Reductions in extracellular volume by magnetic resonance indicate ATTR cardiac amyloid regression with patisiran

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ABSTRACT

Background: Administration of patisiran, a TTR-specific small interfering RNA (siRNA), has been shown to benefit neuropathy in patients with hereditary transthyretin (ATTR) amyloidosis but its effect on cardiomyopathy (ATTR-CM) remains uncertain.

Objectives: To determine the effect of patisiran on the cardiac amyloid load as measured by cardiac magnetic resonance (CMR) and extracellular volume (ECV) mapping in ATTR-CM.

Methods: Patisiran was administered to 16 patients with hereditary ATTR-CM who underwent protocolized assessments at the UK National Amyloidosis Centre, 12 of whom received concomitant diflunisal as a 'TTR stabilising' drug. Patients underwent serial monitoring with CMR, echocardiography, cardiac biomarkers, bone scintigraphy and 6-minute walk test (6MWT). The findings were compared with 16 patients who were retrospectively group matched on the basis of amyloid type and ECV-CMR.

Results: Patisiran was well tolerated. Median serum TTR knockdown among treated patients was 86% (IQR 82-90%); 82% cases showed >80% knockdown. Patisiran was typically associated with a reduction in ECV (adjusted mean difference between groups -6.2% [95% CI -9.5% to -3.0%]; p=0.001) and accompanied by a fall in NT-proBNP (adjusted mean difference between groups -1342 ng/L [95% CI -2363 to -321]; p=0.012) and an increase in 6MWT distance (adjusted mean difference between groups 168 meters [95% CI 57 to 2800]; p=0.004) after 12 months of therapy as well as a median reduction in cardiac uptake by bone scintigraphy of 19.6% (IQR 9.8-27.1%).

Conclusions: Reductions in ECV by CMR provide evidence for ATTR cardiac amyloid regression in a proportion of patients receiving patisiran.

Keywords: ATTR, amyloidosis, patisiran, RNAi

LIST OF ABBREVIATIONS

ATTR amyloidosis: Transthyretin amyloidosis
ATTR-CM: Transthyretin cardiomyopathy
CMR: Cardiac magnetic resonance
ECV: Extracellular volume
6MWT: 6 minute walking test
LGE: Late gadolinium enhancement
LVH: Left ventricular hypertrophy
NT-proBNP: N-terminal pro-brain natriuretic peptide
DPD: ^{99m}Tc-3,3-diphosphono-1,2-propanodicarboxylic acid
EAP: early access programme

INTRODUCTION

Cardiac transthyretin amyloidosis (ATTR-CM), in which amyloid deposits are derived from plasma transthyretin, is an under-recognized cause of heart failure. Until recently, ATTR-CM was untreatable and inexorably progressive. However, therapy with tafamidis, a TTR stabilizer, was recently shown to be associated with slowing of disease progression, improved survival and a reduction in hospitalizations among patients with ATTR-CM (1). Other drugs also show promise in ATTR amyloidosis. Diflunisal, another TTR stabilizer (2), along with two novel TTR gene 'silencers', the small interfering RNA (siRNA) patisiran (3) and the anti-sense oligonucleotide inotersen (4), have all been shown in randomized placebo-controlled trials to slow, halt or indeed improve neuropathy (ATTR-PN) in patients with hereditary ATTR amyloidosis; some are currently being tested in ATTR-CM in phase 3 clinical trials

However, the efficacy and specific mechanism of action of these disease-modifying therapies on the myocardium in ATTR-CM remain uncertain. Furthermore, since no markers of treatment response have been identified, patients who fail to respond to treatment, cannot be identified. There is therefore a need to identify markers of treatment response at a myocardial level. Serum concentration of N-terminal pro-brain natriuretic peptide (NT-proBNP) and echocardiographic parameters are the most commonly used clinical tools for assessing cardiac response to therapy in amyloidosis, but neither directly quantifies the amyloid burden. At diagnosis, both NT-proBNP and myocardial strain predict prognosis in ATTR-CM (5) but they represent processes downstream of amyloid deposition. Furthermore, NT-proBNP concentration is confounded by renal impairment, fluid overload and neurohormonal activation (5,6) and myocardial strain is not well standardized and affected by changes in cardiac preload and afterload. Cardiovascular magnetic resonance (CMR) with tissue characterization is a sensitive tool for characterizing myocardial amyloid deposits. Cardiac magnetic resonance (CMR) can visualize, with late gadolinium enhancement (LGE), and measure, with T1

mapping, the continuum of cardiac amyloid deposition (7,8). The extent of LGE and the elevation in native T1 and extracellular volume (ECV) correlate with amyloid burden and provide incremental information on outcome (7,9,10). It is postulated that the novel disease-modifying therapies reduce the rate of accumulation of new ATTR amyloid which, in the context of a constant rate of amyloid clearance, may result in overall amyloid regression, but this remains unproven.

We sought to determine the effect of patisiran on cardiac amyloid load as measured by cardiac magnetic resonance (CMR) and extracellular volume (ECV) mapping in ATTR-CM.

METHODS

Patients

All patients with hereditary ATTR amyloidosis, enrolled into a UK patisiran Early Access Programme (EAP) between July 2017 and November 2018, were invited to participate in a prospective protocolized clinical follow-up programme comprising comprehensive investigations and functional assessments at commencement of therapy and on an annual basis thereafter including routine biochemistry, cardiac biomarkers, measurement of TTR concentration, echocardiography, CMR, ^{99m}Technetium (^{99m}Tc) labelled 3,3-diphosphono-1,2-propanodicarboxylic acid (DPD) scintigraphy and 6-minute walk test (6MWT). Diagnosis of ATTR-CM was established in all cases on the basis of accepted diagnostic criteria (11,12), and all patients underwent sequencing of the TTR gene.

All patients received 0.3 mg/kg patisiran by infusion every 3 weeks. Two patients had no evidence of ATTR-CM, 7 patients had permanent pacemakers in situ and 3 patients refused the protocolized assessments. Analyses were conducted in the remaining 16 patients, 12 of whom received concomitant diflunisal, a TTR stabilizing drug, at a dose of 250 mg twice daily, throughout the duration of assessment. The findings at 12 months were compared with 16 patients who were retrospectively group matched with treated patients on the basis of amyloid type and cardiac amyloid burden at baseline (ECV by CMR),but did not receive disease-modifying therapy.

Patients were managed in accordance with the Declaration of Helsinki and provided written informed consent with approval from the Royal Free Hospital ethics committee (ref: 06/Q0501/42).

Echocardiography

All echocardiograms were reviewed by experienced operators blinded to the therapy and analysed as previously described (13).

CMR protocol and image analysis

All participants underwent standard CMR on a 1.5-T clinical scanner (Aera, Siemens Healthcare, Erlangen, Germany). A standard volume and LGE study was performed. The gadolinium-based contrast agent used was 0.1 mmol/kg gadoterate meglumine. LGE imaging was acquired with magnitude reconstruction and phase sensitive inversion recovery (PSIR) reconstruction in all patients. For native T1 and post-contrast mapping, basal, mid and apical ventricular short-axis and 4-chamber long-axis images were acquired by the modified Look-Locker inversion recovery (MOLLI) after regional shimming before the administration of contrast, as previously described in literature (see supplemental material for sequence details) (14). At fifteen minutes after the bolus of gadoterate meglumine and standard LGE imaging (standard fast low-angle shot inversion recovery or balanced steady-state free-precession sequence with magnitude reconstruction and PSIR reconstruction), the T1 measurement was repeated with the MOLLI sequence and inline extracellular volume maps (ECV) were automatically generated (15).

All CMR images and maps were analyzed offline and blinded to therapy. The endo- and epicardial borders were manually delineated for each basal, mid-ventricular and apical short-axis T1 map and T1 and ECV values for the segmented myocardium were subsequently averaged. A change in ECV was defined as an absolute increase or decrease of more than 0.03. The LGE pattern was classified into characteristic for cardiac amyloidosis and negative for cardiac amyloidosis.

^{99m}Tc-DPD bone scintigraphy protocol and image analysis

Images were acquired via General Electric's (GE) Discovery NM/CT 670 hybrid gamma camera (GE Healthcare, Chicago, IL, USA). Patients were intravenously administered with approximately 700 MBq (18.9 mCi) of ^{99m}Tc-DPD (Teceos®). The protocol consisted of a planar whole-body acquisition performed at 3 hours post injection followed immediately by single photon emission computed tomography (SPECT) imaging of the thorax. A low-dose, non-contrast CT scan of the heart was also performed for the purposes of attenuation correction and anatomical localization generating a volume of interest (VOI) for the whole heart. This VOI was then copied to the SPECT dataset for quantitation. Final results were expressed as percentage of injected dose, as previously described (16).

Functional assessments, cardiac biomarkers and plasma TTR concentration

N-Terminal pro-B-type natriuretic peptide was measured with an electrochemiluminescence sandwich immunoassay on the Elecsys system 2010 (Roche Diagnostics). TTR concentration was measured using Optilite Pre-Albumin assay. Six-minute walk tests were performed as per standard protocol.

Statistics

The variables are presented as median and interquartile range. Baseline variables were compared in the treated and untreated patients using the non-parametric Mann Whitney U test. The median follow-up times were also compared using the Mann Whitney U test. The treatment effect at the 12-month assessment for each variable was estimated by performing a linear regression analysis with the outcome variable being the 12 month value and the explanatory variables being the baseline value of that variable, group (treated = 1, untreated = 0) and age (which was found to differ significantly between groups). The estimated regression coefficient for group in the regression analysis represented the difference in means

(treated minus untreated) at 12 months, after adjusting for the baseline value of the variable and age. Although there is no requirement for the underlying variables to be normally distributed in a regression analysis, it is assumed that the residuals are approximately normally distributed and there is homoscedascitity. The latter assumption was checked by plotting the residuals against the predicted values, and a random scatter of points was observed in each regression analysis, indicating that the constant variance assumption was satisfied. If the residuals are not normally distributed this may affect the ensuing p-value. A regression analysis is reasonably robust to a violation of the assumption of normality of the residuals, but for any analysis in which this was questionable, consideration was given to the proximity of the p-value to the significance level, and conclusions drawn from the analysis only if the p-value was distant from the significance level.

The significance level for all hypothesis tests was taken as 0.025 to adjust for multiple testing. All data were analysed using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp.).

RESULTS

Patients

Baseline variables in the 16 treated patients and the 16 untreated patients are shown in Table 1. In the treated group, the TTR variants were T60A (p.T80A; n=6), V30M (p.V50M; n=3), A97S (p.A117S; n=1); and F33I (p.F53I), H90D (p.H110D), S77Y (p.S97Y), Y69F (p.Y89F), G47V (p.G67V) and D38V (p.D58V) in one case each. In the control group nine had wild-type ATTR 5 had T60A (p.T80A; n=11), one A97S (p.A117S; n=2) and one V122I (p.V142I).

Two out of 16 patients in the treatment group and 5/16 in the control group were on beta-blockers, 2/16 patients in the treatment group and 5/16 in the control group were on ACE-I/ARB and 7/16 patients in the treatment group and 8/16 in the control group were on diuretics. One patient in the treated group had a decrease in furosemide of 20 mg and two patients in the control group increased their furosemide by 20 mg during the 12 months of follow-up.

Median follow-up time among treated patients was 12.4 (IQR 8.8-13.6) months and among control subjects was 12.3 (IQR 9.5-13.1) months (p=0.8).

Tolerability

Both diflunisal and patisiran were well tolerated. No patient discontinued either drug during the period of assessment. Infusion-related reactions occurred in 4 patients and were mild and self-limiting in each case. Diflunisal, a non-steroidal inflammatory drug was accompanied by prophylactic H2 receptor antagonist therapy in all cases and was not complicated by gastro-intestinal bleeding in any such case. There were 8 serious adverse events (SAEs) during the period of assessment, none thought to be related to either patisiran or diflunisal.

Efficacy

Median serum TTR knockdown among treated patients was 86% (IQR 82-90%). There was no difference in percentage knockdown between patients receiving diflunisal (median 86%) and those receiving patisiran without concomitant diflunisal (median 87%; p>0.999).

Among treated patients, cardiac amyloid burden diminished in 6/16 (38%), was unchanged across the period of assessment in 7/16 (48%), and increased in 3/16 cases (19%). Among 16 control subjects, amyloid burden remained unchanged in 7/16 (44%) cases and increased in 9/16 (56%) cases. No untreated patient achieved a reduction in ECV (Figure 1A). The adjusted difference in mean ECV at 12 months in the treated and untreated groups was -6.2% (95% CI -9.5% to -3.0%), indicating that the mean in the treated group was 6.2% lower than that in the untreated group, after adjusting for baseline ECV and age (Table 2; p=0.001). There was no evidence of an association between time from baseline to follow-up CMR and reduction in ECV by linear regression analysis. There was no significant difference between the group means at 12 months in native T1 or T2. CMR findings were accompanied by a median (IQR) reduction in cardiac uptake by Tc-DPD scintigraphy of 19.6% (9.8-27.1%) in treated patients, with 15/16 (94%) showing a reduction in cardiac uptake and the remaining patient showing no change. The adjusted difference in means between the groups in NT-proBNP at 12 months was -1342 ng/L (95% CI -2363 to -321), with the mean in the control group being greater than that in the treated group (Figure 1B; p=0.012). There was a significant improvement in 6MWT in the treated group compared to the control group, with an adjusted difference in the mean 6MWT distance at 12 months of 168 meters (95% CI 57 to 280) (Figure 1C; p=0.004). Although the PND scores were higher at baseline in the treated group (5 PND I, 6 PND II, 5 PND III) than in the control group (4 PND I, 1 PND II, 0 PND III), it is noteworthy that only 1 patient in the treated group and no patient in the untreated group changed their PND score over the 12 month assessment period.

Among the 16 treated patients, the adjusted difference in mean NT-proBNP at 12 months in the 6 cases with a reduction in ECV compared to the remaining 10 cases with unchanged or increased ECV was -768 ng/L (95% CI -1456 to -80), indicating that the mean NT-proBNP in 'regressors' was 768 ng/L lower than that in 'non-regressors' after adjusting for baseline NT-proBNP and age (p=0.032). The adjusted difference in mean E/E' at 12 months in the 'regressors' and 'non-regressors' was -5.5 (95% CI -11.2 to 0.2), but did not reach statistical significance (p=0.056). There were no differences between treated 'regressors' and treated 'non-regressors' in any other imaging parameters.

DISCUSSION

This is the first demonstration of reductions in ECV in patients with ATTR-CM and provides evidence for ATTR cardiac amyloid regression. The natural history of cardiac ATTR amyloidosis in the absence of disease-modifying therapy has been well documented, and is one of inexorable amyloid accumulation culminating in death within 2-10 years (17). Remarkable recent therapeutic developments in ATTR amyloidosis have been associated with improved clinical outcomes in some patients with both ATTR-CM (18) and ATTR-PN (3). Although the mechanisms underpinning these clinical responses have not yet been identified, it seems likely that, as in other amyloidosis syndromes, the equilibrium between rate of amyloid formation and rate of amyloid clearance will have been altered by halting or slowing amyloid accumulation. Imaging evidence of cardiac organ response in amyloidosis has historically been determined by echocardiography, but significant improvement in any particular echocardiographic parameter has not been demonstrated in ATTR-CM patients treated with tafamidis whilst among those receiving patisiran, only a very marginal improvement in some echocardiographic parameters has been demonstrated (19). The emergence of advanced myocardial tissue characterization by magnetic-resonance, specifically T1 mapping with native T1 and ECV measurements has made it possible to estimate cardiac amyloid burden *in vivo* for the first time. The serial CMR studies we report here strongly suggest that regression of cardiac amyloid can occur in a proportion of patients and that disease stabilization occurs in others following treatment with patisiran and diflunisal. Whilst disease-modifying therapy was associated with an average reduction in ECV among the treated group when compared to the control group, individual responses varied substantially. Among treated patients, cardiac amyloid burden diminished in 38%, was unchanged in 48%, and increased in 19% of cases after 12 months of therapy. This is entirely consistent with other types of amyloidosis, in which the equilibrium between rate of

amyloid accumulation and rate of amyloid regression is altered to different degrees in different individuals by therapy such that some show overall amyloid regression, others show stable amyloid deposits, and others merely show a slowing of disease progression (3,20-24). These findings only serve to highlight the importance of assessing change in amyloid burden at an individual level which is uniquely possible with CMR with T1 mapping. Whilst reduction in ECV could, in part, be related to reduction in myocardial oedema, which has been postulated to contribute to remarkably high native T1 values during phases of rapid amyloid accumulation (12), the reduction in ECV in the absence of changes in T2 and native T1 observed in this cohort of patients, provides strong evidence that this was not the case here. Similarly, it is not possible with CMR and multiparametric mapping to distinguish between increase in ECV due to amyloid or due to fibrosis. However, histological studies of cardiac and indeed extra-cardiac amyloid have consistently demonstrated very little or no fibrosis in patients with amyloidosis, particularly ATTR type (25). The same is true following histological regression of amyloid in mouse models, which is remarkable for the complete absence of fibrosis and return to normal tissue architecture (26). Furthermore, patients with cardiac AL amyloidosis who achieve a reduction in ECV following chemotherapy (27) typically have accompanying improvement in organ function, biomarkers, and functional status, supporting the histological analyses. Lastly, the degree of reduction in ECV was very extensive in some of the patients in our cohort, which one would not expect to see were it due to fibrosis alone. On the basis of all the available evidence therefore, we feel confident that the reduction in ECV is overwhelmingly likely to represent cardiac amyloid regression rather than reduction in fibrosis.

Our CMR findings are also supported by serial evaluation of NT-proBNP and 6MWT distance, although improvement in the latter is likely to have been contributed to by amyloid-related peripheral neuropathy. The improvement in the NT-proBNP is in line with the

published reports in patients with AL amyloidosis, where a reduction in amyloid deposition is associated with improvement in plasma NT-proBNP concentration. However, several mechanisms co-exist and may interact to influence NT-proBNP concentration, functional status (NYHA) and 6MWT distance including peripheral neuropathy, autonomic dysfunction, neuro-hormonal activation and kidney disease each of which vary between individuals depending on comorbidities, disease type and response to treatment thereby making these markers relatively non-specific. This highlights the essential requirement for a specific marker of amyloid burden to evaluate efficacy of novel therapies that specifically target ATTR amyloid production.

It is noteworthy, that all treated patients had a reduction in cardiac uptake of ^{99m}Tc-DPD after 12 months of therapy apart from one in whom cardiac uptake was unchanged. Furthermore, the reduction in cardiac uptake was typically accompanied by a reduction in muscle and soft tissue uptake and a corresponding increase in bone signal as illustrated in Figure 3. However, whilst the reduction in cardiac uptake on ^{99m}Tc-DPD imaging after 12 months of therapy unequivocally indicates a biological effect of disease-modifying therapy and has never before been seen among patients undergoing serial ^{99m}Tc-DPD scintigraphy who are not receiving disease-modifying therapy (16), the authors would advise caution in interpreting such images and exclusively ascribing changes to cardiac ATTR amyloid burden in the absence of cardiac MRI or other imaging, biochemical or functional evidence of direct cardiac benefit. The dynamics and kinetics of ^{99m}Tc-DPD binding in the bones, soft tissues (including muscles) and hearts of patients with cardiac ATTR amyloidosis differ and a change in uptake in any one of these three compartments will affect the appearance and calculation of percentage cardiac uptake (16).

The ability to detect treatment response at an individual level in terms of amyloid burden has the potential to have an important impact on the management of patients with ATTR

amyloidosis in the near future. Several disease-modifying drugs are in late phase development and are likely to be available for ATTR-CM in the next few years; being able to evaluate individual patients' responses will be key to individualizing treatment strategies, acknowledging that long term studies are required to correlate changes in cardiac amyloid load with overall survival.

The cohort is too small to indicate the relative benefit of TTR stabilization versus TTR knockdown. One can merely postulate that the combination of knockdown and stabilization may be synergistic which would not be surprising given the need for both thyroxine binding pockets of the transthyretin tetramer to be occupied in order to achieve 'complete' tetramer stabilization *in vivo*. The relative concentration of stabilizer in relation to TTR protein in the serum is several fold higher in subjects with substantial TTR knockdown than in those who have normal serum TTR concentration. Further studies will be required to elucidate the relative benefits of each individual therapeutic modality and whether there is indeed mechanistic synergy.

In summary, we demonstrate here for the first time reductions in ECV among a proportion of patients receiving patisiran over the course of 12 months providing evidence for cardiac ATTR amyloid regression, accompanied by scintigraphic, biochemical and functional evidence of clinical benefit. Further larger studies are required to elucidate the individual variations in response to these two therapeutic modalities and the relative contribution of each to the benefit on cardiac structure and function among patients with ATTR-CM.

Limitations

This study has several limitations. Only a small number of treated patients were included due to limited access to the patisiran Early Access Programme. The control group were matched

to treated patients retrospectively on the basis of amyloid type and amyloid burden (ECV) by CMR at baseline. However, due to the rarity of certain mutations among the treated group, not all patients were matched for genotype, and there were statistically significant differences in age and 6MWT distance between the control and treated groups. However, to account for this, the analysis of change in ECV was adjusted for age and all comparisons were also adjusted for the baseline value of each variable. A further limitation is the absence of serial Tc-DPD scans in the control group, although it is important to highlight that reduction in cardiac uptake on serial Tc-DPD imaging has never been described (16).

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CLINICAL PERSPECTIVES

Competency in Medical Knowledge: Measurement of extracellular volume, easily acquired during a routine clinical CMR scan, can provide evidence for ATTR cardiac amyloid regression in patients receiving disease-modifying therapy.

Translational outlook: Future studies using CMR with ECV mapping could help to stratify individual treatment responses to the new disease-modifying therapies in patients with ATTR-CM.

Table 1. Baseline characteristics, biomarkers, echocardiographic and CMR parameters in patients with ATTR amyloidosis.

Characteristics	Patients on treatment	Patients not on treatment	P value
Age (y)	62 (54;67)	69 (62;80)	0.023
Gender (M/F)	13/3	14/2	0.632
6MWT (m)	337(188;472)	463(362;486)	0.039
NT-proBNP (ng/L)	805 (264;2206)	1659 (587;2347)	0.402
eGFR	78 (62;88)	71 (55;79)	0.168
Echocardiographic parameters			
IVSd (mm)	16 (14;17)	16 (15;17)	0.564
PWTd (mm)	16 (14;17)	16 (15;17)	0.468
RWT	0.70 (0.67;0.82)	0.75 (0.66;0.85)	0.592
E' Lat (cm/s)	0.07 (0.05;0.09)	0.06 (0.05;0.07)	0.294
E' Sept (cm/s)	0.05 (0.03;0.06)	0.04 (0.04;0.05)	0.343
E wave (cm/s)	0.73(0.60;0.90)	0.77(0.64;1.04)	0.346
A wave (cm/s)	0.67(0.47;0.85)	0.56(0.36;0.85)	0.554
E/A	1.03(0.75;1.64)	1.60(0.88;2.08)	0.270
E/E'	10.8(7.4;16.5)	13.3(9.7;18.0)	0.184
MAPSE (cm/s)	10(7;13)	9(7;10)	0.515
TAPSE (cm/s)	18(14;21)	17(13;19)	0.468
PASP (mmHg)	36(30;42)	43(36;46)	0.164
MCF (%)	0.20 (0.17;0.36)	0.17(0.14;0.23)	0.175
LS (%)	-13.7(-16;-10.5)	-12.6(-15.4;10.8)	0.956
CMR parameters			
LVEDVi (mL/m2)	77(70;81)	75(66;81)	0.724
LVESVi (mL/m2)	27(20;31)	31(24;41)	0.184
LAA (cm2)	25(23;34)	29(24;31)	0.780
RAA (cm2)	23(17;30)	24(19;30)	0.539
LV mass (g/m2)	104(88;141)	126(101;145)	0.149
LVSVi (mL/m2)	49(42;58)	43(38;52)	0.184
LVEF (%)	63(59;75)	60(55;65)	0.119
MCF (%)	0.44(0.34;0.62)	0.33(0.29-0.44)	0.057
Native T1 (ms)	1142(1105;1177)	1124(1091;1172)	0.515
Native T2 (ms)	50(48;52)	51(49;53)	0.439
ECV (%)	46(43;53)	49(41;51)	0.724

All variables are presented as median (interquartile range). NT-proBNP, N-terminal pro-brain natriuretic peptide; IVSd, interventricular septum diameter; PWTd posterior wall thickness diameter; RWT, relative wall thickness; MAPSE, mitral annular plane systolic excursion; TAPSE, tricuspid annular plane systolic excursion; PASP, pulmonary artery systolic pressure; MCF, myocardial contraction fraction; LS, longitudinal strain; LVEDVi, left ventricular end diastolic volume indexed; LVESVi, left ventricular end systolic volume indexed; LV, left ventricular; SV, stroke volume; LVEF, left ventricular ejection fraction; LA left atrium; ECV, extracellular volume.

Table 2. Biomarkers, echocardiographic and CMR changes over time

Characteristics	Patients on treatment		Patients not on treatment		Group regression coefficient (95% CI)*	p value
	Baseline	1 year	Baseline	1 year		
6MWT (m)	337(188;472)	368(265;478)	463(362;486)	322(184-506)	169 (57;280)	**0.004**
NT-proBNP (ng/L)	805 (264;2206)	668(298;2076)	1659 (587;2347)	2477(1062-4615)	-1343 (-2364;-322)	**0.012**
Echocardiographic parameters						
IVSd (mm)	16 (14;17)	17(15;17)	16 (15;17)	16(15;18)	-0.093 (-0.840;0.653)	0.799
PWTd (mm)	16 (14;17)	16(14;18)	16 (15;17)	16(15;18)	0.113 (-0.620;0.846)	0.755
RWT	0.70 (0.67;0.82)	0.77(0.66;0.82)	0.75 (0.66;0.85)	0.75(0.63;0.81)	0.035 (-0.032;0.102)	0.297
E' Lat (cm/s)	0.07 (0.05;0.09)	0.07 (0.05;0.09)	0.06 (0.05;0.07)	0.06 (0.05;0.08)	0.002 (-0.015;0.019)	0.794
E' Sept (cm/s)	0.05 (0.03;0.06)	0.06(0.04;0.07)	0.04 (0.04;0.05)	0.04 (0.04;0.05)	0.011 (-0.002;0.024)	0.101
E wave (cm/s)	0.73(0.60;0.90)	0.87(0.58;1.04)	0.77(0.64;1.04)	0.84 (0.65;1.11)	0.064 (-0.049;0.177)	0.253
A wave (cm/s)	0.67(0.47;0.85)	0.80 (0.44;0.98)	0.56(0.36;0.85)	0.50 (0.38;0.61)	0.093 (-0.119;0.304)	0.371
E/A	1.03(0.75;1.64)	0.98 (0.69;1.53)	1.60(0.88;2.08)	1.67(0.97;2.23)	0.015 (-0.947;0.977)	0.975
E/E'	10.8(7.4;16.5)	11.1(9.5;14.0)	13.3(9.7;18.0)	12.5(9.2;21.4)	-0.757 (-4.781;3.268)	0.703
MAPSE (cm/s)	10(7;13)	9(7;12)	9(7;10)	8(7;11)	0.266 (-1.810;2.342)	0.795
TAPSE (cm/s)	18(14;21)	20(14;23)	17(13;19)	16(12;20)	1.631 (-1.438;4.699)	0.285
PASP (mmHg)	36(30;42)	37(33;39)	43(36;46)	40(32;50)	-7.882 (-22.005;6.242)	0.239
MCF (%)	0.20 (0.17;0.36)	0.23(0.13;0.35)	0.17(0.14;0.23)	0.16(0.15;1.9)	0.49 (-0.018;0.116)	0.146
LS (%)	-13.7(-16;10.5)	-12.6(-14.8;-10.6)	-12.6(-15.4;-10.8)	-11.1(-14.1;-8.6)	-1.705 (-4.500;1.089)	0.221
CMR parameters						
LVEDVi (mL/m^2)	77(70;81)	75(67;76)	75(66;81)	81(72;88)	-6.165 (-12.237;-0.094)	0.047
LVESVi (mL/m^2)	27(20;31)	25(21;36)	31(24;41)	37(26;42)	-3.275 (-7.098;0.548)	0.090
LA area	25(23;34)	26(22;30)	29(24;31)	26(24;32)	-1.117 (-3.806;1.572)	0.402
RA area	23(17;30)	24(20;28)	24(19;30)	26(22;29)	-0.831 (-3.332;1.670)	0.502
LV mass (g/m^2)	104(88;141)	110(86;142)	126(101;145)	127(111;150)	-3.089 (-20.622;14.443)	0.721
LVSVi (mL/m^2)	49(42;58)	48(41;58)	43(38;52)	46(41;55)	-2.009 (-6.122;2.105)	0.326
LVEF (%)	63(59;75)	63(57;73)	60(55;65)	57(53;66)	1.769 (-1.756;5.294)	0.313
MCF (%)	0.44(0.34;0.62)	0.40(0.33-0.56)	0.33(0.29-0.44)	0.38(0.33;0.43)	-0.017 (-0.077;0.043)	0.571
Native T1, ms	1142(1105;1177)	1133(1102;1183)	1124(1091;1172)	1130(1110;1167)	-12.355 (-34.652;9.940)	0.266
Native T2, ms	50(48;52)	50(49;52)	51(49;53)	51(50;54)	0.268 (-1.710; 2.245)	0.783

ECV (%)	46(43;53)	48(41;51)	49(41;51)	53(46;58)	-6.2 (-9.5; -3.0)	**0.001**

All variables are presented as median (interquartile range). * - Group regression coefficient represents the adjusted mean difference between groups at 1 year. NT-proBNP, N-terminal pro-brain natriuretic peptide; IVSd, interventricular septum diameter; PWTd posterior wall thickness diameter; RWT, relative wall thickness; MAPSE, mitral annular plane systolic excursion; TAPSE, tricuspid annular plane systolic excursion; PASP, pulmonary artery systolic pressure; MCF, myocardial contraction fraction; LS, longitudinal strain; LVEDVi, left ventricular end diastolic volume indexed; LVESVi, left ventricular end systolic volume indexed; LV, left ventricular; SV, stroke volume; LVEF, left ventricular ejection fraction; LA left atrium; ECV, extracellular volume.

FIGURES

Figure 1. Change in extracellular volume, NT-proBNP and 6-minute walking test. Dotplots comparing change from baseline to 12 months among treated and untreated patients (solid horizontal lines represent group medians). A) Change in extracellular volume (p=0.001; Mann Whitney U test); B) Change in NT-proBNP comcentration (p<0.001; Mann Whitney U test); C) Change in 6-minute walk test distance (p<0.005; Mann Whitney U test)

Figure 2. ECV maps showing reduction with treatment. ECV maps (from left to write: four-chamber, basal, mid and apical short axis) of a patient at baseline (upper panels) and 12 months after treatment (lower panels).

Figure 3. ^{99m}Tc-DPD scans showing changes in cardiac soft tissue and bone uptake. Serial planar anterior whole body ^{99m}Tc-DPD scans taken 12 months apart in a patient receiving diflunisal and patisiran. At baseline (left) there is extensive cardiac (large arrow) and soft tissue uptake with attenuated bone uptake (small arrow). At 12 months (right), there is an unequivocal reduction in cardiac and soft tissue uptake with a corresponding increase in bone uptake, particularly evident in the long bones.

Central illustration. Cardiac amyloid regression. Cardiac biopsies showing TTR amyloid, serial planar anterior whole body ^{99m}Tc-DPD scans and ECV maps showing cardiac amyloid regression in a patient receiving diflunisal and patisiran.

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