Clinical and Experimental Results on Cardiac Troponin Expression in Duchenne Muscular Dystrophy

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Background: Because of controversial earlier studies, the purpose of this study was to provide novel experimental and additional clinical data regarding the possible reexpression of cardiac troponin T (cTnT) in regenerating skeletal muscle in Duchenne muscular dystrophy (DMD).

Methods: Plasma from 14 patients (mean age, 7.5 years; range, 5.7–19.4 years) with DMD was investigated for creatine kinase (CK), the CK MB isoenzyme (CKMB), cTnT and cardiac troponin I (cTnI), and myoglobin. cTnT concentrations were measured by an ELISA (second-generation assay; Roche) using the ES 300 Analyzer. cTnI, myoglobin, and CKMB were measured by an ELISA using the ACCESS System (Beckman Diagnostics). Troponin isoform expression was studied by Western blot analysis in remnants of skeletal muscle biopsies of three patients with DMD and in an animal model of DMD (mdx mice; n = 6).

Results: There was no relation of cTnT and cTnI to clinical evidence for cardiac failure. cTnT concentrations remained below the upper reference limit in all patients. cTnT was increased (median, 0.11 µg/L; range, 0.06–0.16 µg/L) in 50% of patients. The only significant correlation was found for CK (median, 3938 U/L; range, 2763–5030 U/L) with age (median, 7.5 years; range, 6.8–10.9 years; r = −0.762; P = 0.042). Western blot analysis of human or mouse homogenized muscle specimens showed no evidence for cardiac TnT and cTnI expression, despite strong signals for skeletal muscle troponin isoforms.

Conclusions: We found no evidence for cTnT reexpression in human early-stage DMD and in mdx mouse skeletal muscle biopsies. Discrepancies of cTnT and cTnI in plasma samples of DMD patients were found, but neither cTnT nor cTnI plasma concentrations were related with other clinical evidence for cardiac involvement.

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in skeletal muscle specimens of patients with chronic
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recognized by these antibodies. Thus, there is still debate
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ies with other troponin isoforms was lacking, and it
motion or evaluation concerning the possible cross-reactivi-
blot analysis and immunohistochemistry using antibodies
found evidence of cTnT in biopsies from patients with
patients with end-stage renal disease
was demonstrated in human skeletal muscle from pa-
any developmental stage
and has not been detected in tissues other than heart in
It is, therefore, highly
specific for myocardial injury. TnI and TnT, together with
CtC, form a complex that regulates the calcium-mediated
interaction of actin and myosin in striated muscle. Both
TnI and TnT are encoded by three different genes that are
differentially expressed, namely, the slow- and fast-twitch
skeletal and the cardiac TnI isoforms (12, 13). Moreover,
in the human fetal heart, four cardiac TnT isoforms have
been observed that are generated by combinatorial alter-
native splicing of two 5' exons (14, 15). cTnT isoforms
have also been described in fetal human skeletal muscle,
but during the prenatal period, they are down-regulated
and skeletal isoforms of TnI are up-regulated. Thus, there
are no cTnT isoforms detectable in healthy adult skeletal
muscle (14).

Nevertheless, Baum et al. (16) found increased cTnT in
human serum samples of patients with renal failure, and
in a second study (4), they found increased cTnT in 8 of
33 patients with DMD without cardiac involvement. Sim-
ilar results have been reported by Kobayashi et al. (17),
who demonstrated increased cTnT concentrations in pa-
ients with polymyositis/dermatomyositis without car-
diac failure.

Similarly to CKMB, fetal isoforms of cTnI may be
reexpressed in regenerating skeletal muscle (17, 18). cTnT
was found in regenerating adult rat skeletal muscle after
injury or denervation (18), and cTnT protein expression
was demonstrated in human skeletal muscle from pa-
ients with end-stage renal disease (19). Bodor et al. (20)
found evidence of cTnT in biopsies from patients with
DMD, polymyositis, or renal failure. However, the results
of the above-mentioned studies were based on Western
blot analysis and immunohistochemistry using antibodies
that were not well characterized. For example, informa-
tion or evaluation concerning the possible cross-reactivi-
ties with other troponin isoforms was lacking, and it
remains unclear which epitopes of the TnI isoforms were
recognized by these antibodies. Thus, there is still debate
and residual uncertainty on possible reexpression of cTnT
in skeletal muscle specimens of patients with chronic
myopathies.

Because of controversial earlier studies, the purpose of
this study was to provide novel experimental and addi-
tional clinical data regarding possible reexpression of
cTnT in regenerating skeletal muscle. DMD was chosen as
the prototype of myopathies with ongoing cycles of
muscle fiber degeneration and regeneration. DMD is a
degenerative X-chromosome-linked disease of skeletal
muscle that usually causes death around the age of 20 via
cardiomyopathy (CMP) or respiratory failure (21). This
severe muscle-wasting disease results from mutations in
the gene encoding for dystrophin, a cytoskeletal protein
under the sarcolemma of skeletal and cardiac muscle
fibers. The absence of dystrophin is proposed to lead to
sarcolemmal instability and muscle cell necrosis (21, 22).
We tested plasma and muscle biopsies of patients with
DMD and skeletal muscle specimens of adult mdx mice, a
widely studied animal model for DMD, for cTnT and cTnI
using commercially available immunoassays and immu-
noblotting with well-characterized antibodies.

Materials and Methods

Samples

mdx mice. Musculus quadriceps femoris specimens of six
male mdx mice (8–10 weeks of age) were obtained from
the Department of Anatomy, University of Vienna. There,
PCR-restriction fragment length polymorphism analysis
was done to verify the mutation in the dystrophin gene,
and histochemical analysis confirmed the typical muscle
fiber degeneration pattern in skeletal muscle samples that
were used for Western blot analysis. Skeletal muscle and
heart muscle samples of C57 B1/10 mice were used as
controls.

Patients. EDTA blood was drawn from 14 patients (medi-
an age, 7.5 years; minimum–maximum, 5.7–19.4 years)
with DMD attending the Department of Pediatrics at the
University of Freiburg. The diagnosis of DMD was based
on standard clinical protocols of the Departments of
Pediatrics at the Universities of Innsbruck and Freiburg
based on the detection of the characteristic mutations in
the dystrophin gene by PCR testing and the work up of
muscle biopsy specimens demonstrating typical morpho-
logical changes and the absence of dystrophin. In addition,
remnants of skeletal muscle biopsies that were taken for
diagnostic purposes during the early stage of disease from
three patients (4.5–6 years; one tissue specimen from the
Department of Anatomy 3, University of Vienna, without
an available blood sample; two specimens from the De-
partment of Pediatrics at the University of Innsbruck)
with DMD were available for Western blot analysis. In all
patients creatinine was within reference values. A careful
clinical history, chest x-ray, electrocardiogram, and echo-
cardiogram were used to assess myocardial function.
Blood samples were immediately centrifuged at 2000g for
15 min at room temperature (25 °C) and frozen at 20 °C
until further analysis. Tissue samples were shock-frozen
in liquid nitrogen after excision and stored at −80 °C.
Healthy tissue from the heart and skeletal muscle and
nongravid uterus were obtained as negative controls at
the autopsy of a 15-year-old victim of an accident. Puri-
fied human cTnT (HyTest) served as positive control. All samples and tissue specimens were collected in accordance with the Helsinki Declaration of 1975 as revised in 1983.

**HOMOGENIZATION AND IMMUNOBLOTTING (WESTERN BLOT)**

Muscle tissue of mdx mice and patients was cut into small pieces in crude-muscle extraction buffer [10 μL of buffer for 1 mg of muscle; buffer composition was as follows: 1.784 g of Na2HPO4·12H2O (40 mmol/L), 0.02 g of MgCl2·6H2O (1 mmol/L), 0.038 g of EGTA (1 mmol/L), 0.002 g of phenylmethylsulfonyl fluoride (0.1 mmol/L), 0.0005 g of Leupeptin, 0.0005 g of Pepstatin A, 0.1 g of soybean trypsin inhibitor, and 100 mL of distilled water] at 4°C and further homogenized with a Potter (B. Braun) for 10 min. The homogenate was centrifuged at 1348 g for 10 min at 4°C, and the supernatant was frozen at -80°C until further analysis.

Protein concentrations were determined by the Bradford dye-binding procedure (cat. no. 500-0006; Bio-Rad Laboratories GmbH). Either 28 μg of mdx tissue homogenate or 34 μg of human tissue homogenate was loaded onto 12% ready-to-use SDS-Minigels (cat. no. 161-0900; Bio-Rad) and separated at 150 V and 14°C for ~90 min. Proteins were then transferred onto nitrocellulose by a MiniTransfer-Blot Electrophoretic Transfer Cell (cat. nos. 170-3930 and 170-3935; Bio-Rad) at 75 V and 4°C for 105 min.

Non-specific binding sites were blocked by incubating the nitrocellulose for 40 h at 4°C in 70 g/L bovine serum albumin (BSA) blocking solution (pH 7.4). The membranes were then washed three times with 1 mL/L Tween 20 (cat. no. 822184; Merck) in phosphate-buffered saline (PBS; pH 7.4). The nitrocellulose membrane was then incubated with one of three different monoclonal antibodies (MAbs) specific for cTnT [MAb M7 (stock solution, 8.5 g/L; gift of Roche, Penzendorf, Germany) at a 1:2000 dilution in PBS with 50 g/L BSA; MAb 7G7 (stock solution, 5.1 g/L; cat. no. 4T19; HyTest) at a 1:12 000 dilution in PBS with 50 g/L BSA; or MAb 1F2 (stock solution, 8 g/L; cat. no. 4T19; HyTest) at a 1:12 000 dilution in PBS with 50 g/L BSA] and one antibody that recognizes all TnT isoforms (Sigma clone JLT-12, cat. no. T-6277; Sigma Immuno Chemicals) at a 1:3000 dilution in PBS with 50 g/L BSA; or MAb 10F2, detection epitope 190–196, which is common to all three TnT isoforms 23] (gift of Sanofi Diagnostics Pasteur, Montpelier, France) at a 1:12 000 dilution for mdx tissue or a 1:6000 dilution for human tissue in PBS with 50 g/L BSA) was then added, and the membrane was incubated for 30 min. An antibody against cardiac and skeletal TnI (biotinylated MAb 10F2, detection epitope 190–196, which is common to all three TnI isoforms) was then added, and the membrane was incubated for 30 min in phosphate-buffered saline (PBS; pH 7.4). The nitrocellulose membrane was then washed three times with Tween 20 (3 mL/L for cTnT and cTnI and 1 mL/L for cTnT) in PBS (pH 7.4), and incubated with peroxidase-labeled secondary rat antibody anti-mouse IgG (cat. no. NA 931; Amersham Life Science) at a 1:6000 dilution for 20 min for all primary antibodies except for MAb 10F2. The membrane was again washed three times with Tween 20 (5 mL/L for cTnT and 3 mL/L for all other antibodies) in PBS (pH 7.4). Finally, the nitrocellulose membrane was incubated for 1 min with ECL™ substrate (cat. no. RPN 2106; Amersham Life Science) and exposed to x-ray films (Hyperlens™ ECL; cat. no. HP79NA; Amersham) for ~20 s. Molecular mass markers [Sigma Marker™ (low-molecular mass range; cat. no. M-3913), biotinylated SDS-MG-marker (cat. no. 100B-Kit; Sigma), or ECL protein molecular mass marker (cat. no. RPN 2107; Amersham)] as well as positive and negative controls were included in each analysis. The bands on x-ray films were evaluated visually as well as by scanning with a densitometer (Elscript 440; ATH Analyzentechnik Hirschmann GmbH) using the two-dimensional evaluation program.

**QUANTIFICATION OF cTnT, cTnI, MYOglobin, CKMB, AND CK**

Measurement of cTnT in plasma of patients was performed by second-generation ELISA on the ES300 analyzer (Roche) with MAb M7, the detection antibody specific for cTnT (4, 24). MAb M7 was also used for immunoblotting (see above). MAb M7 detects epitope 125–131 of cTnT. This epitope is in the middle of the molecule, and differences between human fetal and adult cTnT are found only within the first 30 amino acids. Therefore, MAb M7 recognizes both the adult and fetal isoforms of human cTnT. MAb M7 does not react with the amino acid sequences with other TnT isoforms, and therefore, it recognizes only the cardiac isoform of human TnT without cross-reactivity with the skeletal isoforms of TnT. The detection limit for this assay is 0.03 μg/L, and the upper reference limit in this assay are 0.30 and 6 μg/L, respectively, for CKMB. The reported intraassay imprecision (CV) at the lower end of the measuring range is 3.3% (at 0.27 μg/L), and the interassay imprecision is 5.4% (at 0.26 μg/L).

cTnI, myoglobin, and CKMB were quantified by ELISA on the ACCESS System (Beckman Diagnostics). The detection limit and the upper reference limit in this assay are 0.03 and 0.1 μg/L, respectively, for cTnI; 8.9 and 70 μg/L, respectively, for myoglobin; and <0.3 and 6 μg/L, respectively, for CKMB. The reported intraassay imprecision at the lower end of the measuring range is 5.6% (for 0.36 μg/L) and the interassay imprecision is 6.7% (for 0.30 μg/L). Total CK activity was measured by a standardized...
and optimized kinetic enzymatic method on a Hitachi 717 analyzer (Roche Diagnostics); activities at 25 °C are given.

**STATISTICS**

Nonparametric statistical methods were used to describe parameters, i.e., median, 25th, and 75th percentiles. The Spearman rank correlation coefficient ($r$) was calculated: $r \geq 0.8$ was considered a close correlation, $0.6 < r < 0.8$ a moderate correlation, and $r < 0.6$ a weak correlation. Results were considered statistically significant at $P < 0.05$.

**MDX MICE**

Hematoxylin and eosin staining showed the typical histological signs of myopathy, such as increased endomysial fibrosis and centronucleated fibers, which are characteristic for adult regenerated fibers. The muscle fibers also showed variations in diameter. Dystrophin staining with immunofluorescence confirmed the absence of dystrophin in mdx mice. Western blot analysis revealed no evidence of protein expression of either cTnT (MAb M7) or cTnI (MAb 11E12) in skeletal muscle samples of mdx mice (Fig. 1). Moreover, with non-isoform-specific antibodies for TnT (MAb JLT-12) and TnI (MAb 10F2), no cardiac isoform expression could be demonstrated in the same skeletal muscle samples, whereas the skeletal isoforms of TnT and skeletal TnI bands were clearly seen (Fig. 2).

**HUMAN SPECIMENS**

In human skeletal muscle samples of three DMD patients, no evidence of either cTnT protein (molecular mass ~39 kDa; MAb M7) or cTnI protein expression (molecular mass ~24 kDa; MAb 11E12) could be seen by Western blot analysis (Fig. 3). The absence of cTnT expression was also confirmed by two other anti-cTnT antibodies (MAbs 7G7 and 1F2). However, in the same specimens, we found strong signals for the skeletal isoforms of TnT (molecular mass ~31–33 kDa) and TnI (molecular mass ~21 kDa; Fig. 4). Thorough clinical histories, chest x-rays, electrocardiograms, and echocardiograms showed no evidence of cardiac involvement. The laboratory values for the two DMD patients attending the Department of Pediatrics at the University of Innsbruck were as follows: cTnT, 0.03

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**Fig. 1.** cTnT and cTnI protein expression in skeletal muscle samples from mdx mice.
Western blots of nondiseased murine heart muscle (lane HM), nondiseased murine skeletal muscle (lane SM), and skeletal muscle samples of mdx mice (lanes 1–6) were probed with cTnT-specific MAb M7 (A) and cTnI-specific MAb 11E12 (B). Lane MW, molecular mass marker.

**Fig. 2.** Cardiac and skeletal TnT and TnI protein expression in skeletal muscle samples from mdx mice.
Western blots of nondiseased murine heart muscle (lane HM), nondiseased murine skeletal muscle (lane SM), and skeletal muscle samples from mdx mice (lanes 1, 2, 5, and 6) were probed with nonspecific antibodies: MAb JLT-12 for TnT (A) and MAb 10F2 for TnI (B). Lane MW, molecular mass marker.

**Fig. 3.** cTnT and cTnI protein expression in a representative skeletal muscle biopsy from a patient with DMD.
Western blots of DMD patient 1 (lane 1), purified cTnT (lane cTnT), and nondiseased human heart muscle (lane HM) were probed with cTnT-specific MAb M7 (A) and cTnI-specific MAb 11E12 (B). Lane MW, molecular mass marker.
and 0.03 μg/L; cTnI, 0 and 0.02 μg/L; myoglobin, 1232 and 168 μg/L; CKMB, 526.5 and 133.4 μg/L; CK, 3865 and 1328 U/L, respectively.

Plasma from 14 DMD patients did not reveal any relation of cTnT and cTnI concentrations with clinical evidence for heart failure. One patient had been treated for CMP, and two had early-stage CMP without the necessity for drug treatment. cTnI concentrations remained below the upper reference limit in all patients (0.01–0.1 μg/L). Nine patients without CMP as well as two patients with CMP showed cTnI concentration below the detection limit (<0.03 μg/L). Three patients had detectable cTnI concentrations (two with CMP and one without CMP). The cTnT concentration was increased (median, 0.11 μg/L; maximum–minimum, 0.01–0.20 μg/L) in 50% of patients, and the highest concentration was found in a patient without any signs of cardiac failure. Two patients with early-stage CMP showed cTnT as well as cTnI concentrations within the reference interval. cTnT and cTnI concentrations did not differ between patients with or without CMP (Fig. 5). The only significant correlation was found for CK (median, 3938 U/L; range, 2763–5030 U/L) with age (median, 7.5 years; range, 6.8–10.9 years; r = 0.762, P = 0.004), an established marker of DMD disease severity. In contrast we found no correlation of CKMB, myoglobin, cTnT, and cTnI with age.

Discussion

In this study, we used different antibodies directed against various isoforms of TnT and TnI to evaluate cTnT and cTnI expression in skeletal muscle samples of mdx mice and patients with early-stage DMD. We also measured cTnT and cTnI in plasma of patients with DMD to compare these results with the results of Western blot analysis in tissue specimens. The antibodies used for immunoblotting in the present study are very well char-
characterized and specific for either cTnI (23) or cTnT (4, 25–27). MAb 11E12 recognizes residues 31–34 in the N-terminal part of cTnI, which is present only in the cardiac isoform of TnI, and MAb 10F2 recognizes residues 190–196 of the C-terminal part of cTnI (23). MAb M7 is the detection antibody of the second-generation ELISA for cTnT (ES300 Analyzer; Roche) (4, 24, 27). Because the amino acid sequence of the epitope detected by this antibody is not part of the amino acid sequence shared with the other cTnT isoforms, MAb M7 is very specific for human cTnT with no cross-reactivity with the skeletal muscle isoform. Moreover, the fetal cTnT isoform expressed in skeletal muscle is completely different from the adult isoform, but may be reexpressed in the failing human heart (14, 15).

There was no evidence of either cTnI or cTnT protein expression in skeletal muscle samples of both mdx mice and DMD patients. Additionally, we used three different well-characterized antibodies for cTnT, which showed equal results. However, in the same samples, there was strong evidence of expression of the skeletal muscle isoforms of TnI and TnT. These findings are in contrast to the recent report of Ricchiuti and Apple (28), who found expression of a 150-bp cTnT amplicon in two of five patients with DMD. Although they demonstrated amplification for cTnT (150-bp), they did not show protein expression for cTnT in these DMD patients. Furthermore, patients with DMD were not well characterized in this study and it is not clear whether skeletal muscle biopsies were obtained from early-stage or end-stage DMD patients. Therefore, there is still not sufficient evidence at the protein level (28) that cTnT is reexpressed in DMD patients. In our study, we obtained samples of biopsies that were performed at an early stage of the disease to confirm and establish the diagnosis of DMD. We could not obtain any material from autopsies of patients with DMD or of DMD patients with end-stage disease. We did not find any reexpression of human adult cTnT in skeletal muscle in the early stage of DMD, but we cannot comment on or exclude reexpression of cTnT in regenerating skeletal muscle of DMD patients with the advanced stage of the disease. In our study, we could only investigate skeletal muscle specimens of three DMD patients, but none of them, and none of the six mdx mice, showed any sign of cTnT protein expression in skeletal muscle.

Ricchiuti et al. (24) demonstrated the variable presence of cTnT isoforms in skeletal muscle specimens of patients with chronic renal diseases. They reported the expression of a high-molecular mass isoform of cTnT (39 kDa) detected by MAb M7 in some but not all skeletal muscle biopsies of these patients, and of low-molecular mass cTnT isoforms (~34–36 kDa) in 20 of 45 skeletal muscle biopsies of these patients detected by MAb 11.7, 13-11, and JS-2. In contrast, we could not find any evidence of cTnT expression using three different and well-characterized antibodies against cTnT, neither in human (DMD early stage) nor in mdx mouse (advanced disease stage) skeletal muscle specimens.

Although the murine model is genetically identical to human DMD, a limitation of the mdx model is that it does not strictly parallel the histopathology and progression of human DMD. In contrast to human DMD, in mdx mice there is minimal fibrosis and fatty tissue replacement, and cellular necrosis is long compensated by regeneration of muscle fibers. Necrotic fibers start to appear after 3 weeks and become numerous after 4 weeks. During the disease, a lack of synchronization has been described, i.e., a craniocaudal gradient was found in the appearance of necrosis followed by regeneration. The degeneration and regeneration of mdx muscle is reported to occur essentially between the 3rd and 10th week (21). The mdx mice in our study were 8–10 weeks of age, and the muscle fibers showed the characteristic histological signs of adult regenerated fibers. We demonstrated that the mdx muscle specimens investigated in our study were affected by the disease. Despite that fact, we did not find any reexpression of cTnT in the musculus quadriceps femoris specimens of these mdx mice.

These controversial results of rather small investigations in DMD patients will have to be clarified in larger, preferably multicenter, studies with study populations that should ideally cover the whole spectrum of DMD disease stages. However, our findings, obtained with specific antibodies and with well-characterized DMD patients, may contribute to a better understanding of reexpression of cardiac troponins in DMD. We demonstrated that cardiac troponins are not expressed during the early stages of DMD in humans and are not expressed in end-stage disease in the mdx mouse model.

Nevertheless, the second-generation cTnT assay showed increased cTnT values in >50% of DMD patients without clinical evidence for cardiac involvement. Similar results have been reported by Baum et al. (4) and Müller-Bardorff et al. (27), who found increased cTnT concentration in patients with muscular dystrophy. It was demonstrated by ELISA and Western blot analysis that the antibodies used in the second-generation assay for cTnT reveal no or only extremely minor reactivity with skeletal TnT (27). Thus, cross-reactivity with skeletal muscle TnT cannot be the reason for the increased cTnT concentrations found in patients without evidence of cardiac failure.

Despite the increased plasma cTnT values, we could not demonstrate any reexpression of cTnT in skeletal muscle of patients with early-stage DMD. A possible explanation is that increased cTnT may reflect subclinical myocardial damage that cannot be detected with the currently available clinical methods for diagnosing cardiac involvement. Patients with end-stage DMD are a high-risk group for dilatative CMP (29). Hence, the sensitivity (30, 31) of the cTnT assay or the diagnostic time window of cTnT may exceed the sensitivity of the cTnI assay or the diagnostic time window of cTnI and allow the
detection of minor myocardial damage with greater accuracy. cTnI increases and mostly peaks in parallel to cTnT after acute myocardial infarction (at least 4–5 days), but cTnT stays increased for at least 1 or 2 days longer than cTnI (32). However, we currently do not have convincing evidence for this hypothesis of a greater sensitivity of cTnT compared with cTnI from other clinical settings.

Similar discrepancies and unexpected increases of cardiac troponins have been also reported in patients with end-stage renal failure (33). It was suggested by the authors of that report that this discrepancy may in part be attributable to differences in the precision at the lower end of the measuring ranges of the assays used in their study. However, in contrast to their study, the troponin assays used in our study had a comparable imprecision at the lower end of the measuring range. Our cTnI ELISA on ACCESS (intraassay CV, 5.6% for 0.36 \( \mu \)g/L; interassay CV, 6.7% for 0.30 \( \mu \)g/L) shows a lower imprecision than the Stratus\textsuperscript{®} II analyzer. Therefore, discrepancies between troponin concentrations found in our patients are not likely to be attributable to differences in assay precision. The comparative prognostic value of cardiac troponins in patients on chronic hemodialysis has already been investigated in several smaller studies (34, 35). Both increased cTnT and cTnI appear to predict cardiac complications in this patient population, but the currently available published data do not allow the conclusion that one troponin is superior to the other for cardiac risk stratification in this clinical setting.

In the present study, the cardiac troponins in DMD patients did not correlate with other cardiac markers or with age, which is the best marker for the assessment of disease severity. CK showed a 20- to 50-fold increase in plasma samples of patients with DMD compared with controls and was the only marker that correlated closely with age. In previous studies, maximum activities for CK were observed in patients 2–5 years of age (36, 37), and with progression of the dystrophic process, activities in serum decreased (38). CKMB showed equally strong increases in plasma concentrations. We confirmed the previously reported high percentage of CKMB increases in patients with DMD; CKMB is not a suitable cardiac marker in DMD patients. Both troponins were markedly more specific markers in our patient population. This corroborates with earlier studies that reported increased CKMB in patients with DMD or with polymyositis (39–41). The increase in myoglobin is also consistent with earlier reports that describe higher CK and myoglobin concentrations in patients with DMD (42, 43).

In conclusion, no evidence for troponin reexpression was found in human (early-stage disease) and mdx mouse skeletal muscle (end-stage disease) samples. Discrepancies of cTnT and cTnI in plasma samples of DMD patients showed no relationship between both troponins and other clinical evidence for cardiac involvement. These clinical results obtained in a relatively small population cannot exclude cTnT reexpression and release from skeletal muscle in end-stage or advanced stages of DMD. The prognostic significance of plasma cTnT in patients with DMD remains to be clarified in larger prospective multicenter clinical trials.

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References


