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RESEARCH ARTICLE

Redefining the epidemiology of cardiac amyloidosis. A systematic review and meta-analysis of screening studies

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Aims An algorithm for non-invasive diagnosis of amyloid transthyretin cardiac amyloidosis (ATTR-CA) and novel disease-modifying therapies have prompted an active search for CA. We examined the prevalence of CA in different settings based on literature data.

Methods and results We performed a systematic search for screening studies on CA, focusing on the prevalence, sex and age distribution in different clinical settings. The prevalence of CA in different settings was as follows: bone scintigraphy for non-cardiac reasons (n = 5 studies), 1% (95% confidence interval [CI] 0%–1%); heart failure with preserved ejection fraction (n = 6), 12% (95% CI 6%–20%); heart failure with reduced or mildly reduced ejection fraction (n = 2), 10% (95% CI 6%–15%); conduction disorders warranting pacemaker implantation (n = 1), 2% (95% CI 0%–4%); surgery for carpal tunnel syndrome (n = 3), 7% (95% CI 5%–10%); hypertrophic cardiomyopathy phenotype (n = 2), 7% (95% CI 5%–9%); severe aortic stenosis (n = 7), 8% (95% CI 5%–13%); autopsy series of 'unselected' elderly individuals (n = 4), 21% (95% CI 7%–39%). The average age of CA patients in the different settings ranged from 74 to 90 years, and the percentage of men from 50% to 100%. Many patients had ATTR-CA, but the average percentage of patients with amyloid light-chain (AL) CA was up to 18%.

Conclusions Searching for CA in specific settings allows to identify a relatively high number of cases who may be eligible for treatment if the diagnosis is unequivocal. ATTR-CA accounts for many cases of CA across the different settings, but AL-CA is not infrequent. Median age at diagnosis falls in the eighth or ninth decades, and many patients diagnosed with CA are women.

Keywords Cardiac amyloidosis • Epidemiology • Screening • Diagnosis • Red flags • Heart failure • Hypertrophy • Carpal tunnel syndrome • Scintigraphy • Autopsy

Introduction

Our knowledge of the epidemiology of cardiac amyloidosis (CA) relies mostly on real-world studies using in- or outpatient claims data,[1,2] or registries of diagnosed patients.[3,4] These data have led to classify CA as a rare disorder, namely as a condition affecting fewer than 5 people in 10 000.[5] Over the last years, an algorithm for non-invasive diagnosis of amyloid transthyretin (ATTR) CA and novel disease-modifying therapies have prompted an active search for CA in different clinical settings.

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The diagnostic flowchart can be schematically articulated into three steps: suspicion, definite diagnosis of CA, and identification of the CA subtype.[6–9] The main diagnostic steps are the search for a monoclonal protein, bone scintigraphy with diphosphonate or pyrophosphate tracers, and possibly further histological exams. Several findings may prompt a diagnostic workup for CA. Some of these 'red flags' consist of clinical evidence of extra-cardiac disease (with frequent involvement of tendons, peripheral nerves and kidneys), low QRS voltages despite increased left ventricular (LV) wall thickness on echocardiogram, preserved apical strain despite depressed basal strain on echocardiogram, or Q waves on electrocardiogram (ECG) without evidence of a previous infarction.[10] The identification of these red flags or conditions with a strong epidemiological link with CA, such as aortic stenosis (AS) or heart failure with preserved ejection fraction (HFpEF), may prompt a screening for CA. Several screening studies are reshaping our understanding of CA epidemiology, but their results have never been systematically assessed.

In this systematic review we investigated the prevalence of CA in studies where CA was searched in subjects with one or more 'red flags' for CA[10] (such as unexplained LV thickening and/or carpal tunnel syndrome [CTS]), or in unselected individuals (such as those undergoing bone scintigraphy or in autopsy series).

Methods

We performed a systematic review of published studies searching the PubMed/Medline and EMBASE databases on 21 January 2022 with the following keywords: ('prevalence' OR 'frequency' OR 'epidemiology' OR 'screening') AND ('cardiac amyloidosis' OR 'amyloidosis' OR 'transthyretin' OR 'ATTR' OR 'light-chain' OR 'AL') (field: title; filter: English language). Reference lists of relevant studies were also screened. We included papers meeting all the following criteria: (i) full-length papers or research letters, and (ii) studies where CA (defined as amyloidosis involving at least the left ventricle) was searched. We excluded (i) abstracts, preprint articles or preclinical studies, (ii) studies on isolated valve or atrial amyloidosis, and (iii) studies where the prevalence of CA was estimated based on administrative or healthcare data, without an active search. Two authors (A.A. and G.V.) independently screened the studies for possible inclusion. The process of study selection is reported in online supplementary *Figure S1*. The same authors (A.A. and G.V.) retrieved the following data from each study: inclusion and exclusion criteria, number of subjects enrolled, number of cases of CA (also considering separately ATTR- or AL-CA or other aetiologies), number of men versus women with CA, and age of CA patients. Controversies about study selection or data retrieval were solved through discussion between the two authors (A.A. and G.V.) and with a third author (C.R.).

The studies were classified into the following settings: total body scintigraphy with 'bone tracers' for non-cardiac reasons, HFpEF, heart failure with reduced or mildly reduced ejection fraction (HFrEF/HFmrEF), conduction disorders warranting pacemaker implantation, surgery for CTS or idiopathic trigger finger, hypertrophic cardiomyopathy (HCM) phenotype, AS undergoing surgical or transcatheter valve replacement (SAVR/TAVR), and autopsy series of unselected elderly individuals. For each setting, the prevalence of CA (average and range across studies), mean/median age of CA patients (average and range), and the percentage of men versus women with CA (average and range) were calculated. For settings including more than three studies, a meta-analytic assessment was performed. In particular, we synthesized eligible studies and derived the pooled estimate for the prevalence of CA within each setting. The inverse hyperbolic tangent was used for the r-to-Z transformation of the proportion of patients with CA in the pooled analysis. The confidence intervals (CI) of individual studies were calculated using the exact binomial (Clopper–Pearson) method.[11] The mean prevalence and CI of individual studies were illustrated with forest plots. Heterogeneity was quantified using the I^2 measure.[12] Statistical analysis was performed with STATA v12.1 (StataCorp, College Station, TX, USA). Two-tailed p-values <0.05 were deemed significant. When using a quality assessment tool for prevalence studies,[13] the internal validity was good for all studies, except for scintigraphy studies where a final diagnosis was not reached[14–17]; given the design of the original studies, we could not provide definite answers to questions exploring external validity, e.g. 'Was the study's target population a close representation of the national population in relation to relevant variables?'.[13]

The PRISMA checklist is provided in online supplementary *Appendix S1*. The review was registered on PROSPERO (CRD42022306259).

Results

Thirty-one studies were ultimately selected.[14–44] Six studies had a retrospective design, 21 had a prospective design, and 4 were forensic series (online supplementary *Table S1*). The studies were classified into the following settings: total body scintigraphy with 'bone tracers' for non-cardiac reasons ($n = 5$ studies),[14–18] HFpEF ($n = 7$),[19–25] HFrEF/HFmrEF ($n = 2$),[26,27] conduction disorders warranting pacemaker implantation ($n = 1$),[26] surgery for CTS or idiopathic trigger finger ($n = 3$ and $n = 1$, respectively),[28–31] HCM phenotype ($n = 2$),[32,33] AS undergoing SAVR/TAVR ($n = 7$),[34–40] or autopsy series of elderly individuals ($n = 4$).[41–44] The diagnosis of CA was suspected based on cardiac magnetic resonance ($n = 1$)[34] or bone scintigraphy ($n = 6$),[14–18,39] or was made through multiparametric algorithms ($n = 16$)[19,20,22–33,37,38,40] or histology ($n = 7$).[21,35,36,41–44] Further details are provided in *Table 1*. The prevalence values of CA were as follows: bone scintigraphy for non-cardiac reasons, ~1%; HFpEF, from 2% to 33%; HFrEF/HFmrEF, from 9% to 11%; conduction disorders, 2%; CTS surgery, from 3% to 10%; HCM phenotype, from 5% to 9%; AS undergoing SAVR/TAVR, from 0% to 4% or from 8% to 16%, respectively; autopsy series of elderly individuals, from 4% to 29%. In studies with available data, the average proportion of males ranged from 45% in autopsy series to 100% in HFrEF/HFmrEF, and the average age from 74 to 90 years (*Figure 1*).

Studies on individuals undergoing bone scintigraphy for reasons other than suspected CA are likely those most informative on the prevalence of CA in the general population. Four studies assessed all scans performed for non-cardiac reasons[14,16–18] or for any indication[15]; one study enrolled only elderly subjects.[16] CA was suspected based on tracer uptake on planar scintigraphy in four of five studies[14–17] and was confirmed by single photon emission computed tomography (SPECT) in only one study.[14] Three studies allowed to investigate more thoroughly the relationship between CA prevalence, age and sex,[14,16,18] reporting a higher likelihood in men than women across all age categories, and rising prevalence

Table 1 Screening studies on cardiac amyloidosis in different settings

Author	Indication to screening	Broad setting	Subject, n	Men (%)	Mean/ median age (years)	CA diagnosis	CA prevalence (%)	ATTR-CA (% of CA pts)	AL-CA (% of CA pts)	Other CA (% of CA pts)	Men (% of CA pts)	Mean/median age of CA pts (years)	Notes
Cuscaden[14]	Bone scan for non-cardiac reasons	Bone scan for non-cardiac reasons	6918	N/A	N/A	Scintigraphy (alone)	0.2	N/A	N/A	N/A	94	N/A	SPECT when positive planar scintigraphy; all positive cases >65 years
Bianco[15]	Bone scan for any reason	Bone scan for non-cardiac reasons	4228	N/A	N/A	Scintigraphy (alone)	0.5	N/A	N/A	N/A	78	83	
Longhi[18]	Bone scan for non-cardiac reasons	Bone scan for non-cardiac reasons	12400	37	74	Scintigraphy (subgroup with scintigraphy+: ECG, echo, EMB in selected cases)	0.4 (43 in subgroup further investigated)	N/A (100 in subgroup further investigated)	N/A	N/A	N/A	N/A	Scintigraphy+ in ~1.3% of men and ~0.4% of women ≥81 years
Mohamed-Salem[16]	Bone scan for non-cardiac reasons, ≥75 years	Bone scan for non-cardiac reasons	1114	65	81	Scintigraphy (alone)	2.8	N/A	N/A	N/A	90	85	4.2% in men, 1.0% in women
Kim[17]	Bone scan for non-cardiac reasons	Bone scan for non-cardiac reasons	9581	N/A	N/A	Scintigraphy (alone)	0.1	N/A	N/A	N/A	83	81	
Lindmark[19]	HF clinic, IVS >14 mm	HFpEF	86	57	77	Scintigraphy, monoclonal protein, biopsy when needed	15	100	0	0	88	84	
González-López[20]	HFpEF, LVWT ≥12 mm, ≥60 years	HFpEF	120	41	82	Scintigraphy, monoclonal protein, biopsy when needed	13.3	100	0	0	50	86	
Hahn[21]	HFpEF with unclear aetiology	HFpEF	108	39	66	Histology (EMB)	14	73	20	7	39	66	
AbouEzzeddine[22]	HF, LVEF ≥40%, LVWT ≥12 mm, age ≥60 years	HFpEF[a]	286	50	78	Scintigraphy, monoclonal protein, biopsy when needed	6.3	100	0	0	83	84	Prevalence from 0% (60–69 years) to 21% (≥90 years), and 10.1% in men vs. 2.2% in women
Bennani Smires[23]	HFpEF, ≥65 years, no CAD	HFpEF	49	57	76	CMR, scintigraphy, monoclonal protein, biopsy when needed	33	60	40	N/A	93	77	
Arvanitis[24]	African Americans >60 years with (1) an ICD-9/10 diagnosis of HF and (2) mean LVWT ≥12 mm					HFpEF	99	N/A	N/A	N/A	100	66	A third patient with CMR findings consistent with CA
Devesa[25]	HFpEF, LVWT <12 mm	HFpEF	58	54	79	Scintigraphy, monoclonal protein, biopsy when needed	5.2	100	0	0	67	84	
Lopez-Sainz[26]	(1) CD requiring PM implantation; 2) hospitalized HF, LVEF <50%, >60 years, LVWT ≥12 mm	1) CD 2) HFrEF/HFmrEF	139	57 CD, 57 HF	79 CD, 78 HF	Scintigraphy, monoclonal protein, biopsy when needed	2 CD, 11 HF	100	0	0	50 CD, 100 HF	90 CD, 86 HF	
Goland[27]	Unexplained LV systolic dysfunction	HFrEF/HFmrEF	75	69	65	Scintigraphy, monoclonal protein, biopsy when needed	9.3	100	0	0	100	75	
Vianello[28]	Men undergoing bilateral CTS surgery	CTS	53	100	73	Scintigraphy (+ exclusion of a monoclonal protein)	4	100	0	0	100	79	33% in those with LV thickening
Sperry[29]	CTS surgery in men ≥50 years and women ≥60 years	CTS	98	51	68	Histology+: cardiac screening	10	70	20	10	60	73	
Zegri-Reiriz[30]	CTS surgery, ≥60 years, LVWT ≥12 mm	CTS	101	32	73	Scintigraphy, monoclonal protein, biopsy when needed	3	67	33	0	33	76	6% in LV thickening + bilateral surgery, 14% if occupational factors excluded
Sperry[31]	Surgery for idiopathic trigger finger, ≥50 years	–	100	41	66	Histology of the surgical specimen + 'cardiac screening' if positive histology	0	N/A	N/A	N/A	N/A	N/A	
Damy[32]	Initial diagnosis of HCM	HCM	298	74	62	*TTR* gene testing, then scintigraphy, CMR, biopsy	5	100	0	0	73	74	

Table 1 (Continued)

Author	Indication to screening	Broad setting	Subject, n	Men (%)	Mean/ median age (years)	CA diagnosis	CA prevalence (%)	ATTR-CA (% of CA pts)	AL-CA (% of CA pts)	Other CA (% of CA pts)	Men (% of CA pts)	Mean/median age of CA pts (years)	Notes
Maurizi[33]	Initial diagnosis of HCM	HCM	343	58	60	TTR mutations; if no mutation found but ≥1 red flag, blood/urine tests, abdominal fat biopsy and/or scintigraphy and ApoAI sequencing	9	88	9	3	87	74	
Cavalcante[34]	Moderate/severe AS referred to CMR	AS	113	56	70	CMR (suspected CA)	8	N/A	N/A	N/A	89	88	
Treibel[35]	AS referred to SAVR	AS	146	58	71	Histology (intraoperative biopsy)	4	100	0	0	67	75	Prevalence 6% in calcific AS >65 years
Singal[36]	AS referred to SAVR, ≥65 years	AS	46	70	70	Histology[b]	0 (?)	N/A	N/A	N/A	N/A	N/A	No amyloid in IVS biopsy, only in the valve. Positive scintigraphy in 9%
Nitsche[37]	AS referred to TAVR	AS	191	51	85	Non invasive, bone scintigraphy-based	8	94	6	0	63	84	
Nitsche[38]	AS referred to TAVR	AS	407	50	83	Non invasive, bone scintigraphy-based	12	98	2	0	N/A	N/A	Partial population overlap with another cohort[37]
Castano[39]	AS referred to TAVR	AS	151	68	84	Non invasive, bone scintigraphy-based	16	N/A	N/A	N/A	92	86	
Scully[40]	AS referred to TAVR	AS	101	43	86	Non invasive, bone scintigraphy-based	14	100	0	0	50	88	
Lie[41]	Autopsy 90–105 years	Autopsy	237	39	(≥90)	Histology (Congo red + ≥1 of the following: methyl violet, thioflavine T, and sulfate-alcian blue	21	100	0	0	40	N/A	Grade 3 (26%–50%) and 4 (>50%) deposits in tissue samples
Roberts[42]	Autopsy ≥80 years	Autopsy	490	51	(>80)	Histology (no details provided)	4	N/A	N/A	N/A	N/A	N/A	'Grossly visible amyloid' deposits
Tanskanen[43]	Autopsy >85 years	Autopsy	256	N/A	(≥85)	Histology	5	100	0	0	N/A	N/A	'Clearly detectable areas of amyloid in several visual fields, including vascular deposits': 3%. 'Large amounts of amyloid': 3%
Porcari[44]	Autopsy ≥75 years	Autopsy	56	43	86	Histology	29	50	50	0	50	86	Diffuse amyloidosis in the LV: 29%. Amyloidosis as the main cause of death: 14%

AL-CA, amyloid light-chain cardiac amyloidosis; AS, aortic stenosis; ATTR-CA, amyloid transthyretin cardiac amyloidosis; CAD, coronary artery disease; CD, conduction disorder; CMR, cardiac magnetic resonance; CTS, carpal tunnel syndrome; ECG, electrocardiogram; EMB, endomyocardial biopsy; HCM, hypertrophic cardiomyopathy; HF, heart failure; HFmrEF, heart failure with mildly reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; ICD, International Classification of Diseases; IVS, interventricular septum; LV, left ventricle; LVEF, left ventricular ejection fraction; LVWT, left ventricular wall thickness; N/A, not available; PM, pacemaker; SAVR, surgical aortic valve replacement; SPECT, single photon emission computed tomography; TAVR, transcatheter aortic valve replacement.

[a]These patients were classified by the authors as having HFpEF; their median ejection fraction was 60%. Only the community screening cohort was considered.

[b]Planar scintigraphy was performed as well, with diverging results from cardiac histology.

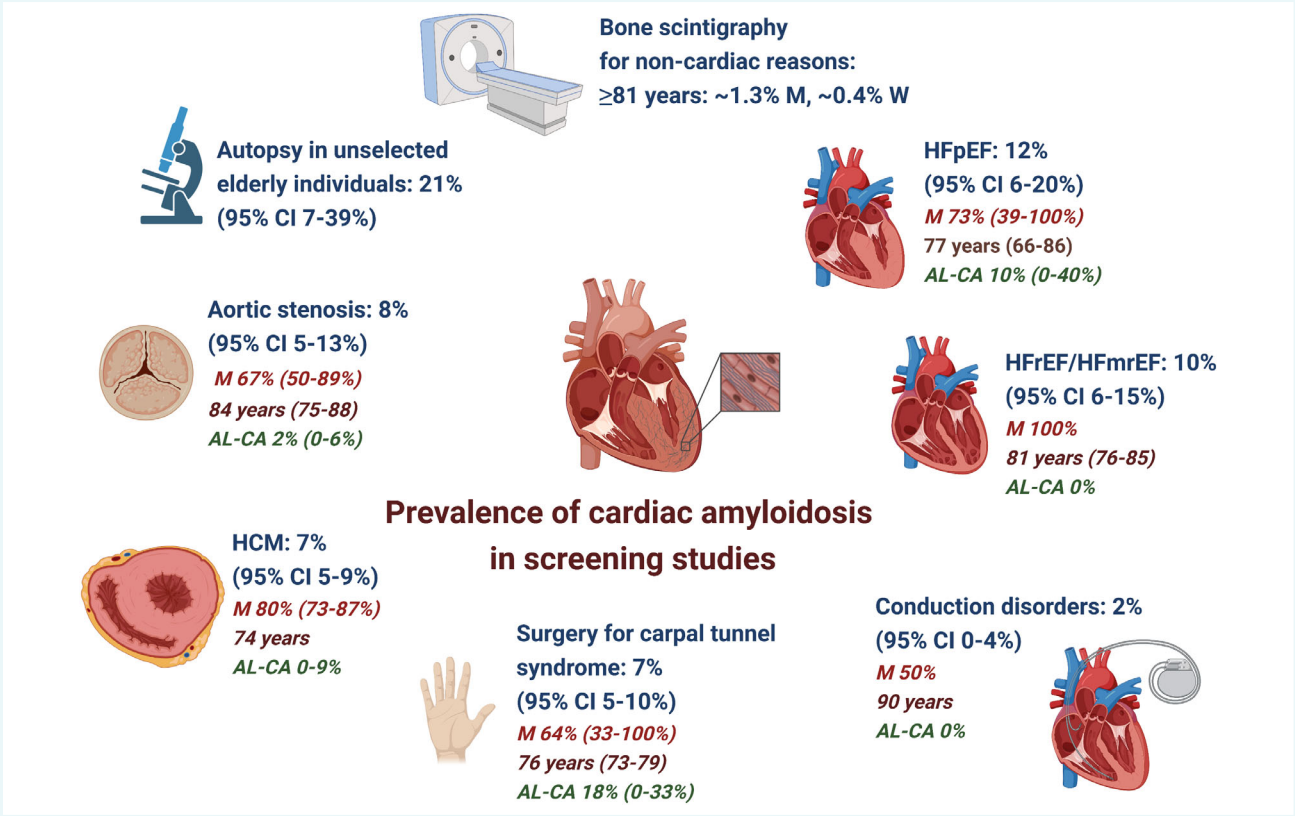

Figure 1 Prevalence of cardiac amyloidosis (CA) in different settings. Except for two specific settings (bone scintigraphy for non-cardiac reasons and autopsy in unselected elderly individuals), the mean and 95% confidence interval (CI) or the range of prevalence of CA across studies (*blue*), the percentage of males out of all patients with CA (average and range; *red*), the mean/median age of patients with CA (*brown*), and the percentage of patients with amyloid light-chain (AL) CA out of all patients with CA (*green*) are reported. Information on the original studies is reported in *Table 1*. HCM, hypertrophic cardiomyopathy; HFmrEF, heart failure with mildly reduced ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; M, men; W, women.

values with age. A meta-analytic assessment of bone scintigraphy studies found a prevalence of 1% (95% CI 0%–1%), with a high degree of heterogeneity (online supplementary *Figure S2*). The estimated prevalence of CA was higher in the smaller studies (*Figure 2*). Only the largest study investigated the relationship between cardiac bone tracer uptake, evidence of structural heart disease and symptoms.[18] In this study, a moderate to strong myocardial uptake was found in 0.36% of subjects with a progressive increase with age, and higher rates in men than women in all age categories, peaking at 1.4% in men in the ninth decade.[18] Thirty-two of 45 patients showing myocardial uptake were offered a comprehensive cardiological evaluation including ECG, echocardiography, and endomyocardial biopsy in selected cases. Only 14 patients agreed to be evaluated. All of them displayed LV thickening (which was unexplained in 10 patients and out of proportion in the remaining 4) and had abnormal ECG findings, 4 were symptomatic for dyspnoea, and 3 had CTS. ATTR-CA was ultimately diagnosed in all these patients.[18]

Five out of seven studies on HFpEF employed a multiparametric diagnostic algorithm including bone scintigraphy and the search for a monoclonal protein,[19,20,22,23,25] while one relied exclusively on myocardial histology[21] and another one screened for the V122I genotype.[24] Prevalence values were remarkably consistent in three of the largest studies (González-López *et al.*,[20] $n = 120$, 13.3%; Hahn *et al.*,[21] $n = 108$, 14%; Lindmark *et al.*,[19] $n = 86$, 15%). On average, 73% of CA patients from all the HFpEF studies were men, and aged 77 years; AL-CA accounted for 12% of cases. Women accounted for up to 50% of patients with CA.[20] Overall, the prevalence of CA in patients with HFpEF was 12% (95% CI 6%–20%) (online supplementary *Figure S3*).

The average prevalence of CA in two studies on HFrEF/HFmrEF was 10% (95% CI 6–15%).[26,27]

Patients with CA often develop high-degree atrioventricular conduction disorders requiring pacemaker implantation. Nonetheless, only one study searched for CA among patients implanted with a pacemaker, finding CA in 2% of patients (50% men, age 90 years).[26]

Carpal tunnel syndrome is an established red flag for CA. Three studies assessed patients operated for CTS, adding further criteria such as bilateral surgery in men,[28] age cut-offs,[29,30] or LV thickening.[30] Patients meeting these criteria underwent either bone scintigraphy and the search for a monoclonal protein,[28,30] or further exams (cardiac biomarkers, ECG, echocardiography and bone

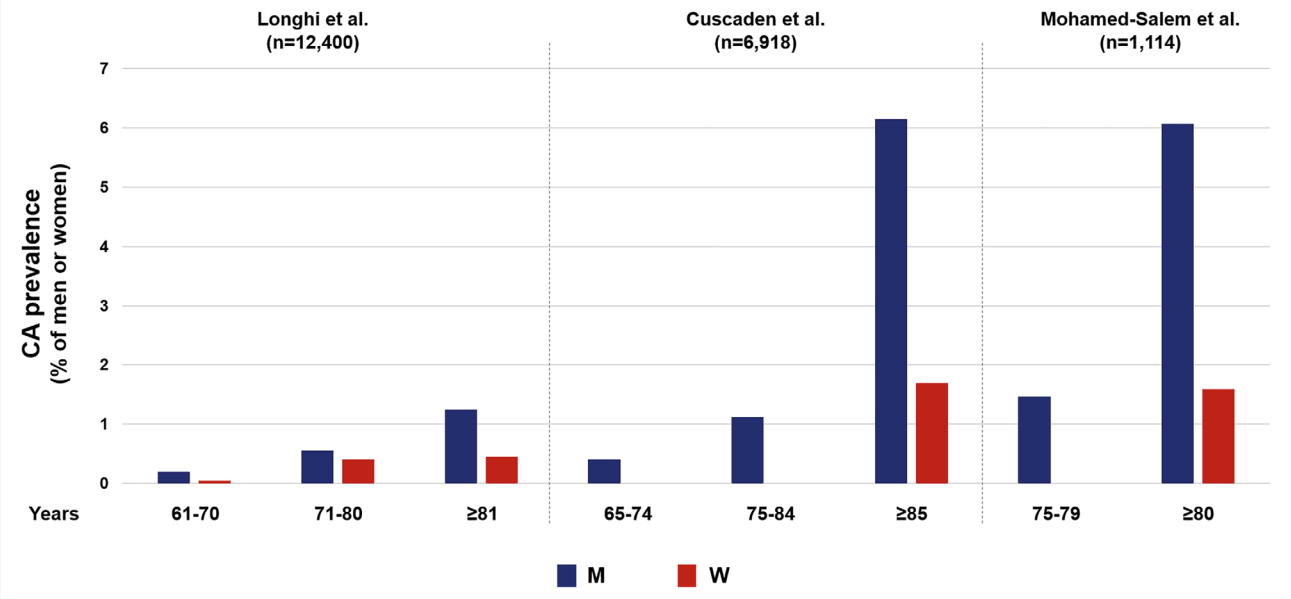

Figure 2 Prevalence of incidental myocardial uptake of bone tracers among men (M) and women (W) from the general population. See *Table 1* for details on the original studies. CA, cardiac amyloidosis.

scintigraphy) only in patients with amyloid deposits on biopsy specimens of tenosynovial tissue.[29] The prevalence of CA was 7% (95% CI 5%–10%). The prevalence rose to 14% in patients undergoing bilateral CTS surgery, aged ≥60 years, with LV thickening, and without occupational risk factors,[30] and to 33% among male patients with LV thickening and undergoing surgery on both hands.[28] Therefore, while epidemiological studies link CTS surgery with the risk of developing CA,[45] a not negligible proportion of patients operated for CTS have already CA. Interestingly, patients with AL-CA accounted on average for 18% of all patients with CA.

Two studies enrolled patients with an initial diagnosis of HCM,[32,33] with a pooled prevalence of CA of 7% (95% CI 5%–9%), and AL-CA in 4.5%. One study just searched for *TTR* mutations.[32] The other study first searched *TTR* mutations, which were found in 3.5% of subjects. Afterwards, individuals with no *TTR* mutations but at least one red flag for CA underwent a diagnostic workup for CA.[33] Not surprisingly, the prevalence of CA was almost double in the second study than in the first one (9% vs. 5%).[32,33] In the first study, all patients with ATTR-CA were older than 63 years.[32] Similarly, the prevalence of CA in the second study increased from 1% in those aged 40–49 years to 26% in those over 80 years.[33]

All but one study on AS[34] enrolled only patients with severe AS referred to surgery[35,36] or percutaneous replacement.[37–40] The prevalence of CA ranged from 0% to 16% (*Figure 3*). A pooled assessment found a prevalence of 8% (95% CI 5%–13%), with a high heterogeneity (online supplementary *Figure S4*) . On average, men accounted for 67% of CA patients, and the mean age was 84 years. Two percent of patients had AL-CA. Singal *et al.*[36] found an intense bone tracer uptake (Perugini 2 or 3) in 9% of patients undergoing SAVR. None of these patients displayed amyloid deposits in biopsy specimens from the antero-basal interventricular septum.

Four studies reported the results of *post-mortem* examinations of unselected elderly individuals.[41–44] Two studies were carried out as part of population studies.[41,43] One study on subjects aged 90–105 years dated back to 1988, and employed Congo red and another amyloid stain; 21% of patients had amyloid in at least one fourth of tissue samples, but the clinical phenotype and the cause of death were not specified.[41] Roberts and Shirani[42] found 'grossly visible amyloid' in 4% of patients and deemed it the cause of death in these patients. Another study used Congo red and then immunohistochemistry for transthyretin only, finding a 5% prevalence of 'clearly detectable' or 'large amounts' of amyloid.[43] In a series of 56 autopsies of subjects aged ≥75 years, Porcari *et al.*[44] found diffuse amyloid deposits in 43% of autopsied hearts, half of them with a positive immunostaining for AL amyloid. Twenty-nine percent of patients had severe amyloid deposits, and CA was identified as the main cause of death in 14% of patients; LV wall thickness in these last two subgroups was not specifically evaluated. A pooled assessment of autopsy studies found a global prevalence of 21% (95% CI 7%–39%) with a high degree of heterogeneity (online supplementary *Figure S5*).

Discussion

This systematic review provides some insight on the prevalence of CA in the general population or in specific conditions such as HFpEF. Each setting must be evaluated separately, and heterogeneous study designs make each attempt of synthetic evaluation challenging. Nonetheless, we may safely conclude that CA is relatively common in several settings (HFpEF, HFmrEF/HFrEF, conduction disorders requiring pacemaker implantation, CTS surgery, HCM phenotype, or severe AS requiring valve replacement) when

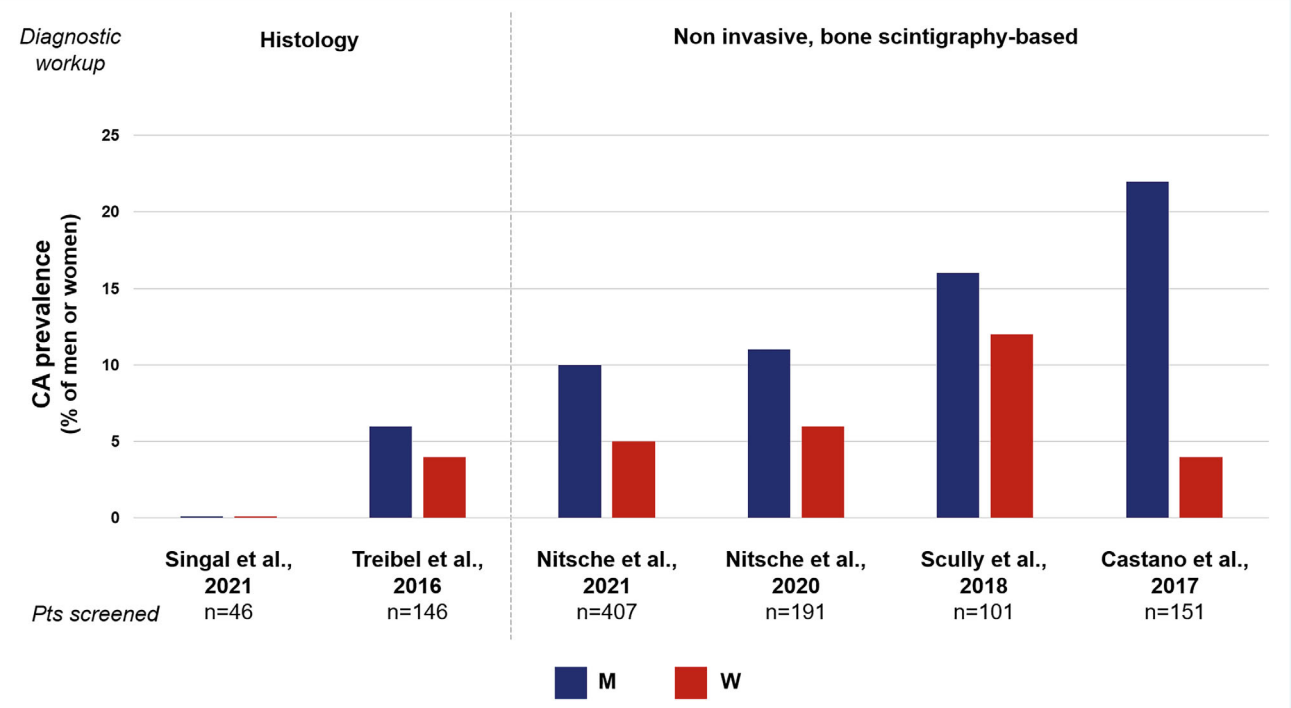

Figure 3 Prevalence of cardiac amyloidosis (CA) in patients with severe aortic stenosis referred to surgical or transcatheter aortic valve replacement. Prevalence values were calculated as the number of cases of CA among men (M) and women (W) divided by the number of M and W, respectively. In the study by Singal *et al.*,[36] no patient had CA diagnosed based on histology. Nitsche *et al.*[38] considered the number of M and W with a Perugini score 2–3 instead of the number of M and W with CA (not provided). See *Table 1* for further details on the original studies.

this condition is actively searched, and that the prevalence of CA increases with age. Furthermore, both men and women may have CA, and the diagnostic workup should include the search for a monoclonal protein[6,8,9,46,47] to avoid missing patients with AL-CA, who have a much shorter survival than those with ATTR-CA if untreated.[48]

Cardiac amyloidosis may be defined as the combination of the following elements: (i) histological evidence of amyloid deposits or (in the case of ATTR-CA) an intense myocardial uptake of bone tracers in the absence of a monoclonal protein, (ii) clear evidence of cardiac disease on echocardiography or cardiac magnetic resonance, and (iii) symptoms that can be attributed to cardiac disease. Treatment for CA is indicated when all these criteria are met.[6,8,9,46,47] A significant knowledge gap, not addressed in current society documents,[6,8,9,46,47] is whether we can diagnose CA and start a specific treatment when bone scintigraphy is positive (grade 2–3 uptake) but there is no clear evidence of cardiac involvement on echocardiography or cardiac magnetic resonance. The same considerations apply for grade 1 uptake without clear signs of cardiac involvement, which might represent either an early disease stage or a false positive finding. SPECT imaging might hold additional diagnostic value to planar scintigraphy, by verifying the presence and intensity of regional tracer uptake. The role of SPECT imaging should be further investigated also because it could avoid the need for histological confirmation in patients with suspected CA and no monoclonal protein, possibly increasing the chances of identifying patients with early disease.

Overall, a proper definition of CA is crucially important when considering screening studies, especially those evaluating asymptomatic individuals, or autopsy series. In these cases, the detection of bone tracer myocardial uptake or the histological demonstration of amyloid should be associated with evidence of structural heart disease (typically LV thickening).

Besides the exclusive reliance on myocardial uptake to define CA, another possible limitation of scintigraphy studies is the use of different tracers (online supplementary *Table S1*). The ^{99m}Tc phosphates currently most often used in Europe are ^{99m}Tc-DPD (3,3-diphosphono-1,2-propanodicarboxylate) and ^{99m}Tc-HMDP (hydroxymethylene). By contrast, ^{99m}Tc-PYP (pyrophosphate) is the only tracer available in the United States, Canada and Japan. The diagnostic criteria for positive planar scintigraphy have not been standardized, and screening studies employed different criteria (online supplementary *Table S1*). Furthermore, SPECT imaging enables a more accurate assessment of tracer uptake in the myocardium and blood pool and is recommended by all societies. It is currently unknown whether the three tracers perform equally well, and whether tomographic imaging adds to planar scintigraphy. All the scintigraphy studies but one[14] did not perform a SPECT acquisition, although SPECT may reduce the likelihood of false positive results by confirming that abnormal

uptake occurs in the myocardium rather than the cardiac blood pool.[49,50] Blood pool issues are quite common with ^{99m}Tc-PYP, and bisphosphonate scintigraphy can show a cardiac uptake even in patients with AL-CA or rarer forms of amyloidosis, but also rib fractures, valvular or annular calcifications, recent myocardial infarction, or hydroxychloroquine cardiac toxicity.[7] False positive results for reasons other than CA are unlikely to have played a significant role in screening studies. Nonetheless, the predictive accuracy of bone scintigraphy is much lower in the general population than in historical cohorts with a higher prevalence of CA.[51,52] This casts some doubts on the estimated prevalence of CA in screening studies, especially given that a definite diagnosis of CA was not made in any studies except for some patients in the study by Longhi *et al.*[18] In addition to scintigraphy studies, the diagnosis of CA was not well established in autopsy studies, where only one study tried to correlate morphological and histological findings with clinical data.[44]

Overall, screening for CA in specific settings allowed to identify a relatively high number of cases, in agreement with the notion that CA is not a rare disorder. Many patients have ATTR-CA, but the percentage of cases with AL-CA is far from negligible, and untreated AL-CA has a grim prognosis. Many studies focused on elderly subjects, given the clear relationship between age and the likelihood of CA. Men accounted for a high proportion of patients with CA identified through an active screening, suggesting that female sex is a protective factor.[53] Nonetheless, women accounted for 27% of patients with HFpEF and 36% of patients with CTS (two settings with a characteristic female preponderance),[54,55] but also for 33% of patients with severe AS. These observations prompt further investigations on the biological relationship between sex and CA.

Cardiac amyloidosis and severe AS are often associated, although the exact relationship between these two conditions has not been clarified so far. Three hypotheses can be formulated: (i) this association could derive from the age-dependent penetrance of both conditions, (ii) amyloid deposition may promote both cardiomyopathy and AS, or (iii) cardiomyopathy could be exacerbated by pressure overload due to severe AS.[56] The higher prevalence of CA in patients with severe AS compared with elderly individuals from the general population (based for example on scintigraphy studies) tends to disprove the first hypothesis. Additionally, a pathogenic role of systemic amyloidosis in AS does not seem highly likely. In a series of 100 stenotic aortic valves surgically removed, amyloid deposits were found in 74 cases. None of the most common amyloid proteins were identified, and amyloid deposition appeared secondary to athero-inflammatory conditions and high shear stress.[53,57] Finally, degenerative AS might represent a mechanical trigger to amyloid deposition by increasing myocardial strain.[58] The study by Singal *et al.*[36] suggests the intriguing perspective of isolated aortic valve amyloidosis, as 72% of explanted valves showed amyloid deposits (ATTR in 58% of all valves), while none of the interventricular septum biopsy specimens had amyloid deposits. Further studies are warranted to investigate this point, also because the discrepancy between negative myocardial biopsy in all cases and positive scintigraphy in 9%[36] might suggests false negative results of myocardial biopsy.

The number of studies on some settings was low, not allowing a meta-analytic assessment, while on other settings the degree of heterogeneity was high. Nonetheless, it is reasonable to conclude that screening strategies are most likely to detect CA in men and women in their 70s or 80s displaying (i) heart failure symptoms or conduction disorders, (ii) CTS with indication for surgery, unexplained or out of proportion LV thickening, or severe AS. There is a crucial need to identify the most cost-effective strategies for CA identification in different settings. Large-scale surveys considering the clinical and echocardiographic phenotype and the combination of red flags, such as the nationwide ongoing AC-TIVE study,[59] are crucial to achieve this goal.

Conclusions

Searching for CA in specific settings allows to identify a relatively high number of cases who may be eligible for treatment if the diagnosis is unequivocal (i.e. when there is a combination of evidence of amyloid deposits, structural heart disease, and symptoms of cardiac disease). ATTR-CA accounts for many cases of CA across the different settings, but AL-CA is not infrequent and should be searched. Median age at diagnosis falls in the eighth or ninth decades. Many patients diagnosed with CA are women (for example, 36% of cases of CA among patients operated for CTS).

Supplementary Information

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

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