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RESEARCH ARTICLE

Evaluation of the 2021 ESC recommendations for family screening in hereditary transthyretin cardiac amyloidosis

Steven A. Muller[1,2,3], Belén Peiró-Aventin[3,4], Giulia Biagioni[5], Giacomo Tini[6], Giulia Saturi[3,7,8], Christina Kronberger[9], Anouk Achten[10], Stephan Dobner[11,12], Wouter P. te Rijdt[3,13], Alessio Gasperetti[14], Anneline S.J.M. te Riele[1,3], Guerino G. Varrà[3,15], Alberto Ponziani[3,7,8], Alexander Hirsch[16,17], Aldostefano Porcari[3,15], Manon G. van der Meer[1,3], Mattia Zampieri[5], Pim van der Harst[1,3], Andreas Kammerlander[9], Elena Biagini[3,7], J. Peter van Tintelen[2,3,18], Emanuele Barbato[6], Folkert W. Asselbergs[3,19,20,21], Silvia Menale[5], Christoph Gräni[11], Marco Merlo[3,15], Michelle Michels[3,16], Christian Knackstedt[3,10], Christian Nitsche[9], Simone Longhi[3,7], Beatrice Musumeci[6], Francesco Cappelli[5], Pablo Garcia-Pavia[3,4,22,23], and Marish I.F.J. Oerlemans[1,3]*

[1]Department of Cardiology, University Medical Center Utrecht, Utrecht, The Netherlands; [2]Netherlands Heart Institute, Utrecht, The Netherlands; [3]Member of the European Reference Network for rare, low prevalence and complex diseases of the heart (ERN GUARD-Heart); [4]Department of Cardiology, Hospital Universitario Puerta de Hierro Majadahonda, IDIPHISA, CIBERCV, Madrid, Spain; [5]Tuscan Regional Amyloid Centre, Careggi University Hospital, Florence, Italy; [6]Cardiology, Department of Clinical and Molecular Medicine, Sapienza University of Rome, Rome, Italy; [7]Cardiology Unit, Cardiac Thoracic and Vascular Department, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy; [8]Department of Experimental, Diagnostic and Specialty Medicine, University of Bologna, Bologna, Italy; [9]Division of Cardiology, Department of Internal Medicine II, Medical University of Vienna, Vienna, Austria; [10]Department of Cardiology, Maastricht University, Cardiovascular Research Institute Maastricht (CARIM), Maastricht, The Netherlands; [11]Department of Cardiology, Inselspital, Bern University Hospital, University of Bern, Bern, Switzerland; [12]3rd Medical Department of Cardiology and Intensive Care Medicine, Clinic Ottakring (former Wilhelminenhospital), Vienna, Austria; [13]Department of Genetics, Erasmus MC, Cardiovascular Institute, Thoraxcenter, Rotterdam, The Netherlands; [14]Division of Medicine, Department of Cardiology, Johns Hopkins University, Baltimore, MD, USA; [15]Center for Diagnosis and Treatment of Cardiomyopathies, Cardiothorarcovascular Department, Azienda Sanitaria Universitaria Giuliano-Isontina, University of Trieste, Trieste, Italy; [16]Department of Cardiology, Erasmus MC, Cardiovascular Institute, Thoraxcenter, Rotterdam, The Netherlands; [17]Department of Radiology and Nuclear Medicine, Erasmus MC, Rotterdam, The Netherlands; [18]Department of Genetics, University Medical Center Utrecht, Utrecht University, Utrecht, The Netherlands; [19]Institute of Cardiovascular Science, Faculty of Population Health Sciences, University College London, London, UK; [20]Amsterdam University Medical Centers, Department of Cardiology, University of Amsterdam, Amsterdam, The Netherlands; [21]Health Data Research UK and Institute of Health Informatics, University College London, London, UK; [22]Universidad Francisco de Vitoria, Pozuelo de Alarcón, Spain; and [23]Centro Nacional de Investigaciones Cardiovasculares (CNIC), Madrid, Spain

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Aims The 2021 European Society of Cardiology (ESC) screening recommendations for individuals carrying a pathogenic transthyretin amyloidosis variant (ATTRv) are based on expert opinion. We aimed to (i) determine the penetrance of ATTRv cardiomyopathy (ATTRv-CM) at baseline; (ii) examine the value of serial evaluation; and (iii) establish the yield of first-line diagnostic tests (i.e. electrocardiogram, echocardiogram, and laboratory tests) as per 2021 ESC position statement.

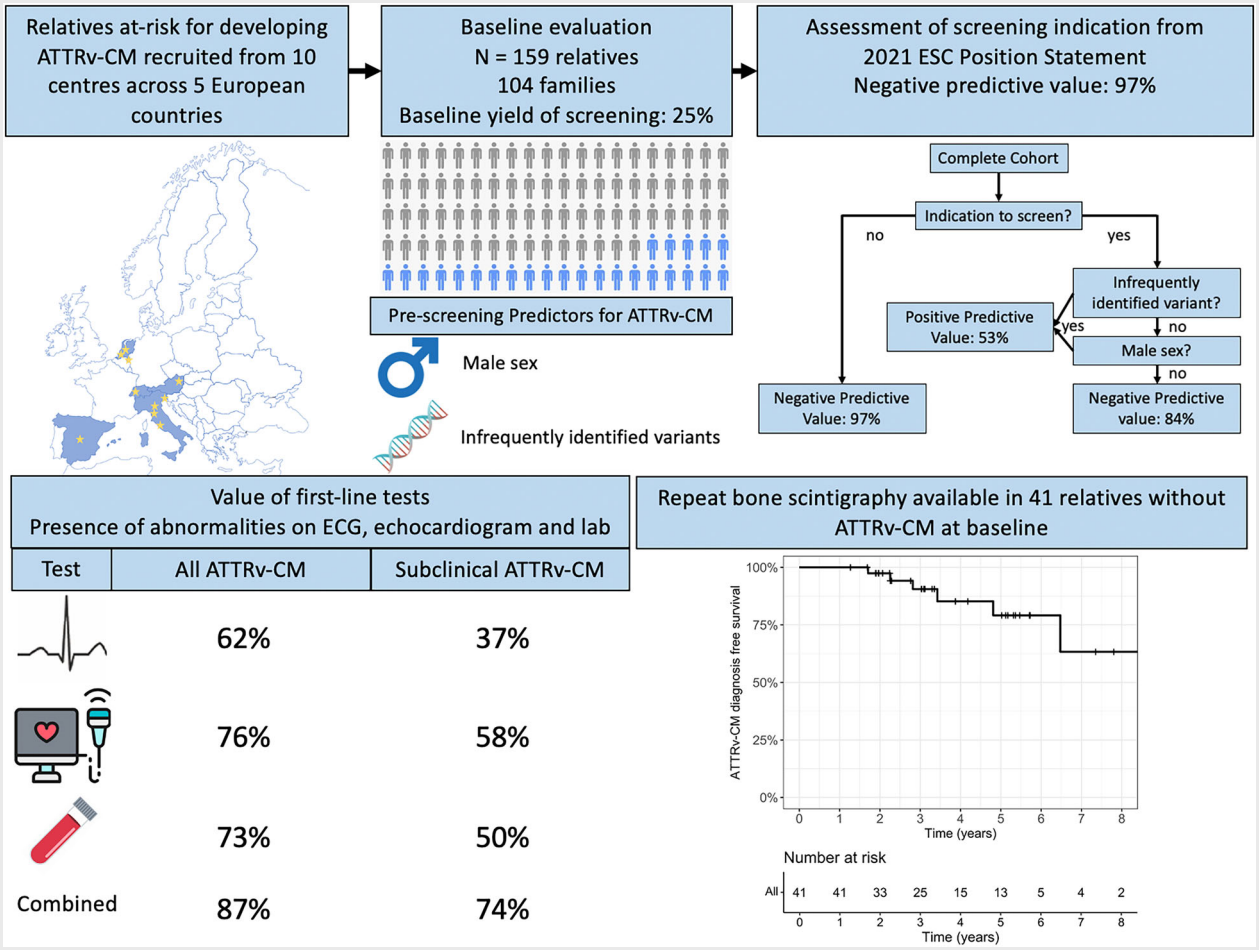
Methods and results We included 159 relatives (median age 55.6 [43.2–65.9] years, 52% male) at risk for ATTRv-CM from 10 centres. The primary endpoint, ATTRv-CM diagnosis, was defined as the presence of (i) cardiac tracer uptake in bone scintigraphy; or (ii) transthyretin-positive cardiac biopsy. The secondary endpoint was a composite of heart failure (New York Heart Association class ≥II) and pacemaker-requiring conduction disorders. At baseline, 40/159 (25%) relatives were

*Corresponding author. Department of Cardiology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht, The Netherlands. Tel: +31 (0)88-7555555; Fax: +31 (0)88-7555423; Email: m.oerlemans@umcutrecht.nl

diagnosed with ATTRv-CM. Of those, 20 (50%) met the secondary endpoint. Indication to screen (≤10 years prior to predicted disease onset and absence of extracardiac amyloidosis) had an excellent negative predictive value (97%). Other pre-screening predictors for ATTRv-CM were infrequently identified variants and male sex. Importantly, 13% of relatives with ATTRv-CM did not show any signs of cardiac involvement on first-line diagnostic tests. The yield of serial evaluation ($n = 41$ relatives; follow-up 3.1 [2.2–5.2] years) at 3-year interval was 9.4%.

Conclusions Screening according to the 2021 ESC position statement performs well in daily clinical practice. Clinicians should adhere to repeating bone scintigraphy after 3 years, as progressing to ATTRv-CM without signs of ATTRv-CM on first-line diagnostic tests or symptoms is common.

Graphical Abstract



Family screening in hereditary transthyretin cardiac amyloidosis. ATTRv-CM, hereditary transthyretin amyloid cardiomyopathy; ECG, electrocardiogram; ESC, European Society of Cardiology.

Keywords Cascade screening • Amyloidosis • ATTRv • Repeat evaluation

Introduction

Transthyretin (TTR) amyloidosis (ATTR) is a progressive infiltrative systemic disease that is caused by deposition of amyloid fibrils predominantly affecting the heart and nervous system.[1] With the significant advances in ATTR therapies,[2–5] an increasing number of patients with ATTR cardiomyopathy (ATTR-CM) are being identified,[6] including patients with a genetic predisposition for developing ATTR-CM. Cardiologists therefore not only need to care for ATTR-CM patients, but also for an increasing number of ATTR variant (ATTRv) carriers at risk for developing hereditary ATTR-CM (ATTRv-CM). Consequently, the 2021 position statement of the European Society of Cardiology (ESC) Working Group on Myocardial and Pericardial Diseases on the diagnosis and treatment of amyloidosis recommends that ATTRv carriers without cardiac involvement are to be routinely evaluated starting 10 years earlier than the youngest member of the family developed phenotype or the usual age at presentation for the specific variant. Recommended evaluation includes yearly electrocardiogram (ECG), echocardiogram, blood tests, and biannual Holter monitoring. Additionally, bone scintigraphy and/or cardiac magnetic resonance (CMR) imaging is recommended every 3 years or if any aforementioned tests is abnormal.[7] This recommended follow-up protocol might result in an increasing burden on clinical resources and has a potential psychosocial impact on patients and their families.

Among ATTRv carriers, disease expression is highly variable, even among those carrying the same variant or those with extracardiac amyloidosis.[1] Although the current position statement has taken this into account by recommending initiation of cardiac screening ~10 years prior to the age of earliest disease onset in affected family members or as soon as extracardiac amyloidosis is present,[7] objective evidence to support this approach is lacking. As such, there is a need to substantiate this recommendation with real-world evidence.[8–10]

The purpose of this study was threefold: (i) to determine the penetrance of ATTRv-CM in relatives at baseline evaluation; (ii) to examine the value of serial evaluation; and (iii) to establish the yield of first-line diagnostic tests (i.e. ECG, echocardiogram, and laboratory tests) in relatives at risk for developing ATTRv-CM. To do so, we collected a carefully genotyped and phenotyped cohort of relatives at risk for ATTRv-CM from 10 referral centres for diagnosis and management of ATTR across five different European countries.

Method

Study population

We recruited our study population from 10 referral centres for diagnosis and management of ATTR across five different European countries (online supplementary *Table S1*). From each centre, we included all individuals who (i) harboured a pathogenic TTR variant, and (ii) had at least one clinical evaluation, as described below. To establish the yield of family screening in ATTRv-CM we excluded all probands, defined as the first individual in a family in whom the pathogenic TTR variant was found. This study followed the Code of Conduct and the Use of Data in Health Research and was exempted from the Medical Research Involving Human Subjects Act (WMO) as per judgement of the Medical Ethics Committee (METC 22/968, Utrecht, The Netherlands).

Clinical evaluation

The medical history of each participant was obtained by review of health records and clinical evaluation. Detailed clinical information regarding demographics, presentation, symptom onset, and (non-)invasive tests was obtained. Participants were divided based on their relationship with the proband as parents, siblings, children, second-degree or third-degree relatives.

We subsequently established if the subjects fulfilled one of the screening criteria as stated in the ESC position statement.[7] In short, an individual fulfilled screening criteria if: (i) cardiac screening was performed 10 years prior to disease onset in the proband (or in other individuals with the same variant if disease onset in the family was unknown); or (ii) had signs of extracardiac amyloidosis as diagnosed by a neurologist or ophthalmologist. If the age of disease onset in the proband was unknown, a cut-off when screening should be initiated was established at 40 and 55 years of age in subjects carrying the Val50Met variant (disease onset >50 years of age) and subjects carrying non-Val50Met variants (disease onset >65 years of age), respectively.[9]

Since the recommendations for systematically screening ATTRv carriers are relatively new, it is most likely that differences in clinical practice between amyloidosis referral centres had occurred in the past. We therefore defined a cardiac evaluation as an evaluation in which ATTRv-CM could be ascertained, as described below.

ATTRv-CM diagnosis

Diagnosis of ATTRv-CM was based on the ESC position statement.[7] In short, ATTRv-CM was confirmed when: (i) bone scintigraphy (^{99m}Tc-DPD or ^{99m}Tc-HMDP) showing cardiac uptake grade II–III by Perugini grading[11]; or (ii) cardiac biopsy-proven TTR amyloidosis. As a recent publication showed that grade I cardiac uptake in bone scintigraphy in absence of monoclonal proteins has a 98% likelihood of being ATTR-CM,[12] we also confirmed (in contrast to the ESC position statement) ATTRv-CM diagnosis in grade I cardiac tracer uptake in bone scintigraphy. Of note, single photon emission computed tomography (SPECT) was performed to rule out false positive tracer uptake (e.g. recent myocardial infarction). Furthermore, we utilized the proposed diagnostic CMR criteria in Phe84Leu carriers, as it has been established that Pheu84Leu carriers do not show tracer uptake on bone scintigraphy.[13]

Study outcomes

The primary outcome was ATTRv-CM diagnosis at baseline evaluation or during follow-up. The secondary endpoint of this study was cardiac symptoms attributed to ATTRv-CM. Cardiac symptoms were defined as a composite of heart failure (New York Heart Association class ≥II) and pacemaker-requiring conduction disorders. By study design, subjects could only meet the secondary endpoint when diagnosed with ATTRv-CM. Therefore, subjects were stratified by the presence of meeting the primary and secondary endpoint: 'subclinical ATTRv-CM' (i.e. only meeting the primary endpoint), or 'clinical ATTRv-CM' (i.e. meeting both the primary and secondary endpoint).

To evaluate the value of first-line diagnostic (i.e. ECG, echocardiography, and laboratory) tests we defined, in accordance with the 2021

position statement, the following to be 'red flags' for ATTRv-CM: (i) for ECG, the presence of conduction disorders, supraventricular arrhythmia, pseudo-infarction, or low-voltage; (ii) for echocardiography, a wall thickness of ≥12 mm in diastole; and (iii) for laboratory parameters, elevated troponin (defined as above the upper limit of normal) or elevated (N-terminal pro-)B-type natriuretic peptide (defined as above the upper limit of normal adjusted for age and gender[14]).

Statistical analysis

Nominal variables were expressed as number (%), and continuous variables as mean ± standard deviation or median [interquartile range]. Comparisons for binary variables were performed by Chi-square or Fisher's exact test. For continuous variables, independent *t*-test or Mann–Whitney U test were used.

To determine the (i) presence of age-related penetrance, (ii) performance of the screening indications of the ESC position statement, and (iii) screening solely on predicted disease onset, we visualized the distribution of no ATTRv-CM, subclinical ATTRv-CM, and clinical ATTRv-CM at baseline evaluation. Demographics were examined using logistic regression to establish a pre-screening likelihood of ATTRv-CM. We subsequently build a clinical pre-screening algorithm, to help cardiologists establish an estimate of the risk of ATTRv-CM before initiating cardiac screening. Next, we reported the prevalence of abnormal tests at baseline evaluation. Last, we assessed and visualized the overall probability of survival free from the respective endpoints using a Kaplan–Meier curve and compared using the log-rank test.

A *p*-value <0.05 was considered statistically significant. Data were analysed using RStudio version 2021.09.01 (Boston, MA, USA).

Results

Study population

Our cohort consisted of 159 individuals from 104 families. Baseline characteristics are shown in *Table 1*. Median age at first evaluation was 55.6 [43.2–65.9] years, 83 (52%) were male, the median years screened before predicted disease onset was 9.4 [−1.0–22.7], and the Val50Met was the most prevalent variant ($n = 55$; 35%). In total, 35 (22%) relatives harboured other, infrequently identified, variants (online supplementary *Table S2*). Overall, most relatives came to attention because of cascade genetic screening ($n = 92$; 58%). Additionally, approximately one-third had known neurologic amyloidosis ($n = 37/103$; 36%), and 16% ($n = 5/31$) had ophthalmological amyloidosis. Most relatives were asymptomatic from a cardiac standpoint ($n = 131$; 82%).

Baseline evaluation

At baseline evaluation, 40 (25%) relatives were diagnosed with ATTRv-CM of whom 50% ($n = 20/40$) had clinical ATTRv-CM (online supplementary *Table S3* for how ATTRv-CM diagnosis at baseline was reached). Relatives diagnosed with ATTRv-CM were significantly older (66.5 [61.0–75.2] vs. 49.2 [40.8–58.8] years, $p < 0.001$) and *Figure 1A* shows the age-related prevalence of ATTRv-CM diagnosis at time of baseline evaluation. Additionally, relatives diagnosed with ATTRv-CM were significantly more often male (28 [70%] vs. 55 [46%], $p = 0.015$), a sibling of the proband (23 [58%] vs. 26 [22%], $p < 0.001$), and more likely to have neurologic involvement (21 [75%] vs. 16 [21%], $p < 0.001$) as compared to those without ATTRv-CM at baseline. Although not statistically significant, relatives with ATTRv-CM at baseline more often carried infrequently identified variants (14 [35%] vs. 21 [18%], $p = 0.051$).

Relatives with ATTRv-CM at baseline were significantly more likely to have cardiac symptoms (19 [48%] vs. 9 [8%], $p < 0.001$) and cardiac red flags (34 [87%] vs. 39 [33%], $p < 0.001$) for ATTRv-CM as compared to relatives without ATTRv-CM at baseline. Of relatives with clinical ATTRv-CM, most had heart failure (90% [$n = 18/20$]) whereas the remaining (10% [$n = 2/20$]) had pacemaker-requiring conduction disorders.

Pre-screening predictors of ATTRv-CM

Figure 1B,C visualizes the yield of screening by recommendations of the 2021 ESC position statement. Screening initiated due to predicted disease onset, extracardiac amyloidosis or the combination of age and extracardiac amyloidosis had a 31% ($n = 17/54$), 18% ($n = 2/11$) and 68% ($n = 19/28$) yield of ATTRv-CM, respectively (*Figure 1B*). In relatives with both predicted disease onset and extracardiac amyloidosis as an indication to screen, clinical ATTRv-CM was most prevalent (74%; $n = 14/19$) (*Figure 1B*). Conversely, clinical ATTRv-CM among relatives screened through predicted disease onset (35%; $n = 6/17$, *Figure 1B*) or with extracardiac amyloidosis (0%; $n = 0/2$, *Figure 1B*) was less prevalent (combined 32%; $n = 6/19$). Importantly, these six relatives with clinical ATTRv-CM at baseline were relatives without extracardiac amyloidosis who were all screened after the predicted disease onset. Consequently, relatives who fulfil early screening criteria (i.e. presence of extracardiac amyloidosis or screened 0–10 years before predicted disease onset) were only diagnosed with subclinical ATTRv-CM. Furthermore, a diagnosis of ATTRv-CM in relatives without an indication to screen was rare (3%; $n = 2/66$) (*Figure 1B*). Consequently, the screening indications of the 2021 ESC position statement had an almost excellent negative predictive value of 97% (95% confidence interval [CI] 89–99%) and a positive predictive value of 41% (95% CI 36–46%). After multivariable analysis, other independent pre-screening predictors for ATTRv-CM were male sex (odds ratio 4.4 [95% CI 1.8–11.6]; $p = 0.002$) and harbouring an infrequently identified variant (odds ratio 3.4 [95% CI 1.3–9.7]; $p = 0.015$) (*Table 2*). *Figure 2* visualizes the implementation of all predictors into a clinical algorithm. In the population with an indication to screen ($n = 93$), this additional algorithm provided a negative predictive value of 84% (95% CI 69–93%) and a positive predictive value of 53% (95% CI 46–60%).

Value of first-line diagnostic tests

As can be appreciated from *Table 1*, abnormalities on ECG (62% [$n = 23/37$]), echocardiography (76% [$n = 28/37$]), and laboratory parameters (73% [$n = 22/30$]) were present in most relatives with ATTRv-CM at baseline. Consequently, 87% ($n = 34/39$) of relatives with ATTRv-CM had at least one abnormality on one of the diagnostic tests, while 13% ($n = 5/39$) of relatives with ATTRv-CM did not have any 'red flags' of ATTRv-CM.

Table 1 Baseline characteristics

	Overall ($n = 159$)	No ATTRv-CM ($n = 119$)	ATTRv-CM ($n = 40$)	*p*-value
Pre-screening demographics				
Age at presentation (years)	55.6 [43.2–65.9]	49.2 [40.8–58.8]	66.5 [61.0–75.2]	<0.001
Years screened before predicted disease onset[a]	9.4 [−1.0–22.7]	16.5 [3.2–26.4]	−0.9 [−7.8–1.43]	<0.001
Male sex	83 (52.2)	55 (46.2)	28 (70.0)	0.015
Relationship to the proband				<0.001
Sibling	49 (31.2)	26 (22.2)	23 (57.5)	
Child	74 (47.1)	71 (60.7)	3 (7.5)	
Parent	4 (2.5)	1 (0.9)	3 (7.5)	
2nd degree	20 (12.7)	16 (13.7)	4 (10.0)	
3rd degree or further	10 (6.4)	3 (2.6)	7 (17.5)	
Amino acid change				0.051
Val50Met	55 (34.6)	43 (36.1)	12 (30.0)	
Ile88Leu	46 (28.9)	34 (28.6)	12 (30.0)	
Val142Ile	23 (14.5)	21 (17.6)	2 (5.0)	
Infrequently identified variants	35 (22.0)	21 (17.6)	14 (35.0)	
Specialism of referral				0.441
Geneticist	92 (57.9)	69 (58.0)	23 (57.5)	
Cardiologist	35 (22.0)	28 (23.5)	7 (17.5)	
Neurologist	28 (17.6)	20 (16.8)	8 (20.0)	
Internal medicine	3 (1.9)	1 (0.8)	2 (5.0)	
Self-referral	1 (0.6)	1 (0.8)	0 (0.0)	
Neurologic amyloidosis ($n = 103$)	37 (35.9)	16 (21.3)	21 (75.0)	<0.001
Carpal tunnel syndrome present ($n = 158$)				0.005
Bilateral	31 (19.6)	18 (15.1)	13 (33.3)	
Unilateral	14 (8.9)	8 (6.7)	6 (15.4)	
Ophthalmological amyloidosis ($n = 31$)	5 (16.1)	5 (18.5)	0 (0.0)	1.000
Cardiac evaluation				
Cardiac symptoms	28 (17.6)	9 (7.6)	19 (47.5)	<0.001
Dyspnoea	20 (12.6)	2 (1.7)	18 (45.0)	<0.001
Palpitations	11 (6.9)	7 (5.9)	4 (10.0)	0.598
(Pre-)syncope	6 (3.8)	2 (1.6)	4 (10.0)	0.056
Clinical ATTRv-CM	20 (12.6)	0 (0.0)	20 (50.0)	<0.001
Any 'red flag' on ECG/Echo/lab ($n = 158$)	73 (46.2)	39 (32.8)	34 (87.2)	<0.001
Any 'red flag' on ECG ($n = 155$)	46 (29.7)	23 (19.5)	23 (62.2)	<0.001
1st degree AV block ($n = 155$)	11 (7.4)	4 (3.5)	7 (21.2)	0.003
2nd degree AV block or higher ($n = 155$)	2 (1.3)	0 (0.0)	2 (5.3)	0.058
Ventricular conduction disorder ($n = 155$)	32 (20.6)	15 (12.7)	17 (45.9)	<0.001
Low voltages ($n = 155$)	21 (13.5)	6 (5.1)	15 (39.5)	<0.001
Pseudo-infarction pattern ($n = 155$)	14 (9.0)	6 (5.1)	8 (21.1)	0.008
LVEF (%) ($n = 153$)	63 [57–66]	64 [60–67]	58 [52–63]	<0.001
IVSd <12 mm ($n = 157$)	112 (71.3)	102 (86.4)	10 (25.6)	<0.001
LVPWd <12 mm ($n = 150$)	127 (84.7)	108 (95.6)	19 (51.4)	<0.001
Both IVSd and LVPWd <12 mm ($n = 150$)	108 (72.0)	99 (87.6)	9 (24.3)	<0.001
Relative wall thickness ($n = 146$)	0.43 [0.37–0.51]	0.40 [0.36–0.46]	0.64 [0.47–0.75]	<0.001
Any 'red flag' in lab ($n = 98$)	28 (28.6)	6 (8.8)	22 (73.3)	<0.001
Elevated troponin ($n = 76$)	10 (13.2)	0 (0.0)	10 (38.5)	<0.001
Elevated NT-proBNP ($n = 98$)	25 (25.5)	6 (8.8)	19 (63.3)	<0.001

ATTRv-CM, hereditary transthyretin amyloid cardiomyopathy; AV, atrioventricular; ECG, electrocardiogram; Echo, echocardiography; IVSd, interventricular septal thickness at end-diastole; LVEF, left ventricular ejection fraction; LVPWd, left ventricular posterior wall thickness at end-diastole; NT-proBNP, N-terminal pro-B-type natriuretic peptide. Variables are expressed as frequency (%), mean ± standard deviation, or median [interquartile range], as appropriate. Total number of patients for a given variable are mentioned if missing data.

[a]A negative number indicates that screening was performed after the predicted disease onset.

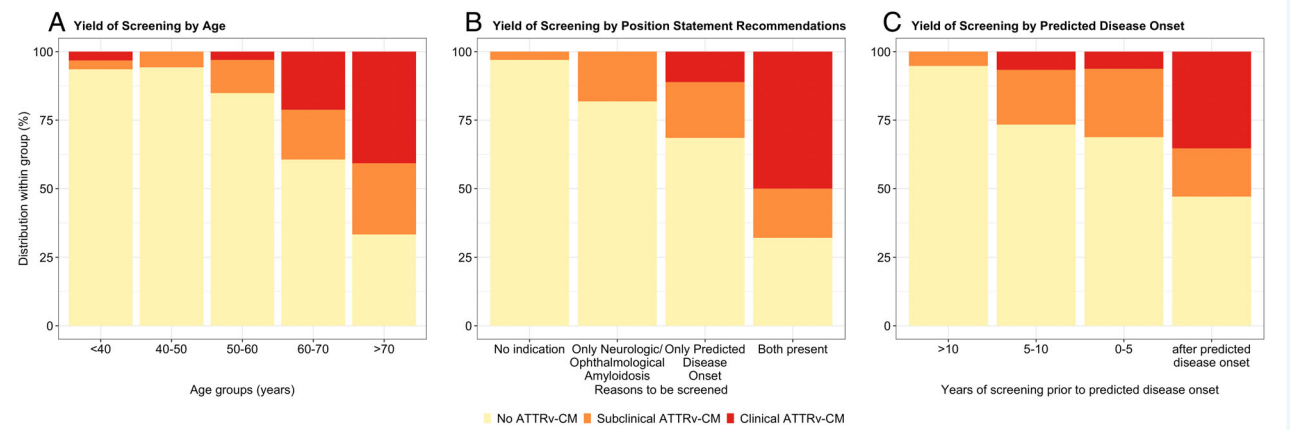


Figure 1 Cardiac amyloidosis at baseline. Prevalence of hereditary transthyretin amyloid cardiomyopathy (ATTRv-CM) among pathogenic transthyretin amyloidosis variant carrying relatives at time of baseline evaluation stratified by (*A*) age, (*B*) screening indication, and (*C*) years of screening prior to predicted disease onset. All 159 relatives are included in every graph. To show the proportion of relatives in each group, every group is scaled to 100%.

Table 2 Pre-cardiac screening predictors for hereditary transthyretin amyloid cardiomyopathy at baseline evaluation

	Univariate		Multivariate	
	Odds ratio (95% CI)	***p*-value**	**Odds ratio (95% CI)**	***p*-value**
Indication for screening	22.1 (6.4–139.8)	<0.001	30.3 (8.1–202.2)	<0.001
Male sex	2.7 (1.3–6.0)	0.011	4.4 (1.8–11.6)	0.002
Infrequently identified variants	2.5 (1.1–5.6)	0.024	3.4 (1.3–9.7)	0.015
Referred by geneticist	0.98 (0.5–2.0)	0.957	–	–

CI, confidence interval.

When stratifying between clinical and subclinical ATTRv-CM (online supplementary *Table S4*), abnormalities on any modality ($n = 20/20$ [100%] vs. $n = 14/19$ [76%]; $p = 0.020$), ECG ($n = 16/18$ [89%] vs. $n = 7/19$ [37%], $p = 0.002$), echocardiography ($n = 17/18$ [94%] vs. $n = 11/19$ [58%], $p = 0.019$), and laboratory parameters ($n = 15/16$ [94%] vs. $n = 7/14$ [50%], $p = 0.012$) were significantly more prevalent in relatives with clinical ATTRv-CM as compared to relatives with subclinical ATTRv-CM. This shows that 26% ($n = 5/19$) of relatives with subclinical ATTRv-CM were diagnosed by positive bone scintigraphy without any 'red flags' of ATTRv-CM on first-line diagnostic tests.

Progression to ATTRv-CM

Of 119 relatives without ATTRv-CM at baseline, 41 (34%) received at least one repeat evaluation. Relatives with repeat evaluation were significantly more likely to be a sibling of the proband ($p = 0.031$), harbour an infrequently identified or Val50Met variant ($p = 0.003$), or suffered from neurological amyloidosis ($p = 0.002$). Additionally, relatives who were referred by a geneticist were less likely to have a repeat evaluation ($p = 0.002$). No differences in cardiac parameters at baseline between relatives with and without follow-up were observed ($p > 0.05$) (online supplementary *Table S5*).

These 41 relatives were followed for a median of 3.1 [2.2–5.2] years. Overall, 7/41 (17%) developed ATTRv-CM. In those seven relatives, median time to ATTRv-CM diagnosis was 3.4 [2.5–5.6] years (*Figure 3*). When utilizing the proposed 3-year screening interval of the 2021 ESC position statement, the yield of screening was 9.4% Although not statistically significant, a visual trend towards faster ATTRv-CM development was observed in relatives with a baseline indication for screening as compared to relatives without a baseline indication for screening ($p = 0.16$, online supplementary *Figure S1*). Importantly, 29% ($n = 2/7$) of relatives progressed towards ATTRv-CM diagnosis without showing 'red flags' on first-line diagnostic tests (online supplementary *Figure S2*).

Progression to clinical ATTRv-CM

In addition to the 41 relatives without ATTRv-CM at baseline with follow-up, 17/20 (85%) of relatives with subclinical ATTRv-CM at baseline had follow-up data available. These 58 relatives were followed for a median of 3.0 [1.9–5.1] years. Progression to clinical ATTRv-CM was observed in seven relatives of whom most had heart failure (71% [$n = 5/7$]) whereas the remaining (29% [$n = 2/7$])

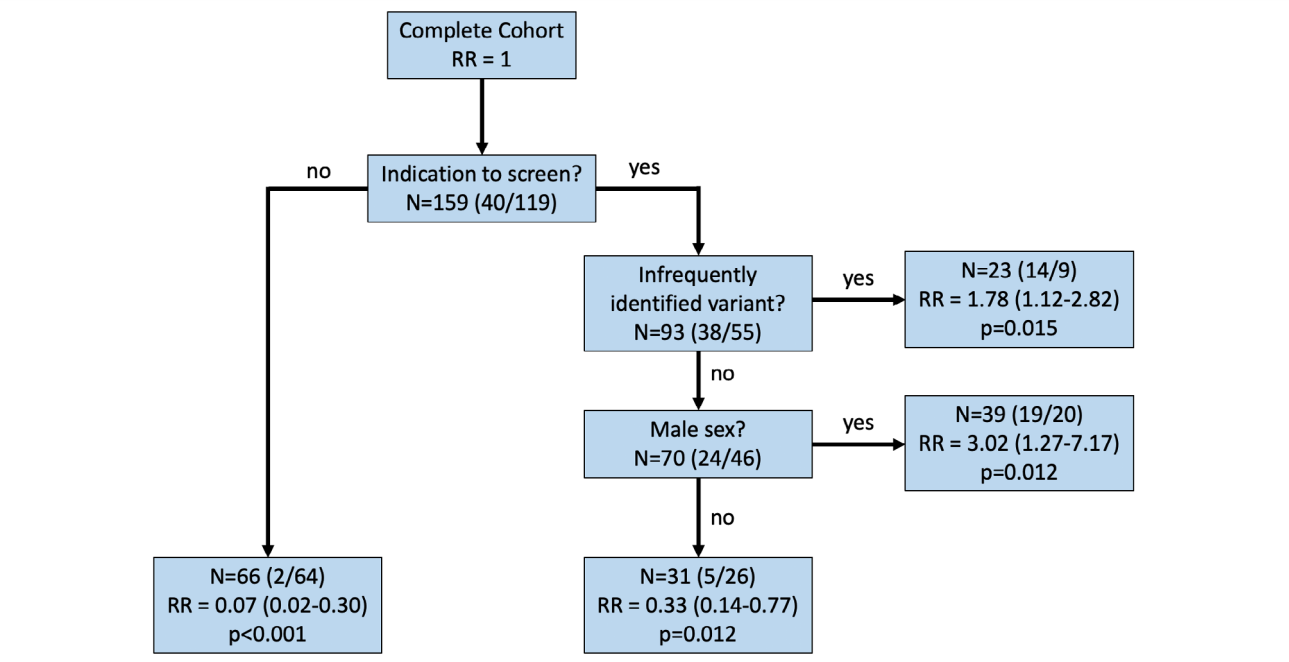


Figure 2 Implementation of pre-screening predictors for hereditary transthyretin amyloid cardiomyopathy (ATTRv-CM) into a clinical applicable algorithm. The total number of relatives at each node is provided; brackets indicate number of patients with/without ATTRv-CM. Relative risk (RR) (95% confidence interval) for ATTRv-CM diagnosis is provided for every terminal node.

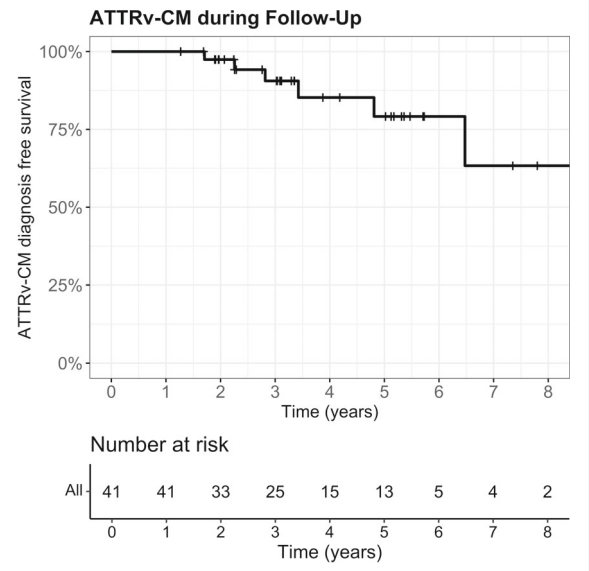


Figure 3 Hereditary transthyretin amyloid cardiomyopathy (ATTRv-CM) development during follow-up. Diagnosis-free survival curve of ATTRv-CM in the 41 patients without ATTRv-CM at baseline who had repeat bone scintigraphy.

had pacemaker-requiring conduction disorders. In those seven relatives, median time to clinical ATTRv-CM was 3.0 [1.3–3.4] years (*Figure 4A*).

As expected, relatives with subclinical ATTRv-CM at baseline progressed more rapidly to clinical ATTRv-CM compared to relatives without ATTRv-CM at baseline (clinical ATTRv-CM reached after 3.0 [0.7–3.4] years vs. 1.9 and 3.4 years, $p < 0.001$) (*Figure 4B*). Except for one relative, all relatives had a positive bone scintigraphy before the heart failure or conduction disorder event. The relative who met the secondary endpoint before ATTRv-CM diagnosis (explained in detail in online supplementary *Figure S2*, patient no. 3 from the top) was at presentation an asymptomatic 47-year-old male harbouring the Val142Ile variant without comorbidities or extracardiac amyloidosis. After 1.89 years since his baseline evaluation, the relative presented with a syncope due to a third-degree atrioventricular block for which he received a pacemaker. Bone scintigraphy at the time of hospitalization proved negative (grade 0). Repeat bone scintigraphy 4.5 years after pacemaker implantation proved positive (grade III).

Discussion

Since the possibility of achieving a non-invasive diagnosis and the introduction of disease-modifying therapies for ATTR-CM, an increasing number of subjects diagnosed with ATTRv-CM has come to clinical attention.[6] Subsequently, the population necessitating pre-symptomatic screening has also increased. This study is the first to (i) scrutinize the approach of cardiac family screening in relatives at risk for developing ATTRv-CM, and (ii) evaluate the performance of the 2021 ESC position statement.

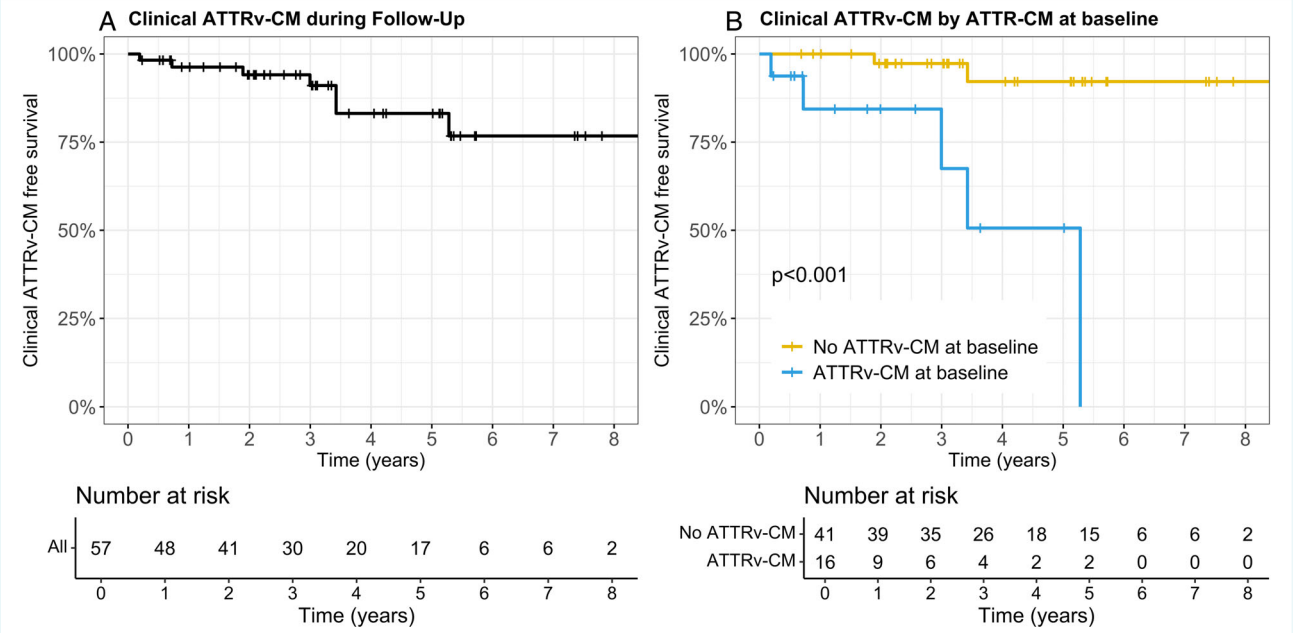


Figure 4 Development of clinical hereditary transthyretin amyloid cardiomyopathy (ATTRv-CM) during follow-up. Clinical ATTRv-CM-free survival curve in the 57 patients without clinical ATTRv-CM at baseline who had follow-up available in (*A*) the overall population and (*B*) stratified by the presence of ATTRv-CM at baseline evaluation.

This study has several interesting findings (*Graphical Abstract*). First, 25% of relatives referred for family screening had ATTRv-CM at baseline evaluation, with an equal distribution between subclinical and clinical ATTRv-CM. Second, the indication for screening as proposed by the 2021 ESC position statement has an almost excellent negative predictive value (97%). Third, independent pre-screening predictors for ATTRv-CM at baseline are infrequently identified variants and male sex. To provide clinicians with a clinically applicable risk algorithm, the absence of infrequently identified variants and female sex in relatives with an indication for screening yielded a good negative predictive value (84%). Fourth, the yield of 3-yearly serial evaluation was 9.4%. Last, 26% of relatives with subclinical ATTRv-CM did not show any 'red flags' of ATTRv-CM on first-line diagnostic tests.

Baseline evaluation

The 2021 ESC position statement for the management and treatment of cardiac amyloidosis[7] incorporated screening recommendations to enable early (i.e. subclinical) ATTRv-CM diagnosis in at-risk relatives. We showed that (i) ATTRv-CM among relatives without an indication to be screened is rare (3%), and (ii) relatives who fulfil criteria for early screening (i.e. presence of extracardiac amyloidosis or 0–10 years before predicted disease onset) are only diagnosed with subclinical ATTRv-CM. This indicates that the proposed ESC 2021 screening recommendations perform well in daily clinical practice.

Our study also explored characteristics that are associated with ATTRv-CM at baseline. We established a sex-specific prevalence in our cohort as previously described in wild-type ATTR-CM.[15] This is further illustrated by our effort to provide clinicians with a clinical algorithm for risk of ATTRv-CM at baseline evaluation, for which sex is an important variable (*Figure 2*). Consequently, adapting sex-specific screening indications could be a logical consequence. However, caution is warranted as we found that if female relatives have ATTRv-CM, they tend to be more severely affected (i.e. clinical ATTRv-CM) than male relatives.

Additionally, our results showed that harbouring an infrequently identified variant is associated with a threefold increased risk of having ATTRv-CM at baseline with a tendency to be more severely affected. Since the criteria to screen involve both extracardiac amyloidosis as well as a predicted age of disease onset,[7] the phenotype of these infrequently identified variants could be predominantly cardiac and to a lesser degree neurologic as well as the precise age of onset in this population is largely unknown. Consequently, this could explain the association between infrequently identified variants and ATTRv-CM at baseline. Therefore, future studies describing variant-specific natural disease history are needed.

Serial evaluation

In our cohort, we found that serial bone scintigraphy yields a 3-year risk of progression to ATTRv-CM of 9.4%. The 3-yearly yield of repeat bone scintigraphy according to the 2021 ESC position statement is similar to the recommended screening strategies for other cardiomyopathies.[16–21] Although a selection bias in our study population was apparent, our data represent an important real-world experience in current clinical practice. Nonetheless,

future studies are necessary to confirm our findings and to identify risk factors associated with ATTRv-CM development.

Of note, one relative exhibited third-degree atrioventricular block necessitating pacemaker implantation without a positive bone scintigraphy at that time. This shows that undetectable low amyloid depositions can severely affect the cardiac conduction system and may remain undetectable for years. As such, relatives presenting with conduction disorders may benefit from a closer follow-up with Holter monitoring.

Value of first-line diagnostic tests

Another important finding was that 13% of relatives with ATTRv-CM did not show any 'red flags' of cardiac amyloidosis on ECG, echocardiogram, and laboratory parameters. When excluding relatives with clinically manifest ATTRv-CM (i.e. those who already had to be diagnosed based on signs and symptoms), this number increased to 26%. This extends previous findings[15] that ATTRv-CM can be present without any 'red flags' while treatment is beneficial in this population.[22] Therefore, baseline and repeat bone scintigraphy with a 3-year interval as proposed by the 2021 ESC position statement is of vital importance to diagnose ATTRv-CM as early as possible.

This finding has also implications outside family screening as diagnostic amyloidosis algorithms use a wall thickness ≥12 mm as a starting point to initiate screening for cardiac amyloidosis.[7,23] Up to 42% of relatives with subclinical ATTRv-CM did not have a wall thickness ≥12 mm. Additionally, 50% of relatives with subclinical ATTRv-CM did not meet previously proposed cut-offs for relative wall thickness for early ATTR-CM detection.[24,25] These findings support an increasing body of evidence that cardiac amyloidosis can be diagnosed before cardiac signs are apparent on conventional diagnostic tests.[15,26]

In contrast, a non-negligible portion (33%) of relatives without ATTRv-CM did show 'red flags' of ATTRv-CM on first-line diagnostic tests with approximately one-third of those relatives showing 'red flags' on ECG. When comparing the relatives without ATTRv-CM at baseline to the general population,[27–29] the prevalence of 'red flags' on first-line diagnostic tests is comparable and/or could be attributed to other causes (e.g. coronary artery disease). This is important as it stresses that careful clinical evaluation by experienced clinicians is warranted to evaluate early cardiac involvement in these at-risk relatives.

Limitations and future perspectives

While our cohort of comprehensively evaluated individuals is the first describing the yield of screening in relatives at risk for ATTRv-CM, the retrospective nature of our study design should be mentioned as a limitation. Additionally, we were underpowered to perform Cox proportional hazard regression to ascertain predictors of ATTRv-CM development. Third, relatives with repeat evaluation were more likely to have extracardiac amyloidosis and were more likely to be referred by a treating specialist. As a result, this selection bias probably resulted in an overestimation of the real progression to ATTRv-CM. Last, our multicentre study design including centres from different European countries may be a limitation given the differences in indications for disease-modifying therapies,[30,31] which potentially could affect screening protocols.

Although a recent publication suggested that treatment in ATTR-CM before the onset of heart failure is may be beneficial,[22] future studies are necessary to determine if treatment of ATTRv-CM before the onset of 'red flags' is beneficial.

Conclusion

In our cohort of at-risk relatives for ATTRv-CM, 25% had ATTRv-CM at baseline evaluation, of whom 50% had clinically manifest ATTRv-CM. Screening recommendations of the 2021 ESC position statement performed well in daily clinical practice. While first-line diagnostic tests help clinicians towards arriving at an ATTRv-CM diagnosis, 25% with subclinical ATTRv-CM do not show 'red flags' for cardiac amyloidosis on ECG, echocardiography, or laboratory testing. Clinicians should therefore adhere to the recommended 3-yearly bone scintigraphy. As pacemaker-requiring conduction disorders may manifest prior to developing detectable amyloid burden on bone scintigraphy, more frequent follow-up with Holter monitoring in relatives with conduction disorders without ATTRv-CM should be considered.

Supplementary Information

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

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References

1. Porcari A, Fontana M, Gillmore JD. Transthyretin cardiac amyloidosis. *Cardiovasc Res* 2023;**118**:3517–3535. https://doi.org/10.1093/cvr/cvac119
2. Rapezzi C, Elliott P, Damy T, Nativi-Nicolau J, Berk JL, Velazquez EJ, *et al.* Efficacy of tafamidis in patients with hereditary and wild-type transthyretin amyloid cardiomyopathy: Further analyses from ATTR-ACT. *JACC Heart Fail* 2021;**9**:115–123. https://doi.org/10.1016/j.jchf.2020.09.011
3. Maurer MS, Schwartz JH, Gundapaneni B, Elliott PM, Merlini G, Waddington-Cruz M, *et al.*; ATTR-ACT Study Investigators. Tafamidis treatment for patients with transthyretin amyloid cardiomyopathy. *N Engl J Med* 2018;**379**:1007–1016. https://doi.org/10.1056/NEJMoa1805689
4. Maurer MS, Kale P, Fontana M, Berk JL, Grogan M, Gustafsson F, *et al.*; APOLLO-B Trial Investigators. Patisiran treatment in patients with transthyretin cardiac amyloidosis. *N Engl J Med* 2023;**389**:1553–1565. https://doi.org/10.1056/NEJMoa2300757
5. Gillmore JD, Judge DP, Cappelli F, Fontana M, Garcia-Pavia P, Gibbs S, *et al.*; ATTRibute-CM Investigators. Efficacy and safety of acoramidis in transthyretin amyloid cardiomyopathy. *N Engl J Med* 2024;**390**:132–142. https://doi.org/10.1056/NEJMoa2305434
6. Brons M, Muller SA, Rutten FH, van der Meer MG, Vrancken AFJE, Minnema MC, *et al.* Evaluation of the cardiac amyloidosis clinical pathway implementation: A real-world experience. *Eur Heart J Open* 2022; 1–9. https://doi.org/10.1093/ehjopen/oeac011
7. Garcia-Pavia P, Rapezzi C, Adler Y, Arad M, Basso C, Brucato A, *et al.* Diagnosis and treatment of cardiac amyloidosis: A position statement of the ESC Working Group on Myocardial and Pericardial Diseases. *Eur Heart J* 2021;**42**:1554–1568. https://doi.org/10.1093/eurheartj/ehab072
8. Ando Y, Adams D, Benson MD, Berk JL, Planté-Bordeneuve V, Coelho T, *et al.* Guidelines and new directions in the therapy and monitoring of ATTRv amyloidosis. *Amyloid* 2022;**29**:143–155. https://doi.org/10.1080/13506129.2022.2052838
9. Conceição I, Coelho T, Rapezzi C, Parman Y, Obici L, Galán L, *et al.* Assessment of patients with hereditary transthyretin amyloidosis – understanding the impact of management and disease progression. *Amyloid* 2019;**26**:103–111. https://doi.org/10.1080/13506129.2019.1627312
10. Lenihan D, Cheng RK. Early treatment of cardiac ATTR: "To remove the weeds, you have to get the roots". *JACC CardioOncol* 2022;**4**:455–457. https://doi.org/10.1016/j.jaccao.2022.08.008
11. Perugini E, Guidalotti PL, Salvi F, Cooke RMT, Pettinato C, Riva L, *et al.* Noninvasive etiologic diagnosis of cardiac amyloidosis using 99mTc-3,3-diphosphono-1,2-propanodicarboxylic acid scintigraphy. *J Am Coll Cardiol* 2005;**46**:1076–1084. https://doi.org/10.1016/j.jacc.2005.05.073
12. Rauf MU, Hawkins PN, Cappelli F, Perfetto F, Zampieri M, Argiro A, *et al.* Tc-99m labelled bone scintigraphy in suspected cardiac amyloidosis. *Eur Heart J* 2023;**44**:2187–2198. https://doi.org/10.1093/eurheartj/ehad139
13. Musumeci MB, Cappelli F, Russo D, Tini G, Canepa M, Milandri A, *et al.* Low sensitivity of bone scintigraphy in detecting Phe64Leu mutation-related transthyretin cardiac amyloidosis. *JACC Cardiovasc Imaging* 2020;**13**:1314–1321. https://doi.org/10.1016/j.jcmg.2019.10.015
14. Braisch U, Koenig W, Rothenbacher D, Denkinger M, Friedrich N, Felix SB, *et al.* N-terminal pro brain natriuretic peptide reference values in community-dwelling older adults. *ESC Heart Fail* 2022;**9**:1703–1712. https://doi.org/10.1002/ehf2.13834
15. González-López E, Gagliardi C, Dominguez F, Quarta CC, de Haro-Del Moral FJ, Milandri A, *et al.* Clinical characteristics of wild-type transthyretin cardiac amyloidosis: Disproving myths. *Eur Heart J* 2017;**38**:1895–1904. https://doi.org/10.1093/eurheartj/ehx043
16. Towbin JA, McKenna WJ, Abrams DJ, Ackerman MJ, Calkins H, Darrieux FCC, *et al.* 2019 HRS expert consensus statement on evaluation, risk stratification, and management of arrhythmogenic cardiomyopathy. *Heart Rhythm* 2019;**16**:e301–e372. https://doi.org/10.1016/j.hrthm.2019.05.007
17. Ommen SR, Mital S, Burke MA, Day SM, Deswal A, Elliott P, *et al.* 2020 AHA/ACC Guideline for the diagnosis and treatment of patients with hypertrophic cardiomyopathy: Executive summary: A report of the American College of Cardiology/American Heart Association Joint Committee on Clinical Practice Guidelines. *Circulation* 2020;**142**:e533–e557. https://doi.org/10.1161/CIR.0000000000000938
18. Vissing CR, Espersen K, Mills HL, Bartels ED, Jurlander R, Skriver SV, *et al.* Family screening in dilated cardiomyopathy: Prevalence, incidence, and potential for limiting follow-up. *JACC Heart Fail* 2022;**10**:792–803. https://doi.org/10.1016/j.jchf.2022.07.009
19. van Velzen HG, Schinkel AFL, Baart SJ, Oldenburg RA, Frohn-Mulder IME, van Slegtenhorst MA, *et al.* Outcomes of contemporary family screening in hypertrophic cardiomyopathy. *Circ Genom Precis Med* 2018;**11**:e001896. https://doi.org/10.1161/CIRCGEN.117.001896
20. Muller SA, Gasperetti A, Bosman LP, Schmidt AF, Baas AF, Amin AS, *et al.* Individualized family screening for arrhythmogenic right ventricular cardiomyopathy. *J Am Coll Cardiol* 2023;**82**:214–225. https://doi.org/10.1016/j.jacc.2023.05.005
21. Arbelo E, Protonotarios A, Gimeno JR, Arbustini E, Barriales-Villa R, Basso C, *et al.* 2023 ESC Guidelines for the management of cardiomyopathies. *Eur Heart J* 2023;**44**:3503–3626. https://doi.org/10.1093/eurheartj/ehad194
22. Gonzalez-Lopez E, Escobar-Lopez L, Obici L, Saturi G, Bezard M, Saith SE, *et al.* Prognosis of transthyretin cardiac amyloidosis without heart failure symptoms. *JACC CardioOncol.* 2022;**4**:442–454. https://doi.org/10.1016/j.jaccao.2022.07.007
23. Arana-Achaga X, Goena-Vives C, Villanueva-Benito I, Solla-Ruiz I, Rengel Jimenez A, Gaspar TI, *et al.* Development and validation of a prediction model and score for transthyretin cardiac amyloidosis diagnosis: T-Amylo. *JACC Cardiovasc Imaging* 2023;**16**:1567–1580. https://doi.org/10.1016/j.jcmg.2023.05.002
24. Davies DR, Redfield MM, Scott CG, Minamisawa M, Grogan M, Dispenzieri A, *et al.* A simple score to identify increased risk of transthyretin amyloid cardiomyopathy in heart failure with preserved ejection fraction. *JAMA Cardiol* 2022;**7**:1036–1044. https://doi.org/10.1001/jamacardio.2022.1781
25. Boldrini M, Cappelli F, Chacko L, Restrepo-Cordoba MA, Lopez-Sainz A, Giannoni A, *et al.* Multiparametric echocardiography scores for the diagnosis of cardiac amyloidosis. *JACC Cardiovasc Imaging* 2020;**13**:909–920. https://doi.org/10.1016/j.jcmg.2019.10.011
26. Aimo A, Tomasoni D, Porcari A, Vergaro G, Castiglione V, Passino C, *et al.* Left ventricular wall thickness and severity of cardiac disease in women and men with transthyretin amyloidosis. *Eur J Heart Fail* 2023;**25**:510–514. https://doi.org/10.1002/ejhf.2824
27. Eriksson P, Hansson PO, Eriksson H, Dellborg M. Bundle-branch block in a general male population: The study of men born 1913. *Circulation* 1998;**98**:2494–2500. https://doi.org/10.1161/01.cir.98.22.2494
28. Desai CS, Ning H, Lloyd-Jones DM. Competing cardiovascular outcomes associated with electrocardiographic left ventricular hypertrophy: The Atherosclerosis Risk in Communities study. *Heart* 2012;**98**:330–334. https://doi.org/10.1136/heartjnl-2011-300819
29. Haataja P, Nikus K, Kähönen M, Huhtala H, Nieminen T, Jula A, *et al.* Prevalence of ventricular conduction blocks in the resting electrocardiogram in a general population: The Health 2000 Survey. *Int J Cardiol* 2013;**167**:1953–1960. https://doi.org/10.1016/j.ijcard.2012.05.024
30. Compendium. Vyndaqel Technical infomation. https://compendium.ch/product/1441791-vyndaqel-kaps-61-mg/mpro (Accessed 30 May 2024)
31. Zorginstituut Nederland. *Farmacotherapeutisch rapport tafamidis (Vyndaqel 61 mg) bij de behandeling van ATTR-CM*. Diemen, the Netherlands: Zorginstituut Nederland; 2021. https://www.zorginstituutnederland.nl/publicaties/adviezen/2021/08/11/gvs-advies-tafamidis-vyndaqel-bij-de-behandeling-van-attr-cm (Accessed 30 May 2024)