Clinical and prognostic implications of capillary density in patients with cardiac light chain amyloidosis

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Abstract

Aims Cardiac involvement is crucial factor determining outcomes of light chain (AL) amyloidosis. This study evaluated whether capillary density (CD) quantified from endomyocardial biopsy is associated with structural and functional parameters of amyloid heart. Also, we investigated whether capillary density improves the prognostic value of the current staging system in AL amyloidosis patients with cardiac involvement.

Methods and results A total of 67 patients with biopsy-proven AL cardiac amyloidosis were prospectively enrolled in this study. All patients underwent transthoracic echocardiography and EBM at the time of diagnosis. Left ventricle global longitudinal strain was evaluated on two-dimensional strain echocardiography. The primary endpoint was all-cause mortality. Amyloid load and capillary density were calculated on endomyocardial biopsy stained immunohistochemically with antibody against amyloid P and CD31, respectively.

Among the study population, 37 patients (55.2%) were classified as Stage IV, and the cumulative incidence of death at 1 year was 34% (23 patients). A total of 65 (97%) patients underwent chemotherapy. CD showed a significant correlation with left ventricle global longitudinal strain ($r = 0.274$, $P = 0.025$), log N-terminal pro-B type natriuretic peptide ($r = -0.311$, $P = 0.005$), and amyloid load ($r = -0.438$, $P < 0.001$). Patients with a CD $\leq 220$/mm² were at significantly higher risk of death than those with a CD $> 220$/mm² ($P = 0.026$ by log-rank test). A model based on the 2012 Mayo staging system and CD showed significantly better discrimination and reclassification ability than a model using the 2012 Mayo staging system alone (C-index 0.582 vs.0.689, $P$ for difference <0.001).

Conclusions Capillary density was significantly related to the severity of amyloid deposits in the myocardium and showed incremental prognostic value in addition to the 2012 Mayo staging system.

Keywords Cardiac amyloid; Capillary density; Prognosis

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Background

Cardiac amyloidosis is currently considered an underestimated cause of heart failure.¹,² Recent studies reported that high prevalence of microvascular dysfunction in cardiac amyloidosis is one of pathophysiological mechanisms of heart failure in cardiac amyloidosis.³⁻⁵ Amyloid deposition in the vessel walls and extrinsic compression from perivascular and interstitial amyloid infiltration have impact on microvascular structure.³ Microvascular dysfunction in cardiac amyloidosis may be attributed to reduced longitudinal strain despite preserved ejection fraction (EF) in cardiac amyloidosis due to increased vulnerability to ischaemia.³

Microvascular structure can be evaluated by assessing capillary density (CD) using quantitative morphometry from myocardial tissues acquired from endomyocardial biopsy (EMB). The prognostic significance of capillary rarefaction has been reported in different clinical settings.⁶,⁷ However,
the associations between CD and structural and clinical parameters in cardiac amyloidosis as well as the prognostic value of CD have never been investigated.

**Aims**

This study evaluated whether CD quantified from EMB is associated with structural and functional parameters of amyloid heart. We also investigated whether capillary density improves the prognostic value of the current staging system in AL amyloidosis patients with cardiac involvement.

**Method**

**Study population and endpoints**

The study population consisted of 67 patients with biopsy-proven AL cardiac amyloidosis enrolled from 2008 to 2018 at Samsung Medical Center. All patients underwent blood sampling including biomarkers, echocardiography, and EMB at the time of diagnosis. EMB was performed at the septum of the right ventricle, from which at least five pieces were obtained. All acquired cardiac tissues were used for histologic analysis. Patients were staged using the 2012 Mayo staging system. The primary endpoint was all-cause mortality during follow-up.

**Echocardiography**

All patients underwent 2D conventional echocardiography with strain analysis using a Vivid 7 or Vivid 9 cardiovascular ultrasound system (GE Medical Systems, Horten, Norway). Standard 2D and Doppler measurements were taken according to current guidelines. Mean left ventricular (LV) wall thickness was calculated as the average of end diastolic interventricular septal wall and LV posterior wall thickness. For 2D strain analysis, images with frame rates set to 60–80 frames per second were analysed offline using customized software (EchoPAC P, version; GE Medical Systems). The absolute value of left ventricle global longitudinal strain (LV GLS) ([LV GLS]) was used in analysis.

**Histological assessment**

Formalin-fixed and paraffin-embedded tissue samples from EMB were used for histologic assessment. Amyloid was detected by Congo red staining, viewed under polarized light for green–yellow–orange birefringence and by immunohistochemical staining against amyloid P (Millipore Sigma, Darmstadt, Germany). To quantify amyloid load, digital images were obtained from sections stained immunohistochemically for amyloid P using a Vectra 3.0 spectral imaging system (PerkinElmer, Waltham, MA, USA). Area percentages of amyloid deposits were evaluated using image analysis software (InForm version 2.4.0, PerkinElmer, Waltham, MA, USA). The area percentage of the amyloid deposit was calculated as the ratio of the stained area to total tissue area. Capillaries on the section of EMB were identified by immunohistochemical staining with a specific antibody against endothelium, CD31 (JC70A, Dako, Glostrup, Denmark). The number of capillaries was counted in whole sections from each case under a microscope, and tissue areas were measured using SPOT advanced software 5.1 (SPOT imaging solutions, Sterling Heights, USA). CD was calculated as the number of the capillaries divided by area (mm²).

**Statistical analysis**

Correlations were analysed with the Spearman rank correlation coefficient. Simple logistic regression analysis was performed to evaluate the probability of cardiac structural and functional parameters being abnormal across the spectrum of CD. Survival analysis was performed using the Kaplan–Meier method with the log-rank test. Receiver-operating characteristic curves were created to determine optimal cut-off values for CD to predict 2 year mortality. Comparisons between the current staging system and CD with the c-statistic were carried out using the procedure proposed by DeLong et al.

**Results**

Among the study population, 37 patients (55.2%) were classified as Stage IV, and the cumulative incidence of death at 1 year was 34% (23 patients) during a median follow-up of 24 (7–53) months. A total of 65 (97%) patients underwent chemotherapy. Detailed baseline characteristics are presented in Table 1.

Capillary density showed a significant positive correlation with [LV GLS] (r = 0.274, P = 0.025) and negative correlations with log N-terminal pro-B type natriuretic peptide (NT-proBNP; r = −0.311, P = 0.005) and amyloid load (r = −0.438, P < 0.001) (Figure 1). In the subgroup of patients who underwent cardiac magnetic resonance imaging (MRI) (n = 43), CD showed significant negative correlations with extracellular volume (r = −0.304, P = 0.048). The probability of structural and functional imaging parameters and NT-proBNP being abnormal is described in Figure 2. As cardiac amyloid load increased, capillary rarefaction progressed, and CD decreased throughout disease process. NT-proBNP, e' velocity, and left atrial volume index showed high probabilities of being abnormal at low amyloid burden with preserved CD. LVEF...
was preserved until very late stage, and the probability of being abnormal gradually increased across the spectrum of CD reduction. [LV GLS] sensitively reflected LV dysfunction in the process of capillary rarefaction as the probability of being abnormal increased.

A CD of 220/mm² provided the best cut-off values to discriminate 2 year mortality (area under the receiver-operating characteristic curve: 0.657, 95% confidence interval: 0.525–0.789, \( P = 0.029 \)). Patients with a CD ≥ 220/mm² were at significantly higher risk of death than those with a CD < 220/mm² (\( P = 0.026 \), Figure 3A). In the multivariable model, CD was an independent predictor of mortality even after adjustment for [LV GLS] and the 2012 Mayo staging system (Table 2). A model based on the 2012 Mayo staging system was significantly associated with mortality (Table 2).

### Table 1 Baseline clinical characteristics

|                                | Entire cohort (n = 67) | CD > 220/mm² (n = 46) | CD ≤ 220/mm² (n = 21) | \( P \) value |
|--------------------------------|------------------------|------------------------|------------------------|--------------|
| Age, years, median (IQR)       | 61 (53–67)             | 62 (54–67)             | 58 (49–69)             | 0.840        |
| Male, n (%)                    | 43 (64)                | 28 (61)                | 15 (71)                | 0.584        |
| Body mass index, kg/m², median (IQR) | 22.6 (20.7–24.7) | 23.4 (21.3–25.2) | 20.9 (19.3–23.1) | 0.034        |
| Other involved organ            |                        |                        |                        |              |
| Kidney, n (%)                  | 22 (33)                | 19 (41)                | 3 (14)                 | 0.048        |
| Liver, n (%)                   | 4 (6)                  | 2 (4)                  | 2 (10)                 | 0.584        |
| Lambda restricted, n (%)       | 51 (76)                | 36 (78)                | 15 (71)                | 0.551        |
| dFLC, mg/dL                    | 25 (12–60)             | 37 (13–72)             | 14 (5–36)              | 0.032        |
| NT-proBNP, pg/m, median (IQR)  | 3,167 (1,692–6,442)    | 2,901 (1,394–4,968)    | 4,678 (3,196–9,792)    | 0.008        |
| Troponin T, ng/mL, median (IQR)| 0.083 (0.047–0.184)    | 0.063 (0.044–0.154)    | 0.274 (0.149–0.456)    | 0.006        |
| eGFR, mL/min/1.73m², median (IQR) | 73 (58–90)             | 82 (58–99)             | 71 (58–81)             | 0.096        |
| 2004 Mayo stage, IIIB        | 8 (12)                 | 5 (11)                 | 3 (14)                 | 0.408        |
| 2012 Mayo stage, IVb       | 37 (55)                | 29 (63)                | 8 (38)                 | 0.259        |
| Chemotherapy                   | 65 (95)                | 46 (100)               | 19 (91)                | 0.095        |
| Chemotherapy regimen          |                        |                        |                        |              |
| MDex                           | 23 (34)                | 21 (48)                | 2 (10)                 | 0.002        |
| Bortezomib-based              | 30 (44)                | 16 (35)                | 4 (67)                 | 0.019        |
| Immunomodulatory drug based   | 12 (18)                | 7 (15)                 | 5 (24)                 | 0.495        |
| LV ejection fraction          | 59 (53–64)             | 61 (54–66)             | 54 (41–58)             | 0.001        |
| LV mean wall thickness         | 12.0 (11.5–13.5)       | 12.0 (11.4–13.1)       | 12.5 (11.8–14.3)       | 0.438        |

ASCT, autologous stem cell transplantation; CD, capillary density; dFLC, difference between involved and uninvolved light chain; estimated glomerular filtration rate; MDex, mephalan–dexamethasone; \( ^a \)2012 Mayo Stage 4: free light chain difference ≥ 18 mg/dL, cTnT ≥ 0.025 ng/mL, and NT-ProBNP ≥ 1800 pg/mL.

\( ^b \)2004 Mayo Stage 3B with European modification: cTnT ≥ 0.035 ng/mL NT-ProBNP threshold of >8500 ng/L.

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**Figure 1** Correlations between capillary density and left ventricle global longitudinal strain (LV GLS), log N-terminal pro-B type natriuretic peptide (NT-proBNP), and amyloid load.
Figure 2 The probability of cardiac structural and functional variables being abnormal across a spectrum of capillary density. [LV GLS], left ventricle global longitudinal strain; NT-proBNP, N-terminal pro-B type natriuretic peptide, LVEF, left ventricular ejection fraction.

Figure 3 (A–C) Kaplan–Meier curves for all-cause mortality according to a capillary density of 220/mm².

system and CD showed significantly better discrimination and reclassification ability than a model using the 2012 Mayo staging system alone (C-index 0.582 vs.0.689, P for difference <0.001) for 2 year mortality (Figure 4). The limitations of our study include the small number of patients, lack of a comparison group with non-amyloid cardiomyopathy, and...
limited cardiac MRI data. Because we performed EMB from the right ventricle, there is a possibility of sampling error; however, CD still showed good correlation with extracellular volume on cardiac MRI despite the limited number of patients that underwent cardiac MRI.

**Conclusions**

To the best of our knowledge, this study is the first study to investigate the clinical and prognostic implications of CD in patients with cardiac AL amyloidosis. Our main findings are as follows: (i) CD was significantly related to amyloid load in the myocardium, (ii) CD was also significantly associated with [LV GLS] and NT-proBNP, and (iii) the addition of CD to the 2012 Mayo staging system improved predictive performance when compared to the 2012 Mayo staging alone for predicting 2 year mortality.

**Table 2** Clinical predictors for all-cause mortality

|                          | Univariable analysis |                       | Multivariable analysis (model 1) |                       | Multivariable analysis (model 2) |
|--------------------------|----------------------|-----------------------|----------------------------------|-----------------------|----------------------------------|
|                          | HR (95% CI)          | P value               | HR (95% CI)                      | P value               | HR (95% CI)                      | P value               |
| Age, year                | 1.01 (0.96–1.10)     | 0.738                 | 1.69 (0.82–3.45)                 | 0.152                 | 2.33 (0.92–5.93)                 | 0.075                 |
| 2012 Mayo Stage 4a       | 1.28 (0.71–2.30)     | 0.409                 |                                  |                       |                                  |                       |
| 2004 Mayo Stage 3B with  | 6.96 (3.55–13.67)    | <0.001                |                                  |                       |                                  |                       |
| European modificationb   | e', cm/s             | 0.68 (0.44–1.06)      | 0.088                            |                       |                                  |                       |
| LA volume index, mL/m²   | 1.02 (0.98–1.106)    | 0.213                 |                                  |                       |                                  |                       |
| Mean LV wall thickness, mm | 1.31 (1.02–1.69)    | 0.033                 | 1.08 (0.95–1.23)                 | 0.243                 | 1.12 (0.98–1.28)                 | 0.107                 |
| [LV GLS], %              | 0.91 (0.82–0.99)     | 0.028                 | 0.94 (0.86–1.03)                 | 0.155                 | 0.95 (0.87–1.05)                 | 0.328                 |
| CD ≤ 220/mm²             | 4.72 (2.58–8.63)     | <0.001                | 3.77 (1.76–8.11)                 | 0.001                 | 2.51 (1.19–5.29)                 | 0.016                 |

CD, capillary density; CI, confidence interval; e', early diastolic tissue velocity at septal mitral annulus; FLC-d, free light chain differential; GLS, global longitudinal strain; HR, hazard ratio; LA, left atrium; LV, left ventricle; NT-proBNP, N-terminal pro-B type natriuretic peptide; TnT, cardiac troponin T.

a2012 Mayo Stage 4: Free light chain difference ≥ 18 mg/dL, cTnT ≥ 0.025 ng/mL, and NT-ProBNP ≥ 1800 pg/mL.
b2004 Mayo Stage 3B with European modification: cTnT ≥ 0.035 ng/mL NT-ProBNP threshold of >8500 ng/L.

**Figure 4** Receiver-operating characteristic analysis comparing predictive performance between 2012 Mayo staging with capillary density (CD) and 2012 Mayo staging alone for 2 year mortality. AUC, area under the receiver-operating characteristic curve; CI, confidence interval.

Previous studies reported a high prevalence of coronary microvascular dysfunction in cardiac amyloidosis in the absence of epicardial coronary artery disease and in comparison with hypertrophic cardiomyopathy. In this study, we directly assessed CD from cardiac tissue and comprehensively described structural and functional cardiac abnormalities associated with process of capillary rarefaction. These findings of our study provide mechanistic insight into the pathological process of capillary rarefaction with infiltration of amyloid and underscore capillary rarefaction as a probable mechanism for diastolic dysfunction and [LV GLS] impairment in cardiac AL amyloidosis. This study also provides histological evidence for [LV GLS] being a prominent prognostic imaging parameter. In conclusion, CD assessed from EMB shows good correlation with amyloid load as well as structural and functional cardiac parameters. CD provides additive prognostic values to the current staging system in patients with cardiac AL amyloidosis.
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Conflict of interest

None declared.

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