Unmasking the prevalence of amyloid cardiomyopathy in the real world: results from Phase 2 of the AC-TIVE study, an Italian nationwide survey

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Aim

To investigate the prevalence of amyloid cardiomyopathy (AC) and the diagnostic accuracy of echocardiographic red flags of AC among consecutive adult patients undergoing transthoracic echocardiogram for reason other than AC in 13 Italian institutions.

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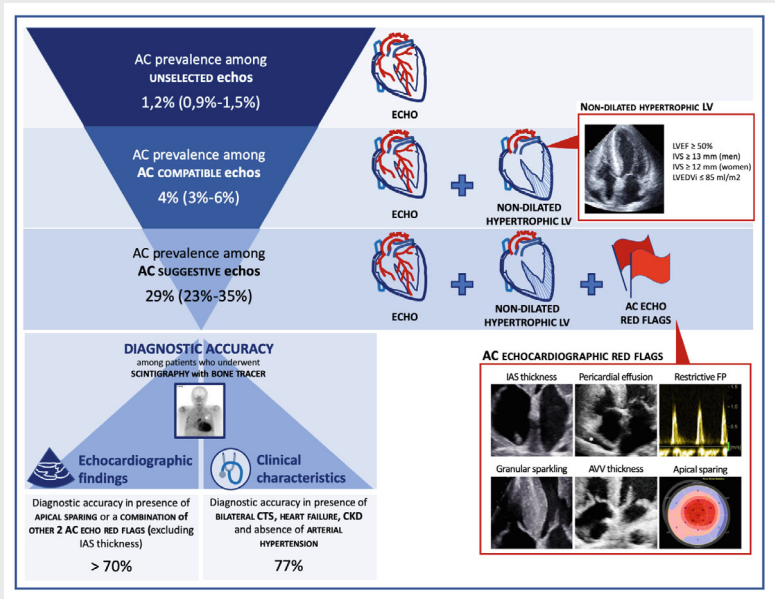
Methods and results

This is an Italian prospective multicentre study, involving a clinical and instrumental work-up to assess AC prevalence among patients ≥55 years old with an echocardiogram suggestive of AC (i.e. at least one echocardiographic red flag of AC in hypertrophic, non-dilated left ventricles with preserved ejection fraction). The study was registered at ClinicalTrials.gov (NCT04738266). Overall, 381 patients with an echocardiogram suggestive of AC were identified among a cohort of 5315 screened subjects, and 217 patients completed the investigations. A final diagnosis of AC was made in 62 patients with an estimated prevalence of 29% (95% confidence interval 23%–35%). Transthyretin-related AC (ATTR-AC) was diagnosed in 51 and light chain-related AC (AL-AC) in 11 patients. Either apical sparing or a combination of ≥2 other echocardiographic red flags, excluding interatrial septum thickness, provided a diagnostic accuracy >70%.

Conclusion

In a cohort of consecutive adults with echocardiographic findings suggestive of AC and preserved left ventricular ejection fraction, the prevalence of AC (either ATTR or AL) was 29%. Easily available echocardiographic red flags, when combined together, demonstrated good diagnostic accuracy.

Graphical Abstract



Amyloid cardiomyopathy (AC) prevalence according to selected group. CKD, chronic kidney disease; CTS, carpal tunnel syndrome; FP, filling pattern; IAS, interatrial septum; IVS, interventricular septum; LV, left ventricle; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction.

Keywords Amyloid cardiomyopathy • Epidemiology • Echocardiography • Light chain amyloidosis • Transthyretin amyloidosis • Red flags

Introduction

Amyloid cardiomyopathy (AC) has been traditionally considered a rare disease with an estimated raw prevalence of <1/100 000 individuals, mostly affecting the elderly and incidentally detected at autopsy.[1–3] In recent years, AC has become an emerging cause of heart failure (HF) and mortality worldwide, due to increased awareness of the disease, identification of clinical and instrumental red flags and cutting-edge diagnostic strategies.[1,4,5] Despite these advances, the echocardiographic identification of AC remains challenging, resulting in significant delays in diagnosis with prognostic implications.[6]

Echocardiography is the first-level imaging technique that most frequently rises the suspicion of AC, revealing peculiar signs

('red flags') of an underlying infiltrative cardiomyopathy.[7,8] Recently, some echocardiographic scores have been published, mainly focusing on highly suspected amyloidosis and employing quantitative parameters, not always easily achievable.[9,10] Thus, we conducted a national survey of prevalence and accuracy of echocardiographic red flags of AC in consecutive patients undergoing routine echocardiography (AC-TIVE study) to determine the usefulness of easily obtainable echocardiographic signs suggestive of AC (red flags) in detecting AC among an unselected population and to estimate the prevalence of the disease.

In Phase 1 of the AC-TIVE study, we showed the distribution of echocardiographic findings suggestive of AC in a cohort of consecutive unselected adults undergoing echocardiography for reasons other than AC.[11] Phase 2 of the AC-TIVE study was designed to actively assess the prevalence of AC among patients with echocardiographic signs suggestive of AC and to fill the knowledge gap in the clinical accuracy and utility of the selected echocardiographic red flags.

Methods

This is a national, multicentre, prospective cohort study performed in 13 tertiary centres across Italy (online supplementary *Figure S1*). Trieste University Hospital acted as coordinating centre of the study. The local Regional Institutional Review Board approved the study (identifier 199_2019), and the participating centres obtained local institutional review board approvals for the collection of prospective anonymous data. The study was conducted according to the Declaration of Helsinki and was registered at ClinicalTrials.gov (NCT04738266).

Study design

The study design included two phases: (1) recording phase (Phase 1) consisting in a screening for echocardiographic red flags among unselected consecutive patients ≥55 years over a 2-week period (excluding patients referred for known or suspected AC), and (2) diagnostic phase (Phase 2) involving a diagnostic work-up for AC to estimate disease prevalence among patients with ≥1 echocardiographic red flag. The study was held between November 2019 and July 2021. Conduction and duration of the study was affected by the intercurrent COVID-19 pandemic.

Phase 1

The initial, recording phase has been recently described.[11] In summary, among all the screened echocardiograms, we selected those reporting hypertrophic (interventricular septum [IVS] thickness ≥12 mm in women and ≥13 mm in men), non-dilated (indexed left ventricular [LV] end-diastolic volume ≤85 ml/m^2) left ventricles with preserved ejection fraction (≥50%). Those echocardiograms were defined as 'AC compatible'. Patients with an 'AC compatible' echocardiogram were screened for echocardiographic red flags suggestive of AC, which were: restrictive filling pattern (RFP), granular sparkling, pericardial effusion, interatrial septum (IAS) thickness >5 mm, atrio-ventricular valve (AVV) thickness >5 mm, and LV apical sparing pattern at two-dimensional speckle-tracking echocardiography. 'AC compatible' echocardiograms plus ≥1 of the aforementioned red flags were defined as 'AC suggestive' echocardiograms. Patients with known or suspected AC or hypertrophic cardiomyopathy (HCM) were excluded. Suspected AC was defined as any patient referred for echocardiographic evaluation, due to the suspicion of underlying cardiac amyloidosis by the referring physician.

Phase 2

Patients with 'AC suggestive' echocardiograms were eligible for Phase 2 of the AC-TIVE study and were contacted to schedule a cardiological evaluation. Those who consented, underwent clinical evaluation, blood and urine tests and scintigraphy with bone tracer. Diagnosis of transthyretin-related AC (ATTR-AC) was made in presence of grade 2 or 3 myocardial uptake of radiotracer on cardiac scintigraphy and absence of clonal dyscrasia with blood and urine tests, according to Gillmore's algorithm[12] and the latest European Society of Cardiology position statement.[2] In the presence of grade 1 myocardial uptake of radiotracer, cardiac magnetic resonance (CMR) and histological analysis were performed to rule out or confirm the diagnosis of AC, and, eventually, to determine the subtype.[2] All patients with light chain-related AC (AL-AC) were histologically proven.

Data collection and diagnostic investigation

Baseline clinical characteristics, laboratory tests and echocardiographic parameters of enrolled patients were collected at each participating centre. History of hypertension was defined as the need for at least one anti-hypertensive drug either in the past or in the present due to blood pressure values >140/90 mmHg. Pseudonecrosis was defined as the presence of pathological Q waves on the electrocardiogram (ECG) in the absence of an ischaemic heart disease. Echocardiographic quantitative parameters were measured based on current international recommendations.[13,14] Blood and urine tests were systematically performed to exclude the presence of monoclonal protein and included immunofixation electrophoresis of serum and urine and serum free light chain assay. Troponin and natriuretic peptides were also routinely assessed. Cardiac scintigraphy with bone tracers (namely ^{99m}Tc-pyrophosphate, ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid, and ^{99m}Tchydroxymethylene diphosphonate) was performed according to acquisition protocols adopted in each participating centre and the current guidelines.[2] Single photon emission computed tomography was available in all centres and it was used to better characterize myocardial uptake and exclude blood pool, according to local protocols. CMR and biopsy were performed in selected cases following local clinical practice.

Statistical analysis

Considering an estimated AC prevalence of approximately 8%–10% reported in the available literature,[4] the required sample size to estimate the frequency of disease with a 95% confidence interval (CI) and an accuracy of ±4% was of at least 216 patients.

Continuous variables are expressed as median with interquartile range (IQR, 25th–75th), according to the distribution shape; categorical variables are expressed as absolute numbers and percentages. Differences between groups were evaluated using Mann–Whitney U test for continuous variables and the Chi-square or Fisher's exact test for dichotomous variables, as appropriate. Prevalence was calculated as the proportion of the study population carrying

the disease ± standard error. Binary logistic regression analysis was performed to assess the predictive clinical parameters associated with the development of AC. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy were calculated using standard techniques. A *p*-value <0.05 was considered statistically significant.

All statistical analyses were performed using IBM SPSS Statistics 24.0 package (New York, NY, USA) statistical software version 20.

Results

Study population

Among a total of 5315 consecutive unselected echocardiograms screened in Phase 1, 1169 exams (22%) were classified as 'AC compatible' (i.e. hypertrophic and non-dilated left ventricle with preserved ejection fraction) and 381 exams (7%) were classified as 'AC suggestive' (i.e. 'AC compatible' plus ≥1 echocardiographic red flag of AC) (*Figure 1*, upper panel). The final population of the study consisted of 217 patients who entered and completed Phase 2 (57% of the 'AC suggestive' echocardiograms). The main reasons why 164 (43%) patients did not enter Phase 2 were refusal to participate (n = 66; 40%) and death (n = 49; 30%) (online supplementary *Figure S2*). Dropout was greater among the elderly (49% of patients aged >75 years vs. 34% of patients aged ≤75 years, p = 0.003).

Clinical characteristics and echocardiographic parameters, including red flag distribution, were widely comparable between patients who completed Phase 2 and those who did not, excluding few items such as sex, history of bilateral carpal tunnel syndrome (CTS), presence of monoclonal protein and number of red flags (online supplementary *Table S1*). However, pre-test probability of AC according to clinical and echocardiographic features was similar among the two cohorts.

Amyloid cardiomyopathy prevalence

Among the 217 patients who completed Phase 2, 62 received a final diagnosis of AC with an estimated prevalence of 28.6% (95% CI 22.5%–34.7%) (*Figure 1*, middle panel). ATTR-AC was diagnosed in 51 and AL-AC in 11 patients, with a prevalence of 23.5% (95% CI 17.8%–29.2%) and 5.1% (95% CI 2.2%–8.0%), respectively. Among the 1169 'AC compatible' echocardiograms, the prevalence of AC was estimated to be at least 4.4% (95% CI 3.2%–5.5%). Considering the whole screened population (i.e. 5315 unselected echocardiograms), the prevalence was at least 1.2% (95% CI 0.9%–1.5%) and, specifically, 1% (95% CI 0.7%–1.2%) and 0.2% (95% CI 0.1%–0.3%) for ATTR-AC and AL-AC, respectively (*Figure 1*, bottom panel).

ATTR-AC diagnosis was mostly achieved through a grade 2 (n = 15) or 3 (n = 34) myocardial uptake at bone scintigraphy (n = 49, 96%) and the absence of monoclonal protein. Only two cases of ATTR-AC had grade 1 myocardial uptake and required endomyocardial biopsy to reach the final diagnosis. Conversely, none among AL-AC patients (n = 0, 0%) showed grade 2 or 3 myocardial uptake at bone scintigraphy and they all required histological confirmation of the diagnosis (*Figure 1*, bottom panel).

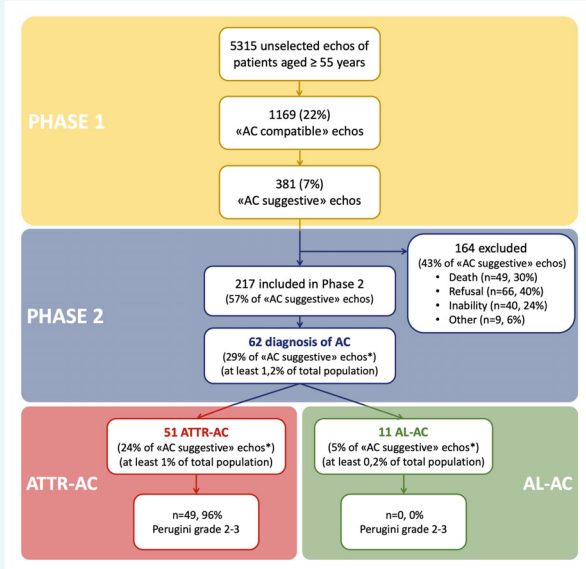


Figure 1 Flow-chart showing amyloid cardiomyopathy (AC) prevalence. Summary of Phase 1 (upper panel) and Phase 2 (middle and bottom panels) of the AC-TIVE study. 'AC compatible' echos, echocardiograms with (i) interventricular septum thickness ≥12 mm in women and ≥13 mm in men, (ii) left ventricular ejection fraction ≥50%, and (iii) indexed left ventricular end-diastolic volume ≤85 ml/m^2; 'AC suggestive' echos, 'CA compatible' echocardiograms plus at least one of the following echocardiographic red flags: (i) restrictive filling pattern (E-wave deceleration time <120 ms or ≤150 ms in presence of E/A ≥2) and/or increased left ventricular diastolic filling pressures (E/E′ ≥15), (ii) granular sparkling appearance of the myocardium, (iii) pericardial effusion of any entity, (iv) interatrial septum thickness >5 mm; (v) atrio-ventricular valve thickness >5 mm, and (vi) left ventricular apical sparing pattern at speckle tracking echocardiography. *The percentage is calculated among the 217 patients who completed Phase 2; however, the substantial comparability of basal characteristics and red flag distribution allowed to generalize the results to the entire cohort of patients with 'AC suggestive' echocardiograms. AL, light chain amyloid; ATTR, transthyretin amyloid.

Among the 26 available genetic tests, only 1 (3.8%) case of variant ATTR-AC (p.Pro31Ala) was identified.

ATTR-AC diagnosis increased with age from 12% (n = 8) in patients <70 years of age to 33% (n = 25) in patients ≥80 years (p = 0.016). Conversely, AL-AC prevalence was not clearly affected by age across the whole population (p = 0.076), even though most of the AL-AC (10 out of 11) were diagnosed in patients aged <80 years (*Figure 2*).

Amyloid cardiomyopathy patient characteristics

In the study population (217 patients who completed Phase 2), the most common clinical indications for echocardiography were HF (n = 53, 24%) and valvular heart disease (n = 48, 22%). Compared

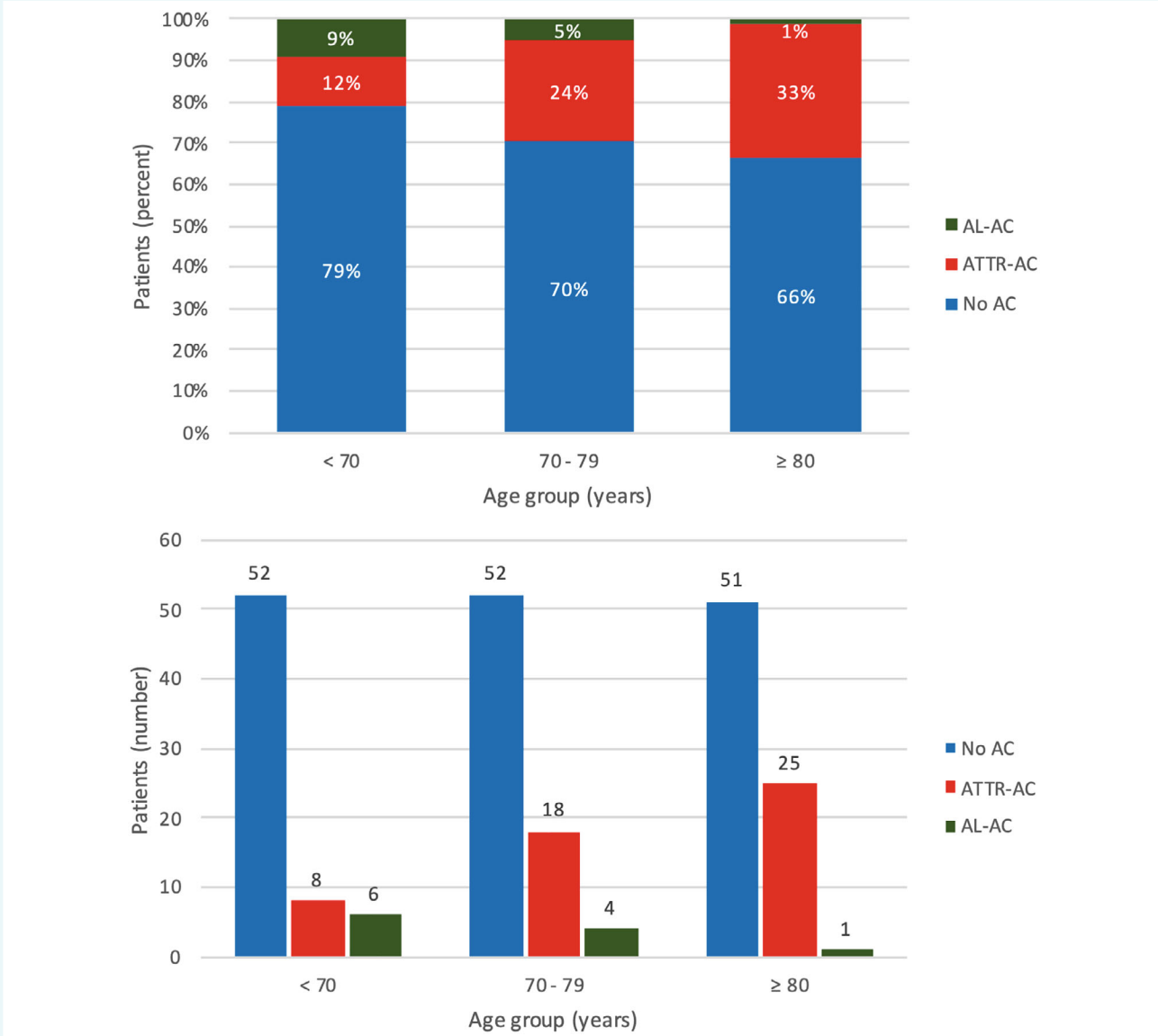


Figure 2 Amyloid cardiomyopathy (AC) prevalence, either transthyretin-related AC (ATTR) or light chain-related AC (AL), according to age (upper panel expressed in percentage, lower panel expressed in absolute number) in the study population.

to non-AC patients, AC patients were 2.0 and 3.6 times more frequently referred to echocardiography for HF (n = 24, 39%) and unexplained LV hypertrophy (n = 11, 18%). Valvular heart disease (valvular prosthesis n = 4, 7% and native valvular disease n = 7, 11%) was the third most frequent clinical indication for echocardiography in AC patients (online supplementary *Figure S3*).

Table 1 shows baseline characteristics of the study population, according to the final presence of AC. Compared to non-AC patients, AC patients (either ATTR or AL) more frequently were non-hypertensive and suffered from bilateral CTS, had higher N-terminal pro brain natriuretic peptide (NT-proBNP) and troponin levels and thicker LV walls ($p < 0.001$ for all). A trend toward higher prevalence of male patients was detected among AC patients versus non-AC patients (p = 0.063). At ECG, AC patients more frequently had low QRS voltage ($p < 0.001$) and pseudonecrosis (p = 0.011). Notably, AL-AC patients were the youngest (67 years, IQR 62–78), had the highest NT-proBNP values (2627 ng/L, IQR 467–4420) and the thickest LV (17 mm, IQR 13–19) and right ventricular (9 mm, IQR 7–12) walls. Conversely, ATTR-AC patients were the oldest (79 years, IQR 72–84), had a history of bilateral CTS in almost half cases (49%) and were more frequently affected by atrial fibrillation (43%).

Univariate and multivariable regression analyses for the final diagnosis of AC and for ATTR-AC, including clinical variables at the time of echocardiographic evaluation, are displayed in *Table 2*. History of arterial hypertension portended a lower likelihood of AC, while history of bilateral CTS and HF conferred higher

Table 1 **Baseline characteristics, laboratory exams and echocardiographic findings of patients who received the final diagnosis of amyloid cardiomyopathy, either transthyretin-related or light chain-related, and those in whom the diagnosis of amyloid cardiomyopathy was excluded**

	Available data	ATTR-AC (*n* = 51)	AL-AC (*n* = 11)	Non-AC (*n* = 155)	*p*-value	*p*-value[a]
Clinical features						
Age (years)	217	79 (72–84)	67 (62–78)	74 (66–81)	**0.008**[b]	**0.010**[b]
Male sex	217	32 (63%)	9 (82%)	81 (52%)	0.091[b]	0.305[b]
Arterial hypertension (mmHg)	217	22 (43%)	5 (46%)	117 (76%)	**<0.001**[b]	1.000[b]
Pacemaker	217	14 (28%)	3 (27%)	29 (19%)	0.321[b]	1.000[b]
Bilateral CTS	216	25 (49%)	2 (20%)	26 (17%)	**<0.001**[b]	0.162[b]
Syncope	169	3 (10%)	0 (0%)	14 (11%)	0.808[b]	0.564[b]
Peripheral neuropathy	168	10 (32%)	1 (10%)	26 (20%)	0.285[b]	0.238[b]
CKD	168	13 (43%)	6 (60%)	39 (31%)	0.085[b]	0.473[b]
Heart failure	217	18 (35%)	6 (55%)	29 (19%)	0.003[b]	0.311[b]
Electrocardiogram						
AF/AFl	217	22 (43%)	3 (27%)	55 (36%)	0.546[b]	0.501[b]
NSVT and SVT	168	7 (23%)	0 (0%)	17 (13%)	0.206[b]	0.174[b]
Low-voltage ECG	168	12 (39%)	3 (30%)	11 (9%)	**<0.001**[b]	0.720[b]
Pseudonecrosis	165	6 (21%)	2 (22%)	7 (6%)	**0.011**[b]	1.000[b]
Laboratory tests						
Haemoglobin (g/dl)	162	13 (11–14)	13 (11–15)	13 (12–14)	0.869[b]	0.756[b]
Creatinine (mg/dl)	162	1.00 (1.00–2.00)	1.00 (1.00–1.25)	1.00 (1.00–1.00)	0.145[b]	0.693[b]
hs-Troponin I (ng/L)	109	58 (21–94)	53 (21–65)	12 (7–27)	**<0.001**[b]	0.555[b]
NT-proBNP (pg/L)	173	1588 (887–3201)	2627 (467–4420)	457 (199–907)	**<0.001**[b]	0.511[b]
Proteinuria	132	2 (9%)	6 (75%)	22 (22%)	**0.001**[b]	**0.001**[b]
Monoclonal protein	217	6 (12%)	11 (100%)	23 (16%)	**<0.001**[b]	**<0.001**[b]
Echocardiographic findings						
LV wall thickness (mm)	217	16 (15–18)	17 (13–19)	14 (13–15)	**<0.001**[b]	0.794[b]
LVEF (%)	217	55 (50–56)	60 (50–65)	57 (54–63)	**0.001**[b]	0.398[b]
LVEDVi (ml/m^2)	214	48 (44–57)	50 (47–56)	52 (45–60)	0.081[b]	0.449[b]
E/E′	197	16 (13–17)	14 (11–23)	13 (10–17)	**0.021**[b]	0.516[b]
LA diameter (mm)	206	45 (41–47)	43 (38–47)	43 (39–47)	0.216[b]	0.211[b]
RV wall thickness (mm)	158	5 (4–7)	9 (7–12)	5 (4–7)	**0.005**[b]	**0.002**[b]
RV FAC (%)	172	38 (33–44)	40 (37–44)	40 (38–47)	**0.044**[b]	0.598[b]
RV dysfunction	214	13 (26%)	0 (0%)	15 (10%)	**0.013**[b]	0.101[b]
Moderate-severe valve disease	217	30 (59%)	3 (27%)	77 (50%)	0.159[b]	0.094[b]
Systolic PAP (mmHg)	198	35 (30–44)	33 (25–55)	32 (28–40)	0.202[b]	0.769[b]
Red flags						
1 RF	217	15 (29%)	3 (27%)	87 (56%)	**0.002**[b]	1.000[b]
2 RF	217	13 (26%)	4 (36%)	45 (29%)	0.712[b]	0.475[b]
≥3 RF	217	23 (45%)	4 (36%)	23 (15%)	**<0.001**[b]	0.742[b]

AC, amyloid cardiomyopathy; AF, atrial fibrillation; AFl, atrial flutter; AL, light chain amyloid; ATTR, transthyretin amyloid; CKD, chronic kidney disease; CTS, carpal tunnel syndrome; ECG, electrocardiogram; FAC, fractional area change; hs, high-sensitivity; LA, left atrial; LV, left ventricular; LVEDVi, left ventricular end-diastolic volume index; LVEF, left ventricular ejection fraction; NSVT, non-sustained ventricular tachycardia; NT-proBNP, N-terminal pro brain natriuretic peptide; PAP, pulmonary artery pressure; RF, red flag; RV, right ventricular; SVT, sustained ventricular tachycardia.

[a]*p*-value for ATTR-AC versus AL-AC patients.

[b]Fisher's exact test.

probability of receiving a diagnosis of AC (*Table 2*). The model achieved an AUC of 0.77 (*Figure 3*, left panel). Clinical variables independently associated with a final diagnosis of ATTR-AC were older age, absence of arterial hypertension and history of bilateral CTS (*Table 2*). A predictive model performed with these variables had an AUC of 0.76 for ATTR-AC diagnosis (*Figure 3*, right panel).

Distribution and diagnostic accuracy of echocardiographic red flags of amyloid cardiomyopathy

As displayed in *Table 1*, non-AC patients more frequently reported a single red flag ($p = 0.002$), while AC patients more frequently showed three or more red flags ($p < 0.001$). Among patients with

Table 2 **Univariate and multivariable logistic regression analyses for amyloid cardiomyopathy and transthyretin-related amyloid cardiomyopathy diagnosis, considering clinical (non-echocardiographic) variables**

Clinical variables	AC			Multivariable *p*-value	ATTR-AC			Multivariable *p*-value
	Odds ratio	95% CI	Univariate *p*-value		Odds ratio	95% CI	Univariate *p*-value	
Age	1.027	0.994–1.061	0.111		1.051	1.013–1.090	**0.008**	**0.006**
Male sex	1.784	0.966–3.293	**0.064**	0.299	1.422	0.747–2.710	0.284	
Arterial hypertension	0.251	0.135–0.466	**<0.001**	**0.032**	0.274	0.142–0.525	**<0.001**	**0.001**
Pacemaker	1.641	0.824–3.268	0.158		1.584	0.767–3.274	0.214	
Bilateral CTS	3.827	1.987–7.371	**<0.001**	**<0.001**	**4.705**	2.376–9.315	**<0.001**	**0.001**
Syncope	0.643	0.175–2.359	0.505		0.949	0.255–3.526	0.938	
Peripheral neuropathy	1.424	0.631–3.216	0.395		1.949	0.819–4.597	0.132	
CKD	2.065	0.999–4.266	**0.050**	0.067	1.580	0.707–3.535	0.265	
AF/AFl	1.229	0.671–2.249	0.505		1.413	0.745–2.677	0.290	
Heart failure	2.744	1.431–5.263	**0.002**	**0.004**	2.042	1.029–4.049	**0.041**	0.208

AC, amyloid cardiomyopathy; AF, atrial fibrillation; AFl, atrial flutter; ATTR, transthyretin amyloid; CI, confidence interval; CKD, chronic kidney disease; CTS, carpal tunnel syndrome.

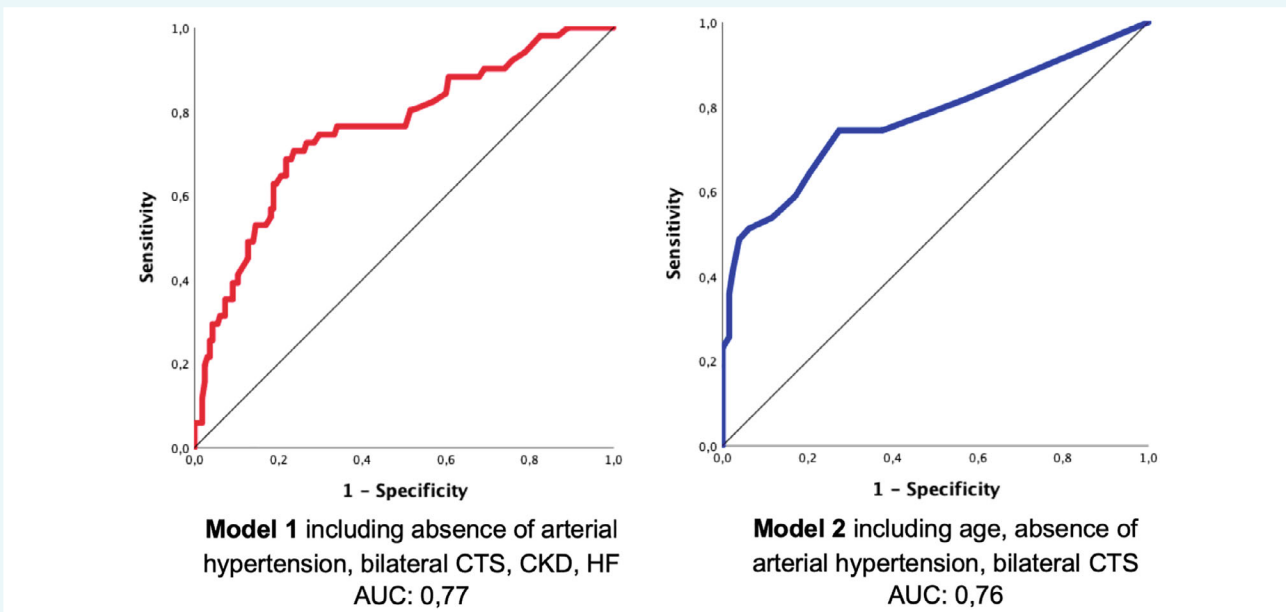


Figure 3 Diagnostic accuracy of clinical models for amyloid cardiomyopathy (*left panel*) and transthyretin-related amyloid cardiomyopathy (*right panel*) measure with receiver operating characteristic curve. AUC, area under the curve; CKD, chronic kidney disease; CTS, carpal tunnel syndrome; HF, heart failure.

a single red flag, similar distribution of each red flag was demonstrated between AC and non-AC patients, except for apical sparing which was more frequently reported in AC patients ($p = 0.048$).

Online supplementary *Figure S4* displays the distribution of echocardiographic red flags of AC in the study population, according to AC diagnosis. RFP ($n = 33$, 53%), apical sparing ($n = 28$, 48%), and granular sparkling ($n = 30$, 48%) were twice as prevalent in AC than non-AC patients. IAS thickness >5 mm was the most diffuse red flag among non-AC patients ($n = 73$, 47%) and the less frequent red flag among AC patients ($n = 25$, 40%), together with AVV thickness >5 mm ($n = 25$, 40%). No significant differences were reported between older (>75 years) and younger (≤75 years) patients, even though IAS thickness >5 mm was slightly prevalent among younger people.

Apical sparing showed the highest PPV (50%, 95% CI 40%–61%) and accuracy (71%, 95% IC 65%–77%) for detecting AC among the six investigated red flags, while IAS thickness >5 mm appeared to be the less useful red flag for diagnosis (PPV 26%, 95% CI 20%–33%; accuracy 50%, 95% CI, 43%–56%) (*Table 3*). Excluding apical sparing and IAS thickness >5 mm, any combination of two among the other

Table 3 Diagnostic performance of the different red flags

Statistic	IAS thickness >5 mm		Apical sparing		RFP + AVV thickness >5 mm + granular sparkling	
	Value	95% CI	Value	95% CI	Value	95% CI
Sensitivity	40%	28%–54%	48%	34%–61%	18%	9%–30%
Specificity	53%	45%–61%	81%	74%–87%	98%	95%–100%
PLR	0.86	0.61–1.22	2.47	1.61–3.80	9.17	2.65–31.75
NLR	1.12	0.87–1.44	0.65	0.50–0.84	0.84	0.75–0.94
PPV	26%	20%–33%	50%	40%–61%	78.57%	51%–93%
NPV	69%	63%–74%	79%	75%–83%	74.88%	73%–77%
Accuracy	50%	43%–56%	71%	65%–77%	75.12%	69%–81%

AVV, atrio-ventricular valve; CI, confidence interval; IAS, interatrial septum; NLR, negative likelihood ratio; NPV, negative predictive value; PLR, positive likelihood ratio; PPV, positive predictive value; RFP, restrictive filling pattern.

red flags provided an accuracy >70% (online supplementary *Table S2*). The highest PPV (79%, 95% CI 51%–93%) and accuracy (75%, 95% CI 69%–81%), together with the highest specificity (98%, 95% CI 95%–100%), were achieved through the combination of RFP, AVV thickness >5 mm and granular sparkling. However, this combination was penalized by a poor sensitivity (18%, 95% CI 9%–30%) (*Table 3*).

Discussion

To the best of our knowledge, this is the first study to determine the prevalence of AC in a consecutive contemporary national cohort of unselected patients aged ≥55 years undergoing echocardiography.

The main findings of the AC-TIVE study (Phase 2) are: (i) at least 1.2% of unselected patients ≥55 years undergoing echocardiography for reasons other than known or suspected AC and near 29% of subjects with non-dilated, hypertrophic hearts with LV ejection fraction (LVEF) ≥50% and ≥1 standard echocardiographic red flag for AC, eventually received a diagnosis of AC, and (ii) apical sparing or a combination of at least two red flags, excluding IAS, provided a diagnostic accuracy >70%, guiding decision-making in proceeding with further tests for AC (*Graphical Abstract*). These data deserve attention as 62 patients received a diagnosis of AC, potentially being candidates to specific disease-modifying therapies without diagnostic delays.

Amyloid cardiomyopathy epidemiology

While the current estimate of AL-AC prevalence is 8 cases per million person-year,[15] the prevalence of ATTR-AC is still unknown.[16] In recent years, ATTR-AC has been found to be more frequent in screening studies of high-risk populations such as patients with unexplained LV hypertrophy at the time of CTS (10%),[17–19] patients hospitalized for HF with preserved LVEF (13%),[19,20] patients with an initial diagnosis of HCM (9%),[19,21] and patients with aortic stenosis referred for transcatheter aortic valve implantation (16%).[19,22] In our study, the prevalence of ATTR-AC ranged from 3.2% to 5.5% among ≥55-year-old subjects with a hypertrophic non-dilated heart with preserved LVEF and from 17.8% to 29.2% in presence of ≥1 echocardiographic red flag of AC. In the whole population of unselected patients ≥55 years undergoing clinically-indicated echocardiography (excluding those referred for infiltrative disease and HCM), we documented a prevalence of at least 1.2% for AC and 1% for ATTR-AC. These data represent a novelty in AC epidemiology, suggesting a significantly higher disease prevalence than previously demonstrated.[23]

Surprisingly, in our study, AL-AC frequency was higher than expected (11 subjects, approximately 5% of the 'AC suggestive' echocardiograms), accounting for 18% of the total AC diagnoses. The 0.2% prevalence of AL-AC found in the general unselected population ≥55 years might be overestimated due to an increased sensitivity for AC among haematologists working in tertiary referral centres. However, this result deserves attention in light of the potential survival benefit from latest treatment strategies in early diagnosis.[24–26]

As expected,[27,28] among patients with 'AC suggestive' echocardiograms, the prevalence of ATTR-AC increased with age, up to 33% in patients ≥80 years, approaching that found in autoptic studies.[3,27,28] However, both ATTR-AC and AL-AC were significantly represented also among younger subjects, affecting 21% of ≤70-year-old individuals, suggesting the opportunity of systematically looking for echocardiographic AC red flags, mostly in high-risk clinical scenarios, also in patients <80 years. Male patients were more frequently affected compared to female patients, even though no statistical difference was detected. Accordingly, a significant proportion of AC patients, higher than expected, were women. Although AC was traditionally considered to affect mainly men,[29] underdiagnosis of wild-type ATTR-AC in women has recently been proposed.[30] The unselected nature of the cohort of patients of our study, not holding a diagnosis or a suspicion of AC, could account for male prevalence's attenuation.

The role of echocardiography laboratories

Patients who finally received a diagnosis of AC were referred for echocardiography mostly for HF, unexplained LV hypertrophy and

valvular heart disease. The detection of ≥1 standard echocardiographic red flag of AC in those scenarios should definitely prompt further evaluation. Furthermore, other clinical features emerged as a possible guide for the physician in screening patients for AC: advanced age, absence of arterial hypertension and a history of bilateral CTS in patients with hypertrophic hearts conferred the highest risk of ATTR-AC (*Table 2* and *Figure 3*). Importantly, obtaining this clinical information at the time of echocardiographic evaluation is easy and not time consuming. Of note, further studies are required for a deeper clinical characterization, AC-oriented, in the diagnostic work-up of AC.

In the present study, a large proportion of the reviewed echocardiograms, i.e. about 7% of the exams performed in subjects aged ≥55 years without suspicion of infiltrative diseases and 33% of those with non-dilated, hypertrophic hearts with preserved LVEF, showed 'AC suggestive' findings.[11] In those patients, we demonstrated that standard echocardiographic red flags emerged as accurate tools in screening patients to raise the suspicion of AC and in orienting specific diagnostic work-up for AC.[2] As expected, the apical sparing pattern at two-dimensional speckle tracking echocardiography examination of LV myocardial deformation was the most accurate single red flag for AC detection. However, in absence of this parameter, mostly for technical and time-consuming issues, the combination of two other red flags (excluding IAS thickness), usually accomplished as part of a standard echocardiogram and not affected by heart rhythm or poor image quality, could provide comparable accuracy (above 70%). Of note, the presence of a single red flag was more frequently reported among non-AC patients, while three or more red flags were clearly prevalent among AC patients (*Table 1*), confirming close attention to patients with non-dilated hypertrophic heart and more than one red flag suggestive of AC.

In light of our results, in patients ≥55 years, mainly with hypertrophic non-dilated hearts with preserved LVEF, it appears reasonable to propose a systematic echocardiographic red flags approach, integrated with information on clinical indications to the exams and looking for high-risk clinical scenarios. Through the systematic screening of myocardial infiltration, this approach would reasonably enhance the opportunity to diagnose AC patients earlier than commonly performed with actual symptom-driven approach, as to benefit as soon as possible from novel disease-modifying therapies for both ATTR-AC (i.e. tafamidis[26]) and AL-AC (i.e. bortezomib and daratumumab[24,25]). Finally, the echocardiographic laboratory should serve as a collector of AC coming from different medical areas (i.e. haematology, surgery, internal medicine), increasing the pool of screened patients beyond those, usually already symptomatic, referred to the cardiologist.

Disease awareness

The main reason for the 164 non-entering patients into Phase 2 of the AC-TIVE study was refusal to participate (40.2%), mainly among patients ≤75 years. This might be exacerbated by the restrictions and the situation due to the contemporary COVID-19 pandemic. Nevertheless, it suggests that people are poorly informed about AC and they do not fear the disease despite the dismal prognosis. Concurrently, the knowledge of the disease is still a prerogative of few specialists, limiting the spread of awareness about AC.

The prevalence of AC found in this survey and the availability of disease-modifying therapies advocates the promotion of educational programmes and networks to increase public and physicians' awareness of the disease and, thus, the likelihood of an earlier diagnosis. An earlier diagnosis of ATTR-AC and, mostly, AL-AC are pivotal for increasing the survival of those conditions.

Limitations

Some limitations should be considered: first, the absence of a core lab, due to the survey nature of the study; second, the inclusion of only third level centres with potential referral bias. For the purposes of the study, we specifically selected standard (except for apical sparing) AC red flags, easily obtainable in all echocardiography laboratories. However, future studies are needed for including first and second level centres and comparing diagnostic accuracy of standard and more specific echocardiographic AC red flags. Apical sparing assessment was limited by technical issues (mainly in case of low-quality images) and it could not be assessed in the whole population, though it was available in most cases (348 out of 381 patients, equal to 91%). AC prevalence among patients with 'AC suggestive' echocardiograms was penalized by high dropout rate. However, the size of the study population and the similar clinical risk profile of AC between the study population and the excluded patients allow us to speculate that AC prevalence was not dissimilar between the two groups. Genetic testing was not available for the whole population even though habitually performed in clinical practice, as genotyping was beyond the scope of the study. Finally, clinical profiling of AC patients needs future external validation.

Conclusions

In a national cohort of consecutive patients ≥55 years with echocardiographic findings suggestive of AC and preserved LVEF, the prevalence of AC ranged from 23% up to 35%. Although ATTR-AC was predominant, AL-AC was diagnosed in a significant number of cases. The combination of standard echocardiographic red flags could accurately orient second level diagnostic work-up for AC.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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