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ΔΙΠΛΩΜΑΤΙΚΗ ΕΡΓΑΣΙΑ

«Systematic Review and Meta-analysis of the Diagnostic and Prognostic Value of Echocardiographic Markers and Scores in Cardiac Amyloidosis»

«Συστηματική Ανασκόπηση και Μετα-ανάλυση της Διαγνωστικής και Προγνωστικής Αξίας των Ηχοκαρδιογραφικών Δεικτών και Σκορ στην Καρδιακή Αμυλοείδωση»

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Abbreviations table

Abbreviation	Full Form	Abbreviation	Full form
AA amyloidosis	Amyloid A amyloidosis	LAScd	Left Atrial Conduit Strain
AFD	Anderson-Fabry Disease	LASct	Left Atrial Contraction strain
AL amyloidosis	Immunoglobulin Light Chain Amyloidosis	LASr	Left Atrial Reservoir Strain
ANF	Atrial Natriuretic Factor	LAVI	Left Atrial Volume Index
AS	Aortic Stenosis	LGE	Late Gadolinium Enhancement
ATTR amyloidosis	Transthyretin Related Amyloidosis	LVEDD	Left Ventricular End Diastolic Diameter
ATTRh	Hereditary ATTR	LVEDV	Left Ventricular End Diastolic Volume
ATTRv	Variant ATTR	LVESV	Left Ventricular End Systolic Volume
ATTRwt	Wild-type ATTR	LVH	Left Ventricular Hypertrophy
CA	Cardiac Amyloidosis	LVMI	Left Ventricular Mass Index
CI	Confidence Interval	LVOT	Left Ventricular Outflow Tract
CMR	Cardiac Magnetic Resonance	LVWT	Left Ventricular Wall Thickness
DPD	3,3-diphosphono-1,2-propanodicarboxylic acid	MAPSE	Mitral Annular Plane Systolic Excursion
DT	Deceleration Time	MCF	Myocardial Contraction Fraction
ECG	Electrocardiogram	MGUS	Monoclonal Gammopathy of Undetermined Significance
ECV	Extracellular Volume	NT-proBNP	N-Terminal pro-B-type Natriuretic Peptide
EF	Ejection Fraction	PET/CT	Positron Emission Tomography/Computed Tomography
EFBSR	Ejection Fraction to Basal Strain Ratio	PWTd	Posterior Wall Thickness (end-diastole)
EFSR	Ejection Fraction to Strain Ratio	PYP	Pyrophosphate
EMB	Endomyocardial Biopsy	RALS	Relative Apical Sparing
ESC	European Society of Cardiology	RBP4	Retinol Binding Protein 4
FLC	Free Light Chain	ROC curve	Receiver Operating Characteristic curve
GCW	Global Constructive Work	RV	Right Ventricular
GLS	Global Longitudinal Strain	RVFAC	Right Ventricular Fractional Area Change
GWE	Global Work Efficiency	RVFWS	Right Ventricular Free Wall Strain
GWI	Global Work Index	RVGS	Right Ventricular Global Strain
H/CL	Heart to Contralateral ratio	RVWT	Right Ventricular Wall Thickness
H/WB	Heart to Whole Body ratio	RWT	Relative Wall Thickness
HCM	Hypertrophic Cardiomyopathy	SAB	Septal Apical to Base ratio
HFpEF	Heart Failure with Preserved Ejection Fraction	SPECT	Single-photon Emission Computed Tomography
HHT	Hypertensive Heart Disease	SPIE	Serum Protein Electrophoresis with Immunofixation
HMDP	hydroxymethylene diphosphonate	SROC curve	Summary ROC curve
IAS thickness	Interatrial Septal Thickness	STE	Speckle Tracking Echocardiography
IVSd	Intraventricular Septum (end-diastole)	SV	Stroke Volume
IWT score	Increased Wall Thickness score	TAPSE	Tricuspid Annular Plane Systolic Excursion
LA	Left Atrium	TAVI	Transcatheter Aortic Valve Implantation
LAA	Left Atrial Area	UPIE	Urine Protein Electrophoresis with Immunofixation
LAEF	Left Atrial Emptying Fraction		

Περίληψη

Εισαγωγή: Η καρδιακή αμυλοείδωση είναι μια προοδευτική, διηθητική νόσος του μυοκαρδίου που εκδηλώνεται με ποικίλα απεικονιστικά και κλινικά ευρήματα. Καθότι συχνά εκδηλώνεται με τη μορφή υπερτροφικής μυοκαρδιοπάθειας είναι ιδιαίτερης σημασίας η έγκαιρη και ακριβής διαφοροδιάγνωση της νόσου από άλλες παθήσεις.

Στόχοι: Στόχος της συγκεκριμένης διπλωματικής εργασίας είναι η συστηματική ανασκόπηση και μετα-ανάλυση της διαγνωστικής και προγνωστικής αξίας υπερηχογραφικών δεικτών και σκορ στην καρδιακή αμυλοείδωση.

Μέθοδοι: Διενεργήθηκε συστηματική ανασκόπηση της βιβλιογραφίας στις βάσεις Pubmed, Scopus και Web of Science έως τον Σεπτέμβριο 2024, ακολουθώντας κατάλληλη στρατηγική αναζήτησης σε κάθε βάση.

Αποτελέσματα: Από σύνολο 6,139 ασθενών με υπερτροφία μυοκαρδίου 2,527 είχαν τελικά διάγνωση αμυλοείδωσης. Από τη μετα-ανάλυση μας προέκυψε ότι το βέλτιστο cutoff σημείο για τον δείκτη RALS ήταν 0,9 (με ευαισθησία 0,63 και ειδικότητα 0,81), ενώ για τον δείκτη SAB ήταν αντίστοιχα 3,2 (με ευαισθησία 0,58 και ειδικότητα 0,85). Συνολικά, σε επίπεδο ειδικότητας 95%, οι δείκτες που έδειξαν καλύτερη ευαισθησία ήταν οι LASr και LAScd (ευαισθησία 0,89 και 0,85 αντίστοιχα), υπερτερώντας έναντι των καθιερωμένων RALS και SAB (ευαισθησία 0,40 και 0,42 αντίστοιχα).

Συμπέρασμα: Ο υπέρηχος είναι ένα χρήσιμο εργαλείο στα αρχικά στάδια της διερεύνησης της καρδιακής αμυλοείδωσης, συμβάλλοντας στον αρχικό αποκλεισμό της νόσου. Επειδή η ευαισθησία των διάφορων δεικτών ποικίλλει σε υψηλά επίπεδα ειδικότητας, θα ήταν προτιμότερη η χρήση συνδυασμού αυτών, συμπεριλαμβάνοντας και νεότερους δείκτες όπως οι LASr, LAScd.

Λέξεις-Κλειδιά: Καρδιακή αμυλοείδωση, Υπερτροφία, Υπερτροφική μυοκαρδιοπάθεια, Υπερηχογραφία, Δείκτης

Abstract

Introduction: Cardiac amyloidosis (CA) is a progressive infiltrative disorder of the heart leading to a variety of imaging features and clinical manifestations. Often presented as left ventricular hypertrophy (LVH), there is an unmet need for accurately and timely distinguishing the disease from other causes of LVH.

Aims: The aim of the current thesis is to present an overview of echocardiographic markers and scores in the diagnosis and prognosis of cardiac amyloidosis and conduct a meta-analysis of diagnostic accuracy on the field.

Methods: A systematic review of the literature was conducted in Pubmed, Scopus and Web of Science from inception till September 2024, using an appropriate search strategy for each database.

Results: From a total of 6.139 patients with LVH, 2.527 had a definite diagnosis of CA. Our meta-analysis showed that the optimal cutoff point for RALS was 0.9 with a pooled sensitivity of 0.63 and a specificity of 0.81, while for SAB the optimal cutoff was 3.2 with

a pooled sensitivity of 0.58 and a specificity of 0.85. Overall, at a fixed level of 95% specificity the indices that demonstrated the best sensitivity were LASr and LAScd (0.89 and 0.85 respectively) over RALS or SAB (sensitivity 0.40 and 0.42 respectively).

Conclusion: Echocardiography can be a useful tool in the first steps of the diagnostic pathway of CA by ruling out the disease. As the sensitivity between the different indices varies, not a single echocardiographic marker should be used but rather a combination, incorporating novel echocardiographic indices.

Keywords: Cardiac amyloidosis, Hypertrophy, Hypertrophic cardiomyopathy, Echocardiography, Index

Introduction

Definition and subtypes of cardiac amyloidosis

The term “amyloidosis” comprises a block of conditions characterized by deposition of misfolded proteins (amyloid fibrils), affecting multiple organs, leading to dysfunction or insufficiency. (1) Cardiac amyloidosis is a serious and progressive infiltrative disorder of the cardiac muscle provoked by the extracellular accumulation of the amyloid fibrils in the heart. Although considered rare, the disease is rather underestimated, due to the low clinical suspicion among clinicians about its existence. In fact, cardiac amyloidosis can be a source of a variety of common clinical conditions in everyday practice, such as heart failure (mostly heart failure with preserved ejection fraction, HFpEF), aortic stenosis and arrythmias, including atrial fibrillation. (2) Cardiac involvement is a key prognostic factor, as it significantly increases mortality(1). Although more than 30 proteins have been identified to form amyloid aggregations, only 9 of them can target the heart leading to severe cardiac disease(2).

The two most common types of cardiac amyloidosis are ATTR and AL amyloidosis, caused by the deposition of the misfolded proteins of transthyretin and light-chain immunoglobulin respectively (2) and accounting for approximately 95% of the cases (3) AL amyloidosis is mainly a hematological disorder due to plasma cell dyscrasia (80% lambda and 20% kappa light chains), most commonly monoclonal gammopathy of undetermined significance (MGUS) (1,4). As reported by Rigopoulos et al. cardiac involvement is present to up to 70% of cases of AL amyloidosis, although isolated cardiac manifestation of the disease is evident in <5% of patients (1)

On the other hand, ATTR is due to the accumulation of transthyretin, a binding protein for thyroxin and retinol, mainly produced by the liver. ATTR amyloidosis could be further divided into hereditary or variant ATTR amyloidosis (ATTRh or ATTRv), carrying an autosomal dominant inheritance pattern and wild-type (formerly known as senile) amyloidosis (ATTRwt), typically evolving after the sixth decade of life (1). ATTRv amyloidosis has been associated with more than 120 gene mutations, with the most common worldwide being the Val30Met (currently named Val50Met) mutation(5). Cardiac involvement is predominant in ATTRwt amyloidosis (100% of patients affected), while for ATTRv it ranges from 30-100% depending on mutation type(2). In both forms of amyloidosis (AL and ATTR) cardiac toxicity is mediated either by direct harmful effects of amyloid deposits to the heart or by indirect, still undetermined, pathways. AL exerts a

stronger and more harmful effect on the heart due to the faster rate of fibril accumulation (4) which is associated with a poorer prognosis (1).

Other, more rare types of amyloidosis include serum amyloid A amyloidosis (AA), Apolipoprotein A1 amyloidosis and atrial natriuretic factor amyloidosis (ANF)(4,5). The disease, especially in the form of ATTR, manifests clinically mostly in older adults, with a male predominance (4,6).

Clinical presentation

Patients suffering from cardiac amyloidosis can present with a diversity of clinical manifestations of cardiac but also extracardiac origin, as isolated cardiac involvement is rather uncommon (1). General, non-specific symptoms such as fatigue and weight loss may be present, regardless of amyloidosis type (5).

Cardiac involvement is exhibited mainly with symptoms of heart failure (usually HFpEF), although in advanced disease, arrythmias (mostly atrial fibrillation), angina (due to infiltration of cardiac vessels) or valvulopathy (aortic stenosis, predominantly low-flow, low-gradient) can also arise (7,8). Therefore, patients who are candidates for TAVI should always be examined with caution.

Extracardiac symptoms include a variety of neurological complications, such as sensorimotor disabilities, as well as autonomic dysfunction (orthostatic hypotension, erectile dysfunction, incontinence) (1,9). Patients can also present with syncope as a result of the above mentioned dysautonomia or arrythmias(7) which is usually an indicator of poorer prognosis (4,9). Other clinical symptoms, typical of AL amyloidosis, consist of macroglossia, periorbital purpura and muscular pseudohypetrophy (10) as well as nephropathy, proteinuria (reaching levels of nephrotic syndrome) and liver involvement (2,5). The first two are highly indicative of AL amyloidosis although not encountered but up to 1/3 of the patients (4). Lumbar spine stenosis, spontaneous biceps tendon rupture and carpal tunnel syndrome (characteristically bilateral) are typical findings in ATTR amyloidosis (2,5,6) Interestingly, carpal tunnel syndrome is present in approximately half of the patients with ATTR amyloidosis and can precede the diagnosis of amyloidosis for many years, and therefore, when combined with heart failure symptoms, should yield suspicion for underlying amyloidosis (1,6). Furthermore, as mentioned by Hafeez et al., over 45% of older patients undergoing spinal stenosis surgery were found to have ATTR deposition, while the prevalence of ATTR deposits was 33% in patients who suffered from spontaneous rupture of the distal biceps tendon(6) The prevalence of extracardiac manifestations of hereditary ATTR has been found to differ slightly depending on the type of mutation (6).

Diagnosis

The definitive diagnosis of cardiac amyloidosis is established through the recognition of amyloid fibrils in heart tissue. According to the position statement of the ESC Working Group on Myocardial and Pericardial Diseases both invasive and non-invasive criteria have been proposed for the diagnosis (2). Invasive diagnosis includes either an endomyocardial biopsy positive for amyloid deposits through Congo red staining or a positive extracardiac biopsy when accompanied by specific echocardiographic or CMR criteria (2). Non-invasive diagnosis may be an appropriate alternative only for ATTR

amyloidosis and consists of grade 2 or 3 of diphosphonate scintigraphy (99mTc-pyrophosphate (PYP), 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) or 99mTc-hydroxymethylene diphosphonate (HMDP) scintigraphy) and exclusion of clonal dyscrasia by negative serum free light chains and negative serum and urine immunofixation (SPIE, UPIE) plus specific echocardiographic or CMR criteria (2). It is further advisable that diagnosis work-up of ATTR amyloidosis is then followed by genetic testing so that the exact type of ATTR is determined (2).

The following sections will provide a detailed analysis of the different tools in the diagnostic pathway of cardiac amyloidosis, highlighting the laboratory and imaging hallmarks of the disease.

ECG

The ECG is abnormal in the vast majority of cases (10). A lack of left ventricular hypertrophy signs or the presence of low-voltage QRS complexes despite LVH confirmed by imaging techniques is a red flag for the diagnosis of CA. Low-voltage QRS (<0.5 mV in extremity leads and < 1 mV in precordial leads) is more common in AL amyloidosis (up to 70%) but is a late feature in the disease course (1,5,7) and thus of worse prognosis (7). Another common sign is the pseudonecrosis or pseudoinfarct pattern, mostly of the anterior leads, (presence of Q waves or slow progression of R waves) which is the most common feature in ATTRwt (1,5) and is present in 47-60% of all CA patients (9). Conduction abnormalities and arrythmias (especially atrial fibrillation) can also be present (1). However, none of these features is pathognomonic for CA, thus their absence should not lead to exclusion of the disease (9).

BIOMARKERS

High levels of troponin T or NT-proBNP disproportionate to the clinical presentation is a common red flag for cardiac amyloidosis often foretelling a worse prognosis (5,7). These cardiac markers have also been used for disease severity stratification in different models (5,7). In discriminating AL from ATTR amyloidosis, SPIE and UPIE are of value in determining the existence of monoclonal light chain gammopathy but should always be followed by verification of quantitative increase of light chain proteins in serum using FLC assay (1). Nonetheless, this is not the case for all AL patients as approximately 2-3% lack positive laboratory testing. Therefore, exclusion of AL amyloidosis should not be relied only on these findings if clinical suspicion is high (5). On the other hand, positive results should be also interpreted with caution as up to 5% of people beyond the age of 65 have MGUS (5). Concerning ATTR, specific biomarkers such as TTR levels, TTR ligand retinol binding protein 4 (RBP4) are currently under investigation (1,6).

ECHOCARDIOGRAPHY

Echocardiography is a widespread imaging technique in clinical settings. Although it cannot establish the diagnosis of CA on its own, it has additive value to the other imaging techniques and can raise clinical suspicion for the disease. There are many traditional as well as novel echocardiographic techniques, markers and scores that can be used for that purpose.

Conventional 2D-echocardiography provides useful information on the cardiac structure and function (9). Granular-sparkling appearance of the myocardium has often been

reported, but is of limited diagnostic value (1,9). Patients with CA most often present with increased thickness of the left ventricle (LVWT >12mm), biatrial dilation and restrictive filling pattern, with reduced EF, increased E/e', TAPSE <14mm (9). Increased thickness of the right ventricle, the interatrial septum or the heart valves can also be present (9). LVH is usually concentric, of higher degree in ATTR patients (1). RV hypertrophy usually follows LVH and, when present, is an indicator of worse prognosis (9,11). As CA typically causes HFpEF, EF is preserved until late stages of the disease (1). Diastolic dysfunction on the other hand is present from early disease stages and progressively evolves to a restrictive filling pattern. Pericardial effusion can also be present and is linked to worse prognosis (9).

2D speckle tracking echocardiography (STE) is a new arrow in the quiver for the diagnosis of cardiomyopathies including CA and for differential diagnosis (9). Strain assessment of the different layers of the LV as well as of the LA is particularly important (9). The relative apical sparing of longitudinal strain (preserved apical strain as compared to reduced mid and basal segment strain)- the so-called "cherry-on-top" sign- is a highly indicative sign of CA (1,5,9) and has a high diagnostic accuracy in differentiating CA from other causes of LVH (12). Different echocardiographic markers, such as EFSR (LVEF to LS ratio), SAB (Septal Apical to Basal ratio) and RELAPS (average apical LS/average basal LS + average mid-LS) have been found to have a good sensitivity and specificity in identifying CA in patients with LVH (13). Furthermore, new echocardiographic scores (AL and IWT scores), incorporating STE parameters have been developed both for AL and ATTR-CA respectively (14). Myocardial work analysis derived from STE has led to new parameters such as Global work index (GWI) and Global work efficiency (GWE) that are used to assess myocardial performance and treatment efficacy (9). Many of these indices are also useful in risk stratification and prognosis of CA (15).

CARDIAC MAGNETIC RESONANCE (CMR)

CMR has a high diagnostic accuracy in diagnosing CA and is a useful tool for CA prognosis (1). Characteristic CMR findings are late gadolinium enhancement (LGE) and T1 relaxation time pattern (1). As reported by Rigopoulos et al. either diffuse subendocardial LGE without coronary distribution or a more localized and patchy LGE with dark mid-wall are both characteristic LGE patterns found even in subclinical cases of CA (1).The diffuse subendocardial pattern is more prominent in AL amyloidosis, while a transmural enhancement pattern is characteristic of ATTR (7,9). Mostly in ATTR, delayed enhancement is marked by apical sparing, similar to the apical sparing of echocardiography (7). A combination of LGE and apical strain sparing has proven to be a reliable diagnostic indicator for CA (1). This late enhancement is unique for CA because in other heart diseases, such as hypertensive heart disease, contrast agent clearance is faster (6). Particular components of LGE may be of clinical significance in differentiating AL from ATTR. More specifically, RV LGE, atrial LGE, global or any transmural LGE are more ATTR specific (1,4).

As people with CA also present with some degree of renal failure, non-contrast T1 CMR has proved to be a good alternative in these patients (1). A newer technique, T1 mapping, is also of great significance for such patients as it ensures quantitative analysis of myocardial relaxation time, without gadolinium administration (5). This technique is also potentially useful in amyloid burden identification and heart response to treatment and thus can be used in treatment guidance (9).

Another useful application is the extracellular volume (ECV) assessment, which is related to the deposition of amyloid, often exceeding 40% in patients with established CA and is more prominent in patients with ATTR (5,7). A combination of ECV and T1 mapping has been deemed useful for recognizing patients in incipient disease stages, even in the absence of LGE (9). Furthermore, in the Look-Locker scout sequence, which can be used for quantifying amyloid burden, an abnormal reverse nulling pattern is observed (1,9).

CMR is also useful in the differential diagnosis of LVH and cardiomyopathies, as CA is characterized by unique signal intensity patterns on imaging, which reflect amyloid deposition and a more rapid clearance of gadolinium. This faster clearance leads to a reduced T1 contrast between the myocardium and the blood pool (16). Additionally, the lack of myocardial edema on T2-weighted black blood imaging can help distinguish CA from inflammatory conditions (1).

SCINTIGRAPHY

Cardiac scintigraphy using sodium pyrophosphate derivates labeled with technetium-99m-99mTc-PYP, 3,3–diphosphono-1,2-propanodicarboxylic acid - DPD or TC-99m-hydroxymethylene diphosphonate-HMDP scintigraphy plays a major role in the diagnosis of ATTR amyloidosis, especially in combination with monoclonal protein assessment for the exclusion of AL form (2), bearing a sensitivity of up to 100 (1,7). The visual Perugini scale for cardiac uptake considers rib uptake for comparison and is determined as follows: 0= absence of uptake, 1=uptake smaller than the costal arches, 2= uptake equal to the costal arches, and 3= high uptake - more intense than bone uptake (7). Heart to contralateral ratio (H/CL) and heart to whole body ratio (H/WB) are new alternative indexes that can be used for quantitative assessment (9,11). As mentioned by Rigopoulos et al. grade 2 or 3 myocardial uptake alone has a very high sensitivity but lower specificity in detecting ATTR due to some degree of myocardial uptake also in AL patients (1). Although of high diagnostic accuracy with a reported combination of sensitivity and specificity >90%, potential causes of false positive and false negative results should be always kept in mind (9). For example, lower sensitivity has been reported in a small group of patients with ATTRv bearing a specific mutation, which could be of significance if validated in larger patient cohorts (5).

SPECT could be used in addition to the above-mentioned techniques in order to avoid misdiagnosis (9). Positron emission tomography is an upcoming technique in the field of CA diagnosis (1). Interestingly, a series of studies has shown that PET scanning exhibited higher diagnostic yield for AL amyloidosis (5). In particular, 18F-Florbetapir PET/CT showed high diagnostic accuracy of AL amyloidosis even in latent stages, which could be of value given the fulminant course of the disease (5).

In case of diagnosis of ATTR-CA by scintigraphy further examination through genetic testing is recommended in order to confirm the exact type (1).

BIOPSY AND HISTOLOGICAL ANALYSIS

Endomyocardial biopsy (EMB) still remains the diagnostic gold standard of the disease and for confirmation of the amyloid type, though invasive and undertaken only by specialized centers (1). Amyloid deposits in tissues can be identified histologically using Congo red staining, which reveals characteristic green birefringence under polarized light. The specific type of amyloid protein is determined through laser microdissection

combined with mass spectrometry (1). If EMB is not feasible, targeted biopsy of another affected, more reachable organ (such as abdominal fat, salivary glands, rectum etc) can be performed (5). Despite being a gold standard, EMB can be complicated by serious events such as tamponade or even death (9). While ATTR-CA diagnosis can be achieved non-invasively, tissue biopsy is necessary for the diagnosis of AL-CA (5).

Differential diagnosis

Differential diagnosis of CA mainly relates to other causes that can lead to cardiac hypertrophy, mostly LVH. These include hypertrophic cardiomyopathy (HCM), storage diseases (Anderson-Fabry disease (AFD), hemochromatosis etc), hypertensive heart disease (HHD), aortic stenosis (AS) and sarcoidosis (1). In the elderly, presence of low flow low gradient aortic stenosis and new-onset LVH should yield the suspicion of CA (17). HCM presents in younger patients with an asymmetrical LVH, frequently with LVOT obstruction as opposed to CA (1).

Prognosis

Prognosis of cardiac amyloidosis is ominous, especially in the case of AL, where median survival lies between 6 to 24 months from diagnosis, whereas in the case of ATTR amyloidosis median survival reaches up to 69 months (2). Since the forecast in most cases of cardiac amyloidosis is poor, there is an unmet need for prompt diagnosis and treatment as early intervention is essential for achieving a better prognosis (1). In this context echocardiography presents as an emerging tool, as it is widely accessible, low-cost and non-invasive when compared to current standard diagnosing techniques. Thus, it can be used as a screening tool in the initial steps in order to rule out CA and guide further diagnostic work-up (1). Moreover, many echocardiographic indexes ranging from conventional to deformation-based parameters have been considered to be significant prognostic markers in the course of the disease (18).

Due to restrictions on timing and size of the thesis, the current thesis will focus on the diagnostic value of different echocardiographic parameters in cardiac amyloidosis.

Methods

This study was carried out in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. We did not use any language restrictions. Conferences abstracts of the ESC congresses were checked for additional studies.

Criteria for considering studies for the reviews

Types of studies

We included prospective and retrospective cohort studies that provide data on the diagnostic accuracy of the index test. We included only studies that provided 2x2 contingency tables of each index against the reference standard or that provided information from which these data could be derived. We excluded non-research articles (reviews, case reports or case series, letters to the editor), non-English articles as well as other systematic reviews and meta-analyses.

Participants

We included studies that evaluated the index tests for cardiac amyloidosis described below in adult patients suspected of having the disease, diagnosed with increased ventricular wall thickness, defined as intraventricular septal thickness in end-diastole 12>mm, or posterior wall thickness in end-diastole 12>mm. This spectrum includes patients with cardiac amyloidosis, hypertrophic cardiomyopathy, Anderson-Fabry disease, aortic stenosis, hypertensive heart disease, athlete's heart.

Index test

We considered the following index tests:
- Simple 2D echocardiographic markers: IVSd, PWTd, RWT, LVEDD, LVEDV, LVESV, LVMI, LVEF, LAA, LAVI, LAEF, IAS thickness, MAPSE, TAPSE, SV, E/A, DT, E/e'

- Speckle tracking echocardiographic markers: MCF, GLS, RALS, SAB, A/B ratio, LASr, LAScd, LASct, EFSR, EFBSR, RVWT, RV FAC, RVGS, RVFWS, GWI, GCW

Echocardiographic scores:

- AMYLI score, defined as the product of RWT and E/e' (RWT x E/e')
- IWT score, defined as the combination of RWT, TAPSE (mm), E/e', LS (%), and septal longitudinal systolic apex-to-base ratio (SAB) with the following cut-offs and scoring (14):

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

Target condition

We included articles targeting any type of cardiac amyloidosis (immunoglobulin light chain amyloidosis - AL, transthyretin cardiac amyloidosis – ATTR or amyloid A amyloidosis – AA, Apolipoprotein A1 amyloidosis, atrial natriuretic factor amyloidosis – ANF).

Reference standard

We considered three reference standards: endomyocardial biopsy (EMB) and two composite reference standards, based on the ESC Myocardial Working Group position paper (2) and ASNC/AHA/ASE/EANM/HFSA/ISA/SCMR/ SNMMI Expert Consensus Recommendations for Multimodality Imaging in Cardiac Amyloidosis (19).

We defined the composite reference standards as follows based on the position paper of Garcia-Pavia et al. and the ASNC/AHA/ASE/EANM/HFSA/ISA/SCMR/SNMMI Expert

Consensus Recommendations for Multimodality Imaging in Cardiac Amyloidosis by Dorbala et al.:

a. Extracardiac biopsy positive for amyloid detection in combination with specific echocardiographic or CMR criteria as stated in the protocol
b. Cardiac uptake on diphosphonate scintrigraphy grade 2 or 3 in combination with exclusion of clonal dyscrasia by negative serum free light chains and negative serum and urine immunofixation (SPIE, UPIE) plus specific echocardiographic or CMR criteria as stated in the protocol.

All reference standards are considered equivalent.

Search methods for identification of studies

Electronic searches

A systematic review of the literature was conducted in the following databases: MEDLINE (Pubmed), SCOPUS (Elsevier) and Web of Science, using a corresponding search strategy for each database, up to September 2024, using the search strategy provided in the Appendix.

Searching other resources

European Society of Cardiology conference proceedings were also examined, using the term “cardiac amyloidosis”.

Data collection and analysis

Selection of studies

Two review authors screened the records based on title and abstract. Any disagreements were resolved through discussion or, if necessary, by consulting a third investigator. Full-text articles of the selected records were then retrieved, which were independently reviewed by two authors. Any disagreements were resolved in the same manner, as previously described. We contacted the corresponding authors in order to clarify any missing information or piece of data needed. The reasons for excluding studies were recorded and clearly outlined. The entire process is appropriately depicted in a PRISMA flow diagram (Figure 1)(20).

Statistical Analysis & Data Synthesis

We conducted separate analyses for each individual index test and target condition, treating all reference standards as equivalent. Sensitivity and specificity along with 95% confidence interval (CI) were estimated from each included study; these estimates were presented on forest plots and in receiver operating characteristic (ROC) space.

We anticipated variability in thresholds used for each index test and expected that some studies would report results for multiple cutoff points. For index tests reporting results at multiple cutoff points, we adopted the models proposed by Steinhauser et al. for modelling multiple thresholds in meta-analysis of diagnostic test accuracy studies, to incorporate all available data and obtain estimates of summary sensitivity and specificity

at the optimal threshold, where highest performance is expected (21). Specifically, we used the common random intercept and common slope model (CICS). The model accounts for across-study heterogeneity and dependence of sensitivity and specificity. For each index test analyzed using this approach, we presented the ROC plot with lines connecting points from the same study, as well as the SROC plot showing confidence regions for sensitivity and specificity, along with the optimal threshold mark. Such analyses were performed using the 'diagmeta' R-package (22).

We used the hierarchical summary receiver operating characteristic (HSROC) model proposed by Rutter and Gatsonis (2001) to estimate a summary curve for index tests in which each study reports results for a single threshold. (23). We included only index tests reported in at least four studies. Convergence was assessed using the Gelman-Rubin statistic. All analyses were performed within a Bayesian framework using the **rjags** R-package.

All analyses were performed using R (R Core Team (2024). _R: A Language and Environment for Statistical Computing_. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/). In all other cases where less than four studies were included, we provided a narrative summary of the study results.

Investigations of heterogeneity

In meta-analyses, heterogeneity in sensitivity and specificity estimates was visually assessed for each test by examining forest plots and ROC plots.

Assessment of reporting bias

We did not conduct a formal assessment of reporting bias using funnel plots or tests for funnel plot asymmetry, as these methods are not recommended for diagnostic test accuracy reviews (24). Instead, to minimize the risk of publication bias, our search strategy included reaching out to experts and relevant organizations to identify unpublished and ongoing studies derived from the ESC congresses registrations.

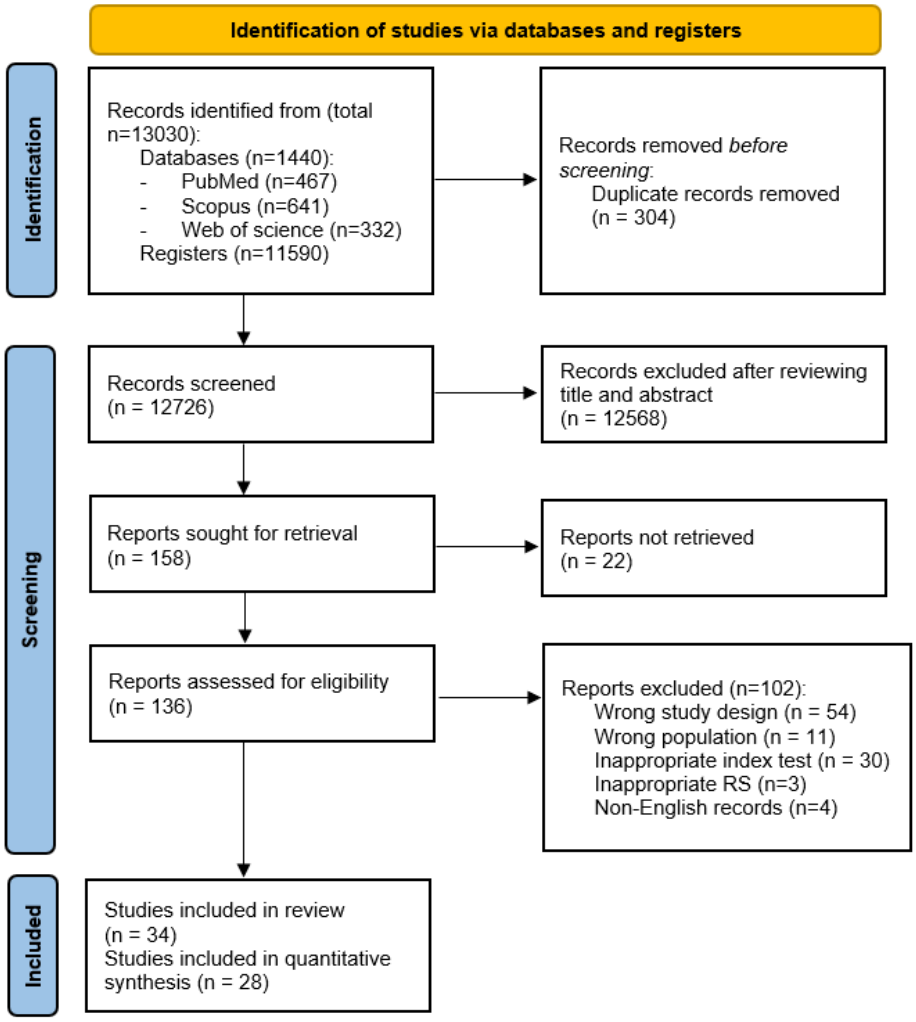
Assessment of methodological quality

The methodological quality of each study was assessed using the QUADAS-2 assessment tool (25). This process was performed by two independent reviewers, who separately assessed risk of bias and applicability concerns of each study.

Results

A total number of 1,440 records from databases and 11,590 records from conference proceedings were screened for inclusion, of which after exclusion based on title and abstract 158 were sought for retrieval and 136 full texts were assessed for eligibility. Finally, 34 reports were included in the review and 28 in quantitative synthesis. The selection process is appropriately depicted in a PRISMA flow diagram (Figure 1).

Figure 1: Prisma Flow Diagram



The total number of patients included in the meta-analysis was 6,139, of which 2,527 had a definite diagnosis of amyloidosis. Details of the included studies and echocardiographic measurements are available on Appendix table 2 and Appendix table 3 of the Appendix.

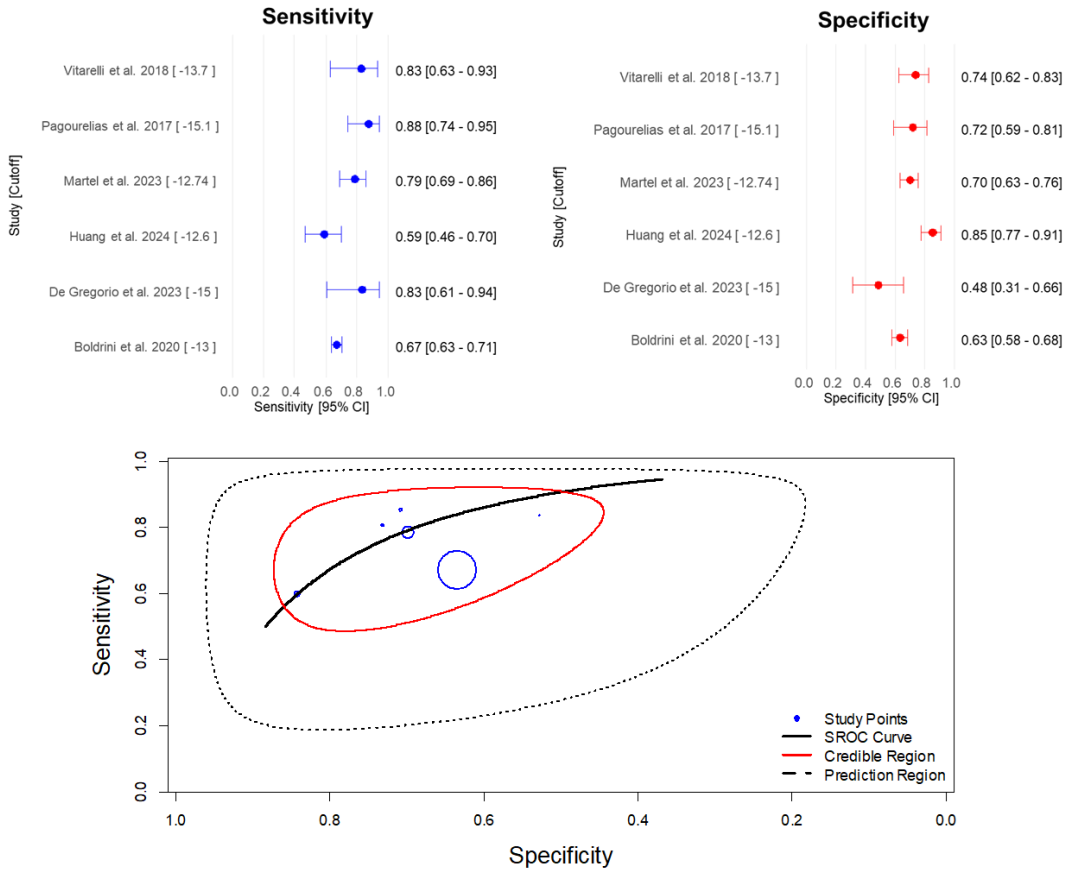
The assessment of the methodological quality of the studies in terms of risk of bias and applicability concerns was evaluated using the QUADAS-2 assessment tool and is depicted on Appendix Figure A and Appendix Figure B of the Appendix. Most studies were considered of high risk of bias due to the retrospective nature of the studies and, for the domain of the index test, due to the lack of prespecified thresholds for each index test.

Between-study heterogeneity was observed, potentially attributed to differences in patient populations and cutoff values, as indicated by lack of CI overlap in forest plots and spread in ROC plots.

Newer deformation markers

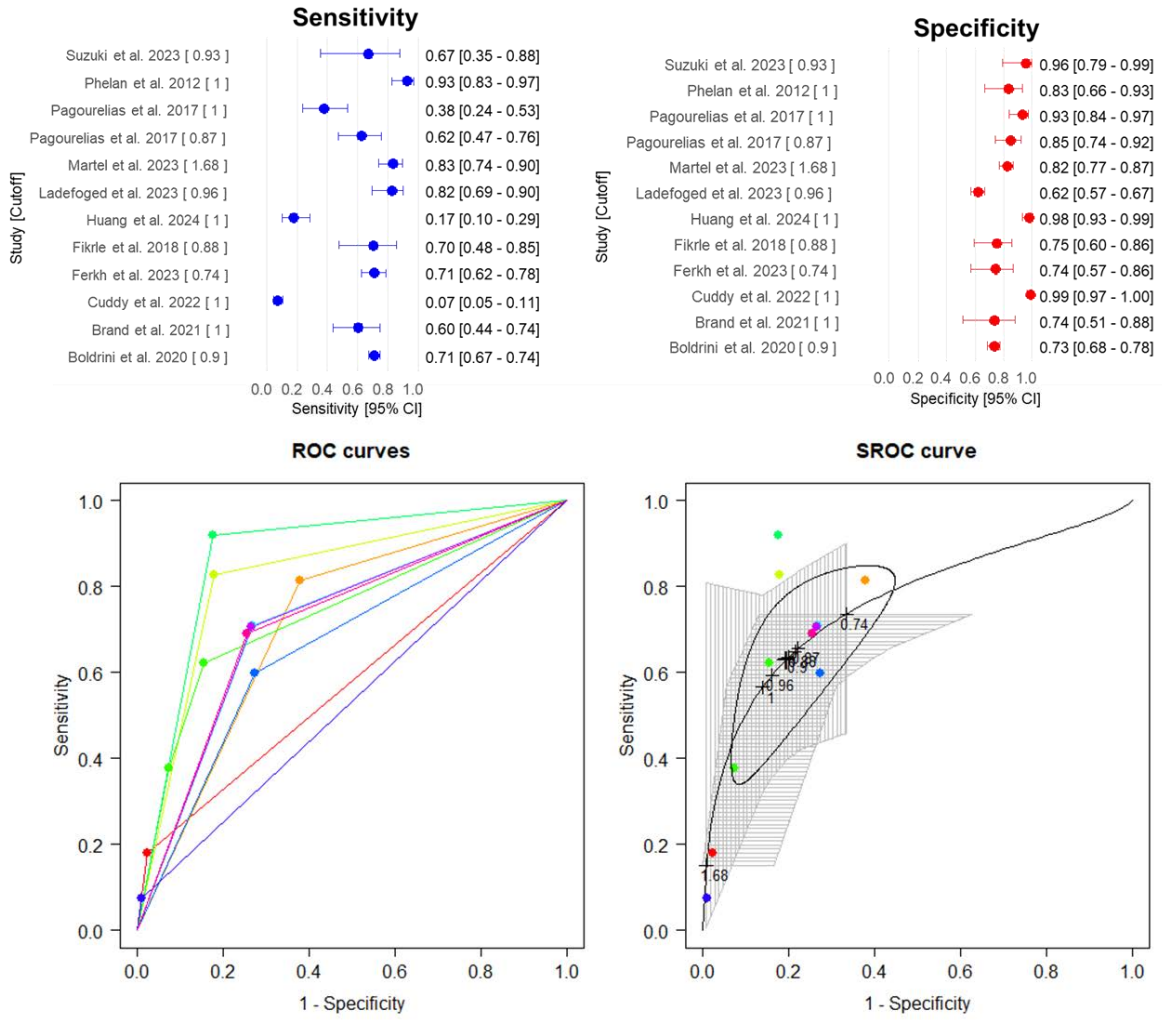
Six studies evaluated **GLS** for the detection of cardiac amyloidosis (6 studies, 1,716 participants, 880 (51.3%) with the target condition). The cutoff points used to define positive results varied across the included studies, ranging from -12.6 to -15.1. Using the HSROC model, the summary curve produced illustrates the expected trade-off between sensitivity and specificity as the threshold varies (Figure 2). For a specificity of 0.95, sensitivity was 0.78 (95% CI 0.61 to 0.92).

Figure 2: GLS



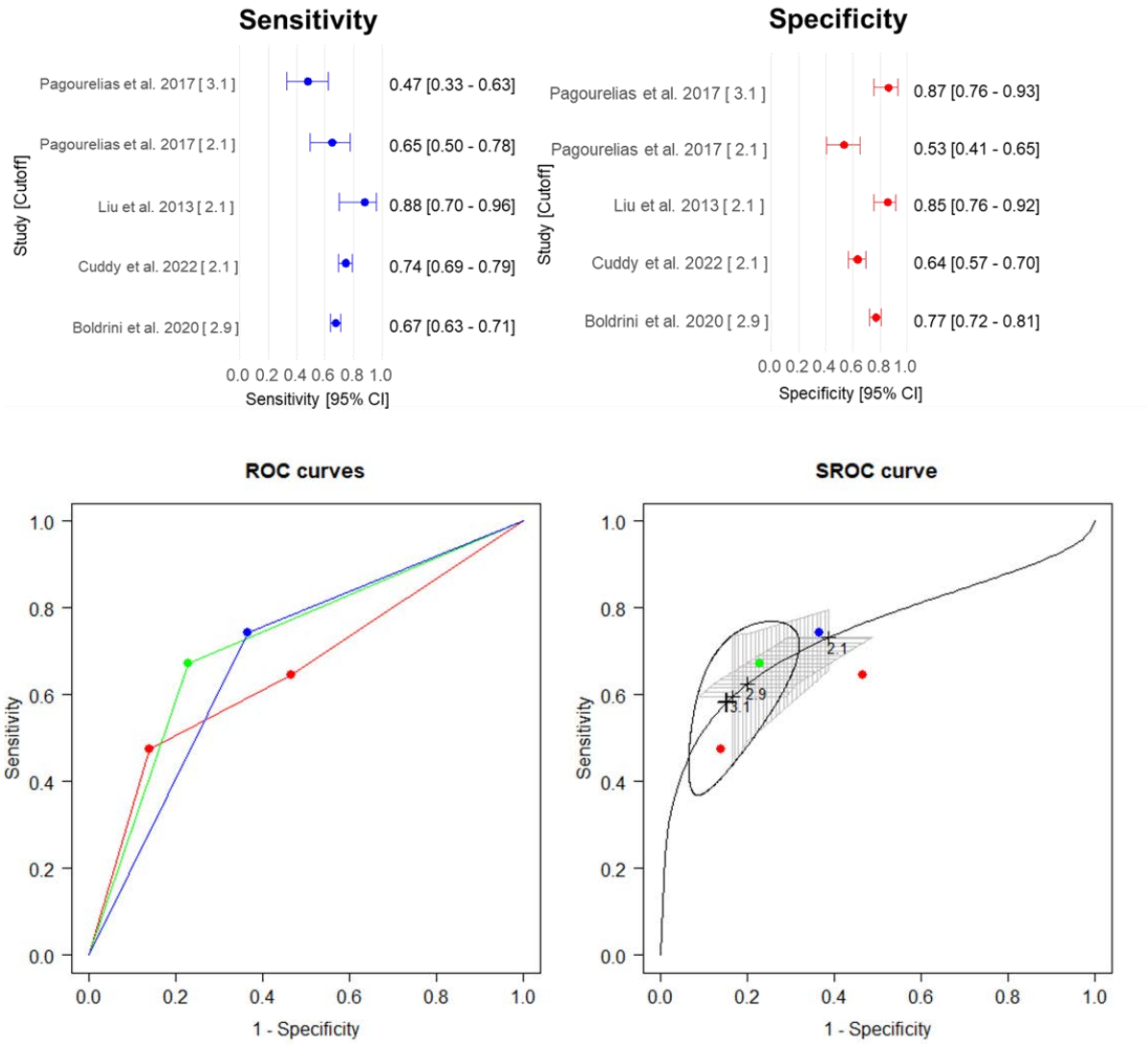
For **RALS**, using the CICS model we identified an optimal cut-off point of 0.909 with an estimated sensitivity of 0.63 (95% CI 0.39 to 0.82) and a specificity of 0.81 (95% CI 0.61 to 0.92) (10 studies, 2,870 participants, 49.5% diseased, AUC: 0.77 [95% CI 0.38 to 0.92], Figure 3). From the analysis, a specificity of 0.95 is achieved at a cutoff value 1.26, where the corresponding sensitivity is 0.38.

Figure 3: RALS



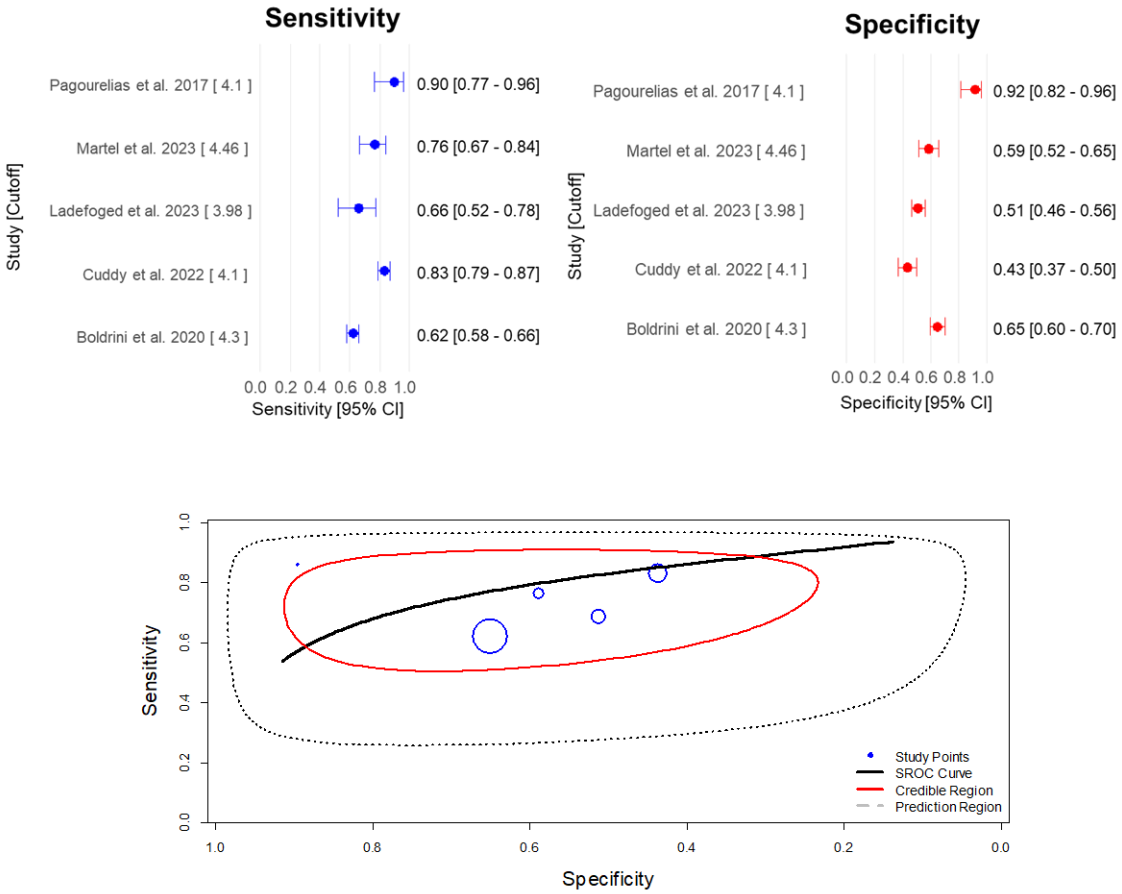
With respect to **SAB**, our model identified an optimal cut-off point of 3.181 with a pooled sensitivity of 0.58 (95% CI 0.41 to 0.74) and a specificity of 0.85 (95% CI 0.72 to 0.92). (3 studies, 1.595 participants, 32.1% diseased, AUC: 0.74 [95%CI 0.57 to 0.86], Figure 4). From the analysis, for achieving a specificity level of 0.95, the cutoff value should be set at 4.25, where the corresponding sensitivity is 0.42.

Figure 4: SAB



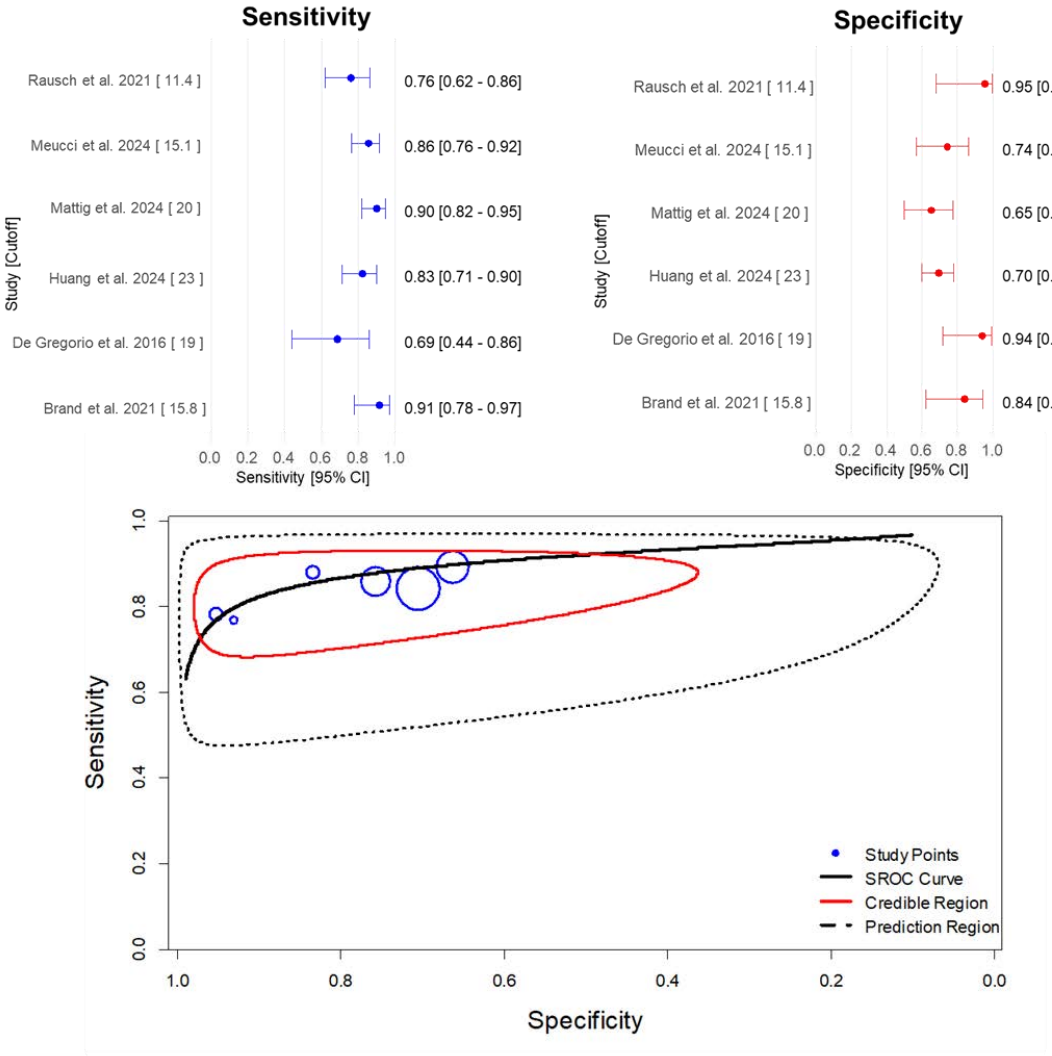
Five studies evaluated **EFSR** for the detection of cardiac amyloidosis (5 studies, 2,297 participants, 1,129 (49.2%) with the target condition, Figure 5). The cutoff points used to define positive results varied across the included studies, ranging from 3.98 to 4.46. Using the HSROC model, the summary curve produced illustrates the expected trade-off between sensitivity and specificity as the threshold varies; For a specificity of 0.95, sensitivity was estimated as 0.78 (95% CI 0.57 to 0.92).

Figure 5: EFSR



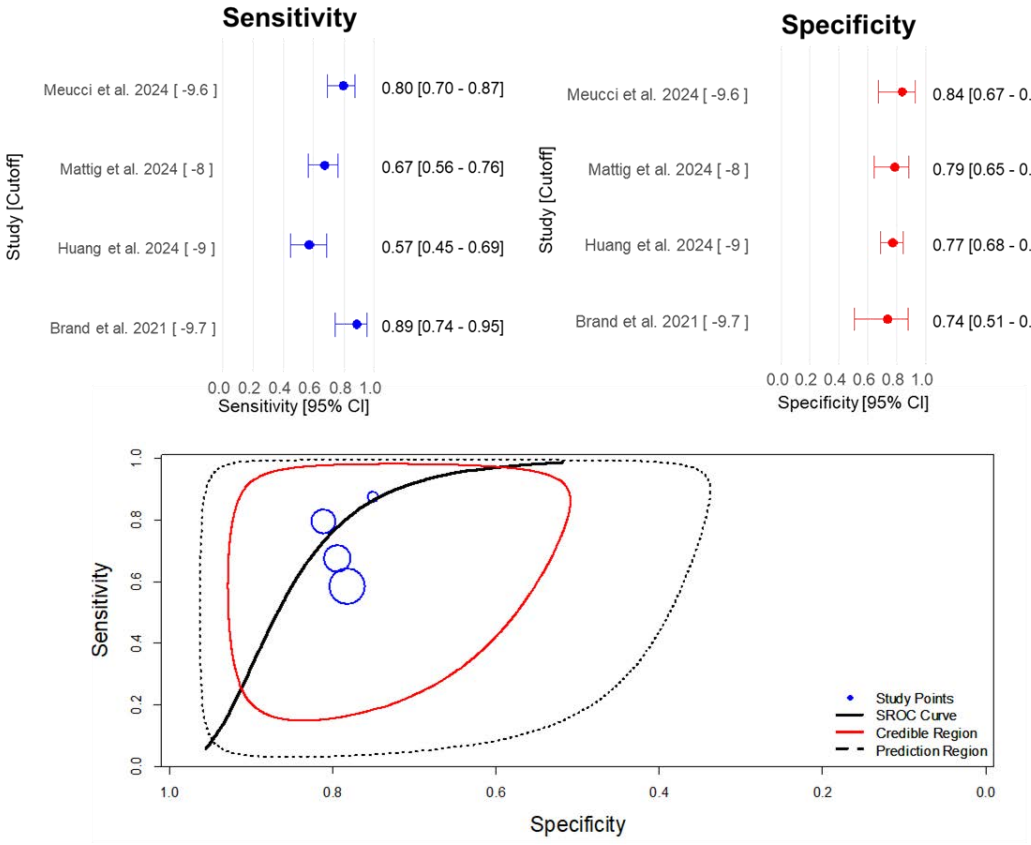
Six studies evaluated **LASr** for the detection of cardiac amyloidosis (6 studies, 594 participants, 322 (54.2%) with the target condition, Figure 6). The cutoff points used to define positive results varied across the included studies, ranging from -11.4 to -23. Using the HSROC model, the summary curve produced illustrates the expected trade-off between sensitivity and specificity as the threshold varies; For a specificity of 0.95, sensitivity was estimated as 0.89 (95% CI 0.73 to 0.99).

Figure 6: LASr



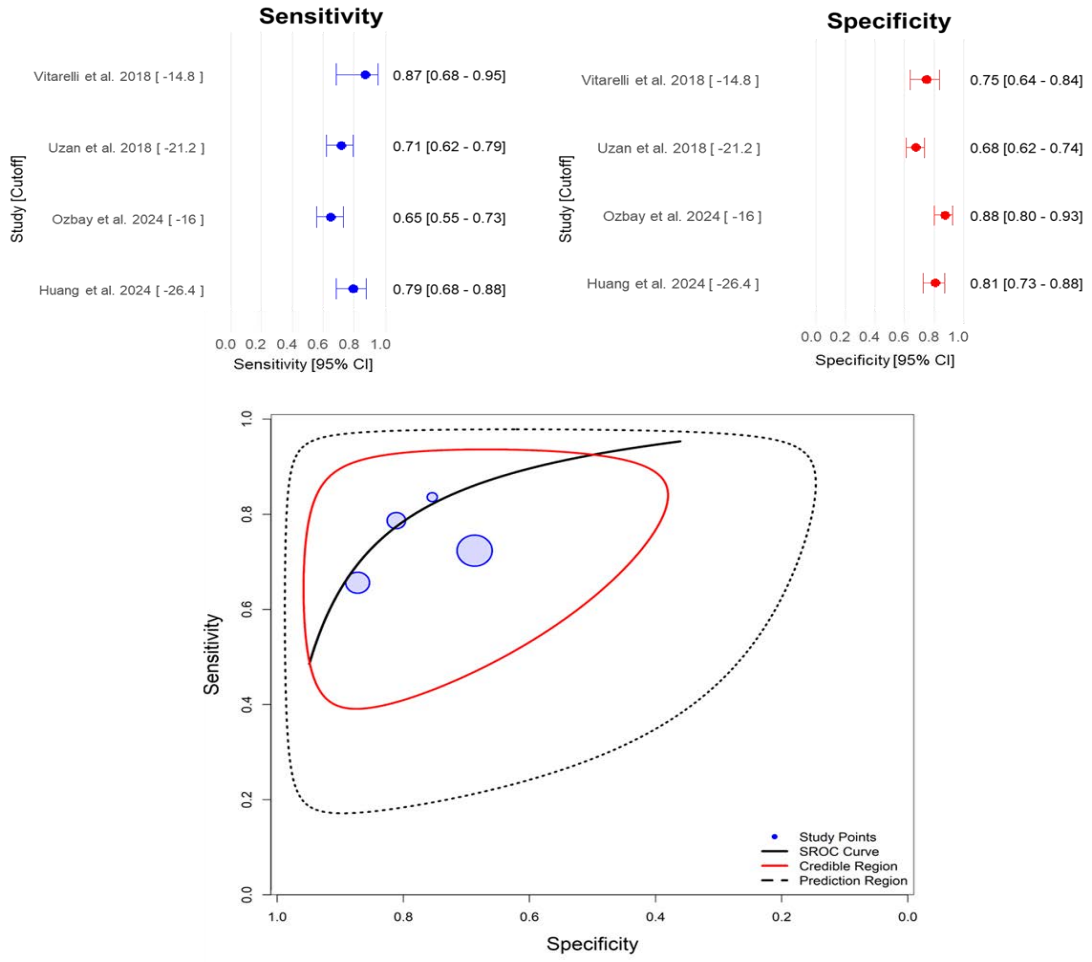
Four studies evaluated **LAScd** for the detection of cardiac amyloidosis (4 studies, 458 participants, 263 (57.4%) with the target condition, Figure 7). The cutoff points used to define positive results varied across the included studies, ranging from -8 to -9.7. Using the HSROC model, the summary curve produced illustrates the expected trade-off between sensitivity and specificity as the threshold varies; For a specificity of 0.95, sensitivity was estimated as 0.85 (95% CI 0.56 to 0.99).

Figure 7: LAScd



Four studies evaluated **RVFWS** for the detection of cardiac amyloidosis (4 studies, 786 participants, 299 (38%) with the target condition, Figure 8). The cutoff points used to define positive results varied across the included studies, ranging from -14.8 to -26.4. Using the HSROC model, the summary curve produced illustrates the expected trade-off between sensitivity and specificity as the threshold varies; for a specificity of 0.95, sensitivity was estimated as 0.78 (95% CI 0.54 to 0.95).

Figure 8: RVFWS



Regarding **EFBSR** we identified only one study presenting a cutoff value of 7.75 with a sensitivity of 0.787 and a specificity of 0.90 (298 participants, 89 (29.9%) diagnosed with cardiac amyloidosis (26) (Figure 12).

We identified 2 studies that evaluated the accuracy of **GWI**. Sensitivity ranged from 0.80 to 0.89, whereas specificity from 0.55 to 0.70 (2 studies, 488 participants, 68 (13.9%) with cardiac amyloidosis; Figure 12).

Regarding **GCW** we identified only one study presenting a cutoff value of 1,541 with a sensitivity of 0.86 and a specificity of 0.70 (166 participants, 83 (50%) diagnosed with cardiac amyloidosis (27) (Figure 12).

Regarding **LASct** we identified one study presenting a cutoff value of -10 with a sensitivity of 0.65 (95% CI 0.53 to 0.76) and a specificity of 0.87 (95% CI 0.79 to 0.92) (165 participants, 63 (38.2%) diagnosed with cardiac amyloidosis) (28)(Figure 13).

We identified 2 studies that evaluated the accuracy of **MCF**. Sensitivity ranged from 0.56 to 0.62, whereas specificity from 0.75 to 0.97 (2 studies, 1,078 participants, 687 (63.7%) with cardiac amyloidosis; Figure 15).

Regarding **RV FAC** we identified only one study presenting a cutoff value of 39 with a sensitivity of 0.65 and a specificity of 0.58 (115 participants, 92 with LVH of which 83 (25%) diagnosed with cardiac amyloidosis (29)(Figure 15).

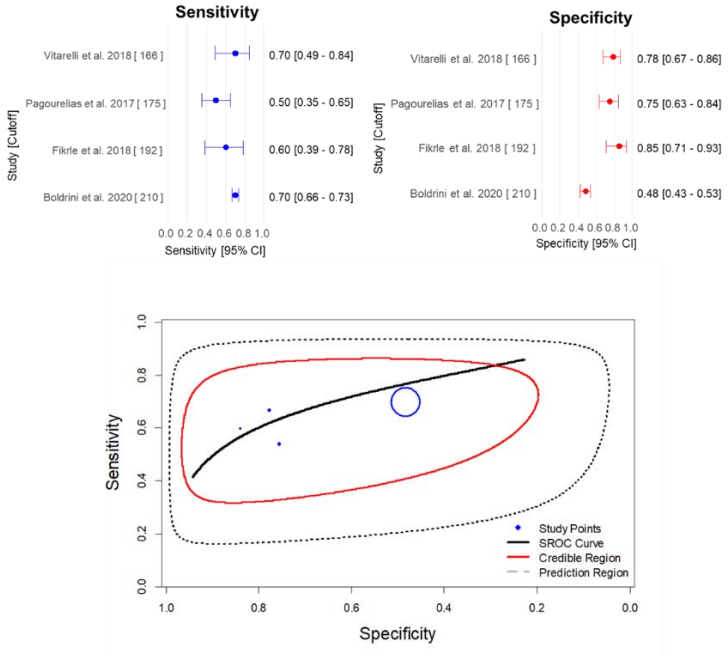
We identified 2 studies that evaluated the accuracy of **RVGS**. Sensitivity ranged from 0.78 to 0.81, whereas specificity from 0.75 to 0.81 (2 studies, 280 participants, 86 (30.7%) with cardiac amyloidosis; Figure 15).

We identified 2 studies that evaluated the accuracy of **A/B ratio**. Sensitivity ranged from 0.71 to 0.74, whereas specificity from 0.63 to 0.82 (2 studies, 519 participants, 73 (14.1%) with cardiac amyloidosis; Figure 16).

Simple 2D echocardiographic markers

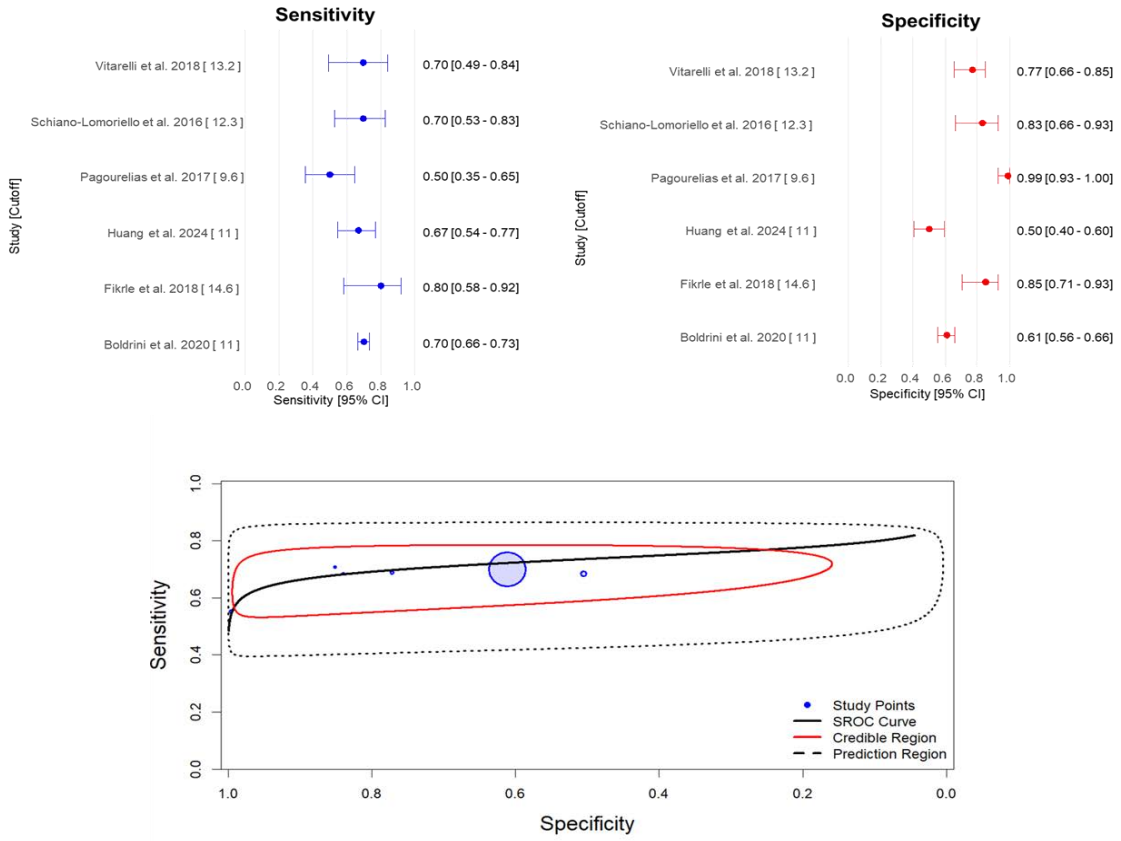
Four studies evaluated **DT** for the detection of cardiac amyloidosis (5 studies, 1,230 participants, 730 (59.4%) with the target condition, Figure 9). The cutoff points used to define positive results varied across the included studies, ranging from 166 to 210. Using the HSROC model, the summary curve produced illustrates the expected trade-off between sensitivity and specificity as the threshold varies; for a specificity of 0.95, sensitivity was estimated as 0.64 (95% CI 0.36 to 0.86).

Figure 9: DT



Six studies evaluated **E/e'** for the detection of cardiac amyloidosis (6 studies, 1,458 participants, 826 (56.7%) with the target condition). The cutoff points used to define positive results varied across the included studies, ranging from 9.6 to 14.6. Using the HSROC model, the summary curve produced illustrates the expected trade-off between sensitivity and specificity as the threshold varies (Figure 10); For a specificity of 0.95, sensitivity was estimated as of 0.72 (95% CI 0.50 to 0.90).

Figure 10: E/e'



We identified 2 studies that evaluated the accuracy of **E/A**. Sensitivity ranged from 0.65 to 0.74, whereas specificity from 0.75 to 0.98 (2 studies, 1,038 participants, 667 (64.3%) with cardiac amyloidosis; Figure 12).

We identified 2 studies that evaluated the accuracy of **LAA**. Sensitivity ranged from 0.68 to 0.77, whereas specificity from 0.45 to 0.78 (2 studies, 1,050 participants, 683 (65.1%) with cardiac amyloidosis; Figure 13).

Concerning **LAVI** we identified only one study presenting a cutoff value of 47 with a sensitivity of 0.436 (95% CI 0.278-0.604) and a specificity of 0.927 (95% CI 0.82-0.98) (100 participants, 40 (40%) diagnosed with cardiac amyloidosis (13)(Figure 13).

For **LAEF** we identified only one study presenting a cutoff value of 36 with a sensitivity of 0.82 and a specificity of 0.80 (127 participants, 34 (26.8%) diagnosed with cardiac amyloidosis (30)(Figure 13).

In relation to **IAS** thickness we identified only one study presenting a cutoff value of 5 with a sensitivity of 0.40 (95% CI 0.28-0.54) and a specificity of 0.53 (95% CI 0.45-0.61) (217 participants, 62 (28.6%) diagnosed with cardiac amyloidosis (31)(Figure 13).

We identified 2 studies that evaluated the accuracy of **IVSd**. Sensitivity ranged from 0.64 to 0.68, whereas specificity from 0.59 to 0.79 (2 studies, 1,079 participants, 670 (62.1%) with cardiac amyloidosis; Figure 13)

As for **LVEDD** we identified only one study that reported a cutoff value of 46 with a sensitivity of 0.65 (95% CI 0.61-0.69) and a specificity of 0.57 (95% CI 0.52-0.63) (978 participants, 647 (66.2%) diagnosed with cardiac amyloidosis (14)(Figure 14).

In regard to **LVEDV** only one study was located, with a cutoff value of 79 with a sensitivity of 0.65 (95% CI 0.61-0.69) and a specificity of 0.52 (95% CI 0.46-0.59) (978 participants, 647 (66.2%) diagnosed with cardiac amyloidosis (14)(Figure 14).

For **LVESV** only one study reported results on test accuracy. Using a cutoff value of 52, a sensitivity of 0.17 (95% CI 0.14-0.21) and a specificity of 0.73 (95% CI 0.68-0.79) were reported (978 participants, 647 (66.2%) diagnosed with cardiac amyloidosis (14)(Figure 14).

Regarding **LVMI** we identified a single study presenting a cutoff value of 141 with a sensitivity of 0.64 (95% CI 0.61-0.68) and a specificity of 0.65 (95% CI 0.59-0.70) (978 participants, 647 (66.2%) diagnosed with cardiac amyloidosis (14)(Figure 14).

We identified 3 studies that evaluated the accuracy of **LVEF**. Sensitivity ranged from 0.641 to 0.742, whereas specificity from 0.51 to 0.70 (3 studies, 1,376 participants, 776 (56.4%) with cardiac amyloidosis; Figure 14).

For **MAPSE** we identified only one study presenting a cutoff value of 11 with a sensitivity of 0.72 (95% CI 0.69-0.76) and a specificity of 0.60 (95% CI 0.55-0.65) (978 participants, 647 (66.2%) diagnosed with cardiac amyloidosis (14)(Figure 14).

For the index test **PWTd** we identified a single study presenting a cutoff value of 13 with a sensitivity of 0.82 (95% CI 0.79-0.85) and a specificity of 0.73 (95% CI 0.68-0.78) (978 participants, 647 (66.2%) diagnosed with cardiac amyloidosis (14)(Figure 15).

Regarding **RWT** we identified only one study presenting a cutoff value of 0.6 with a sensitivity of 0.79 (95% CI 0.76-0.82) and a specificity of 0.72 (95% CI 0.67-0.77) (978 participants, 647 (66.2%) diagnosed with cardiac amyloidosis (14)(Figure 16).

For **RVWT** we identified only one study presenting a cutoff value of 6.4 with a sensitivity of 0.66 and a specificity of 0.61 (115 participants, 23 (20%) diagnosed with cardiac amyloidosis) (29)(Figure 16).

We identified 2 studies that evaluated the accuracy of **TAPSE**. Sensitivity ranged from 0.67 to 0.77, whereas specificity from 0.63 to 0.64 (2 studies, 1,070 participants, 670 (62.6%) with cardiac amyloidosis; Figure 16).

With respect to **SV**, we identified only one study presenting a cutoff value of 42 with a sensitivity of 0.70 (95% CI 0.66-0.74) and a specificity of 0.55 (95% CI 0.49-0.62) (978 participants, 647 (66.2%) diagnosed with cardiac amyloidosis (14)(Figure 16).

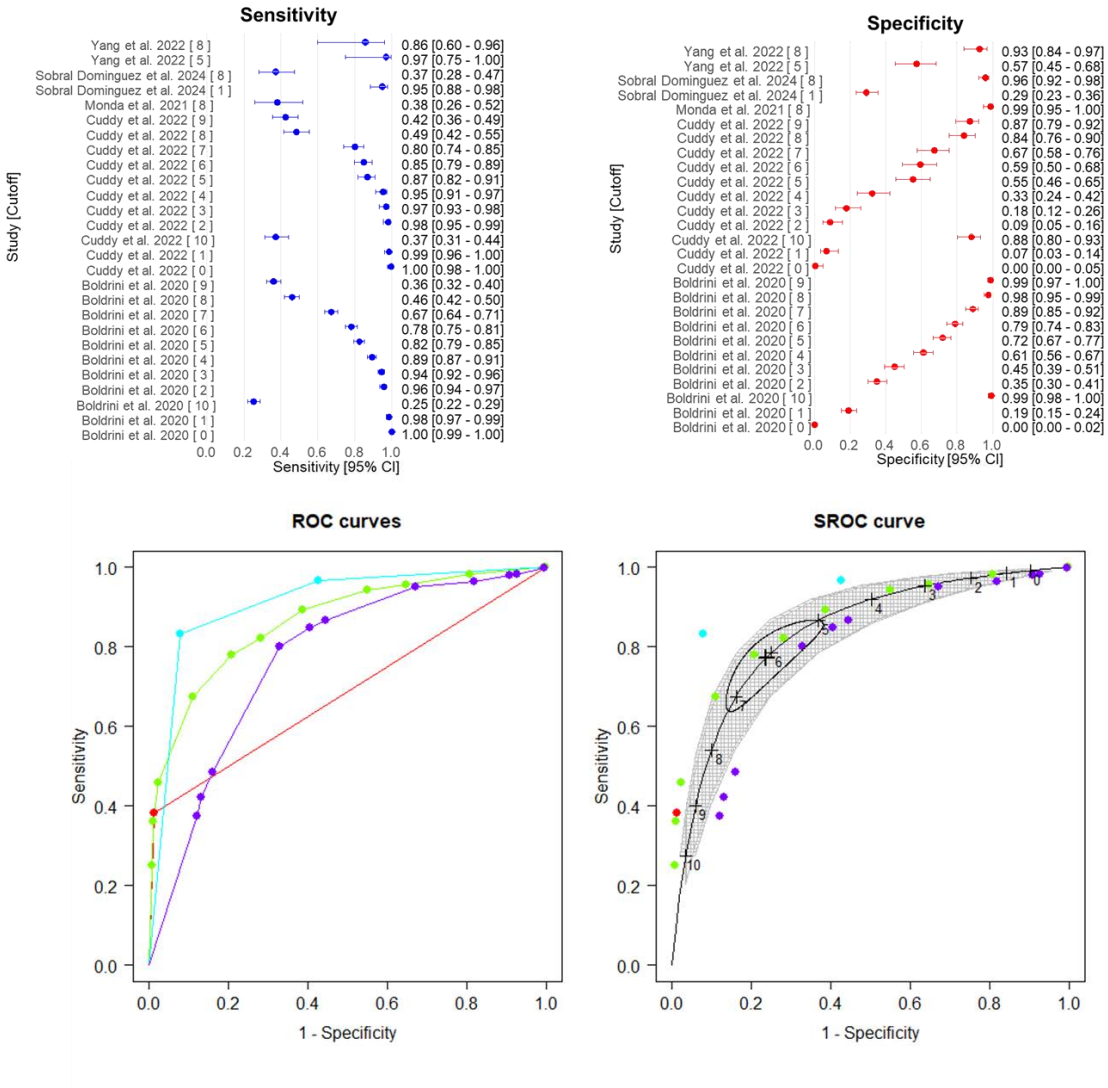
Echocardiographic scores

We identified 2 studies that evaluated the accuracy of the **AMYLI score**. Sensitivity ranged from 0.51 to 0.71, whereas specificity was 0.83 in both studies (2 studies, 393 participants, 199 (50.6%) with cardiac amyloidosis; Figure 12).

For the **IWT score**, our model identified an optimal cut-off point of 6 with a pooled sensitivity of 0.77 (95% CI 0.67 to 0.85) and a specificity of 0.76 (95% CI 0.65 to 0.85). (4 studies, 1,464 participants, 60.5% diseased, AUC: 0.83 [95%CI 0.78 to 0.88], Figure 11).

In order to achieve a specificity of 0.95, the corresponding cutoff value is 9, where sensitivity is 0.35.

Figure 11: IWT score



In the following forest plots (Figures 12-16), the study results for each index test, where only a narrative synthesis was conducted, are summarized.

Figure 12

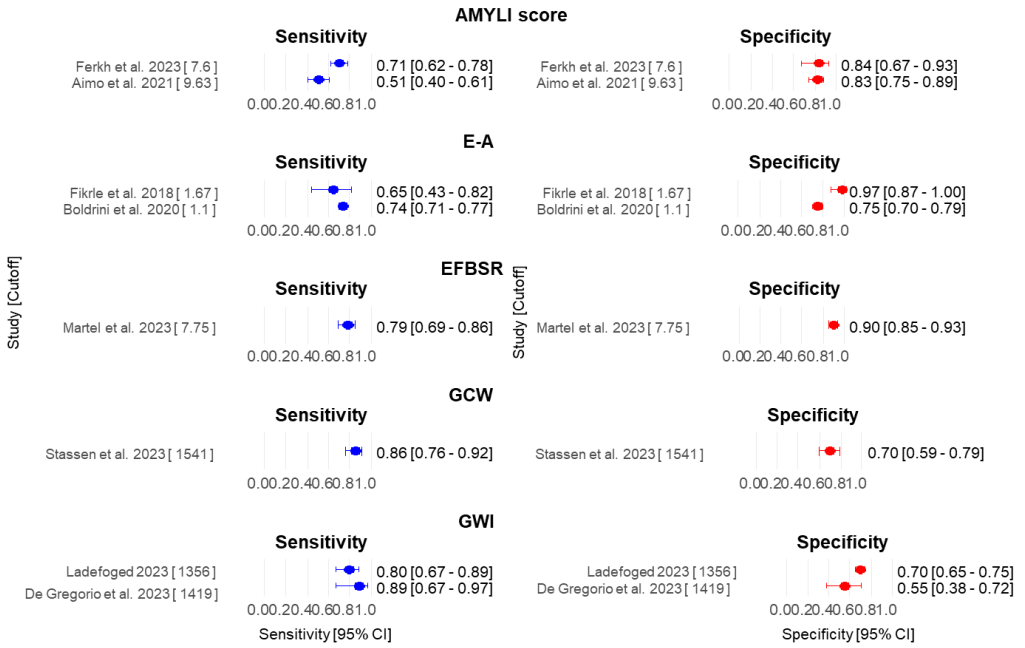


Figure 13

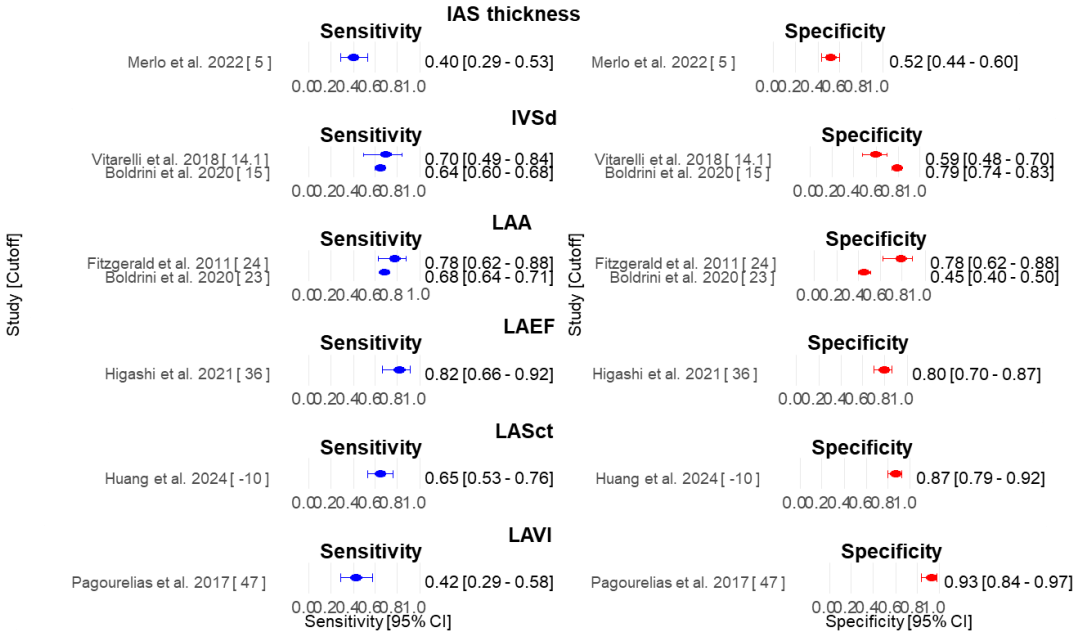


Figure 14

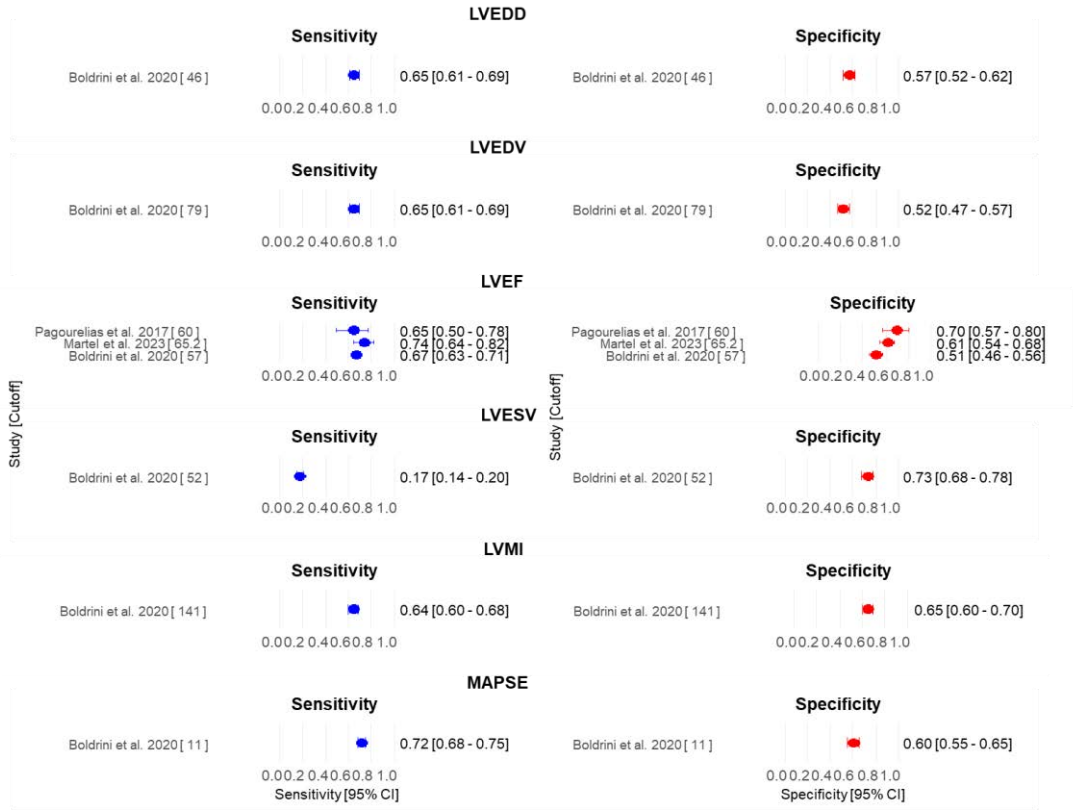


Figure 15

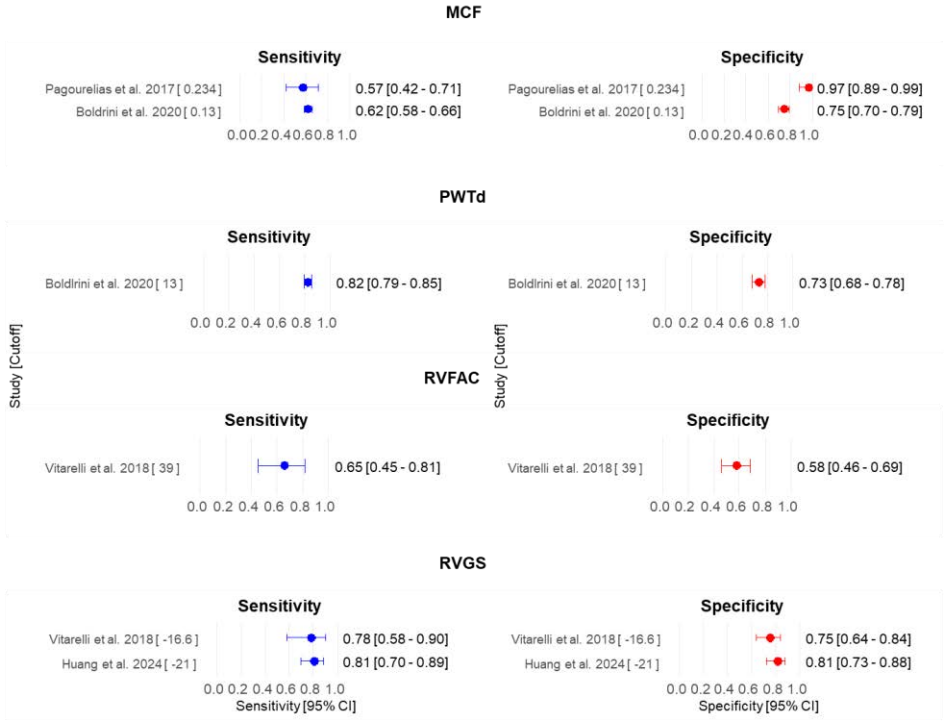
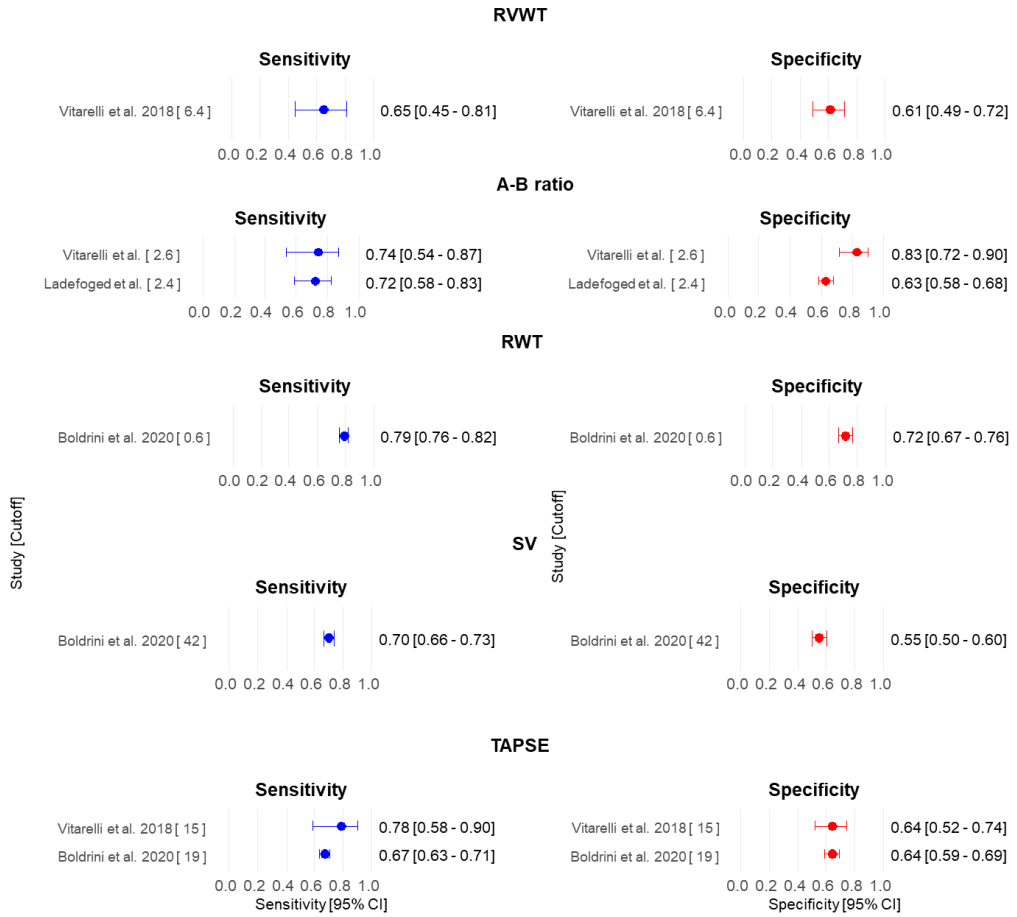


Figure 16



Discussion

This is the first systematic review and meta-analysis on the field summarizing the findings of studies of diagnostic accuracy studies (12–14,26–47), using different statistical approaches in order to use all the available information collected from the extraction of data. It should be emphasized that echocardiography remains a first line approach in the management of patients as it is readily and widely available to every center. As a result, it can be a useful screening tool during the first steps of the diagnostic pathway of cardiac amyloidosis, by ruling out the disease and thus avoiding unnecessary, more expensive or not widely accessible tests (CMR, scintigraphy, biopsy). In the current meta-analysis traditional as well as novel echocardiographic indices were examined, highlighting the importance of other indices rather than the established RALS, EFBSR, SAB. LASr and LAScd presented a higher sensitivity (0.89 and 0.85 respectively) at a specificity 0.95 than the former widely used echocardiographic markers. Furthermore, at a high specificity level, speckle tracking echocardiography markers present greater diagnostic accuracy than traditional 2D echocardiographic markers. At a specificity level of 0.95 sensitivity ranged from 0.42 to 0.89 among different indices, indicating that not a single

echocardiographic marker should be used in the diagnostic pathway of the disease, but rather a combination of different indices.

Conclusion

Our study provides key insights into the diagnostic accuracy of echocardiographic markers and scores in detecting cardiac amyloidosis among patients with cardiac hypertrophy.

Strengths

To our knowledge, this is the first systematic review and meta-analysis to evaluate echocardiographic markers and scores for the diagnosis of cardiac amyloidosis in patients with cardiac hypertrophy, as well as the first meta-analysis of diagnostic accuracy to address this topic. In addition, all studies included in the meta-analysis are relatively recent (from 2010 onward).

For the different index tests explored, our aim was to make use of all available information in its entirety to provide a comprehensive assessment of their diagnostic performance. To this end, we used models that can accommodate multiple thresholds per study and allow estimate the optimal thresholds for maximizing sensitivity and specificity per index test considered.

Limitations

This meta-analysis has also some limitations. First, echocardiography, which serves as an index test in this study, is also part the composite reference standard based on current guidelines. This could introduce incorporation bias and bring over overfitting issues; therefore, a sensitivity analysis could help assess its potential impact on the results.

Then, considerable heterogeneity was observed across studies, likely due to differences in thresholds, and study designs or characteristics. This variability may pose difficulties to the generalizability of the findings and their interpretation.

In addition, several echocardiographic markers were assessed in only one or two studies, making it difficult to derive clear conclusions about their diagnostic accuracy. Future studies with larger sample sizes and standardized thresholds are needed.

Finally, a comparison between the diagnostic markers and scores was not feasible; more advanced statistical approaches, such as meta-regression or network meta-analysis models for diagnostic test accuracy studies would be required for such analysis, but these methods exceed the scope of the current thesis.

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Appendix

A. Search strategy in different databases

PubMed

Concept 1: Cardiac hypertrophy

#1 “Cardiomegaly”[Mesh]

#2Hypertrophy[tw] OR hypertrophic[tw] OR hypetroph*[tw]

#3Cardiomyopath*[tw]

#4 #2 AND #3

#5Ventricle*[tw] OR ventricular [tw]

#6 #2 AND #5

#7 Septum[tw] OR septal[tw]

#8 #2 AND #7

#9 #1 OR #4 OR #6 OR #8

Concept 2: Echocardiographic markers and scores

#10 "Echocardiography"[Mesh] OR “Echocardiograph*”[tw] OR 2D-echocardiography[tw] OR 3D-echocardiography[tw]

Concept 3: Cardiac amyloidosis

#11 "Amyloidosis"[Mesh] OR "Amyloid"[Mesh] OR “cardiac amyloidosis”[tw] OR “transthyretin cardiac amyloidosis”[tw] OR “transthyretin amyloidosis cardiomyopathy”[tw] OR “transthyretin amyloid cardiomyopathy”[tw] OR “ATTR amyloidosis”[tw] OR “wild-type ATTR amyloidosis”[tw] OR “wt-ATTR amyloidosis”[tw] OR “hereditary ATTR amyloidosis”[tw] OR “hereditary transthyretin amyloidosis”[tw] OR “mutant transthyretin amyloidosis”[tw] OR “ATTR cardiac amyloidosis”[tw] OR “senile systemic amyloidosis”[tw] OR “senile cardiac amyloidosis”[tw] OR “immunoglobulin light chain amyloidosis”[tw] OR “light chain amyloidosis”[tw] OR “systemic AL amyloidosis”[tw] OR “AL cardiac amyloidosis”[tw] OR “secondary amyloidosis”[tw] OR amyloid*[tw]

Final search: #9 AND #10 AND #11

Scopus

(TITLE-ABS-KEY ("cardiac hypertroph*" OR "hypertrophic cardiomyopath*" OR "ventricular hypetroph*" OR "septal hypertroph*") AND TITLE-ABS-KEY (echocardiograph*) AND TITLE-ABS-KEY (amyloidosis OR "cardiac amyloidosis" OR " ATTR amyloidosis" OR "AL amyloidosis"))

Web of science

Concept 1: Cardiac hypertrophy

#1 TS= ("cardiac hypertroph*" OR "hypertrophic cardiomyopath*" OR "ventricular hypertroph*" OR "septal hypertroph*")

Concept 2: Echocardiographic markers and scores

#2 TS= echocardiograph*

Concept 3: Cardiac amyloidosis

#3 TS= (“cardiac amyloidosis” OR “transthyretin cardiac amyloidosis” OR “transthyretin amyloidosis cardiomyopathy” OR “transthyretin amyloid cardiomyopathy” OR “ATTR amyloidosis” OR “wild-type ATTR amyloidosis” OR “wt-ATTR amyloidosis” OR “hereditary ATTR amyloidosis” OR “hereditary transthyretin amyloidosis” OR “mutant transthyretin amyloidosis” OR “ATTR cardiac amyloidosis” OR “senile systemic amyloidosis” OR “senile cardiac amyloidosis” OR “immunoglobulin light chain amyloidosis” OR “light chain amyloidosis” OR “systemic AL amyloidosis” OR “AL cardiac amyloidosis” OR “secondary amyloidosis” OR amyloid*)

Final search: #1 AND #2 AND #3

B. Study protocol

Background

The term “amyloidosis” comprises a block of conditions characterized by deposition of misfolded proteins (amyloid fibrils), affecting multiple organs, leading to dysfunction or insufficiency (Rigopoulos et al., 2019).

Target condition being diagnosed

Cardiac amyloidosis (CA) is a serious and progressive infiltrative disorder of the cardiac muscle provoked by the extracellular accumulation of amyloid fibrils. Although considered rare, the disease is rather underestimated, due to the low clinical suspicion

among clinicians about its existence. In fact, cardiac amyloidosis can mimic common clinical conditions in everyday practice, such as heart failure with preserved ejection fraction (HFpEF), aortic stenosis and arrythmias, including atrial fibrillation (Garcia-Pavia et al., 2021). Cardiac involvement is a key prognostic factor, as it significantly increases mortality(1). Although more than 30 proteins have been identified to form amyloid aggregations, only 9 of them can target the heart leading to severe cardiac disease(2).

The two most common types of cardiac amyloidosis are ATTR and AL amyloidosis, caused by the deposition of the misfolded proteins of transthyretin and light-chain immunoglobulin respectively (2) and accounting for approximately 95% of the cases (Gościniak et al., 2022).

Other, more rare types of amyloidosis include serum amyloid A amyloidosis (AA), Apolipoprotein A1 amyloidosis and atrial natriuretic factor amyloidosis (ANF) (4,5). The disease, especially in the form of ATTR, manifests clinically mostly in older adults, with a male predominance (4,6).

Index test

The index tests that will be used are different echocardiographic markers and scores derived from conventional echocardiography and speckle tracking echocardiography.

The following tests will be considered:

- Simple 2D echocardiographic markers: IVSd, PWTd, RWT, LVEDD, LVEDV, LVESV, LVMI, LVEF, LAA, LAVI, LAEF, IAS thickness, MAPSE, TAPSE, SV, E/A, DT, E/e'

- Speckle tracking echocardiographic markers: MCF, GLS, RALS, SAB, A/B ratio, EFSR, EFBSR, LASr, LAScd, LASct, RVWT, RV FAC, RVGS, RVFWS, GWI, GCW

Echocardiographic scores:

- AMYLI score, defined as the product of RWT and E/e' (RWT x E/e')
- IWT score, defined as the combination of RWT, TAPSE (mm), E/e', LS (%), and septal longitudinal systolic apex-to-base ratio (SAB) with the following cut-offs and scoring (Boldrini et al., 2020)(Boldrini et al., 2020):

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

Objectives

The objectives of the current systematic review and meta-analysis will be to examine the diagnostic accuracy of different echocardiographic markers and scores in the diagnosis of cardiac amyloidosis in patients with left ventricular hypertrophy.

Methods

Criteria for considering studies for the reviews

Types of studies

We will include prospective and retrospective cohort studies that provide data on the diagnostic accuracy of the index test. We will include only studies that provide 2x2 tables reporting true positives, false negatives, false positives, true negatives or that provide information from which these data can be derived.

We will exclude non-research articles (reviews, case reports or case series, letters to the editor) as well as other systematic reviews and meta-analyses.

Participants

We will include studies that evaluated the index tests for cardiac amyloidosis described below in adult patients suspected of having the disease, diagnosed with increased ventricular wall thickness, defined as intraventricular septal thickness in end-diastole 12>mm, or posterior wall thickness in end-diastole 12>mm. This spectrum includes patients with cardiac amyloidosis, hypertrophic cardiomyopathy, Anderson-Fabry disease, aortic stenosis, hypertensive heart disease, athlete's heart.

Index test

We will consider the following index tests:
- Simple 2D echocardiographic markers: IVSd, PWTd, RWT, LVEDD, LVEDV, LVESV, LVMI, LVEF, LAA, LAVI, LAEF, IAS thickness, MAPSE, TAPSE, SV, E/A, DT, E/e'

- Speckle tracking echocardiographic markers: MCF, GLS, RALS, SAB, A/B ratio, LASr, LAScd, LASct, EFSR, EFBSR, RVWT, RV FAC, RVGS, RVFWS, GWI, GCW

Echocardiographic scores:

- AMYLI score, defined as the product of RWT and E/e' (RWT x E/e')
- IWT score, defined as the combination of RWT, TAPSE (mm), E/e', LS (%), and septal longitudinal systolic apex-to-base ratio (SAB) with the following cut-offs and scoring (14):

Appendix Table 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

Target condition

We will include reviews targeting any type of cardiac amyloidosis (immunoglobulin light chain amyloidosis - AL, transthyretin cardiac amyloidosis – ATTR or amyloid A amyloidosis – AA, Apolipoprotein A1 amyloidosis, atrial natriuretic factor amyloidosis – ANF).

Reference standard

We will consider three reference standards: endomyocardial biopsy (EMB) and two composite reference standards, based on the ESC Myocardial Working Group position paper (2) and ASNC/AHA/ASE/EANM/HFSA/ISA/SCMR/ SNMMI Expert Consensus Recommendations for Multimodality Imaging in Cardiac Amyloidosis (19).

Endomyocardial biopsy (EMB) still remains the diagnostic gold standard of the disease and for confirmation of the amyloid type, though invasive and undertaken only by specialized centers (1). Amyloid deposits in tissues can be identified histologically using Congo red staining, which reveals characteristic green birefringence under polarized light. The specific type of amyloid protein is determined through laser microdissection combined with mass spectrometry (1).

We define the composite reference standards as follows based on the position paper of Garcia-Pavia et al. and the ASNC/AHA/ASE/EANM/HFSA/ISA/SCMR/SNMMI Expert Consensus Recommendations for Multimodality Imaging in Cardiac Amyloidosis by Dorbala et al.:

c. Extracardiac biopsy positive for amyloid detection in combination with specific echocardiographic or CMR criteria as stated below
d. Cardiac uptake on diphosphonate scintigraphy grade 2 or 3 in combination with exclusion of clonal dyscrasia by negative serum free light chains and negative serum and urine immunofixation (SPIE, UPIE) plus specific echocardiographic or CMR criteria as stated below.

Echocardiographic criteria for the composite diagnosis of CA:

A. Unexplained LV thickness (≥12 mm) plus 1 or 2:

1. Characteristic echocardiography findings (≥2 of a, b, and c have to be present):

a. Grade 2 or worse diastolic dysfunction

b. Reduced tissue Doppler s', e', and a' waves velocities (<5 cm/s)

e. Decreased LV GLS (absolute value < -15%).

2. IWT score ≥8

B. Any of the following features in the absence of alternative causes of LVH:

1. LVWT >12mm
2. Relative apical sparing of global LS ratio >1
3. ≥ Grade 2 diastolic dysfunction

CMR criteria for the composite diagnosis of CA:

Characteristic CMR findings (a and b have to be present or any of the following in the absence of alternative causes of LVH):

a. Diffuse subendocardial or transmural LGE

b. Abnormal gadolinium kinetics (myocardial nulling preceding or coinciding with the blood pool)

c. ECV >0.40% (strongly supportive, but not essential/diagnostic)

For the diagnosis of AL amyloidosis we further define the criteria listed below as stated by Dorbala et al.:

a. Extracardiac biopsy positive for AL amyloidosis AND
b. Typical imaging features as listed above OR
c. Abnormal cardiac biomarkers (NT-proBNP, Troponin) when alternative causes for abnormal values have been excluded

Search methods for identification of studies

Electronic searches

We will search the following databases: MEDLINE (Pubmed), SCOPUS (Elsevier) and Web of Science, using a corresponding search strategy for each database, up to September 2024, using the search strategy provided in the Appendix.

Searching other resources

We will also examine the European Society of Cardiology conference proceedings, using the term "cardiac amyloidosis".

Data collection and analysis

Selection of studies

Two review authors will screen the records based on title and abstract. Any disagreements will be resolved through discussion or, if necessary, by consulting a third investigator. We will then retrieve full-text articles of the selected records, which will be independently reviewed by two authors. Any disagreements will be resolved in the same manner, as previously described. We will contact corresponding authors in order to clarify any missing information or piece of data needed. The reasons for excluding studies will be recorded and clearly outlined. The entire process will be appropriately depicted in a PRISMA flow diagram(20).

Data extraction and management

We will design a data extraction form. Two independent authors will perform the data extraction, and any discrepancies will be resolved through discussion or by a third reviewer as needed. The data extraction items will include the following:

Study details: First author, year of publication, country where the study was performed, study design, follow-up time

Study participants: details about participants screened, including total number, number of participants with CA, number and subcategories of participants without CA, baseline characteristics (age, male sex, BMI, BSA, systolic and diastolic blood pressure, eGFR, NT-proBNP, echocardiographic measurements)

Target condition: cardiac amyloidosis, type of amyloidosis (transthyretin cardiac amyloidosis – ATTR, light chain amyloidosis – AL, Amyloid A amyloidosis – AA amyloidosis.

Reference standards: type of reference standard/diagnostic method used

Index test: details of index test, including time from index to reference standard, thresholds

Outcomes: data from 2x2 tables (TP, FN, FP, TN), cut-off values, sensitivity and specificity for each index test, number of missing or unavailable results.

Assessment of methodological quality

We will assess the methodological quality of each study using the QUADAS-2 assessment tool (25). This process will be performed by two independent reviewers, who will separately assess risk of bias and applicability concerns of each study.

Statistical analysis and data synthesis

We will conduct separate analyses for each individual index test and target condition under consideration. All reference standards will be treated as equivalent. We will estimate sensitivity and specificity from each included study along with 95% confidence interval (CI) and present the estimates on forest plots and in receiver operating characteristic (ROC) space.

We anticipate that different thresholds will have been used for each index test, and we expect that each study will report results for multiple cutoff points. For index tests reporting results at multiple cutoff points, we will apply the models proposed by Steinhauser et al. for modelling multiple thresholds in meta-analysis of diagnostic test accuracy studies, to incorporate all available data and obtain estimates of summary sensitivity and specificity at the cutoff point, where optimal performance can be expected (21). We will perform such analyses using the 'diagmeta' package in R (22).

For index tests where each study provides results for a single threshold, and there is variation in threshold used for test positivity across studies, we will use the HSROC model, suggested by Rutter and Gatsonis to estimate a summary curve (23). We will conduct these analyses within a Bayesian framework using the 'rjags' package in R.

Investigations of heterogeneity

We will visually assess heterogeneity in sensitivity and specificity estimates for each test by examining forest plots and SROC plots.

Assessment of reporting bias

We will not conduct a formal assessment of reporting bias using funnel plots or tests for funnel plot asymmetry, as these methods are not recommended for diagnostic test accuracy reviews (24). Instead, to minimize the risk of publication bias, our search strategy includes reaching out to experts and relevant organizations to identify unpublished and ongoing studies derived from the ESC congresses registrations

C. Tables

Appendix table 2: Baseline characteristics

Author	Year	Study design	Population	Follow-up (y)	Ix for CA	Total pts	CA	Type(n)	Non-CA	HCM	FD	AS	HTN	Age (y)	Male, n(%)
Aimo et al.	2021	Retrospective	Increased LVWT	11	CMR, Biopsy, scintigraphy	251	111	ATTR (79)	140	11	N/A	16	33	81±5.9 N/A	123 (67) N/A
Boldrini et al.	2020	Prospective	Increased LVWT	10	CMR, Biopsy, scintigraphy	1187	647	ATTR (339) AL (308)	331	57	3	30	183	72±11 71±12	485 (75) 215 (65)
Brand et al.	2021	Retrospective	Unclear LVH	7	Biopsy	54	35	ATTR (14) AL (20) AA (1)	19	12	N/A	N/A	2	65.5±12 53.5±14.5	20 (57) 10 (53)
Cuddy et al.	2022	Retrospective	Referral at amyloidosis centers	10	Scintigraphy+absence of MP	598	324	ATTR (324)	274	N/A	N/A	N/A	N/A	76±7.5 69.2±14.9	283 (87.1) 186 (67.6)
De Gregorio et al.	2023	Prospective	Patients with ATTR amyloidosis	1	CMR, scintigraphy	83	32	ATTR (32)	51	29	N/A	N/A	22	70±11 54.9±13.3	23 (72) 34 (66.7)
Ferkh et al.	2023	Retrospective	Patients with CA, FD, HTN	13	Biopsy, scintigraphy	209	120	ATTR (58) AL(62)	89	N/A	31	N/A	58	68.3±12 62.1±15.2	95 (79.2) 62 (69.7)
Fikrle et al.	2018	Retrospective	Patients with AL-CA, FD, HTN	2	Biopsy	60	20	AL (20)	40	N/A	20	N/A	20	64±12 58±12.3	14 (70) 28 (70)
Higashi et al	2021	Retrospective	Patients with CA, HCM	N/A	Biopsy	127	34	N/A	93	93	N/A	N/A	N/A	75.3±9.3 65.3±12.8	26 (76) 66 (71)
Fitzgerald et al	2011	Retrospective	Patients at Cleveland Clinic, USA	4	Biopsy	72	36	N/A	36	N/A	N/A	N/A	N/A	54 56	22 (61) 23 (64)
Huang et al	2024	Retrospective	Patients with AL-CA, HCM, HTN	11	Biopsy	198	63	AL (63)	135	51	N/A	N/A	51	59±7 54±11.1	42 (66.7) 80 (78.4)

Appendix table 2: Baseline characteristics (continued)

Author	Year	Study design	Population	Follow-up (y)	Ix for CA	Total pts	CA	Type (n)	Non-CA	HCM	FD	AS	HTN	Age (y)	Male, n (%)
Ladefoged et	2023	Retrospective	Patients with ATTR+AS, AS only	3	Biopsy, scintigraphy+absence of MP	566	212	ATTR (212)	354	N/A	N/A	354	N/A	81.7±6.2 79.7±6.8	190 (89.6) 170 (48)
Martel et al.	2023	Retrospective	CA, other hypertrophic CM	2	N/A	298	89	N/A	209	137	N/A	52	20	74.5±10.6 57.3±20	72 (80.9) 144 (68.9)
Mattig et al.	2024	Retrospective	CA, FD	3	CMR, Biopsy, Scintigraphy	135	88	ATTR (80)	47	N/A	47	N/A	N/A	78.7±9 52.3±13	67 (79) 25 (53)
Merlo et al.	2022	Prospective	Patients >55 yo, echo suggestive of CA	2	CMR, Biopsy, Scintigraphy	217	62	ATTR (51) AL (11)	155	N/A	N/A	N/A	N/A	76.7±10.6 73.7±11.2	41 (61.1) 81 (52)
Meucci et al.	2024	Prospective	ATTR-CA, FD	2.5	CMR, Scintigraphy+absence of MP	114	83	ATTR (83)	31	N/A	31	N/A	N/A	77±9.1 57.7±15.5	69 (83) 21 (68)
Monda et al.	2021	Retrospective	Increased LVWT	2	Biopsy, scintigraphy+absence of MP	152	50	ATTR (26) AL (24)	102	52	N/A	25	25	69.3±14.1 60.1±16.2	34 (68) 63 (62)
Ozbay et al.	2024	Retrospective	Suspicion of CA	N/A	CMR, Biopsy, Scintigraphy	214	108	ATTR (108)	106	17	N/A	14	75	77.9±9.1 70.3±14.2	96 (89) 65 (61)
Pagourelias et al.	2017	Retrospective	CA, HCM, HTN	10	Biopsy	100	40	ATTR (14) AL (26)	60	40	N/A	N/A	20	65.5±10.8 60.8±12.9	26 (65) 50 (83)
Phelan et al.	2012	Retrospective	Suspicion of CA, increased LVWT	1.5	CMR, Biopsy, Echocardiography	85	55	N/A	30	N/A	N/A	N/A	N/A	68±10 61±16	47 (85) 21 (70)

Appendix table 2: Baseline characteristics (continued)

Author	Year	Study design	Population	Follow-up	Ix for CA	Total pts	CA	Type (n)	Non-CA	HCM	FD	AS	HTN	Age (y)	Male, n (%)
Rausch et al.	2021	Retrospective	Cases of CA diagnosed at one center	15	Biopsy, Scintigraphy, laboratory findings	79	44	ATTR (33) AL (11)	35					76.4±24.2	35 (79.5)
									35	N/A	N/A	N/A	25	65±34	22 (88)
Schiano-Lomoriello et al.	2016	Prospective	Primary CA, HTN, controls	N/A	Biopsy+echocardiography	93	33	AL (33)						62±11.9	22 (67)
									60	N/A	N/A	N/A	30	60±8.1	20 (67)
Stassen et al.	2023	Retrospective	CA, HCM	17	CMR, Biopsy, Scintigraphy	166	83	N/A						59±12	N/A
									83	83	N/A	N/A	N/A	59±12	N/A
Uzan et al.	2018	Retrospective	AL-CA, HCM, HTN	3.5	CMR, Biopsy, Scintigraphy	315	105	AL (105)						67.7±10.8	72 (68.5)
									210	105	N/A	N/A	105	63.7±14.6	131 (62.4)
Vitarelli et al.	2018	Prospective	AL-CA, HCM, HTN, ATHL	3	Biopsy, monoclonal protein+echo	115	23	AL (23)						62.1±10.6	12 (52)
									92	23	N/A	N/A	23	48.7±18.1	36 (52)
Yang et al.	2022	Prospective	Suspected HF	3	Scintigraphy+absence of MP	82	14	ATTR (14)						N/A	N/A
									68	N/A	N/A	N/A	N/A	N/A	N/A
De Gregorio et al.	2016	Retrospective	ATTR-CA, HCM	2	CMR, Scintigraphy+absence of MP	47	16	ATTR (16)						57.7±9.8	13 (81)
									31	16	N/A	N/A	N/A	57.6±19.9	12 (75)
Sobral-Dominguez et al.	2024	Retrospective	Increased LVWT	N/A	CMR, Biopsy, Scintigraphy	295	97	ATTR (97)						N/A	N/A
									198	70	N/A	128	N/A	N/A	N/A
Suzuki et al.	2023	Retrospective	LVH	3,5	Biopsy	32	9	ATTR (9)						N/A	N/A
									23	14	N/A	N/A	9	N/A	N/A

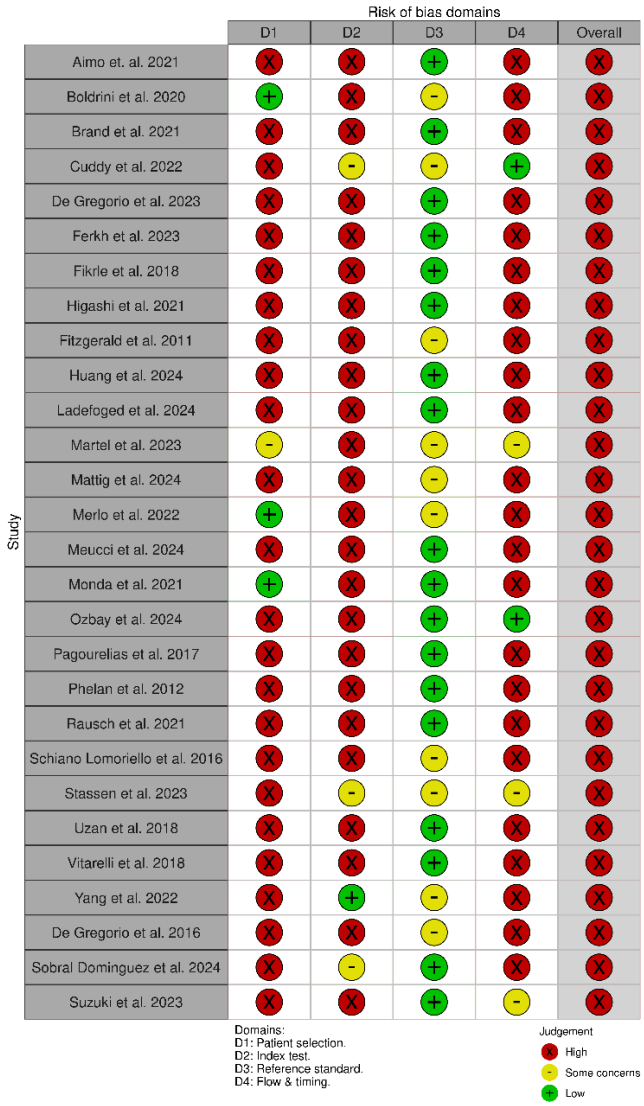
Author	Group (n)	LVEF (%)	IVSd (mm)	PWTd (mm)	LAVI (ml/m2)	E/A	E/e'	Mitral DT (ms)	GLS (%)	SAB	RALS	EFSR	LASr (%)	LAScd (%)
Aimo et al.	LVH (184)	45,7±14,9	N/A	N/A	N/A	N/A	12,8±5,5	N/A	-12±6	1,3±0,9	N/A	N/A	N/A	N/A
Boldrini et al.	CA (647)	51±13	16,4±2,7	15,9±2,7	N/A	2,1±1,2	16±8	186±66	-11±4	6,1±7	1,26±0,55	4,9±1,7	N/A	N/A
	LVH (331)	55±13	14±2,1	12,7±2,3	N/A	1,2±2,2	12±7	194±106	-15±5	2,9±1,9	0,81±0,35	4,1±1,4	N/A	N/A
Brand et	CA (35)	50,2±8,6	17,7±2,9	17±3,1	44,5±12,3	2,8±2,1	18,1±5,6	N/A	-10,6±3,5	N/A	1,37±0,94	N/A	9,7±5,2	-6,5±3,5
	LVH (19)	52,5±7,7	17,9±4,3	16,6±5,1	45,7±16,3	1,2±0,7	13,2±5,3	N/A	-12,8±3,7	N/A	0,86±0,29	N/A	22,7±7,8	-13,9±5,2
Cuddy et al.	CA (324)	48,2±11,7	17,1±3,2	16,5±3,4	46,4±13,6	2,2±1,4	18±6,6	183±53	-8,4±2,9	4,5±4,8	0,9±0,3	5,8±1,8	N/A	N/A
	LVH (274)	51,5±12,5	13,8±3,6	12,6±3,1	47,0±21,1	1,1±0,8	15,2±7,2	192±61	-11,6±4,1	2,2±2,1	0,6±0,2	4,7±1,5	N/A	N/A
De Gregorio et al.	CA (32)	49±10	N/A	N/A	47±13	N/A	17±6	N/A	-12±4	N/A	N/A	N/A	N/A	N/A
	LVH (51)	62,3±6,5	N/A	N/A	38,7±18,4	N/A	8,5±3,7	N/A	-16,2±4,1	N/A	N/A	N/A	N/A	N/A
Ferkh et al.	CA (120)	53,7±7,5	N/A	N/A	49,3±15	1,25±0,79	15,9±6,7	N/A	-12,7±3,9	N/A	0,88±0,29	4,19±1,12	N/A	N/A
	LVH (89)	60,6±4,7	N/A	N/A	33,3±10	0,98±0,33	10,5±3,1	N/A	-17,9±2,8	N/A	0,64±0,13	3,43±0,55	N/A	N/A
Fikrle et al.	CA (20)	59±9	15,5±2,2	N/A	43±9	2,61±1,60	22,1±10,1	187±50	N/A	N/A	1,23±0,64	N/A	N/A	N/A
	LVH (40)	64±8	15,1±2,3	N/A	42,5±11,6	1,01±0,3	10,8±4,4	244±53,5	N/A	N/A	0,75±0,2	N/A	N/A	N/A
Higashi et al.	CA (34)	52,7±10,1	14,3±3,87	12,3±3,87	50±20,9	1,5±0,9	25±8,5	N/A	-9,3±3,87	N/A	N/A	N/A	10±6,2	N/A
	HCM (93)	66,7±8,3	14±4,52	9,7±2,26	40,7±13,6	0,9±0,4	11,7±1,5	N/A	-14±4,52	N/A	N/A	N/A	14,3±5,3	N/A
Fitzgerald et al.	CA (36)	54	16,9	15,7	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	HTN (36)	56	16,8	15,4	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Huang et al.	CA (63)	52±11,4	15±3	14,3±2,3	35,6±10,9	0,98±0,44	13,3±5,7	N/A	-12,4±4,9	N/A	0,71±0,17	N/A	14,7±9,9	-7,7±4,6
	LVH (102)	59,5±6,9	16,9±3,8	12,2±2,1	33,5±11	0,84±0,33	11,7±4,3	N/A	-16,3±3,8	N/A	0,72±0,2	N/A	27,5±7,4	-12±5,5
Ladefoged et al.	CA (212)	47,8±10,6	16,7±3,0	14,1±3,1	46,6±17	1,5±1,1	12,4±4,8	N/A	-11,3±3,6	N/A	1,2±0,5	4,3±1,03	N/A	N/A
	AS (354)	50,9±11,9	14,2±2,2	12,8±2,5	42,5±15,6	0,9±0,5	13,9±5,7	N/A	-13,0±4,0	N/A	0,9±0,3	4±0,8	N/A	N/A
Martel et al.	CA (89)	56,2±12,8	18,3±3,5	15,1±3,5	52,1±18,3	N/A	N/A	N/A	-10,5±3,4	N/A	2,5±1,2	5,8±1,8	N/A	N/A
	LVH (209)	66,1±11,8	17±5,7	11,7±2,9	41,7±23,8	N/A	N/A	N/A	-14,8±4,4	N/A	1,3±0,4	4,9±2,3	N/A	N/A
Mattig et al.	CA (88)	50±9	18±4	N/A	48±15,1	1,8±1,4	16±7,5	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	FD (47)	57±8	15±4	N/A	40±17,6	1,1±0,5	9,3±3,8	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Merlo et al.	CA (62)	57,2±12,2	N/A	N/A	N/A	N/A	15,4±5	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	LVH (155)	58±6,7	N/A	N/A	N/A	N/A	13,3±5,2	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Meucci et al.	CA (83)	54±12,8	N/A	N/A	N/A	1,5±1,1	17,5±5,2	N/A	12,8±4,5	N/A	N/A	N/A	8,9±8,5	5,1±3,7
	FD (31)	61,7±9,3	N/A	N/A	N/A	0,98±0,36	11,2±5,8	N/A	15,6±4,1	N/A	N/A	N/A	21,3±10,9	14,1±7,3
Monda et al.	CA (50)	50,7±8,9	16,9±3,5	15,4±3,4	N/A	N/A	18,1±8,4	N/A	10,5±3,3	3,8±3,1	N/A	N/A	N/A	N/A
	LVH (102)	59,8±7,2	16,6±4,5	12,4±1,8	N/A	N/A	11,7±5,1	N/A	16,9±4,5	1,4±0,6	N/A	N/A	N/A	N/A
Ozbay et al.	CA (108)	48,1±12,4	N/A	N/A	42,8±12,9	N/A	19,5±10,0	N/A	-9,8±3,2	N/A	1,2±0,4	-5,2±1,8	N/A	N/A
	LVH (106)	54±9,9	N/A	N/A	48,1±20,8	N/A	15,2±6,4	N/A	-13,3±3,7	N/A	0,7±0,3	-4,3±1,1	N/A	N/A
Pagourelias et al.	CA (40)	56±12,7	17,6±3,9	15±3,2	41±17	2±1,4	18,4±11	188,3±51	-11±4,1	3,7±3,3	0,97±0,3	5,5±1,5	N/A	N/A
	LVH (60)	63,7±8,6	16,5±4	11,1±2	35,4±16,8	1,02±0,4	7,9±3,3	211,6±61,5	-18,3±2,6	2,5±1,8	0,73±0,17	3,5±0,5	N/A	N/A
Phelan et al.	CA (55)	47±12	N/A	N/A	39,3±10,1	2,20±1,1	24,1±12,7	183±45	-8,9±3,7	N/A	N/A	N/A	N/A	N/A
	LVH (30)	55±13	N/A	N/A	42,2±13,9	1,22±0,65	17,8±8,4	226±66	-14,9±4,4	N/A	N/A	N/A	N/A	N/A

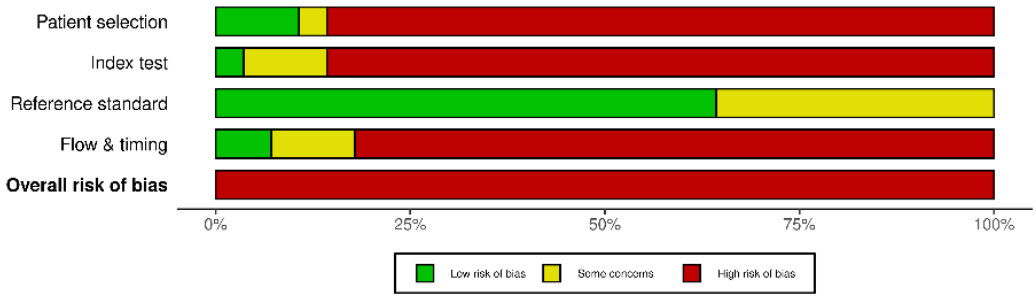
Rausch et al.	CA (44)	48,8±12,6	17±3,4	15,3±3,2	56,3±17,8	1,8±1,3	21,6±8,1	194,5±79	N/A	N/A	N/A	N/A	10,1±7,4	6,7±4,1
	HTN (25)	60±5,9	14,4±2	14,2±3	41±12	1,05±0,4	13,2±5,7	200±45	N/A	N/A	N/A	N/A	24,8±6,4	13,1±3,9
Schiano-Lomoriello et al.	CA (33)	57,5±7,9	12,8±1,0	10,7±1,6	36,5±12,3	1,35±0,90	15,3±5,6	213,3±64,2	-14,9±5,4	N/A	N/A	N/A	N/A	N/A
	HTN (30)	57,8±5,6	12,2±1,6	10,7±1,3	37,5±8,7	1,03±0,32	11,1±3,0	214,7±34,7	-16,9±2,9	N/A	N/A	N/A	N/A	N/A
Stassen et al.	CA (83)	53±13	17±3	N/A	45±16	N/A	17,7±9	N/A	12±5	N/A	N/A	N/A	N/A	N/A
	HCM (83)	63±13	17±3	N/A	36±14	N/A	13±6.8	N/A	14±5	N/A	N/A	N/A	N/A	N/A
Uzan et al.	CA (105)	58,2±11,8	14,7±2,4	13,9±3,6	36,3±12,4	1,63±1,1	15,3±7,6	N/A	-13,38±5,3	N/A	N/A	N/A	N/A	N/A
	LVH (210)	59,9±10,8	15,7±3,1	11,6±2,8	35,1±13,6	1,16±0,7	11,3±5,2	N/A	-14,95±4,3	N/A	N/A	N/A	N/A	N/A
Vitarelli et al.	CA (23)	62±7	14,8±1,7	13,4±1,8	N/A	N/A	16,4±2,9	151±43	-12,8±2,3	N/A	N/A	N/A	N/A	N/A
	LVH (69)	65±5,95	14,3±2,3	12,8±1,5	N/A	N/A	9,6±5,2	242±53,1	-16,5±4,2	N/A	N/A	N/A	N/A	N/A
Yang et al.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
De Gregorio et al.	CA (16)	56±9	16,4±2,8	13,3±2,3	38,0±10,6	1,35±0,71	15,6±7,6	151,4±56,9	-11,5±3,2	N/A	N/A	N/A	14,1±4,7	N/A
	HCM (16)	68±8	18,7±2,8	10,7±2,3	37,5±13,4	1,07±0,59	10,4±4,1	205,7±59,8	-13,0±3,4	N/A	N/A	N/A	20,0±5,6	N/A
Sobral-Dominguez et al.	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
Suzuki et al.	CA (9)	53,6±7,9	16,9±4,0	15,9±3,9	62,6±28,5	N/A	20,9±14,5	N/A	13,3±4,0	N/A	0,96±0,22	N/A	N/A	N/A
	LVH (23)	55,5±7	15±3	11,7±2,2	45,3±22,2	N/A	17,2±7,5	N/A	15,2±3,2	N/A	0,7±0,2	N/A	N/A	N/A

Appendix table 3: Echocardiographic measurements. AS: Aortica Stenosis, CA: Cardiac amyloidosis, FD: Fabry Diseease, HCM: Hypertrophic Cardiomyopathy, HTN: Hypertension, LVH: Left Ventricular Hypertrophy, N/A: Not Applicable

D. Figures

Appendix Figure A: Risk of bias





Appendix Figure B: Applicability concerns