Tako-Tsubo cardiomyopathy: intraindividual structural analysis in the acute phase and after functional recovery

Holger M. Nef1*,†, Helge Möllmann1†, Sawa Kostin2, Christian Troidl2, Sandra Voss2, Michael Weber1, Thorsten Dill1, Andreas Rolf1, Roland Brandt1, Christian W. Hamm1, and Albrecht Elsaesser1

1Department of Cardiology, Kerckhoff Heart Center, Benekestr. 2–8, D-61231 Bad Nauheim, Germany; and 2Max-Planck-Institute for Heart and Lung Research, D-61231 Bad Nauheim, Germany

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Aims To gain more insight into the phenomenon of Tako-Tsubo cardiomyopathy (TTC), the purpose of the present study was to investigate the myocardial structure in the acute phase of TTC and after functional recovery.

Methods and results We studied eight patients presenting with TTC diagnosed by coronary angiography, ventriculography, magnetic resonance imaging, and echocardiography. Serial myocardial biopsies were taken during the phase of severely impaired left ventricular function and after functional recovery. Specimens were examined by light and electron microscope as well as immunohistochemistry. Additionally, specific methods detecting different types of cell death and measurements of virus titer were performed.

All patients showed the typical contractile pattern of TTC and complete functional recovery within 12 ± 3 days. In ‘acute’ biopsies, many vacuoles of different size were found contributing to cellular hypertrophy. PAS staining revealed intracellular accumulation of glycogen. Additionally, structural deteriorations characterized by disorganization of contractile and cytoskeletal proteins could be detected. The extracellular matrix proteins were increased. Signs of oncotic and apoptotic cell death were absent. After functional recovery, all described alterations showed a nearly complete reversibility.

Conclusion TTC is accompanied by severe morphological alterations potentially resulting from catecholamine excess followed by microcirculatory dysfunction and direct cardiotoxicity. However, the affected myocardium represents a high potential of structural reconstitution which correlates with the rapid functional recovery.

KEYWORDS
Apical ballooning; Tako-Tsubo cardiomyopathy; Structural alterations

Introduction
A new cardiac syndrome which is characterized by a transient akinesia of the apex and compensatory basal hypertkinesis has been recently reported.1 Most reports originate from Japan, where the pattern of left ventricular (LV) dysfunction has been referred to as Tako-Tsubo cardiomyopathy (TTC).1 More recently, the term ‘transient LV apical ballooning’ has been used to describe similar cardiac contractile abnormalities in patients after emotional or physical stress.2–5 The clinical characteristics of this phenomenon have been described as follows: (i) acute onset of reversible LV apical wall motion abnormalities (ballooning) with chest symptoms, (ii) electrocardiographic (ECG) changes (i.e. ST-elevation), (iii) minimal myocardial enzyme release, and (iv) no significant stenosis of the epicardial vessels on coronary angiography.6

To date, several groups have investigated endomyocardial biopsies from both, right and left ventricle revealing myocyte injury and slight increase of connective tissue.1,4,6,8 However, a systematic investigation including electron microscopy and immunohistochemistry in order to specify these structural alterations is still lacking. More important, serial investigations of these morphological alterations in the acute phase and after functional recovery have not yet been performed. Thus, in the present study, we analyzed for the first time intraindividual biopsies taken in the acute phase and after functional recovery from eight patients.

Methods
Patient data and study protocol
Between March 2005 and April 2006, in 14 patients referred to our hospital with a suspected acute coronary syndrome TTC was diagnosed. All patients showed an acute onset of chest pain associated with transient ST-elevation and mild increase of cardiac enzymes. Coronary artery disease (CAD) was excluded angiographically and all patients showed the typical contractile pattern with severely
depressed contractile function due to apical akinesia diagnosed by ventriculography and echocardiography.

Among these patients, nine patients were included in this investigation after documenting right ventricular (RV) involvement by means of echocardiography and magnetic resonance imaging (CMR). In one patient, CMR could not be performed due to an implanted pacemaker. Four patients without RV involvement were excluded from the study. One patient refused participation in the study. Endomyocardial biopsies of RV were finally taken in eight patients during the acute phase of TTC and after recovery of contractile function. These patients had given written informed consent to their participation in the study, and approval of the Institutional Review Board had been obtained. The investigation conforms to the principles outlined in the Declaration of Helsinki.

Clinical methods

Coronary angiography and ventriculography

Coronary angiography was performed upon admission in Judkins technique with a 6F catheter. Standard projections were obtained. CAD was defined as >50% reduction in the lumen diameter. LV ejection fraction (LVEF), end-diastolic volume (EDV), and end-systolic volume (ESV) were calculated by means of the area-length method using a quantitative LV analysis (QLVA, CMS, Medis Medical Imaging Systems, Netherlands).

Transthoracic echocardiography

All examinations were performed using a Philips Medical Systems SONOS 5500 with a 2.5 MHz transducer or Philips Medical Systems IE33 for real-time 3D (RT3D) echocardiography. Consecutive measurements were carried out every 12 h until reconstitution of normal function. From the apical 2- and 4-chamber views, LVEF was calculated using the modified biplane Simpson method. For evaluation of regional wall abnormalities, the LV was divided into 16 segments according to the American Society of Echocardiography. RV systolic function was also assessed visually using standard apical and subcostal views.

CMR imaging

A 1.5-T scanner (Siemens Sonata®, Erlangen, Germany) and a six element phased-array surface coil were used for all CMR studies. The patients were positioned supine, head first. After survey localizer sequences, cine images of the heart were acquired in standard 2-chamber, 4-chamber, LV outflow tract and short-axis orientations using a fast, ECG-gated breath-hold steady-state free precession sequence (TE 1.58 ms, TR 41.08 ms, flip angle >60°, slice thickness 6 mm). Wall motion abnormalities of LV were evaluated according to the 16-segment model. The RV chamber was assessed in an eight-segment model. On a stack of short-axis views (SLT 10 mm, no gap) spanning the entire LV, endo- and epicardial contours were drawn in each slice using commercially available software (Argus, Siemens®, Erlangen, Germany) for analysis of functional parameters such as EF, EDV, ESV, and stroke volume (SV) by multiplying the area with slice thickness according to Simpson’s method.

For viability imaging, contrast agent (0.1 mmol Gadolinium-DTPA/kg bodyweight, Magnevist®, Schering, Germany) was injected and after 10–15 min, imaging was performed by use of an inversion recovery 3D-TurboFLASH sequence (TR 440 ms, TE 1.25 ms, Flip angle 10°, optimized TI 270–310 ms, SLT 5–6 mm, 14 slices, 39 segments) covering the entire myocardium in 2-chamber, 4-chamber, and short axis planes.

Measurements of cardiac biomarkers

Upon admission, and subsequently every 12 h until discharge all patients gave blood samples from an antecubital vein into gel-filled tubes. Serum concentrations of N-terminal pro-brain natriuretic peptide (NT-proBNP) and troponin T (TnT) were measured using a one-step enzyme immunoassay based on electrochemiluminescence technology (Elecsys proBNP/Troponin T STAT Elecsys, Roche Diagnostics, Germany). The measurements of creatine kinase (CK) and CK-MB were performed following standard procedures.

Endomyocardial biopsies

Endomyocardial biopsies were obtained from each patient on the day of admission in the phase of severely depressed contractile function. Six biopsies ('acute' biopsies) were taken from the involved right side of the apical septum with a flexible bioprobe (Cordis, USA) via a right internal jugular venous approach. Additionally, two biopsies were taken from a non-affected septal region of the right ventricle (RV Con). Extraction of biopsies was monitored by RT3D echocardiography (Philips Medical Systems, IE33, Germany) facilitating an accurate and reproducible placement of the bioprobe within the RV. After reconstitution of myocardial function (12 ± 3 days) as documented by echocardiography and CMR repeated endomyocardial biopsies were gained from the same segments ('recovered' biopsies). The tissue was either immediately fixed in glutaraldehyde buffered with 0.1 mol/L Na cacodylate (pH 7.4, 440 mOsmol) for electron microscopy or immersed in liquid nitrogen for immunohistochemistry, RT-PCR, and the TUNEL method.

Control tissue

The control tissue was gained from donor heart for which suitable recipients were not found at the time of surgery. The age of the donors was 26, 31, and 39 years. Two donors were female, one male. All donors did not have chronic illness. They died after accidents. Histological findings of these hearts have been completely normal.

Detection of viral genomes

DNA and RNA were extracted simultaneously from frozen heart muscle tissue probes. RT-PCR was performed for detection of enteroviruses (EV) including coxsackieviruses and echoviruses, adenoviruses (ADV), parvovirus B19 (PVB19), human cytomegalovirus (CMV), Epstein-Barr-virus (EBV), chlamydia pneumonialae, and influenza virus A and B. As a control for successful extraction of RNA, primer sequences were chosen from the sequence of the glyceraldehyde-3-phosphate dehydrogenase gene.

Light and electron microscopy

Small tissue samples were embedded in Epon following routine procedures. Semi-thin sections were stained with periodic acid-Schiff’s reagent (PAS) for glycogen and evaluated in the light microscope. Ultra-thin sections were stained with uranyl acetate and lead citrate and both viewed and photographed in a Philips CM 10 electron microscope.

Immunohistochemistry

The tissue samples were mounted with Tissue Tek (Sakura Fine-tec) and cryosections were air-dried and fixed in acetone. All sections were incubated for 1 h at room temperature. Incubation with the first antibody (α-actinin EAS3, 1:100, Sigma; titin 12, 1:50, Sigma; titin z1/2, 1:100, Sigma; dystrophin Mandb8, 1:100; Sigma; dystrophin H300, 1:100, SantaCruz; fibronecin, 1:100, ICN; collagen-I, 1:100, Sigma; CD68 EBM11, 1:50, Dako; CD3 T3-8BS, 1:50, Dako; phallaidin, 1:100, Sigma; ubiquitin, 1:100, Dako; complement-9, 1:50, Novo Castra) was followed by treatment with biotinylated second antibody when non-directly labelled antibodies were used. The directly labelled antibodies were conjugated to Cy3. The last incubation was carried out with fluoresceisothiocyanate-linked streptavidin-cy2 (Rockland, USA). Nuclei were stained with Draq-5 (Molecular probes, USA). The sections were viewed in a Leica TCS SP laser scanning confocal laser
microscope (Leica, Germany) equipped with appropriate filter blocks using a Silicon Graphics Octane workstation (Silicon Graphics, USA) and three-dimensional multichannel image processing software (Bitplane, Germany).

Quantification of fibrosis
In laser microscopy (Leica Microsystems, Germany) fibrosis was quantified from fibronectin stained representative sections of the biopsies using Image J software version 1.35. Seven fields of vision, 40×, were randomly chosen. Contents were expressed in % of total myocardium.

TUNEL
For in situ detection of apoptosis, an In Situ Detection Kit (Roche, Basel, Switzerland) was used as described previously. The labelling procedure using a mixture of terminal deoxynucleotidyl transferase and reaction buffer containing digoxigenin-labelled dUTP was carried out according to the kit’s instruction.

Statistical analysis
All results are expressed as median and interquartile range (IQR). A non-parametric test for paired samples (Wilcoxon Rank Test) was used to compare the variables age, EF, EDV, ESV, and SV. The degree of fibrosis has been compared using a Friedman Test followed by Wilcoxon Rank Tests for multiple post-hoc testing. Because of the exploratory nature of this study, we made no adjustment to the significance level to account for multiple testing. The Pearson correlation coefficient was used to estimate the correlations between the differences of baseline and discharge serum levels of NT-proBNP and EF values. The statistical analysis was performed with SPSS 12 (SPSS, Chicago, IL, USA). A two-sided P-value of less than 0.05 was considered statistically significant.

Results
Patients' characteristics
The patients (six females and two males) were 70.5-years-old (IQR 62.0–76.0; Table 1). Prior cardiovascular history was uneventful, with no CAD, chest pain, myocardial infarction, valvular heart disease, or heart failure (Table 1). Each patient experienced a stressful incident on the day of admission. All patients reported sudden onset of chest pain mimicking an acute coronary syndrome. Symptoms rapidly improved within several hours after admission. The electrocardiogram revealed a significant ST-elevation initially in the anterior leads changing to T-wave inversion on day 1 after admission. All patients showed a marked prolongation of the QT interval (QTc 463 ms, IQR 431–490).

Clinical data
Echocardiography
Initial echocardiography showed a severely reduced LV function (EF 27.5%, IQR 22.5–30.0). A dynamic intraventricular obstruction with LV intracavitary pressure gradient was excluded in all patients. After 12 ± 3 days complete functional recovery could be documented (EF 62.5%, IQR 57.5–67.5; P = 0.0002). The qualitative evaluation of RV function in the subcostal projections documented impaired RV contractility in the apical and septal regions in all patients.
Coronary angiography
Upon admission coronary angiography revealed no or only a diffuse CAD without obstructive stenoses (>50%), or spontaneous vasospasm in all patients. Left ventriculography showed akinesia in the anterolateral, apical, diaphragmatic, and septal areas as well as a hypercontractile base. The median EF of the LV was 30.4% (IQR 27.3–36.0) as measured by quantitative LV analysis.

Magnetic resonance imaging
CMR was performed in all patients on the day of admission. According to the 16-segments LV model in all patients akinesia was present in segment 13, 14, 15, and 16; hypo- to akinesia was documented in segment 7, 8, 9, 10, 11, and 12. Normal function was present in segment 1, 2, 3, 4, 5, and 6. In the phase of severely depressed function (EDV 115.0 mL, IQR 111.0–117.5; ESV 69.0 mL, IQR 66.0–72; SV 45.0 mL, IQR 41.5–48.0) the late-enhancement CMR-imaging showed no increased signal intensity ruling out myocardial infarction or myocarditis. Evaluation of RV wall motion abnormalities revealed in all patients hypo- to akinesia predominantly in the apico-lateral segment (segment 1) according to the eight-segment model of RV function. Within 12 ± 3 days, complete recovery of functional parameters were present as documented by a significant decrease of EDV (EDV 93.0 mL, IQR 91.5–104.0, \( P = 0.006 \)) and ESV (ESV 64.0 mL, IQR 59.0–70.0; \( P = 0.002 \)). Concordantly, at follow-up RV function also showed complete restitution in all patients.

Time course of cardiac enzymes and biomarkers
Upon admission, in all patients TnT ranged from 0.04 to 0.51 with a median of 0.18 ng/mL (IQR 0.09–0.28; normal value, <0.03 ng/mL). In the following days, the measured peak TnT ranged from 0.21 to 1.67 with a median of 0.58 ng/mL (IQR 0.45–1.17). The maximum for CK varied from 148 to 454 U/L (normal value, <174 U/L), and for CK-MB from 24 to 51 U/L (normal value, <24 U/L), respectively, and declined to normal levels within 4 days. The peak NT-proBNP ranged from 1215 to 13747 ng/L, with a median of 4524 ng/L (IQR 2626–9239) and showed a gradual decrease in the following days. The decline of NT-proBNP serum levels at discharge showed a mild inverse correlation (\( r = -0.589 \)) to improvement of EF but did not reach statistical significance (\( P = 0.218 \)).

Detection of viral genomes
Viral genomes, such as EV, ADV, PVB19, CMV, EBV, chlamydia pneumoniae, and influenza virus A and B, were excluded by nested-PCR in all patients.

Light microscopy
All biopsies demonstrated structural alterations, showing different-sized myocytes, many of which were hypertrophied (>20 μm). PAS staining revealed large intracytoplasmic areas filled with glycogen in ‘acute’ biopsies. The positive staining for PAS was markedly reduced in ‘recovered’ biopsies (Figure 1); no positive staining was documented in LV-Con and RV-Con, respectively.

Electron microscopy
In electron microscopy, the main alterations observed in ‘acute’ biopsies included numerous vacuoles of different size and content in comparison with LV-Con and RV-Con leading to an enlarged diameter of the myocytes. Several vacuoles were filled with cellular debris, myelin bodies, and degradation products. The specific arrangement of cytoskeletal and contractile proteins was dissolved. The content of contractile material was reduced, and prevalently detected in the border area of the myocytes. Contraction bands were sporadically found. Clusters of mitochondria with abnormalities in size and shape and areas of non-specified cytoplasm were observed. The nuclei typically appeared rounded or oval either in the middle or in the border area of the cells. Cell swelling associated with a damage of the basal lamina, electronlucent nuclei clumped with chromatin, or damaged mitochondria with flocculent densities as typical signs of oncotic cell death were absent. Additionally, the interstitial space was widened and contained fibrotic material, including collagen fibrils, formations of cell debris, macrophages, and an increased number of fibroblasts. The myocytes in the ‘recovered’ biopsies showed a normal size, only few small vacuoles, a nearly normal rearrangement of the intracellular structures, and a regular composition of the myocardium (Figure 2).

Immunohistochemistry
Intracellular proteins
α-Actinin: In ‘acute’ biopsies, the protein was primarily located in the border area of the myocytes, whereas in particular around the nuclei of myocytes (arrows) were documented (B).
the ‘recovered’ biopsies, RV-Con, and LV-Con a regular distribution was documented (Figure 3A/B, data not shown for RV-Con, LV-Con).

Actin: In ‘acute’ biopsies, the amount of the contractile protein actin was reduced and a regional accentuated lack of specific labelling was observed (Figure 3C,E,G). Actin was displaced to the border area of the myocytes. In the ‘recovered’ tissue (Figure 3D,F,H), a homogeneous labelling was found resembling the normal findings in RV-Con and LV-Con (data not shown).

Dystrophin: In ‘acute’ biopsies, reduced staining with the dystrophin antibody specific for the amino-terminal dystrophin was documented in comparison with ‘recovered’ biopsies (Figure 3C/D), RV-Con, and LV-Con (data not shown). No difference was found using an antibody labelling the carboxy-terminal dystrophin (Figure 3E/F).

Connexin-43: The myocardial organization in ‘acute’ biopsies was disturbed and a profound loss of gap junctions was observed. In the ‘recovered’ tissue, the specific labelling for connexin-43 showed a reorganization of the cell–cell contact (Figure 3G/H).

Titin: ‘Acute’ biopsies were characterized by disappearance of titin from some myocytes and displayed a punctuate labelling instead of typical cross-striated pattern.

Figure 2  Electron microscopy of ‘acute’ biopsies showing numerous vacuoles of different sizes and contents (myelin bodies, residual cellular products), loss of contractile material, and areas of non-specified cytoplasm (A). The interstitial space was widened containing formation of cellular debris (B). In the ‘acute’ phase, formation of myelin bodies could be documented (C). In TTC contraction bands of sarcomeres were found (D). ‘Recovered’ biopsies showed a nearly complete rearrangement of contractile material with regularly distributed sarcomeres, normal nuclei, and mitochondria (E, F). vac, vacuole; svac, small vacuoles; N, nucleus; cyt, cytoplasm; mit, mitochondria; cd, cellular debris; mb, myelin bodies; sarc, sarcomeres; cb, contraction band.
After functional recovery, titin showed a homogeneous cross-striated sarcomeric pattern in most of the myocytes (Figure 4A–D).

### Extracellular proteins

**Fibronectin:** The ‘acute’ biopsies showed a widened interstitial space with large amounts of fibronectin separating the remaining myocytes from each other (Figure 5A/B).

**Collagen-1:** The amount of collagen fibrils was increased in ‘acute’ biopsies corresponding to fibronectin. In the ‘recovered’ biopsies, a slight reduction could be documented (Figure 5C/D), but collagen-1 was still increased in comparison to LV-Con and RV-Con.

### Quantification of fibrosis

The ‘acute’ biopsies showed a significant enlargement of the extracellular matrix (ECM) staining positively for collagen-1 (6.8%, IQR 5.1–7.7) in comparison with the control tissue (LV-Con: 2.4%, IQR 0.8–2.5, \( P = 0.012 \); RV-Con: 2.0%, IQR 0.95–2.6, \( P = 0.001 \)). After functional recovery, the amount of collagen-1 was significantly reduced to 3.4% (IQR 2.9–3.8, \( P = 0.001 \)) in comparison with the acute phase.

Quantification of fibrosis by fibronectin staining revealed in ‘acute’ biopsies 25.1% (IQR 21.9–27.9) and 17.5% (IQR 14.1–20.1, \( P = 0.007 \)) in ‘recovered’ biopsies. LV-Con (12.3%, IQR 8.3–13.2, \( P = 0.012 \)) and RV-Con (9.5%, IQR 8.8–12.2, \( P = 0.003 \)) showed a significantly lesser amount of fibronectin in comparison with the ‘acute’ biopsies.

### Inflammatory cells

Immunohistochemical staining for macrophages (CD68) showed several extracellular clusters in ‘acute’ biopsies, which were also detected in the ‘recovered’ myocardium (Figure 5E/F).

Accordingly, an increased number of T-lymphocytes (CD3) was detected in ‘acute’ biopsies in comparison to ‘recovered’ biopsies (Figure 5G/H), RV-Con, and LV-Con (data not shown).

### Protein degradation

In ‘acute’ biopsies several ubiquitin-positive cells were detected, whereas no large accumulations of ubiquitin-protein complexes were found. No differences were found in ‘recovered’ biopsies, LV-Con, and RV-Con (data not shown).

### Cell death

Complement-9 was neither found in ‘acute’ nor in ‘recovered’ biopsies, RV-Con, and LV-Con (data not shown).

### TUNEL

TUNEL-positive myocytes were neither detected in the ‘acute’ and ‘recovered’ biopsies nor in LV-Con and RV-Con (data not shown).

### Discussion

Several pathomechanisms have been proposed to explain the syndrome of the TTC but the underlying pathophysiological concept remains unclear. The idea that excess catecholamines could potentially trigger myocardial dysfunction has received significant attention in the past. The temporal relationship between a stressful event and the onset of the clinical syndrome seems to substantiate a significant link between catecholamines and TTC.

Since Wittstein et al.\textsuperscript{15} reported increased levels of plasma catecholamines and stress-related neuropeptides marking a sympathetic hyperactivity in patients presenting with TTC, the possibility of ischemia resulting from...
epicardial coronary arterial vasospasm or microvascular dysfunction has to be considered as a possible causative factor. Earlier studies suggest also a direct myocardial toxicity due to catecholamines as another pathophysiological mechanism.16,17

On the basis of our results, we conclude that in TTC, both catecholamine-mediated microcirculatory disturbance inducing ischemia and direct toxicity of catecholamines might be responsible for the observed alterations:

The increase of cardiac biomarkers TnT and CK as cytoplasmic proteins at the time of severe functional depression strongly suggest a damage of cardiomyocytes most probably resulting from ischaemia. Our findings of large cellular areas filled with glycogen indicate a severe energy deprivation which may also result from ischaemia.13 Moreover, the occurrence of multiple vacuoles and of ubiquitin demonstrates a disturbance in protein metabolism. These vacuoles might represent a compensatory mechanism protecting myocytes from self-destruction by eliminating deleterious material.18 However, we found no signs for cell death, such as apoptosis and oncosis as evidenced by TUNEL, C9 staining, and electron microscopy.19

A fact supporting a microcirculatory disturbance is the previously reported diminished coronary flow reserve using a Doppler guide wire.20 Additionally, a reduced coronary flow velocity in the absence of relevant coronary artery stenosis was also observed as measured by TIMI frame count method immediately after onset of TTC.21,22

In this study, the observed contraction bands, the increase in fibrosis, and the regional accumulation of inflammatory cells may account to the sympathetic overdrive. In TTC, plasma catecholamines were elevated at the time of presentation and significant higher when compared with patients presenting with Killip III myocardial infarction.15 It has been shown that oxidation of catecholamines results in the formation of highly toxic substances and free radicals causing intracellular calcium overload and myocardial cell damage.23

Our findings are in good correspondence to the typical histological signs of catecholamine toxicity which are described as focal mononuclear inflammatory, areas of fibrotic response, and characteristic contraction bands.24 Contraction bands have been reported in several clinical settings of extensive catecholamine production such as pheochromocytoma25 or subarachnoid haemorrhage,26 showing that catecholamines may be an important link between emotional stress and cardiac injury.15

Most notably, Khullar et al.27 investigated the short-term catecholamine effect on myocardium in an animal model (rhesus monkey). The histopathological examination revealed myofibrillar degeneration, myocytolysis and vacuolization with aggregation of lymphomononuclear cells.27

In our investigation, angiographic evidence for multiple spontaneous epicardial vasospasm was absent. A provocative test has not been performed given the poor clinical setting of the enrolled patients at the time of coronary angiography and

Figure 4 Immunolabelling for titin was performed using T12 (A, B) and Tz1/Z2 (C, D). F-actin (red) was visualized with TRITC—conjugated phalloidin and nuclei (blue) were counterstained with Draq-5. Note that titin in the 'acute' stage (A, C) is either absent in the central parts of the myocytes or shows a punctuated pattern as compared with a clear cross-striated pattern of labelling and higher expression levels in the 'recovery' phase (B, D).
the fact that the region of wall motion abnormalities did not correspond to the perfusion territory of a single coronary artery making this explanation very unlikely. This fact is also supported by other investigations documenting only a minority of patients presenting with spontaneous vasospasms in multiple epicardial coronary arteries in TTC. Additionally, Tsuchihashi et al. observed only in 21% of patients with TTC epicardial vasospasms inducible by provocative substances like acetylcholine in TTC.

The presented data demonstrate for the first time that TTC is also accompanied by reversible structural alterations. The rapid recovery of the contractile function within several days was accompanied with an equally fast reconstitution of the myocardial integrity as could be shown by a nearly complete normalization of the intracellular structural arrangement (α-actinin, actin, titin).

The observed accumulation of ECM in the myocardium potentially contributing to contractile dysfunction and to electrophysiological abnormalities seems to occur very rapidly but showed reduction already after an interval of several days. Most notably, the content of fibrosis in TTC did not reach a critical value not allowing to maintain the structural integrity. As published previously, a degree of fibrosis could be identified as a structural cut-off point making a functional recovery possible. Although the observed fibrosis during the acute phase may contribute to contractile dysfunction, the reduced degree in the latter biopsy explains that it does not affect the long-term regional wall motion.

In neonatal rat cardiac fibroblasts norepinephrine stimulated cellular proliferation through TGF-β up-regulation, which was followed by enhanced protein expression of fibronectin and collagen-1. In contrast, a norepinephrine infusion was also accompanied by transient increases of anti-fibrotic factors MMP-2 and TIMP-2 resulting subsequently in activated ECM turnover. Additionally, increased natriuretic peptides as evidenced in this study and others may have an anti-proliferative effect upon stimulated fibroblasts contributing to the dynamic process of ECM turnover which we documented in this study.

The normalization of the N-terminal dystrophin provides evidence for the rapid reverse remodelling by a reduction of mechanical stress. This reduction of cardiac wall stress is also reflected by the rapid decrease of NT-proBNP serum levels which were initially markedly increased.

In this investigation, only patients showing typical wall motion abnormalities of both, the left and the right ventricles were included. As previously documented by others, TTC is not restricted to LV alone but also RV involvement is commonly present. Therefore, we conclude that the biopsies taken from the RV side of the septum are equally valuable to investigate morphological alterations of TTC like LV biopsies.

In summary, this study shows for the first time that TTC is accompanied by severe morphological alterations potentially resulting from both, catecholamine-mediated microcirculatory disturbance followed by ischaemia and from direct toxicity of catecholamines. However, the affected myocardium represents a high potential of structural reconstitution which correlates to the rapid functional recovery.

**Limitations**

The presented study is rather of descriptive nature and has the inherent limitations of any small, observational case series. Although our study does not claim a full clarification of the pathomechanism, it still provides strong arguments for the previously proposed underlying mechanisms.

The non-diseased LV was tissue derived from three donor hearts for which suitable recipients were not found at the time of surgery. Although these donors were not completely healthy and considerably younger than the TTC collective in this study, the hearts are nonetheless valuable controls as...
evidenced by normal immunohistological and electron microscop-ic findings. However, the described findings were consistently found in eight patients in whom TTC was diagnosed using a broad spectrum of independent diagnostic tools.

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References


