Comparison of right ventricular longitudinal strain imaging, tricuspid annular plane systolic excursion, and cardiac biomarkers for early diagnosis of cardiac involvement and risk stratification in primary systematic (AL) amyloidosis: a 5-year cohort study

Diego Bellavia¹, Patricia A. Pellikka¹, Angela Dispenzieri², Christopher G. Scott³, Ghormallah B. Al-Zahrani¹, Martha Grogan¹, Francesco Pitrolo⁴, Jae K. Oh¹, and Fletcher A. Miller Jr.¹*

¹Division of Cardiovascular Diseases, Mayo Clinic, 200 First Street SW, Rochester, MN 55905, USA; ²Division of Hematology, Mayo Clinic, Rochester, MN, USA; ³Division of Biomedical Informatics and Biostatistics, Mayo Clinic, Rochester, MN, USA; and ⁴Division of Cardiovascular Diseases, Ospedale Cervello, Palermo, Italy

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Aims
To determine the role of assessing right ventricular (RV) function, using standard echocardiography and Doppler myocardial imaging (DMI), in the early diagnosis of cardiac amyloidosis and in the prediction of mortality.

Methods and results
Patients with primary systemic (AL) amyloidosis seen at our institution from 1 February 2004 through 31 October 2005 (N = 249) were categorized by left ventricular thickness and E’ velocity and compared with 38 age- and sex-matched controls. Standard echocardiographic and DMI examination were used to measure echocardiographic parameters of RV function: systolic tissue velocity, strain rate, and strain were determined for basal and middle RV free wall segments. Patients were followed up for the endpoint of mortality. RV tricuspid annular plane systolic excursion (TAPSE) and all DMI measurements were lower in patients with AL amyloidosis and normal echocardiography results (AL-normal-echo group) than controls. A bivariate model including strain of the basal segment of the RV free wall and TAPSE was the best for distinguishing AL-normal-echo patients from controls. Male sex [hazard ratio (HR), 2.2; P = 0.005], brain natriuretic peptide levels (HR 1.4; P = 0.003), troponin T levels (HR 1.6; P = 0.01), pleural effusion (HR 3.6; P < 0.001), E/A ratio (HR 1.3; P = 0.006), RV systolic pressure (HR 1.02; P = 0.01), and RV strain rate of the middle segment (HR 1.3; P = 0.02) were independent predictors of death.

Conclusion
DMI measures of the RV can identify early impairment of cardiac function or stratify risk of death in patients with AL amyloidosis. Further studies with longer follow-up are warranted to confirm these results.

Keywords
AL amyloidosis • Cardiac biomarkers • Right ventricular function • Standard echocardiography • Strain rate imaging

* Corresponding author. Tel: +1 507 284 3682; fax: +1 507 284 3968, Email: miller.fletcher@mayo.edu

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Introduction

Congestive heart failure is seen in ~25% of patients with primary systemic (AL) amyloidosis.1 Cardiac involvement of amyloidosis (CA) is the main contraindication to peripheral blood autologous stem cell transplant, which has been effective in increasing 5-year survival to 47%.2 Some evidence suggests that assessment of right ventricular (RV) function is useful for determining the prognosis of patients with CA.3,4 The aims of this study were: (1) to determine the role of different measures of RV function by standard echocardiography and by longitudinal systolic Doppler imaging (DMI) for identifying RV dysfunction in patients with AL amyloidosis who have no echocardiographic evidence of cardiac involvement, by comparison with control patients; (2) to determine the role of different measures of RV function for identifying RV dysfunction in patients with advanced CA compared with patients with AL amyloidosis and no abnormalities by standard echocardiography; and (3) to identify independent predictors of all-cause mortality in this study population, considering demographic, clinical, and cardiovascular data, including cardiac biomarkers and echocardiographic parameters of RV function.

Methods

Study population

This study was approved by our institutional review board. We prospectively considered 276 consecutive patients with AL amyloidosis who underwent complete echocardiographic and Doppler examinations at Mayo Clinic, Rochester, MN, USA, from 1 February 2004 through 31 October 2005. The diagnosis of AL amyloidosis was made by biopsy of subcutaneous fat or an involved organ with demonstration of typical Congo red birefringence under polarized light. AL amyloidosis was further confirmed by the presence of a monoclonal protein in the serum or urine and/or a monoclonal population of plasma cells in the bone marrow and immunohistochemical staining of the amyloid fibrils.

Once a diagnosis of AL amyloidosis was ascertained, all patients received a thorough clinical evaluation by a haematologist and were referred to the cardiology service for a complete assessment of cardiovascular performance, including blood tests and echocardiography. Patients who had familial or secondary amyloidosis; senile amyloidosis; history of any grade of systemic or pulmonary hypertension; coronary artery disease detected by history of typical symptoms, as well as by regional hypokinesia or akinesia of myocardial segments by standard echocardiography, or induced by exercise; or history of moderate or greater valvular heart disease were excluded. After exclusion of 27 patients with such conditions, 249 patients were enrolled in the study. Most of them were initially referred to our institution for further work-up and management and were admitted for the first time. Patients provided written informed consent to participate in the study.

The patients were categorized according to left ventricular (LV) wall thickness (the average of the thickness of the ventricular septum and posterior wall)—an ‘advanced-CA’ group (thickness >12 mm for men and >11 mm for women) and an ‘AL-normal-wall-thickness’ group (thickness ≤12 mm for men and ≤11 mm for women). For the purpose of identifying early systolic RV dysfunction in patients with AL amyloidosis with normal two-dimensional (2D) and Doppler echocardiography results, compared with healthy controls (aim 1), we did not include in the analysis the advanced-CA group or patients in the AL-normal-wall-thickness group with: (i) Doppler E’ velocity <7 cm/s for the medial mitral annulus; (ii) restrictive diastolic filling (diastolic dysfunction grade ≥3); (iii) chronic atrial fibrillation; or a low-voltage or pseudo-infarct pattern on electrocardiography. The remaining patients with AL amyloidosis but without evidence of cardiac involvement by standard 2D echocardiography and Doppler criteria (‘AL-normal-echo’ group) constituted the study population for aim 1. They were compared with 38 age- and sex-matched healthy subjects identified from patients referred to our laboratory for the assessment of cardiac performance as pre-operative work-up for non-cardiac surgery (n = 13), for atypical chest pain (n = 8), or for the investigation of a cardiac murmur (n = 17).

Patients were followed up for the endpoint of all-cause mortality. The vital status of each patient was confirmed by review of medical records and the Social Security Death Index. Patients who had no record of death were censored at the time they were last known to be alive. Follow-up was closed on 5 April 2010.

Biomarkers

In patients with AL amyloidosis, blood was collected at the time of echocardiography for the measurement of brain natriuretic peptide (BNP), N-terminal pro-BNP, cardiac troponin T, creatinine, alkaline phosphatase, albumin, and uric acid levels, and glomerular filtration rate. Protein was measured from 24 h urine collection. All were measured as previously described.5

Electrocardiography

Standard 12-lead resting electrocardiography was performed in all patients at the time of the echocardiographic examination, as previously described.6

Standard echocardiography

All echocardiographic examinations were performed with a commercially available instrument (Vivid 7 System; Vingmed, General Electric, Milwaukee, WI, USA). Standard LV systolic and diastolic parameters from 2D and Doppler echocardiography (including transmitral and pulmonary vein flow analysis), as well as pulsed-wave tissue Doppler imaging of the mitral medial annulus, were acquired and measured as previously described.5 RV wall thickness and RV end-diastolic diameters at basal and mid-ventricular levels were measured using the subcostal view. RV end-diastolic and end-systolic four-chamber areas were derived by manually tracing the endocardial borders at end diastole (onset of the QRS complex) and end systole (smallest ventricular area). RV fractional area change was calculated as:

$$100 \times \frac{\text{RV diastolic area} - \text{RV systolic area}}{\text{RV diastolic area}}$$

RV dysfunction was defined by a fractional area change of <40%.

Right index of myocardial performance (RIMP) was measured as previously described.7 To determine tricuspid annular plane systolic excursion (TAPSE), the apical four-chamber view was used, and an M-mode cursor was placed through the lateral tricuspid annulus in real time. TAPSE was measured as the total displacement of the tricuspid annulus (in cm) from end diastole to end systole. RV systolic pressure was calculated by inserting the tricuspid regurgitation velocity, obtained with continuous-wave Doppler, into the simplified Bernoulli equation.8 Velocity of the tricuspid regurgitation jet was assessed, comparing the quality and peak measurement of the continuous-wave Doppler waves, obtained either in the apical four-chamber view or the parasternal long-axis view (the RV was visualized by tilting the probe
anteriorly). The wave with the highest quality and deepest peak was considered the most accurate.

Off-line analysis of standard echocardiographic variables was performed with the use of dedicated software (ProSolv CardioVascular, Indianapolis, IN, USA); three consecutive beats were measured and averaged for each measurement.

**DMI data acquisition and analysis**

A narrow-sector view of the RV free wall was acquired using an apical four-chamber view, to maintain a frame rate of greater than 200 frames per second. Colour 2D digital data from three cardiac cycles were analysed off-line with the use of dedicated software (EchoPAC; GE Healthcare, Fairfield, CT, USA).

Because of increased RV motion and RV apex anatomy, longitudinal DMI measures of the RV apex would have been difficult to obtain, with likelihood of unreliability. Therefore, two sample volumes (offset 12 mm) were placed at the basal and middle segments of the RV free wall. Systolic tissue velocity, systolic strain rate (sSR), and systolic strain (sS) along the longitudinal axis were determined.

**Intra-observer and inter-observer variability**

To examine intra-observer variability (repeatability), a sample of 10 echocardiographic examinations was randomly selected for masked review by the same investigator. To examine inter-observer variability, a co-investigator blinded to the clinical information and to the results of the first investigator examined 10 randomly selected echocardiographic studies. Intraclass correlation coefficients (ICCs) for the same observer and different observers were calculated using previously described formulae for single segments and for the global mean of each DMI modality.

**Statistical analysis**

Statistical analyses were performed with a commercially available software program (SAS v8.2; SAS Institute, Inc., Cary, NC, USA). Comparisons between groups for continuous variables were made with the non-parametric Wilcoxon rank-sum test, and comparisons for categorical variables were performed using the Fisher exact test. Logistic regression and the derived receiver operating characteristic (ROC) curves were used to determine whether a combination of echocardiographic parameters of RV function, including TAPSE, RIMP, and DMI measures, might be a better discriminator than each index alone in distinguishing AL-normal-echo patients from controls.

The natural logs of BNP, N-terminal pro-BNP, creatinine, 24 h urine protein, and alkaline phosphatase values were used in the construction of univariable and multivariable survival models to decrease skewness. Covariate information (clinical data, electrocardiography, biomarkers, and echocardiographic variables) was collected at the time of the echocardiographic examination in all enrolled patients. Observations with missing values for any of the covariates were excluded from the analysis.

Many of the patients in the study were first referred to Mayo Clinic some months after their initial diagnosis. A naive computation of the survival curve for time from diagnosis will be biased upwards in this case.

Left truncation was appropriately taken into account for the survival curves and Cox regression analysis by not considering a subject to be in the risk set until the time of echocardiography. Univariable and multivariable analyses of the time to events were performed with use of Cox proportional hazards models, with demographic, clinical, electrocardiographic, standard echocardiographic, and RV DMI variables as independent variables. Multivariable models were created for each group of variables sequentially (clinical and electrocardiography, biomarkers, standard echocardiography, parameters of RV function by standard and DMI echocardiography) using a bootstrap approach. Candidate variables for the bootstrap selection were those with a univariable P-value <0.20. One thousand bootstrap samples of size N = 249 were selected with replacement from the data set. For each bootstrap sample, a stepwise model selection technique with P-value for selection and retention of 0.05 was applied, and the number of times each candidate variable appeared in the final model was tallied. Variables that appeared in the greatest percentage of models were then placed into the model together. This was repeated for each group of variables sequentially, with those variables from the previous group being retained in subsequent models even if a variable lost significance as additional variables were added.

The final model was constructed with clinical, electrocardiographic, biochemical, standard echocardiographic parameters of LV and RV function, and RV DMI measures to see whether standard echocardiographic parameters of RV function, including DMI measures, add information to the model. We used the survival C-statistic, a measure of concordance between observed and predicted survival from Cox proportional hazards models, to evaluate the discrimination ability of the model. This measure is similar to the area under the curve (AUC) for binary endpoints, but the survival C-statistic is interpreted as the probability of correctly ordering event times using the covariate risk score. Standard errors for C-statistics were obtained from 1000 bootstrap samples and used to construct 95% CIs. These standard errors were also used to formulate a test for differences between model C-statistics. A confirmatory analysis was then performed by fitting the final multivariable model obtained in the overall analysis on the subgroup of patients who had a diagnosis of AL amyloidosis within 3 months of echocardiography. Cut-point values for independent predictors in the final model were determined by the method of Contal and O’Quigley using log-rank statistics.

Data are presented as mean (SD) or number (percentage). P-values <0.05 were considered statistically significant. In the multivariate models, a variable was considered a significant predictor of survival if the P-value was less than 0.05.

**Results**

A total of 249 patients with AL amyloidosis met the inclusion criteria and were prospectively studied (Figure 1). Of these, 143 patients met the criteria for the advanced-CA group (8 patients had atrial fibrillation), and 106 were in the AL-normal-wall-thickness group. In the AL-normal-wall-thickness group, 47 had low Doppler E’ velocity (2 with atrial fibrillation) and 6 had a low-voltage (n = 5) or pseudo-infarct pattern (n = 1) by electrocardiography. The 53 remaining patients without evidence of cardiac involvement by standard 2D echocardiography and Doppler criteria (AL-normal-echo group) constituted the study population for aim 1.

**AL-normal-echo group vs. controls**

Clinical variables, electrocardiography characteristics, biomarkers, and standard echocardiography findings are shown in Tables 1 and 2. All 53 patients and 38 controls were in New York Heart Association class I. Thirty-four patients (64%) underwent stem cell transplant. RV ejection time and TAPSE were significantly decreased in AL-normal-echo patients compared with controls. All RV DMI modalities (systolic tissue velocity, sSR, and sS)
measured at the basal and middle RV free wall also were significantly decreased in the patient group (Table 3).

Of the three individual DMI measurements, sS of the basal RV free wall was the most accurate for distinguishing AL-normal-echo patients from controls (AUC 0.73; 95% CI 0.62–0.84) (Figure 2A), although sS of the middle segment and TAPSE reached nearly equal diagnostic accuracy (AUC 0.69 for both measures; comparison of the ROC curves, \( P = 0.72 \); RV basal sS, \( P = 0.005 \); TAPSE, \( P = 0.02 \)). RIMP did not significantly improve the predictive ability of the model (\( P = 0.69 \)). When using basal sS and TAPSE together, the ROC AUC was 0.81. The optimal ROC-derived cut-off values were –30% and 2.3 cm for basal sS and TAPSE, respectively (sensitivity 73%; specificity 67%; positive predictive value 75%; negative predictive value 64%).

**Advanced-CA group vs. AL-normal-wall-thickness group**

Most of the systolic and diastolic function parameters of LV and RV function by standard and DMI echocardiography were abnormal in the advanced-CA group compared with the AL-normal-wall-thickness group (Tables 2 and 3).

TAPSE was the most accurate measure to distinguish patients in the advanced-CA group from the AL-normal-wall-thickness group (AUC 0.73; 95% CI 0.65–0.80) (Figure 2B), although sS of the middle segment of the RV free wall had similar accuracy (AUC 0.71; comparison of the ROC curves, \( P = 0.80 \)). The most predictive bivariable model included RV free wall thickness with TAPSE or middle segment sS interchangeably (AUC for both: 0.81). The optimal ROC-derived cut-off value for RV free wall thickness was 7 mm, 1.9 cm for TAPSE, and \( 25\% \) for sS of the middle segment. The model considering RV free wall thickness and TAPSE had slightly greater specificity and negative predictive value (70 and 66%, respectively), whereas the model with RV free wall thickness and middle segment sS had greater sensitivity and positive predictive value (81 and 79%, respectively).

**Survival analysis**

The median follow-up time for censored patients was 53 months (range 0.6–75 months), with a total of 126 deaths (51%): 40 in the AL-normal-wall-thickness group (32%) and 86 in the advanced-CA group (68%). Survival curves are shown in Figure 3. Results of the univariable survival analysis are reported in Table 1–3.

Results from four multivariable models developed in a stepwise fashion are detailed in Table 4. When all variables including demographic and clinical information, electrocardiography, cardiac...
biomarkers, standard echocardiography, and parameters of RV function (including longitudinal DMI) were considered together, the significant independent predictors of survival were male sex (hazard ratio [HR] 2.17; \( P = 0.005 \)), ln BNP level (HR 1.37; \( P = 0.003 \)), troponin T level (HR 1.58; \( P = 0.01 \)), pleural effusion (HR 3.56; \( P = 0.001 \)), E/A ratio (HR 1.23; \( P = 0.006 \)), RV systolic pressure (HR 1.02; \( P = 0.01 \)), and RV sSR of the middle segment (HR 1.3; \( P = 0.02 \)) (Table 4). The global C-statistic obtained combining these variables was 0.78 (95% CI 0.73–0.83). This was higher than, although not significantly different from, the models that did not include DMI measures (\( P = 0.54 \)).

Optimal cut-points for the independent predictors of survival in the final model included BNP level 486 pg/mL, troponin T level 0.02 ng/mL, E/A ratio 1.4, RV systolic pressure 36 mmHg, and RV sSR (middle segment) −1.37/s (Figure 3).

**Table 1 Clinical variables, ECG characteristics, and biomarkers**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (( n = 38 ))</th>
<th>AL-normal-echo (( n = 53 ))</th>
<th>P-value</th>
<th>AL-normal-wall-thickness (( n = 106 ))</th>
<th>Advanced-CA (( n = 143 ))</th>
<th>P-value</th>
<th>HR(^b) (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>60 (13)</td>
<td>59 (10)</td>
<td>0.84</td>
<td>62 (10)</td>
<td>65 (10)</td>
<td>0.04</td>
<td>1.03 (0.006)</td>
</tr>
<tr>
<td>Men</td>
<td>18 (47)</td>
<td>27 (51)</td>
<td>0.83</td>
<td>62 (58)</td>
<td>93 (65)</td>
<td>0.29</td>
<td>NS</td>
</tr>
<tr>
<td>Heart rate, bpm</td>
<td>69 (13)</td>
<td>73 (14)</td>
<td>0.13</td>
<td>74 (12)</td>
<td>77 (14)</td>
<td>0.06</td>
<td>1.02 (0.008)</td>
</tr>
<tr>
<td>NYHA class</td>
<td></td>
<td></td>
<td>0.19</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.23</td>
<td>10 (9)</td>
<td>43 (30)</td>
<td>&lt;0.001</td>
<td>3.1 (0.001)</td>
</tr>
<tr>
<td>II</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>&gt;0.99</td>
<td>9 (8)</td>
<td>48 (34)</td>
<td>&lt;0.001</td>
<td>1.4 (0.04)</td>
</tr>
<tr>
<td>III</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.57</td>
<td>7 (7)</td>
<td>23 (16)</td>
<td>0.02</td>
<td>3.2 (&lt;0.001)</td>
</tr>
<tr>
<td>IV</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.12</td>
<td>20 (19)</td>
<td>61 (43)</td>
<td>&lt;0.001</td>
<td>1.9 (&lt;0.001)</td>
</tr>
<tr>
<td>Stem cell transplant</td>
<td>NA</td>
<td>34 (64)</td>
<td>0.25</td>
<td>60 (57)</td>
<td>69 (48)</td>
<td>0.5</td>
<td>0.5 (0.005)</td>
</tr>
<tr>
<td>Biomarkers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BNP, pg/mL</td>
<td>NA</td>
<td>NA</td>
<td>197</td>
<td>185</td>
<td>0.32</td>
<td>1.0</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>NT-proBNP, pg/mL</td>
<td>NA</td>
<td>NA</td>
<td>934</td>
<td>834</td>
<td>0.64</td>
<td>1.0</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Troponin T, ng/mL</td>
<td>NA</td>
<td>NA</td>
<td>0.01</td>
<td>0.04</td>
<td>&lt;0.001</td>
<td>2.9</td>
<td>(&lt;0.001)</td>
</tr>
<tr>
<td>Creatinine, mg/dL</td>
<td>NA</td>
<td>NA</td>
<td>1.1</td>
<td>1.2</td>
<td>0.88</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>GFR, mL/min/BSA</td>
<td>NA</td>
<td>NA</td>
<td>57</td>
<td>63</td>
<td>0.53</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Values are mean (SD) or \( n \) (%).

\(^b\)Survival analysis considers all patients with AL amyloidosis (\( N = 249 \)).

BNP, brain natriuretic peptide; BSA, body surface area; ECG, electrocardiography; GFR, glomerular filtration rate; HR, hazard ratio; NA, not applicable; NS, not significant; NT-proBNP, N-terminal pro-BNP; NYHA, New York Heart Association.

Subgroup analysis

In the group of patients who were enrolled at the time the diagnosis was ascertained and who completed laboratory, standard echocardiographic, and RV DMI assessment within 3 months (\( n = 106 \)), there were 52 deaths in a median follow-up of 53 months. HRs and significant predictors in the multivariable survival analysis were similar for these patients compared with the entire cohort (Table 5). The C-statistic of the model was not different, as demonstrated by the extensive overlapping of the 95% CIs obtained by applying the same models to the two samples of patients. When independent predictors of survival defined in the overall analysis were applied to this subgroup of patients, considering all the groups of variables together, male sex, ln BNP level, RV systolic pressure, and sSR of the RV middle segment were no longer significant at the 0.05 level. However, HRs (95% CIs) and regression coefficients and their standard errors were similar to those obtained in the overall analysis.

Intra-observer and inter-observer variability

The ICC for intra-reader reproducibility was greater for peak longitudinal systolic tissue velocity [0.91 (95% CI 0.79–1.02)], sS [0.98 (0.96–1.00)], and TAPSE [0.96 (0.90–1.01)] than for sSR [0.84 (0.58–1.04)]. The ICC for inter-reader reproducibility was also higher for systolic tissue velocity [0.92 (0.82–1.01)], sS [0.92 (0.82–1.02)], and TAPSE [0.94 (0.86–1.01)] than for sSR [0.83 (0.53–0.93)].

Discussion

To our knowledge, this is the first investigation to test the usefulness of a comprehensive assessment of RV function, including TAPSE and RV DMI measures, for the early diagnosis of RV myocardial dysfunction and for estimating prognosis in patients with AL.
amyloidosis. The current investigation had several main findings. DMI measures revealed evidence of abnormal RV systolic function in patients with AL amyloidosis compared with healthy subjects, despite normal standard 2D echocardiographic measurements and normal E' velocity of the mitral annulus. TAPSE and DMI modalities also demonstrated more profound RV dysfunction in patients with overt CA compared with patients with apparently normal cardiac performance. Combined assessment of sS of the basal segment of the RV free wall and TAPSE was the most accurate strategy for the early diagnosis of RV dysfunction in patients with AL amyloidosis. Finally, RV systolic pressure and RV sSR of the middle segment were independent predictors of outcome by multivariable survival analysis, which included clinical information, electrocardiography findings, biochemical data, standard echocardiographic variables, and parameters of RV function by standard and DMI echocardiography.

TAPSE is an excellent measure of RV longitudinal systolic function with high feasibility and reproducibility. In our population, its diagnostic accuracy was comparable with that of sS for the early detection of longitudinal systolic RV impairment in AL-normal-echo patients, and it had incremental value for this purpose. In addition, RV free wall thickness and TAPSE (or sS of the middle segment) were the most accurate measures for distinguishing between the advanced-CA and AL-normal-wall-thickness groups. Finally, TAPSE was a significant predictor of outcome in univariable analysis, although it was not an independent predictor in the final multivariable model—longitudinal sSR of the RV free wall was a better prognostic factor.

We have previously highlighted the importance of pleural effusion and high BNP levels as independent negative prognostic factors in patients with AL amyloidosis during short-term follow-up. In the present study, we confirmed their usefulness with longer follow-up duration and more events. Consistent with a previous report from our group, we also confirmed the importance of troponin T level as an independent predictor of mortality. BNP and troponin levels provide complementary prognostic information; therefore, combining these two biomarkers is an efficient strategy to better define risk in patients with AL amyloidosis. It is noteworthy that, after considering female sex, the presence of pleural effusion, and abnormally high BNP and troponin T levels,
the only standard echocardiographic measures to provide useful prognostic information were transmirtal E/A ratio and RV systolic pressure. The prognostic usefulness of diastolic transmitral flow Doppler analysis has been previously reported in patients with AL amyloidosis, but the striking role of RV systolic pressure in risk stratification is a new observation. The prognostic value of systolic pressure assessed in the RV was so high in our population as to overcome well-established predictors by LV standard echocardiography, such as LV thickness/mass, pulsed-wave tissue Doppler imaging, and other parameters of diastolic dysfunction. This observation underscores that, although pulmonary artery hypertension is not common in AL amyloidosis, high RV systolic pressure in these patients is an ominous finding.

Finally, consistent with our previous findings on LV function in AL amyloidosis, longitudinal sS was also the most sensitive diagnostic DMI modality in the RV. It was able to accurately discriminate not only between AL patients with apparently normal cardiac function and healthy subjects, but also between patients with overt CA and those at earlier stages of disease (AL-normal-wall-thickness). Surprisingly, and different from our previous observations in the LV, longitudinal sSR of the RV free wall, not sS, was the most significant DMI modality to help in predicting survival in our population. In particular, sSR of the middle RV free wall segment was an independent predictor of mortality, showing statistical significance only slightly higher than sSR of the RV base, but definitely greater than sS. It is therefore tempting to hypothesize that sS and sSR imaging offer different (but equally substantial) pathophysiological information when considering LV and RV myocardial fibres. In a pressure pump with high afterload and low wall excursion, such as the LV, assessment of total deformation of the longitudinally oriented fibres (i.e. strain) provides the most helpful data in defining risk of death, whereas in a volume pump with high preload, low afterload, and high wall excursion, such as the RV, assessment of rate of deformation (i.e. strain rate) is apparently more prognostically useful than the total deformation (i.e. strain).

### Limitations

Because the subgroup of patients enrolled around the time of diagnosis (n = 106) is likely most representative of patients being encountered by clinicians and cardiologists working in primary or secondary referral centres, we tested the generalizability of the final multivariable model by applying the same predictors to this subgroup. HRs, regression coefficients, and overall C-statistics were similar for this subgroup compared with the entire cohort, suggesting that patients enrolled around the time of diagnosis do not have different characteristics than the whole study population and that the same criteria can be used to stratify their risk of death. However, statistical power in this relatively small subset was limited.

Several variables were tested in this investigation for their potential role as prognostic factors. Although the follow-up period and number of events were adequate, some of the predictors omitted from our multivariable analysis could still be useful, as has been reported by other researchers. In particular, we did not compare the prognostic usefulness of RV DMI parameters with DMI values obtained from the LV, although the RV parameters have been extremely promising in a recent analysis completed by our group. This decision was made after careful evaluation to avoid the risk of defining falsely independent

### Table 3: RV measures

<table>
<thead>
<tr>
<th>Variable</th>
<th>Controls (n = 38)</th>
<th>AL-normal-echo (n = 53)</th>
<th>P-value</th>
<th>AL-normal-wall-thickness (n = 106)</th>
<th>Advanced-CA (n = 143)</th>
<th>P-value</th>
<th>HR* (P-value)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EDD middle, mm</td>
<td>21 (9)</td>
<td>19 (5)</td>
<td>0.11</td>
<td>19 (4)</td>
<td>19 (5)</td>
<td>0.75</td>
<td>NS</td>
</tr>
<tr>
<td>FAC, %</td>
<td>51 (12)</td>
<td>45 (8)</td>
<td>0.06</td>
<td>45 (9)</td>
<td>45 (10)</td>
<td>0.74</td>
<td>NS</td>
</tr>
<tr>
<td>TR, cm/s</td>
<td>2.52 (0.1)</td>
<td>2.52 (0.3)</td>
<td>0.92</td>
<td>2.49 (1)</td>
<td>2.71 (0.5)</td>
<td>&lt;0.001</td>
<td>1.1 (0.01)</td>
</tr>
<tr>
<td>RVSP, mmHg</td>
<td>31 (6)</td>
<td>32 (7)</td>
<td>0.49</td>
<td>32.3 (11)</td>
<td>39.26 (12)</td>
<td>&lt;0.001</td>
<td>1.04 (0.001)</td>
</tr>
<tr>
<td>Ejection time, ms</td>
<td>307 (37)</td>
<td>286 (43)</td>
<td>0.03</td>
<td>281 (39)</td>
<td>267 (37)</td>
<td>0.009</td>
<td>NS</td>
</tr>
<tr>
<td>RIMP</td>
<td>0.26 (0.2)</td>
<td>0.3 (0.2)</td>
<td>0.20</td>
<td>0.32 (0.2)</td>
<td>0.4 (0.2)</td>
<td>0.003</td>
<td>NS</td>
</tr>
<tr>
<td>TAPSE, cm</td>
<td>2.7 (0.5)</td>
<td>2.3 (0.5)</td>
<td>0.008</td>
<td>2.2 (0.6)</td>
<td>1.7 (0.6)</td>
<td>&lt;0.001</td>
<td>0.9 (0.002)</td>
</tr>
<tr>
<td>sTV basal, cm/s</td>
<td>10.56 (2)</td>
<td>9.1 (3)</td>
<td>0.02</td>
<td>9.11 (3)</td>
<td>8.26 (3)</td>
<td>0.006</td>
<td>NS</td>
</tr>
<tr>
<td>sTV middle, cm/s</td>
<td>7.59 (2)</td>
<td>5.94 (3)</td>
<td>0.006</td>
<td>5.95 (3)</td>
<td>5.52 (3)</td>
<td>0.09</td>
<td>NS</td>
</tr>
<tr>
<td>sSR basal, L/s</td>
<td>−2.39 (1)</td>
<td>−2.06 (1)</td>
<td>0.04</td>
<td>−2.04 (1)</td>
<td>−1.44 (1)</td>
<td>&lt;0.001</td>
<td>1.3 (0.001)</td>
</tr>
<tr>
<td>sSR middle, L/s</td>
<td>−2.21 (1)</td>
<td>−1.8 (1)</td>
<td>0.01</td>
<td>−1.79 (1)</td>
<td>−1.47 (1)</td>
<td>0.007</td>
<td>NS</td>
</tr>
<tr>
<td>sS basal, %</td>
<td>−34.77 (5)</td>
<td>−28.26 (9)</td>
<td>&lt;0.001</td>
<td>−27.1 (9)</td>
<td>−21.02 (9)</td>
<td>&lt;0.001</td>
<td>1.04 (0.002)</td>
</tr>
<tr>
<td>sS middle, %</td>
<td>−34.09 (7)</td>
<td>−28.46 (8)</td>
<td>0.005</td>
<td>−27.88 (8)</td>
<td>−20.96 (9)</td>
<td>&lt;0.001</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Values are mean (SD).

**Survival analysis considers all patients with AL amyloidosis (N = 249).

EDD, end-diastolic diameter; FAC, fractional area change; HR, hazard ratio; NS, not significant; RIMP, right index of myocardial performance; RVSP, right ventricular systolic pressure; sS, systolic strain; sSR, systolic strain rate; sTV, systolic tissue velocity; TAPSE, tricuspid annular plane systolic excursion; TR, tricuspid regurgitation.
predictors of mortality in our study population, obtained merely because too many statistical inferences were considered together (i.e. the ‘multiple comparisons’ problem).

Although we excluded all patients with a history of coronary artery disease, as well as patients having symptoms (e.g. chest pain) or echocardiographic evidence of myocardial ischaemia (e.g. wall-motion abnormalities), stress testing or coronary angiography was not performed for all our patients. This was in part for ethical considerations, but mainly because pre-test probability of coronary artery disease is very low in patients with AL amyloidosis with an unremarkable past medical history who are asymptomatic and have normal wall motion on echocardiography.

For this investigation, RV sS and sSR were measured by DMI. A recently published consensus statement of the European and American societies of echocardiography stated clearly that ‘in the majority of areas, this methodology [i.e. DMI] is not yet ready for routine clinical use’ and that ‘additional testing is needed in multicenter settings to better establish the diagnostic accuracy of the different parameters and their reproducibility in various disease states.’ We agree with these considerations and, although DMI measures collected in the present study have high reproducibility, our results should be interpreted with caution. We are looking forward to better standardization of parameters among manufacturers and believe that our observations will be confirmed by other groups.

**Conclusions**

In this study, we observed that patients with AL amyloidosis and apparently normal 2D and Doppler echocardiography results have early impairment of RV longitudinal systolic function, primarily detected by sS of the basal segment of the RV free wall and TAPSE. Moreover, we compared for the first time clinical, laboratory, and...
Table 4  Multivariate analysis: independent predictors of survivala

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)b</th>
<th>P-value</th>
<th>HR (95% CI)c</th>
<th>P-value</th>
<th>HR (95% CI)d</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.03 (1.02–1.05)</td>
<td>&lt;0.001</td>
<td>1.05 (1.03–1.07)</td>
<td>&lt;0.001</td>
<td>1.04 (1.01–1.06)</td>
<td>0.007</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.96 (1.28–2.98)</td>
<td>0.002</td>
<td>2.16 (1.35–3.47)</td>
<td>0.001</td>
<td>2.17 (1.26–3.74)</td>
<td>0.005</td>
</tr>
<tr>
<td>BNP (ln)</td>
<td>1.28 (1.1–1.5)</td>
<td>0.001</td>
<td>1.32 (1.11–1.57)</td>
<td>0.002</td>
<td>1.37 (1.12–1.68)</td>
<td>0.003</td>
</tr>
<tr>
<td>Troponin T (ln)</td>
<td>1.99 (1.53–11.46)</td>
<td>&lt;0.001</td>
<td>1.78 (1.32–2.39)</td>
<td>&lt;0.001</td>
<td>1.58 (1.11–2.27)</td>
<td>0.01</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>3.97 (2.42–6.52)</td>
<td>&lt;0.001</td>
<td>3.56 (1.98–6.41)</td>
<td>&lt;0.001</td>
<td>1.23 (1.06–1.43)</td>
<td>0.006</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.27 (1.11–1.44)</td>
<td>&lt;0.001</td>
<td>1.02 (1.00–1.04)</td>
<td>0.01</td>
<td>1.3 (1.04–1.62)</td>
<td>0.02</td>
</tr>
<tr>
<td>RV systolic pressure</td>
<td>1.02 (0.98–1.05)</td>
<td>0.03</td>
<td>1.65 (0.81–3.36)</td>
<td>0.17</td>
<td>1.65 (0.81–3.36)</td>
<td>0.17</td>
</tr>
<tr>
<td>RV middle sSR</td>
<td>1.78 (1.32–2.39)</td>
<td>&lt;0.001</td>
<td>1.58 (1.11–2.27)</td>
<td>&lt;0.001</td>
<td>1.58 (1.11–2.27)</td>
<td>0.01</td>
</tr>
</tbody>
</table>

4Stepwise multivariable Cox proportional hazards models. Endpoint is death from any cause. The models were built with a stepwise (backward) approach, initially considering all 249 patients together, and were validated through the bootstrap technique.

5Clinical data, biomarkers, and standard echocardiography parameters were considered together. Model C-statistic: 0.79 (95% CI 0.72–0.86).

6Clinical data, biomarkers, and standard echocardiography parameters were considered together. Model C-statistic: 0.78 (95% CI 0.73–0.83); P = 0.54 (vs. previous model).

BNP, brain natriuretic peptide; CI, confidence interval; HR, hazard ratio; RV, right ventricular; sSR, systolic strain rate.

Table 5  Independent predictors of survival: subgroup multivariable analysis for patients with diagnosis of AL amyloidosis within 3 months of echocardiography and other testinga

<table>
<thead>
<tr>
<th>Variable</th>
<th>HR (95% CI)b</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1.04 (1.01–1.08)</td>
<td>0.02</td>
</tr>
<tr>
<td>Male sex</td>
<td>2.09 (0.8–5.45)</td>
<td>0.13</td>
</tr>
<tr>
<td>BNP (ln)</td>
<td>1.36 (0.94–1.96)</td>
<td>0.1</td>
</tr>
<tr>
<td>Troponin T (ln)</td>
<td>1.95 (1.20–3.14)</td>
<td>0.006</td>
</tr>
<tr>
<td>Pleural effusion</td>
<td>2.93 (1.07–8.01)</td>
<td>0.04</td>
</tr>
<tr>
<td>E/A ratio</td>
<td>1.25 (1.02–1.53)</td>
<td>0.03</td>
</tr>
<tr>
<td>RV systolic pressure</td>
<td>1.02 (0.98–1.05)</td>
<td>0.37</td>
</tr>
<tr>
<td>RV middle sSR</td>
<td>1.65 (0.81–3.36)</td>
<td>0.17</td>
</tr>
</tbody>
</table>

4Subgroup multivariable Cox proportional hazards model. The final model shown in Table 4 is applied to the population of patients enrolled at the time of diagnosis (n = 106; 52 total deaths).

5Model C-statistic: 0.79 (95% CI 0.72–0.86).

BNP, brain natriuretic peptide; CI, confidence interval; HR, hazard ratio; RV, right ventricular; sSR, systolic strain rate.

Conflict of interest: none.

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References


