Multimodal Validation and Disease Stage Dependency of ATTR Cardiomyopathy Red Flags in Patients With LV Thickening

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Structured Abstract:

Introduction

Timely diagnosis of transthyretin amyloid cardiomyopathy (ATTR-CM) is critical to improving outcomes and remains challenging. Guidelines recommend assessing for red flags (RFs) to facilitate ATTR-CM detection, but their diagnostic validation remains incomplete. This study aimed to multidimensionally validate these RFs in patients with left ventricular thickening (LVT).

Methods

Prospective registry study including all patients with LVT and standardized workup at tertiary amyloidosis center. For validated RFs, "high-risk" (specificity for ATTR-CM>90%) and "low-risk" (false-negative rate<5%) findings were predefined.

Results

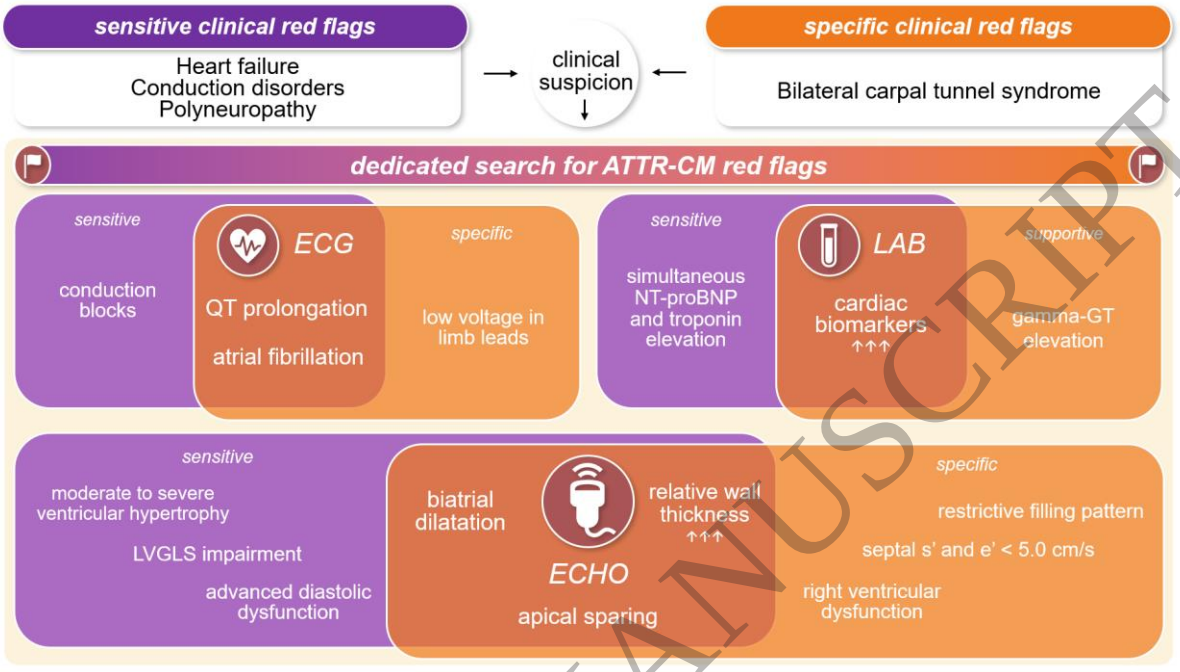
425 patients (321 ATTR-CM,104 control group) were included. Heart failure (97%) and polyneuropathies (78%) were among most sensitive clinical findings, while bilateral carpal tunnel syndrome (bCTS) was more specific (80%) but less sensitive (45%). Diverse ECG changes were common, yet mostly unspecific. Marked elevations of NT-proBNP and troponin were most discriminative among non-imaging RFs (Youden's index[YI]:59%). Apical sparing (Apical-to-Basal-strain-ratio≥2.0) was the most effective, stage-independent echocardiographic parameter (YI62%), followed by biatrial enlargement, advanced diastolic dysfunction, and LVGLS impairment (YI48-55%). Normal ECG without conduction disorders history and normal levels of both NT-proBNP and hs-troponin-T constituted "low-risk" findings. Heart failure with concomitant bCTS, peripheral low voltage, right ventricular dysfunction, 5-5-5 sign, restrictive filling pattern and apical sparing with LVGLS impairment represented "high-risk" RFs. Contrary to most non-imaging RFs, most echocardiographic RFs showed association with advancing disease stage ($p<0.05$). All aforementioned RFs were associated with ATTR-CM independently of age, sex, and LVT-severity ($p<0.05$).

Conclusions

ATTR-CM is frequently associated with validated RFs that differ in their diagnostic characteristics, which should be considered when evaluating for ATTR-CM.

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Graphical Abstract:



Graphical Abstract: Multimodal Validation and Disease Stage Dependency of ATTR Cardiomyopathy Red Flags in Patients With LV Thickening
ATTR-CM = transthyretin amyloid cardiomyopathy, GT = glutamyltransferase, LVGLS = left ventricular global longitudinal strain

ALT-TEXT: This graphical abstract provides an overview of the red flags associated with transthyretin amyloid cardiomyopathy across different diagnostic modalities. These findings are classified as predominantly sensitive, predominantly specific (or supportive for laboratory parameters), or overlapping. The evaluated modalities included clinical examination, ECG, laboratory assessment, and echocardiography.

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Introduction:

Once considered an orphan disease, cardiac amyloidosis (CA) is now recognized as relatively common, with reported prevalence of CA as high as 13.7% in heart failure patients.(1) Transthyretin amyloid cardiomyopathy (ATTR-CM) typically presents with ventricular thickening and is responsible for a major number of CA cases, representing an underdiagnosed, yet treatable cause of heart failure.(2) The systemic nature of the disease frequently results in heterogeneous clinical presentations, regularly leading to significant diagnostic delays in clinical practice.(3) To aid in timely identification, essential for maximizing therapeutic benefits and improving outcomes, numerous cardiac and extracardiac "red flags" (RFs) have been proposed and incorporated into the guidelines for CA and ATTR-CM diagnostics.(4,5) Nevertheless, prior research that shaped the current understanding of RFs in ATTR-CM has primarily focused on individual parameters or included small patient cohorts with limited generalizability. Furthermore, the diagnostic value of these RFs has not yet been multimodally validated in a real-world, contemporary population of patients presenting with suspected disease. Therefore, the present study aimed to validate these RFs and to identify those that most effectively discriminate patients with ATTR-CM among patients with unexplained left ventricular wall thickening. Moreover, we sought to determine which RFs are typically present in early stages of ATTR-CM and to assess whether their prevalence is associated with advancing disease stage. Finally, guidelines recommend screening for CA in patients with a left ventricular wall thickness of ≥12 mm when accompanied by RFs, but the optimal approach to such screening remains unclear.(5) Therefore, based on sensitivity and specificity of validated RFs, we aimed to identify those that represent particularly "high" and "low" risk findings for ATTR-CM, and may in turn support diagnostic pathways in patients with unclear left ventricular wall thickening.

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Methods:

Study Design

In this prospective registry study, we enrolled all patients with left ventricular thickening (LVT) who had been referred to the Amyloidosis Center Charité Berlin (ACCB) for diagnostic clarification of suspected CA between May 2019 and October 2024. ATTR-CM patients were compared with control group (CG) without CA but with LVT. LVT was defined as diastolic interventricular septum or posterior wall thickness of ≥12mm. Prespecified inclusion criteria for the analysis comprised age ≥ 18 years, result of genetic testing for transthyretin (TTR) gene mutation, completion of the standardized diagnostic protocol at the ACCB (described below), and technetium-99m, 3,3-diphosphono-1,2-propanodicarboxylic acid (99mTc-DPD) scintigraphy and/or myocardial biopsy leading to the definitive diagnosis of ATTR-CM or exclusion of CA in accordance with the current guidelines.(5) Patients were included in the study only if they met all of the prespecified inclusion criteria. Accordingly, patients with other CA types or without completed multimodal workup were excluded. Analyzed RFs were selected based on the available literature, including the aforementioned guidelines.(4,5) All analyzed RFs are routinely evaluated as part of standardized protocol in our centre. In the ACCB catchment area, the vast majority of patients with suspected CA (predominantly ATTR-CM) is routinely referred to our centre from all levels of the healthcare system for diagnostic evaluation and characterization. Consequently, the study cohort should reflect a contemporary, real-world population at risk of disease.

Left ventricular global longitudinal strain (LVGLS) impairment was defined as a LVGLS reduction below -15.0%.(5) Apical sparing was evaluated using Relative Apical Sparing Pattern (RELAPS) and Apical-to-Basal Strain Ratio (ABR) approaches. In accordance with most widely used

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thresholds, apical sparing was defined as a RELAPS value over 1.00 and – consistently with the RELAPS approach – an ABR value of 2.00 or more.(6)

RELAPS was calculated as:

$$RELAPS = \frac{mean\ apical\ LVLS}{(mean\ midventricular\ LVLS + mean\ basal\ LVLS)}$$

LVLS = left ventricular longitudinal strain

ABR was calculated as:

$$ABR = \frac{mean\ apical\ LVLS}{mean\ basal\ LVLS}$$

LVLS = left ventricular longitudinal strain

Ancillary definitions of parameters are provided in Supplementary Table 1. All evaluated data were independently reviewed by a researcher not involved in ACCB board decisions.

All included patients provided informed written consent for the participation in the ACCB patient registry study (DRKS00032002). The study complies with Declaration of Helsinki, Good Clinical Practice, and the laws and regulations applicable in Germany. The institutional review board of the Charité – Universitätsmedizin Berlin approved the use of the data for the purposes of the present study (EA1/014/20).

Baseline characteristics and the results of the following modalities comprised standardized diagnostic protocol: multidisciplinary clinical examination, dedicated neurological assessment, genetic and laboratory testing (including confirmation or exclusion of monoclonal component presence), 12-lead electrocardiogram (ECG), transthoracic echocardiography (TTE), 99mTc-DPD

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scintigraphy and/or myocardial biopsy. Neurological assessment included detailed evaluation of sensory and motor function, involving nerve conduction studies, electromyography, sensory evoked potentials, and quantification using the Neuropathy Impairment Score. TTE examinations and measurements were performed using either the Vivid E9 system (GE Vingmed, Horton, Norway) or with Vivid E95 system (GE Vingmed, Horton, Norway) with an M5S and/or an 4Vc-D transducer. In single cases, single measurements were performed on a Philips system or analysed offline using TomTec version TTA2.51 (TomTec, Unterschleißheim, Germany). Imaging data quality was rigorously evaluated, and only measurements meeting the quality criteria according to the current guidelines(7,8) were included in the study. Furthermore, data were collected exclusively from examinations performed as part of the initial diagnostic assessment.

Statistical Analysis

Quantitative data are presented as means ± standard deviation or median (interquartile range). For the normally distributed quantitative variables, the assumption of homoscedasticity was assessed using Levene's test. When Levene's test yielded a significant result, the Welch-adjusted t test was used instead of conventional independent samples t test. Qualitative data are presented as counts (percent) or percent alone. Due to binary nature of numerous RFs, overall diagnostic effectiveness of RFs was compared using Youden's index (YI; YI = sensitivity + specificity – 100%), with cut-off values for quantitative parameters derived from the aforementioned literature. In case of two laboratory variables ("marked" NT-proBNP and high sensitivity troponin T elevations), receiver operating characteristic (ROC) curve analysis was performed to identify most discriminative cut-offs due to lack of corresponding cut-offs in the literature. Multivariable logistic regression analysis was performed to evaluate whether the discriminative value of RFs remained independent after adjustment for demographic and clinical variables which were chosen based on clinical

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considerations, including age, sex, and the severity of LVT. LVT severity was analyzed using polynomial terms due to violating the linearity assumption in corresponding analyses. Furthermore, logistic regression was utilized to investigate the association between advancing disease stage, as defined by the National Amyloidosis Centre (NAC) staging system(9), and the increasing prevalence of RFs within the cohort of patients with ATTR-CM. Finally, we classified RFs into "low" and "high" risk findings based on their sensitivity and specificity. Because missing the diagnosis of ATTR-CM may be more grave than the potentially unnecessary diagnostic clarification, we predefined "low-risk" findings as possessing < 5.0% probability of incorrectly excluding ATTR-CM (false-negative rate<5%), whereas "high risk" findings were characterized by a specificity for ATTR-CM exceeding 90.0% in the present study. Statistical analysis was conducted using SPSS Statistics for Windows version 29.0 (IBM Corp., Armonk, N.Y., USA). Statistical significance was defined as $p < 0.05$.

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Results:

General Characteristics

Of the 451 patients with suspected CA and LVT enrolled in the ACCB registry study by October 2024, 425 (94%) met inclusion criteria and were included in the analysis. Among these, 321 (75%) patients were diagnosed with ATTR-CM, while it was excluded in 104 (25%) cases which formed the CG.

Wild type ATTR (ATTRwt) represented 94% (n=302) of ATTR-CM cases, whereas hereditary ATTR (ATTRv) comprised 6% (n=19). According to the NAC staging system, 52% (n=167) of ATTR-CM patients were classified as Stage I, 31% (n=100) as Stage II, and 17% (n=54) as Stage III.

Patients with ATTR-CM were older on average and more frequently male. The groups did not differ significantly with respect to the prevalence of baseline cardiovascular risk factors and major adverse cardiovascular events. General characteristics of the study population are summarized in Table 1.

Non-Imaging Red Flags

Heart failure, conduction disorders and polyneuropathy of various severities emerged as the most overall effective and early-stage clinical RFs. On the other hand, bilateral carpal tunnel syndrome was more specific and less sensitive in the examined context. Conversely, the frequencies of biceps tendon rupture (3.4% vs. 2.9%, $p = 0.788$) and newly diagnosed aortic valve stenosis in individuals aged ≥ 65 years (15.3% vs. 14.4%, $p = 0.835$) did not differ significantly between the groups. All but one ATTR-CM patient presented with at least one clinical RF. Concomitant presence of

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bilateral carpal tunnel syndrome and heart failure was the most specific clinical presentation, 7 times more common in ATTR-CM compared to CG (42.7% vs. 5.8%, $p < 0.001$).

In patients with heart failure, the both groups did not significantly differ in respect to the distribution of heart failure phenotypes (heart failure with preserved/mildly reduced/reduced ejection fraction: HFpEF 58.1% vs 72.7%, HFmrEF 29.9% vs. 20.5%, HFrEF 12.0% vs. 6.8%; $p = 0.176$) or the prevalence of moderate to severe dyspnoea burden (New York Heart Association class III/IV: 40.5% vs. 29.5%, $p = 0.165$).

On ECG, QT interval prolongation and atrial fibrillation were the most generally effective findings, with the latter demonstrating a highly significant, 74% increase in prevalence across advancing NAC stages in ATTR-CM. Only 8 (2.5%) ATTR-CM patients had normal ECG and no conduction disease.

Biomarkers analysis revealed that marked elevations of NT-proBNP (optimal ROC-analysis cut-off: >1000 ng/L) and high sensitivity troponin T (optimal ROC-analysis cut-off: 2 x norm) were the most effective non-imaging RFs in this study. The combination of normal high sensitivity troponin T (<14.0ng/L) and NT-proBNP (<180 ng/L) correctly ruled out ATTR-CM in all but one case.

Table 2 presents the diagnostic characteristics of non-imaging RFs that were significantly more frequent in ATTR-CM, while table 3 presents the prevalence of validated and independently associated RFs in ATTR-CM across NAC stages.

Echocardiographic Red Flags

Relative wall thickness (RWT) of > 0.6 was the most effective strictly morphologic RF in ATTR-CM, while advanced—grade II-III—diastolic dysfunction proved to be the most diagnostically

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effective strictly functional RF. Serving as both structural and functional marker, biatrial dilatation yielded the second highest YI among single-parameter echocardiographic parameters at 54.9%. However, these findings occurred with only moderate prevalence in NAC stage I, where moderate to severe left ventricular wall thickening, left atrial enlargement, and LVGLS reduction represented the most common, but nonspecific early-stage disease manifestations.

Among deformation imaging parameters, ABR-based apical sparing (ABR ≥ 2.0) proved to be most diagnostically effective and NAC stage-independent echocardiographic marker for ATTR-CM observed in three-fourths of NAC-I ATTR-CM patients. In contrary, another apical sparing marker – RELAPS – was found to be significantly associated with advancing NAC stages in ATTR-CM and was present in less than half of NAC-I ATTR-CM patients. When ABR ≥ 2.0 was combined with LVGLS reduction below -15.0%, the YI increased slightly compared to sole ABR analysis. This combination demonstrated the highest overall diagnostic effectiveness among all evaluated parameters in the study, with YI of 64.0%.

Table 4 presents the diagnostic characteristics of echocardiographic RFs that were significantly more frequent in ATTR-CM, while table 5 presents the prevalence of validated and independently associated RFs in ATTR-CM across NAC stages.

None of initially significant non-imaging and echocardiographic RFs were reclassified after applying Benjamini-Hochberg procedure. Validated "high" and "low risk" RFs that reached the prespecified specificity and false-negative rate thresholds for ATTR-CM were summarized in Fig. 1.

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Discussion:

The present study is the first to directly compare, validate, and examine the stage-dependence of a broad range of red flags for ATTR-CM across modalities commonly used as primary diagnostic tools, based on a large, contemporary real-world cohort at risk of disease. Our analysis demonstrated that the majority of these findings serve as important age- and sex-independent warning signs of ATTR-CM across the entire hypertrophic spectrum. However, some of the classic RFs, such as biceps tendon rupture, did not occur more frequently in patients with ATTR-CM than in those in the control group. In general, the diagnostic yield of the RFs strongly depends on the modality employed, the organ system involved and disease stage. Importantly, since most red flags can be detected by easily available, routine examinations, integrating our findings into clinical practice should be straightforward.

Clinical Evaluation: Raising Suspicion

Clinical signs and resulting symptom burden often serve as the major trigger for initiating the diagnostic cascade. Therefore, raising clinical suspicion is a crucial component for establishing the diagnosis of underlying ATTR-CM. Deleterious effects of amyloid plaques(10) explain the near-universal occurrence of heart failure of varying severity, as well as frequent polyneuropathic and cardiac conduction disorders already present in early NAC-stages, as demonstrated in our study. These clinical signs are not specific on an individual level; however, since amyloidosis is a systemic disorder, their simultaneous occurrence should prompt a serious consideration of ATTR-CM. Notably, all these clinical RFs represent distinct conditions that can be readily identified in clinical practice. Our findings suggest that, in the context of unexplained LVT, the combination of bilateral carpal tunnel syndrome and heart failure constitutes a particularly high-risk clinical situation. Importantly, carpal tunnel syndrome can precede cardiac involvement by more than ten

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years(11), highlighting the need for close interdisciplinary care to facilitate earlier diagnosis of ATTR-CM.

Not all well-known clinical signs proved to be independent ATTR-CM predictors. For instance, spinal stenosis was more common in ATTR-CM, but not independently associated after adjustment, leaving the definitive role of this classic finding(4,5,12,13) uncertain. Accordingly, our results are consistent with the mixed evidence in the literature regarding its direct association with CA.(14–18) Notably, there was no significant difference in the prevalence of biceps tendon rupture across the groups in our study. Importantly, biceps tendon rupture is associated with multiple amyloid-unrelated risk factors that are widely present in the general population(19). Considering that this association was originally reported in a relatively small patient cohort(20) and that biceps tendon rupture can occur in CA independently of amyloid deposition(21), it is plausible that this finding may not hold substantial diagnostic value for ATTR-CM in a broad population.

ECG and Biomarkers: Substantiating Suspicion

ECG analysis and laboratory evaluation represent widely accessible, low-threshold diagnostic modalities that enable effective reinforcement of clinical suspicion in daily practice. This study confirms that a completely normal ECG renders ATTR-CM less likely(22) and demonstrates that it represents a low-risk finding if no concomitant conduction disease is present. Furthermore, although various conduction blocks were common in early-stage ATTR-CM, our study demonstrates that no individual ECG finding is consistently present during the early stages of the disease. Generally, low voltage in limb leads is considered to be the principal hallmark of ATTR-CM on ECG. Indeed, it exhibited very good specificity in our study but was present in only about every third ATTR-CM patient, which aligns well with previous report on low voltage prevalence

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in CA(23), warranting further caution also in cases with inconspicuous voltages. Overall, QT interval prolongation and atrial fibrillation were among the most effective findings for ATTR-CM, which however, are not specific.

A combination of normal NT-proBNP and troponin levels has been proposed to make CA unlikely(24), a finding corroborated by the present study. Although nonspecific, concurrent elevation of these biomarkers represents a particularly sensitive and early indicator of the disease, as demonstrated in our study. Moreover, our findings show that their specificity increases with rising plasma levels, warranting attention in cases of unexplained severe elevations of both NT-proBNP and troponin. Interestingly, the greater prevalence of gamma-glutamyltransferase elevation among ATTR-CM patients may reflect both congestive liver dysfunction and subtle hepatic involvement(25–27), providing additional clues in challenging cases.

Echocardiography: Corroborating Suspicion

Due to its availability and cost-effectiveness, echocardiography is often the imaging modality of choice for the initial assessment of ATTR-CM risk. Our findings confirm the important role of apical sparing – particularly when quantified using the ABR method. ABR-based apical sparing exhibited better overall diagnostic performance than the RELAPS approach due to considerably higher sensitivity. This suggests that ABR may serve as a more precise indicator of ATTR-CM, particularly in earlier disease stage – possibly without severe midventricular involvement. This hypothesis aligns with the findings of Henein et al.(28), whose smaller and predominantly older ATTRv-CM study population exhibited a uniform RELAPS presence, possibly reflecting more advanced disease compared to ATTRwt-CM patients. Consistently with this hypothesis, ABR-based apical sparing was frequent in our study—also in early-stage disease—and observed with relatively constant prevalence across advancing NAC stages. In contrast, the RELAPS-positivity

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was seen in less than half of NAC stage I patients and was associated with increasing disease stage. Overall, apical sparing is neither pathognomonic, nor capable of detecting all ATTR-CM cases, but it remains an effective, yet definition-dependent RF.

Similarly, several additional echocardiographic findings exhibited meaningful diagnostic performance. Due to their indirect nature, they should always be interpreted holistically in conjunction with other markers. Among them, biatrial enlargement was confirmed to be particularly useful. It highlights the role of atrial myopathy in ATTR-CM, which most likely occurs early in disease course.(29–31) Other "high risk" findings included advanced diastolic dysfunction; expectedly, specificity increased along with dysfunction severity, exceeding 95% for the restrictive filling pattern. Further fairly specific findings included substantial right ventricular dysfunction and markedly reduced septal tissue doppler velocities, confirming its role as a typical cardiomyopathic feature.(32) Importantly, these observations underscore the impact of amyloid infiltration on both ventricles and across all phases of the cardiac cycle, including systole. Accordingly, moderate to severe left ventricular wall thickening and LVGLS reduction emerged as sensitive findings that were frequently present in early-stage disease, as shown in our study.

While other classic echocardiographic RFs were also more prevalent in the ATTR-CM group, their overall diagnostic effectiveness was limited, rendering them primarily supportive findings.

Results of this study underscore the important diagnostic role of comprehensive echocardiographic evaluation in the recognition and risk assessment for ATTR-CM, integrating left ventricular strain, morphological and diastolic indices, as well as right ventricular assessment (Fig. 2).

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Disease Stage Dependence

Contrary to most non-imaging RFs, the prevalence of atrial fibrillation increased significantly with advancing disease stage in patients with ATTR-CM. This finding may reflect the greater sensitivity of atrial fibrillation to the progression of diffuse amyloid deposition, with the occurrence of arrhythmogenic foci becoming increasingly likely due to the advancing architectural disruption of the atria. Hence, the de novo onset of this condition might potentially serve as supportive clinical marker of disease progression. In contrast to non-imaging RFs and ABR-based apical sparing, the prevalence of most echocardiographic RFs increased to varying degrees with advancing NAC stages in ATTR-CM. This finding might suggest that echocardiography may more effectively capture changes along the disease course, whereas most non-imaging RFs and ABR may serve best as binary diagnostic indicators, with greater independence of cardiac involvement severity. Nevertheless, these hypotheses are of explorative nature and warrant further investigation.

Practical Considerations

Based on the particularly sensitive and specific validated RFs, this study identified findings that could be considered as particularly "high" and "low-risk" for ATTR-CM in patients with unexplained LVT. Importantly, their assessment may potentially support rendering disease presence less likely or reinforce suspicion, particularly when consistent with the overall patient presentation. Therefore, a RFs-based supportive pathway for stepwise ATTR-CM risk guidance in patients with unexplained LVT may potentially be proposed (Fig. 3). It may help to address the expanding need for initial screening in patients presenting with clinical features suggestive of the condition – specifically, left ventricular thickening in combination with at least one clinical RF, as

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outlined in current guidelines.(5) Even though primarily supportive and of schematic character, this pathway should be compatible with a range of diagnostic modalities and could therefore be readily implementable in routine clinical practice. Accordingly, a key strength of this approach may lie in its broad applicability across diverse clinical settings. Primary care physicians and general internists could use it to initiate structured risk assessment and determine the need for further evaluation. Likewise, cardiologists can promote multidisciplinary care and refer to the multimodal "high-" and "low-risk" findings even in cases where clinical data might be scarce. Because the pre-test probability of ATTR-CM is setting-dependent, the absolute positive and negative predictive values of RFs are likely to differ across different healthcare environments and clinically distinct subgroups. Accordingly, patients' demographic and clinical characteristics, as well as their comorbidities, should be considered whenever RFs are present to assess the likelihood of alternative aetiological explanations. Therefore, the holistic perception of RFs as complementary and mutually reinforcing should be promoted. Accordingly, contextual, multimodal assessment and multidisciplinary care should be regularly pursued, which may be supported by the utilization of the proposed pathway. Finally, given the growing role and robust diagnostic performance of cardiac magnetic resonance imaging (CMR) in providing aetiologic clarification, the proposed pathway incorporates this modality as an important component in the evaluation of ATTR-CM – particularly in otherwise inconclusive cases. Overall, the findings of this study may foster broader, interdisciplinary awareness and support earlier diagnosis of this underrecognized condition. In turn, it may directly contribute to improving outcomes by preventing the progression to advanced heart failure.

Study Limitations

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Limitations of this study should be acknowledged. Even though this real-world study recruited a comparatively high number of comprehensively characterized patients and was designed to lower the risk of bias, nature of registry studies and tertiary referral structure may inherently introduce selection bias. However, in this study, all patients were prospectively characterized according to a standardized protocol and were evaluated in an unselected manner. This should mitigate the potential selection bias, albeit not eliminate it entirely. Similarly, since in the study region the diagnostic clarification of suspected ATTR-CM is routinely and almost entirely performed in our centre, the analyzed patients originated from all levels of healthcare. Therefore, referral and spectrum bias should also be lowered and the study cohort should reflect a contemporary, real-world population at risk of disease. Nevertheless, the prevalence of ATTR-CM is substantially lower in primary care settings, which should be considered when interpreting the absolute predictive performance of "high-risk RFs" and "low-risk findings". Similar effect modification is also likely in distinct clinical subgroups, such as patients with end-stage renal disease or severe aortic stenosis, in whom the specificity estimates of individual RFs might be lower. RFs were not evaluated in other subtypes of CA due to limited number of such patients at our centre. Therefore, further studies are needed to perform multimodal validation of RFs in other subtypes of CA, particularly light chain CA. In contrast to other RFs, no definite pathophysiological rationale or corresponding reference values for the identified optimal cut-off values of NT-proBNP (1000 ng/L) and hsTnT (2 x norm) are available in the literature. Therefore, these two thresholds should primarily be interpreted as hypothesis-generating and further examined in other cohorts. Similarly, the association between NAC stage and "marked NT-proBNP elevation" should be interpreted with caution, since NT-proBNP is itself a component used in defining NAC stage. Even though prespecified, based on validated findings, and following a clinically driven rationale, the sensitivity

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and specificity cut-offs for "high" and "low-risk" findings for ATTR-CM in the LVT setting were determined at the discretion of the study team and should therefore be considered primarily supportive. For these reasons, the proposed RFs-based pathway to stepwise ATTR-CM risk guidance should also be regarded as primarily supportive and schematic, without claiming to be all-embracing. Therefore, future studies should investigate whether machine learning can contribute to creating RFs-based risk scores or algorithms that provide additional benefit over currently available scoring systems. Although CMR is an important and robust imaging modality for CA, we did not evaluate the effectiveness of CMR findings in this study, which should be investigated in further studies. Even though left ventricular wall thickening is a hallmark characteristic of ATTR-CM, future studies should investigate how the RFs perform in a cohort of patients without increased left ventricular wall thickness.

Conclusion

ATTR-CM is frequently associated with cardiac and extracardiac warning signs that enable well-founded suspicion of this disease in patients with LVT in routine clinical practice. Increased awareness of these red flags may facilitate earlier diagnosis, allowing timely initiation of disease-modifying treatments and thereby improving patient prognosis by hindering the progression or development of severe heart failure.

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Conflict of interests

E.R.D. received consulting fees, advisory board fees, travel grants and lecture fees from Edwards Lifesciences, General Electrics, GlaxoSmithKline and Boehringer Ingelheim, which were not related to this project. I.M. has received research grants from Pfizer Pharmaceuticals and Sanofi; and has received lecture fee and financial reimbursement for advisory board activities from Sanofi, Takeda, Pfizer Pharmaceuticals, BridgeBio, and Chiesi Farmaceutici S.p.A outside the

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submitted work. B.H. is inventor on patents that use RNA for diagnosis of myocarditis. Patent protection is in process for MCG for diagnosis and measurement of therapy response in inflammatory cardiomyopathy and for cytokines for targeted therapy in inflammatory cardiomyopathy and heart failure. B.H. received speaker fees from Pfizer Pharmaceuticals Inc. and AstraZeneca, as well as research grant from Pfizer Pharmaceuticals Inc. unrelated to this project. S.M.W. has received travel support by Pfizer Pharma GmbH. H.F.P. has received travel support and speaker fees by Alnylam Pharmaceuticals Inc. K.H. received financial reimbursement for consulting, advisory board activities, speaker fees and/or contributions to congresses and travel support to attend scientific meetings by Akcea Therapeutics Inc., Alnylam Pharmaceuticals Inc., Amicus, AstraZeneca, GSK, Hormosan, Takeda Pharmaceutical Inc., Pfizer Pharmaceuticals Inc., and Swedish Orphan Biovitrum Inc and ViiV Healthcare GmbH. K.H. further received research funding by the foundation Charité (BIH clinical fellow), Alnylam Pharmaceuticals Inc., and Pfizer Pharmaceuticals. G.B. received lecture fees, research funding and travel reimbursement from Alnylam Pharmaceuticals, Intellia Therapeutics and AstraZeneca. S.S. received consulting fees, advisory board fees, lecture fees, research funding, and travel grants for participation in scientific meetings from Pfizer Pharma GmbH, Alnylam Netherlands BV, Bayer Vital GmbH, Novartis Pharma GmbH, Boehringer Ingelheim Pharma GmbH, Edwards Lifesciences, and AstraZeneca GmbH, which were not related to this project. All other authors have nothing to disclose.

Authors' Contributions

JP, JG, GB and SS wrote the manuscript. All authors contributed to data set definition, data collection and revision of the manuscript. SS and KH provided resources for the study conduction and supervised the manuscript.

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Data Availability

The data underlying this article are available in the article and in its online supplementary material.

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Tables

Table 1: General Characteristics of the Study Population

Parameter	ATTR-CM	Control group	p-value
Age (years)	80 ± 7	69 ± 14	<0.001
BMI (kg/m^2)	25.7 ± 3.8	28.5 ± 5.6	<0.001
Male	284 (88.5%)	65 (62.5%)	<0.001
Female	37 (11.5%)	39 (37.5%)	<0.001
Arterial hypertension	235 (73.2%)	84 (81.6%)	0.121
Hyperlipoproteinemia	218 (67.9%)	67 (64.4%)	0.511
Diabetes mellitus	81 (25.2%)	29 (27.9%)	0.592
Myocardial infarction	29 (9.0%)	6 (5.8%)	0.292
Peripheral artery disease	20 (6.2%)	7 (6.7%)	0.801
Chronic obstructive pulmonary disease	23 (7.2%)	6 (5.8%)	0.624
Valvular intervention/surgery	34 (10.6%)	14 (13.5%)	0.422
Recurring syncope	15 (4.7%)	2 (1.9%)	0.214
Stroke/transient ischemic attack	37 (11.5%)	9 (8.7%)	0.413
High sensitivity troponin T (ng/L)	46 (29-70)	15 (8-24)	<0.001
NT-proBNP (ng/L)	2422 (1329-4410)	309 (84-939)	<0.001
eGFR (ml/min/1.73m^2)	58 ± 21	68 ± 24	<0.001
Echocardiography			
Interventricular septum thickness (mm)	18 ± 3	15 ± 3	<0.001
Left ventricular enddiastolic diameter (mm)	46 ± 6	44 ± 6	0.012
Posterior wall thickness (mm)	16 ± 3	12 ± 2	<0.001
Left ventricular mass index (g/m²)	168 ± 39	121 ± 37	<0.001
Left atrial volume index (ml/m²)	49 ± 16	36 ± 18	<0.001
Left ventricular ejection fraction (%)	51 ± 9	57 ± 7	<0.001
Stroke volume index (ml/m²)	33 ± 11	39 ± 11	<0.001
Cardiac index (L/min/m²)	2.32 ± 0.74	2.73 ± 0.8	<0.001
TAPSE (mm)	17 ± 5	22 ± 5	<0.001
Right ventricular s' velocity (cm/s)	10.5 ± 2.9	13.1 ± 3.7	<0.001
Left ventricular mean e' velocity (cm/s)	5.8 ± 1.8	7.3 ± 2.2	<0.001
E/e' mean	16.3 ± 6.7	11.2 ± 5.0	<0.001
Left ventricular global longitudinal strain (%)	-11.7 ± 3.3	-16.0 ± 3.6	<0.001

ATTR-CM = transthyretin amyloid cardiomyopathy, BMI = body mass index; NT-proBNP = N-terminal pro-B-type natriuretic peptide; eGFR = estimated glomerular filtration rate; TAPSE = tricuspid annular systolic plane excursion

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Table 2: Non-Imaging Red Flags

Red flag	Sensitivity	Specificity	p-value	adjusted p-value†	YI [%]	Odds Ratio† (95%CI)
Clinical Presentation						
Heart failure	96.6%	56.9%	<0.001	**<0.001**	**53.5**	10.1 (4-24)
Conduction disorders	93.1%	51.9%	<0.001	**<0.001**	**45.0**	6.0 (3-12)
Polyneuropathy	77.6%	53.8%	<0.001	**0.008**	**31.4**	2.3 (1-4)
Bilateral CTS	44.5%	79.8%	<0.001	**<0.001**	**24.3**	4.2 (2-9)
Spinal stenosis	26.2%	85.6%	0.014	0.157	–	–
ECG						
QT interval prolongation	65.0%	76.1%	<0.001	**<0.001**	**41.1**	3.1 (2-6)
Atrial fibrillation	62.4%	78.6%	<0.001	**0.002**	**41.0**	2.9 (1-6)
Pseudoinfarction pattern	54.7%	82.6%	<0.001	**<0.001**	**37.3**	3.6 (2-7)
Low voltage in limb leads	31.7%	94.6%	<0.001	**0.003**	**26.3**	5.8 (2-18)
Any conduction block	75.9%	55.1%	<0.001	0.088	–	–
AV block	*44.9%*	*76.3%*	*<0.001*	*0.173*	–	–
Nonspecific intraventricular conduction delay	*16.3%*	*96.7%*	*0.001*	***0.012***	***13.0***	*6.6 (2-29)*
Complete LBBB/RBBB	*26.5%*	*84.9%*	*0.024*	*0.595*	–	–
Bi-/Trifascicular block	*14.3%*	*94.6%*	*0.023*	*0.912*	–	–
Biomarker Analysis						
Marked NT-proBNP elevation (>1000 ng/L)	81.6%	77.8%	<0.001	**<0.001**	**59.4**	5.7 (3-11)
Marked hsTnT elevation (≥ 2 x norm)	79.7%	79.4%	<0.001	**<0.001**	**59.1**	4.7 (2-9)
Simultaneous hsTnT and NT-proBNP elevation	96.7%	56.8%	<0.001	**<0.001**	**53.5**	6.8 (3-18)
Gamma-GT elevation	50.9%	79.2%	<0.001	**0.020**	**30.1**	2.2 (1-5)
eGFR CKD-EPI < 60 ml/min/1.73m^2	54.5%	63.7%	0.001	0.928	–	–

Independently associated red flags are sorted after Youden's index within modalities. † adjusted for age, sex, and increasing left ventricular wall thickness; CI = confidence interval; CTS = carpal tunnel syndrome; AV = atrioventricular; LBBB/RBBB = left/right bundle branch block; hsTnT = high sensitivity troponin T (elevation: >14.0ng/L); NT-proBNP = N-terminal pro-B-type natriuretic peptide (elevation: >180ng/L); GT = glutamyltransferase; eGFR = estimated glomerular filtration rate; CKD-EPI = Chronic Kidney Disease Epidemiology Collaboration

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Table 3:
Prevalence of Validated Non-Imaging Red Flags in ATTR-CM Patients Across NAC-Stages

Parameter	Prevalence in NAC-Stage (%)			p-value for association*	Odds Ratio (95% CI) **
	I	II	III		
Clinical Presentation					
Heart failure	93.4	100.0	100.0	0.994	–
Conduction disorders	90.4	95.9	96.4	0.069	–
Polyneuropathy	72.5	83.7	82.1	0.052	–
Bilateral CTS	45.5	45.9	39.3	0.501	–
ECG					
QT interval prolongation	62.5	65.0	73.3	0.207	–
Atrial fibrillation	47.9	75.8	83.3	**<0.001**	2.7 (2-4)
Pseudoinfarction pattern	47.3	69.6	53.3	0.092	–
Low voltage in limb leads	26.5	41.5	31.1	0.194	–
NICD	14.5	20.2	14.9	0.658	–
Biomarker Analysis					
*Marked NT-proBNP elevation (>1000 ng/L)****	*66.5*	*96.9*	*100.0*	*<0.001*	*17.1 (5-54)*
Marked hsTnT elevation (≥ 2 x norm)	66.5	92.4	98.0	**<0.001**	5.7 (3-11)
Simultaneous hsTnT and NT-proBNP elevation	94.9	97.8	100.0	0.084	–
Gamma-GT elevation	34.4	64.5	75.5	**<0.001**	2.7 (2-4)

* p-value for association between increasing NAC-stage and the prevalence of the examined red flag; ** for RF-occurrence and one-stage-increase *** association may be influenced by the role in definition of NAC-stages; CI = confidence interval; CTS = carpal tunnel syndrome, NICD = nonspecific intraventricular conduction delay; hsTnT = high sensitivity troponin T (elevation: >14.0ng/L); NT-proBNP = N-terminal pro-B-type natriuretic peptide (elevation: >180ng/L); GT = glutamyltransferase;

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Table 4: Echocardiographic Red Flags

Red flag	Sensitivity	Specificity	p-value	adjusted p-value†	YI [%]	Odds Ratio† (95%CI)
Echocardiography						
Ventricular Morphology						
Relative wall thickness > 0.6	69.6%	77.5%	<0.001	**<0.001**	**47.1**	3.8 (2-8)
Max. wall thickness ≥ 15mm‡	90.0%	56.7%	<0.001	**<0.001**	**46.7**	–
Thickened atrioventricular valve	53.5%	76.9%	<0.001	**0.004**	**30.4**	2.5 (1-5)
Right ventricular thickening	87.8%	45.0%	<0.001	0.114	–	–
Moderate/severe VHD	42.3%	71.2%	0.015	0.697	–	–
Ventricular Function: Non-Strain						
Diastolic dysfunction II-III°	79.3%	69.0%	<0.001	**0.004**	**48.3**	2.9 (1-6)
Septal s' and e' < 5.0 cm/s	46.3%	93.8%	<0.001	**<0.001**	**40.1**	5.8 (2-16)
Increased filling pressures (E/e' mean > 14.0)	61.6%	77.6%	<0.001	**<0.001**	**39.2**	3.5 (2-7)
Restrictive filling pattern	39.7%	97.8%	<0.001	**<0.001**	**37.5**	19.4 (4-94)
Right ventricular dysfunction	42.8%	91.2%	<0.001	**<0.001**	**34.0**	5.0 (2-13)
5-5-5 sign	26.2%	95.8%	<0.001	**0.030**	**22.0**	3.7 (1-13)
Atrial Morphology						
Biatrial enlargement	78.1%	76.8%	<0.001	**<0.001**	**54.9**	4.5 (2-8)
Left atrial enlargement	88.5%	59.6%	<0.001	**<0.001**	**48.1**	4.0 (2-8)
Ventricular Function: Strain						
LVGLS impairment + ABR ≥ 2.0	69.7%	94.3%	<0.001	**<0.001**	**64.0**	15.2 (6-42)
ABR ≥ 2.0	80.8%	81.4%	<0.001	**<0.001**	**62.2**	8.5 (4-17)
RELAPS > 1.0	58.1%	94.4%	<0.001	**<0.001**	**52.5**	11.6 (4-36)
LVGLS impairment	84.6%	63.7%	<0.001	**<0.001**	**48.3**	4.3 (2-9)

Independently associated red flags are sorted after Youden's index within categories; right ventricular dysfunction was defined as TAPSE <16mm if consistent with RV s' value; LVGLS impairment was defined as LVGLS below -15.0%(5) † adjusted for age, sex, and left ventricular wall thickness (in ‡ left ventricular wall thickness omitted. Therefore, no odds ratios were reported in this case); CI = confidence interval; VHD = valvular heart disease; LVGLS = left ventricular global longitudinal strain; ABR = Apical-to-Basal strain ratio; RELAPS = relative apical sparing pattern;

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Table 5:

Prevalence of Validated Echocardiographic Red Flags in ATTR-CM Patients Across NAC-Stages

Parameter	Prevalence in NAC-Stage (%)			p-value for association*	Odds Ratio (95% CI) **
	I	II	III		
Echocardiography					
Ventricular Morphology					
Relative wall thickness > 0.6	62.6	75.0	81.5	**0.004**	1.7 (1-2)
Max. wall thickness ≥ 15mm	87.3	92.9	92.9	0.145	–
Thickened atrioventricular valve	45.7	61.3	63.6	**0.007**	1.5 (1-2)
Ventricular Function: Non-Strain					
Diastolic dysfunction II-III°	70.5	88.5	87.8	**0.003**	2.0 (1-3)
Septal s' and e' < 5.0 cm/s	29.9	58.6	72.5	**<0.001**	2.6 (2-4)
Increased filling pressures (E/e' mean > 14.0)	51.7	70.5	75.0	**<0.001**	1.8 (1-3)
Restrictive filling pattern	29.5	53.7	66.7	**<0.001**	2.3 (1-4)
Right ventricular dysfunction	22.7	62.9	66.0	**<0.001**	3.0 (2-4)
5-5-5 sign	17.6	34.5	48.4	**<0.001**	2.1 (1-3)
Atrial Morphology					
Biatrial enlargement	68.1	86.5	92.9	**<0.001**	2.7 (2-4)
Left atrial enlargement	83.0	93.3	96.2	**0.005**	2.5 (1-5)
Ventricular Function: Strain					
LVGLS impairment + ABR ≥ 2.0	59.7	82.1	77.8	**0.002**	1.8 (1-3)
ABR ≥ 2.0	75.0	90.5	81.5	0.086	–
RELAPS > 1.0	48.8	73.2	59.5	**0.040**	1.5 (1-2)
LVGLS impairment	78.3	88.5	96.3	**0.001**	2.4 (1-4)

* p-value for association between increasing NAC-stage and the prevalence of the examined red flag; ** for RF-occurrence per one-stage-increase; Right ventricular dysfunction was defined as TAPSE <16mm if consistent with RV s' value; LVGLS impairment was defined as LVGLS below -15.0%(5); CI = confidence interval; VHD = valvular heart disease LVGLS = left ventricular global longitudinal strain; ABR = Apical-to-Basal strain ratio; RELAPS = relative apical sparing pattern;

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Figures

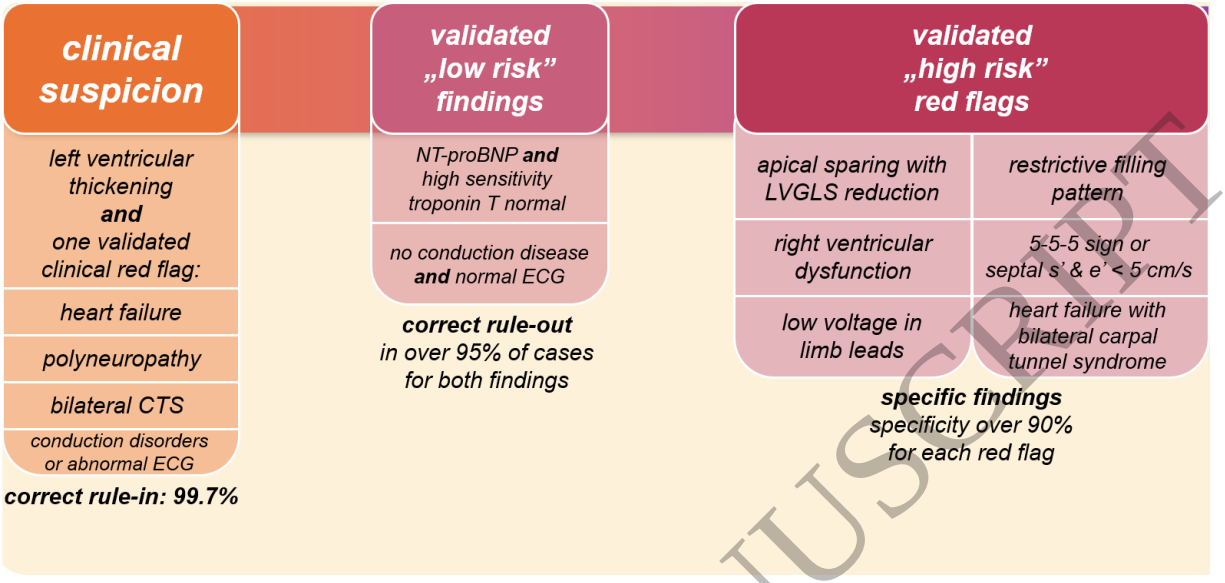


Figure 1: Validated Low Risk Findings and High Risk Red Flags for ATTR-CM in Patients With Unexplained Left Ventricular Thickening

CTS = carpal tunnel syndrome; LVGLS = left ventricular global longitudinal strain, ATTR-CM = transthyretin amyloid cardiomyopathy

ALT-TEXT: Flowchart summarizing "low-" and "high-risk" findings for ATTR-CM in patients with unexplained left ventricular thickening. The first column, "clinical suspicion" combines left ventricular thickening with at least one validated clinical red flag. The second and third columns list validated "low-risk" findings and "high-risk" red flags for ATTR-CM, respectively.

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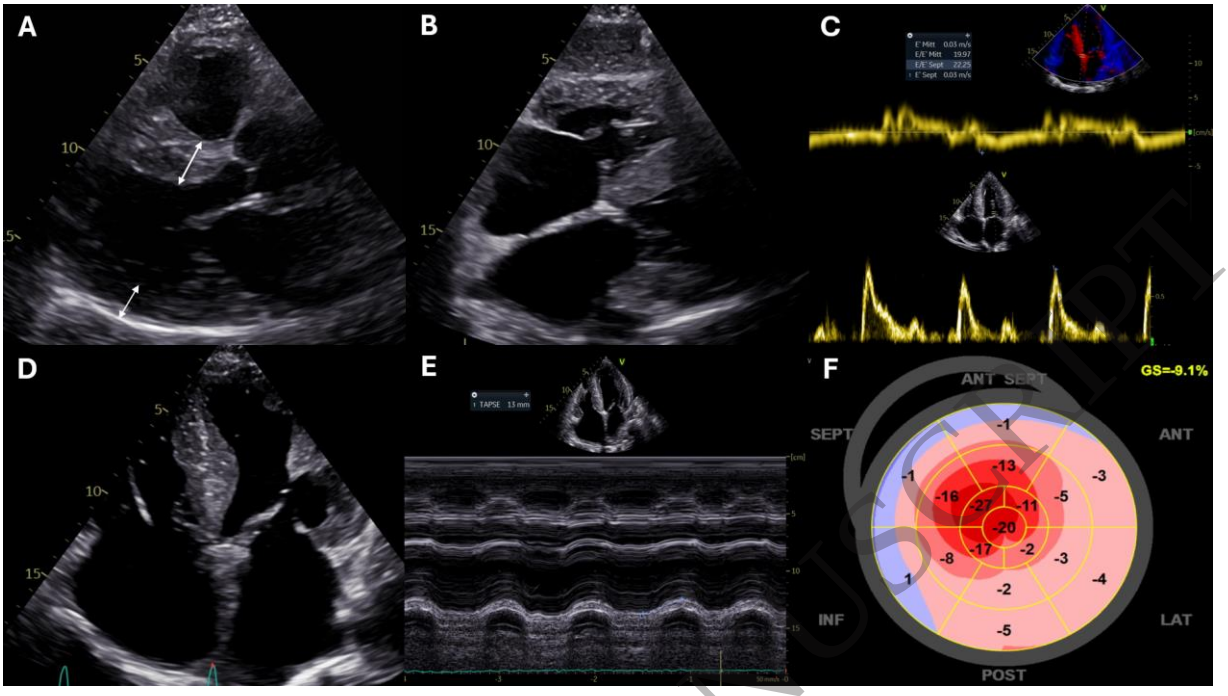


Figure 2: "Amyloidosis-Echo": validated findings on echocardiography in ATTR-CM. A – parasternal long axis, white arrows: prominent LV-wall thickening; B – subcostal view, prominent wall thickening; C – doppler imaging, mean E/e' > 14.0 indicating increased filling pressures, septal e' and s' waves velocities < 5 cm/s, restrictive filling pattern; D – apical four chamber view, biatrial dilatation, LV-wall thickening, mitral valve thickening; E – TAPSE < 16 mm; F – Apical sparing (ABR 6.0), significant left ventricular global longitudinal strain reduction.

ALT-TEXT: Six echocardiographic panels labelled A through F displaying cardiac imaging views, Doppler waveforms, M-mode analysis, and a strain analysis bullseye plot to illustrate validated diagnostic findings for transthyretin amyloid cardiomyopathy.

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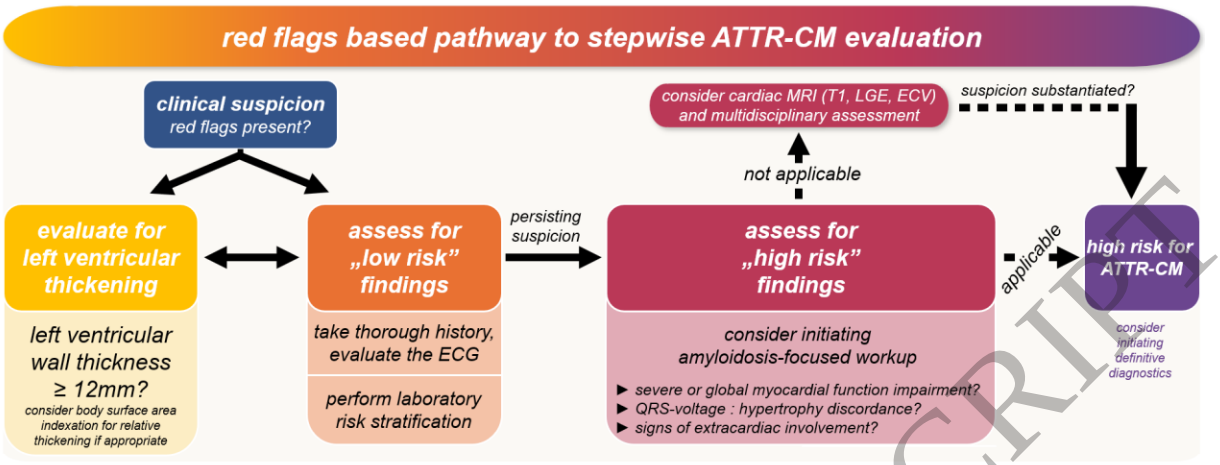


Figure 3: Supportive Red Flags Based Pathway To Stepwise Evaluation of Potential ATTR-CM in Patients With Unexplained Left Ventricular Thickening

ATTR-CM = transthyretin amyloid cardiomyopathy, MRI = magnetic resonance imaging, LGE = late gadolinium enhancement, ECV = extracellular volume

ALT-TEXT: Stepwise, red flags based flowchart to support risk stratification of transthyretin amyloid cardiomyopathy in patients with unexplained left ventricular thickening. Flowchart includes assessment of left ventricular wall thickness, "low-risk" findings, "high-risk" red findings in patients with clinical suspicion of the disease. Additionally, flowchart proposes utilization of cardiac magnetic resonance imaging and multidisciplinary assessment.

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