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ORIGINAL RESEARCH

# Plasma Hepatocyte Growth Factor for Diagnosis and Prognosis in Light Chain and Transthyretin Cardiac Amyloidosis

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## ABSTRACT

**OBJECTIVES** This study determined the diagnostic and prognostic usefulness of hepatocyte growth factor (HGF) in light chain and transthyretin cardiac amyloidosis.

**BACKGROUND** Delays in diagnosis of cardiac amyloidosis are common, usually resulting from nonspecific findings on clinical examination and testing. A discriminatory plasma biomarker could result in earlier diagnosis and improve prognosis assessment.

**METHODS** A total of 188 patients with cardiac amyloidosis, amyloidosis without cardiac involvement, symptomatic heart failure with left ventricular hypertrophy (LVH), or heart failure with a reduced ejection fraction (HFrEF) were enrolled prospectively. Serum biomarkers were measured at study enrollment, and all patients with amyloidosis were followed for all-cause mortality, cardiac transplantation, or left ventricular assist device implantation. Multinomial logistic regression and Kaplan-Meier survival estimates tested the association of biomarker levels with cardiac amyloidosis and clinical outcomes, respectively. Harrell's C-statistic and the likelihood ratio test compared the prognostic accuracy of plasma biomarkers.

**RESULTS** HGF was significantly higher in patients with cardiac amyloidosis ($p < 0.001$). An HGF level of 205 pg/ml discriminated cardiac amyloidosis from LVH and HFrEF with 86% sensitivity, 84% specificity, and an area under the curve of 0.88 (95% confidence interval: 0.83 to 0.94). In patients with amyloidosis, elevated HGF levels were associated with worse event-free survival over a median follow-up of 2.6 years ($p < 0.001$) with incremental prognostic accuracy over N-terminal pro-B-type natriuretic peptide and troponin T ($p < 0.001$).

**CONCLUSIONS** HGF discriminates light chain and transthyretin cardiac amyloidosis from patients with symptomatic heart failure with LVH or HFrEF and is associated with worse cardiac outcomes. Confirmation of these findings in a larger, multicenter study that is enrolling suspected cases of cardiac amyloidosis is underway.


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Cardiac amyloidosis is a restrictive cardiomyopathy that results from the intramyocardial deposition of amyloid fibrils, which are composed of misfolded light chain or transthyretin proteins in >95% of cases. Although light chain amyloidosis (AL) and hereditary transthyretin amyloidosis (ATTR) are uncommon conditions, wild-type ATTR is likely underdiagnosed, particularly among patients with heart failure with preserved ejection fraction and calcific aortic stenosis (1–5). Traditionally, treatment options for AL and ATTR have been limited, and median survival has been only 2 to 4 years from time of diagnosis (6,7). Recently, several therapeutics were approved for the treatment of ATTR, whereas multiple other agents are in late stage drug development for both AL and ATTR (8). Accordingly, earlier diagnosis of cardiac amyloidosis is an important clinical priority.

The presenting signs, symptoms, and findings on routine cardiac testing for patients with cardiac amyloidosis are nonspecific. As a result, a potential delay of up to 36 months is common for patients with AL and ATTR cardiac amyloidosis, with more advanced disease documented at presentation in those with longer delays in diagnosis (9,10). Transthoracic echocardiography (TTE) is widely available and may be useful to differentiate cardiac amyloidosis from other causes of symptomatic heart failure. Specifically, the ratio of left ventricular ejection fraction (LVEF) to global longitudinal strain and a relative apical sparing pattern in longitudinal strain have been shown to be diagnostically useful (11,12). However, a plasma biomarker that discriminates cardiac amyloidosis among patients with symptomatic heart failure has yet to be identified.

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Hepatocyte growth factor (HGF) is a pro-angiogenic, mitogenic, and morphogenic cytokine that is primarily expressed in mesenchymal cells (13). Small studies, including preliminary data from our own group, have shown that HGF is elevated in patients with amyloidosis, particularly in those with cardiac involvement (14–16). The β-pleated sheet structure of amyloid fibrils has been shown to activate tissue-type plasminogen activator, which increases production of HGF due to structural homology of pro-HGF with plasminogen (17,18). In addition, HGF mediates mesenchymal-epithelial interactions during the process of tissue repair, which may be triggered by amyloid fibril deposition (13,14). The usefulness of HGF to differentiate cardiac amyloidosis from other causes of symptomatic heart failure is not known.

Fibrosis and inflammation are additional processes that may mediate the pathophysiological effects of amyloid fibril deposition (1,19). Galectin-3 and interleukin (IL)-6 are known biomarkers of fibrosis and inflammation, respectively, which may have diagnostic and prognostic usefulness for cardiac amyloidosis.

The primary objective of this study was to assess the diagnostic performance of HGF for AL and ATTR cardiac amyloidosis among patients with symptomatic heart failure, and to determine the prognostic usefulness of HGF for adverse cardiac events in patients with AL and ATTR amyloidosis. We also explored the diagnostic usefulness of galectin-3 and IL-6 for AL and ATTR cardiac amyloidosis.

## METHODS

**STUDY PARTICIPANTS.** Consecutive patients referred to the Vanderbilt Amyloidosis Multidisciplinary Program for initial evaluation of cardiac amyloidosis were enrolled prospectively into the Main Heart Registry from 2010 to 2017. The diagnosis of cardiac amyloidosis was established by 1 of 2 criteria: 1) positive endomyocardial biopsy by Congo red stain; or 2) positive noncardiac biopsy by Congo red stain with increased wall thickness on TTE, low voltage or pseudo-infarction on electrocardiogram, or characteristic delayed myocardial enhancement on cardiac magnetic resonance imaging. All positive biopsy specimens were sent to the Mayo Clinic reference laboratory for mass spectrometry subtyping of the amyloid protein. Radionuclide bone scintigraphy was not used for diagnosis because it was not routinely available over the entire study.

TTE, electrocardiography, and cardiac magnetic resonance studies were performed as part of routine patient care. Increased wall thickness was defined according to American Society of Echocardiography guidelines for left ventricular hypertrophy (LVH) as a left ventricular mass index of >115 g/m$^2$ in men and >95 g/m$^2$, in women with relative wall thickness of >0.44 (20). Low voltage on electrocardiography was defined as QRS amplitude <5 mV in all limb leads or <10 mV in all precordial leads (21). Late gadolinium enhancement on cardiac magnetic resonance was evaluated using standard sequences.

Detailed clinical and demographic data were obtained at the time of study entry using a standardized questionnaire administered by study personnel, with verification via medical records. All patients with

### ABBREVIATIONS AND ACRONYMS

**AL** = light chain amyloidosis

**ATTR** = transthyretin amyloidosis

**ATTR-CA** = transthyretin cardiac amyloidosis

**CI** = confidence interval

**CV** = coefficient of variation

**eGFR** = estimated glomerular filtration rate

**HFrEF** = heart failure with reduced ejection fraction

**HGF** = hepatocyte growth factor

**IL** = interleukin

**IVSd** = interventricular septal diameter in diastole

**LVAD** = left ventricular assist device

**LVEF** = left ventricular ejection fraction

**LVH** = left ventricular hypertrophy

**NT-proBNP** = N-terminal pro-B-type natriuretic peptide

**ROC** = receiver-operating characteristic

**TTE** = transthoracic echocardiography

amyloidosis were followed for the combined outcome of all-cause mortality, cardiac transplantation, or left ventricular assist device (LVAD) implantation.

As a control or comparator group, consecutive patients referred to the Heart Failure Clinic at the Vanderbilt Heart and Vascular Institute from 2010 to 2012 with symptomatic heart failure and either LVH or heart failure with reduced ejection fraction (HFrEF) were enrolled into the Main Heart Registry. LVH was defined using the previously described American Society of Echocardiography guidelines, and reduced ejection fraction was defined as LVEF ≤30% and New York Heart Association functional class III or IV. All control patients underwent a baseline assessment and were deemed to be free of amyloidosis by clinical evaluation.

This study was approved by the Vanderbilt University Medical Center Institutional Review Board. All participants provided written informed consent.

**LABORATORY ANALYSES.** Blood samples were collected at the time of study enrollment, and plasma was separated by centrifugation for 15 min at 1,500*g* at 4°C. Plasma aliquots were stored at −80 °C before use in assays.

HGF and IL-6 were measured using the Milliplex Map Kit (EMD Millipore Corporation, Billerica, Massachusetts), which is a 2-step chemiluminescent microsphere immunoassay. The limit of detection of the HGF assay is 6.8 pg/ml, with intra- and inter-assay coefficients of variation (CV) of 8.7% and 8.8%, respectively. The limit of detection of the IL-6 assay is 0.2 pg/ml with intra-assay and interassay CVs of 9.3% and 5.5%, respectively.

Galectin-3 was measured using a 2-step, sandwich enzyme chemiluminescent immunoassay using Quantikine technology (BioTek Instruments, Winooski, Vermont). The limit of detection of galectin-3 ranged from 0.003 to 0.085 ng/ml (mean: 0.016 ng/ml). The intra assay and interassay CVs ranged from 3.0% to 4.4% and 6.8% to 8.6%, respectively.

N-terminal pro-B-type natriuretic peptide (NT-proBNP) and troponin-T were measured on the Cobas e 411 analyzer using Elecsys immunoassays (Roche Diagnostics, Indianapolis, Indiana), which uses 2-step, sandwich electrochemoluminescence technology. The limit of detection of the NT-proBNP assay is 5.0 pg/ml with intra- and interassay CVs ranging from 1.8% to 4.6% and 1.3% to 4.2%, respectively. The limit of detection of the troponin-T assay is 5 ng/l with intra- and inter-assay CVs ranging from 1.4% to 10.3% and 0.7% to 5.6%, respectively.

For patients with symptomatic heart failure and either LVH or HFrEF, BNP and troponin-I were measured in the clinical laboratory using commercial fluorescence immunoassays as part of routine clinical care.

**STATISTICAL ANALYSIS.** Baseline data were summarized as median (25th and 75th percentile range [interquartile range]) for continuous variables and counts (percentages) for categorical data. Categorical variables among the 4 groups were compared using the chi-square test or Fisher's exact test. Continuous variables with approximately normal distributions were compared with 1-way analysis of variance, accounting for unequal variances and using the Tukey-Kramer adjustment for between group comparisons. The Kruskal-Wallis test was used to compare continuous variables where the normality assumption was not met. Approximate normality was determined graphically by reviewing the histograms and Q-Q plots for each variable's distribution.

Multinomial logistic regression tested the association of biomarker levels with cardiac amyloidosis compared with the other groups (amyloidosis without cardiac involvement, LVH, and HFrEF) after adjusting for age, left ventricular interventricular septal diameter at end-diastole (IVSd), and LVEF, which were selected based on clinical judgment. Log transformation of continuous variables was used for linearity assumption. Receiver-operating characteristic (ROC) analysis was performed to identify an optimal HGF cutoff for the diagnosis of cardiac amyloidosis using Youden's index. Further ROC analysis explored the relative discriminative ability of HGF, NT-proBNP, and troponin-T to predict cardiac involvement in patients with amyloidosis. All biomarker data were subjected to natural logarithmic transformation for the regression models, including the ROC analyses.

Kaplan-Meier survival estimates for the combined outcome of all-cause mortality, cardiac transplantation, or LVAD implantation were compared between those with normal and abnormal biomarker values using the log-rank test. We determined abnormal levels of HGF through our ROC analysis, whereas abnormal levels of previous biomarkers were defined by cutoffs that were used in published staging models for AL and ATTR cardiac amyloidosis (NT-pro-BNP: 332 pg/ml; troponin-T: 35 ng/l, and estimated glomerular filtration rate [eGFR]: 45 ml/min/1.73 $m^2$) (7,22).

Harrell's C-statistic was used to compare the predictive ability of abnormal biomarker values for the combined outcome by area under the curve analysis, accounting for censoring (23). The discriminative ability of NT-proBNP, troponin-T, and HGF for

**TABLE 1 Baseline Clinical and Echocardiographic Characteristics**

| | Cardiac Amyloidosis (n = 72) | Amyloidosis Without Cardiac Involvement (n = 30) | Heart Failure With Left Ventricular Hypertrophy (n = 44) | Heart Failure With Reduced Ejection Fraction (n = 42) | p Value* |
|---|---|---|---|---|---|
| Age (yrs) | 65 (56–71)§ | 59 (52–70) | 60 (46–70) | 56 (49–67) | 0.005 |
| Male (%) | 60 | 50 | 59 | 57 | 0.83 |
| Systolic BP (mm Hg) | 112 (101–123)†‡ | 119 (110–132) | 122 (104–132) | 107 (100–128) | 0.009 |
| Diastolic BP (mm Hg) | 70 (62–78)† | 77 (72–82) | 70 (62–78) | 68 (64–72) | 0.001 |
| eGFR <45 ml/min/1.73 $m^2$ (%) | 35 | 37 | 30 | 31 | 0.38 |
| NYHA functional class III/IV (%) | 44‡§ | 0 | 16 | 100 | <0.001 |
| Medications (%) | | | | | |
| ACEI/ARB | 36 | 37 | 32 | 57 | 0.073 |
| Aldosterone antagonist | 58†‡ | 13 | 11 | 50 | <0.001 |
| Beta-blocker | 38‡§ | 27 | 73 | 86 | <0.001 |
| Loop diuretic | 78†‡ | 27 | 25 | 81 | <0.001 |
| Digoxin | 11§ | 0 | 2 | 36 | <0.001 |
| Echocardiography | | | | | |
| LVEF (%) | 55 (40–60)†‡§ | 60 (55–62) | 60 (60–65) | 20 (15–25) | <0.001 |
| IVSd (cm) | 1.5 (1.2–1.7)†‡§ | 1.1 (1.0–1.2) | 1.6 (1.4–2.1) | 1.1 (0.9–1.2) | <0.001 |

Values are median (interquartile range) or %. Categorical variables among the 4 groups were compared using the chi-square test or Fisher's exact test. Continuous variables with approximately normal distributions were compared with 1-way analysis of variance, accounting for unequal variances and using the Tukey-Kramer adjustment for between group comparisons. The Kruskal-Wallis test was used to compare continuous variables when the normality assumption was not met. *Compared across all groups. †$p < 0.05$ compared with amyloidosis without cardiac involvement. ‡$p < 0.05$ compared with left ventricular hypertrophy. §$p < 0.05$ compared with heart failure with reduced ejection fraction.

ACEI = angiotensin-converting enzyme inhibitor; ARB = aldosterone receptor blocker; BP = blood pressure; eGFR = estimated glomerular filtration rate; IVSd = interventricular septal thickness in diastole; LVEF = left ventricular ejection fraction; NYHA = New York Heart Association.

prediction of the combined outcome was further evaluated using Akaike information criterion, with smaller values indicating better fit, and the likelihood ratio test to compare the difference between models (24).

All analyses were performed using complete case analysis. As a sensitivity analysis, we conducted 10 iterations of multiple imputation to estimate missing HGF values using fully conditional specification. Covariates in the regression model were selected based on clinical judgment and included age, sex, use of cardiac medications, LVEF, IVSd, blood pressure, IL-6, and galectin-3 at baseline. We then used the 10 imputed datasets to confirm the association of HGF with the diagnosis of cardiac amyloidosis.

All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, North Carolina) with a 2-sided alpha set at 0.05.

## RESULTS

**PATIENT CHARACTERISTICS.** Baseline clinical and echocardiographic characteristics of the 188 patients included in this study are shown in Table 1. Of the 102

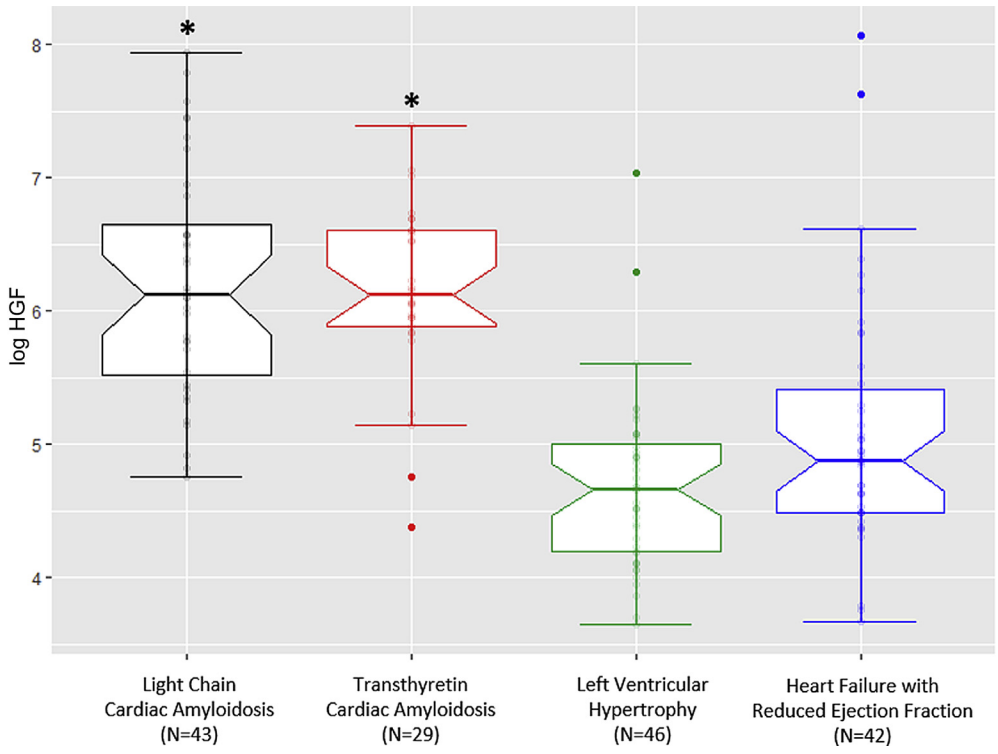
**TABLE 2 Baseline Biomarker Characteristics**

| | Cardiac Amyloidosis (n = 72) | Amyloidosis Without Cardiac Involvement (n = 30) | Heart Failure With Left Ventricular Hypertrophy (n = 44) | Heart Failure With Reduced Ejection Fraction (n = 42) | p Value* |
|---|---|---|---|---|---|
| HGF (pg/ml) | 455 (323–735)†‡§ | 247 (124–490) | 105 (66–151) | 131 (89–234) | <0.001 |
| Galectin-3 (ng/ml) | 14 (10–18)‡§ | 13 (11–21) | 19 (14–29) | 22 (15–26) | <0.001 |
| IL-6 (pg/ml) | 4.1 (3.2–5.5)‡ | 4.3 (2.7–4.7) | 3.0 (1.5–4.3) | 3.8 (2.2–6.2) | <0.003 |
| NT-proBNP (pg/ml) | 2,363 (735–5,634)† | 204 (53–559) | — | — | <0.001 |
| BNP (pg/ml) | — | — | 357 (135–775) | 945 (256–1,495) | — |
| Troponin-T (ng/l) | 43 (14–72)† | 12 (9–23) | — | — | <0.001 |
| Troponin-I (ng/ml) | — | — | 0.04 (0.02–0.14) | 0.04 (0.03–0.51) | — |

Values are median (interquartile range). The Kruskal-Wallis test was used to compare biomarker values across groups. *Compared across all groups. †$p < 0.05$ compared with amyloidosis without cardiac involvement. ‡$p < 0.05$ compared with left ventricular hypertrophy. §$p < 0.05$ compared with heart failure with reduced ejection fraction.

HGF = hepatocyte growth factor; IL = interleukin; NT-proBNP = N-terminal pro-B-type natriuretic peptide.

**FIGURE 1 HGF Is Elevated in Light Chain and Transthyretin Cardiac Amyloidosis**




Box and whisker plots show median and interquartile range for normalized hepatocyte growth factor (HGF) values for patients with light chain cardiac amyloidosis, transthyretin cardiac amyloidosis, left ventricular hypertrophy (LVH), and heart failure with reduced ejection fraction (HFrEF). HGF was significantly higher in light chain and transthyretin cardiac amyloidosis compared with LVH and HFrEF ($p < 0.001$). *$p < 0.001$ compared with LVH and HFrEF.

patients with amyloidosis, 69 had AL and 33 had ATTR; 90 were white and 12 were African American. Seventy-two patients had amyloidosis with cardiac involvement (40 diagnosed by endomyocardial biopsy, 32 diagnosed by extracardiac biopsy with supportive cardiac imaging findings) and 30 patients had amyloidosis without cardiac involvement (26 with AL, 4 with ATTR). In our control group, 44 patients had LVH and 42 had HFrEF. There were no significant differences in sex or prevalence of renal dysfunction across the 4 groups.

Patients with cardiac amyloidosis tended to be older than those with LVH ($p = 0.052$) and HFrEF ($p < 0.05$). The LVEF was lower compared with those with amyloidosis without cardiac involvement and LVH and higher compared with those with HFrEF ($p < 0.05$). Patients with cardiac amyloidosis had increased IVSd compared with those with amyloidosis without cardiac involvement and HFrEF but had decreased IVSd compared with those with LVH ($p < 0.05$).

**BASELINE BIOMARKER CHARACTERISTICS.** Baseline biomarker levels for the study population are shown in Table 2. HGF, galectin-3, and IL-6 were measured in ≥90% of patients with cardiac amyloidosis and ≥83% of patients with amyloidosis without cardiac involvement. NT-proBNP and troponin-T were measured in 97% of patients with cardiac amyloidosis and 90% of patients with amyloidosis without cardiac involvement. HGF and galectin-3 were measured in the entire control group.

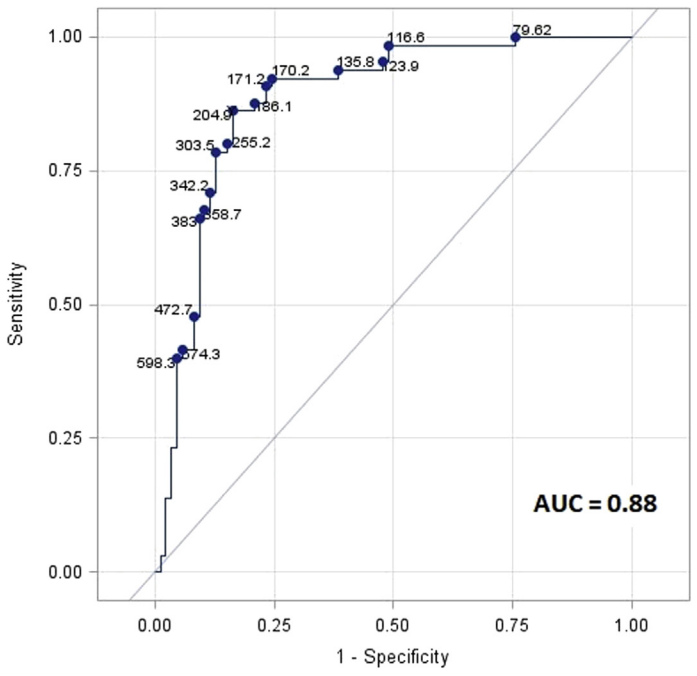
HGF was significantly higher in patients with cardiac amyloidosis compared with patients with amyloidosis without cardiac involvement, LVH, and HFrEF ($p < 0.001$) (Figure 1, Table 2). In multinomial logistic regression analysis, HGF remained significantly associated with cardiac amyloidosis after adjusting for age, IVSd, and LVEF ($p < 0.001$). In sensitivity analysis using 10 imputed datasets, this multivariable association remained significant ($p < 0.001$). There was no significant difference in HGF between the AL and ATTR cardiac amyloidosis

groups (p = 0.87). Compared with the LVH and HFrEF control subjects, HGF showed good discrimination for cardiac amyloidosis by ROC analysis with an area under the curve of 0.88 (95% CI: 0.83 to 0.94). An HGF cutoff of 205 pg/ml was optimal for the diagnosis of cardiac amyloidosis in this population, with 86% (95% CI: 78% to 94%) sensitivity and 84% (95% CI: 76% to 92%) specificity (Figure 2).

Galectin-3 was lower in patients with cardiac amyloidosis compared with the control subjects with LVH and HFrEF in the univariable (p < 0.001) and multivariable analyses (p = 0.002). There was no difference in galectin-3 compared with those with amyloidosis without cardiac involvement (p = 0.99). A cutoff of 18.4 ng/ml was optimal in discriminating cardiac amyloidosis from the control subjects with LVH and HFrEF with an area under the curve of 0.71 (95% CI: 0.64 to 0.78). IL-6 was higher in patients with cardiac amyloidosis compared with the control subjects with LVH in the univariable (p < 0.01) and multivariable analysis (p = 0.014), with no difference compared with those with amyloidosis without cardiac involvement (p = 0.66) and HFrEF (p = 0.36).

Among patients with amyloidosis, NT-proBNP and troponin-T were significantly higher in those with cardiac involvement in univariable (p < 0.001 for both) and multivariable analyses (p = 0.002 and 0.008, respectively). NT-proBNP and troponin-T data were not available for the LVH and HFrEF groups, although BNP and troponin-I were provided as a reference (Table 2).

**FIGURE 2 HGF Has Diagnostic Usefulness for Cardiac Amyloidosis Compared With Symptomatic Heart Failure With LVH or HFrEF**




Receiver-operator characteristic analysis was used to evaluate the diagnostic performance of HGF for cardiac amyloidosis compared with symptomatic heart failure with LVH hypertrophy or HFrEF. An HGF level of 205 pg/ml was optimal and identified light chain or transthyretin cardiac amyloidosis with 86% sensitivity, 84% specificity, and area under the curve (AUC) of 0.88. Other abbreviations as in Figure 1.

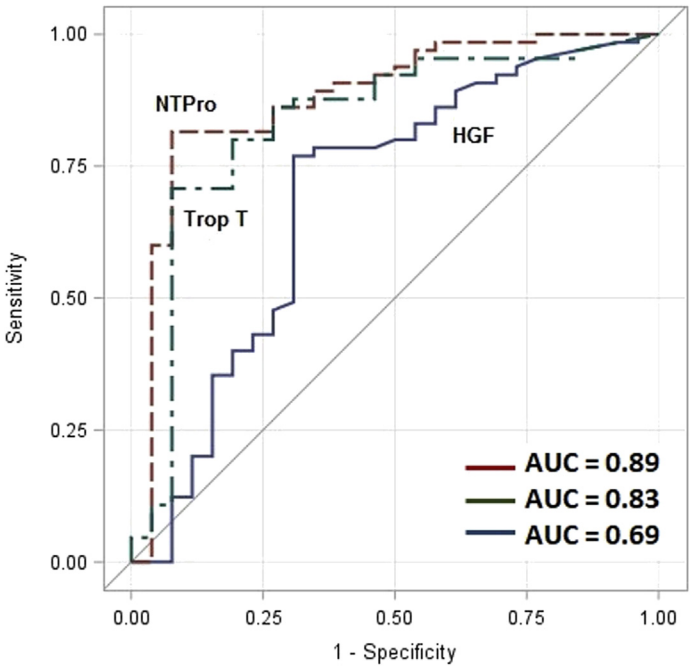
**CONFIRMATORY USEFULNESS OF HGF FOR CARDIAC INVOLVEMENT IN AMYLOIDOSIS.** Among the 102 patients with amyloidosis, we evaluated the confirmatory usefulness of elevated HGF for cardiac involvement with amyloidosis. In this cohort, the optimal HGF cutoff for discriminating cardiac involvement was 320 pg/ml, which yielded 77% (95% CI: 67% to 87%) sensitivity and 69% (95% CI: 51% to 87%) specificity with an area under the curve of 0.69 (95% CI: 0.55 to 0.83). An HGF level >320 pg/ml predicted cardiac involvement with amyloidosis in univariable (p < 0.001) and multivariable (p = 0.004) analyses. In this cohort with known amyloidosis, NT-proBNP and troponin-T showed better discrimination for cardiac involvement by the c-statistic with areas under the curve of 0.89 (95% CI: 0.80 to 0.97) and 0.83 (95% CI: 0.73 to 0.93), respectively (Figure 3).

**PROGNOSTIC USEFULNESS OF HGF IN AMYLOIDOSIS.** Over a median of 2.6 years (interquartile range: 1.9 to 3.1 years) of follow-up, there were 27 deaths, 2 cardiac transplantations, and 1 LVAD implantation among the 102 patients with amyloidosis. All patients had at least 18 months of follow-up. We evaluated the prognostic usefulness of abnormal HGF, NT-proBNP, troponin-T, and eGFR for all-cause mortality, cardiac transplantation, and LVAD implantation within this group. Cutoffs used were HGF 320 pg/ml as derived from our amyloidosis cohort, and NT-proBNP 332 pg/ml, troponin-T 35 ng/l, and eGFR 45 ml/min/1.73 $m^2$ cutoffs that were used in published staging models for AL and ATTR cardiac amyloidosis (7,22).

By Kaplan-Meier analysis, elevated HGF, NT-proBNP, and troponin-T were associated with worse event-free survival (p < 0.001 for all), whereas abnormal eGFR was not (Figure 4). An HGF level of 676 pg/ml optimally discriminated those with the combined outcome with 69% (95% CI: 52% to 86%) sensitivity and 79% (95% CI: 70% to 87%) specificity by ROC analysis.

Individually, elevated HGF, NT-proBNP, and troponin-T above their respective cutoffs each showed modest predictive accuracy for the combined

**FIGURE 3 NT-proBNP, Troponin-T, and HGF Are Useful for Confirming Cardiac Involvement in Patients With Light Chain and Transthyretin Amyloidosis**




Receiver-operator characteristic analysis was used to evaluate the confirmatory usefulness of biomarkers for cardiac involvement with light chain and transthyretin amyloidosis. Curves are shown for N-terminal pro-B-type natriuretic peptide (NT-proBNP) **(red)**, troponin-T **(green)**, and HGF **(blue)**. NT-proBNP had the strongest confirmatory usefulness for cardiac involvement with amyloidosis (AUC 0.89), followed by troponin-T (AUC 0.83) and HGF (AUC = 0.69). Abbreviations as in Figures 1 and 2.

outcome with areas under the curves of 0.68 (95% CI: 0.60 to 0.75), 0.64 (95% CI: 0.59 to 0.69), and 0.65 (95% CI: 0.56 to 0.74), respectively (Table 3). In combination, NT-proBNP and troponin-T had similar predictive accuracy (area under the curve: 0.69; 95% CI: 0.61 to 0.77). The addition of HGF to NT-proBNP and troponin-T significantly improved the predictive model with an area under the curve of 0.75 (95% CI: 0.68 to 0.83) ($p < 0.001$ compared with NT-proBNP and troponin-T by the likelihood ratio test).

As a sensitivity analysis, we evaluated the incremental prognostic value of elevated HGF for the combined outcome in the AL and ATTR groups separately, using established staging systems for each (7,22). In the 69 patients with AL, the addition of HGF to a model of NT-proBNP and troponin-T improved the area under the curve from 0.74 (95% CI: 0.64 to 0.84) to 0.81 (95% CI: 0.71 to 0.91). By the likelihood ratio test, the discriminatory ability of the predictive model was significantly improved with the addition of HGF ($p < 0.001$). In the 33 patients with ATTR, the addition of HGF to a model of NT-proBNP and eGFR improved the area under the curve from 0.61 (95% CI: 0.47 to 0.74) to 0.65 (95% CI: 0.52 to 0.78), although the discriminatory ability of the model was not significantly improved by the likelihood ratio test ($p = 0.14$).

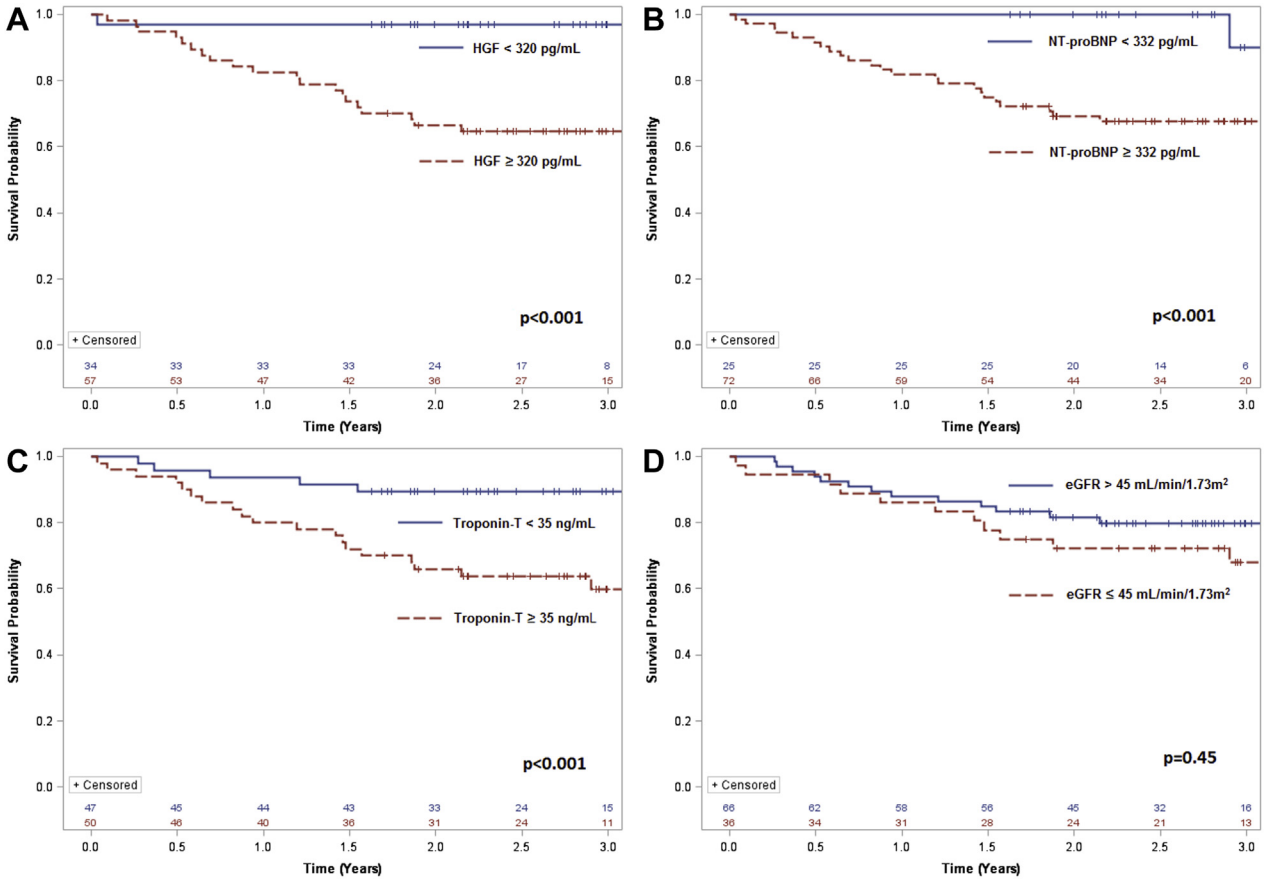
## DISCUSSION

In this single-center prospective cohort study, we found that HGF was discriminatory for AL and ATTR cardiac amyloidosis among patients with symptomatic heart failure. Among patients with known amyloidosis, NT-proBNP was a stronger indicator of cardiac involvement than HGF or troponin-T. In addition, elevated HGF was associated with the combined outcome of all-cause mortality, cardiac transplantation, and LVAD implantation with incremental prognostic value in addition to NT-proBNP and troponin-T (Central Illustration).

We found that HGF identified cardiac amyloidosis in a population with symptomatic heart failure, both with preserved and reduced ejection fraction. To date, no other circulating biomarker has shown diagnostic usefulness in this setting. These data extended our previously reported analysis in a larger sample size and validated the previous findings (16). Additional studies are required in a broad population of patients with symptomatic HF to further validate the usefulness of HGF for diagnostic purposes in cardiac amyloidosis. In contrast, NT-proBNP appeared to be indicative of cardiac involvement in the setting of known amyloidosis based on our findings and published reports in AL amyloidosis (25). In the heart failure setting, relative apical sparing of longitudinal strain and cardiac magnetic resonance are currently used as noninvasive screening tools for cardiac amyloidosis (12,26), although image quality, cost, and accessibility represent important barriers to the use of these techniques as screening tools. With additional validation, HGF may represent a reliable, inexpensive, and accessible initial screening test for cardiac amyloidosis that would direct the use of downstream confirmatory testing, including TTE with strain, cardiac magnetic resonance, technetium pyrophosphate bone scintigraphy, or endomyocardial biopsy.

Although HGF showed good discrimination for cardiac amyloidosis compared with symptomatic heart failure, it was less useful in differentiating cardiac amyloidosis from amyloidosis without cardiac involvement. This supported the hypothesis that HGF elevation in amyloidosis was specific to the

**FIGURE 4** **Elevated HGF, NT-proBNP, and Troponin-T Are Associated With Worse Event-Free Survival in Light Chain and Transthyretin Cardiac Amyloidosis**




Patients with amyloidosis were followed for a median of 2.6 years (interquartile range: 1.9 to 3.1 years) for the combined clinical outcome of all-cause mortality, cardiac transplantation, or left ventricular assist device implantation. The prognostic usefulness of **(A)** HGF, **(B)** NT-proBNP, **(C)** troponin-T, and **(D)** estimated glomerular filtration rate (eGFR) were evaluated using the following cutoffs: HGF 320 pg/ml as derived from our amyloidosis cohort, and NT-proBNP 332 pg/ml, troponin-T 35 ng/l, and eGFR 45 ml/min/1.73 $m^2$ as used in published staging models for light chain and transthyretin cardiac amyloidosis. By Kaplan-Meier analysis, elevated HGF, NT-proBNP, and troponin-T were associated with worse event-free survival ($p < 0.001$ for all), whereas abnormal eGFR was not. Abbreviations as in Figures 1 to 3.

process of extracellular amyloid fibril deposition. Mechanistically, HGF elevation may result from a role in tissue repair because the mitogenic HGF cytokine is produced by mesenchymal cells, whereas its receptor, c-met, is expressed on epithelial cells (13,14). Alternatively, elevated HGF may result because the β-pleated sheet structure of amyloid proteins activates tissue-type plasminogen activator, which then increases production of HGF due to structural homology of pro-HGF and plasminogen (17,18). Consistent with the findings by Abraham et al. (15), we observed higher HGF levels in patients with amyloidosis with cardiac involvement compared with patients without cardiac involvement. It remains unclear why cardiac

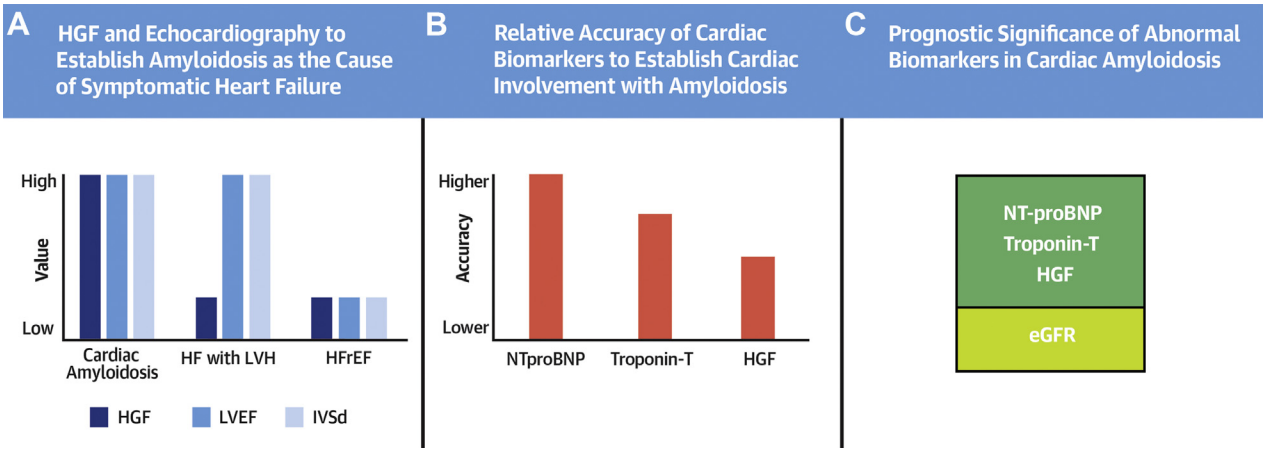
**TABLE 3** **Predictive Ability of Elevated HGF, NT-proBNP, and Troponin-T for All-Cause Mortality, Cardiac Transplantation, and LVAD Implantation in Patients With Light Chain and Transthyretin Amyloidosis**

| Biomarker | C-Statistic (95% CI) | AIC | LRT p Value |
|---|---|---|---|
| HGF | 0.68 (0.60–0.75) | 181.3 | — |
| NT-proBNP | 0.64 (0.59–0.69) | 221.3 | — |
| Troponin-T | 0.65 (0.56–0.74) | 223.6 | — |
| NT-proBNP + troponin-T | 0.69 (0.61–0.77) | 219.8 | 0.061* |
| NT-proBNP + troponin-T + HGF | 0.75 (0.68–0.83) | 175.0 | <0.001† |

Biomarkers were incorporated into models using cutoff values derived from our amyloidosis cohort (HGF of 320 pg/ml) or used in published staging models for light chain and transthyretin cardiac amyloidosis (NT-proBNP of 332 pg/ml, troponin-T of 35 ng/l). *Compared with NT-proBNP alone. †Compared to NT-proBNP + troponin-T.

AIC = Akaike information criterion; CI = confidence interval; LRT = likelihood ratio test; LVAD = left ventricular assist device; other abbreviations as in Table 2.

**CENTRAL ILLUSTRATION** **Clinical Usefulness of Biomarkers in Light Chain and Transthyretin Cardiac Amyloidosis**




**Zhang, K.W. et al. J Am Coll Cardiol CardioOnc. 2020;2(1):56-66.**

Our findings indicate that plasma biomarkers including hepatocyte growth factor (HGF), N-terminal pro-B-type natriuretic peptide (NT-proBNP), and troponin-T may have clinical usefulness for diagnosis and prognosis in light chain and transthyretin cardiac amyloidosis. **(A)** Compared with patients with symptomatic heart failure due to left ventricular hypertrophy (LVH) or heart failure with reduced ejection fraction (HFrEF), HGF is higher in patients with cardiac amyloidosis. **(B)** Among patients with known systemic amyloidosis, NT-proBNP and troponin-T are better indicators of cardiac involvement with amyloidosis compared with HGF. **(C)** NT-proBNP, troponin-T, and HGF are associated with worse event-free survival in patients with cardiac amyloidosis, whereas the estimated glomerular ejection fraction (eGFR) is not. HF = heart failure; IVSd = interventricular septal thickness (diastole).

involvement with amyloidosis would lead to higher HGF levels.

Our results indicated that low galectin-3 might discriminate cardiac amyloidosis from symptomatic heart failure with LVH or HFrEF, whereas IL-6 did not. This suggested that the processes of tissue fibrosis and ventricular remodeling that characterize chronic systolic heart failure are less active in cardiac amyloidosis (27), whereas inflammation is equally prominent in both disease processes. Additional study in larger patient cohorts is needed to confirm these findings.

We found that elevated HGF was associated with adverse cardiovascular outcomes with incremental prognostic value in addition to NT-proBNP and troponin-T in a mixed population with AL and ATTR amyloidosis. Additional study in larger populations is needed to confirm the prognostic usefulness of HGF in AL and ATTR, individually. Current staging models for AL incorporate circulating free light chains, troponin-T, and NT-proBNP (22,28), highlighting the clinical importance of cardiac involvement with amyloidosis. Published staging models for ATTR cardiac amyloidosis (ATTR-CA) use NT-proBNP and troponin-T in wild-type ATTR-CA, and NT-proBNP and eGFR in mixed populations of wild-type and hereditary ATTR-CA (7,29). Abnormal eGFR was not associated with the combined outcome in our study; this might be due to our inclusion of patients with AL amyloidosis, as well as disproportionate enrollment of whites compared with African Americans. By comparison, the population studied by Gillmore et al. (7) consisted of 36% of patients with hereditary ATTR-CA, with 64% of those patients having the V122I mutation. Furthermore, eGFR is not specific to the disease process of cardiac amyloidosis and may reflect differences in comorbidities or ethnicity as opposed to cardiac amyloidosis disease stage. Future efforts to establish prognostic models for cardiac amyloidosis should include the cardiac-specific biomarkers NT-proBNP and troponin-T and confirm our findings regarding HGF in larger patient populations with AL and ATTR.

**STUDY LIMITATIONS.** First, this was a single-center study with a limited sample size and small group assignments that raise the risk of type I error. Our findings should therefore be seen as preliminary. Confirmation of our findings in a larger, multicenter prospective study that enrolled patients with confirmed and suspected cardiac amyloidosis is underway as part of the ACE-MOVE (Prospective Characterization of Patients with Amyloidosis Focusing on Diagnosis and Innovative Treatment to Improve Outcomes) Registry. Similarly, we were limited in our

adjustment for potential confounders. Second, only 56% of cardiac amyloidosis cases were diagnosed by endomyocardial biopsy, which remains the gold standard for diagnostic testing. Nonbiopsy diagnostic criteria used for cardiac amyloidosis were in accordance with clinically accepted and published standards (29,30). Third, we combined AL and ATTR cardiac amyloidosis in our outcome analyses due to sample size limitations. Additional studies in separate AL and ATTR populations are needed to confirm our findings regarding the prognostic usefulness of HGF. Finally, NT-proBNP and troponin-T were not available for the LVH or HFrEF control groups, which precluded comparison to HGF for the diagnosis of cardiac amyloidosis in this population. The diagnostic performance of HGF compared with NT-proBNP and troponin-T for cardiac amyloidosis will be explored in the prospective, previously mentioned ACE-MOVE Registry.

## CONCLUSIONS

HGF is elevated in patients with AL and ATTR cardiac amyloidosis and differentiates patients with cardiac amyloidosis from those with other etiologies of symptomatic heart failure. Furthermore, elevated HGF was associated with risk of all-cause mortality, cardiac transplantation, and LVAD implantation with incremental prognostic value over NT-proBNP and troponin-T. Additional study in a larger, multicenter cohort of patients with confirmed and suspected cardiac amyloidosis is underway to validate our findings.

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## PERSPECTIVES

**COMPETENCY IN MEDICAL KNOWLEDGE:** Elevated HGF is suggestive of the diagnosis of light chain or transthyretin cardiac amyloidosis as well as adverse clinical outcomes in this population.

**TRANSLATIONAL OUTLOOK:** Further study is underway to validate the role of HGF as a biomarker. Future studies are also needed to define the pathophysiological basis of HGF elevation in cardiac amyloidosis.

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**KEY WORDS** biomarkers, cardiac amyloidosis, hepatocyte growth factor