# Cardiac Transthyretin-derived Amyloidosis

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# Cardiac Screening of Amyloid TTR Pathogenic Variant Carriers: Complementary value of Echocardiographic Global Longitudinal Strain Imaging vs Bonescintigraphy

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## Abstract

*Objectives:*
To investigate the value of left ventricular global longitudinal strain (LV-GLS) for screening for cardiac involvement in a population of TTR pathogenic variant carriers.

*Background:*
Cardiac involvement is the major determinant of mortality in hereditary transthyretin-derived amyloïdose (ATTRv). Treatment options have recently become available, which make early diagnosis important. Several imaging modalities for screening are proposed including bonescintigraphy, conventional and strain echocardiography but data on their relative value is limited in this population.

*Methods:*
Fifty-three TTR pathogenic variant carriers from 2 academic hospitals underwent transthoracic echocardiography and bonescintigraphy as part of routine out-patient care to assess cardiac involvement. LV-GLS was measured by 2 blinded reviewers. Abnormalities in LV-GLS (cut-off -21.5%) and conventional TTE (cTTE) parameters were evaluated in bonescintigraphy positive (≥ grade 1) and negative cases.

*Results:*
Carriers with a positive bonescintigraphy (n=20) were 16 years older than those with a negative scan (n=33). When bonescintigraphy was negative, LV-GLS was abnormal in 11 cases (38%). Three out of them had diabetes and/or hypertension as potential additional explanation to ATTRv for the abnormal LV-GLS. In the remaining 11 carriers, cardiac ATTRv was the sole most likely cause of the abnormal LV-GLS. In 85% (17/20) of patients with a positive bonescintigraphy, LV-GLS was equally sensitive in determining the presence of cardiac ATTR as the combination of all cTTE parameters.

*Conclusions:*
LV-GLS and bonescintigraphy are complementary in screening TTR pathogenic variant carriers for cardiac involvement and should be used in conjunct. LV-GLS can play a significant role in repeated assessment in this population and our results suggest that LV-GLS may detect early disease onset before bonescintigraphy.

## Introduction

Hereditary transthyretin-derived amyloidosis (ATTRv) is a systemic disease characterised by deposition of misfolded tranthyretin (TTR) proteins in multiple organ systems.[1] Cardiac involvement is the major determinant of mortality in ATTRv.[2]

New treatment options have recently been introduced that either halt production of or stabilize the TTR precursor protein, preventing further amyloid deposition in organs.[3,4] Herewith, disease progression can be slowed or halted, but end-organ damage may not be reversed due to already existing depositions.[5] Therefore, early diagnosis of cardiac involvement with limited changes is crucial to improve patient prognosis in ATTRv pathogenic variant carriers.[6,7]

The best modality to facilitate early detection of cardiac involvement is under discussion. Bonescintigraphy is considered the imaging modality of first choice, but its value was recently questioned in ATTRv patients as it was less sensitive for cardiac involvement in carriers of the p.(Phe64Leu) pathogenic variant.[8,9] Also, radiation exposure in a relatively young population where repeated assessments are needed makes bonescintigraphy less attractive. The gold standard of cardiac biopsy is an invasive procedure with potential complications and limited availability.

Transthoracic echocardiography (TTE) is the most used imaging modality in clinical cardiology, including use in patients with cardiomyopathies.[10] However, changes in left ventricular ejection fraction (LVEF) or wall thickness occur rather late in the course of disease.[11] Speckle tracking derived deformation imaging of the LV - or strain-imaging - is known to detect early myocardial disease[12] and is hypothesized as a sensitive screening modality for cardiac amyloidosis.[13] On the other hand, strain-imaging may be less specific and can indicate other cardiac pathology besides ATTRv related changes.

The objective of this study was to describe cardiac involvement in TTR pathogenic variant carriers using multi-modality imaging with focus on the complementary value of TTE derived global longitudinal strain-imaging (GLS) of the LV.

## Methods

### Study population

We retrospectively analyzed patients with an established pathogenic variant (ACMG/AMP class 5; established by DNA analysis)[2,14] in TTR, the gene encoding transthyretin (TTR) who were screened for development of cardiac phenotypical changes. Patients were either index patients referred for cardiac screening (i.e. with an unknown cardiac status; for example presenting with a neurologic phenotype) or family members of index patients with ATTRv identified as part of family screening. Patients were included

and screened at the Amyloidosis Center of Expertise of the University Medical Center Groningen from 2012 onward and the Amyloidosis Center of Expertise of the University Medical Center Utrecht from 2018 onward.[2,15] This study confirms to the Principles of the Declaration of Helsinki and was approved by the local ethics committees.

Patients were included in the current analysis when at least one TTE with sufficient imaging quality for LV-GLS analysis was available within 6 months before or after a bonescintigraphy or in-between two negative or two positive bonescintigraphies (see flowchart in Figure 1). Some patients had more than one evaluation since patients were screened longitudinally as part of their routine follow-up; in this case the first available evaluation was used for analysis. Demographic, clinical, laboratory and bonescintigraphy data were collected from patient charts. One cardiologist (SvW) checked the chart transcript in a blinded fashion to decide upon cardiac complaints including but not limited to dyspnea, chest pain, peripheral edema and palpitations.

**Electrocardiography (ECG)**

ECG abnormalities were defined as: conduction delay (incomplete or complete bundle branch block or any degree AV block), low QRS voltage (defined as QRS amplitude <=0.5 mV in all limb leads or <= 1 mV in all precordial leads), pseudo-infarct pattern or a rhythm other than sinus rhythm.[16]

**TTE**

TTE images were routinely obtained from standard parasternal, apical and subcostal views using Philips or GE machines as a part of clinical care. Conventional TTE (cTTE) parameters were evaluated according to the recommendations of the European Association of Cardiovascular Imaging[17] and verified at the central echolab at MUMC by a single, blinded reviewer (SvW). The following cut-offs were used for determining cardiac pathology: LVEF <50%, interventricular septum (IVST) or posterior wall thickness (PWT ) >12 mm, e' lateral <10 cm/sec, e' septal <7 cm/sec, E/e' ratio >14, TR-velocity >=2.8 m/s, TAPSE <17 mm, right ventricular (RV) S' <9.5 cm/s, LAVI >34 ml/m2. Diastolic dysfunction was graded according to accepted algorithms, grade 2 or more was deemed pathological.[18]

Analysis of LV function with speckle tracking–based GLS imaging was performed at the central echolab, using a dedicated software package (AutoSTRAIN, TOMTEC-ARENA*1.2, TOMTEC Imaging Systems GmbH, Unterschleissheim, Germany) with a recently published algorithm.[12] Apical 2-, 3- and 4-chamber views of all available TTEs were uploaded and reviewed for image quality and completeness by two independent reviewers (JW, CK). Incomplete TTEs or studies of insufficient quality were excluded. Regional and global longitudinal peak systolic strain was calculated following contour detection by the software's algorithm; although these suggested contours were revised and corrected if deemed necessary by the reviewers. Reviewers were blinded to all

other data. For this analysis, we used a vendor-specific cutoff value of -21.5% to define abnormal LV-GLS, as previously described.[12]

**Bonescintigraphy**

Bonescintigraphies were performed using 99mTc-HDP tracer at a dose of 700 MBq (intravenously administered, scan was performed 3h after injection of tracer) as previously described.[19] All images were visually scored by an experienced specialist in nuclear medicine according to the Perugini scale, a positive bonescintigraphy was defined as ≥ grade 1 uptake.[20]

**Statistics**

Data are presented as medians with interquartile ranges or as frequencies with percentages. Between-group comparisons of characteristics were tested using the Mann-Whitney U test or Fisher's exact test, as appropriate. A P-value of 0.05 was deemed statistically significant. All analysis were performed with SPSS version 26 (IBS Statistics).

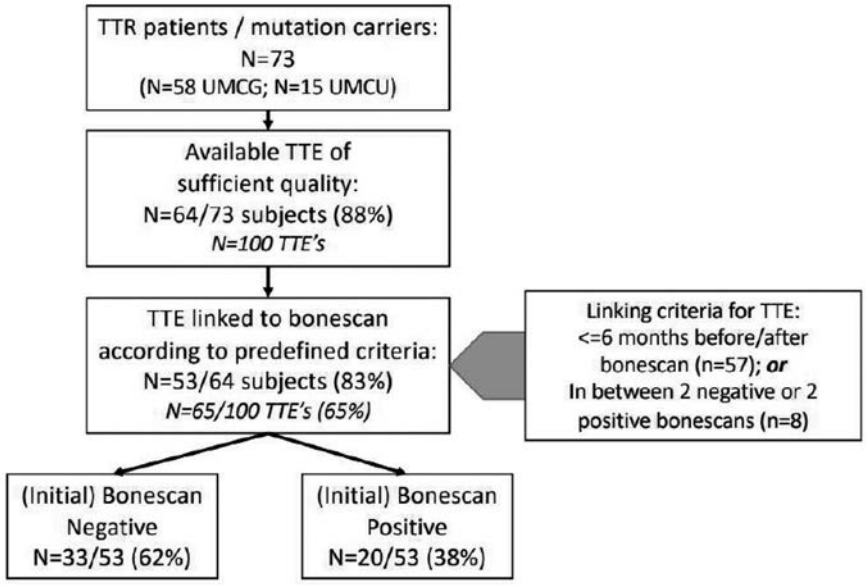


**Figure 1: Study design flowchart** TTE = transthoracic echocardiography; TTR = transthyretin; UMCG = University Medical Center Groningen; UMCU = University Medical Center Utrecht.

## Results

### Baseline characteristics

Fifty-three patients originating comprised the study cohort. Figure 1 shows the selection procedure. At baseline, their median age was 57 years.[46-67] Twenty-four (45%) patients were female. Genotype distribution was: TTRV30M (p.(Val50Met); n=32; 60%); followed by TTRE89K (p.(Glu109Lys); n=5; 9.4%), TTRT114C (p.Tyr134Cys); n=5; 9.4%), TTRV122I (p.(Val142Ile); n=4; 7.5%), TTRV71A (p.(Val91Ala); n=4; 7.5%), TTRG47E (p.(Gly67Glu); n=1), TTRI107V (p.(Ile127Val); n=1), TTRS23N (p.(Ser43Asn); n=1).

Patients were divided by their first bonescintigraphy result. Twenty patients (38%) presented with a positive bonescintigraphy of whom n=4 grade 1, n=14 grade 2 and n=2 grade 3. Patients with a positive bonescintigraphy were 16 years older at the time of evaluation compared to those with a negative bonescintigraphy (n=33; 62%). They also had higher heart rates, NT-proBNP and troponin-T levels and more often showed ECG abnormalities (Table 1). There was no statistical difference in terms of genotype, although the T114C (0% vs 15%) and V30M (55% vs 64%) pathogenic variants were numerically less prevalent in patients with an positive bonescintigraphy. Classical cardiovascular (CV) risk factors including hypertension, diabetes mellitus type 2, smoking and familial CV disease were equally prevalent in patients with a positive versus a negative bonescintigraphy (Table 1).

Echocardiographic parameters differed between patients with a positive versus negative bonescintigraphy; patients with a positive bonescintigraphy had lower LV-GLS, higher LV wall thickness and more severe diastolic dysfunction reflected by lower tissue doppler imaging (e') and higher E/e' ratio (Table 2). LVEF was similar in both groups.

**Table 1:** Baseline characteristics - stratified by initial bone scintigraphy

| | Valid N | Bonescintigraphy negative (n=33) | Valid N | Bonescintigraphy positive (n=20) | P-value |
|---|---|---|---|---|---|
| Age (years) | 33 | 49 (44-57) | 20 | 65 (64-74) | <0.001 |
| Female sex | 33 | 18 (55%) | 20 | 6 (30%) | 0.10 |
| TTR mutation | | | | | |
| V30M | | 21 (64%) | | 11 (55%) | |
| T114C | | 5 (15%) | | 0 | |
| E89K | 33 | 2 (6.1%) | 20 | 3 (15%) | 0.21 |
| V71A | | 2 (6.1%) | | 2 (10%) | |
| V122L | | 2 (6.1%) | | 2 (10%) | |
| Other (G47E, I127V, S23N) | | 1 (3.0%) | | 2 (10%) | |
| Hypertension | 33 | 3 (9.1%) | 20 | 4 (20%) | 0.41 |

**Table 1:** Baseline characteristics - stratified by initial bone scintigraphy

| | Valid N | Bonescintigraphy negative (n=33) | Valid N | Bonescintigraphy positive (n=20) | P-value |
|---|---|---|---|---|---|
| Diabetes | 33 | 2 (6.1%) | 20 | 0 | 0.52 |
| Familial CVD | 33 | 7 (21%) | 20 | 6 (30%) | 0.52 |
| Cardiac complaints | 33 | 6 (18%) | 20 | 3 (15%) | 0.54 |
| Systolic BP (mmHg) | 32 | 130 (115-140) | 20 | 137 (130-149) | 0.09 |
| Diastolic BP (mmHg) | 32 | 75 (71-85) | 20 | 80 (73-85) | 0.42 |
| Heart rate (bpm) | 33 | 68 (60-78) | 19 | 80 (70-90)) | 0.01 |
| Heart rhythm:<br>-Sinus<br>-AF / AT<br>-Pacemaker | 33 | 32 (97%)<br>0<br>1 (3.0%) | 20 | 18 (90%)<br>2 (10%)<br>0 | 0.31 |
| ECG abnormality | 33 | 4 (12%) | 20 | 10 (50%) | 0.004 |
| NT-proBNP (pg/ml) | 33 | 79 (115-140) | 18 | 550 (317-1173) | <0.001 |
| Hs-TnT (ng/L) | 27 | 5 (3-10) | 16 | 26 (13-53) | <0.001 |
| eGFR (ml/min) | 32 | 91 (80-103) | 20 | 92 (76-99) | 0.60 |

Data are presented as medians with interquartile ranges. CVD = cardiovascular disease; BP = blood pressure; AF = atrial fibrillation; AT = atrial tachycardia; Hs-TNT = High sensitive troponin-T; eGFR = estimated glomerular filtration rate.

**Table 2:** Echocardiographic parameters - stratified by initial bone scintigraphy

| | Valid N | Bonescintigraphy negative (n=33) | Valid N | Bonescintigraphy positive (n=20) | P-value |
|---|---|---|---|---|---|
| LVEF (%) | 33 | 56 (53-63) | 20 | 58 (49-66) | 0.61 |
| LV-GLS | 33 | 22.1 (21.0-23.7) | 20 | 16.1 (14.2-19.5) | <0.001 |
| LVEDD (mm) | 33 | 46 (42-51) | 20 | 44 (39-46) | 0.06 |
| IVST (mm) | 33 | 9.0 (8.0-10.0) | 20 | 15.0 (13.0-17.3) | <0.001 |
| PWT (mm) | 33 | 8.0 (7.0-9.6) | 20 | 13.8 (10.3-14.2) | <0.001 |
| LAVI (ml/m2) | 30 | 28 (25-33) | 18 | 33 (27-43) | 0.02 |
| MV E velocity (cm/s) | 33 | 65 (53-86) | 19 | 90 (57-114) | 0.07 |
| E/A ratio | 33 | 1.1 (0.83-1.37) | 19 | 1.02 (0.84-1.48) | 0.78 |
| E' lateral (cm/s) | 33 | 13.0 (10.6-14.8) | 20 | 6.7 (5.0-9.1) | <0.001 |
| E' septal (cm/s) | 33 | 10.3 (7.8-12.3) | 20 | 4.8 (3.3-6.5) | <0.001 |
| E/e' ratio | 33 | 5.9 (5.3-7.4) | 19 | 13.0 (8.3-25.9) | <0.001 |
| TAPSE (mm) | 32 | 24 (23-29) | 19 | 21 (18-24) | 0.004 |
| RV S' (cm/s) | 23 | 14.2 (12.7-15.3) | 13 | 11.8 (10.9-13.4) | 0.04 |

**Table 2:** Echocardiographic parameters - stratified by initial bone scintigraphy

| | Valid N | Bonescintigraphy negative (n=33) | Valid N | Bonescintigraphy positive (n=20) | P-value |
|---|---|---|---|---|---|
| TR peak velocity (m/s) | 23 | 2.2 (2.0-2.4) | 11 | 2.6 (2.5-2.7) | 0.001 |
| Diastolic dysfunction<br>-None<br>-Grade 1<br>-Grade 2+ | 26 | 21 (81%)<br>3 (12%)<br>2 (7%) | 18 | 4 (22%)<br>7 (39%)<br>7 (39%) | <0.001 |

Data are presented as medians with interquartile ranges. LVEF = left ventricular ejection fraction; LV-GLS = left ventricular global longitudinal strain; LVEDD = left ventricular end-diastolic dimension; IVST = interventricular septal thickness; PWT = posterior wall thickness; LAVI = left atrial volume index; MV = mitral valve; TAPSE = tricuspid annular plane systolic excursion; RV = right ventricle; TR = tricus pid regurgitation.

### Comparing imaging modalities: LV-GLS and cTTE versus bonescintigraphy

The classification of patients by LV-GLS and cTTE versus bonescintigraphy is visualized in Figure 2 A + B. The majority of patients with a positive bonescintigraphy had abnormal LV-GLS (n=17/20; Fig 2A) of whom 15 (88%) had grade 2 or more cardiac tracer uptake. All but one of them also had cTTE abnormalities (n=16/17; Fig 2B). In the patients with a positive bonescintigraphy and normal LV-GLS (n=3/20; Fig 2A), cTTE parameters were normal in 2 and abnormal in 1. Tracer uptake was grade 1 in 2 patients and grade 2 in 1 patient.

In case of a negative bonescintigraphy (n=33), LV-GLS was abnormal in 11 cases (Fig 2A). In 6 of them cTTE was also abnormal (Fig 2B). An additional 8 cases had cTTE abnormalities only (Fig 2B). Thus, in 58% of cases (19/33) with a negative bonescintigraphy either LV-GLS or cTTE showed abnormalities (Fig 2B).

When using grade 2+ myocardial uptake as cut-off for a positive bone-scintigraphy sensitivity of LV-GLS would have been 94% (15/16 patients).

### Clinical phenotype versus Imaging Classification

All patients with hypertension and/or diabetes (n=8) had abnormal LV-GLS. Figure 3 shows specific clinical parameters (3A) and cTTE parameters (3B) of patients divided by their LV-GLS and bonescintigraphy result (both abnormal, either one abnormal, both normal).

Patients with both a positive bonescintigraphy and an abnormal LV-GLS (n=20, median age 67 [62-74] years) had the most severe clinical phenotype (Fig 3A, $P<0.05$) in terms of elevated cardiac biomarkers (>75%) and ECG abnormalities (53%) and polyneuropathy

(88%). Yet the prevalence of cardiac complaints was limited (12%) and did not differ between groups, neither did genotype or CTS. This group entailed least females (24%). Four patients had a history of hypertension. These patients also had significantly more abnormalities on cTTE, most often being abnormal e' followed by increased LV wall thickness (Fig 3B).

Interestingly, the other 3 groups did not differ significantly in terms of clinical or cTTE parameters but numbers were small. More specifically, of the three patients with a positive bonescintigraphy yet normal LV-GLS (median age 65 [54-81] years; 67% female), one had elevated NT-proBNP levels, cardiac complaints, abnormal e', abnormal LV wall thickness and abnormalities on ECG; one had elevated NT-proBNP levels only and the remaining 1 patient had no abnormalities whatsoever. Patients with a negative bonescintigraphy and a normal LV-GLS (n=22, median age 48 [45-56] years; 59% female) were all free from diabetes or hypertension, yet 2 patients had elevated cardiac biomarkers (9%) and 2 had ECG abnormalities (14%). Of the patients with a negative bonescintigraphy yet an abnormal LV-GLS (n=11, median age of 51 [42-61] years, 46% female); 3 (27%) suffered from hypertension, diabetes or both. Two had elevated NT-proBNP levels (18%); 5 (50%) had abnormal e'.

**Longitudinal screening for Cardiac Involvement**

Ten patients had >1 available pair of investigations with a maximum of 3 evaluations per patient. Only one patient showed progression from a negative to a positive scintigraphy after two years follow-up; whereas LV-GLS remained normal. In 6 patients the consecutive scintigraphies remained negative; although three of them developed abnormal LV-GLS over time. Three patients had consecutive positive scintigraphies over time: n=1 with both abnormal cTTE and LV-GLS from the first evaluation; n=1 with abnormal cTTE and LV-GLS from the second evaluation and n=1 with normal cTTE and LV-GLS at all timepoints.

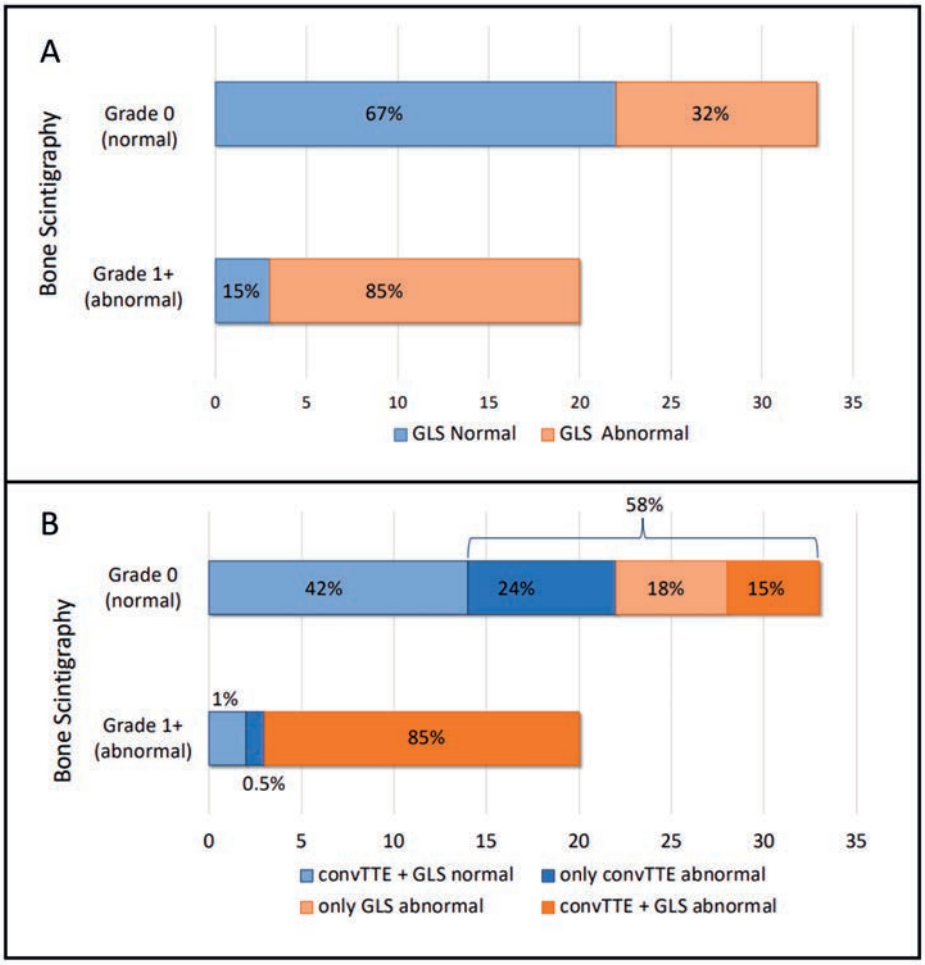


**Figure 2:** Bonescintigraphy versus LV-GLS (A) and conventional TTE (B) in TTR pathogenic variant carriers.

GLS = global longitudinal strain (of the LV); convTTE = conventional transthoracic echocardiography.

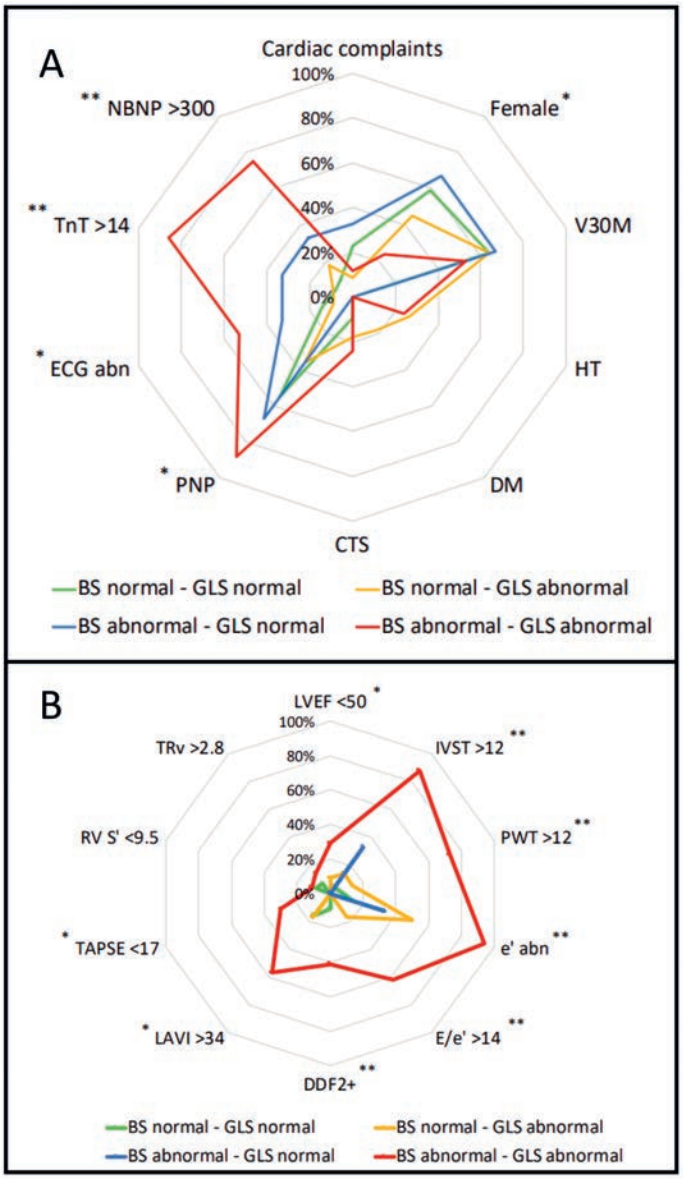


**Figure 3:** Clinical characteristics (A) and specific cTTE parameters (B) versus bonescintigraphy and LV-GLS results in TTR pathogenic variant carriers.

BS = bonescintigraphy; GLS = global longitudinal strain (of the LV); HT = hypertension; DM = diabetes; CTS = carpal tunnel syndrome; PNP = polyneuropathy; ECG = electrocardiogram; TnT = troponin T; NBNP = N-terminal pro-B-type natriuretic peptide; LVEF = left ventricular ejection fraction; IVST = interventricular septum thickness; PWT = posterior wall thickness; abn = abnormal; DDF = diastolic dysfunction; LAVI = left atrial volume indexed; TAPSE = tricuspid annular plane systolic excursion; RV = right ventricular; TRv = tricuspid regurgitation velocity.

## Discussion

Cardiac screening in a cohort of TTR pathogenic variant carriers showed that i) LV-GLS is the most sensitive TTE parameter for predicting a positive bonescintigraphy – equally sensitive as a combination of all conventional TTE parameters; and ii) echocardiographic abnormalities - either LV-GLS or conventional - were highly prevalent in patients with a normal bonescintigraphy in this otherwise healthy and young population, suggesting that bonescintigraphy may miss some early cases. Our results reveal the challenges of detecting cardiac involvement in clinical practice and support multimodality imaging when evaluating patients for the presence of cardiac ATTRv.

Compared to bonescintigraphy, LV-GLS was the most sensitive single TTE measure in confirming a positive bonescintigraphy. In other words, LV-GLS detected cardiac involvement by bonescintigraphy as good as all other cTTE parameters combined. This makes LV-GLS a very attractive parameter for cardiac screening, as GLS and requires an apical TTE view only, making it an easily obtained measure that is ideal for repeated screenings – even more so with the upcoming 3D-imaging probes and automated contour detection software such as we used in the current study.

Being the best single echocardiographic parameter, sensitivity of LV-GLS (at -12.5%) was 85% in our cohort. A recent study found a lower sensitivity of 79%,[16] as to be expected since a less sensitive cut-off of -17% was used. In our study, 3/20 cases with a positive bonescintigraphy had a normal LV-GLS, of whom 2 were female. Females are known for their greater LV-GLS values[21] which may explain 'false-negative results', supporting use of gender-specific cutoffs for LV-GLS. In 1 patient with abnormal bonescintigraphy and normal LV-GLS there was not any other cardiac abnormality whatsoever including biomarkers, ECG, cTTE and clinical evaluation and Perugini grade was 1. This case may reflect a false-positive bonescintigraphy – reported to be up to 10%[8] – or may still be an early form of cardiac ATTRv. We deliberately chose a grade 1+ tracer uptake as being positive in this specific population at high risk of developing cardiac ATTRv. When choosing a grade 2+ cut-off, sensitivity of LV-GLS would have increased to 94%, but we then would have re-classified 5 patients with grade 1 scans as 'negative' that are – in our view – very suspected of cardiac ATTRv.

The other way around, approximately one third of cases with a negative bonescintigraphy showed abnormalities in terms of LV-GLS, conventional TTE, or both. This is considerably different compared to a recent publication,[16] stating that none of the patients with a negative bonescintigraphy had cTTE or LV-GLS abnormalities. However, only 6/40 patients had a negative bonescintigraphy in this study (compared to 33/53 in ours) and their mean LV-GLS of -19.6% would have been considered abnormal by our definition. Also, their study population and methods were different from ours. First, they included wild-type and hereditary ATTR patients alongside asymptomatic TTR variant carriers. Second, their definition of a positive bonescintigraphy (heart:blood pool

ratio >1.1) was different from ours (Perugini grade 1 or more). It can be argued that the abnormal echocardiographic findings in bonescintigraphy negative patients should be considered 'false positive' and not indicative of cardiac ATTR. However, this subset of our cohort was young (median 51 years) with a low prevalence CV risk factors, not providing a good alternative explanation for the abnormal LV-GLS in most cases.[17,22] Hence early cardiac disease related to their TTR pathogenic variant is in our opinion likely in these patients despite a negative bonescintigraphy defined as a Perugini score of 0. The longitudinal evaluation available in a small subset of patients also showed that 3 patients developed abnormal LV-GLS over time despite bonescintigraphy still being negative.

Although bonescintigraphy is considered the test of first-choice to demonstrate cardiac ATTR involvement, the landmark publication in this regard[8] investigated a cohort composed of patients from tertiary referral centers in which patients had evident cardiac involvement (LV wall thickness mean 15mm). One may question whether the sensitivity of bonescintigraphy assessed on the basis of the Perugini score can be extrapolated to cases with very early cardiac involvement – the actual moment when we need to diagnose and start therapy. Strain-derived echocardiography is shown to be one the of earliest affected measures in different forms of myocardial disease/injury.[23] For example, asymptomatic relatives of patients with a dilated cardiomyopathy had a significantly higher prevalence of reduced LV-GLS despite normal LVEF and absence of symptoms compared with matched control subjects, and independent of genotype,[13] suggesting an early and pre-clinical manifestation of cardiomyopathy. In addition, abnormal LV-GLS was associated with myocardial deterioration, cardiac hospitalization, and death.[13] Similarly, in ATTR cardiomyopathy, LV-GLS was one of the parameters with a high probability of being abnormal already at low cardiac amyloid burdens.[14] Correspondingly, strain parameters seem sensitive to differentiate ATTR- cardiomyopathy cases from controls in several reports.[19,23,22] Although higher age and the presence of comorbidities can trouble interpretation when using LV-GLS to evaluate the presence of cardiac amyloidosis – i.e. being not disease specific – LV-GLS is a strong predictor of outcome irrespective of the underlying cardiac disease. Supporting this notion, we also found that patients with a positive bonescintigraphy and normal LV-GLS had a clinically less severe phenotype than those patients with both abnormal LV-GLS and bonescintigraphy. Thus, LV-GLS can be considered complementary to bonescintigraphy in screening TTR pathogenic variant carriers for evaluating the presence and severity of cardiac disease.

Although we did not evaluate cardiac magnetic resonance (CMR) imaging in our study (due to limited availability), CMR should be prompted in the diagnostic workup as it combines anatomy, function, tissue characteristics (late enhancement, extracellular volume, T1 mapping) and nowadays software also allows strain imaging. Many CMR parameters have excellent diagnostic and prognostic value.[11] However, CMR and the specific software packages for the functionalities mentioned are not available at all

centers and CMR is not suitable for all patients (e.g. patients carrying metal implants/ cardiac devices, patients with claustrophobia).

Our data are insufficient to support a specific screening algorithm, but based on our results, our experience, available literature and practical considerations, we would suggest to combine different imaging modalities at first evaluation a TTR pathogenic variant carriers and preferable also thereafter (taking into account radiation exposure). LV-GLS is an accurate, sensitive and practical tool for longitudinal evaluation of TTR pathogenic variant carriers. An abnormal LV-GLS should not instantly prompt therapy for ATTR – because of insufficient disease specificity – but should warrant closer monitoring and further evaluation for both ATTR cardiomyopathy and other cardiovascular risk factors/diseases. Further research is needed to investigate if and how a screening algorithm incorporating multimodality imaging can support clinical decision making; i.e. to determine when to start ATTR targeted therapy.

## Limitations

Some cases were excluded in this analysis because of limited image quality. However, we are not aware of image quality being related to cardiac disease or substrate and as such these exclusions are considered to be at random. A second limitation is the absence of endomyocardial biopsy, which is the gold standard, but was not available in this observational cohort study. Particularly in patients with a discordant imaging result this would have been helpful. Finally, our cohort size is small, but ATTRv is a rare disease.

## Conclusion

LV-GLS and bonescintigraphy are complementary in screening TTR pathogenic variant carriers for cardiac involvement and should be used in conjunct. LV-GLS can play a significant role in repeated assessment of this population and may pick-up early disease before bonescintigraphy.

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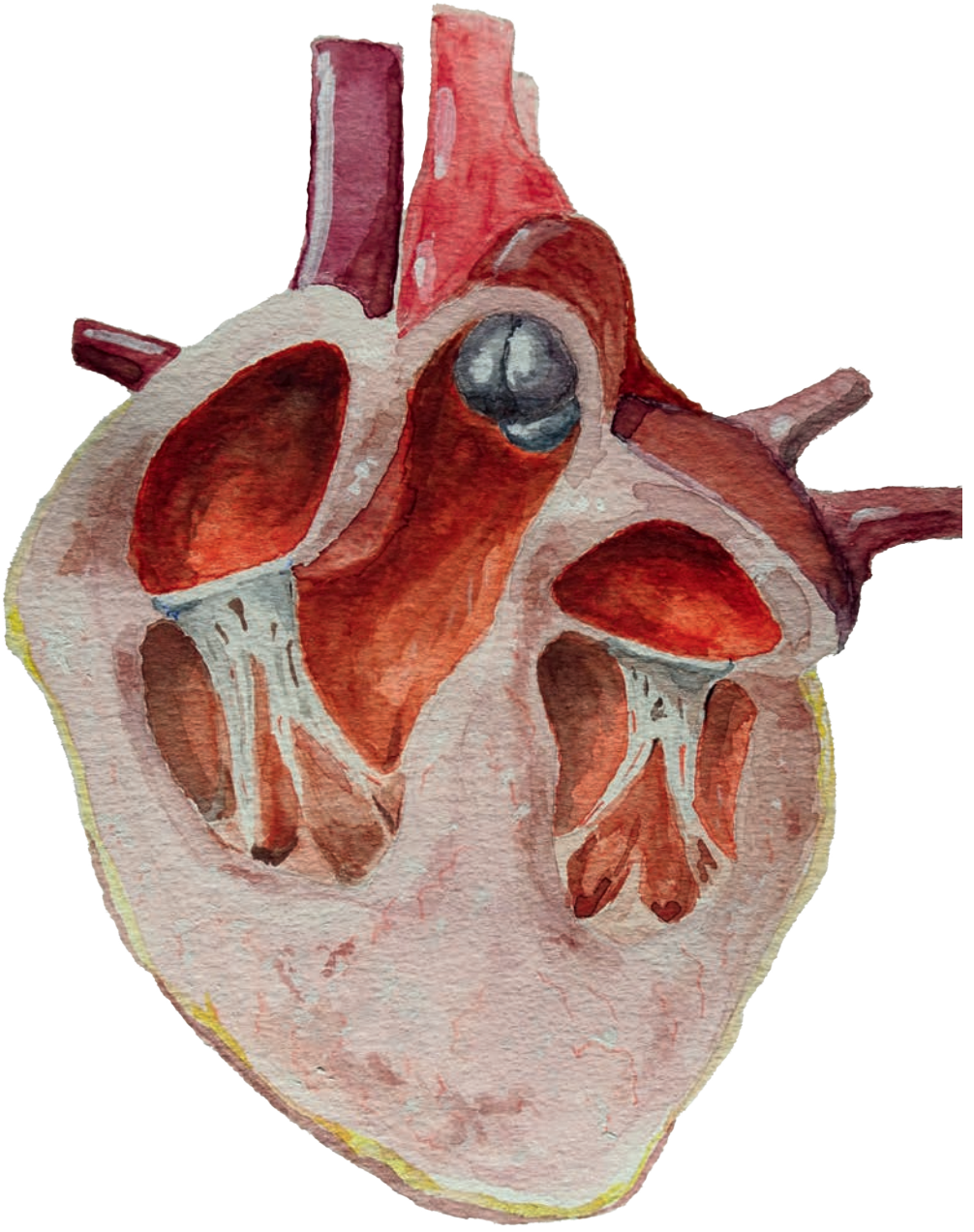

# Part 2

Wildtype Transthyretin-derived amyloidosis