Transpl Int (1998) 11 [Suppl 1]: S502–S507

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Assessment of troponin-T for detection of clinical cardiac rejection

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Abstract Non-invasive detection of cardiac rejection still remains a challenge after heart transplantation. We assessed troponin-T as a new serum marker to diagnose cardiac rejection. Twenty-five heart transplant patients (Berne) were monitored prospectively for up to 2 years, and compared to 89 retrospectively assessed patients (Stanford). Blood samples (392 Berne and 320 Stanford) were analyzed (creatine kinase, isoenzymes MB activity and MB mass, troponin-T and troponin-I). Regression analysis between the results of these blood samples and cardiac rejection grading from simultaneously performed endomyocardial biopsies was carried out. Troponin-T tests done in two different laboratories showed a good correlation ($r = 0.91$; $P < 0.0001$), whereas tropinin-T versus troponin-I showed a lower correlation ($r = 0.53$; $P < 0.0001$). Troponin-T and -I in contrast to other enzymes were elevated for a longer period (up to 4 weeks before returning to baseline) after transplantation than during conventional cardiac surgery. Beyond 3 months the following correlations were found between troponin-T (new or old test) and the other enzymes (creatine kinase: $r = 0.26$, MB activity: $r = 0.4$, and MB mass: $r = 0.68$). The correlation between the degree of rejection and the enzyme release is poor, however, the best results were obtained for troponin-T ($r = 0.22$; $P < 0.001$). We found a low correlation between troponin-T and the degree of rejection beyond 3 months after heart transplantation. Despite a troponin-T elevation in some patients with rejection, the new test is not sensitive enough to be used alone for the non-invasive diagnosis of cardiac rejection.

Key words Troponin-T · Cardiac rejection · Heart transplantation · Cardiac enzymes

Introduction

The diagnosis of acute cardiac rejection remains a major problem after cardiac transplantation. According to the international ISHLT registry, mortality of cardiac rejection in the early phase after transplantation is still high [1]. Furthermore, the differentiation between cardiac rejection and infection can be difficult. So far the diagnosis of rejection has been mainly based on the invasive endomyocardial biopsy technique first described by Caves followed by histological evaluation of the biopsy material [2, 3]. This method is invasive, time consuming, and cost intensive particularly since it has to be performed regularly in the early phase after transplantation.

Several non-invasive methods for detecting cardiac rejection have been assessed, but so far none is reliable enough to eliminate endomyocardial biopsy completely. These methods include echocardiography, nuclear magnetic resonance imaging and spectroscopy, electrocardiography with frequency analysis, cytoimmunological

monitoring, and a wide variety of serum assays [4–10]. Only echocardiography is widely applied in the postoperative monitoring of patients after cardiac transplantation, but when signs consistent with rejection are found endomyocardial biopsy is still recommended. Myocardial serum markers such as creatine kinase and its isoenzymes have been used in the past, but are considered bad predictors for the diagnosis of rejection [10]. For some years troponin-T, and more recently troponin-I, have been used as a standard new specific assay for detecting myocardial damage [11, 12]. Troponin is a myocardial protein of the contractile apparatus and has a high sensitivity and specificity for myocardial necrosis and even for myocardial ischemia [13]. Several authors have reported a higher specificity and sensitivity when compared to conventional myocardial enzymes such as creatinine kinase and its isoenzymes or myoglobin even perioperatively [12, 14].

Our group has published early experimental results showing a significant troponin-T elevation during cardiac rejection in the rat [15]. Several authors have assessed troponin-T clinically and found contradictory results [16–18]. Most groups report that the diagnosis of mild cardiac rejection is not reliable enough with troponin-T or troponin-I [16, 18]. The aim of this study was to assess troponin-T and -I before, during, and after cardiac transplantation and to correlate it to the patient's cardiac rejection score during the immediate and medium-term postoperative follow up. In addition, a comparison to other myocardial serum markers was performed.

Patients and methods

Prospective study at the University of Berne

Twenty-five heart transplant patients were monitored peri- and postoperatively for up to 2 years. Blood samples were drawn before transplantation, 6 h after, on days 1, 3, and 7 postoperatively, and thereafter each time an endomyocardial biopsy was performed. Thus 392 blood samples and 297 biopsies were available for evaluation.

This study had received institutional approval.

Retrospective study of patients at Stanford University

Three hundred and twenty blood samples of 89 patients all beyond 3 months after cardiac transplantation were sent to us for evaluation.

Assessment of serum markers

The following myocardial markers were used; their upper normal value at our institution is indicated in brackets:

1. Creatine kinase (CK 200 U/l, Boehringer Mannheim, Germany)
2. Creatine kinase isoenzyme MB (CKMB activity 20 U/l; Boehringer Mannheim, Germany)
3. CKMB mass (CK mass 4 µg/l, Stratus, Boehringer Mannheim, Germany)
4. Troponin-T (TNT 0.2 µg/l, Enzymun test; Boehringer Mannheim, Germany)
5. Troponin-T new (TNT 0.1 µg/l, Stat-test; Boehringer Mannheim, Germany)
6. Troponin-I (TNI 0.6 µg/l; Dade, USA)

Blood samples were all stored at –80 °C degrees and all tests were performed in duplicate. The diagnosis of cardiac rejection was assessed on specimens from endomyocardial biopsy. They were graded according to the ISHLT classification and the Texas score [3]. The calculations were performed using the Texas grading system only since it is linear from 0–10.

All patients received a triple regimen of immunosuppression (cyclosporine, Imuran, prednisone) after an antithymocyte globulin (ATG) induction therapy of 5 days. Mild rejections (1 a) were not treated. If a rejection episode was ongoing or more severe (grade 1 b ISHLT) treatment with 500–1000 mg Solumedrol i.v. on 3 consecutive days was administered. In three cases mycophenolate mofetil (Cellcept) was used for the treatment of acute rejection. One patient required plasmapheresis because of a presumed vascular rejection with severe hemodynamic compromise of the graft. There were 1.2 moderate rejection episodes per patient in the first 3 months. One patient died from organ failure and another from progressive severe rejection.

The Stanford patients were treated with triple therapy and showed very low grades of rejections (< 1 b ISHLT) since they were beyond 3 months after the cardiac transplantation, and none required treatment for rejection during the time of the study.

Statistics

All values are expressed as mean values ± 1 SD with the range in brackets. Regression analyses were performed using stata (Santa Monica, USA). Specificity and sensitivity tests were omitted since results of the regression analysis were poor.

Results

Prospective patients at the University of Berne

Table 1 shows the mean values of the myocardial markers and the rejection grading according to the Texas score during the 1st and 2nd, and beyond the 2nd, month after transplantation. All values during the 1st month were higher than the upper normal value for each assay. They dropped during the 2nd month and were always normal after the 2nd month. In contrast, the mean degree of rejection was slightly elevated and showed no change over time (Texas 1.6 ± 1.3). However, there were 0.4 episodes per month and patient with acute rejection requiring treatment during the first 3 months.

There was a significant correlation between troponin-T and the other myocardial markers overall or beyond 3 months after transplantation. The best correlation was found for the two troponin-T assays ($r = 0.88$ and 0.91) followed by troponin-T versus creatine kinase

Table 1 Myocardial serum markers after cardiac transplantation: variation with time for the patients in Berne (n = 392) (*POD* Postoperative day, *TNT* troponin-T, *CK* creatine kinase, *CKMB* creatine kinase MB)

Time	< 30 POD	> 30 POD	> 60 POD
TNT (μg/l)	1.75 ± 1.9	0.07 ± 0.4	0.03 ± 0.1
TNT new (μg/l)	1.55 ± 1.8	0.11 ± 0.6	0.019 ± 0.6
CK (U/l)	476 ± 738	112 ± 240	141 ± 273
CKMB (activity; U/l)	42.57 ± 44.5	14.8 ± 16.6	15.5 ± 18.9
CKMB (mass; μg/l)	22.99 ± 55.6	1.9 ± 3.7	1.94 ± 4
Texas score (0–10)	1.68 ± 1.58	1.64 ± 1.3	1.46 ± 1.2

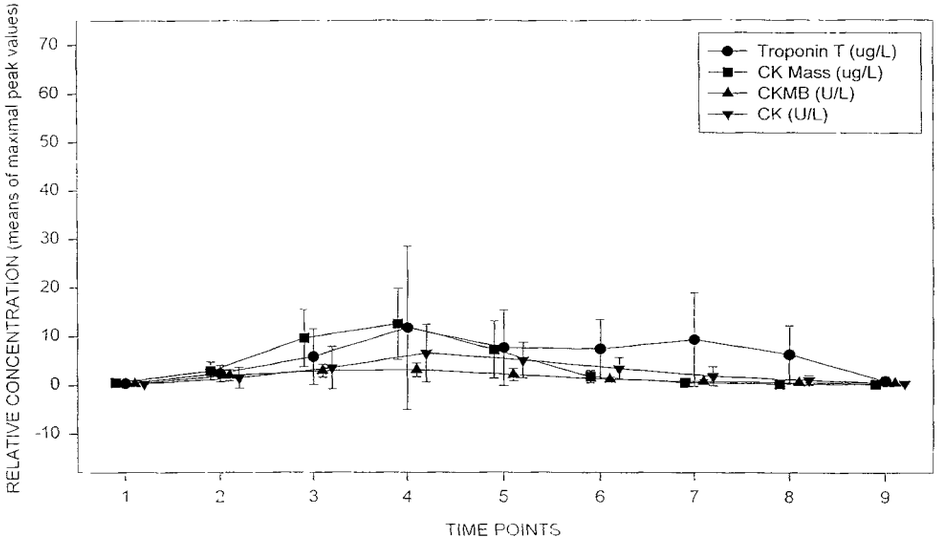
Table 2 Correlation (r) and significance (P) of troponin with other myocardial serum markers (*TNI* Troponin-I)

TNT vs serum marker	r	$P <$
University of Berne (n = 392) after 3 months		
TNT vs TNT new	0.88	0.0001
TNT vs CK	0.26	0.001
TNT vs CKMB (activity)	0.4	0.001
TNT vs CKMB (mass)	0.68	0.0001
Stanford University (n = 320) after 3 months		
TNT vs TNT new	0.91	0.0001
TNT vs TNI	0.44	0.001
TNT new vs TNI	0.53	0.0001
TNT vs CK	0.13	0.02
TNT vs CKMB (activity)	0.04	0.51

MB (CKMB) mass (r = 0.68), CKMB activity (r = 0.4) and creatine kinase (r = 0.26). The correlation between troponin-T and troponin-I was low (r = 0.53 and 0.44). (Table 2). Troponin-T values showed a prolonged and higher elevation after cardiac transplantation than after conventional cardiac surgery. In the latter case, if there is no significant perioperative ischemia, baseline values are reached within a few days and the curve is only rarely biphasic (see Fig. 1). In contrast, after transplantation these values often show a relative elevation of 10–24-fold and a biphasic curve, and return to normal values only after 2–3 weeks. We did not find a significant difference between the two troponin-T assays (Figs. 2, 3).

Correlation between the degree of cardiac rejection and the elevation in serum markers is poor despite most being significant in the regression analysis. The highest is found for troponin-T (r = 0.22; $P < 0.001$), followed by creatine kinase, CKMB activity, and CKMB mass (Table 3). Comparing patients with mild rejection (Texas score 0–3) to patients with moderate (Texas score ≥ 3) rejection, we found a significant difference with up to tenfold higher troponin-T values in the patient with moderate rejection. The difference in value of the other cardiac enzymes is not significant (Table 4). In three patients there was an elevation of both troponin assays which preceded, or were simultaneous with, the onset of endomyocardial rejection (Figs. 2, 3).

Fig. 1 Perioperative time course of mean values of cardiac markers for 29 patients undergoing heart surgery without ECG changes, at the following time points: *1* preoperative, *2* 1 h on cardiopulmonary bypass, *3* on arrival in the intensive care unit, *4* 6 h postoperatively, *5* first postoperative day (POD), *6* second POD, *7* third POD, *8* fifth POD, *9* at hospital discharge. Represented are the relative concentrations (discrimination = 1) of the mean values of maximum peak levels for troponin-T (μg/l), creatine kinase (*CK*; U/l), creatine kinase MB (*CKMB*) activity (U/l), CKMB mass (*CK Mass*; μg/l)



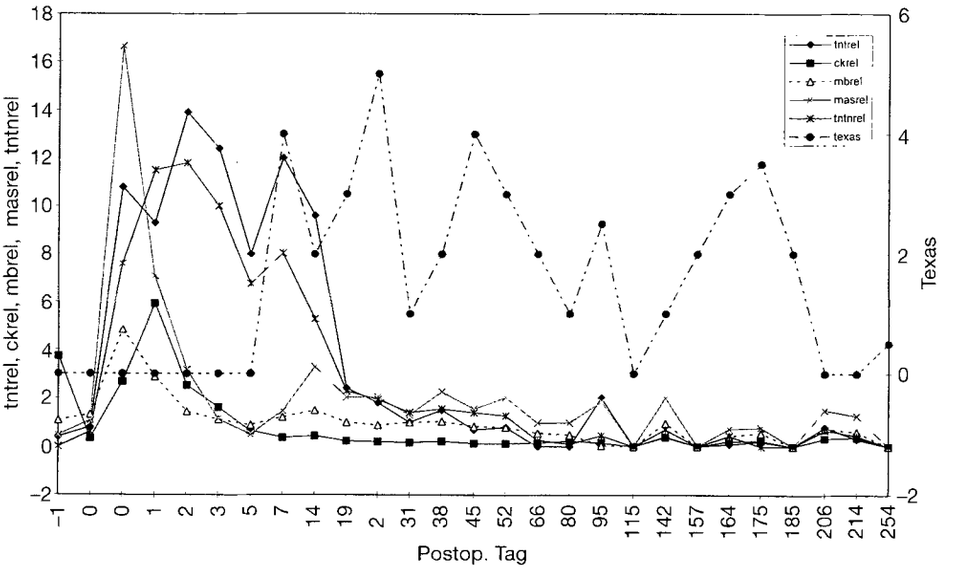


Fig. 2 A marked 10–14-fold elevation of the normal troponin-T and troponin-T new values is seen in the first days after transplantation with a biphasic character. On day 7 both troponin-T and troponin-T new show a de novo increase which goes along with a moderate rejection on biopsy. The decay is slowed down and takes up to 2 months until the values are normal. On day 95 and on day 206, troponin-T rises with documented ongoing or preceding an onset of acute cardiac rejection

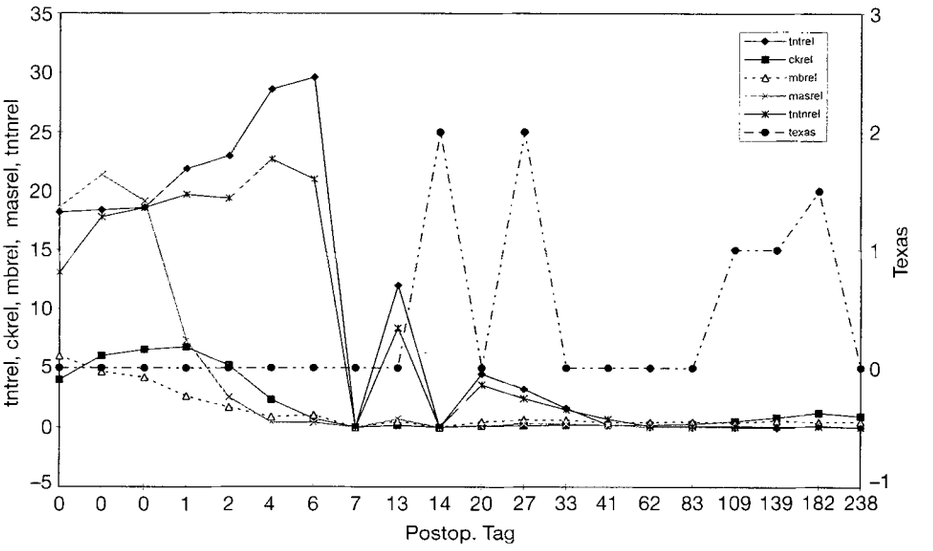


Fig. 3 Shows a patient course with a 30-fold increase of troponin-T and a 20-fold increase of the new troponin-T with an early normalization of these values at 1 week. This is followed by two de novo increases at 2 and 3 weeks postoperatively. Simultaneously there is a positive biopsy result of mild rejection

Figs. 2, 3 Time course of myocardial serum markers and degree of cardiac rejection. The *x-axis* represents postoperative days, the *left ordinate* the relative elevation for troponin-T (*TNTrel*), creatine kinase (*ckrel*), creatine kinase MB activity (*mbrel*), creatine kinase MB mass (*masrel*) and Troponin-T new (*tntnrel*); the *right ordinate* indicates the degree of rejection according to the Texas score. A score above 3 is considered to be moderate rejection

Results of the patients from Stanford University

The results of 320 blood samples drawn 3 months after transplantation were all in the normal range if the mean values are considered. The simultaneously diagnosed mean rejection grading was 0.6 ± 0.93 according to the Texas score (yielding a correlation of $r = 0.22$; $P < 0.001$). Similar correlation factors as described above were found among the myocardial enzymes. Thus a poor relationship was found between myocardial markers and the degree of rejection as described above. (Tables 2, 3).

Discussion

Troponin-T, and more recently troponin-I, has demonstrated the highest predictive value for myocardial ischemia with or without necrosis [13, 14]. A significant elevation was found in an experimental heterotopic

Table 3 Correlation (*r*) of the degree of rejection (Texas score) with various serum markers for the patients at Berne and Stanford Universities

Serum marker	Berne	Stanford
TNT	0.2*	0.22*
TNT new	0.17*	0.22*
TNI	–	0.19*
CK	0.18*	0.13*
CKMB (activity)	0.1*	0.02
CKMB (mass)	0.03	–

* $P < 0.001$ (regression analysis)

Table 4 Values of serum markers according to the degree of rejection (Texas) for the Berne patients ($n = 392$)

Serum marker	Texas score 0–3	Texas score > 3
TNT (μg/l)	0.02 ± 0.05*	0.22 ± 0.8*
TNT new (μg/l)	0.04 ± 0.24*	0.21 ± 0.7*
CK (U/l)	78.4 ± 105	237 ± 454
CKMB (activity; U/l)	12.4 ± 6.1	23.3 ± 32.2
CKMB (mass; μg/l)	1.4 ± 2.3	3.8 ± 6.4

* $P < 0.05$

transplantation model with an increasing degree of rejection [15]. This finding contradicts some clinical trials where no correlation between rejection and troponin-T elevation could be found [16, 18]. It must be emphasized that all degrees of rejection in the experimental paper showed histologically some degree of myocytolysis [15]. It is probable that the currently available troponin-T and -I tests do require myocardial cell membrane damage to release the cytosolic or later the bound troponin fraction in order to be detectable in the serum. Despite using two troponin-T assays (one being four times more sensitive than the commercially available test) the correlation between cardiac rejection and troponin-T did not improve. For this reason, calculations of sensitivity, specificity, and prediction value were not performed. Probably the use of mean values in this study at various time points after transplantation might have reduced the relevance of detection of cardiac rejection. If one considers the longitudinal course as shown in Figs. 2 and 3, troponin-T may well be an indicator for monitoring rejection. This is important especially since in three of our patients the test showed an elevation simultaneously or 1 week before the biopsy indicated cardiac rejection. This finding is in accordance with Hossein et al. who were able to show different levels of troponin-T according to the severity of rejection [17]. More recently, Dengler et al. have used a non-commercially available highly sensitive troponin ELISA test in 422 serum samples from 95 heart transplant recipients: their results showed a sensitivity of 78% and a specificity of 61% [19]. However, so far no other centers have been able to duplicate their results.

Comparing troponin-T to other myocardial serum markers we always found significant regressions, but suboptimal correlations. This is due to the fact that the kinetics and the specificity of myocardial damage show great variability depending on the markers used. The best correlation existed between troponin-T and CKMB mass ($r = 0.68$) followed by troponin-I ($r = 0.53$). Equally the best correlation was found, although poor, between troponin-T and rejection ($r = 0.22$). A further severe limitation of the use of troponin-T or troponin-I for monitoring patients after cardiac transplantation results from the fact that these markers stay elevated for a relatively long period after transplantation before returning to normal values as shown in this paper and by others [18]. This occurs normally only after 1 month and only if there is no ongoing rejection. Thus the test cannot be used in the critical early phase after transplantation when patients suffer the most frequent and severe episodes of rejection. On the other hand, patients beyond 3 months after transplantation rarely show acute rejection and if so, mainly mild which can hardly be detected with this test. Since the present test does not permit the diagnosis of mild rejection episodes, its value as a routine longitudinal screening test is questionable. On the other hand the value of troponin-T for screening potential heart transplant donors has been reported [20].

In conclusion, troponin-T and troponin-I show a low correlation to cardiac rejection. Thus they can hardly be recommended for the detection of rejection after human cardiac transplantation. Furthermore, soon after transplantation troponin-T or -I are highly elevated for a prolonged ill-defined period. Nevertheless, they showed the best correlation, although poor, to the degree of rejection compared to other myocardial serum markers. In patients with suspected rejection, troponin-T can be of great help since in three of our patients it was elevated before or during the onset of moderate cardiac rejection. More sensitive assays may improve this screening test in the future, allowing a non-invasive monitoring of cardiac rejection.

Acknowledgements The authors would like to express their thanks to Mrs H. Bockhoff for performing all the tests and to the staff of the Cardiology, Transplantation, and Clinical Chemistry Units.

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