Multiparametric echocardiography scores for the diagnosis of cardiac amyloidosis

Michele Boldrini, MD[a,d], Francesco Cappelli, MD[i], Liza Chacko, MD[a], Maria Alejandra Restrepo-Cordoba, MD[e,f], Angela Lopez-Sainz MD, PhD[e,f], Alberto Giannoni, MD, PhD[b,c], Alberto Aimo, MD[c], MRCP[a], Andrea Baggiano, MD[l], Ana Martinez-Naharro, MD[a], Carol Whelan, MD[a], Cristina Quarta, MD, PhD[a], Claudio Passino, MD[b,c], Vincenzo Castiglione, MD[c], Vladyslav Chubuchnyi, MD[b], Valentina Spini, MD[b], Claudia Taddei, BSc[b], Giuseppe Vergaro, MD, PhD[b,c], Aviva Petrie, BSc (Hons)[g], Luis Ruiz-Guerrero, MD[e], Vanessa Moñivas, MD, PhD[g], Susana Mingo-Santos, MD, PhD[g], Jesus G Mirelis, MD, PhD[e,f], Fernando Dominguez, MD, PhD[e,f], Esther Gonzalez-Lopez, MD, PhD[e,f], Stefano Perlini, MD, PhD[d], Gianluca Pontone, MD, PhD[l], Julian Gillmore, MD, PhD[a], Philip N Hawkins, PhD, FMedSci[a], Pablo Garcia-Pavia, MD, PhD[e,f,g], Michele Emdin, MD, PhD[b,c], Marianna Fontana, MD, PhD[a].

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[a]National Amyloidosis Centre, University College London, Royal Free Campus, London, UK.
[b]Fondazione Toscana Gabriele Monasterio, Pisa, Italy.
[c]Institute of Life Sciences, Scuola Superiore Sant'Anna, Pisa, Italy.
[d]Emergency Department, Internal Medicine Department, Amyloidosis Research and Treatment Center, IRCCS Policlinico San Matteo Foundation, Pavia, Italy.
[e]Department of Cardiology, Hospital Puerta de Hierro Majadahonda, Madrid, Spain.
[f]CIBERCV, Madrid, Spain.
[g]University Francisco de Vitoria (UFV), Pozuelo de Alarcón, Madrid, Spain.
[h]Eastman Dental Institute, University College London, Grays Inn Road, London, UK.
[i]Tuscan Regional Amyloid Centre, Careggi University Hospital, Florence, Italy.
[l]Centro Cardiologico Monzino, IRCCS, Milan, Italy.

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Address for correspondence:
Dr Marianna Fontana,
Cardiac MRI Unit, National Amyloidosis Centre,
University College London, Royal Free Campus,
Rowland Hill Street, London, NW3 2PF, UK
E-mail: m.fontana@ucl.ac.uk
Phone No: +44 2074332764
Fax No: +44 2044332817

ABSTRACT

Background: Cardiac amyloidosis (CA) is a serious though increasingly treatable cause of heart failure. Diagnosis is challenging and frequently unclear at echocardiography, which remains the most often used imaging tool.

Objectives: We aimed to study the accuracy of a broad range of echocardiographic variables to develop multiparametric scores to diagnose CA in patients with proven light chain (AL) amyloidosis or those with increased heart wall thickness (IWT) in whom amyloid was suspected. We also aimed to further characterise structural and functional changes associated with amyloid infiltration.

Methods: We studied 1187 consecutive patients evaluated at 3 referral centres for CA and analysed morphological, functional and strain-derived echo parameters with the aim of developing a score-based diagnostic algorithm. Cardiac amyloid burden was quantified using extracellular volume measurements at cardiac magnetic resonance.

Results: 332 patients were diagnosed with AL amyloidosis and 339 patients with transthyretin (ATTR) CA. Concentric remodelling and strain-derived parameters displayed the best diagnostic performance. A multivariable logistic regression model incorporating relative wall thickness, E/e'ratio, longitudinal strain and tricuspid annular plane systolic excursion had greatest diagnostic performance in AL amyloidosis (area under the curve - AUC- 0.90[95% confidence interval 0.87-0.92]), whilst addition of septal apical- to -base ratio yielded the best diagnostic accuracy in the IWT group (AUC 0.87[0.85-0.9]).

Conclusions: Specific functional and structural parameters characterize different burdens of CA deposition with different diagnostic performances, and enable to define two scores that are sensitive and specific tools to diagnose or exclude CA.

CONDENSED ABSTRACT

Echocardiography is a first-line imaging examination when cardiac amyloidosis(CA) is suspected, but no systematic and standardized approach to a cumulative assessment of echocardiographic parameters has ever been proposed. We studied a broad range of echocardiographic variables to develop a multiparametric approach to CA diagnosis in patients with proven light chain amyloidosis and those with increased heart wall thickness in whom amyloid was suspected. We identified specific functional and structural parameters that become abnormal with different burdens of CA deposition, allowing to define echocardiographic scores that represent accurate tools to diagnose CA.

Key Words:
Cardiac amyloidosis, echocardiography, hypertrophy, wall thickness, global longitudinal strain.

Abbreviations:
AL=Light chain
ATTR=Transthyretin
CA=Cardiac Amyloidosis
CMR=Cardiovascular magnetic resonance
ECV=extracellular volume
EF=Ejection Fraction
IVSd=Interventricular septum in diastole
IWT=Increased wall thickness
LAA=Left atrium area LS=Longitudinal Strain

INTRODUCTION

Cardiac amyloidosis (CA) is a progressive disorder and an under-diagnosed cause of heart failure. Both its poor prognosis compared to other hypertrophic phenocopies, and the increasing availability of disease-modifying treatments, underscores the importance of early diagnosis. Endomyocardial biopsy has traditionally been the gold standard for diagnosis of CA, but its invasive nature has curtailed its usage, placing much dependence on cardiac imaging. Cardiovascular magnetic resonance (CMR) and diphosphonate scintigraphy have lately been validated as reliable tools for diagnosis of CA, and hence have great potential for transforming the diagnostic pathway in suspected CA(1-3), but their availability remains limited. By contrast, echocardiography remains the first-line imaging technique for patients presenting with heart failure, but diagnosis of CA is frequently delayed or missed, due in part to the limited sensitivity and specificity of the wall thickness alone, or wall thickness plus NT-proBNP, as recommended by the current diagnostic criteria.(4,5) Echocardiographic findings that have been proposed for diagnosis of CA range from conventional LV remodelling parameters to those evaluating diastolic function and deformation(5-20). However, the diagnostic accuracy of these indices has only been tested either in studies focusing on single variables(9,18,21) or small retrospective studies(22-28), leaving a knowledge gap in the clinical utility of the various echocardiographic parameters or their combination in patients with a clinical suspicion of CA.

Two fundamental clinical scenarios can be identified: firstly, in patients with confirmed systemic AL amyloidosis, in whom the structural and functional changes of early cardiac amyloid involvement can be extremely subtle, and yet strongly influence prognosis and therapeutic decisions; secondly, as a differential diagnosis among patients with a hypertrophic phenotype, which is far more commonly due to hypertensive heart disease, aortic stenosis or hypertrophic cardiomyopathy.

The aims of the present study were to: (1) assess the relative diagnostic accuracy of a broad range of echocardiographic variables in a large multicentre cohort of consecutive patients referred for suspected CA; (2) develop a refined multiparametric approach to enhance diagnosis of CA inthe two challenging clinical scenarios of patients with known systemic AL amyloidosis and patients presenting with a hypertrophic phenotype; (3) further characterise functional and structural cardiac abnormalities that occur in CA across a wide spectrum of cardiac amyloid burden.

METHODS

Patients. The study subjects comprised consecutive patients referred for suspected CA between 2009 to 2018 to 3 specialized centres: National Amyloidosis Centre (NAC), London, United Kingdom (n=899 subjects); Fondazione Toscana Gabriele Monasterio, Pisa, Italy (n=180); Hospital Puerta de Hierro in Madrid, Spain (n=108). The study population was categorised for analysis into two groups: patients with proven systemic AL amyloidosis (n=494) and patients with increased LV wall thickness on echo (IVSd or PWTd ≥12mm) that had suggested the possibility of amyloidosis (n=978).

All patients underwent a complete diagnostic workup including clinical evaluation, echocardiography, serum and urine biochemistry comprising N-terminal pro-B-type natriuretic peptide (NT-proBNP), serum free light chain assay along with serum and urine immunofixation-electrophoresis and in some selected cases myocardial or non-myocardial biopsy. CMR was performed in 85% of patients according to standardized protocols. Patients with suspected ATTR CA also underwent diphosphonate scintigraphy. The study received ethics approval at each participating centres.

Diagnosis of CA. Cardiac AL amyloidosis was defined either by the combination of typical features on CMR(1,3) and histologically proven systemic AL amyloidosis using a non-cardiac biopsy(1) (n=314) or a cardiac biopsy containing AL amyloid (n=18). Cardiac ATTR amyloidosis was defined as either the combination of typical features on CMR, grade 2 or 3 cardiac uptake on DPD-scintigraphy in the absence of monoclonal gammopathy (n=295)(2), or in the presence of monoclonal gammopathy a cardiac biopsy containing ATTR amyloid (n=44)(29).

The echocardiographic and CMR protocols are reported in the supplemental material.

Statistical Analysis. Baseline characteristics are expressed as mean (standard deviation) when normally distributed and median (interquartile interval) when non-normally distributed.

Variables with normal distribution and equal variances were compared with Student's *t* test whilst variables with unequal variances were compared with the Welch's *t* test.

Diagnostic accuracy. The diagnostic endpoint was defined as the presence of CA, defined as above(1). The overall study population was divided into the two groups of interest: patients with systemic AL (n=494) and patients with increased LV wall thickness (n=978). Frequencies of disease were calculated in both samples. Discrimination was evaluated by drawing the receiver operating curve (ROC) and determining the area under it (AUC or *c*-statistic).

Model creation. Variables potentially predictive for the diagnosis from the univariable analyses showing statistical significance at the 10% level were selected for multivariable logistic regression analysis, avoiding combinations of variables that would lead to collinearity. Among the variables independently associated with the diagnosis we selected those most clinically relevant and tested their accuracy to diagnose CA by calculating the area under the curve (AUC) values. The optimal cut off value was defined as the point with the highest sum of sensitivity and specificity. Then each variable was dichotomized according to the optimal cut off value and two models were built with a multivariable logistic regression approach. Each variable in the two models was weighted by its coefficient from the multivariate logistic regression analysis. The two models were: AL Score for the systemic AL subpopulation, and increased wall thickness (IWT) Score for the increased wall thickness subpopulation. The selected variables were: relative wall thickness (RWT), tricuspid annular plane systolic excursion (TAPSE), E/e' and longitudinal strain (LS) for AL Score, and RWT, TAPSE, E/e', LS and septal longitudinal systolic apex to base ration (SAB) for IWT Score. For each subpopulation, we performed a second logistic regression analysis using the dichotomized explanatory variables and then the coefficient of each of these variables was divided by the smallest coefficient in the model and allocated a weight based on rounding this

to the nearest integer. The overall risk score for a subpopulation was obtained by summing the weights so obtained from all coefficients. Bar charts were drawn to show the stepwise increase in risk of CA for increasing values of the score.

Cut-offs for all points of the scores were created. For each cut-off, the following parameters were calculated after a logistic regression analysis, with a binary explanatory variable defined by the relevant cut off and the diagnostic endpoint as the outcome: sensitivity, specificity, true and false positives, true and false negatives.

Model validation. Bootstrap validation was used to determine the performance of each of the two prediction models on a hypothetical set of new patients. For each model, 200 bootstrap data sets were created by sampling 'with replacement' from the original data set. The model evaluated in one bootstrap sample provided the bootstrap AUC and when that model was evaluated in the original data set, it provided the test AUC. The difference between the two gave an estimate of the optimism in the fit for that bootstrap sample. The mean of these differences over all 200 bootstrap samples provided a stable estimate of the optimism. Finally, the optimism-adjusted AUC, estimating the internally validated AUC, was determined by subtracting the optimism from the apparent AUC, where the apparent AUC was the AUC evaluated using the model derived from the original data set. The distribution of the values of optimism from the 200 bootstrap samples was visually represented in a box plot. The scoring system used for each of the two models was validated by creating a bar chart showing the percentage of patients with CA for each overall score. A useful scoring system is characterized by a stepwise increase in the percentage with CA with increasing values.

Probability of cardiac structural and functional variables being abnormal across the spectrum of ECV. Univariable logistic regression analysis was performed for evaluating the probability of each of the selected cardiac structural and functional variables being abnormal

across the range of ECV. Cut-offs for defining abnormalities in these variables were chosen according to published reference guidelines: IVSd, PWTd$\geq$12mm(30), RWT>0.42, MCF$\leq$0.234, E/A>2, E/é>8, LAA>20cm^2, LVEF<55%, MAPSE<11mm, TAPSE<16mm, LS>-20%, SAB>2.1, RALS>1, EFSR>4.1(9,18,22).

Two-sided tests were used for all analysis and a p<0.05 was considered significant unless otherwise specified. The data were analysed using Stata (StataCorp. 2015. Stata Statistical Software: Release 14; StataCorp LP, College Station, TX, USA).

RESULTS

The study population comprised 1187 patients, categorised for the analysis into those with systemic AL amyloidosis (n=494, with or without cardiac involvement) or those with increased LV wall thickness (n= 978, with or without CA, regardless of amyloid type). The overall prevalence of CA was 57%. 332 patients had AL CA and 339 patients had ATTR CA, of whom 200 had wild-type ATTR CA and 139 patients had variant ATTR CA. Among the remaining patients, 172 had systemic AL amyloidosis without cardiac involvement; 57 had hypertrophic cardiomyopathy; 64 had other types of amyloidosis or related disorders without cardiac involvement (AA, n=6; localizedAL, n=36;light chain deposition disease, n=20; LECT2, n=2); 30 had severe aortic stenosis; 183 had hypertensive remodelling; 97 had other types of heart disease (sarcoid heart n=6, myo-pericarditis n=25, dilated cardiomyopathy n=24, Fabry's disease n=3, heart failure with preserved ejection fraction n=5, ischaemic heart disease n=28, pulmonary hypertension n=2, arrhythmogenic right ventricular cardiomyopathy n=4); and 27 had normal cardiac structure. Among patients with systemic AL amyloidosis, 114 had concomitant non-amyloid heart disease. Baseline characteristics are shown in Table 1.

Diagnostic accuracy.

The main echocardiographic findings are summarized in Table 1. In both groups of patients with systemic AL amyloidosis and IWT, the presence of CA was characterised by a greater increased wall thickness and LV mass, greater extent of concentric remodelling, a higher degree of diastolic dysfunction, lower MCF, and more severe reduction in LVEF, MAPSE and TAPSE.

In patients with systemic AL amyloidosis, the variables associated with the best diagnostic accuracy were those related to concentric hypertrophy and geometry (PWTd, IVSd, RWT), LS and NT-proBNP. In patients with increased wall thickness, the variables associated with

the best diagnostic accuracy were those reflecting concentric hypertrophy (PWTd, IVSd, RWT), but also diastolic dysfunction (E/A) and strain parameters assessing the presence of relative apical sparing (RALS, SAB) (Table 2).

Model performance. The model including RWT, E/e', LS and TAPSE as explanatory variables showed the best diagnostic accuracy in patients with systemic AL amyloidosis ("AL Score"). The apparent AUC was 0.909 and the optimism was 0.004 so that the optimism corrected AUC, estimating the internally validated AUC, was 0.905.. The model with RWT, E/e', LS, TAPSE and SAB as explanatory variables showed the best diagnostic accuracy in patients with IWT (IWT score). The apparent AUC was 0.870 and the optimism was 0.006 so that the optimism corrected AUC, estimating the internally validated AUC, was 0.864. The AL and IWT models both showed good calibration as assessed by the Hosmer-Lemeshow goodness-of-fit test ($p = 0.64$, and $p = 0.11$, respectively). Their Loess smoothed calibration curves were very close to the diagonal and having a slope of 1.

Diagnostic score. A score including RWT, E/e', LS and TAPSE showed the best diagnostic accuracy to detect CA in patients with systemic AL amyloidosis (AL score). A score including RWT, E/e', LS, TAPSE and SAB showed the best diagnostic accuracy to detect CA in patients with increased wall thickness (IWT score). To each variable were attributed points when positive for the optimal diagnostic cut-off. For the AL score we weighted the variables attributing two points to E/e' and RWT, one point to LS and TAPSE, with a final score ranging from 0 to 6 (points). For the IWT score we weighted the variables attributing three points to RWT and SAB, two points to TAPSE, one point to E/e' and LS, with a final score ranging from 0 to 10 (points). See Table 3 for details about the cut off values for each variable in the AL and IWT scores.

For each score, the diagnostic performance of the different score points was calculated (Table 4).

The AL and IWT scores could not be calculated in 3.4% and 5.6% of the respective subpopulations, because one of the variables was missing for inadequate quality.

Relationship between traditional/strain-derived parameters and ECV

Increasing myocardial amyloid burden, as described by increasing ECV values, was associated with several alterations in different echocardiographic variables, either traditional or related to strain evaluation (Figure 1). This approach allowed to group selective alterations of different echocardiography variables by the probability of becoming abnormal at low or high disease burdens – as assessed by ECV as a marker of cardiac infiltration/burden - or to be gradually distributed across the spectrum of myocardial disease. In detail, IVSd, PWTd, RWT, MCF, E/e' and LS had higher probability of being abnormal at low cardiac amyloid burdens (ECV ≥40% and <51%;). Conversely, TAPSE and LVEF only became abnormal at high burdens of cardiac infiltration (ECV ≥70%). Finally, the probability of LAA, MAPSE, E/A, SAB, RALS and EFSR becoming abnormal gradually increased across the spectrum of ECV values (ECV ≥51%<70%).

Additionally, ECV values displayed a good correlation with the points of both AL and IWT score (Figure 2).

DISCUSSION

Herein, we report the results of a large multi-centre study evaluating the diagnostic accuracy of a combination of traditional (non-deformation) and strain-derived echocardiographic variables in patients with suspected CA. We derived two simple multiparametric scores to either diagnose or exclude CA in two important clinical scenarios, i.e. among patients with proven systemic AL amyloidosis or in patients with a "hypertrophic" cardiac phenotype. Finally, we identified the functional and structural changes across the spectrum of severity of amyloid deposition.

In patients with systemic AL amyloidosis, the variables individually associated with the highest diagnostic accuracy were structural variables in keeping with concentric hypertrophy (PWTd, IVSd, RWT), reduction in the LS and increased plasma NT-proBNP. The good diagnostic accuracy of variables indicating concentric hypertrophy in this population is not surprising, as increase in LV mass with concentric remodelling in the presence of systemic AL amyloidosis is likely driven by amyloid infiltration. However, wall thickness alone, or wall thickness plus NT-proBNP, as recommended by the current diagnostic criteria(5), are sensitive but poorly specific markers (table 4). This casts some doubt on the current use of these criteria, as markers with high sensitivity and low specificity should be used to rule out CA rather than to confirm the diagnosis. LS and NT-proBNP are also characterized by a good diagnostic accuracy underscoring the importance of markers of early disease in patients with systemic AL amyloidosis, where typically the amyloid burden remains relatively small even in advanced disease, as corroborated by relatively lower ECV values in AL than ATTR patients(31).

In patients with a hypertrophic phenotype, the variables associated with best diagnostic accuracy were structural variables reflecting concentric hypertrophy (PWTd, IVSd, RWT), but with significantly higher cut-offs in terms of increase in LV wall thickness, evidence of

diastolic dysfunction (E/A, E/e') and strain variables assessing the relative apical sparing (RALS, SAB). The high diagnostic accuracy of E/A, which reflects the diastolic alterations that are typically associated with CA, and variables able to assess the relative apical sparing, a characteristic phenomenon associated with amyloid infiltration, is in keeping with the importance, in the group of patients with myocardial hypertrophy, of more specific markers, able to differentiate amyloid infiltration from other causes of increased LV mass.

The high sensitivity of some functional and structural metrics was also confirmed when these variables were assessed against progressively increasing levels of cardiac amyloid infiltration. Echocardiographic structural and functional metrics were divided into 3 groups that became sequentially abnormal with increasing cardiac amyloid burden, as assessed by ECV quantification. Variables associated with concentric hypertrophy, LS, and also MCF and E/e' showed a high probability of becoming abnormal at low levels of cardiac infiltration, confirming their role as early disease markers. Interestingly, strain parameters reflecting the relative apical sparing (SAB, RALS and EFSR) fell in the intermediate group of variables with a probability of being abnormal rising progressively across the spectrum of increasing amyloid burden. This stands in agreement with the utility of these markers forthe differential diagnosis of myocardial hypertrophy, when the amyloid burden is typically at least moderate, rather than detection of cardiac involvement in patients with systemic AL amyloidosis, where the whole spectrum can be typically seen, including very early stages of infiltration. Finally, we confirmed that EF and TAPSE tend to be preserved until higher burdens of cardiac infiltration, reflecting the most advanced disease stages.

While these analyses confirm that the various echocardiographic variables on their own are associated with a variable degree of diagnostic accuracy, a combination of parameters reflecting structural and functional changes has the potential to increase the diagnostic accuracy of echocardiography in the two challenging clinical scenarios examined.

In patients with systemic AL amyloidosis, a score ("AL score") including RWT, E/e', LS and TAPSE showed a very good diagnostic accuracy in identifying patients with CA with an AUC of 0.90. The usefulness of this score would be to allow CA diagnosis without performing a cardiac biopsy. However, because confirming or excluding CA has a significant impact in treatment strategies, we propose to use highly sensitive and highly specific cut-offs (AL score <1 and ≥5) to exclude or confirm the diagnosis of CA, and restrict the use of second level imaging modality such as CMR or undergo endomyocardial biopsy to patients with intermediate probability. Using these cut-offs, the diagnosis or the exclusion of CA in patients with systemic AL amyloidosis could be obtained in 15% and 35% respectively without the need for further investigations, carrying immediate implications on patient management. For example, in patients with cardiac involvement, bortezomib, one of the most widely used chemotherapy agents, is given at a reduced dose as the presence of cardiac involvement is associated with increased mortality.(32,33) The current widely accepted standard to assess cardiac involvement(32,33) lacks specificity (ranging from 47 to 67% in our study). The algorithm proposed in the present study is associated with a significantly higher diagnostic accuracy and, if adopted, would have immediate implications in terms of treatment strategies. In patients with myocardial hypertrophy a score including RWT, E/e', TAPSE, LS, and SAB showed a very good diagnostic accuracy in identifying patients with CA (IWT score) with an AUC of 0.87. For both scores the AL and IWT scores, higher values were associated with higher amyloid burden, as measured by ECV (Figure 2). Several studies have shown recently that ATTR CA is frequent in common cardiac diseases that exhibit IWT. For example, 13.3% of elderly patients admitted with HFpEF, 16% of patients with aortic stenosis treated with TAVI and 5% of outpatients with IWT ≥15 mm have been reported to have CA(34,35). In patients with a hypertrophic phenotype, we acknowledge that further confirmatory testing will be required to definitively confirm a diagnosis of cardiac

amyloidosis, however we strongly believe that the application of a score based approach in patients with undifferentiated LVH will prove to be a valuable tool for sonographers and clinicians, serving to support earlier clinical suspicion of cardiac amyloidosis (even early disease), subsequent earlier diagnosis, and recognition of cardiac amyloidosis as an important and under-recognised cause of restrictive cardiomyopathy both in a hospital and pre-hospital setting. Because it is essential to avoid misdiagnosis of CA as effective treatments are now available, the primary aim of the different cut-offs provided (including the ones highly specific and highly sensitive) is to guide the diagnostic algorithm and the use of second-level tests in the most efficient way. When the score points denote a very high likelihood of CA, searching for a clonal dyscrasia and performing a diphosphonate scintigraphy should be the next diagnostic steps, to confirm CA and help differentiate AL from ATTR subtypes (38). CMR or endomyocardial biopsy should however be considered if AL is in the differential, i.e. in a patient who has a hypertrophic phenotype and a clonal gammopathy. By contrast, when the probability of CA is very low, CMR should be considered as the next diagnostic test of choice, helping clinicians to re-define diagnosis across the spectrum of disorders presenting with a hypertrophic phenotype.

Study limitations

Because of the study design, the population was composed of patients referred to specialised amyloid centres for suspected CA. Whilst this does not represent an unselected population, we believe that our algorithm will be applicable to the general population when there is a suspicion of CA. However, we do not propose that this algorithm is applied immediately to the general population as a screening tool for cardiac amyloidosis, as further studies are required to validate these findings within the general unselected cardiology population. Moreover, LS and its derived variables have been performed in a 4-chamber view. Four-chamber longitudinal strain was used instead of GLS to minimize the number of patients who

would have been excluded from strain evaluation due to inadequate acoustic windows and/or acoustic drop out in multiple LV segments. Acquisition of good quality imaging without segment drop out is by far more challenging in 2- or 3-chamber apical views compared to 4-chamber views.(36) Furthermore, it has been demonstrated that GLS in the three apical views and the 4-chamber longitudinal strain are not significantly different, have a very good correlation and minimal bias.(36) Finally, we acknowledge that strain analysis was performed using the two worldwide diffuse vendor software, which could introduce an inter-software variability bias. However, with the recent vendor-specific software for strain analysis used in the present paper, it was demonstrated that mean LV GLS values were not significantly different between the two analyzed vendors (GE and Philips), showed good correlation and minimal bias.(37)

CONCLUSION

The diagnosis of CA has increased exponentially during the past few years, undoubtedly also because of a significant increase in disease awareness. Current echocardiographic diagnostic criteria, developed and validated only for AL amyloidosis, have reasonable sensitivity, but a relatively low specificity. No systematic approach has hitherto been available for patients with a hypertrophic phenotype to stratify the probability of CA. We provide here a multiparametric echocardiographic approach for the two challenging clinical scenarios of patients with systemic AL amyloidosis or a hypertrophic phenotype. Using highly sensitive and highly specific cut-offs in patients with systemic AL amyloidosis, amyloidosis can be excluded or confirmed in 50% of patients without the need of further tests. Although not obviating the need for confimatory testing, in patients with a hypertrophic phenotype, highly specific or highly sensitive cut-offs can be used to guide the diagnostic algorithm, avoid

unnecessary tests, and limit the time to diagnosis.

CLINICAL COMPETENCIES, TRANSLATIONAL OUTLOOK IMPLICATIONS

Competency in Medical Knowledge: Thanks to a combination of structural and functional variables, a multi-parametric approach echocardiographic allows to reliably rule out or guide further diagnostic examinations in cases of suspected cardiac amyloidosis.

Translational Outlook: The diagnostic performance of echocardiographic scores should be evaluated also outside of specialized centres. Furthermore, the possibility to track disease progression and the response to treatment through multi-parametric echocardiographic scores deserve consideration in future studies.

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FIGURES

Central illustration. Diagnostic algorithms with echocardiographic scores in two different clinical scenarios. Proposed diagnostic algorithms with highly sensitive and highly specific cutoffs to diagnose or exclude cardiac amyloidosis in patients with systemic AL amyloidosis (AL score) (upper panel) and in patients with increased wall thickness (IWT score) (lower panel).

Figure 1. Abnormal echocardiographic parameters according to ECV values.

The probability of cardiac structural and functional variables being abnormal across the spectrum of cardiac amyloid burden (as defined by ECV). Variables can be categorized into 3 groups according to their likelihood of being abnormal: either predominantly at low, intermediate or highburden of amyloid infiltration. E/A=Ewave/Awave ratio; E/e'=Ewave/e'wave; ECV=Extracellular volume; EFSR=Ejection fraction/LS ratio; IVSd=diastolic interventricular septum; LAA=Left atrium area; LVEF=Left ventricular ejection Fraction; LS=Longitudinal Strain; MAPSE=Mitral annular plane systolic excursion; MCF=Myocardial contraction fraction; PWTd=diastolic posterior wall thickness; RALS=Relative apical longitudinal sparing; RWT=Relative wall thickness; SAB=Systolic apex to base ratio; TAPSE=Tricuspid annular plane systolic excursion.

Figure 2. ECV and scores.

Box plots showing the increasing median ECV values according to the points in each score. Upper panel: AL score. Lower panel: IWT score.

TABLES

Table 1. Baseline characteristics of the overall population divided in the two clinically

relevant subgroups.

	Systemic AL			Increased Wall Thickness		
	CA (n=322)	no CA (n=172)	*p value*	CA (n=647)	no CA (n=331)	*p value*
Age,*years*	66(10)	68(11)	0.01	72(11)	71(12)	0.18
Sex	M62%, F38%	M57%, F43%	0.3	M75%, F25%	M65%, F35%	0.0007
BSA,*m²*	1.9(0.2)	1.9(0.2)	0.082	1.9(0.2)	1.9(0.3)	0.053
sBP, *mmHg*	112(22)	133(24)	<0.001	120(24)	137(24)	<0.001
dBP, *mmHg*	70(13)	77(12)	<0.001	72(13)	77(12)	<0.001
NT-proBNP,*ng/mL*	5772(6803)	1386(4108)	<0.001	5252(6694)	2715(5179)	<0.001
eGFR, *mL/min/1.73m²*	72(24)	69(27)	0.368	67(22)	64(26)	0.096
IVSd, *mm*	14.8(2.6)	11.4(2)	<0.001	16.4(2.7)	14(2.1)	<0.001
PWTd,*mm*	14.5(2.6)	11.2(2)	<0.001	15.9(2.7)	12.7(2.3)	<0.001
LVEDD, *mm*	43(6)	47(7)	<0.001	44(7)	48(8)	<0.001
RWT	0.69(0.17)	0.48(0.11)	<0.001	0.75(0.26)	0.55(0.14)	<0.001
LVEDV, *mL*	68(26)	69(24)	0.65	75(28)	89(45)	<0.001
LVESV,*mL*	34(20)	28(13)	0.025	39(21)	44(34)	0.014
LVMi, *g/m²*	150(45)	111(30)	<0.001	182(51)	137(42)	<0.001
MCF	0.15(0.07)	0.23(0.09)	<0.001	0.13(0.06)	0.2(0.09)	<0.001
LAA, *cm²*	23(6)	19(6)	<0.001	26(6)	25(8)	0.059
LVEF, *%*	55(12)	63(10)	<0.001	51(13)	55(13)	<0.001
MAPSE, *mm*	10(3)	14(3)	<0.001	9(3)	12(4)	<0.001
TAPSE, *mm*	17(5)	22(5)	<0.001	17(5)	20(5)	<0.001
SV, *mL*	35(16)	41(15)	0.007	36(15)	46(19)	<0.001
E/a	1.8(1)	1.1(2.3)	<0.001	2.1(1.2)	1.2(2.2)	<0.001
DT,*ms*	190(60)	238(71)	<0.001	186(66)	194(106)	0.21
E/e'	16(8)	9(4)	<0.001	16(8)	12(7)	<0.001
LS, *%*	13(5)	19(4)	<0.001	11(4)	15(5)	<0.001
SAB	5.1(7.1)	2.3(1.3)	<0.001	6.1(7)	2.9(1.9)	<0.001
RALS	0.84(0.25)	1.18(0.49)	<0.001	1.26(0.55)	0.81(0.35)	<0.001
EFSR	4.7(1.6)	3.5(1)	<0.001	4.9(1.7)	4.1(1.4)	<0.001

Variables are expressed as mean (standard deviation). AL amyloidosis=light chain amyloidosis; Increased Wall Thickness=IVSd or PWTd ≥12mm; BSA=Body Surface Area; sBP=Systolic Blood Pressure; dBP=Diastolic Blood Pressure; NTproBNP=N-terminal pro b-type natriuretic peptide; eGFR=Estimated glomerular filtration rate; IVSd=interventricular septum in diastole; PWTd=posterior wall thickness in diastole; LVEDD=Left ventricular end diastolic volume; RWT=Relative wall thickness; LVEDV=Left ventricular end diastolic volume; LVESV=Left ventricular end systolic volume; LVMi=LV mass index; MCF=Myocardial contraction fraction; LAA=Left atrium area; LVEF=left ventricular ejection fraction; MAPSE=Mitral annular plane systolic excursion; TAPSE=Tricuspid annular plane systolic excursion; SV=Stroke volume; E/A=E wave/A wave ratio;

DT=Deceleration Time; E/e'=E wave/e'wave; LS=Longitudinal Strain; SAB=Systolic apex to base ratio; RALS=Relative apical longitudinal sparing; EFSR=Ejection fraction/LS ratio.

Table2. Diagnostic accuracy of echocardiographic variables for the detection of CA in patients with systemic AL amyloidosis (upper panel) and in patients with Increased Wall Thickness (lower panel).

	Systemic AL amyloidosis					
	AUC (95% CI)	Cut off	Sens, % (95% CI)	Spec, % (95% CI)	+LR (95% CI)	-LR (95% CI)
NT-proBNP, *ng/mL*	0.85 (0.75-0.81)	>1302	75 (69-80)	81 (74-87)	3.99 (2.9-5.5)	0.31 (0.2-0.4)
IVSd, *mm*	0.85 (0.82-0.88)	> 13	72 (67-77)	87 (81-91)	5.36 (3.6-7.9)	0.33 (0.3-0.4)
PWTd, *mm*	0.85 (0.82-0.88)	>13	76 (71-80)	84 (78-89)	4.82 (3.4-6-8)	0.29 (0.2-0.4)
LVEDD, *mm*	0.68 (0.64-0.72)	≤ 42	50 (44-55)	80 (73-85)	2.44 (1.8-3.3)	0.63 (0.6-0.7)
RWT	0.86 (0.84-0.90)	> 0.52	85 (80-88)	75 (68-81)	3.39 (2.6-4.4)	0.20 (0.2-0.3)
LVEDV, *mL*	0.51 (0.47-0.58)	≤ 65	53 (47-60)	56 (45-67)	1.21 (0.9-1.6)	0.83 (0.7-1.0)
LVESV, *mL*	0.58 (0.52-0.63)	>36	36 (30-43)	82 (72-90)	2.04 (1.3-3.3)	0.77 (0.7-0.9)
LVMi, *g/m²*	0.75 (0.70-0.78)	>125	57 (51-62)	82 (75-88)	3.11 (2.2-4.4)	0.53 (0.5-0.6)
MCF	0.75 (0.70-0.80)	≤ 0.15	62 (56-68)	78 (68-87)	2.86 (1.9-4.4)	0.48 (0.4-0.6)
LAA, *cm²*	0.69 (0.64-0.73)	> 19	73 (68-78)	58 (49-67)	1.74 (1.4-2.2)	0.46 (0.4-0.6)
LVEF, *%*	0.70 (0.66-0.74)	≤ 60	67 (60-74)	67 (60-74)	2.06 (1.6-2.6)	0.49 (0.4-0.6)
MAPSE, *mm*	0.81 (0.77-0.85)	≤ 11	65 (60-70)	84 (78-89)	4.14 (2.9-5.9)	0.41 (0.4-0.5)
TAPSE, *mm*	0.74 (0.70-0.78)	≤ 19	67 (61-72)	76 (69-82)	2.78 (2.1-3.7)	0.44 (0.4-0.5)
SV, *mL*	0.61 (0.55-0.66)	≤ 39	68 (62-74)	54 (42-65)	1.46 (1.1-1.9)	0.60 (0.5-0.8)
E/A	0.75 (0.71-0.79)	> 1.2	60 (55-66)	84 (78-90)	3.85 (2.7-5.6)	0.47 (0.4-0.5)
DT, *ms*	0.72 (0.67-0.76)	≤ 225	78 (73-82)	58 (50-66)	1.86 (1.5-2.3)	0.38 (0.3-0.5)
E/e'	0.81 (0.78-0.85)	>10	70 (64-75)	81 (74-86)	3.60 (2.6-4.9)	0.38 (0.3-0.5)
LS,*%*	0.82 (0.78-0.85)	≥ -14	63 (57-68)	87 (81-91)	4.68 (3.2-6.9)	0.43 (0.4-0.5)
SAB	0.73 (0.69-0.77)	> 3.1	51 (45-56)	88 (82-92)	4.13 (2.7-6.3)	0.56 (0.5-0.6)
RALS	0.75 (0.71-0.79)	> 1	58 (53-64)	83 (77-88)	3.47 (2.5-4.9)	0.50 (0.4-0.6)
EFSR	0.78 (0.74-0.82)	> 3.8	71 (66-76)	73 (66-80)	2.76 (2.1-3.4)	0.39 (0.3-0.5)
AL score	0.90 (0.87-0.92)	≥ 3	83 (79-87)	85 (78-90)	5.45 (3.8-7.8)	0.20 (0.2-0.3)

	Increased Wall Thickness					
	AUC (95% CI)	Cut off	Sens, % (95% CI)	Spec, % (95% CI)	+LR (95% CI)	-LR (95% CI)
NT-proBNP, *ng/mL*	0.74 (0.70-0.77)	> 1452	76 (72-80)	62 (55-68)	1.98 (1.7-2.3)	0.39 (0.3-0.5)
IVSd, *mm*	0.77 (0.74-0.80)	> 15	64 (60-68)	79 (74-83)	3.08 (2.5-3.8)	0.45 (0.4-0.5)
PWTd, *mm*	0.83 (0.81-0.85)	> 13	82 (79-85)	73 (68-78)	3.02 (2.5-3.6)	0.25 (0.2-0.3)
LVEDD, *mm*	0.64 (0.61-0.67)	≤ 46	65 (61-69)	57 (52-63)	1.52 (1.3-1.7)	0.61 (0.5-0.7)
RWT	0.83 (0.80-0.85)	> 0.6	79 (76-82)	72 (67-77)	2.84 (2.4-3.4)	0.29 (0.2-0.3)
LVEDV, *mL*	0.59 (0.55-0.63)	≤ 79	65 (61-69)	52 (46-59)	1.37 (1.2-1.6)	0.67 (0.6-0.8)
LVESV, *mL*	0.50 (0.47-0.54)	> 52	17 (14-21)	73 (68-79)	0.64 (0.5-0.9)	1.13 (1.0-1.2)
LVMi, *g/m²*	0.68 (0.64-0.71)	> 141	64 (61-68)	65 (59-70)	1.84 (1.6-2.2)	0.55 (0.5-0.6)
MCF	0.74 (0.70-0.76)	≤ 0.13	62 (58-67)	75 (69-80)	2.45 (2.0-3.1)	0.51 (0.4-0.6)
LAA, *cm²*	0.56 (0.52-0.59)	> 23	68 (64-72)	45 (39-51)	1.24 (1.1-1.4)	0.71 (0.6-0.8)
LVEF, *%*	0.60 (0.57-0.63)	≤ 57	67 (63-71)	51 (46-57)	1.38 (1.2-1.6)	0.64 (0.5-0.7)
MAPSE, *mm*	0.70 (0.67-0.73)	≤ 11	72 (69-76)	60 (55-65)	1.82 (1.6-2.1)	0.46 (0.4-0.5)
TAPSE, *mm*	0.70 (0.67-0.73)	≤ 19	67 (64-71)	64 (58-69)	1.86 (1.6-2.2)	0.51 (0.4-0.6)
SV, *mL*	0.66 (0.62-0.69)	≤ 42	70 (66-74)	55 (49-62)	1.57 (1.3-1.8)	0.54 (0.5-0.6)
E/A	0.80 (0.77-0.83)	> 1.1	74 (70-78)	75 (70-81)	3.01 (2.4-3.7)	0.35 (0.3-0.4)
DT, *ms*	0.57 (0.53-0.60)	≤ 210	70 (66-73)	48 (42-54)	1.34 (1.2-1.5)	0.63 (0.5-0.7)
E/e'	0.69 (0.66-0.72)	>11	70 (66-74)	61 (56-67)	1.81 (1.6-2.1)	0.49 (0.4-0.6)
LS,*%*	0.69 (0.66-0.72)	≥ -13	67 (63-71)	63 (57-68)	1.79 (1.5-2.1)	0.53 (0.5-0.6)
SAB	0.77 (0.74-0.80)	> 2.9	67 (63-71)	77 (72-81)	2.89 (2.4-3.5)	0.43 (0.4-0.5)
RALS	0.77 (0.75-0.80)	> 0.9	71 (67-74)	73 (68-78)	2.67 (2.2-3.2)	0.40 (0.3-0.5)
EFSR	0.67 (0.64-0.70)	> 4.3	62 (58-66)	65 (60-70)	1.77 (1.5-2.1)	0.59 (0.5-0.7)
IWT score	0.87 (0.85-0.90)	≥ 6	78 (75-81)	79 (74-84)	3.75 (3.0-4.7)	0.28 (0.2-0.3)

AL amyloidosis=light chain amyloidosis; Increased Wall Thickness=IVSd or PWTd ≥12mm; NT proBNP=N-terminal pro b-type natriuretic peptide; IVSd=interventricular septum in diastole; PWTd=posterior wall thickness in diastole; LVEDD=Left ventricular end diastolic volume; RWT=Relative wall thickness; LVEDV=Left ventricular end diastolic volume; LVESV=Left ventricular end systolic volume; LVMi=LV mass index; MCF=Myocardial contraction fraction; LAA=Left atrium area; LVEF=Left ventricular ejection fraction; MAPSE=Mitral annular plane systolic excursion; TAPSE=Tricuspid annular plane systolic excursion; SV=Stroke volume; E/A=E wave/A wave ratio; DT=Deceleration Time; E/e'=E wave/e'wave; LS=Longitudinal Strain; SAB=Systolic apex to base ratio; RALS=Relative apical longitudinal sparing; EFSR=Ejection fraction/LS ratio.

Table 3. Variable cut-offs and details about calculation of the AL score (upper panel) and the IWT score (lower panel).

	AL score	
	Cut off	**Points**
RWT	> 0.52	2
E/e'	> 10	2
TAPSE, *mm*	≤ 19	1
LS, *%*	≥ -14	1

	IWT score	
	Cut off	**Points**
RWT	> 0.6	3
E/e'	>11	1
TAPSE, *mm*	≤ 19	2
LS, *%*	≥ -13	1
SAB	> 2.9	3

RWT= Relative wall thickness; E/e'=E wave/e'wave; TAPSE=Tricuspid annular plane systolic excursion; LS=Longitudinal Strain; SAB=Systolic apex to base ratio

Table 4. Diagnostic accuracy of different cut-offs of AL score and IWT score for the diagnosis of CA as well as diagnostic accuracy of current diagnostic criteria for CA.

	Sensitivity, % (95% CI)	Specificity, % (95% CI)	FP	FN	TP	TN
Systemic AL amyloidosis **Current diagnostic criteria (septum)** *n=504*						
Septum>12 or NT-proBNP> 322	94 (91-96)	47 (42-53)	93 (18.5%)	16 (3.5%)	314 (62%)	81 (16%)
Septum>12	84 (79-86)	67 (63-73)	57 (11%)	53(10.5%)	279 (56%)	115 (22.5%)
Systemic AL amyloidosis **AL score(AUC 0.9) RWT, E/e', TAPSE, LS** *n=487*						
≥0	100 (99-100)	0 (0-2)	170 (35%)	0	317 (65%)	0
≥1	97 (94-98)	42 (34-50)	99 (20%)	11 (2%)	306 (63%)	71 (15%)
≥2	93 (89-95)	56 (48-64)	75 (15%)	23 (5%)	294 (60%)	95 (20%)
≥3	83.3 (79-87)	85 (78-90)	26 (5%)	53 (11%)	264 (54%)	144 (30%)
≥4	71 (66-76)	91 (85-95)	16 (3%)	92 (19%)	225 (46%)	154 (32%)
≥5	54 (48-59)	98 (95-100)	3 (0.6%)	147 (30%)	170 (35%)	167 (34.4%)
=6	39 (34-45)	99 (96-100)	2 (0.4%)	192(39%)	125 (26%)	168 (34.6%)
Increased Wall Thickness **IWT score (AUC 0.87) RWT, E/e', TAPSE, LS, SAB** *n=923*						
≥0	100 (99-100)	0 (0-1)	307 (33%)	0	616 (67%)	0
≥1	98 (97-99)	19 (15-24)	248 (27%)	11 (1%)	605 (66%)	59 (6%)
≥2	96 (94-97)	35 (30-41)	199 (21%)	27 (3%)	589 (64%)	108 (12%)
≥3	94 (92-6)	45 (39-51)	169 (18%)	35 (4%)	581 (63%)	138 (15%)
≥4	89 (87-92)	61 (55-67)	119 (13%)	66 (7%)	550 (60%)	188 (20%)
≥5	82 (79-85)	72 (67-77)	86 (9%)	109 (12%)	507 (55%)	221 (24%)
≥6	78 (75-81)	79 (74-84)	64(7%)	135(15%)	481(52%)	243(26%)
≥7	67 (63-71)	89 (85-92)	34(4%)	201 (22%)	415 (45%)	273 (29%)
≥8	46 (42-50)	98 (95-99)	7(0.8%)	333 (36%)	283(31.2%)	300 (32%)
≥9	36 (33-40)	99 (97-100)	4 (0.4%)	391 (42.7%)	213(23.1%)	303 (33.8%)
=10	25 (22-29)	99 (98-100)	2 (0.2%)	461 (50%)	155 (16.8%)	305 (33%)

AL amyloidosis=light chain amyloidosis; Increased Wall Thickness=IVSd or PWTd ≥12mm; AUC=area under curve; RWT=relative wall thickness; E/e'=E wave/e'wave; TAPSE=tricuspid annular plane systolic excursion; LS=Longitudinal Strain; SAB=Systolic apex to base ratio; FP=False Positives; TP=True Positives; FN=False Negatives; TN=True Negatives.