Assessment of disease severity and outcome in patients with systemic light-chain amyloidosis by the high-sensitivity troponin-T assay

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Short title: High-sensitivity-TnT in AL amyloidosis

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Abstract

Cardiac biomarkers provide prognostic information in light-chain amyloidosis (AL). Recently a high sensitivity cardiac troponin T (hs-TnT) assay was developed that allows detection of minimal myocardial injury. We hypothesized that hs-TnT may improve risk stratification. Hs-TnT was assessed in 163 patients newly diagnosed AL. Blood levels were higher in AL patients with cardiac (83.7±11.0ng/L) than renal (28.6±6.7ng/L; p<0.05) or other (11.3±3.2ng/L; p<0.05) organ involvement and were related to the severity of cardiac involvement. 34 patients had TnT <0.01µg/L by the 4th generation assay, but hs-TnT above 14ng/L; however, this increased sensitivity was not associated with survival benefit. Forty-seven (28.8%) patients died during follow-up (22.3±1.0 months). Non-survivors had higher hs-TnT than survivors (112.8±16.0 vs. 52.5±7.4ng/L; p<0.001). Outcome was worst if hs-TnT ≥50ng/L and best <3ng/L. Survival of patients with hs-TnT 3-14ng/L did not differ from patients with moderately increased hs-TnT (14-50ng/L), but was worse if IVS was ≥15mm in these patients. Discrimination according to the Mayo staging system was only achieved by the use of the hs-TnT assay, but not by the 4th generation troponin T assay in this patient cohort. Multivariate analysis revealed plasma levels of hs-TnT, NT-proBNP as well as LV impairment as independent risk factors for survival. Hs-TnT and NT-proBNP remained independent predictors of survival after patients with markedly impaired renal function were excluded. Plasma levels of the hs-TnT assay are associated closely with the clinical, morphological and functional severity of cardiac AL amyloidosis in newly diagnosed patients and could provide useful information for clinicians on cardiac involvement and outcome.
Introduction

Light-chain amyloidosis (AL) is a plasma cell dyscrasia characterized by extracellular deposition of pathological insoluble beta-fibrillar immunoglobulin light-chains in diverse organs. Cardiac and renal involvement occurred in more than half of the patients diagnosed with AL. The extent of cardiac amyloidosis (CA) is the most important determinant of clinical outcome resulting in a median survival of about 6 months without therapy. About two-thirds of the patients with CA die from sudden death or congestive heart failure within the first year after cardiac symptoms have been occurred. Moreover, CA may be considered one of the most therapeutically refractory forms of heart failure since medical treatment strategies for heart failure are neither effective nor well tolerated and patients are ineligible for treatment approaches that are capable to rapidly stop the production of the amyloidogenic light chains, e.g. high-dose melphalan chemotherapy and autologous stem cell support.

Due to the potential impact of cardiac involvement for the prognosis of systemic AL several parameters describing the cardiac morphology and function have been described for risk assessment. In addition, cardiac biomarkers, e.g. plasma levels of cardiac troponin T and N-terminal pro-brain natriuretic peptide (NT-proBNP), provided potent prognostic information in patients with AL. Cardiac troponin T is a highly specific and sensitive marker of myocardial injury, while NT-proBNP may be considered a sensitive indicator of cardiac overload.

Hitherto commercially available cardiac troponin assays (4th generation) had a limit of detection of 0.01 µg/L with a recommended diagnostic threshold of 0.03 µg/L. Recently, improvements in the technology of cardiac troponin assays have allowed to provide fully automated assays with a limit of detection that is below the 99th percentile of the 4th generation troponin assays (3 ng/L) in a normal reference populations with a recommended diagnostic threshold of 50 ng/L. Even minor elevations in patients with acute coronary syndrome are associated with an increased risk of an adverse outcome and the use of the novel high-sensitivity troponin assays in patients with chest pain substantially improved the
early diagnosis and risk stratification of acute myocardial infarction by a single troponin measurement\textsuperscript{17,18}.

Thus, it is possible that even minor cardiac troponin elevations below the conventional limit of previous assays improve risk stratification in AL. Therefore, we retrospectively assessed a single measurement of plasma troponin T levels by the high-sensitivity assay (hs-TnT) in 163 patients newly diagnosed with AL to test the significance of the very low cardiac troponin T levels for risk assessment in patients with AL.

Methods

Between December 2005 and November 2008 163 patients (92 male, 71 female) were assessed at the Heidelberg Amyloidosis Center within 3 months after diagnosis of systemic AL. All patients included in the present analysis had biopsy-proven AL confirmed by Congo red staining and immunohistochemistry of any tissue specimen. In all patients plasma cell dyscrasia was documented by serum/urine immunofixation electrophoresis and serum free light-chain test (Binding Site GmbH, Schwetzingen, Germany).

Cardiac involvement was defined as an endomyocardial biopsy specimen containing amyloid (n=41) or by a history of congestive heart failure with myocardial wall thickening on cardiac ultrasound in the absence of arterial hypertension or valvular or coronary disease in a patients with extracardiac proven amyloidosis (n=61). Patients with extra-cardiac proven AL amyloidosis, but no definite exclusion as well as definite diagnosis of cardiac involvement by non-invasive methods were grouped as suspected cardiac involvement (n=24). Due to potential risk of pericardial effusion and lack of clinical consequence endomyocardial biopsies were not performed in this subgroup of patients to obtain definite diagnosis. Further organ involvement was defined according to the guidelines of amyloidosis\textsuperscript{19}.

Patients were evaluated by a detailed history, physical examination, standard blood tests (including estimated glomerular filtration rate according to the modified diet in renal disease formula\textsuperscript{20}), 12-lead electrocardiography, and echocardiography. Electrocardiography was analyzed for low voltage pattern that was considered present if no QRS complex deflection
was below 0.5 mV in any limb lead or the sum of the S-wave deflection in lead V1 and R-wave deflection in lead V5–6 was less than 1.5 mV\textsuperscript{21}. All transthoracic echocardiograms were analyzed for surrogate markers of cardiac amyloidosis (CA), e.g. left atrial diameter, diastolic interventricular septum thickness, diastolic posterior wall thickness, end-diastolic LV cavity diameter, LV end-systolic cavity diameter, LV systolic function, and pericardial effusion\textsuperscript{19}. LV function was assessed by echocardiography according to standard definitions\textsuperscript{22} and was considered markedly impaired at <45% by the Simpson method (2D/4D). Myocardial mass was computed by the Devereux formula and indexed to body surface area\textsuperscript{23}. An additional plasma sample of each patient was frozen at −80°C until it was thawed and immediately used for the hs-TnT assay.

Approval for the study was obtained from the Medical University of Heidelberg institutional review board, conforming to the Declaration of Helsinki\textsuperscript{24}. Informed consent forms were obtained from all patients prior to the investigation.

**Quantification of cardiac biomarkers**

Cardiac troponin T was measured using the 4\textsuperscript{th} generation assay and a novel high sensitivity assay (Roche Diagnostics, Mannheim, Germany) on an ELECSYS 2010 automated analyzer that uses chemiluminescence technology. The interassay coefficient of variation of the high sensitivity assay is 8% at 10 ng/L and 2.5% at 100 ng/L; the intraassay coefficient of variation is 5% at 10 ng/L and 1% at 100 ng/L. The diagnostic range of this assay is 3 to 10000 ng/L\textsuperscript{25,26}. The lower limit of detection for the 4\textsuperscript{th} generation assay is 0.01µg/L. The interassay coefficients of variation were 20% at 0.015µg/L, 10% at 0.03µg/L, and 5% at 0.08µg/L. NTproBNP was measured using an electrochemiluminescence immunoassay (Elecsys proBNP, Roche Diagnostics, Mannheim, Germany). The measurement range extends from 5 to 35.000pg/ml. The minimal detectable concentration is 5pg/ml, and the coefficient of variation is 5.7% at 64pg/ml.
Statistical analysis

Continuous data were expressed as mean±standard error of the mean (SEM). Categorical variables were expressed as absolute numbers and percentages. To test for significant differences between means, nonparametric Mann-Whitney U test or analysis of variance (ANOVA) with Newman-Keuls post test as appropriate after testing for normal distribution by the Kolmogorov-Smirnov test. Linear regression models were used to assess the influence of variables on hs-TnT. All tests were two-tailed and a p-value of less than 5% was regarded as statistically significant.

Receiver operating characteristics (ROC) curves for diagnosis of CA and all-cause mortality were used to assess the predictive accuracy of hs-TnT on the presence of CA and overall survival. Differences in overall survival, defined as the time between diagnosis and death from any cause, was assessed using log-rank analysis with right-censoring. Differences in overall survival were analysed using uni- and multivariable Cox-proportional hazard models and displayed by the Kaplan–Meier product limit method. In multivariate analyses, the main models were adjusted for variables that were associated with the clinical course of AL patients. A separate multivariate analysis excluding patients with markedly impaired renal function has been performed. Statistical analyses were performed using StatView (Version 5.0, SAS Institute, Cary, NC, USA).

Results

Patient Characteristics

The main characteristics of the 163 AL patients included in the study are shown in detail in table 1. In more than half of the patients three or more organs were involved at diagnosis of AL. Cardiac involvement was established in 102 (62.6%) and was suspected in further 24 (14.7%) of the patients. Renal involvement was present in 107 (65.6%) patients. Eighty-two patients (50.3%) had an IVS thickness of 15 mm or more. Mean troponin T plasma level was 69.7±7.2 ng/L by the hs-TnT assay and 0.07±0.01µg/L by the 4th generation troponin assay. A strong correlation between hs-TnT and TnT was observed (r=0.884; p<0.001). Among the
72 patients with negative TnT (<0.01µg/L), 33 had hsTnT plasma values above the 99th percentile of the reference population (14 ng/L). Remarkably, only in 10 (6.1%) patients hs-TnT plasma level were lower than the detection limit of the hs-TnT (3 ng/L).

High-sensitivity cardiac troponin T and cardiac involvement

Troponin levels in blood show a consistent increase with amyloidosis. Even in the patients without evidence of CA by clinical indices the median hs-TnT levels were above the 99th percentile of a normal reference population. In patients with suspected CA independent of further organ involvement mean hs-TnT plasma levels were significantly higher as compared to patients without CA, but lower than in patients with CA (figure 1). Highest levels were observed in patients with cardiac (and additional renal) involvement (figure 1). Mean plasma hs-TnT levels of patients with isolated renal involvement did not differ from patients with non-cardiac organ involvement, but were significantly lower than in patients with predominant CA (figure 2) and increased with deterioration of glomerular filtration rate (figure 3).

Hs-TnT plasma levels were associated with a number of different clinical indices reflecting cardiac AL mass and heart failure severity, such as New York Heart Association functional class (figure 4 A), LV systolic dysfunction (figure 4 B) as well as interventricular septum thickness (figure 4 C). Hs-TnT plasma levels were also higher in patients with more advanced CA as indicated by low voltage pattern (no LVP 53.4±7.0ng/L vs. LVP, n=37; 125.0±20.8ng/L; p<0.001), granular sparkling (n=72; 90.2±12.8ng/L vs. 56.3±7.9ng/L; p<0.05), or pericardial effusion (n=54; 99.2±10.5ng/L vs. 58.4±4.7ng/L; p<0.05). The association of plasma hs-TnT levels with the clinical variables were shown in table 2. These correlations remained significant after adjustment for renal function (data not shown).

The best cut-off value of hs-TnT for the diagnosis of cardiac involvement in AL amyloidosis was 20.1pg/L (ROC-AUC 0.80; 95% CI, 0.72 to 0.88). With this cut-off, sensitivity was 70.3%, specificity was 81.4%. Based on the prevalence of cardiac involvement observed in the present cohort (62.6%), the positive and negative predictive values were 86.4% and 62.1%, respectively.
Ninety-four (57.7%) patients were treated with potentially curative high-dose melphalan chemotherapy and autologous stem cell transplantation within 4.9±0.4 months after diagnosis of AL amyloidosis. These patients had lower hs-TnT plasma levels at diagnosis as compared to patients who were deemed ineligible for this treatment (104.6±112.6ng/L vs. 34.6±43.2ng/L; p<0.001).

Survival analyses

Forty-seven patients (28.8%) died during mean overall follow-up of 22.3±1.0 months. The mean follow-up for the survivors was 27.2±1.1 months and 10.7±1.5 months for the deceased patients. Deceased patients had a significantly higher plasma level of hs-TnT at diagnosis than survivors (52.5±79.3ng/L vs. 112.8±109.4ng/L; p<0.001). The hs-TnT cut-off value discriminating survivors and non-survivors with highest sensitivity (81.25%) and specificity (59.5%) was 70.9ng/L. The area under the ROC curve for the death was 0.71 (95% CI, 0.61 to 0.81). Outcome was worst in patients with plasma levels ≥50ng/L and best with plasma levels below the detection limit (<3ng/L). Interestingly, survival of the patients was similar survival with plasma hs-TnT from 3-14ng/L as compared to patients with hs-TnT plasma levels between 14 and 50ng/L, and in trend – but not significantly – different from the survival curve of patients with hs-TnT <3ng/L (figure 5 A). Additional information for risk assessment of this subgroup is given by the interventricular septum thickness (Figure 5 B). A second analysis excluding patients on dialysis revealed similar survival data (data not shown). Among the patients with troponin T below the 99th percentile of the 4th generation assay (<0.01µg/L) additional 34 patients had a troponin plasma level above the 99th percentile of the hs-TnT assay (>14ng/L). Outcome of these patients identified by the hs-TnT assay did not differ from survival of patients with plasma levels below the 99th percentile of the hs-TnT assay. A discrimination of a patient cohort at intermediary risk for survival according to the Mayo staging system10 was only achieved by the use of the hs-TnT assay, but not by the use of the 4th generation troponin T assay in this independent patient cohort (Figure 6 A and B).
In univariate analysis, age, autologous stem cell transplantation, glomerular filtration rate, LV ejection fraction, NYHA class, NT-proBNP, LVP, myocardial mass index, pericardial effusion, significantly affected overall survival. The Cox univariate model and the multivariate model with the highest prognostic power, according to model validation statistics are reported in table 3. The multivariate model with highest statistical power revealed NT-proBNP, hs-TnT, and LV impairment as independent parameters of survival (table 3, model 1). Both biomarkers remained independent predictors of survival in a separate multivariate analysis excluding patients with markedly impaired renal function (table 3, model 2). When troponin plasma levels assessed by the 4th generation assay were included in the multivariate analysis instead of hs-TnT values NT-proBNP, troponin T, LV myocardial mass and glomerular filtration rate were independent predictors of survival at diagnosis of AL amyloidosis (table 3, model 3).
Discussion

Risk stratification of patients with AL amyloidosis (and cardiac involvement) is crucial. In the past multiple clinical indices have been reported to be useful as indicators of an adverse outcome such as electrocardiographic, echocardiographic, and laboratory parameters. In the present study the diagnostic and prognostic power of a novel troponin T assay with higher sensitivity and lower limit of detection was evaluated in 163 consecutive patients with AL amyloidosis. We observed that a single hs-TnT determination at the time of diagnosis provides important information on morphology and function, as well as even prognosis of patients with AL. These findings also point to the clinical significance of very low detectable levels of troponin release (beneath the detection limit of the currently available 4th troponin T assay).

Cardiac troponin T has become the preferred markers for the diagnosis of acute myocardial infarction as it correlates with acute myocardial infarction risk and with infarct size. Furthermore, even small elevations in patients with non ST-deviation acute coronary syndrome as measured by the 4th generation troponin T assay are associated with an increased risk of an adverse outcome. The use of a novel high-sensitivity cardiac troponin (I and T) assay increases the diagnostic accuracy and discrimination for the early diagnosis of myocardial infarction by a single measurement as compared with a conventional troponin T assay and other markers of myocardial necrosis. In patients with clinically stable coronary artery disease cardiac troponin T levels measured by the hs-TnT assay were elevated in 21% and significantly associated with the incidence of cardiovascular death and heart failure.

These data were in line with reports from the ValHeft trial indicating a dose relationship of a single hs-TnT measurement with adverse outcome in patients with heart failure. Here we show another clinical cause of non-ischemic troponin elevation, namely AL amyloidosis. Cardiac involvement was detected in more than half of the patients with AL. The extent of CA is the most important determinant of clinical outcome. Plasma troponin levels were associated with poor outcome of patients with AL amyloidosis. Therefore, it seems
plausible that cardiac troponin levels below the conventional limit of detection may indicate early cardiac involvement and may further discriminate between subjects at high risk and those at low risk for survival.

High-sensitivity troponin T and severity of cardiac amyloidosis

As some of the tools for diagnosis of CA require very much experience for early detection of cardiac involvement, assessment of the severity of CA by a single blood test offers a valuable benefit for clinicians. In the present study plasma troponin T levels determined by a novel assay with improved sensitivity and lower detection limit was strongly associated with the presence and severity of cardiac involvement. Due to the increased sensitivity 34 patients with troponin T lower than the detection limit had troponin T above the reference value of healthy reference population by the hs-TnT assay most likely indicating minor cardiac amyloid deposition.

Interestingly, even in patients with AL amyloidosis but no apparent cardiac involvement by clinical criteria, mean troponin T levels were above the 99th percentile of normal reference populations. This might be explained by the low sensitivity of current clinical indices to detect cardiac abnormalities in AL\textsuperscript{1,31}. Patients presenting with renal, but no clinical evidence for cardiac involvement did not reveal significantly higher hs-TnT plasma level than patients with other non-cardiac organ involvement. Therefore, even slightly increased hs-TnT might indicate cardiac involvement escaping detection by echocardiography or electrocardiography. Endomyocardial biopsy which is still regarded as gold standard for diagnosis of CA, was performed in only 25% of the study patients. We recently demonstrated that a large number of patients with normal septum thickness had amyloid depositions in their endomyocardial biopsy specimens\textsuperscript{32}. Unfortunately, in this study we could not relate histological analyses to micro elevations of troponin T. This, however, needs further evaluation as improvement in the early diagnosis of cardiac involvement in such patients is of critical importance to initiate causative treatment early, e.g. high-dose melphalan chemotherapy and autologous stem cell transplantation\textsuperscript{33}. 
As demonstrated in the present study hs-TnT is associated in concentration dependent fashion with clinical, morphological, and functional state of the disease severity in a single parameter. Due to its wide measurement, the excellent reproducibility, and low costs, hs-TnT quantitation appears to be a valuable marker in the clinical routine for the evaluation of patients with AL amyloidosis. Since the extent of cardiac involvement is an adverse factor for autologous stem cell transplantation\textsuperscript{34-36}, hs-TnT may aid in patient selection to avoid high treatment-related mortality\textsuperscript{37}.

\textit{High-sensitivity troponin T and prognosis of patients with AL amyloidosis}

Determining levels of circulating cardiac biomarkers has been demonstrated to be a powerful tool for the clinical management of patients with AL\textsuperscript{38}. The data of the present study demonstrated that even minimally raised plasma cardiac troponins are associated with poorer prognosis of patients with AL amyloidosis that surpassed well established predictors of survival in patients with AL\textsuperscript{4-6,8,39}. Interestingly, outcome of patients with any detectable hs-TnT above the detection limit of 3ng/L but even below the 99\textsuperscript{th} percentile of normal reference populations was associated with a similar outcome as observed in patients with hs-TnT plasma levels between 14 and 50ng/L. Interventricular septum thickness is necessary for risk stratification of these patients with intermediary hs-TnT (3-50ng/L). Finally, there is a slight, but not significant, difference from the survival curve of patients with hs-TnT <3ng/L, most likely due to the limited patient number in this subgroup. Furthermore, by the use of the hs-TnT, but not 4\textsuperscript{th} generation troponin T assay in the present analysis a group of patients with intermediary risk for survival was identified according to the Mayo staging system\textsuperscript{10}. Theses differences might be explained due to the fact that Dispenzieri et al. excluded patients evaluated several years ago who did not undergo peripheral blood stem cell transplantation\textsuperscript{10}.

This emphasizes the impact of the use of the hs-TnT assay in patients with AL amyloidosis and indicates that in such highly selected and well characterized patients the hs-TnT method
may even be analyzed below the 99th percentile since even the levels in the upper range of a normal distribution seems to carry prognostic information.

Limitations

Many of the AL patients were first referred to our Amyloidosis Center several months after their initial diagnoses. Thus, there might be a bias in the present patient cohort as indicated by a high rate of patients with cardiac involvement in previous studies\textsuperscript{31} as well as the present study most likely due to the heart transplant program\textsuperscript{40} or due to poor health with inability to visit our Amyloidosis Center. To avoid upward bias in the survival curves only patients with diagnosis within the last three months were included in the present study.

The best sensitivity and specificity according to the ROC-AUC analysis is low; however, with a decrease in the diagnostic cut-off by implementation of more sensitive and precise assay, sensitivity is further increased, but specificity is decreased owing to detection of more acute, subacute, and chronic cardiac diseases\textsuperscript{29,30}. However, a multitude of differential diagnoses of elevated troponin T levels are excluded in the present study by the pre-selection of patients with AL amyloidosis; thus, there is no need for a high specificity in contrast to the diagnosis of myocardial infarction when the troponin assay is used as a diagnostic criterion. Serial measurement of cardiac biomarkers for the response to chemotherapeutic treatment is of paramount interest for risk stratification as it has been demonstrated for NT-proBNP\textsuperscript{11}; however, the present study was to evaluate the prognostic value of a single measurement of hs-TnT as it has been demonstrated for patients with stable coronary artery disease\textsuperscript{29} and stable heart failure\textsuperscript{30}. The impact of serial measurement of hs-TnT for risk prediction needs to be addressed in future studies.

Conclusions

In conclusion, this is the first report on the use of a novel hs-TnT assay in a large cohort of patients with newly diagnosed AL amyloidosis. Our data show that plasma hs-TnT values in AL patients are invariably associated with the severity of cardiac involvement. Additionally, this high-sensitivity assay could provide useful information for clinicians, particularly for the
improvement of risk stratification of AL patients according to the Mayo staging system as well as patients with insignificant troponin release, and probably may improve the early diagnosis of CA. Further studies are needed to evaluate hs-TnT plasma levels for monitoring the effect of treatment on cardiac involvement.

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**Author contributions**

Arnt V. Kristen analyzed and interpreted data, performed statistical analysis, wrote the manuscript

Evangelos Giannitsis contributed vital new reagents or analytical tools, wrote the manuscript

Stephanie Lehrke analyzed and interpreted data, performed statistical analysis

Ute Hegenbart performed research, collected data

Matthias Konstandin collected data

David Lindenmaier collected data

Corina Merkle collected data

Stefan Hardt analyzed and interpreted data

Philipp A. Schnabel analyzed and interpreted data

Christoph Röcken analyzed and interpreted data

Stefan O. Schonland designed research

Anthony D. Ho designed research

Thomas J. Dengler designed research, wrote the manuscript

Hugo A. Katus designed research, contributed vital new reagents or analytical tools
Conflict of Interest Statements:

EG has received financial support for clinical trials from Roche Diagnostics, Germany. He is consultant to Roche Diagnostics and receives honoraria for lectures from Roche Diagnostics. HAK has developed the cTnT assay and holds a patent jointly with Roche Diagnostics. He has received grants and research support from several companies, and has received honoraria for lectures from Roche Diagnostics.

References


(38) Dispenzieri A, Gertz MA, Kyle RA et al. Prognostication of survival using cardiac troponins and N-terminal pro-brain natriuretic peptide in patients with primary


Table 1: Clinical characteristics of the study patients

<table>
<thead>
<tr>
<th>AL patients (n=163)</th>
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<tbody>
<tr>
<td>age (years)</td>
<td>61.0±0.7</td>
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<tr>
<td>male/female</td>
<td>92 (56.4%) / 71 (43.6%)</td>
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<tr>
<td>body mass index (Kg x m$^{-2}$)</td>
<td>24.7±0.3</td>
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<td>lambda/kappa</td>
<td>128 (56.4%) / 35 (43.6%)</td>
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<tr>
<td>amyloid organ involvement</td>
<td>2.7±0.1</td>
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<tr>
<td>1 organ involved</td>
<td>23 (14.1%)</td>
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<tr>
<td>2 organs involved</td>
<td>55 (33.7%)</td>
</tr>
<tr>
<td>3 or more organs involved</td>
<td>85 (52.2%)</td>
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<td>dominant organ involvement</td>
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<tr>
<td>kidney</td>
<td>79 (48.5%)</td>
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<tr>
<td>heart</td>
<td>64 (39.3%)</td>
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<tr>
<td>liver</td>
<td>15 (9.2%)</td>
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<tr>
<td>peripheral nervous system</td>
<td>5 (3.1%)</td>
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<tr>
<td>soft tissues</td>
<td>14 (8.6%)</td>
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<tr>
<td>gastro-intestinal</td>
<td>7 (4.3%)</td>
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<tr>
<td>clinical evidence of kidney involvement</td>
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<td>EGFR (ml x s$^{-1}$ x 1.73m$^{-2}$)</td>
<td>61.8±2.7</td>
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<tr>
<td>EGFR $\leq$60 ml x s$^{-1}$ x 1.73m$^{-2}$</td>
<td>81 (49.7%)</td>
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<tr>
<td>eGFR $\leq$30 ml x s$^{-1}$ x 1.73m$^{-2}$</td>
<td>39 (23.97%)</td>
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<td>dialysis at study inclusion</td>
<td>27 (16.6%)</td>
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<tr>
<td>clinical evidence of heart involvement</td>
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<tr>
<td>heart failure NYHA class</td>
<td>1.7±0.1</td>
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<tr>
<td>NYHA I</td>
<td>31 (19.0%)</td>
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</table>
NYHA II  48 (29.5%)
NYHA III  33 (20.2%)
NYHA IV  1 (0.6%)
LA diameter (mm)  40.6±0.5
IVS thickness (mm)  15.5±0.3
myocardial mass index (g x m$^{-2}$)  61.7±1.5
fractional shortening (%)  33.2±0.8
ejection fraction <45%  30 (18.4%)
granular sparkling  72 (44.2%)
NT-proBNP (pg x mL$^{-1}$)  7796±1274

**Table 2: Correlation of hs-TnT with cardiac parameters in patients with cardiac amyloidosis**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Correlation co-efficient</th>
<th>p value</th>
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<tbody>
<tr>
<td>LA</td>
<td>0.321</td>
<td>&lt;0.001</td>
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<tr>
<td>IVS</td>
<td>0.239</td>
<td>0.027</td>
</tr>
<tr>
<td>LV-EDD</td>
<td>0.038</td>
<td>0.6411</td>
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<td>LV-ESD</td>
<td>0.073</td>
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<tr>
<td>LV mass index</td>
<td>0.371</td>
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<tr>
<td>LV dysfunction</td>
<td>0.300</td>
<td>&lt;0.001</td>
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<tr>
<td>FS</td>
<td>0.123</td>
<td>0.1828</td>
</tr>
<tr>
<td>NT-proBNP</td>
<td>0.706</td>
<td>&lt;0.001</td>
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</table>

LA left atrial diameter; IVS diastolic interventricular septum thickness; PW diastolic posterior wall thickness; LV-EDD left ventricular end-diastolic cavity diameter; LV-ESD left ventricular end-systolic cavity diameter; LV systolic function FS fractional shortening.
Table 3: Univariate and multivariate Cox proportional hazard models for overall survival in cardiac amyloidosis

<table>
<thead>
<tr>
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<th>multivariate model 3</th>
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<td></td>
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<td>p-value</td>
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<td>p-value</td>
</tr>
<tr>
<td>gender (male)</td>
<td>0.502</td>
<td>0.479</td>
<td>0.284</td>
<td>0.594</td>
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<tr>
<td>age (years)</td>
<td>4.103</td>
<td>0.043</td>
<td>1.982</td>
<td>0.159</td>
</tr>
<tr>
<td>number of organs involved</td>
<td>0.256</td>
<td>0.613</td>
<td>0.010</td>
<td>0.919</td>
</tr>
<tr>
<td>Λ-type of light-chain</td>
<td>1.061</td>
<td>0.303</td>
<td>2.171</td>
<td>0.141</td>
</tr>
<tr>
<td>LV ejection fraction (&lt;45%)</td>
<td>18.541</td>
<td>&lt;0.001</td>
<td>5.725</td>
<td>0.017</td>
</tr>
<tr>
<td>NYHA (&lt;3)</td>
<td>50.617</td>
<td>&lt;0.001</td>
<td>2.267</td>
<td>0.132</td>
</tr>
<tr>
<td>eGFR</td>
<td>5.696</td>
<td>0.017</td>
<td>3.644</td>
<td>0.054</td>
</tr>
<tr>
<td>hs-TnT plasma level</td>
<td>21.402</td>
<td>&lt;0.001</td>
<td>6.349</td>
<td>0.012</td>
</tr>
<tr>
<td>Troponin T (4th generation)</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
<td>XX</td>
</tr>
<tr>
<td>NT-proBNP plasma level</td>
<td>17.814</td>
<td>&lt;0.001</td>
<td>19.397</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>myocardial mass</td>
<td>7.065</td>
<td>0.008</td>
<td>1.800</td>
<td>0.180</td>
</tr>
</tbody>
</table>

Multivariate analysis model 1: all patients, hs-TnT

Multivariate analysis model 2: eGFR >30 mL/min*1.73, hs-TnT

Multivariate analysis model 3: all patients, 4th generation assay TnT
Figure legends

Figure 1  Comparison of high-sensitivity troponin T (hs-TnT) plasma levels of patients with light-chain amyloidosis and apparent (black bar), without cardiac involvement (white bar) or suspected cardiac involvement (grey bar). Data are mean±SEM. * p<0.05; ** p<0.01.

Figure 2  Comparison of high-sensitivity troponin T (hs-TnT) plasma levels of patients with light-chain amyloidosis and sole cardiac (black bar), sole renal (grey bar) as well as both, cardiac and renal (mixed bar) involvement in comparison to patients with involvement other than heart and kidneys (white bar). Data are mean±SEM. * p<0.05; ** p<0.01.

Figure 3  Comparison of high-sensitivity troponin T (hs-TnT) plasma levels of patients with light-chain amyloidosis according to the severity of renal impairment assessed by estimated glomerular filtration rate. Data are mean±SEM. * p<0.05; ** p<0.01; *** p<0.001.

Figure 4  Comparison of high-sensitivity troponin T (hs-TnT) plasma levels of patients with light-chain amyloidosis according to the severity of heart failure indicated by A) New York Heart Association functional class, B) impairment of left ventricular ejection fraction, and C) interventricular septum thickness. Data are mean±SEM. * p<0.05; ** p<0.01; *** p<0.001.

Figure 5  Association of hs-TnT plasma levels of patients with light-chain amyloidosis with survival stratified by 4 subgroups of patients (A). The subgroup of patients with hs-TnT plasma level between 3 and 50ng/L were stratified by the interventricular septum thickness above vs. below 15mm (B).

Figure 6  Association of troponin plasma levels of patients with light-chain amyloidosis with survival according to the Mayo staging system using the 4th generation (A) or the novel high-sensitivity (B) troponin assay.
Figure 1

hs-TnT plasma level (ng/L)

cardiac involvement

absent (n=37)  suspected (n=24)  apparent (n=102)

**  *  *

absent (n=37)  suspected (n=24)  apparent (n=102)
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Figure 2

Figure 2

hs-TnT plasma level (ng/L)

organ involvement

other (n=10)  

kidneys (n=27)  

heart (n=38)  

heart + kidneys (n=64)

*  

**  

*  

*
Figure 3

hs-TnT plasma level (ng/L)

≥ 90 (n=37)

59-30 (n=42)

≤ 14 (n=21)

29-15 (n=18)

60-89 (n=45)

estimated glomerular filtration rate (ml/min)

200

175

150

125

100

75

50

25

0
Figure 4A

hs-TnT plasma level (ng/L)

NYHA I (n=31)
NYHA II (n=48)
NYHA III (n=33)
NYHA IV (n=1)

***

0 25 50 75 100 125 150 175 200 225
Figure 4B

hs-TnT plasma level (ng/L)

≥ 55% (n=103)
35-44% (n=19)
≤ 34% (n=11)

left ventricular ejection fraction

*  
***  
**  
*  

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Figure 4C

hs-TnT plasma level (ng/L)

≤11 mm (n=19)

12-14 mm (n=62)

≥15 mm (n=82)

interventricular septum thickness
Figure 5A

Cumulative survival (%)

- hs-TnT < 3 ng/L (n=10)
- hs-TnT 3-14 ng/L (n=35)
- hs-TnT 14-50 ng/L (n=55)
- hs-TnT > 50 ng/L (n=63)

Time (months)

p<0.01
n.s.
Figure 5B

For patients with hs-TnT 3-50 ng/L:
- IVS <15 mm (n=58)
- IVS ≥ 15 (n=32)

Cumulative survival (%) vs time (months)

p<0.05
Figure 6A

cumulative survival (%)

cut-off cTnT 0.035µg/L, NT-proBNP 332 pg/mL

- stage I (n=39)
- stage II (n=65)
- stage III (n=59)

p<0.001

n.s.
Figure 6B

cut-off hs-TnT 50ng/L, NT-proBNP 332 pg/mL
logrank p<0.001

- stage I (n=37)
- stage II (n=55)
- stage III (n=71)
Assessment of disease severity and outcome in patients with systemic light-chain amyloidosis by the high-sensitivity troponin-T assay

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