bis–tris gel using 3-Morpholinopropionic acid, sodium acid running buffer according to the manufacturer’s instructions (Invitrogen). One-dimensional SDS-PAGE gel lanes were cut into 1-mm slices using an automatic gel slicer and subjected to in-gel reduction with dithiothreitol, alkylation with chloroacetamide, and digestion with trypsin (Promega, sequencing grade). Nanoflow LC–MS/MS was performed on an ACQUITY UPLC system (Waters) coupled to either an Orbitrap Fusion Trubrid mass spectrometer (Thermo) operating in positive ion mode. Tryptic peptide separation was performed on a BEH C18 column (1.7 μm, 75 μm×250 mm) at 10000 psi using a linear gradient from 0% to 80% B (A=0.1% formic acid, B=80% v/v acetonitrile, 0.1% formic acid) in 70 minutes and at a constant flow rate of 200 nL/min.
The column eluent was directly sprayed into the ESI source of the mass spectrometer. Mass spectra were acquired in continuum mode, and fragmentation of the peptides was performed in data-dependent mode. The MS raw data were analyzed using the MaxQuant software (version 1.5.2.8). A false discovery rate of 0.01 for proteins and peptides and a minimum peptide length of 6 amino acids were required. The Andromeda search engine was used to search the MS/MS spectra against the Uniprot database (taxonomy: Homo sapiens, release February 2015) concatenated with the reversed versions of all sequences. A maximum of 2 missed cleavages were allowed and enzyme specificity was set to trypsin. Further modifications were cysteine carbamidomethylation (fixed) as well as protein N-terminal acetylation, methionine oxidation, and phosphor (STY; variable).

Statistical Analysis

The data were analyzed using SPSS 21. Data are presented as medians with interquartile ranges or numbers with percentages. We calculated the proportions of Pompe patients with increased cTnT, cTnl, CK, and CK-MB levels relative to the 99th percentiles of these parameters in the general population and the corresponding 95% confidence intervals (CIs). To compare differences in cTnT levels about sex, use of ERT, wheelchair use, and hypertension, the Mann–Whitney test was used. Median differences of cTnT levels before and after 2 years of ERT were compared using the Wilcoxon signed-rank test for paired samples. To calculate the relationship between cTnT and CK levels, the Spearman correlation coefficient $\rho$ was used. A $P$ value <0.05 was considered statistically significant.

Results

Study Population

The characteristics of 122 patients with Pompe disease are summarized in Table 1. All patients with the classic infantile form and the majority of patients with nonclassic Pompe disease received ERT at a median period of 5 years. None of the patients had renal failure, whereas 39% of the adult patients had hypertension.

Plasma Parameters and Cardiac Evaluation

One adult patient experienced exercise-induced chest pain, but cardiac evaluation did not reveal any abnormalities. Nevertheless, the cTnT level of this patient was elevated (36 ng/L, normally <14 ng/L) and remained stable throughout the next day. None of the other patients reported cardiac symptoms indicative of AMI. This event triggered this study to evaluate plasma and other parameters to monitor cardiac function in a large number of patients. The results are summarized in Table 2 and on an individual basis in Table II in the Data Supplement.

When compared with the 99th percentile, plasma cTnT levels were elevated in 82% (95% CI, 75%–88%) of all patients with Pompe disease. cTnT levels were elevated in 79% (95% CI, 56%–95%) of patients with classic infantile Pompe disease, 39% (95% CI, 16%–64%) of childhood-onset patients and 88% (95% CI, 82%–94%) of adult-onset patients. When using the sex-specific 99th percentile limits, cTnT levels were still elevated in 77% (95% CI, 66%–86%) of male patients and 88% (95% CI, 78%–95%) of female patients. In only 1 patient, who was asymptomatic, the cTnT level was below the limit of detection. Next, other parameters of cardiac abnormalities, including cTnl and CK-MB levels, were assessed. cTnl values were below the 99th percentile in all patients. When compared with the sex-specific 99th percentile limits, CK-MB levels were elevated in 54% (95% CI, 40%–68%) of male patients and 66% (95% CI, 54%–78%) of female patients.

In addition, ECG and echocardiography were assessed. In the adult-onset patient population, 73% of tested patients showed normal ECGs, whereas 89% of tested patients showed a normal LVMI assessed by echocardiography. Similar outcomes were seen for childhood-onset patients (77% with normal ECGs and 100% with a normal LVMI). In classic infantile patients, 79% of tested patients showed normal ECGs and 69% showed a normal LVMI. The other parameters that were measured by echocardiography are shown in Table 2. Overall, 87% of tested patients showed a normal LVMI, 98% showed normal values of LV end diastolic dimension and LV end systolic dimension, 82% showed a normal interventricular septum, 95% showed normal LV posterior wall, and finally 97% showed normal percentages of fractional shortening.

To determine to what extent cardiac abnormalities could cause elevated plasma cTnT, values were compared between patients with and without cardiac abnormalities. In adulthood, cTnT values did not differ between patients with normal and abnormal ECGs (31 versus 30 ng/L, $P=0.24$). The 4 adult patients with LVH on echocardiography showed cTnT levels of 7, 14, and twice 19 ng/L, whereas 28 of 31 adult-onset patients without LVH contained values >19 ng/L. Rhythm or conduction abnormalities without LVH were detected in 2 childhood-onset patients, with cTnT levels of

| Table 1. Patient Characteristics |
|---------------------------------|
| Total Group | Classic Infantile | Childhood Onset | Adult Onset |
| No. of patients (%) | 122 (100) | 14 (11) | 13 (11) | 95 (78) |
| No. of males (%) | 65 (53) | 8 (57) | 9 (69) | 48 (51) |
| Age, median (IQR), y | 48 (31–62) | 5 (2–10) | 14 (10–21) | 55 (44–64) |
| Disease duration, median (IQR), y | 14 (8–22) | 5 (2–10) | 9 (4–12) | 16 (12–25) |
| No. of patients on ERT (%) | 102 (84) | 14 (100) | 11 (85) | 77 (81) |
| Duration of ERT, median (IQR), y | 5 (4–6) | 5 (2–10) | 6 (3–8) | 5 (4–6) |
| No. of wheelchair-dependent patients (%) | 33 (27) | 5 (36) | 0 (0) | 28 (29) |
| No. of ventilator-dependent patients (%) | 35 (29) | 2 (21) | 0 (0) | 33 (35) |
| Systolic blood pressure, median (IQR), mm Hg | 127 (112–146) | 99 (95–107) | 109 (102–119) | 133 (120–150) |
| Diastolic blood pressure, median (IQR), mm Hg | 76 (67–87) | 57 (54–61) | 63 (60–67) | 79 (74–89) |

ERT indicates enzyme replacement therapy; and IQR, interquartile range.
Table 2. Plasma Parameters and Cardiac Evaluation

|                                | Total Group (n=122) | Classic Infantile (n=14) | Childhood Onset (n=13) | Adult onset (n=95) |
|--------------------------------|---------------------|--------------------------|------------------------|------------------|
| **cTnT levels, median (IQR), ng/L** | 27 (18–39)          | 22 (14–31)               | 9 (6–39)               | 29 (21–40)       |
| No. of patients with cTnT >14 ng/L (%) | 100 (82)            | 11 (79)                  | 5 (38)                 | 84 (88)          |
| **cTnT levels, median (IQR), µg/L** | <0.01 (<0.01 to <0.01) | <0.01 (<0.01 to <0.01) | <0.01 (<0.01 to <0.01) | <0.01 (<0.01 to <0.01) |
| CK levels, median (IQR), U/L | 358 (198 to 682)    | 779 (372 to 1165)        | 677 (350 to 839)       | 326 (183 to 530)  |
| No. of patients with CK >ULN (%) | 95 (78)             | 13 (93)                  | 12 (92)                | 70 (74)          |
| **cTnI levels, median (IQR), g/L** | <0.01 (<0.01 to <0.01) | <0.01 (<0.01 to <0.01) | <0.01 (<0.01 to <0.01) | <0.01 (<0.01 to <0.01) |
| **μCK levels, median (IQR), U/L** | 358 (198 to 682)    | 779 (372 to 1165)        | 677 (350 to 839)       | 326 (183 to 530)  |
| No. of patients with CK-MB >99th percentile (%) | 72 (59)             | 10 (71)                  | 5 (38)                 | 57 (60)          |
| **CK-MB levels, median (IQR), µg/L** | 7.4 (4.5 to 11.5)   | 11.2 (8.4 to 14.3)       | 4.5 (3.2 to 21.3)      | 7.1 (4.6 to 10.8) |
| ECG, number |                         |                          |                        |                  |
| Normal | 90                   | 11                      | 10                     | 69               |
| Abnormal | 21                   | 2                      | 2                      | 17               |
| Ischemic changes | 0                   | 0                      | 0                      | 0               |
| Left ventricular hypertrophy | 7                   | 1                      | 0                      | 6               |
| Rhythm or conduction disturbance | 14                  | 1                      | 2                      | 11              |
| Missing | 11                  | 1                      | 1                      | 9               |
| Echocardiography, number | 61                   | 13                     | 13                     | 35              |
| Left ventricular mass index (IQR), g/m² || 74 (63 to 87) | 74 (66 to 93) | 66 (58 to 82) | 78 (59 to 88) |
| Left ventricular end diastolic dimension (IQR), mm §| 45 (40 to 50) | 36 (28 to 41) | 47 (42 to 57) | 46 (41 to 51) |
| Left ventricular end systolic dimension (IQR), mm ‖| 28 (25 to 34) | 23 (18 to 25) | 31 (27 to 36) | 30 (27 to 34) |
| Interventricular septum (IQR), mm** | 8 (7 to 10) | 8 (7 to 10) | 7 (6 to 8) | 9 (8 to 10) |
| LVPW (IQR), mm** | 9 (7 to 10) | 6 (6 to 9) | 7 (6 to 8) | 9 (9 to 10) |
| Fractional shortening (IQR), %†† | 34 (31 to 41) | 37 (33 to 43) | 34 (33 to 40) | 33 (29 to 40) |

CK indicates creatine kinase; CK-MB, creatine kinase–myocardial band; cTnT, cardiac troponin T; FS, fractional shortening; IQR, interquartile range; IVS, interventricular septum; LoD, limit of detection; LVID ED, left ventricular end diastolic dimension; LVID ES, LV end systolic dimension; LVMI, LV mass index; LVPW, LV posterior wall; and ULN, upper limit of normal.

*99th percentile <14 ng/L in the general adult population, LoD, 5 ng/L.
†99th percentile <0.04 µg/L in the general adult population, LoD, 0.01 µg/L.
‡References ranges in our hospital: children (2–13 years) <230 U/L, male adolescents (13–17 years) <270 U/L, female adolescents (13–17 years) <123 U/L, male adults (>17 years) <200 U/L, and female adults (>17 years) <170 U/L.
§Reference ranges FS: an FS <25% was considered as insufficient shortening of the ventricle for every group.

21 and 30 ng/L. However, 3 other childhood-onset patients without abnormalities contained strongly elevated cTnT levels of 48, 50, and 138 ng/L. One classic infantile patient showed a conductance disturbance on ECG (cTnT, 28 ng/L), and 4 other patients (numbers 7, 8, 9, and 12 in Table II in the Data Supplement) showed an increased LVMI assessed by echocardiography. Patients 7 and 12 had just started with ERT and showed elevated cTnT levels, whereas patients 8 and 9 had long-term LVH with elevated cTnT levels. Other patients in this group without detectable cardiac abnormalities contained strongly elevated cTnT levels ranging from 20 to 59 ng/L. Taken together, the cardiac abnormalities observed in a subset of patients with Pompe disease could not explain the elevated cTnT levels in the majority of patients. This suggested an alternative source of elevated cTnT in patients with Pompe disease independent of myocardial muscle damage.

**Plasma cTnT Levels Correlate With CK Levels**

Most patients with limb-girdle muscle weakness, including Pompe disease, have increased CK levels resulting from skeletal muscle damage. Figure 1 shows that CK and cTnT levels correlated in all the 3 subgroups (P<0.001). In adult patients, a slight negative correlation was found between cTnT levels and age (P=−0.29, R=0.005). cTnT levels did not differ when adult patients were grouped according to sex (P=0.10), treatment with ERT (P=0.29), wheelchair dependency (P=0.57), or hypertension (P=0.76). These findings suggested that similar to CK, cTnT is released from damaged skeletal muscle into the circulation in patients with Pompe disease.

**Expression of cTnT in Skeletal Muscle Tissue of Patients With Pompe Disease**

To determine whether cTnT is expressed in skeletal muscle tissue, reverse transcription qPCR analysis was performed. Primers were used that recognize a C-terminal sequence present in all known isoforms. Cloning of the reverse transcription qPCR product followed by sequence analysis confirmed the identity of the product (Figure IA and IB in the Data Supplement). Strong expression of cTnT was detected in normal heart.
tissue, whereas cTnT was not expressed in the skeletal muscle biopsies taken from healthy controls of several ages (Figure 2). In contrast, 2 of the 3 skeletal muscle biopsies taken from adult Pompe patients with elevated circulating cTnT showed strong expression of cTnT mRNA. The cardiac-specific marker MYBPC3 was expressed in heart but not in the Pompe skeletal muscle biopsies. Surprisingly, Albumin, which was highly expressed in liver tissue, was expressed in the skeletal muscle biopsy of Pompe patient 1, which was the same biopsy that failed to express cTnT. We speculate that this biopsy may have contained adipose tissue, known to express albumin.

cTnT is known to be alternatively spliced. Notably, isoform 6 is expressed in normal adult heart, isoforms 1, 7, and 8 are expressed in fetal heart, and isoform 7 is also expressed in diseased heart. To determine which cTnT isoform is expressed in skeletal muscle of patients with Pompe disease, N-terminal primers that flank the region of alternative splicing were used in reverse transcription qPCR. Agarose gel electrophoresis showed a single product of 129 nt for reverse transcription qPCR of normal cardiac and Pompe-derived skeletal muscle biopsies (data not shown). Cloning and sequencing revealed that the Pompe skeletal muscle product was isoform 6 (Figure IB in the Data Supplement), which is the cTnT isoform expressed in healthy heart.

To determine whether the presence of cTnT mRNA also leads to cTnT protein expression in skeletal muscle from patients with Pompe disease, mass spectrometry was used. In an unbiased proteomic screen, the cTnT-specific tryptic peptide DLNELQALIEAHFENR was unambiguously identified among many other peptides specific for fast and slow skeletal muscle troponin (Figure II in the Data Supplement). In conclusion, patients with Pompe disease have elevated mRNA expression of cTnT in skeletal muscle that is translated into cTnT protein, consistent with the possibility of leakage of cTnT protein from skeletal muscle into the circulation as a result of muscle damage.

**Effect of ERT on Plasma cTnT Levels**

ERT has been shown to improve skeletal muscle function in adult patients with Pompe disease. To determine the effect of
ERT on cTnT levels, 7 adult patients with Pompe disease were tested just before the start of ERT, after 1 and 3 months of treatment, and after 2 years. In 6 patients, cTnT levels decreased after the start of ERT (Figure 3A). Interestingly, CK levels closely followed cTnT levels in these patients (Figure 3B). At group level, median decreases of cTnT and CK during 2 years of ERT were 8 ng/L (P=0.03) and 136 U/L (P=0.02), respectively (Figure III in the Data Supplement).

Discussion

We found that plasma cTnT but not cTnI levels were increased in the majority of patients with Pompe disease, and that cTnT levels correlated with CK levels. Elevated cTnT levels could not be explained by cardiac abnormalities. cTnT was expressed in skeletal muscle biopsies taken from patients with Pompe disease, but not from healthy individuals. We conclude that increased plasma cTnT levels in patients with Pompe disease are most likely the result of skeletal muscle damage, rather than myocardial damage.

Cardiac troponins are the preferred biomarkers for detection of AMI. According to an expert consensus document for the universal definition of myocardial infarction, AMI should be diagnosed if a rise or fall of cardiac biomarkers is detected (preferably cTnT or cTnI), with at least 1 value above the 99th percentile cut-off value. These changes need to be accompanied by symptoms of ischemia, ECG changes, imaging evidence of recent loss of viable myocardium, or new regional wall motion abnormalities, and identification of an intracoronary thrombus by angiography or autopsy. The next generation of high-sensitivity assays for detecting markers of myocardial damage in blood has improved the early detection of AMI. However, by increasing the sensitivity for detection of AMI, the specificity of detection has decreased leading to more false-positive results caused by pathologies potentially not related to AMI.

Increased plasma concentrations of cTnT have been described in individual cases and small sample size reports of patients with various myopathies. In some skeletal muscle myopathies such as Duchenne muscular dystrophy, the heart is known to be affected, which might explain increased cTnT levels. Hypertrophic cardiomyopathy is a characteristic feature in patients with classic infantile Pompe disease (but not in the childhood and adult forms of Pompe disease) and in most cases it is known to respond well to ERT. In our study, all classic infantile patients were already being treated with ERT (median, 5 years; interquartile range, 2–10 years), but in 4 patients LVH was not (yet) resolved at the time cTnT levels were measured. Patient 12 (Table II in the Data Supplement) had only received 2 infusions of ERT (cTnT, 39 ng/L) and patient 7 was treated for 6 months (cTnT, 20 ng/L). Both the patients showed normalization of LVMI values on prolonged ERT. Patient 9 (cTnT, 27 ng/L) started with ERT at the age of 7 months and was treated for 13 years. At the start of treatment, she already had a severe hypertrophic cardiomyopathy, which persisted over the years. Patient 8 showed an increased LVMI after 4 years of ERT (cTnT, 23 ng/L), but the LVMI was normalized after an additional 2 years of ERT. For these patients, the source of plasma cTnT levels, being heart or skeletal muscle derived, is unknown.

The high degree of similarity between members of the troponin family, especially between fast skeletal, slow skeletal, and cardiac troponin (http://www.uniprot.org), complicates the identification of cTnT in adult muscle biopsies using single antibodies because of potential cross-reactivity. Indeed, our initial experiments using 3 antibodies for cTnT yielded inconclusive results. Reports in the literature using antibody-based detection suggested that cTnT can be expressed in diseased skeletal muscle. Here, we used sequence-based techniques to exclude detection of troponin paralogs. This showed induced mRNA expression of the cTnT isoform 6 in skeletal muscle of patients with Pompe disease, which is normally expressed in the heart of healthy adults, rather than reexpression of an embryonic splice form. Mass spectrometry analysis confirmed expression of cTnT at the protein level. Although the mass spectrometry method is not quantitative and it is difficult to predict the ability to detect certain peptides, a higher number of cTnT peptides was anticipated based on the high-mRNA expression of cTnT in diseased skeletal muscle. This may indicate protein instability or a low efficacy of protein translation. A genome-wide mRNA expression study indicated elevated cTnT expression in skeletal muscle biopsies taken from untreated classic infantile patients with Pompe disease, who have LVH. cTnT expression in skeletal muscle from adult, childhood onset, and treated classic infantile patients with Pompe disease, who lack a cardiac phenotype,

Figure 3. Effects of enzyme replacement therapy (ERT) on plasma cardiac troponin T (cTnT) and creatine kinase (CK) levels. Seven adult Pompe patients with elevated plasma cTnT (A) and CK (B) levels at baseline and after start of ERT. Individual patients are indicated by a single color.
has not been reported to date. Accumulating evidence indicates that cTnT expression in skeletal muscle is a general phenomenon in several neuromuscular diseases. Because these disorders can have various causes, it is likely that a more general signal such as muscle tissue injury, rather than a disease-specific signal plays a role in induction of expression. Future experiments should elucidate how reexpression of cTnT in diseased skeletal muscle is regulated and whether it serves a biological function or is merely an aberrant side effect.

cTnI and CK-MB were measured as 2 other biomarkers for possible cardiac damage. It has been described that cTnI has a higher specificity for cardiac tissue than cTnT and that cTnI is not expressed in healthy or diseased skeletal muscle.6,13,40 Indeed, we did not find increased cTnI levels in our patients with Pompe disease. Therefore, cTnI might be a better biomarker for AMI in Pompe disease and possibly also other neuromuscular disorders, as suggested previously.9 CK-MB levels were increased in 59% of patients with Pompe disease. Elevation of CK-MB in patients with neuromuscular diseases of various pathogeneses without evidence of acute myocardial injury has previously been described as a result of regeneration after skeletal muscle damage, providing a possible explanation why these levels are also elevated in patients with Pompe disease.5,9,41

Our study has some limitations. First, although all findings point to skeletal muscle damage as being the cause of increased plasma cTnT levels in Pompe disease, we cannot fully rule out that there may have been subclinical myocardial damage in some patients. However, we consider all the evidence together sufficiently convincing to state that diseased skeletal muscle tissue is the source of elevated cTnT levels in patients with Pompe disease who do not have signs of cardiac involvement. Second, the reference values for cTnT measured with high-sensitivity assays are based on measurements in healthy adults, raising the question how these relate to values in childhood onset and infantile patients. Recent studies showed that cTnT levels measured with high-sensitivity assays are lowest in individuals <18 years of age and that cTnT levels increase with age, suggesting that the number of classic infantile and childhood-onset patients with elevated cTnT levels may even be underestimated in this study.42 Another limitation is that we did not use the high-sensitivity troponin I assay that is more sensitive than the assay we used. However, cTnI levels were low in our patient population (median <0.01 µg/L), whereas the 99th percentile limit in the general adult population is 0.04 µg/L, which makes it unlikely that the assay used here showed false-negative outcomes. A final limitation of our study is that we did not use magnetic resonance imaging to evaluate myocardial damage. We did not use this technique because a large proportion of the patients with Pompe disease had a decreased forced vital capacity, particularly in supine position, making it difficult for them to remain in this position in the magnetic resonance imaging for 30 to 60 minutes. Moreover, a recent study showed that there was no cardiac involvement on magnetic resonance imaging in a small group of adult patients with Pompe disease.42

Conclusions
In the majority of patients with Pompe disease, plasma cTnT levels are increased because of skeletal muscle damage, whereas myocardial damage is unlikely. Not only in Pompe disease, but likely also in other neuromuscular diseases, it is important to take this in consideration and to avoid unnecessary and potentially harmful cardiac interventions.

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