Left Atrial Morphology, Size and Function in Patients With Transthyretin Cardiac Amyloidosis and Primary Hypertrophic Cardiomyopathy
– Comparative Strain Imaging Study –
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Background: We sought to assess left atrial (LA) morphology and function in patients with transthyretin cardiac amyloidosis (TTR-CA) and hypertrophic cardiomyopathy (HCM). Primarily, longitudinal deformation (reservoir) and pump function were the focus of vector-velocity strain echocardiography imaging.

Methods and Results: The study group comprised 32 patients (mean age 57.7±15.4 years, 16 in each group), and 15 healthy controls. Diagnosis of TTR-CA was based on echocardiography and either gadolinium-enhanced (LGE) cardiac magnetic resonance (cMRI) or radionuclide imaging. At baseline, there were no differences in age, body surface area, blood pressure and risk factors among the groups. Left ventricular (LV) mass was greater in patients than in controls, and slight LA dilatation was found in the TTR-CA group. LA reservoir was 14.1±4.7% in TTR-CA, 20.0±5.6% in HCM, and 34.0±11.8% in controls (<0.001). In addition, LA pump function chiefly was impaired in the former group, irrespective of LA chamber size and LV ejection fraction. LGE in the atrial wall was seen in 9/10 TTR-CA versus 0/8 HCM patients undergoing cMRI (P<0.001). LA reservoir ≤19% and pump function ≤−1.1% best discriminated TTR-CA from HCM patients in the receiver-operating characteristic analysis.

Conclusions: LA reservoir and pump function were significantly impaired in both TTR-CA and HCM patients compared with controls, but mainly in the former group, irrespective of LA volume and LV ejection fraction, likely caused by a more altered LA wall structure. (Circ J 2016; 80: 1830–1837)

Key Words: Amyloidosis; Atrial function; Hypertrophic cardiomyopathy; Strain echocardiography

Strain echocardiography (strain) imaging is a modern and valuable technique for recognizing left ventricular (LV) dysfunction in several cardiomyopathies, including rare diseases such as cardiac amyloidosis (CA) and hypertrophic cardiomyopathy (HCM).1–3

Despite the absence of software tailored for the atria, recent studies suggest this technique is a powered detector of left atrial (LA) mechanics, and both speckle tracking and vector-velocity (feature tracking) modalities have been used for that purpose.4–6

CA represents a cause of LV wall thickening and dysfunction, gradually leading to heart failure as a consequence of amyloid deposits in intramural coronary arteries and endomyocardium. However, noninvasive diagnostic tools of cardiac involvement are challenging when the clinical diagnosis is deficient, and misdiagnoses can be potentially harmful in some patients, because of a failure to differentiate CA from overlapping cardiomyopathies, with different prognosis.7–9

Among all the subtypes of CA, there is a variant caused by mutations in the genes encoding for transthyretin (TTR), a tetrameric protein rich in β-strands highly present in human serum and tissues. Both familial and acquired (wild-type or senile variant) TTR-amyloidosis account for 8–10% of all forms. The familial variant approximately involves 1:100,000 individuals of the general population in the USA, but it is more frequent in some geographic areas, such as Italy.

Clinical expression varies from initial (isolated polyneuropathy without cardiac involvement) to advanced (amyloid deposits into myocardial wall, TTR-CA), the latter usually considered at poor prognosis. Hence, the early identification of the cardiac involvement is a crucial issue in the clinical management of such patients.7–10

Furthermore, HCM represents a dramatic, potentially fatal, cardiac disease often complicated by myocardial fibrosis and
subsequent functional impairment, in which the fibers’ disarray, microvasculature impairment, abnormal collagen deposits, ischemic spots and chronic LV pressure overload are, in turn, important prognosticators.\(^{11,12}\)

In both conditions, there would be a clinical advantage in recognition of functional impairment of the LA, probably earlier than LV dysfunction, and recent studies indicate strain imaging as the most valuable noninvasive technique to disclose subclinical atrial dysfunction.\(^{8,13}\) LA morphofunctional changes are emerging as prognosticators in various cardiac diseases, but only scanty literature is available on comparative strain studies between TTR-CA and HCM patients, being all cardiac chambers target organs for either amyloid deposits or interstitial fibrosis.\(^{6,13-16}\)

In the present study we sought to evaluate and compare the distinctive features of LA size and function in these 2 clinical conditions using vector-velocity strain imaging.

**Methods**

**Patient Population**

All patients consecutively admitted to the University Hospital of Messina for TTR-CA or primary HCM from January 2013 to May 2015 were enrolled. Fine acoustic window for ultrasound investigation was a stringent inclusion criterion, and the exclusion conditions were as follows: (a) systemic hypertension; (b) previous myocardial infarction, ischemic heart disease or stroke; (c) dilated or endstage cardiomyopathy; (d) permanent/persistent atrial fibrillation; (e) severe mitral regurgitation; (f) aortic valve stenosis; (g) chronic lung disease; and (h) severe renal or hepatic dysfunction.

Diagnosis of TTR-amyloidosis had been made 39±13 months before the cardiac study, on the basis of neurological stadiation and genomic testing in all patients. Also, green birefringence under cross-polarized light following Congo red staining was demonstrated in biopsied fat pad tissue in 10 of them (62%). Cardiac involvement was then investigated by Doppler echocardiography in the whole study population in combination with either cardiac magnetic resonance imaging (cMRI) or \(^{99m}\)Tc-DPD scintigraphy.

Nobody from this group had evidence of monoclonal protein in the serum or urinalysis, nor monoclonal population in the plasma cells or in the bone marrow, thus excluding light-chain amyloidosis. All subjects had clinical evidence of polyneuropathy.

Primary HCM was established according to current ACCF/AHA guidelines,\(^{13}\) using family history, electrocardiography, echocardiography, and cMRI if necessary. Stringent echocardiographic criteria were LV wall thickness of \(\geq 15\) mm (\(\geq 17\) mm for posterior septum) in a non-dilated LV chamber, without any possible hemodynamic cause of hypertrophy. All patients complaining of angina or equivalent symptoms underwent exercise ECG or stress-echocardiography in order to rule out underlying active coronary artery disease.

**Study Design**

This was a single-center, case-control imaging study aimed at evaluating LA morphology and function in patients with TTR-CA and primary HCM, comparing findings with 15 apparently healthy control subjects, matched for age, body surface area (BSA) and blood pressure (BP). Ultrasound studies were interpreted by a skilled cardiologist, blinded to the subjects’ grouping.

Because of budget restrictions in the original proposal, cMRI and \(^{99m}\)Tc-DPD scintigraphy were performed only in some TTR-CA patients, especially when echocardiography was not conclusive.

cMRI was also carried out in HCM patients highly suspected of having myocardial fibrosis (severe LV hypertrophy on echocardiography, repetitive ventricular beats and/or non-sustained tachycardia).

Enrolment of patients complied with the Declaration of Helsinki and informed consent was given by all participants. Data were collected anonymously according to the Italian Health System regulation.

**Echocardiography**

All subjects underwent high-resolution ultrasound study. Quantitative findings were indexed to BSA, according to our laboratory protocols and current guidelines.\(^{12,17}\) LV end-diastolic and systolic volumes were achieved by both 4- and 2-chamber apical views, and ejection fraction (EF) was calculated with the biplane Simpson rule method. LA systolic (maximum) and diastolic (minimum) volumes were measured as a mean value from both 4- and 2-chamber apical views, and fractional emptying calculated as follows: (maximum−minimum)/maximum volume percent change. LA volume index \(\leq 29\) ml/m\(^2\) was considered as the upper normal limit, in both men and women. LV diastolic function was evaluated by PW Doppler sampling at the mitral valve inflow (E/A velocity ratio, E-wave deceleration time) and by tissue Doppler velocity (E’ velocity) at the lateral annulus, expected to be less impaired in hypertrophied patients, and the E/E’ ratio calculated. More than mild diastolic dysfunction was defined as LA volume >34 ml/m\(^2\), E/A ratio ≥2, E-wave deceleration time ≤150 ms and E/E’ ratio >12.\(^{17,18}\)
Quantitative Assessment of LA Function
Vector-velocity strain imaging was performed using a commercial ultrasound unit and with a dedicated Mylab platform system (Esaote, Florence, Italy) that allowed measurement of both LV and LA mechanics in an offline modality on digitally stored images.

Global longitudinal strain (εsys) was achieved by average measurements from the 4- and two-chamber apical views, as suggested. LA strain was accomplished by clockwise point-to-point placement on the endocardial border from a foreground apical view of the atrial chamber. Pointing was manually adjusted in order to avoid interference of empty areas like pulmonary vein ostia. Typically, global εsys was a negative value from the LV and positive from the LA chamber. (Hereinafter, peak LA εsys (PALS) will be termed “reservoir”.) Potential interference of heart rate (HR) on strain measurements was also limited by normalizing LA εsys to RR cycle. Atrial pump (contractile) function was identified as the small negative peak velocity following the conduit phase.1,4–6

cMRI and Radionuclide Imaging
cMRI was performed on a 1.5-Tesla cardiac-dedicated clinical system (Gyroscan NT; Philips Medical Systems, Best, The Netherlands) with phased-array coil and vectorcardiogram synchronization. Breath-hold sequenced parameters were: time repetition/time echo 3.8/1.92 ms; slice thickness 8 mm; matrix size 192/512; field of view 300 mm, rectangular field of view 80%; number of phases 30; the late gadolinium-enhanced (LGE) protocol consisted of a functional study devoted to acquiring ECG-gated T1 and T2 analyses and steady-state free-precession cine-imaging, and 3 standard long-axis slices and a stack of contiguous short-axis slices (10 mm each, 30 phases/RR-interval) were acquired. Delay enhancement within the LV and LA myocardium was recognized. In the TTR-CA patients, subendocardial circumferential enhancement was considered a highly sensitive finding for the identification of amyloid deposits, whereas focal patterns were considered to be more specific for HCM. Thickened atrial wall (>3 mm) was also considered a high sensitive finding for the identification of amyloid deposits, whereas focal patterns were considered to be more specific for HCM. Thickened atrial wall (>3 mm) was also considered a sign of TTR deposits or fibrosis (Figure 1).

99mTc-DPD accumulation was evaluated on a whole-body scan (anterior and posterior projections) using a dual-headed gamma camera (Millennium VG; GE Healthcare, Milwaukee, Wisconsin), detected 3h after intravenous injection of 740MBq of 99mTc-DPD. Moreover, a thoracic single photon emission computed tomography scan was attained soon after the whole-body scan using the same machine.

Statistical Analysis
Values are reported as mean±standard deviation (SD) or number and percent (%).

The distribution of qualitative variables was checked by chi-square, whereas continuous variables were investigated at ANOVA testing for independent groups. A post-hoc Scheffé analysis was also performed for between-group differences. Receiver-operating characteristic (ROC) curves were generated to identify cut-off values for both LA reservoir and negative peak strain in order to discriminate patients in the 2 study groups and between both hypertrophic phenotypes and controls.

The null hypothesis was rejected at 2 tails for P<0.05. Statistical analysis was performed by SPSS release 15 (SPSS Inc, Chicago, IL, USA) and MedCalc 6.00.014 (MedCalc Software, Mariakerke, Belgium).

Results
Patients’ Characteristics
Of the 20 TTR-CA and 30 HCM patients initially examined, only 16 in each group met the inclusion criteria; 2 TTR-CA (10%) and 3 HCM (10%) patients were excluded because of systemic hypertension; chronic respiratory insufficiency was found in 2 and 3 patients, respectively; permanent atrial fibrillation in 4 HCM (13%) patients and active coronary artery disease in 4 more HCM patients. Therefore, the study population consisted of 32 patients, mean age 57.7±15.4 years, and their demographic and clinical characteristics are summarized in Table 1. Overall, there were no significant differences in age, sex, BSA and office BP measurements among the groups. However, TTR-CA patients were more symptomatic than HCM patients and both groups more than controls. A higher proportion of HCM patients were on β-blockers, angiotensin-converting enzyme inhibitors and aspirin.

On gene mapping, all TTR-CA patients were carriers of exon 3 mutations, as defined by protein amino acid (and DNA nucleotide) changes according to the standard nomenclature of the Human Genomic Variation Society19 as follows:

<table>
<thead>
<tr>
<th>TTR-CA (n=16)</th>
<th>HCM (n=16)</th>
<th>Controls (n=15)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>13 (81)</td>
<td>12 (75)</td>
<td>8 (53.3) NS</td>
</tr>
<tr>
<td>Age (years)</td>
<td>57.7±9.8</td>
<td>57.6±19.9</td>
<td>57.9±12.7 NS</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.8±0.22</td>
<td>1.79±0.18</td>
<td>1.79±0.16 NS</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>75.1±9.6</td>
<td>69.6±8.7</td>
<td>68.1±7.0 NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>129±7</td>
<td>132±12</td>
<td>132±6   NS</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>74±6</td>
<td>79±9</td>
<td>77±5    NS</td>
</tr>
<tr>
<td>NYHA functional class</td>
<td>1.6±0.8</td>
<td>1.4±0.5</td>
<td>1.0±0.0 &lt;0.01</td>
</tr>
<tr>
<td>LDL-C &gt;130mg/dl</td>
<td>3 (19)</td>
<td>3 (19)</td>
<td>2 (13)  NS</td>
</tr>
<tr>
<td>β-blockers</td>
<td>2 (12)</td>
<td>10 (62)</td>
<td>0       &lt;0.01</td>
</tr>
<tr>
<td>ACEI</td>
<td>2 (12)</td>
<td>4 (25)</td>
<td>0       NS</td>
</tr>
<tr>
<td>Aspirin</td>
<td>0</td>
<td>6 (37)</td>
<td>0       &lt;0.05</td>
</tr>
<tr>
<td>Other drugs</td>
<td>3 (19)</td>
<td>4 (25)</td>
<td>0       NS</td>
</tr>
</tbody>
</table>

Values are mean±SD or number and percent (%). ACEI, angiotensin-converting enzyme inhibitor; BSA, body surface area; CV, cardiovascular; DBP, diastolic blood pressure; HCM, hypertrophic cardiomyopathy; LDL-C, low-density lipoprotein cholesterol; NS, not significant; SBP, systolic blood pressure; TTR-CA, transthyretin cardiac amyloidosis.
Glu89Gln (c.325G>C) in 8 patients (50%), Phe64Leu (c.250T>C) in 6 (37%) and Thr49Ala (c.205A>G) in 2 more patients (12%).

Diagnosis of HCM was based on individual family history and confirmed by both electrocardiographic and echocardiographic findings in 100% of cases. cMRI was carried out in 94% vs. 5 HCM patients (31%), but in none of the controls.

Using ROC curve analyses, LA reservoir behavior was observed after excluding patients with mildly impaired LVEF and HR-normalized LA reservoir confirmed such a trend in the TTR-CA group (Table 2).

Likewise, pump function was more depressed in TTR-CA patients than in HCM patients and controls (P<0.01).

Matching LA size to strain measurements, both the reservoir phase and pump function were lower in TTR-CA patients, irrespective of whether the LA chamber size was normal or dilated (Figure 3). Using ROC curve analyses, LA reservoir ≤20.05% (AUC=0.906; 95% confidence interval (CI) 0.785–0.972, P<0.0001) and pump function ≤−1.4% (AUC=0.777; 95% CI 0.632–0.885, P<0.0001) were the best cut-offs discriminating hypertrophic phenotype from controls, whereas the values of 19% and −1.1%, respectively, distinguished TTR-CA from HCM patients (Figure 4).

### Comparison of cMRI and Strain Imaging

Table 3 shows the characteristics of both subgroups undergo-
Typical endocardial LGE distribution was found in 90% of patients with TTR-CA, whereas there were intramural spots in 62% of those with HCM. Moreover, LGE in the LA wall was present in the former group, together with wall thickening occurring in 70% vs. 12% of cases, respectively.

Patients from the TTR-CA subgroup were confirmed to have much lower values for LA reservoir and atrial pump function, as well as lower LVEF.

**Figure 2.** Strain measurement of the atrial wall in a patient with TTR-CA (A) or HCM (C). (B) Graphical vector-velocity imaging. Columns reporting average values of (D) LA reservoir (reservoir) and (E) pump function in panel E, in each study group (ALL) and in patients with preserved left ventricular ejection fraction (LVEF ≥55%). HCM, hypertrophic cardiomyopathy; LA, left atrium; TTR-CA, transthyretin cardiac amyloidosis.

**Figure 3.** Comparison of atrial functional characteristics among the study groups, related to normal-sized (LAVi ≤29 ml/m²) or dilated (LAVi >29 ml/m²) LA chamber. *P<0.05 (TTR-CA vs. HCM and HCM vs. controls); †P<0.01 and ‡P<0.001 (TTR-CA vs. controls). HCM, hypertrophic cardiomyopathy; LAVi, left atrial volume index; TTR-CA, transthyretin cardiac amyloidosis.
Atrial Function in TTR-CA and HCM

The main findings from the present study indicate that LA dysfunction can be found in a large proportion of patients with a hypertrophic phenotype from either TTR-CA or HCM, but a greater impairment occurs in the former group, irrespective of body mass index, LV mass and function, and LA fractional emptying.

Strain echocardiography has been confirmed as a significantly helpful technique to investigate advanced atrial functional components such as reservoir phase and booster pump work (contractile performance), which are otherwise difficult to investigate noninvasively.4,6,14–16

Also, our study indicates that both markers are greatly impaired in the TTR-CA patients irrespective of LA size and LVEF.

In the ROC curve analyses, LA reservoir ≤19% best discriminated between the 2 groups of patients. In fact, it was found in approximately 94% of TTR-CA patients vs. 31% of HCM patients, respectively, but not in controls.

We have already demonstrated adverse LA remodeling in patients with amyloidosis, possibly related to increased wall stiffness as a consequence of the continuing dumping of insoluble amyloid fibrils in the atrial wall, as well as the ventricular...
wall, indicating cMRI as the gold standard for detecting deposits by LGE technique. On the other hand, interstitial fibrosis has been demonstrated to occur in a variable proportion of HCM patients, but with a different pathogenesis being LGE as the consequence of reiterate microcirculatory injuries, often limited to the ventricular myocardium, at least in the initial stages of the cardiomyopathy.

In view of the relationships among myocardial structure, function and mid-term outcomes, a large body of literature indicates LV fibrosis as an additional cardiovascular prognosticator in HCM patients, particularly in those otherwise classified at a lower risk.11,13,20-22

More recently, Hen et al23 found that progression of LGE on cMRI was related to increased wall thickness, decreased contractility, and reduced intraventricular pressure gradient in a Japanese population. The same group also demonstrated a greater incidence of atrial fibrillation in such patients, underlying a possible relationship between LA function and LV disarray.23

Just recently the attention of clinicians has been placed on the atrial chambers. The clinical effect of LA dilatation has been already shown by Losi et al24 in HCM patients, in whom either systolic volume >27 ml/m² and/or a fast dilating atrial chamber were predictors of unfavorable outcomes.

Badran et al,14 using vector-velocity strain imaging, found that LA reservoir and conduit functions were more impaired in HCM than in hypertensive patients, leading to different clinical outcomes. However, LA reservoir was much higher in their HCM patients (25±15%) than in ours (20±6%), even if their patients had greater LA size and LV ejection fraction impairment, likely suggesting a variability in apparently similar cardiac diseases.

LA reservoir is a marker of LV diastolic function, and its impairment indicates a rise in LV stiffness, harbinger of the upstream transmission of LV chamber filling pressure to the lung venous system, often commensurate with clinical deterioration. However, when considering the interplay of atrial and ventricular chambers because of displacement of the shared atrioventricular plane, it is not easy to ascertain which chamber is first impaired in patients with hypertrophied hearts.

Also of interest, we did not find relevant differences in LA function when excluding patients with LVEF <55%. This can be explained by considering that a greater diastolic dysfunction is expected in patients with impaired LA reservoir, but such an assumption is not obvious in those presenting with mildly reduced LVEF caused by variable ventricular stiffness adaptation.12-16,25-27

In our patients undergoing cMRI, LA function significantly correlated with the wall structure, because LGE was more frequent in TTR-CA patients than in HCM patients. This likely suggests that amyloid deposits in CA patients occur earlier (or are heavier) than fibrosis in HCM patients. Moreover, it could also be hypothesized that in uncomplicated HCM patients, such as the nonobstructive variant, free of interstitial fibrosis and severe mitral regurgitation, an increased LA pump and conduit function may be valid compensatory mechanisms in order to preserve LA function, at least in the early stages of the disease. In contrast, in patients with severe disarray of the wall structure these features might be lacking, matching the high levels of NT-proBNP previously demonstrated in CA patients.6-9

This study also demonstrates that a preclinical LA dysfunction may occur in both groups irrespective of chamber dilatation, with poorer values in TTR-CA patients. It is not easy to give true mechanistic insights on this finding, but it could be theorized that the quality and/or density of tetrameric proteins resulting in amyloid deposits may vary significantly among the patients, being more toxic or fast-storing in those with rapid impairment of atrial function even in the absence of dilatation. This could be leading to higher NT-proBNP serum levels, untested in this but found in previous studies.8,10 Furthermore, it has to be considered that some of our patients were evaluated in the early stage of CA, when LA dilatation cannot yet be present. Therefore, wider studies on atrial chamber adaptation to infiltrative storage diseases should be encouraged.

The present findings must be interpreted in the light of previous studies on the prognostic effect of LA dysfunction. For instance, in 312 individuals from a general population Cameli et al25 showed that a severely reduced LA reservoir (<18.8%) was an independent predictor of cardiovascular events, including atrial fibrillation and stroke, with 78% sensitivity and 85% specificity. Approximately the same value (19%) discriminated between the more and less compromised patients in our series.

This likely indicates that TTR-CA patients, and also those with HCM with low ejection fraction values, may be at risk of arrhythmic disorders, such as atrial fibrillation. In fact, Habibi et al found that hypertensive patients with LA enhancement were more inclined to persistent or permanent atrial fibrillation.29 Therefore, our study adds to the current knowledge by providing new functional issues in patients with similar LV hypertrophy, but different pathophysiology, in an attempt to improve the therapeutic approach to these difficult conditions.

Study Limitations
Several limitations should be considered in the interpretation of the present study. First of all, familial TTR is such a rare variant of amyloidosis, compared with other forms, that it involved a limited sample in the present study, also related to our stringent inclusion criteria. Thus, our results might not be representative of the general patient population suffering from CA.

It is not surprising that multiple determinants can impair LA reservoir and pump function in hypertrophic patients, such as LA chamber dilatation, myocardial stretching in response to pressure overload, dynamic and fixed obstruction to outflow and mitral valve regurgitation,1-3,11,14,16 but not all these factors were investigated.

Among many possible markers of atrial dysfunction, we just studied the reservoir phase and contractile function, based on the most significant clinical studies.4-6,14-16,28 Therefore, we cannot exclude a discriminant role also for the LA conduit phase.

Regarding technical limitations, strain studies of LA function are affected by the fact that available software packages are dedicated to LV not LA chamber analysis. Discrepancies are then expected to be found in view of such differences, as well as the methods used for digital acquisition. It is worth remarking that vector-velocity strain imaging allows detection of LA wall deformation by sparing empty areas such as the pulmonary vein outlet and thin or floating atrial septa. In contrast, the speckle-tracking technique is more valuable for recognizing global chamber deformation, but blank areas are usually included.1,2,5,16,28

Of note, it should be considered that our MRI algorithms were not appropriately powered enough for detecting LA fibrosis in HCM patients, and the most recent T1 mapping techniques are more promising for this purpose.30 Finally, large studies are needed in order to confirm the
present cut-off values for both LA reservoir phase and pump function as discriminators between less and more compromised patients, as well as whether such values are case-sensitive or limited to hypertrophic cardiomyopathies.

Conclusions
The present study results indicate that the LA reservoir phase and contractile function are impaired in a high proportion of TTR-CA and HCM patients in comparison with healthy controls. Greater functional impairment was demonstrated in the former group, likely because of amyloid deposits in the atrial wall being more significant than fibrosis in HCM, irrespective of LA chamber size. Further study is encouraged in order to better ascertain the mechanistic difference among the various infiltrative markers and whether strain-derived functional markers can be endorsed from experimental models to an integrated individual care management and treatment approach.

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Disclosures
No conflicts of interest declared.

References