A new staging system for cardiac transthyretin (ATTR) amyloidosis

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Abstract

Aims: Cardiac transthyretin (ATTR) amyloidosis is an increasingly recognised, progressive and fatal cardiomyopathy, the natural history of which remains unclear. We sought to establish and validate a new prognostic staging system applicable to patients with both wild-type (ATTRwt) and hereditary variant (ATTRv) ATTR amyloid cardiomyopathy.

Methods and Results: Eight hundred and sixty-nine patients with cardiac ATTR amyloidosis (553 with ATTRwt and 316 with ATTRv) attending the UK National Amyloidosis Centre, were stratified into 3 disease stages at baseline on the basis of cut points in two universally measured biomarkers, NT-proBNP and estimated GFR (eGFR). Stage I was defined as NT-proBNP ≤3000 ng/L and eGFR ≥45 ml/min, Stage III was NT-proBNP >3000 ng/L and eGFR <45 ml/min; the remainder were Stage II. The staging system was validated in a cohort of 318 patients with cardiac ATTR amyloidosis from France. Median survival among 393 (45%) Stage I patients was 69.2 months, 334 (38%) Stage II patients was 46.7 months, and 142 (16%) Stage III patients was 24.1 months ($p<0.0001$). After adjusting for age, compared to Stage I the hazard ratio (HR) for death for Stage II was 2.05 (CI: 1.54-2.72, $p<0.001$) and for Stage III was 3.80 (CI: 2.73-5.28, $p<0.001$). HRs and statistical significance were little altered by *TTR* genotype, and were maintained in the validation cohort.

Conclusions: This simple, universally applicable staging system stratifies patients with both ATTRwt and ATTRv amyloid cardiomyopathy into prognostic categories. It will be of value in the design of forthcoming clinical trials of novel amyloid-specific therapies.

Keywords: Amyloid, Amyloidosis, Transthyretin, TTR, Cardiomyopathy, Staging

Introduction

Cardiac transthyretin (ATTR) amyloidosis is an increasingly recognised cause of heart failure among older individuals.[1] It is an inexorably progressive and eventually fatal restrictive cardiomyopathy. The causative amyloid deposits in the heart may be composed of either wild-type (non-mutated) transthyretin or variant transthyretin (TTR), the latter associated with numerous mutations in the TTR gene.

Until recently, diagnosis of cardiac ATTR amyloidosis required demonstration of amyloid deposits within an endomyocardial biopsy.[2] However, advances in diagnostic imaging techniques, including cardiac magnetic resonance imaging (CMR)[3,4] and bone scintigraphy with technetium-labelled DPD,[5,6] HMDP,[7] and PYP[8] have enabled non-invasive, non-histological diagnosis of cardiac ATTR amyloidosis, resulting in a greater than 30 fold increase in diagnosis of this condition in our centre during the past decade. The non-biopsy algorithm for diagnosis of cardiac ATTR amyloidosis was recently validated in an international multi-centre study.[9]

Wild-type ATTR amyloidosis is a condition of older, usually male, individuals.[10] The commonest genetic variants associated with hereditary ATTR amyloid cardiomyopathy are TTR V122I, present in 3-4% of African Americans,[11] and TTR T60A, present in many populations with a frequency of up to 1% in one North Western Irish study.[12] Numerous other rare TTR variants are also associated with ATTR amyloid cardiomyopathy.[13] The diagnosis is made at different stages of the natural history of the disease, reflecting awareness within families and physicians, imaging and other resources, and heterogeneous presentations, but the course of both wild-type[10] and variant ATTR cardiac amyloidosis is ultimately that of gradually progressive cardiac failure and death within 10 years. Current management is symptomatic, centred on rhythm control, diuretics and careful regulation of fluid balance. Although there are no specific therapies as yet, a number of targeted anti–

amyloid agents, designed either to inhibit ATTR amyloid deposition or to clear away existing cardiac amyloid deposits, are now in phase 2 and 3 clinical trials and are showing great promise.[14-17]

Currently, there is no substantiated staging system for cardiac ATTR amyloidosis. Grogan and colleagues recently proposed a staging system using N-terminal pro-B-type natriuretic peptide (NT-proBNP) and troponin T (TnT) for wild-type ATTR amyloidosis but this has not been validated and has not been applied to variant ATTR amyloidosis.[18] Further, this system is confounded by current worldwide use of five generations of troponin assays made by various manufacturers, with different centres favouring different assays. These include newer high sensitivity troponin T (hs-TnT) assays, which not only differ in sensitivity but also give different numerical results to older assays, and troponin I assays.[19] We recently identified NT-proBNP and estimated GFR (eGFR), along with age, to be independent prognostic factors in cardiac ATTR amyloidosis[20] and sought to investigate and validate a staging system for cardiac ATTR amyloidosis which is applicable to both wild-type and variant ATTR amyloid cardiomyopathy based on these two simple, universally employed, biomarkers.

Methods

Patients

A retrospective analysis of 869 patients diagnosed with cardiac ATTR amyloidosis on the basis of the presence of heart failure in conjunction with either endomyocardial biopsy histology confirming ATTR amyloid or previously established non-biopsy diagnostic criteria,[9] who were routinely followed at the UK National Amyloidosis Centre, was performed to establish the staging system (test cohort). All patients had undergone sequencing of the *TTR* gene on the basis of which 553 were diagnosed with wild-type ATTR

cardiac amyloidosis (sub-group 1), 201 were diagnosed with V122I-associated cardiac ATTR amyloidosis (sub-group 2), and 115 were diagnosed with cardiac ATTR amyloidosis in association with other pathogenic TTR variants (sub-group 3); T60A (p.T80A) (n=86), S77Y (p.S97Y) (n=12), D38V (p.D58V), D38Y (p.D58Y), A97S (p.A117S), E42D (p.E62D), E54K (p.E74K), E54L (p.E74L), E89K (p.E109K), E89Q (p.E109Q), F44L (p.F64L), G53A (p.G73A), I107F (p.I127F), I68L (p.I88L), I73V (p.I93V), I84S (p.I104S), I84T (p.I104T), L12P (p.L32P), and Y69F (p.Y89F) in one patient each. Patients with V30M-associated ATTR amyloidosis, which often has a predominantly neuropathic phenotype without cardiomyopathy, were excluded from all analyses.

All patients were managed in accordance with the Declaration of Helsinki and provided informed consent for anonymous publication of scientific data.

Biomarkers

NT-proBNP was measured with an electrochemiluminescence sandwich immunoassay on the Elecsys system 2010 (Roche Diagnostics). GFR was estimated (eGFR) according to the standard MDRD formula (including the correction for race). High-sensitivity troponin T assay was performed with a second-generation assay after 16th December 2015, and prior to that, with a first generation troponin T assay.

Statistical Methods

The mortality endpoint was defined as time to death from baseline for all deceased patients and time to Censor date, 18th May 2017, from baseline among the remainder. Date of baseline was the same as date of diagnosis in >95% of patients and was within one month of diagnosis in nearly all remaining patients, such that median time from diagnosis to baseline was 0 months. Accuracy of occurrence and date of death among deceased patients,

and ongoing survival among those who were censored, was ensured on the basis of UK death certificate data from the UK Office of National Statistics.

In order to define the staging system, optimal cut points for relevant variables were chosen on the basis of a receiver operating characteristic curve and then by the Youden method. For NT-proBNP the optimal cut was 3806 ng/L which gave a sensitivity of 56% and specificity of 68%. A cut point of 3000 ng/L gave a sensitivity of 67% and specificity of 57%. The optimal cut point for eGFR was 49 ml/min/1.73m^2 (sensitivity 78%, specificity 38%) and a cut point of 45 ml/min/1.73m^2 (eGFR <45 ml/min/1.73m^2 defines chronic kidney disease stage 3b) gave a sensitivity of 84% and specificity of 32%. Median NT-proBNP in the test cohort was ~3000 ng/L and this cut point along with NT-proBNP of 4000 ng/L were both good discriminators of survival by log rank tests. Different cut points for eGFR alone (according to established CKD stages) were then tested for their discriminatory value for survival, and both 45 ml/min and 30 ml/min were discriminatory. Given the Youden method results, models using both NT-proBNP of 3000 ng/L and 4000 ng/L and eGFR 45 ml/min/1.73m^2 were then tested for their discriminatory ability and proportional representation. The eGFR cut point of 45 ml/min/1.73m^2 when combined with the NT-proBNP cut point of 3000 ng/L enhanced the discriminatory ability of all models of BNP alone (including an NT-proBNP tertile model), eGFR alone (including an eGFR tertile model) whilst dividing an acceptable proportion of patients into each of three stages. Kaplan-Meier plots were used to view survival from baseline in different groups. Cox proportional hazards regression analysis was used to compare the hazard ratios (HR) for the three stages in the whole cohort and separately for each of the three amyloid sub-groups, with age as a covariate. Results of the age-adjusted models are summarised using estimated hazard ratios with their 95% confidence interval (CI). Harrell's c-statistic was calculated to measure the discriminatory ability of each model.

The staging system was validated using a cohort of 318 patients, comprising all patients diagnosed with cardiac ATTR amyloidosis and followed at Mondor Amyloidosis Network, Henri Mondor Teaching Hospital, Créteil, France.

Two-sided tests were used for all analyses, and $P<0.05$ was considered significant. The data were analysed using Stata (StataCorp. 2015. *Stata Statistical Software: Release 14*. College Station, TX: StataCorp LP). The HRs from the previously published staging system using troponin T were determined by Cox proportional hazards regression analysis and compared to those of the currently proposed staging system. The unavoidable methodological limitations of this comparison, due to the replacement of an earlier generation troponin T assay by a high sensitivity troponin T assay on 16th December 2015, are acknowledged.

Results

Baseline characteristics of all patients in the test cohort are shown in Table 1. Seven hundred and thirty-five patients (85%) were male, and median age at baseline was 77 years. As expected, the majority of patients with wild-type ATTR and non-V122I-associated variant ATTR amyloidosis were Caucasian and nearly all of those with V122I-associated ATTR amyloidosis were of African ancestry.

Median follow up in the whole test cohort using a reversed survival model was 32.2 months (range 0.1-116 months) and median survival from baseline by Kaplan Meier analysis was 57 months (95% CI: 49.1 – 60.4 months). Two-hundred and eight-one patients died during follow up. Univariable and multivariable analyses of factors associated with death are shown in Supplementary Tables 1 and 2 respectively.

By univariable analyses, NT-proBNP >3000 ng/L and eGFR <45 ml/min/1.73m^2 were each found to be significantly associated with death (log rank test, $p<0.0001$; Figure 1). A

staging system was then created using biomarker cutoffs of 3000 ng/L for NT-proBNP and 45 ml/min/1.73m^2 for eGFR. Stage I was defined as NT-proBNP below or equal to the cutoff and eGFR above or equal to the cutoff, Stage III was defined as NT-proBNP above the cutoff and eGFR below the cutoff and the remainder were classified as Stage II. Three hundred and ninety-three (45%) patients were classified as Stage I, 334 (38%) patients were Stage II and 142 (16%) patients were Stage III. Survival probabilities by Kaplan-Meier analysis stratified by Stage for the whole test cohort are shown in Figure 2. Stage I patients had a median survival of 69.2 months (95% CI: lower limit 62.9 months, upper limit indeterminable), Stage II patients had a median survival of 46.7 months (95% CI: 40.2–57.0 months) and Stage III patients had a median survival of 24.1 months (95% CI: 21.2-29.6 months ($p<0.0001$ for Stage I vs II and $p< 0.0001$ for Stage II vs III). After adjusting for age, compared to Stage I, the hazard ratio for death was 2.05 (95% CI: 1.54-2.72, $p<0.001$) for Stage II, and 3.80 (95% CI: 2.73-5.28, $p<0.001$) for Stage III patients. The hazard ratio for death in patients with Stage III cardiac ATTR amyloidosis compared to Stage II cardiac ATTR amyloidosis was 1.86 (95% CI: 1.38-2.48, $p<0.0001$). Harrell's c statistic was 0.69 (Table 2).

Among the subset of 553 patients with wild-type ATTR cardiac amyloidosis (sub-group 1), 234 (42%) were classified as stage I, 219 (40%) were stage II, and 100 (18%) patients were stage III. Survival in wild-type ATTR amyloidosis by Kaplan-Meier analysis, stratified by Stage is shown in Figure 3. Stage I patients had a median survival that was indeterminable, Stage II patients had a median survival of 49.2 months (95% CI: lower limit 41.3 months, upper limit indeterminable) and Stage III patients had a median survival of 32.7 months (95% CI: 23.4-37.0 months) ($p<0.0001$ for Stage I vs II and $p=0.0003$ for Stage II vs III). After adjusting for age in wild-type ATTR amyloidosis, compared to Stage I, the hazard

ratio for death was 2.26 (95% CI: 1.51-3.36, p<0.001) for Stage II, and 4.37 (95% CI: 2.80-6.83, p<0.001) for Stage III (Table 2).

Among the subset of 201 patients with V122I-associated ATTR amyloidosis (sub-group 2), 89 (44%) were classified as stage I, 79 (40%) were Stage II, and 33 (16%) patients were Stage III. Survival in V122I-associated ATTR amyloidosis by Kaplan-Meier analysis, stratified by Stage is shown in Figure 4. Stage I patients had a median survival of 54.4 months (95% CI: lower limit 31.1 months, upper limit indeterminable), Stage II patients had a median survival of 28.8 months (95% CI: 23.6–45.1 months) and Stage III patients had a median survival of 17.7 months (95% CI: 11.5–22.3 months) (p=0.006 for Stage I vs II and p=0.013 for Stage II vs III). After adjusting for age in V122I-associated ATTR amyloidosis, compared to Stage I, the hazard ratio for death was 1.91 (95% CI: 1.17-3.12, p=0.009) for Stage II, and 3.48 (95% CI: 1.94-6.27, p<0.001) for Stage III patients (Table 2).

Among the subset of 115 patients with cardiac ATTR amyloidosis associated with variants other than V122I (sub-group 3), 70 (61%) were classified as Stage I, 36 (31%) were Stage II, and 9 (8%) patients were Stage III. Survival in non-V122I-associated variant ATTR amyloidosis by Kaplan-Meier analysis, stratified by Stage is shown in Figure 5. Stage I patients had a median survival of 76.7 months (95% CI: lower limit 69.0 months, upper limit indeterminable), Stage II patients had a median survival of 54.0 months (95% CI: 28.6–74.6) and Stage III patients had a median survival of 24.1 months (95% CI: lower limit 6.3 months, upper limit indeterminable) (p<0.02 for Stage I vs II and p<0.03 for Stage II vs III). After adjusting for age in non-V122I-associated variant ATTR amyloidosis, compared to Stage I, the hazard ratio for death was 2.28 (95% CI: 1.04-4.98, p=0.039) for Stage II, and 4.05 (95% CI: 1.54-10.64, p=0.005) for Stage III patients (Table 2).

The staging system was applied to the validation cohort of 318 patients with cardiac ATTR amyloidosis; this cohort consisted of 186 patients with wild-type ATTR amyloidosis,

68 patients with V122I-associated ATTR amyloidosis and 64 patients with 21 other known pathogenic TTR variants resulting in cardiac ATTR amyloidosis, including 16 variants that were not included in the test cohort. The baseline characteristics of the validation cohort are shown in Table 1. Despite the 'unselected' nature of the validation cohort, formal calibration with the test cohort indicated remarkable consistency across all three disease stages and a calibration coefficient of 0.999. One hundred and fifty (47%) patients were classified as Stage I, 118 (37%) patients were Stage II and 50 (16%) patients were Stage III. Survival by Kaplan-Meier analysis stratified by stage is shown in Figure 6 and the results from Cox proportional hazards regression analysis, adjusting for age, are shown in Table 2. Stage I patients had a median survival of 69.2 months, Stage II patients had a median survival of 35.0 months and Stage III patients had a median survival of 20.5 months ($p<0.0001$ for Stage I vs II and $P=0.01$ for Stage II vs III). After adjusting for age, compared to Stage I, the hazard ratio for death was 3.36 (95% CI: 1.79-6.29, $p<0.001$) for Stage II, and 6.92 (95%CI: 3.45-13.87, $p<0.001$) for Stage III patients. The hazard ratio for patients with Stage III cardiac ATTR amyloidosis compared to Stage II was 2.06 (95% CI: 1.12-3.79, $p=0.020$). Harrell's c statistic was 0.71 (Table 2).

Comparison of hazard ratios for death between this and the previously reported staging system which used Troponin T and NT-proBNP is shown in Table 2.

Discussion

We present a staging system that is applicable to both wild-type ATTR and variant ATTR cardiac amyloidosis, using two simple and universally measured serum biomarkers, NT-proBNP and eGFR. Patients are diagnosed with cardiac ATTR amyloidosis at different stages in its natural history and this staging system, which is highly informative on prognosis and will be relevant for stratifying the enrolment of patients into forthcoming clinical trials of

novel therapies, represents an important advance, particularly given its simplicity and wide applicability. The staging system discriminates between the ~20% patients with cardiac ATTR amyloidosis who have a median survival of approximately 2 years, the 40% who have a median survival of about 4 years, and the ~40% who have a median survival of around 6 years. Whilst our data validate the previously proposed staging system in wild-type ATTR amyloidosis, using Troponin T and NT-proBNP measurement, this cardiac biomarker based system in our own large dataset was less discriminatory and less consistent with respect to hazard ratios of death across different genotypes than our NT-proBNP and eGFR system, the performance of which was remarkably similar across all pathogenic TTR genotypes and between the test and validation cohorts (Table 2). This, coupled with the widespread availability and consistent inter-assay performance of both the eGFR and NT-proBNP biomarkers used in our staging system, leads us to recommend widespread adoption of this staging system in patients with cardiac ATTR amyloidosis.

Interestingly, a smaller proportion of patients with non-V122I-associated hereditary cardiac ATTR amyloidosis had Stage III disease at the time of diagnosis compared with other types of cardiac ATTR amyloidosis studied here. This is perhaps not surprising since patients with most other pathogenic TTR variants usually have additional autonomic and peripheral nerve amyloidosis, i.e., familial amyloid polyneuropathy,[21] along with a family history of a similar disease phenotype in a first degree relative such that they may conceivably seek medical attention with earlier cardiac involvement than patients with wild-type ATTR amyloidosis or V122I-associated cardiac ATTR amyloidosis who are usually diagnosed after developing overt cardiac symptoms.[22] Nonetheless, with the notable exception of V30M-associated ATTR amyloidosis which frequently presents with isolated neuropathic symptoms in the absence of cardiomyopathy (hence exclusion of such patients from this study), by the time patients with non-V122I hereditary ATTR amyloidosis are

diagnosed, there is invariably evidence of the characteristic amyloid cardiomyopathy and heart failure on investigation using biomarkers, imaging and histology.

It is also noteworthy that despite there being a similar proportion of patients with V122I-associated cardiac ATTR amyloidosis and wild-type ATTR cardiac amyloidosis classified with Stage I, Stage II and Stage III disease, the prognosis of ATTR V122I cardiac amyloidosis was slightly poorer in all three stages. In looking for a possible explanation of this, we noted that eGFR values within each disease stage were similar between patients with V122I and wild-type ATTR amyloidosis, but median NT-proBNP concentration was higher in Stage II and Stage III patients with V122I (median 5176 ng/L and 9527 ng/L respectively) compared to wild-type cases (4212 ng/L and 7776 ng/L respectively). This suggests that the V122I patients may have had slightly more advanced disease at baseline, which is supported further by a higher proportion of this group having NYHA class III/IV heart failure at baseline (Table 1). However, poorer survival of patients with V122I-associated ATTR amyloidosis might also reflect a more aggressive disease phenotype or poorer efficacy of supportive heart failure treatment in black individuals. Further studies to determine which of these factors is responsible for the prognostic differences highlighted here will be greatly enhanced by use of this staging system.

Although there is a correlation between NT-proBNP concentration and eGFR in the general population, the NT-proBNP concentrations that were observed in this patient group and, more importantly, the cutoff of 3000 ng/L is higher than that expected in patients with end-stage renal disease who are euvolaemic and free of significant cardiac disease. The relationship between these two biomarkers in patients with cardiac ATTR amyloidosis, a cause of type 2 cardio-renal syndrome, is complex and reflects a combination of factors including degree of cardiac infiltration, fluid status, hormonal axes, diuretic requirements and

renal perfusion potentially influenced by renovascular amyloid, thus justifying their combined use within the same model.

Until recently there was no therapy known to alter the natural history of cardiac ATTR amyloidosis. However, several promising treatments have lately emerged for neuropathic ATTR amyloidosis, including antisense oligonucleotide and siRNA therapies, which inhibit TTR production by 80-90% and appear to substantially diminish disease progression.[14,23] These therapies are currently in phase 3 clinical trials in patients with hereditary, predominantly neuropathic ATTR amyloidosis and will require evaluation in cardiac ATTR amyloidosis. Although a study of revusiran, the first of these agents to be tested in cardiac ATTR amyloidosis, was discontinued prematurely due to a mortality imbalance, further studies of TTR-lowering therapy in cardiac ATTR amyloidosis are likely to follow. Similarly, diflunisal and tafamidis, small molecule drugs that stabilise the circulating TTR tetramer, have shown clinical benefit in neuropathic ATTR amyloidosis[24,25] and tafamidis is currently undergoing evaluation in a phase 3 study in patients with cardiac ATTR amyloidosis. Lastly, a novel antibody therapy, which has been shown to clear hepatic and renal amyloid deposits,[16] is shortly to be evaluated in patients with cardiac ATTR amyloidosis. The existence of a validated staging system for ATTR amyloid cardiomyopathy, applicable to patients with both wild-type and variant ATTR amyloidosis, analogous to the 'Mayo' disease staging system that has very successfully been employed to stratify patients with systemic AL amyloidosis,[26] will undoubtedly facilitate the clinical development of these promising novel therapies.

In summary, we present here a simple, novel staging system for cardiac ATTR amyloidosis, based on two universally measured biomarkers, which stratifies patients with both wild-type and variant ATTR amyloid cardiomyopathy into prognostic categories. This

staging system represents an important advance in the management of cardiac ATTR amyloidosis.

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Conflict of interest

None to declare

References

1. Mirzoyev SA, Edwards WD, Mohammed SF, Donovan JL, Roger VL, Grogan DR, Redfield MM. Cardiac Amyloid Deposition is Common in Elderly Patients with Heart Failure and Preserved Ejection Fraction. *Circulation* 2010;**122**:A17926.

2. Pepys MB. Amyloidosis. *Annu Rev Med* 2006;**57**:223-241.

3. Fontana M, Banypersad SM, Treibel TA, Maestrini V, Sado DM, White SK, Pica S, Castelletti S, Piechnik SK, Robson MD, Gilbertson JA, Rowczenio D, Hutt DF, Lachmann HJ, Wechalekar AD, Whelan CJ, Gillmore JD, Hawkins PN, Moon JC. Native T1 mapping in transthyretin amyloidosis. *JACC Cardiovasc Imaging* 2014;**7**:157-165.

4. Fontana M, Pica S, Reant P, Abdel-Gadir A, Treibel TA, Banypersad SM, Maestrini V, Barcella W, Rosmini S, Bulluck H, Sayed RH, Patel K, Mamhood S, Bucciarelli-Ducci C, Whelan CJ, Herrey AS, Lachmann HJ, Wechalekar AD, Manisty CH, Schelbert EB, Kellman P, Gillmore JD, Hawkins PN, Moon JC. Prognostic Value of Late Gadolinium Enhancement Cardiovascular Magnetic Resonance in Cardiac Amyloidosis. *Circulation* 2015;**132**:1570-1579.

5. Perugini E, Guidalotti PL, Salvi F, Cooke RM, Pettinato C, Riva L, Leone O, Farsad M, Ciliberti P, Bacchi-Reggiani L, Fallani F, Branzi A, Rapezzi C. Noninvasive etiologic diagnosis of cardiac amyloidosis using 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy. *J Am Coll Cardiol* 2005;**46**:1076-1084.

6. Rapezzi C, Quarta CC, Guidalotti PL, Longhi S, Pettinato C, Leone O, Ferlini A, Salvi F, Gallo P, Gagliardi C, Branzi A. Usefulness and limitations of 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy in the aetiological diagnosis of amyloidotic cardiomyopathy. *Eur J Nucl Med Mol Imaging* 2011;**38**:470-478.

7. Glaudemans AW, van Rheenen RW, van den Berg MP, Noordzij W, Koole M, Blokzijl H, Dierckx RA, Slart RH, Hazenberg BP. Bone scintigraphy with (99m)technetium-

hydroxymethylene diphosphonate allows early diagnosis of cardiac involvement in patients with transthyretin-derived systemic amyloidosis. *Amyloid* 2014;**21**:35-44.

8. Maurer MS. Noninvasive Identification of ATTRwt Cardiac Amyloid: The Re-emergence of Nuclear Cardiology. *Am J Med* 2015;**128**:1275-1280.

9. Gillmore JD, Maurer MS, Falk RH, Merlini G, Damy T, Dispenzieri A, Wechalekar AD, Berk JL, Quarta CC, Grogan M, Lachmann HJ, Bokhari S, Castano A, Dorbala S, Johnson GB, Glaudemans AW, Rezk T, Fontana M, Palladini G, Milani P, Guidalotti PL, Flatman K, Lane T, Vonberg FW, Whelan CJ, Moon JC, Ruberg FL, Miller EJ, Hutt DF, Hazenberg BP, Rapezzi C, Hawkins PN. Nonbiopsy Diagnosis of Cardiac Transthyretin Amyloidosis. *Circulation* 2016;**133**:2404-2412.

10. Pinney JH, Whelan CJ, Petrie A, Dungu J, Banypersad SM, Sattianayagam P, Wechalekar A, Gibbs SDJ, Venner CP, Wassef N, McCarthy CA, Gilbertson JA, Rowczenio D, Hawkins PN, Gillmore JD, Lachmann HJ. Senile Systemic Amyloidosis: Clinical Features at Presentation and Outcome. *J Am Heart Assoc* 2013;**2**:e000098.

11. Jacobson DR, Pastore RD, Yaghoubian R, Kane I, Gallo G, Buck FS, Buxbaum JN. Variant-sequence transthyretin (isoleucine 122) in late-onset cardiac amyloidosis in black Americans. *N Engl J Med* 1997;**336**:466-473.

12. Reilly MM, Staunton H, Harding AE. Familial amyloid polyneuropathy (TTR ala 60) in north west Ireland: a clinical, genetic, and epidemiological study. *J Neurol Neurosurg Psychiatry* 1995;**59**:45-49.

13. Rowczenio DM, Noor I, Gillmore JD, Lachmann HJ, Whelan C, Hawkins P, N. Obici L, Westermark P, Grateau G, Wechalekar AD. Online registry for mutations in hereditary amyloidosis including nomenclature recommendations. *Hum Mutat* 2014;**35**:E2403-E2412.

14. Coelho T, Adams D, Silva A, Lozeron P, Hawkins PN, Mant T, Perez J, Chiesa J, Warrington S, Tranter E, Munisamy M, Falzone R, Harrop J, Cehelsky J, Bettencourt BR,

Geissler M, Butler JS, Sehgal A, Meyers RE, Chen Q, Borland T, Hutabarat RM, Clausen VA, Alvarez R, Fitzgerald K, Gamba-Vitalo C, Nochur SV, Vaishnaw AK, Sah DW, Gollob JA, Suhr OB. Safety and efficacy of RNAi therapy for transthyretin amyloidosis. *N Engl J Med* 2013;**369**:819-829.

15. Dubrey S, Ackermann E, Gillmore J. The transthyretin amyloidoses: advances in therapy. *Postgrad Med J* 2015;**91**:439-448.

16. Richards DB, Cookson LM, Berges AC, Barton SV, Lane T, Ritter JM, Fontana M, Moon JC, Pinzani M, Gillmore JD, Hawkins PN, Pepys MB. Therapeutic Clearance of Amyloid by Antibodies to Serum Amyloid P Component. *N Engl J Med* 2015;**373**:1106-1114.

17. Benson MD, Ackermann EJ, Monia BP. Treatment of transthyretin cardiomyopathy with a TTR-specific antisense oligonucleotide (IONIS-TTRRx). *Amyloid* 2017;**24**:134-135.

18. Grogan M, Scott CG, Kyle RA, Zeldenrust SR, Gertz MA, Lin G, Klarich KW, Miller WL, Maleszewski JJ, Dispenzieri A. Natural History of Wild-Type Transthyretin Cardiac Amyloidosis and Risk Stratification Using a Novel Staging System. *J Am Coll Cardiol* 2016;**68**:1014-1020.

19. Garg P, Morris P, Fazlanie AL, Vijayan S, Dancso B, Dastidar AG, Plein S, Mueller C, Haaf P. Cardiac biomarkers of acute coronary syndrome: from history to high-sensitivity cardiac troponin. *Intern Emerg Med* 2017;**12**:147-155.

20. Hutt DF, Fontana M, Burniston M, Quigley AM, Petrie A, Ross JC, Page J, Martinez-Naharro A, Wechalekar AD, Lachmann HJ, Quarta CC, Rezk T, Mahmood S, Sachchithanantham S, Youngstein T, Whelan CJ, Lane T, Gilbertson JA, Rowczenio D, Hawkins PN, Gillmore JD. Prognostic utility of the Perugini grading of 99mTc-DPD scintigraphy in transthyretin (ATTR) amyloidosis and its relationship with skeletal muscle and soft tissue amyloid. *Eur Heart J Cardiovasc Imaging* 2017;[Epub ahead of print]..

21. Sattianayagam PT, Hahn AF, Whelan CJ, Gibbs SD, Pinney JH, Stangou AJ, Rowczenio D, Pflugfelder PW, Fox Z, Lachmann HJ, Wechalekar AD, Hawkins PN, Gillmore JD. Cardiac phenotype and clinical outcome of familial amyloid polyneuropathy associated with transthyretin alanine 60 variant. *Eur Heart J* 2012;**33**:1120-1127.

22. Gillmore JD, Hawkins PN. V122I transthyretin variant in elderly black Americans. *N Engl J Med* 2015;**372**:1769.

23. Ackermann EJ, Guo S, Booten S, Alvarado L, Benson M, Hughes S, Monia BP. Clinical development of an antisense therapy for the treatment of transthyretin-associated polyneuropathy. *Amyloid* 2012;**19 Suppl 1**:43-44.

24. Coelho T, Maia LF, Martins da Silva A, Waddington Cruz M, Plante-Bordeneuve V, Lozeron P, Suhr OB, Campistol JM, Conceicao IM, Schmidt HH, Trigo P, Kelly JW, Labaudiniere R, Chan J, Packman J, Wilson A, Grogan DR. Tafamidis for transthyretin familial amyloid polyneuropathy: a randomized, controlled trial. *Neurology* 2012;**79**:785-792.

25. Berk JL, Suhr OB, Obici L, Sekijima Y, Zeldenrust SR, Yamashita T, Heneghan MA, Gorevic PD, Litchy WJ, Wiesman JF, Nordh E, Corato M, Lozza A, Cortese A, Robinson-Papp J, Colton T, Rybin DV, Bisbee AB, Ando Y, Ikeda S, Seldin DC, Merlini G, Skinner M, Kelly JW, Dyck PJ. Repurposing diflunisal for familial amyloid polyneuropathy: a randomized clinical trial. *JAMA* 2013;**310**:2658-2667.

26. Dispenzieri A, Gertz M, Kyle R, Lacy M, Burritt MF, Therneau TM, Greipp PR, Witzig TE, Lust JA, Rajkumar SV, Fonseca R, Zeldenrust SR, McGregor CG, Jaffe AS. Serum cardiac troponins and N-terminal pro-brain natriuretic peptide: a staging system for primary systemic amyloidosis. *J Clin Oncol* 2004;**22**:3751-3757.

Figure Legends

Figure 1. Kaplan Meier curves showing survival probabilities in patients with cardiac ATTR amyloidosis. A) Stratified by eGFR ≥ 45 ml/min/1.73m^2 vs < 45 ml/min/1.73m^2 (Log rank test, p<0.0001). B) Stratified by NT-proBNP ≤ 3000 ng/L vs > 3000 ng/L (Log rank test, p<0.0001).

Figure 2. Kaplan Meier curves showing survival probabilities in 869 patients with cardiac ATTR amyloidosis stratified by disease stage (Log rank test; Stage I vs Stage II, p<0.0001; Stage II vs Stage III, p<0.0001).

Figure 3. Kaplan Meier curves showing survival probabilities in 553 patients with wild-type ATTR cardiac amyloidosis stratified by disease stage (Log rank test; Stage I vs Stage II, p<0.0001; Stage II vs Stage III, p=0.0003).

Figure 4. Kaplan Meier curves showing survival probabilities in 201 patients with V122I-associated cardiac ATTR amyloidosis stratified by disease stage (Log rank test; Stage I vs Stage II, p=0.006; Stage II vs Stage III, p=0.013).

Figure 5. Kaplan Meier curves showing survival probabilities in 115 patients with non-V122I-associated variant ATTR cardiac amyloidosis stratified by disease stage. In order to satisfy the proportional hazards assumption, separate analyses were performed before and after 18 months, the time at which the curves cross (Before 18 months: Log rank test; Stage I vs Stage II, p=0.72; Stage II vs Stage III, p<0.001. From 18 months: Log rank test; Stage I vs Stage II, p=0.02; Stage II vs Stage III, p=0.25).

Figure 6. Kaplan Meier curves showing survival probabilities in 318 with cardiac ATTR amyloidosis (validation cohort) stratified by disease stage (Log rank test; Stage I vs Stage II, $p<0.0001$; Stage II vs Stage III, $p=0.01$).

Table 1. Characteristics of 869 patients in test cohort and 318 patients in validation cohort

	ATTR amyloidosis **(Whole test cohort)** **N=869**	**Wild-type ATTR amyloidosis** **(Sub-group 1)** **N=553**	**V122I-associated ATTR amyloidosis** **(Sub-group 2)** **N=201**	**Non-V122I-associated variant ATTR amyloidosis** **(Sub-group 3)** **N=115**	**ATTR amyloidosis** **(Validation cohort)** **N=318**
Median (Range) Age at diagnosis (years)	77 (41-95)	78 (51-95)	77 (47 - 90)	66 (41 - 82)	77 (30-96)
Male Sex (%)	737 (85%)	522 (94%)	143 (71%)	70 (61%)	258 (81%)
Median (Range) Creatinine (µmol/L)	108 (34 – 441)	110 (53 – 357)	119 (46 - 441)	78 (34 – 186)	102 (11-344)
Median eGFR (ml/min)	61 (12 - >90)	58 (15 - >90)	62 (12 - >90)	83 (23 - >90)	65 (12 - >90)
CKD stage I (n)	99	37	16	46	59
CKD stage II (n)	358	221	89	48	121
CKD stage III (n)	370	265	86	19	129
CKD stage IV (n)	39	30	7	2	8
CKD stage V (n)	3	0	3	0	1
Median NT-proBNP (ng/L)	3036 (34 – 45642)	3087 (51 – 34910)	3332 (34 – 45642)	2106 (34 – 30479)	2784 (22-40492)
NT-proBNP ≤2000 (n)	288	170	63	55	126
NT-proBNP 2001-3000 (n)	145	96	33	16	40
NT-proBNP 3001-4000 (n)	108	81	20	7	41
NT-proBNP >4000 (n)	328	206	85	37	111

Median (Range) Troponin T (ng/L)		60 (3 – 843)	60 (4 – 340)	82 (10 – 843)	38 (3 – 161)	60 (3-372)*
NYHA Class (n (%))	**II**	656 (75)	440 (80)	119 (59)	97 (84)	147 (46)
	III	205 (24)	110 (20)	79 (39)	16 (14)	100 (31)
	IV	8 (1)	3 (<1	3 (1)	2 (2)	23 (7)
	Missing data	0	0)	0	0	48 (15)
Median (Range) Systolic Blood Pressure (mmHg)		121 (72-198)	122 (72 - 184)	120 (83 - 198)	117 (91 - 158)	Data unavailable
Median (Range) IVSd (mm)		17 (10-25)	17 (11 – 25)	17 (11 – 24)	16 (10 – 23)	18 (11-29)[¥]
Median (Range) Age at death/censor (years)		79 (42 - 96)	81 (52 – 96)	79 (48 – 92)	69 (42 - 87)	78 (30-97)
Heart Rhythm	**Sinus Rhythm (n)**	481	262	137	82	206
	AF (n)	303	235	44	24	53
	Pacemaker (n)	39	24	9	6	43
	Missing data (n)	46	32	11	3	16
Ischaemic Heart Disease	**Yes (n)**	138	116	15	7	21
	No (n)	685	405	175	105	282
	Missing data (n)	46	32	11	3	15

* = high sensitivity troponin T only; AF = atrial fibrillation; [¥] = data missing in 64 patients

Table 2. Staging of cardiac ATTR amyloidosis. Cox proportional hazards regression analysis, adjusting for age and including comparison of staging systems

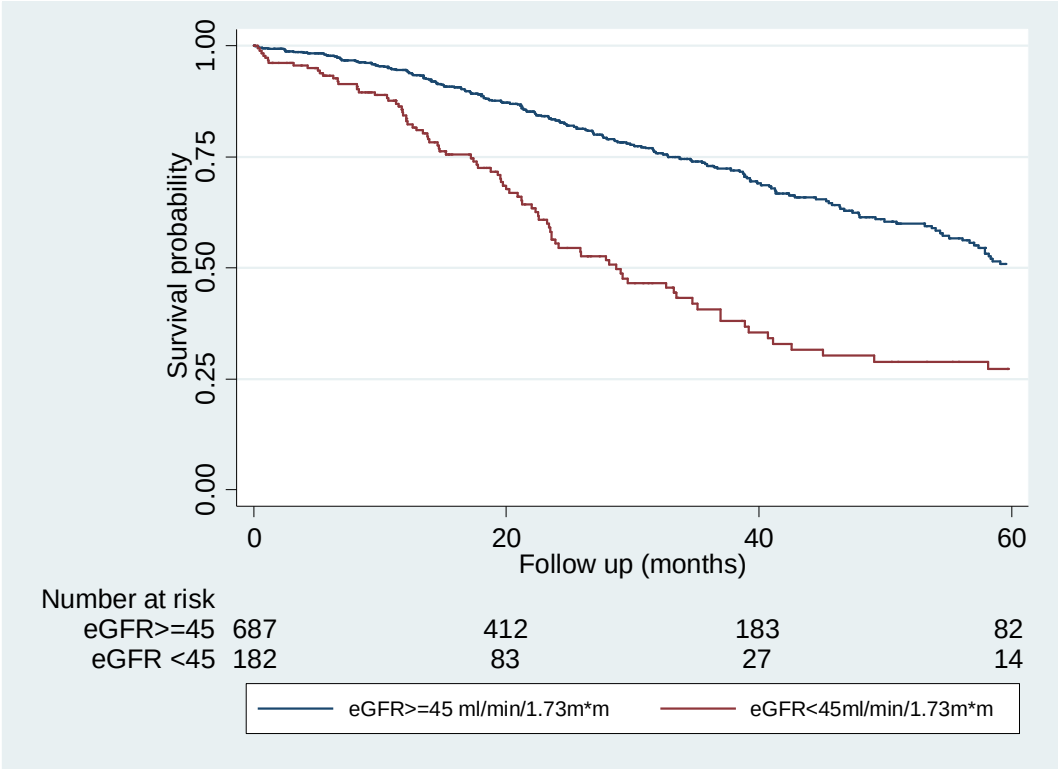
Group	Model item	Results	eGFR and NT-proBNP Test Cohort	eGFR and NT-proBNP Validation Cohort	TnT and NT-proBNP Test Cohort
All cardiac ATTR amyloidosis	Stages II v I	HR (95%CI) P -value	2.05 (1.54 –2.72) <0.001	3.35 (1.79-6.29) <0.001	2.11 (1.45-3.04) <0.001
	Stages III v I	HR (95%CI) P -value	3.80 (2.73-5.28) <0.001	6.91 (3.45-13.87) <0.001	3.33 (2.35-4.71) <0.001
	Stages III v II	HR (95%CI) P -value	1.86 (1.39-2.48) <0.001	2.05 (1.12-3.79) 0.020	1.58 (1.20-2.08) 0.001
	Age	HR (95%CI) P -value	1.02 (1.01-1.04) 0.004	1.02 (0.99-1.05) 0.132	1.02 (1.01-1.05) 0.002
		Harrell's c	0.69	0.71	0.69
Wild type ATTR cardiac amyloidosis (Sub-group 1)	Stages II vs I	HR (95%CI) P -value	2.26 (1.51-3.36) <0.001	2.51 (1.09-5.73) 0.030	1.83 (1.11-3.00) 0.017
	Stages III v I	HR (95%CI) P -value	4.37 (2.80-6.83) <0.001	12.03 (4.47-32.41) <0.001	3.29 (2.11-5.12) <0.001
	Stages III v II	HR (95%CI) P -value	1.94 (1.34-2.81) <0.001	4.79 (2.08-11.02) <0.001	1.80 (1.22-2.64) 0.003
	Age	HR (95%CI) P -value	1.02 (0.99-1.04) 0.16	0.99 (0.94-1.05) 0.794	1.03 0.039
		Harrell's c	0.70	0.67	0.69
V122I-associated cardiac ATTR amyloidosis (Sub-group 2)	Stages II v I	HR (95%CI) P -value	1.91 (1.17-3.12) 0.009	7.28 (1.69-32.59) 0.009	1.48 (0.70-3.15) 0.304
	Stages III v I	HR (95%CI) P -value	3.48 (1.94-6.27) <0.001	1.87 (0.44-7.98) 0.009	2.75 (1.33-5.65) 0.006
	Stages III v II	HR (95%CI) P -value	1.82 (1.06-3.13) 0.030	0.26 (0.05-1.42) 0.120	1.85 (1.16-2.96) 0.010
	Age	HR (95%CI) P -value	1.02 (0.99-1.06) 0.194	1.08 (0.98-1.17) 0.108	1.02 (0.99-1.06) 0.145
		Harrell's c	0.69	0.80	0.68
Non-V122I-associated variant cardiac ATTR amyloidosis (Sub-group 3)*	Stages II v I	HR (95%CI) P -value	2.28 (1.04-4.98) 0.039	5.60 (1.54-20.31) 0.009	3.43 (1.46-8.07) 0.005
	Stages III v I	HR (95%CI) P -value	4.05 (1.54-10.64) 0.005	12.28 (2.47-60.90) 0.002	3.11 (1.12-8.58) 0.029
	Stages III v II	HR (95%CI) P -value	1.78 (0.67-4.71) 0.247	2.19 (0.96-1.05) 0.305	0.91 (0.38-2.18) 0.826
	Age	HR (95%CI) P -value	1.11 (1.01-1.04) <0.001	1.01 (0.96-1.05) 0.768	1.13 1.06-1.21) <0.001
		Harrell's c	0.72	0.76	0.74

eGFR – estimated MDRD GFR, NT-proBNP – N-terminal pro-B-type natriuretic peptide, TnT – Troponin T

*Since the curves in figure 5 for sub-group 3 cross at 18 months, analyses for FU of < and ≥18 months were also performed separately in order to satisfy the proportional hazards assumption. Before 18 months: II v I HR 1.45 (95% CI: 0.20-10.75), III v I HR 14.70 (1.46-148.32), III v II HR 10.11 (0.91-1.12). Harrell's c 0.62; After 18 months: II v I HR 2.55 (1.07-6.07), III v I HR 3.24 (0.98-10.65), III v II HR 1.27 (0.38-4.23). Harrell's c 0.73

Figure 1

A)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0.25
0.50
0.75
1.00
0
20
40
60
Follow up (months)
Number at risk
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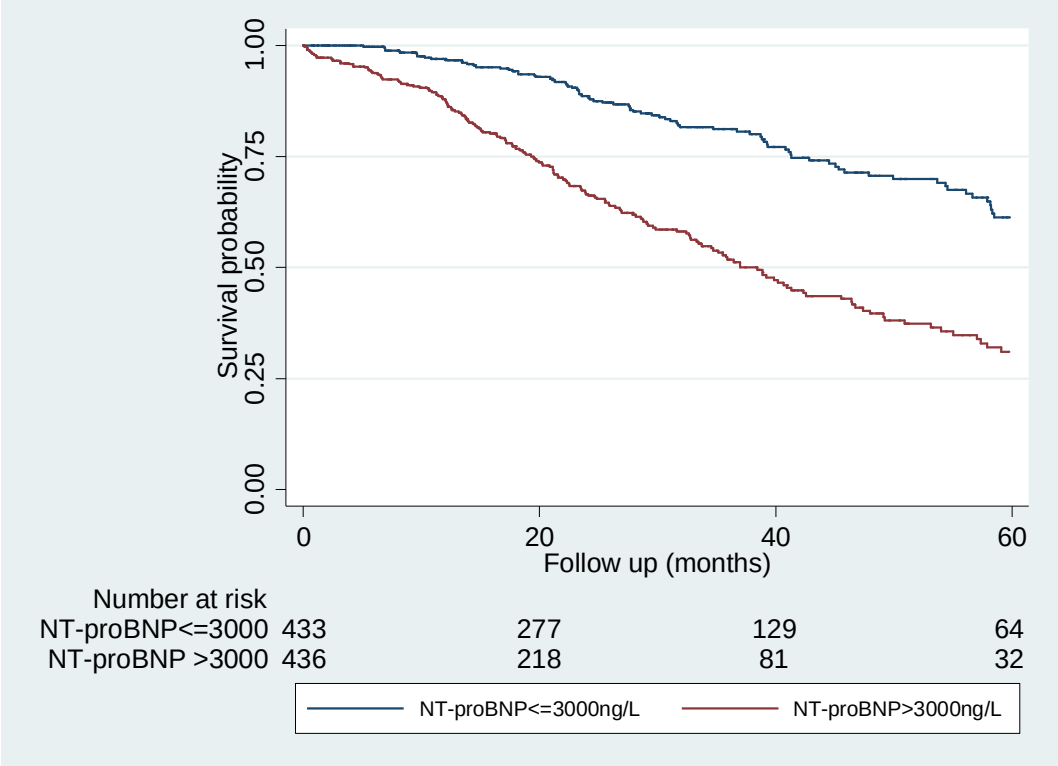
eGFR <45 182 83 27 14
eGFR>=45 ml/min/1.73m*m
eGFR<45ml/min/1.73m*m

B)


Survival probability
0.00
0.25
0.50
0.75
1.00
0
20
40
60
Follow up (months)
Number at risk
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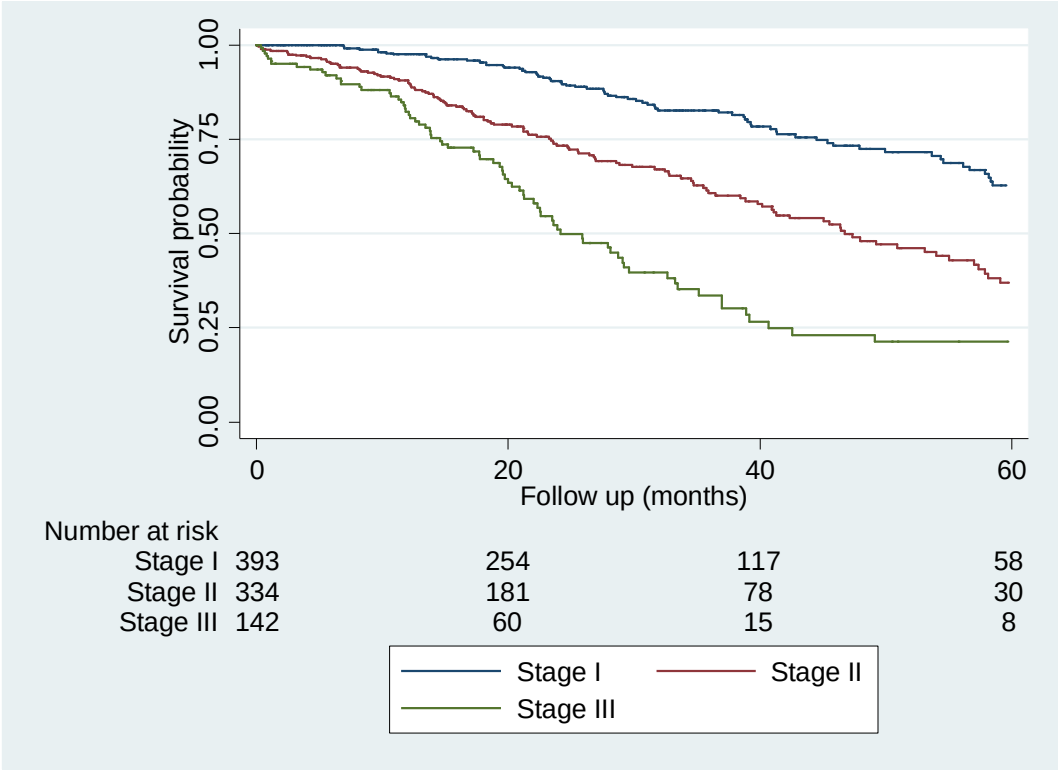
NT-proBNP >3000 436 218 81 32
NT-proBNP<=3000ng/L
NT-proBNP>3000ng/L

Figure 2


Survival probability
0.00
0.25
0.50
0.75
1.00
0
20
40
60
Follow up (months)
Number at risk
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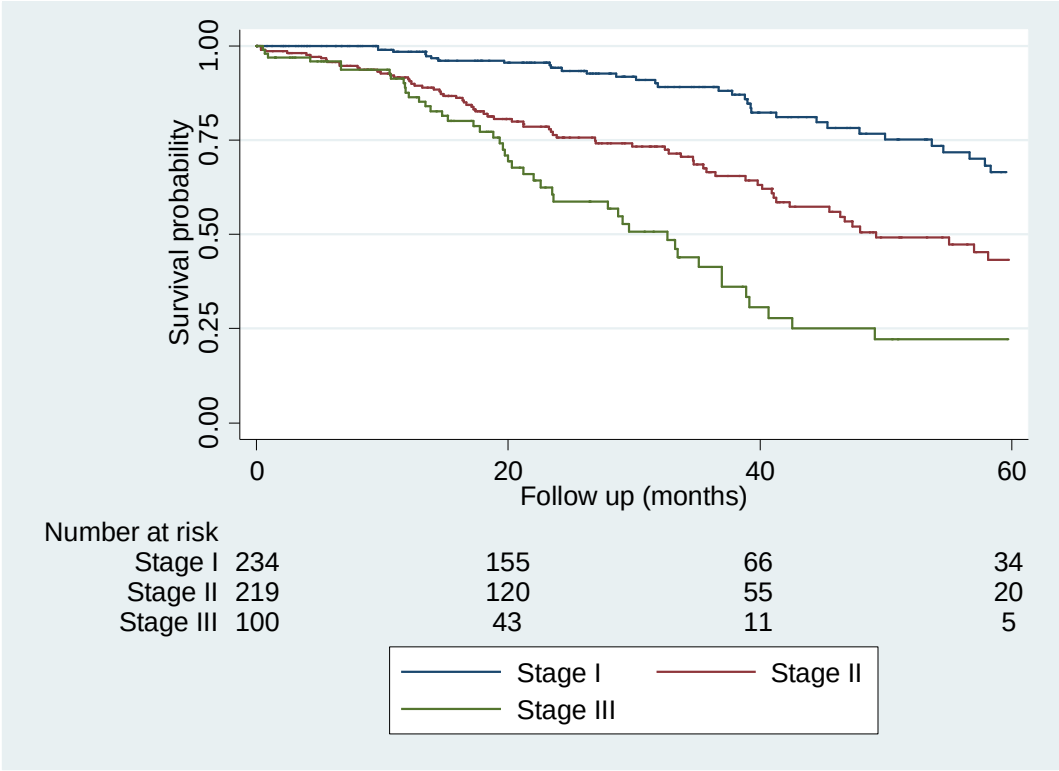
Stage II 334 181 78 30
Stage III 142 60 15 8
Stage I
Stage II
Stage III

Figure 3


Survival probability
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0.25
0.50
0.75
1.00
0
20
40
60
Follow up (months)
Number at risk
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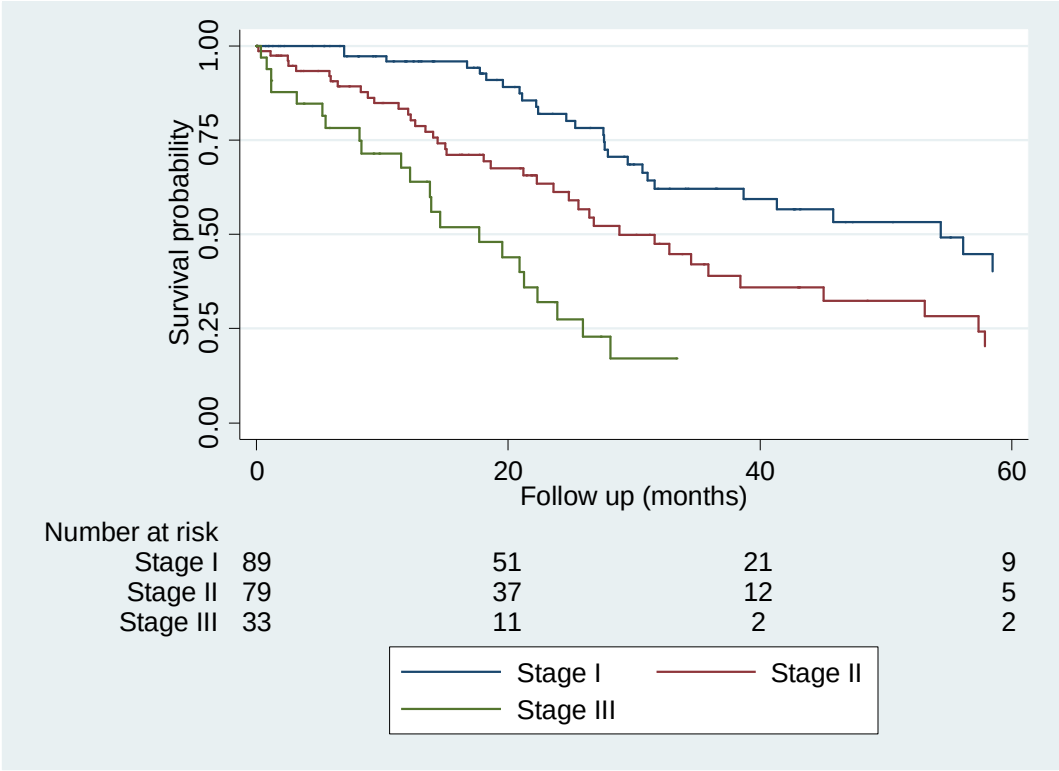
Stage II 219 120 55 20
Stage III 100 43 11 5
Stage I
Stage II
Stage III

Figure 4


Survival probability
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0.25
0.50
0.75
1.00
0
20
40
60
Follow up (months)
Number at risk
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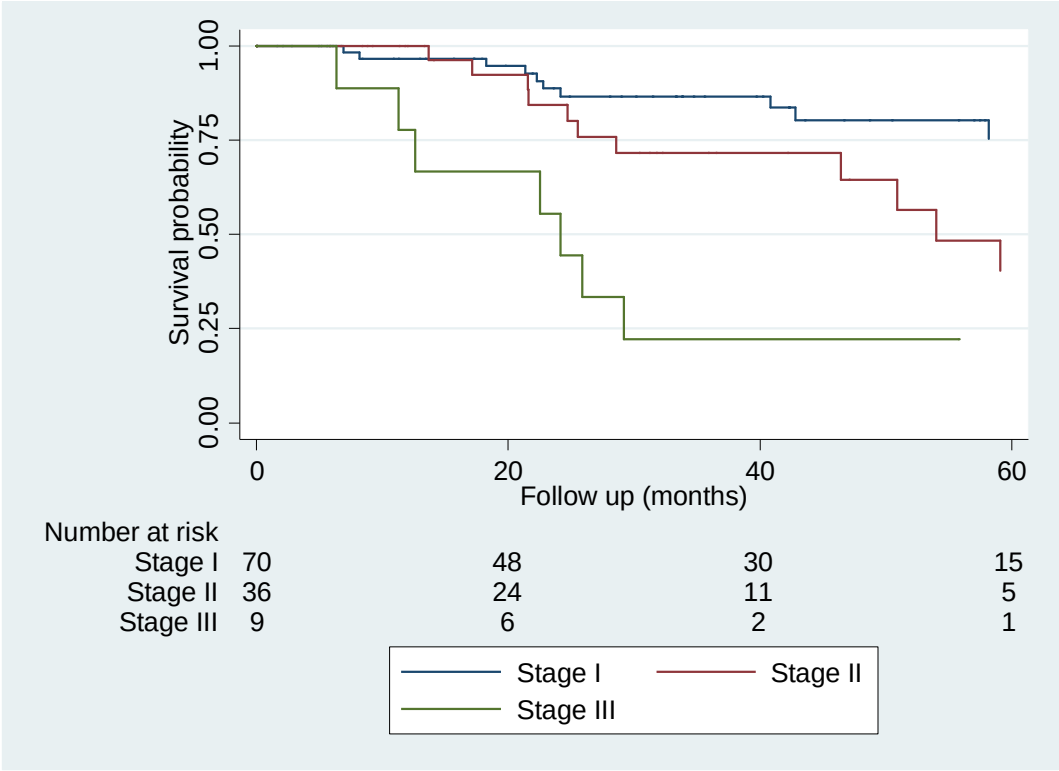
Stage II 79 37 12 5
Stage III 33 11 2 2
Stage I
Stage II
Stage III

Figure 5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0.50
0.75
1.00
0
20
40
60
Follow up (months)
Number at risk
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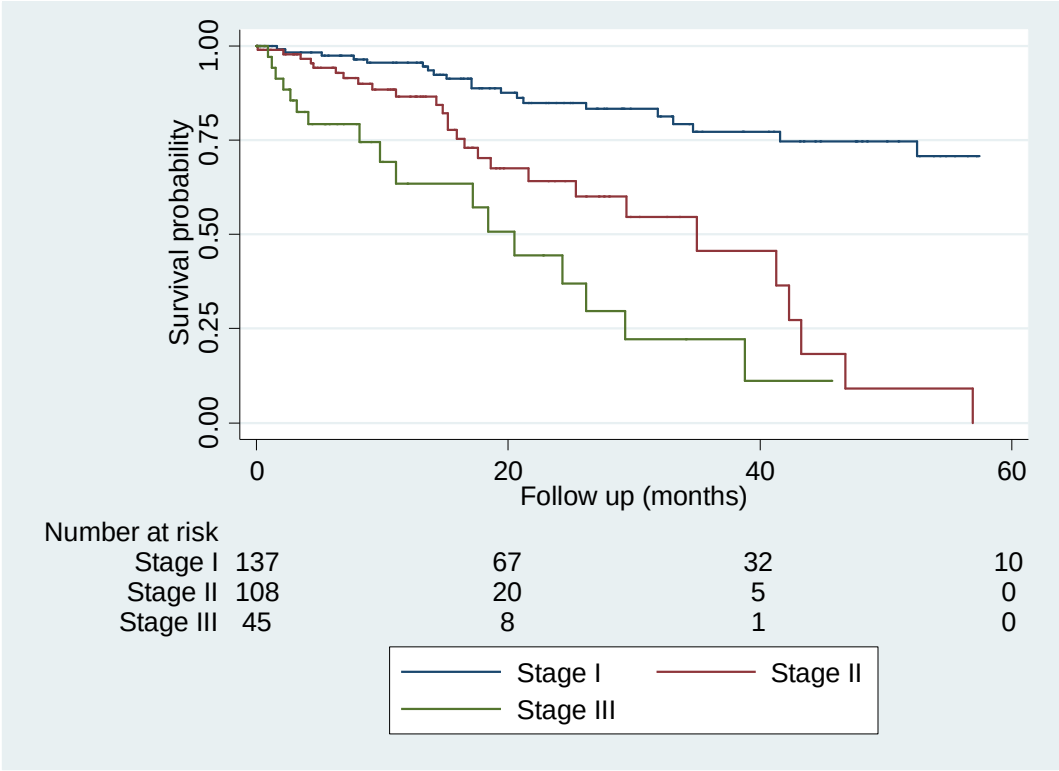
Stage II 36 24 11 5
Stage III 9 6 2 1
Stage I
Stage II
Stage III

Figure 6


Survival probability
0.00
0.25
0.50
0.75
1.00
0
20
40
60
Follow up (months)
Number at risk
Stage I 137 67 32 10
Stage II 108 20 5 0
Stage III 45 8 1 0
Stage I
Stage II
Stage III