ORIGINAL ARTICLE

Impaired right ventricular function as a predictor of early mortality in patients with light-chain cardiac amyloidosis assessed in a cardiology department

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KEY WORDS

cardiac amyloidosis, early mortality, light-chain amyloidosis, right ventricle

ABSTRACT

INTRODUCTION Light-chain (AL) amyloidosis is the most common cardiac amyloidosis. Despite progress in treatment, early mortality remains a substantial problem in these patients.

OBJECTIVES The aim of this study was to determine a clinical profile of patients diagnosed with AL amyloidosis in a cardiology department, as well as to define the cut-off point for early mortality and identify predictors of early mortality in this population.

PATIENTS AND METHODS The study included 30 patients (14 women; median age, 61.5 years) with AL amyloidosis confirmed by echocardiography and biopsy of 2 organs.

RESULTS Six patients were diagnosed with stage II amyloidosis according to the Mayo 2004 classification, and 24 patients—with stage III. Early mortality was defined as death during 102 days after diagnosis and was observed in 14 patients. Patients who died earlier were younger and more frequently reported a weight loss of more than 10 kg and orthostatic hypotension than patients who died later. Moreover, they had higher concentrations of high-sensitivity troponin T and N-terminal pro-B-type natriuretic peptide (NT-proBNP) and worse left and right ventricular (RV) contractility. In the Cox models, the age of less than 64 years, NT-proBNP levels exceeding 4968 pg/ml, RV end-diastolic diameter of less than 34 mm, and tricuspid annular plane systolic excursion lower than 13 mm were significant predictors of mortality within 102 days after diagnosis.

CONCLUSIONS We presented the results of the first Polish prospective noninterventional study on AL amyloidosis diagnosed in the cardiology department. We found that patients have advanced disease at the time of diagnosis. Younger age, impaired RV function, and higher concentrations of cardiac markers are predictors of worse prognosis.

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INTRODUCTION Amyloidosis is a disease in which the deposition of amorphous protein-derived substance in the extracellular compartment results in damage to numerous organs. There are at least 30 proteins that may become precursors to amyloid deposits.[1] Light-chain (AL) amyloidosis is the most common type of amyloidosis.[2] It has also the worst prognosis.[3] Not only amyloid itself but also the misfolded free light chains are toxic for human cells.[4,5] An amyloidogenic plasma cell clone producing lambda (λ) free light chains is more common than the kappa (κ) clone.[6] The heart

has little tolerance for amyloid deposition. Cardiac involvement determines prognosis and limits life duration.[7,8] Diagnosis is often delayed because disease symptoms are nonspecific. To make the situation even more difficult, in Poland there are no referral centers for amyloidosis. Although several case reports[9-11] and reviews[12-14] of AL amyloidosis are available, there is only one original paper describing 3 Polish patients with different types of cardiac amyloidosis.[15]

A histopathological demonstration of amyloid deposits comprising light chains in tissue biopsy and abnormal concentration of serum-free light chains are required for the diagnosis of AL amyloidosis.[6,16] It is not necessary to perform a cardiac biopsy to confirm heart involvement,[6,17,18] although it may be helpful in revealing the coexistence of different types of amyloidosis.[19] Cardiac amyloidosis mainly causes a restrictive cardiomyopathy.[20] However, cardiac wall thickness may increase markedly; therefore, the amyloidosis has also been recently classified as a hypertrophic cardiomyopathy.[21] Hyperechogenicity of the myocardium revealed by echocardiography or a characteristic pattern of late gadolinium enhancement on cardiac magnetic resonance may be sufficient to confirm heart involvement.[22,23] Other features of cardiac amyloidosis are thicker interatrial septum and valve leaflets, as well as decreased longitudinal left ventricular (LV) function with preserved ejection fraction.[24] Moreover, pericardial effusion and atrial thrombi are frequent.[25] Increased LV wall thickness coexistent with low voltage in the limb leads of an electrocardiogram remains a very sensitive and specific marker of amyloidosis.[26]

High-sensitivity troponin T (hs-TnT) as well as N-terminal pro-B-type natriuretic peptide (NT-proBNP) play an important role in amyloidosis management. These cardiac markers have been incorporated in the Mayo staging system since 2004.[27] According to current criteria, NT-proBNP levels exceeding 332 pg/ml in the absence of renal failure define cardiac involvement, while a therapy-related reduction in NT-proBNP levels (>30% and >300 pg/ml) from a starting level of 650 pg/ml or higher is a criterion for identifying cardiac response.[28]

Standard treatment for heart failure slightly relieves the symptoms, but it does not improve the prognosis. Chemotherapy has the potential to destroy a plasma cell clone, and thus to stop further amyloid deposition. Hence, early diagnosis and an immediate initiation of causal treatment are essential. The hematologist plays a major role in choosing the best therapy, with consideration of the patient's performance status at presentation. Available clinical trials suggest that patients treated with chemotherapy live longer and may achieve clinical improvement, as compared with those who received no causal therapy. However, large randomized trials are still lacking.[6,29] Apart from quite effective older agents such as cyclophosphamide, glucocorticoids, and melphalan, several new medications have been discovered recently, including bortezomib and lenalidomide. Novel strategies, including doxycycline, focus on dissolving amyloid deposits, but they have been tested only in clinical trials.[6,29,30] Great treatment results have been reported for high-dose chemotherapy with autologous peripheral blood stem cell transplant, especially in young patients with low-risk disease.[31]

Despite medical progress, there is still a group of patients who die just after they have been diagnosed,[16,32] which is partly due to delay in disease detection, because the median time from the first symptoms to diagnosis is 8 months, even in referral amyloidosis centers.[8] Early mortality may also be caused by high therapy toxicity, especially in the case of liver involvement or congestive heart failure.[6] The risk factors predictive for early mortality include advanced age, low systolic blood pressure (SBP), high concentrations of hs-TnT and NT-proBNP at presentation, a considerable difference between the levels of involved and uninvolved serum immunoglobulin free light chain (dFLC), increased LV wall thickness, and impaired creatinine clearance.[6,33,34]

The aim of our study was to determine a clinical profile of patients diagnosed with AL amyloidosis in a cardiology department, as well as to define the cut-off point for early mortality and identify predictors of early mortality in this population.

PATIENTS AND METHODS

Disease diagnosis

The study included patients newly diagnosed with AL amyloidosis in the Department of Cardiomyopathy, Institute of Cardiology, Warsaw, Poland, from November 2011 to July 2016. Every suspicion of amyloidosis based on transthoracic echocardiography (TTE) or cardiac magnetic resonance (CMR) imaging was confirmed by a histological examination of at least 2 organs. The following histological stainings were performed: hematoxylin and eosin, Congo red, phenol Congo red,[35] and Syrius staining. A type of amyloid was identified by immunohistochemical reactions using a panel of commercially available primary antibodies against 4 major amyloid fibril proteins: immunoglobulin λ and κ light chains, serum amyloid A protein, and transthyretin (all produced by Dako, Glostrup, Denmark).

Blood tests The concentrations of the cardiac markers hs-TnT and NT-proBNP were measured. The serum concentrations of free light chains were assessed using the Binding Site test (Birmingham, United Kingdom). Apart from routine blood tests such as the measurement of creatinine, potassium, and sodium levels, we also analyzed serum albumin and total protein concentrations and lactate dehydrogenase activity.

Cardiac imaging In TTE, the diastolic thickness of the cardiac walls and LV end-diastolic diameter were measured in the parasternal long-axis view

in the M-mode presentation. The right ventricular (RV) end-diastolic diameter (RVEDD) was measured in the apical 4-chamber view at the level of papillary muscles, as recommended by the European Society of Cardiology.[36] Atrial areas were obtained by tracing their endocardial borders at the end of the ventricular systole in the apical 4-chamber view. The LV ejection fraction (LVEF) was estimated by the Simpson method. The tricuspid annular plane systolic excursion (TAPSE) was used to estimate RV contractility and was measured by TTE and CMR. A standard CMR examination was performed with a 1.5 Tesla scanner (Sonata and Avanto, Siemens, Erlangen, Germany) for the LV and RV volume, atrial areas, and LV mass measurements. Late gadolinium enhancement images in the long- and short-axis imaging planes were obtained with a breath-hold segmented inversion recovery sequence performed 10 minutes after contrast injection (gadobutrol, Gadovist, Bayer, Germany and gadoteridol, Prohance, Takeda, Japan).

Clinical presentation and treatment All patients except one were able to estimate the duration of cardiac symptoms such as limited exercise tolerance, exertional dyspnea, chest pain, orthopnoea, or edema. The period from the onset of cardiac symptoms to diagnosis was assessed as the history time. The date of TTE or CMR that confirmed cardiac amyloidosis was the diagnosis time. All patients were followed from the diagnosis time to October 14, 2016.

The performance status of every patient was assessed with the Eastern Cooperative Oncology Group (ECOG) scale. Heart failure and amyloidosis were assessed according to the New York Heart Association (NYHA) classification and 2004 Mayo amyloidosis staging system, respectively. Pulmonary congestion and pleural effusion were evaluated in the posterior-anterior and lateral chest X-ray imaging. The treatment of heart failure, including diuretics, β-adrenergic blocking agents and angiotensin-converting enzyme inhibitors, was planned with caution according to cardiac symptoms and blood pressure. Hematological treatment depended on the patient's performance status at presentation; it was based on the current guidelines[6,16,37] and limited by medication availability in Poland.

The study conformed to the principles of the Declaration of Helsinki and was approved by a local bioethics committee. All patients provided written informed consent to participate in the study.

Statistical analysis A linear regression was used to assess different variables as possible survival prognostic factors. Uncorrelated variables were used as risk factors in the receiver operating characteristic (ROC) curve analysis. The variables with the strongest influence on survival were used to find the cut-off point for early mortality. The empirical probability density function (PDF) of survival together with the fitted Poisson distribution revealed a similar expected survival time for patients with early mortality. Therefore, patients were divided into 2 groups, according to the estimated cut-off point for early mortality. Univariate and multivariate Cox proportional models for censored data were performed for the modeling of the patients' survival time. Uncorrelated variables with a P value of 0.05 or less were included in the multivariate analysis, which confirmed the significance of risk factors previously found with the ROC analysis. The ROC analysis with the estimated cut-off point (between non–early and early mortality) was also used to find the thresholds for parameters considered significant predictors of mortality. The quantitative variables of the 2 mortality groups were compared with the Mann–Whitney test. Due to the lack of normal distribution (according to the Kolmogorov–Smirnov test), the quantitative variables were presented as medians with an interquartile range. For cases in which categorical variables of both the early and non–early-mortality groups were compared, the χ^2 test with the Yates' correction was used. The survival of the total cohort and of patients assessed by the Mayo staging system of amyloidosis was illustrated using the Kaplan–Meier curves. The Matlab version R2016 and Statistica version 12.0 programs were used for statistical analysis.

RESULTS

Clinical profile of patients The study included 30 patients (39–75 years; median age, 61.5 years); the median follow-up was 3.4 months (73 days to 26.2 months). The performance status at diagnosis was grade 1 in 7 patients, grade 2 in 13 patients, grade 3 in 3 patients, and grade 4 in 7 patients, according to the ECOG scale. The clinical profile, laboratory and imaging test results, and treatment details for the total cohort and patients divided into 2 groups according to the cut-off point for early mortality (as defined later in the text) are given in TABLE 1. Patients who died early were significantly younger, had lower blood pressure, and had higher concentrations of hs-TnT and NT-proBNP than those who died later. The median serum creatinine concentration, estimated glomerular filtration rate, and serum albumin and total protein concentrations for the total cohort were 99 μmol/l (interquartile range [IQR], 84–123 μmol/l), 60 ml/min/1.73 m^2 (IQR, 47.2–74.1 ml/min/1.73 m^2), 38.15 g/l (IQR 34.6–42 g/l), and 62 g/l (IQR, 55.7–66 g/l), respectively. There were no significant differences between the groups in the results of these blood tests. Moreover, the groups did not differ significantly in the incidence of orthopnea, chest pain, dyspepsia, edema, and ascites.

Heart failure and cardiac imaging There were 6, 20, and 4 patients with NYHA class 2, 3, and 4 dyspnea, respectively. The symptoms of RV heart failure such as lower limb edema or ascites were

TABLE 1 Characteristics of the total cohort and the study groups divided according to the cut-off point for early mortality of 3.4 months (continued on the next page)

Variables	Total cohort n = 30	Early mortality n = 14[a]	Non–early mortality n = 14[a]	*P* value
Demographic and clinical characteristics				
Age, y	61.5 (52–66)	54.5 (50–62)	66 (60.25–68.5)	0.008
Men, n (%)	16 (53.33)	8 (57.14)	6 (42.86)	0.7
History time, mo	11 (6.75–12.5)	9 (5.75–12)	12 (8–14.25)	0.1
BMI, kg/m^2	25.23 (23.53–27.89)	24.8 (22.31–27.34)	25.25 (23.53–28.09)	0.4
Weight loss >10 kg, n (%)	21 (70)	12 (85.71)	7 (50)	0.04
SBP, mm Hg	98 (90–114)	95 (89–100)	111 (90–129)	0.004
DBP, mm Hg	66.5 (60–75)	63 (60–70)	70 (61–77.5)	0.03
Orthostatic hypotension, n (%)	18 (60)	11 (78.57)	5 (35.71)	0.03
Congestion[b], n (%)	18 (60)	11 (78.57)	6 (42.86)	0.1
Pleural effusion[b], n (%)	20 (66.67)	12 (85.71)	6 (42.86)	0.049
NYHA class II–IV, n (%)	24 (80)	13 (92.86)	9 (64.29)	0.2
History of hypertension, n (%)	7 (23)	1 (7)	6 (42.86)	0.03
Laboratory results				
NT-proBNP, pg/ml	8444 (3185–16 082)	11 017 (933–17 282)	3281 (2620.75–6605.25)	0.001
hs-TnT, µg/l	134.5 (70.75–164.5)	163 (142.4–180)	77 (47–134.5)	0.002
dFLC, mg/l	287.2 (151.76–682.91)	536.1 (233.26–764.75)	219.27 (25.58–555.92)	0.2
iFLC, mg/l	302.4 (166.41–694.48)	435.8 (244.93–774.23)	234.89 (44.63–784)	0.2
LDH[c]	1.1 (0.88–1.61)	1.23 (0.84–1.64)	1.08 (0.9–1.67)	0.99
Plasmocytes in the bone marrow, %	20 (10–25.75)	20 (10–25)	20 (9.25–31.25)	0.9
Echocardiography results				
PW, mm	15 (14–18.25)	15 (14.75–19)	16 (14–17)	0.9
IVS, mm	17 (16–19)	17 (15.75–19)	18 (16–19)	0.7
RVWT, mm	8 (6–9)	8 (6–9)	8 (6–9)	0.4
LAA, cm^2	27 (22.5–29)	26.5 (21–28.5)	27 (23.75–29.75)	0.08
RAA, cm^2	20.5 (19–25.5)	23.5 (18.5–25.5)	20 (19.25–25.75)	0.7
LVEDD, mm	43 (39–46)	42.5 (39–45)	43 (39–46)	0.3
RVEDD, mm	35.5 (33–40)	33.5 (31–38)	36 (35–40.75)	0.05
LVEF, %	53 (44–60)	45 (30–53)	60 (50.75–70)	0.03
TAPSE, mm	15 (10.75–16.25)	13 (7–15)	15 (12.5–17)	0.03
Pericardial effusion, n (%)	17 (56.67)	10 (71.43)	6 (42.86)	0.2
Cardiac magnetic resonance results				
PW, mm	14.5 (14–16)	15 (14–16)	14 (12.25–14.75)	0.06
IVS, mm	17 (14.25–19.75)	18 (14.5–19.75)	15 (14.5–18.5)	0.6
LAA, cm^2	29.5 (26.5–33.5)	29.5 (26–34.8)	30 (27–33)	0.8
RAA, cm^2	28.5 (23.5–32.55)	30 (25–34)	26 (23–30)	0.6
LVDV, ml	144 (119–155.5)	143 (108–156)	144 (127–155)	0.2
RVDV, ml	149 (122.75–162.25)	144 (121.25–167)	149 (140–156)	0.7
LVEF, %	53 (39.75–64)	41 (37.5–54.5)	56.5 (53–64)	0.2
TAPSE, mm	12 (9.75–14.25)	10 (7–14)	13 (12–15)	0.3
LV mass, g	164 (130.75–212)	197.5 (139–224)	160 (124–191)	0.8
Treatment				
β-blocker, n (%)	19 (63.3)	8 (57.14)	11 (78.57)	0.2
ACEI, n (%)	8 (26.67)	1 (7)	7 (50)	0.01
Diuretic, n (%)	24 (80)	13 (92.86)	11 (78.57)	0.3
Chemotherapy, n (%)	18 (60)	7 (50)	11 (78.57)	0.1

TABLE 1 Characteristics of the total cohort and the study groups divided according to the cut-off point for early mortality of 3.4 months (continued from the previous page)

Data are presented as medians with interquartile range unless stated otherwise.

a The follow-up of 1 patient was shorter than the early mortality cut-off point, and the follow-up of another patient was not available; both patients were excluded from the analysis.

b Assessed by chest X-ray

c The baseline serum LDH level was normalized to the institutional reference upper limit of the normal range.

Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; BMI, body mass index; DBP, diastolic blood pressure; dFLC, difference between involved and uninvolved serum immunoglobulin free light chain levels; hs-TnT, high-sensitivity troponin T; iFLC, involved free light chains; IVS, interventricular septum; LAA, left atrial area; LDH, lactate dehydrogenase; LVEDD, left ventricular end-diastolic diameter; LV, left ventricle; LVDV, left ventricular diastolic volume; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PW, posterior wall of the left ventricle; RAA, right atrial area; RVWT, right ventricular wall thickness; RVDV, right ventricular diastolic volume; RVEDD, right ventricular end-diastolic diameter; SBP, systolic blood pressure; TAPSE, tricuspid annular plane systolic excursion

present in 19 patients (63%). LV heart failure defined as the presence of orthopnea and pulmonary congestion confirmed by radiography was diagnosed in 12 patients (40%). On TTE, 28 patients (93%) had a marked increase in LV wall thickness, defined as 14 mm or higher. A marked increase in RV wall thickness, defined as 7 mm or higher, was revealed in 20 patients (66.7%). A LVEF below 40% was observed in 5 patients (16.7%). However, longitudinal LV dysfunction was detected in 26 patients (86.7%). Decreased RV contractility defined as a TAPSE lower than 17 mm was observed in 22 patients (73%).

A comparison of the early mortality and non–early-mortality groups revealed no differences in echocardiographic parameters, apart from LVEF and TAPSE (TABLE 1), which were significantly lower in the early-mortality group. There were no significant differences in CMR results between the groups. CMR revealed the late gadolinium enhancement areas typical for cardiac amyloidosis in all patients.

Nineteen patients (63%) had no history of cardiac disease. Arterial hypertension was more frequent in the non–early-mortality group, and this seems to explain why these patients received angiotensin-converting enzyme inhibitors more often. There were no differences in the frequency of stable ischemic heart disease (1 case in the early-mortality group vs 4 cases in the other group). According to medical records, patients with ischemic heart disease had presented no symptomatic heart failure or LV dysfunction before they developed symptoms attributed to amyloidosis.

Amyloidosis management Twenty-eight patients with newly diagnosed AL amyloidosis had no history of hematological disease. Two patients with multiple myeloma had been sent to a cardiology department for evaluation of cardiac involvement, and they were diagnosed with AL amyloidosis. The following histological examinations were performed: cardiac biopsy (6 patients), minor salivary gland biopsy (6 patients), gastric biopsy (15 patients), kidney biopsy (1 patient), fat tissue biopsy (11 patients), trephine biopsy (23 patients), and autopsy (6 patients). Twenty-one patients were diagnosed with λ light-chain amyloidosis, and 6 patients—with κ-derived one. The autopsy specimens of 3 patients revealed light chains in amyloid deposits, but were not sufficient to differentiate between the 2 types of light chain. Newly diagnosed multiple myeloma was present in 14 patients (60.9%) of the 23 patients who underwent a trephine biopsy. Five patients from the early-mortality group died before a hematological consultation. According to the Mayo amyloidosis staging system, there were no patients in stage I in the study group. Six patients were in stage II and most patients (n = 24; 80%) were assessed as stage III. During the follow-up, 2 patients were diagnosed with relapsed multiple myeloma. The first patient received melphalan and dexamethasone, and the other was administered lenalidomide and dexamethasone as the second-line therapy.

Cut-off point for early mortality The variables considered as valuable risk factors were tested against linear regression (TABLE 2). The ROC analysis of these variables was prepared, and the area under the ROC curve (AUC) of each variable was maximized by changing the cut-off point for mortality. This yielded a cut-off point for early mortality of 3.4 months (102 days). FIGURE 1 illustrates the relationship between the AUC for these variables and different thresholds for early mortality. It shows that the power of tests increased dramatically for the cut-off point of about 3 months. The PDF of survival, namely, P(X=survival), is demonstrated in FIGURE 2. The PDF was skewed; therefore, the median predicted the expected survival time, which was 3.4 months (just like the optimal cut-off point). A similar expected survival time was achieved with the Poisson distribution fit, namely, $\lambda = 3.55$ months, which worked best with the early-mortality group.

Prognostic factors and survival analysis The univariate Cox analysis showed a decrease of 8.7% in the expected hazard with every year of increase in age; an increase of 6.1%—with every mm Hg decrease in SBP; an increase of 13.1%—with every 1000 pg/l increase in NT-proBNP; a decrease of 10.9%—with every mm increase in

TABLE 2 Linear regression model *P* values of variables considered significant survival parameters

Variables	Age	SBP	DBP	dFLC	NT-proBNP	hs-TnT	RVED	EF	TAPSE	LV mass
SBP	0.2	–	–	–	–	–	–	–	–	–
DBP	0.3	0.00004 (0.6)	–	–	–	–	–	–	–	–
dFLC	0.2	0.4	0.6	–	–	–	–	–	–	–
NT-proBNP	0.9	0.3	0.0006 (0.2)	0.7	–	–	–	–	–	–
hs-TnT	0.4	0.2	0.02 (0.2)	0.3	0.0005 (0.5)	–	–	–	–	–
RVEDD	0.2	0.3	0.9	0.5	0.9	0.9	–	–	–	–
LVEF	0.2	0.1	0.5	0.7	0.4	0.2	0.3	–	–	–
TAPSE	0.6	0.2	0.5	0.2	0.5	0.6	0.2	0.002 (0.3)	–	–
LV mass[a]	0.4	0.3	0.4	0.2	0.3	0.1	0.5	0.8	0.1	–
LVEF[a]	0.04 (0.2)	0.04 (0.2)	0.1	0.9	0.1	0.1	0.9	0.0001 (0.5)	0.4	0.1

The coefficients of determination for correlated variables (R^2) are placed in brackets.

a Measured by cardiac magnetic resonance

Abbreviations: see TABLE 1

the RVEDD; and an increase of 12.9%—with every mm decrease in TAPSE (TABLE 3). The hazard ratio for various variables (at 3.4 months referred to the baseline) indicates the survival decrease for these variables. Age, SBP, NT-proBNP, RVEDD, and TAPSE were considered in the multivariate analysis. The *P* values resulting from the log-likelihood analysis of the multivariate model indicated that the Cox models including age, NT-proBNP, and TAPSE provided a significant prediction of survival. However, it was worth adding the RVEDD according to the sum of squared errors of prediction. Although the log-likelihood *P* value suggested that SBP had improved the model prediction properties, *P* values for separate b parameters showed the lack of significance for the 5-parameter Cox model (2 *P* values exceeded 0.05).

The effect of chemotherapy on survival in the univariate analysis was ambiguous, according to the b parameter and its unacceptable *P* value. However, in the multivariate analysis, the Cox model including age and chemotherapy provided a significant prediction of survival. It was better than the Cox model including age and NT-proBNP.

The survival ratio in the subsequent months is shown in FIGURE 3. It illustrates the fitting of the individual Cox models and the total cohort survival curve. The results of the ROC analysis of the variables evaluated as significant predictors of early mortality are presented in TABLE 4.

Nineteen patients (63%) died during the study: 6 patients due to ventricular fibrillation, and another 6 patients—due to pulseless electrical activity. Profuse bleeding resulted in the death of 2 patients (cerebral and gastrointestinal hemorrhage). Because of previous thrombotic events, these patients received low-molecular-weight heparins, but heparin doses were reduced according to the increased bleeding risk in amyloidosis. The median survival for the total cohort was 3.45 months; however, the observed survivals ranged from 1 day to 26.2 months. The median survival for the early-mortality group was 2 months (interquartile range, 1–2.8 months), and for the non–early-mortality group, 8.15 months (interquartile range, 5.7–12.7 months). The Kaplan–Meier survival functions for the total cohort and patients divided into groups according to the Mayo amyloidosis staging system are shown in FIGURE 4.

DISCUSSION

The Polish aspect

According to our knowledge, this is the first Polish prospective noninterventional study focused on AL cardiac amyloidosis. The study group consisted of patients with advanced disease. Considering the median history time, a proper diagnosis is made relatively late in Poland.[8] Patients already have poor performance status when they consult hematologists, which limits opportunities for therapy. The median thickness of cardiac walls confirms an advanced stage of amyloidosis.[3,38,39] In comparison with data from amyloidosis centers, our patients had a higher median concentration of NT-proBNP.[8,39]

We are aware that our study group was relatively small. It seems necessary to create an amyloidosis center in Poland that would offer patients the highest standard of care and gather clinical data on this rare disease. In addition, efforts should be made to educate cardiologists, internal medicine physicians, general practitioners, as well as other specialists on amyloidosis and its leading symptoms, in order to facilitate an early diagnosis that would translate into a better outcome. It is recommended that patients with suspected amyloidosis should be sent to a referral center.[6]

Analysis of prognostic factors

We assessed little-known prognostic factors and checked their utility for prognosis of early mortality. The cut-off point for early mortality was determined using statistical methods and is unique for our study group. Therefore, owing to the small sample size, our

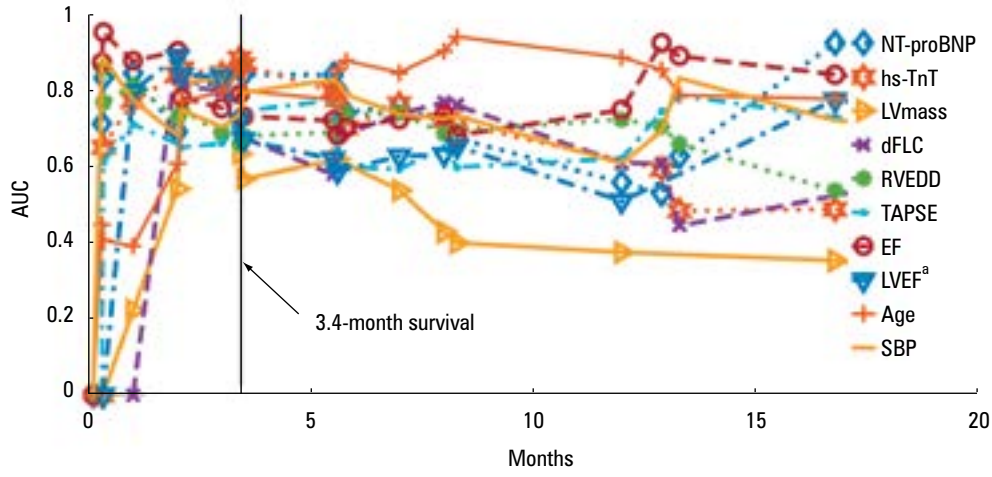


FIGURE 1 Area under the receiver operating characteristic curves of the various parameters for different survival cut-off points. Most parameters proved good classifiers when the total cohort was divided by 3.4-month survival.
a Measured by cardiac magnetic resonance
Abbreviations: AUC, area under the curve; others, see **TABLE 1**

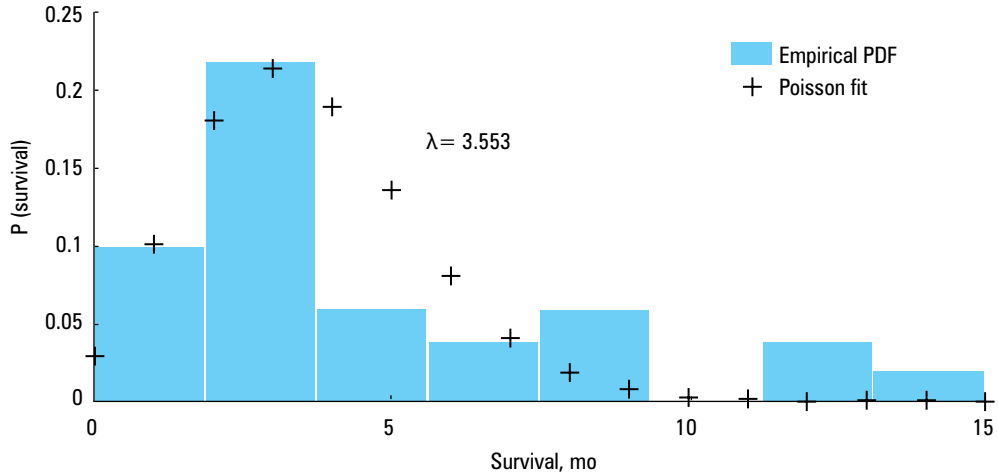


FIGURE 2 Empirical probability density function (PDF) of survival, ie, P(X=survival) and Poisson fit

results should be treated with caution. The study may constitute a good starting point for planning further research in Poland, especially now as the interest in this disease is high, up-to-date therapy is available, and the experience of hematologists in autologous peripheral blood stem cell transplant in amyloidosis has been growing rapidly.

The negative impact of the high levels of hs-TnT and NT-proBNP on survival is well documented,[28,29] and our study provided similar results. However, we did not observe dFLC levels at presentation to be a significant prognostic factor. The parameter was assessed only in 20 patients (66.67%). A recent genetic study has revealed several types of λ chains that are associated with poor prognosis.[40] This may explain why some patients have severe organ damage although their dFLC levels at presentation are low.[41] The genetic tests of light chains might have enhanced the value of our study. Serum lactate dehydrogenase is a well-established biomarker of multiple myeloma activity,[42] and it has been recently analyzed in patients with AL amyloidosis.[43] It was not a significant prognostic factor in our study group, which may have been a result of the small sample size.

In contrast to our results, RV dilation was previously reported as a negative prognostic factor.[38] Ghio et al[39] reported RV dysfunction instead of RV dimension as a relevant factor for poor outcome. It was also observed that RV dilation occurs with progression of cardiac amyloidosis.[44] In our study, early mortality was associated with a smaller dimension of the right ventricle measured by echocardiography. This apparent discrepancy may be explained by the fact that patients with shorter survival had less time to develop RV dilation. Previous studies used mainly the long-axis parasternal view to estimate the RV diameter. We preferred the 4-chamber apical view and considered the measurements of the right ventricle performed at the level of papillary muscles the most repeatable. Moreover, the function of the right ventricle assessed by TAPSE on TTE was an even more relevant negative prognostic factor in our study. This observation is convergent with literature data.[39] Neither TAPSE nor RV volume measured by CMR influenced survival in our study. This suggests that further studies using CMR are necessary.

Advanced age has been reported as a negative prognostic factor of survival.[3,6,34] In our study, younger patients had a shorter survival than older ones. They also had a shorter history time, although this difference was not significant. This may confirm that AL amyloidosis, similarly to other neoplastic diseases, takes a more aggressive course in younger people.

TABLE 3 Univariate and multivariate Cox proportional models

Variable	Univariate analysis			
	HR = exp(b) (95% CI)	Relative effect, %	HR (3.4 months)	*P* value
Age	0.9207 (0.8681–0.9765)	–8.7	1.7330	0.005[a]
SBP	0.9418 (0.9036–0.9817)	–6.1	2.3444	0.004[a]
dFLC	0.9999 (0.9994–1.0003)	0.0	2.0944	0.6
NT-proBNP	1.1309 (1.0555–1.2118)	13.1	1.5748	0.0003[a]
RVEDD	0.9017 (0.8142–0.9986)	–10.9	1.6840	0.04[a]
TAPSE	0.8856 (0.7915–0.9909)	–12.9	1.8174	0.03[a]
LV mass[b]	1.0026 (0.9897–1.0157)	0.26	1.3798	0.7
Chemotherapy[c]	0.44 (0.16–1.19)	–	1.85	0.098
LDH[d]	0.81 (0.27–2.44)	–	1.72	0.7

Variables (1–5)	Multivariate analysis							
	HR = exp(b_n) (95% CI)	SSE	*P* value					Log-likelihood *P* value
			1	2	3	4	5	
Age and NT-proBNP	0.92 (0.87–0.98)	0.414	0.01	0.05	–	–	–	0.05
Age and chemotherapy	0.32 (0.11–1.97)	0.396	0.004	0.047	–	–	–	0.003
Age, NT-proBNP, and TAPSE	0.96 (0.72–1.17)	0.174	0.01	0.01	0.04	–	–	0.00 001
Age, NT-proBNP, TAPSE, and RVEDD	0.83 (0.59–1.17)	0.174	0.02	0.02	0.08	0.2	–	0.09
Age, NT-proBNP, TAPSE, RVEDD, and SBP	0.78 (0.53–1.14)	0.268	0.03	0.6	0.05	0.5	0.04	0.03

a Variables included in the multivariate analysis due to an acceptable *P* value and lack of correlation with other variables (see TABLE 2).

b Measured by cardiac magnetic resonance

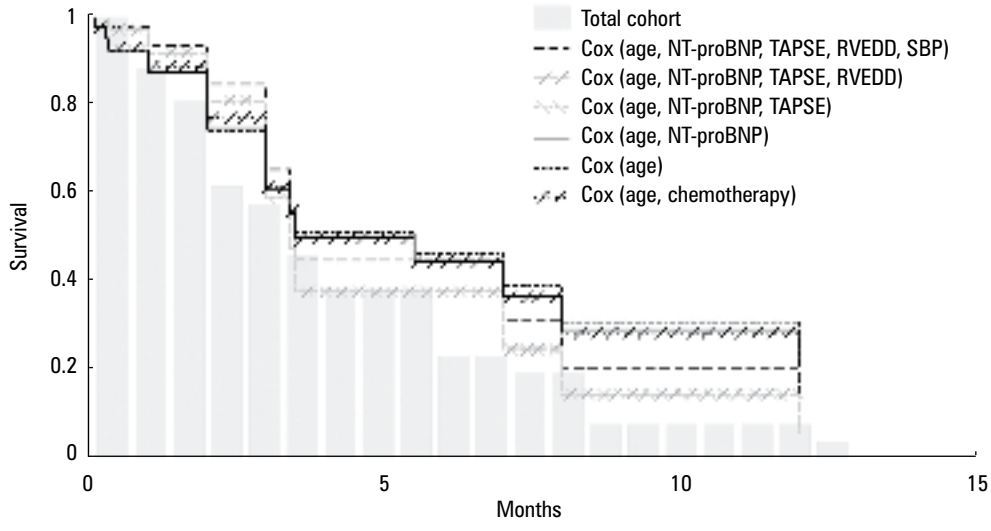
c Chemotherapy was assessed as a binary variable and included in the multivariate analysis despite its *P* value exceeding 0.05.

d LDH activity above the upper limit of normal was assessed as a binary variable.

The *P* values for the likelihood ratio statistics are shown for the multivariate Cox model. The sum of squared errors (SSE) of the Cox model survival prediction and the total cohort survival is shown in the SSE column. *P* values (1–5) in the multivariate analysis correspond to HR and b parameters of the 1st, 2nd, 3rd, 4th, and 5th added variable, respectively.

Abbreviations: CI, confidence interval; HR, hazard ratio; others, see TABLE 1

FIGURE 3
Kaplan–Meier survival curves for the total cohort and the corresponding multivariate Cox models; log-rank P values: see TABLE 3; abbreviations: see TABLE 1



LV thickness and end-diastolic dimension did not influence mortality in our study. There have been studies that confirmed the importance of LV wall thickness as a survival factor.[35,45] However, normal wall thickness was observed in several patients with poor prognosis.[46] Amyloid load, defined as the percentage of the amyloid area in immunohistochemically stained slides of endomyocardial biopsy, has recently been reported as an independent predictor of patient survival.[47]

TABLE 4 Receiver operating characteristic curve analysis for early or non–early mortality (3.4 months)

Variables	Cut-off point	AUC (95% CI)	*P* value	Sensitivity, %	Specificity, %	PPV, %	NPV, %	Accuracy, %
Age[a], y	<64	0.803 (0.641–0.965)	0.001	66.7	85.7	83.3	70.6	75.9
NT-proBNP[a], pg/ml	>4968	0.853 (0.709–0.998)	0.0007	92.8	66.7	72.2	90.9	79.3
SBP[a], mm Hg	<100	0.741 (0.559–0.923)	0.005	66.7	71.4	71.4	66.7	69
hs-TnT[a], µg/l	>134.5	0.8311 (0.638–1)	0.0004	87.5	68.8	58.3	91.8	75
LV mass[a]	>187	0.815 (0.597–1)	0.02	85.7	75	66.7	90	78.9
dFLC, mg/dl	–	–	0.9	100	0	0	45	45
RVEDD[a], mm	<34	0.672 (0.473–0.871)	0.05	85.7	47	60	77.8	65.5
TAPSE[a,b], mm	<13	0.737 (0.55–0.92)	0.007	78.6	50	61.1	70	64.3
TAPSE[c], mm	–	0.659 (0.416–0.903)	0.1	100	0	42.9	–	42.9
LVEF[a], %	<45	0.778 (0.608–0.949)	0.0007	86.7	42.8	61.9	75	65.5
LVEF[a,c], %	<50	0.715 (0.489–0.941)	0.03	80	54.5	61.4	75	66.7
Ortopnea	1	0.572 (0.358–0.787)	0.3	100	0	53.5	–	53.6

a A variable acting as a significant predictor used in further analysis

b Measured by echocardiography

c Measured by cardiac magnetic resonance

Abbreviations: AUC, area under the curve; NPV, negative predictive value; PPV, positive predictive value; others, see TABLES 1 and 3

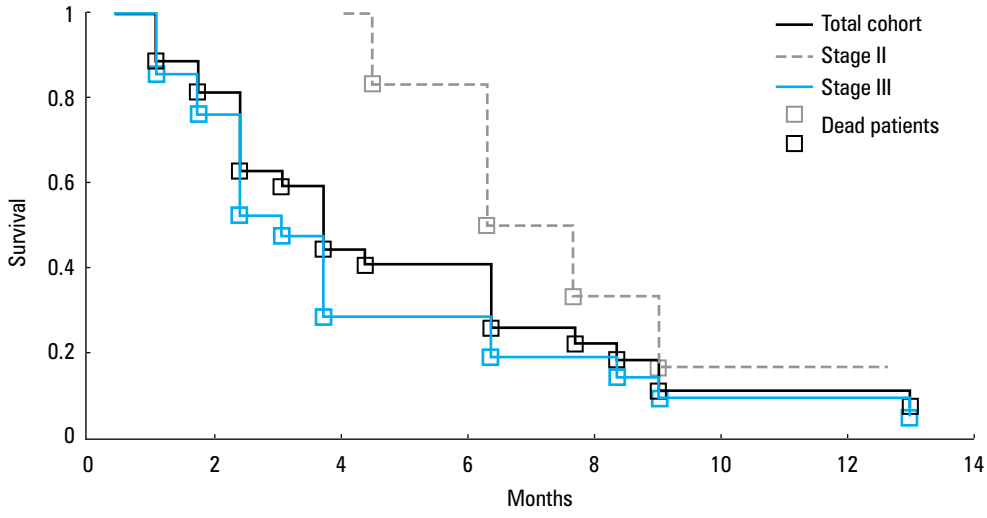


FIGURE 4
Kaplan–Meier survival curves for the total cohort and patients assessed as stage II and stage III amyloidosis according to the 2004 Mayo staging system; log-rank $P < 0.05$

The amyloid deposition was observed to be accompanied by a progressive loss of cardiomyocytes. This may explain why in some cohorts LV wall thickness was not directly associated with higher mortality. Amyloid load of 20% or higher was associated with no survival benefit for chemotherapy even in patients with hematological response, which underlines why early diagnosis is crucial for patient outcome.

Study limitations Although CMR is quite a new diagnostic method, it appears to be very helpful in cardiac amyloidosis imaging. It is recommended as a noninvasive diagnostic option.[21] CMR was not performed in 8 patients due to their performance status or for technical reasons. All TTE examinations were evaluated by an expert in the diagnosis of infiltrative cardiomyopathies.

Although mass spectroscopy is considered a current gold standard for identification of amyloid deposits,[6] it is still not available in Poland as a diagnostic method. Immunohistochemical reactions were performed instead and every biopsy was analyzed by at least 2 pathologists with substantial experience in the diagnosis of AL amyloidosis. To improve amyloid detection, the phenol Congo red staining was used.[35] In 3 cases, light chains were revealed in amyloid deposits by immunohistochemical reactions in autopsy specimens, but it was not possible to differentiate the type of light chain. Patients had clinical characteristics and imaging typical for AL amyloidosis, so they were included in the analysis.

It was our intention to spare patients the endomyocardial biopsy. The low percentage of patients with amyloidosis confirmed by endomyocardial biopsy (6 patients, 20%) may be

considered a limitation of the study. However, it is accepted to diagnose cardiac amyloidosis if a patient with a typical echocardiographic picture has the amyloid deposits in tissue more available for biopsy and an abnormal serum concentration of free light chains. On the other hand, the biopsy of a more available tissue may yield false negative results and delay a proper diagnosis.[48] We preferred a salivary gland biopsy over a gingival biopsy.[29] Instead of fat pad aspiration, we performed a surgical fat tissue biopsy obtaining a specimen of 1-cm abdominal fat tissue. Gastric mucous membrane samples collected during endoscopy appeared to be particularly valuable material. Gastric biopsy confirmed amyloidosis in 13 of the 15 patients who underwent a gastroscopy (86.7%). Gastric biopsy was performed every time even if the mucous membrane seemed to remain unaffected, as recommended by Said et al.[49]

Evaluation of the hematological treatment in our study group requires caution. Different chemotherapy regimens were used according to the patient performance status and current guidelines.[37] Furthermore, pharmacological legislation has recently been revised in Poland. Therefore, some medications, such as bortezomib, have become more available to patients with amyloidosis. This may explain the ambiguous result of the univariate analysis for chemotherapy.

Conclusions Polish patients with AL cardiac amyloidosis diagnosed in the cardiology department have advanced disease at the time of diagnosis. Younger age, impaired RV function, and higher concentrations of cardiac markers are associated with worse prognosis. Proper education of physicians would enable early diagnosis, which is crucial for an improvement in disease prognosis.

Contribution statement JAS and JG conceived the idea for the study. JAS and JD-S contributed to the design of the research. All authors but PZW were involved in data collection. PZW and JAS analyzed the data and PZW performed the statistical analysis. JAS, JD-S and PZW drafted and edited the article. PM, ŁM, WWJ, JD-T, and JG provided critical revision of the article. All authors approved the final version of the manuscript.

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