Clinical Investigation

Clinical Experience With the Use of Doxycycline and Ursodeoxycholic Acid for the Treatment of Transthyretin Cardiac Amyloidosis

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ABSTRACT

Background: The tolerability and utility of combination doxycycline and ursodeoxycholic acid (ursodiol) amyloid fibril disruption therapy for transthyretin cardiac amyloidosis (ATTR CA) in clinical practice is poorly described.

Methods and Results: We report the clinical experience of 53 ATTR CA patients treated with doxycycline and ursodiol. Six patients (11%) did not tolerate the therapy owing to dermatologic and gastrointestinal effects. Of those remaining, the median follow-up was 22 months (range 8−30), mean age was 71 ± 11 years, 41 (87%) were male, and 42 (89%) had wild-type and 5 (11%) mutant ATTR. Five patients (11%) died during follow-up. There was no significant change in New York Heart Association (NYHA) functional class, cardiac biomarkers, or echocardiographic parameters during follow-up. Left ventricular (LV) global longitudinal systolic strain (GLS) improved in 16 patients (38%) (−12 ± 4% to −17 ± 4%; $P < .01$). Patients whose LV GLS improved were significantly younger and had lower NYHA functional class, troponin-T, N-terminal pro−B-type natriuretic peptide (BNP), and baseline LV GLS levels compared with those whose LV GLS did not improve. Troponin-T improved in follow-up for patients whose LV GLS improved (35 ± 21 to 20 ± 14 ng/L; $P = .06$).

Conclusions: Doxycycline and ursodiol therapy for treatment of ATTR CA was tolerable and was associated with stabilized markers of disease progression. LV GLS improved in patients with less advanced disease. (*J Cardiac Fail 2019;25:147−153*)

Key Words: Transthyretin cardiac amyloidosis, doxycycline, ursodiol, tolerability, efficacy.

Transthyretin cardiac amyloidosis (ATTR CA) is characterized by extracellular myocardial deposition of amyloid fibrils derived from either wild-type or mutant forms of the transthyretin protein (TTR).[1−3] ATTR CA represents an important cause of progressive heart failure which is now increasingly recognized with improved modern diagnostic strategies.[4] Previously, treatment options for ATTR CA were limited to supportive care, with the possibility of cardiac transplantation for a small number of eligible patients with advanced heart failure.[5] Recent research into different disease-modifying therapeutic approaches has identified multiple potential options, including suppression of hepatic TTR synthesis,[6,7] stabilization of the TTR homotetramer to prevent its dissociation into monomers and subsequent misassembly into amyloid fibrils,[5,8,9] and disruption of amyloid fibril deposits in end-organ tissues by enhancing breakdown and resorption.[10−12] Although these novel therapies show promise and are in various stages of development, clinical availability and experience for the treatment of ATTR CA currently remain limited.

The combination of the tetracycline antibiotic doxycycline and the antiapoptotic agent tauroursodeoxycholic acid (TUDCA) has been demonstrated to synergistically reduce amyloid fibril burden in a mouse model[10] and to attenuate disease progression in limited clinical trials.[11] Despite these

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Manuscript received August 21, 2018; revised manuscript received January 7, 2019; revised manuscript accepted January 15, 2019.

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See page 152 for disclosure information.

1071-9164/$ - see front matter

https://doi.org/10.1016/j.cardfail.2019.01.006

reports, clinical experience with this therapeutic approach remains limited and of uncertain utility. Ursodeoxycholic acid (ursodiol) is a bile acid typically used to treat certain cholestatic syndromes with an efficacy similar to TUDCA, and it also has been studied in combination with doxycycline for its antiamyloid fibril effects.[12] Our center has used doxycycline and ursodiol therapy for the treatment of patients with ATTR CA, and the purpose of the present study is to report our clinical experience regarding its tolerability and patient outcomes using this approach.

Methods

Study Population

Patients followed at our center with a diagnosis of ATTR CA from November 2014 to the time of writing were included in this retrospective analysis if they met the following criteria: 1) evidence of ATTR CA by either right ventricular (RV) endomyocardial biopsy or positive technetium-99m pyrophosphate nuclear scintigraphy defined by grade 2−3 myocardial uptake or heart−contralateral lung ratio >1.5, as previously described[4]; 2) either wild-type or mutant ATTR CA based on genetic testing or proteomic analysis by means of mass spectrometry performed on biopsy tissue samples; and 3) exclusion of light chain amyloidosis by means of serum and urine immunofixation electrophoresis and serum-free light chain assay. Clinical, medication, biochemical, and transthoracic echocardiography data were collected at baseline and at the time of last follow-up. This study was approved by the University of Calgary Research Ethics Board, and the requirement for informed written patient consent was waived.

Antiamyloid Therapy With the Use of Doxycycline and Ursodiol

Eligible patients were initiated on a combination of 100 mg doxycycline twice daily and 250 mg ursodiol 3 times daily according to the dosing regimen used by a previous phase II open-label clinical trial.[11] Patients with hepatic transaminases, alkaline phosphatase, or bilirubin levels higher than twice the upper limit of normal or a history of calcified cholesterol gallstones or active pancreatic or biliary tree disorders were not eligible. After initiation, patients were clinically monitored for medication tolerance and underwent baseline laboratory testing and follow-up within 4−8 weeks. Therapy was discontinued if any of the following occurred: an increase of hepatic transaminases or alkaline phosphatase or bilirubin levels higher than twice the upper limit of normal. Patients were then followed with clinical appointments every 6 months or as clinically indicated. Patients were also followed with the use of echocardiography and cardiac biomarkers troponin-T and N-terminal pro−B-type natriuretic peptide (NT-proBNP) every 12 months or as clinically indicated after initiation of therapy.

Echocardiography

Comprehensive transthoracic echocardiography examinations were performed with the use of a commercially available ultrasound system equipped with a 1−5-MHz transducer (iE33; Philips Medical Systems, Andover, Massachusetts). Imaging acquisitions and measurements were performed according to current guidelines.[13] Offline quantitative analysis was performed blinded to all other subject data. Routine 2-dimensional cine loops and Doppler imaging acquisitions from 3 consecutive beats in sinus rhythm and 5 in atrial fibrillation were obtained for measurement of left ventricular (LV) chamber size, wall thickness, and calculation of LV mass index. LV end-diastolic and end-systolic volumes were measured and used to calculate ejection fraction (EF) by means of the biplane method of disks summation. Measured LV diastolic function parameters included left atrial volume index, pulsed-wave Doppler early (E) and late (A) diastolic mitral valve inflow velocities, and averaged diastolic septal and lateral mitral valve annular e′ tissue Doppler velocities.[14] Right ventricular (RV) function was assessed by tricuspid annular plane systolic excursion (TAPSE) and tissue Doppler peak systolic velocity (S′) of the tricuspid valve annulus in the apical 4-chamber view. RV systolic pressure was estimated from the peak continuous-wave Doppler velocity of tricuspid valve regurgitation plus the estimated right atrial pressure. Right atrial pressure estimation was based on interrogation of the inferior vena cava diameter and distensibility, and the right atrial area was measured from an apical 4-chamber view.

The LV global longitudinal peak systolic strain (GLS) was calculated with the use of speckle-tracking echocardiography software analysis (Qlab version 10.3; Philips Medical Systems). Three focused views of the LV from the apical imaging window (4-, 3-, and 2-chamber views) were acquired, and the frame rate was optimized to >40 frames/s. A myocardial region of interest was placed by tracing endocardial borders on an end-systolic frame as defined by the electrocardiographic QRS complex and adjusting for myocardial thickness accordingly. The software uses an algorithm to track acoustic markers throughout the cardiac cycle, and myocardial contours can be manually adjusted to optimize tracking if necessary. Peak GLS was computed automatically as the percentage of myocardial systolic shortening relative to the original length, expressed as a negative percentage. For patients in sinus rhythm, analyses were performed on a single cardiac cycle; for patients in atrial fibrillation, strain values were calculated as the average of 3 cardiac cycles.

Statistical Analysis

Categoric variables are presented as absolute values and percentages, and continuous variables are expressed as the mean ± standard deviation. Categoric variables were compared by means of the Fisher exact test, and comparisons for continuous data were performed using the 2-sample independent *t* test or Wilcoxon rank-sum test as

appropriate. The Wilcoxon signed-rank test or paired *t* test was used to compare changes in variables from baseline to follow-up as appropriate. All-cause mortality and cardiovascular hospitalization data were collected. The frequency and reason for therapy discontinuation were collected, along with measures of disease severity that included change from baseline to most recent follow-up in New York Heart Association (NYHA) functional class, troponin-T, NT-proBNP, and echocardiographic parameters. The frequency of an improvement in LV GLS from baseline to follow-up was also collected and was defined as an improvement of ≥3%, based on a previously reported standard deviation from normative values of LV GLS of 2%−3%.[13] All statistical analyses were performed with the use of commercially available software (Stata version 14.2; Statacorp, College Station, Texas). A 2-sided *P* value of <.05 was considered to be statistically significant.

Results

Patient Population and Characteristics

A total of 56 patients were eligible for inclusion. Three patients declined doxycycline and ursodiol therapy and were excluded from the analysis. Six patients (11%) did not tolerate therapy—4 because of increased photosensitivity or erythema/pruritus, and 2 because of abdominal discomfort—for a final study population of 47 patients. No other adverse effects were reported. Clinical, medication, and biochemical characteristics are presented in Table 1. The majority of patients were male (41, 87%) and had wild-type ATTR CA (42, 89%). The mutations among the 5 patients (11%) with mutant ATTR included Val30Met, Thr60Ala, Val20Ile, and Val122Ile (n = 2). Fourteen patients (30%) had RV endomyocardial biopsy confirmation of amyloidosis and 40 (85%) positive cardiac technetium-99m pyrophosphate scan.

Table 1. Baseline Patient Characteristics (n = 47)

Clinical characteristics	
Age (y)	71 ± 11
Male	41 (87%)
Body surface area (m^2)	1.7 ± 0.4
Body mass index (kg/m^2)	26 ± 3
Heart rate (beats/min)	73 ± 14
Systolic BP (mm Hg)	112 ± 19
Diastolic BP (mm Hg)	78 ± 12
NYHA functional class III−IV	22 (47%)
Atrial fibrillation	13 (28%)
Transthyretin amyloidosis type	
Wild-type	42 (89%)
Mutant	5 (11%)
Medications	
Diuretics	33 (70%)
Anticoagulation	16 (34%)
Biochemical	
Troponin-T (ng/L)	64 ± 18
NT-proBNP (ng/L)	1716 ± 1002
eGFR ($mL{\cdot}min^{-1}{\cdot}1.73\ m^{-2}$)	51 ± 17

BP, blood pressure; eGFR, estimated glomerular filtration rate; NT-proBNP, N-terminal pro−B-type natriuretic peptide; NYHA, New York Heart Association.

No patients were taking other prescribed or investigational antiamyloid disease-modifying therapy, including diflunisal.

Clinical and Biomarker Follow-Up

The median follow-up was 22 months (range 8−30). During the follow-up period, 5 patients (11%) died; including 3 from end-stage heart failure, 1 from advanced dementia, and 1 from intra-abdominal sepsis. Five patients (11%) were hospitalized for cardiovascular causes, including 2 for heart failure (including 1 who subsequently died), 1 for permanent pacemaker implantation, 1 for atrial fibrillation, and 1 for chest pain. From baseline to the most recent follow-up there was no significant change in NYHA functional class (47% to 49% class III−IV; *P* = .53), troponin-T (64 ± 18 to 59 ± 23 ng/L; *P* = .29), and NT-proBNP (1716 ± 1002 to 1646 ± 1195 ng/L; *P* = .37), respectively.

Echocardiographic Follow-Up

Baseline and follow-up echocardiographic findings are presented in Table 2, demonstrating no statistically significant changes in any echocardiographic variables. Image quality was adequate for LV GLS calculation in 42 patients. LV GLS improved in 16 patients (38%) (−12 ± 4% to −17 ± 4%; *P* < .01), with no significant change demonstrated among the remaining patients. Figure 1 demonstrates the change in LV GLS and duration from baseline to follow-up assessment for all patients demonstrating improvement, and Figure 2 provides an example of a patient whose LV GLS improved from baseline to follow-up. Baseline characteristics of those patients whose LV GLS improved compared with those whose did not are presented in Table 3. This shows that age, NYHA functional class, troponin-T, NT-

Table 2. Echocardiographic Parameters at Baseline and Follow-Up

Parameter	Baseline	Follow-Up	*P* Value
LV IVS thickness (mm)	16 ± 4	15 ± 4	.56
LV PW thickness (mm)	15 ± 3	15 ± 4	.44
LV mass index (g/m^2)	159 ± 52	147 ± 51	.13
LV end-diastolic volume (mL)	116 ± 21	120 ± 23	.28
LV end-systolic volume (mL)	57 ± 17	55 ± 14	.41
LV ejection fraction (%)	51 ± 12	53 ± 11	.18
LA volume index (mL/m^2)	47 ± 25	49 ± 26	.63
E/A	1.5 ± 0.3	1.4 ± 0.5	.77
Average mitral annular e′ velocity (cm/s)	4 ± 2	5 ± 3	.82
Average E/e′	17 ± 9	17 ± 6	.74
TAPSE (mm)	13 ± 6	17 ± 4	.09
RV S′ (cm/s)	12 ± 3	15 ± 4	.22
Estimated RVSP (mm Hg)	47 ± 18	42 ± 21	.19
Estimated RAP (mm Hg)	12 ± 6	11 ± 6	.67
RA area (cm)	23 ± 6	21 ± 5	.51
LV GLS (%)	−10 ± 5	−13 ± 6	.09

E/A, ratio of early to late (atrial contraction) mitral valve diastolic inflow velocity; E/e′, ratio of early mitral valve diastolic inflow to early diastolic mitral valve annular tissue Doppler velocity; GLS, peak global longitudinal systolic strain; IVS, interventricular septum; LV, left ventricle; PW, posterior wall; RA, right atrium; RAP, right atrial pressure; RV, right ventricle; RVSP, right ventricular systolic pressure; S′, lateral tricuspid valve annular systolic tissue Doppler velocity; TAPSE, tricuspid valve annular plane systolic excursion.

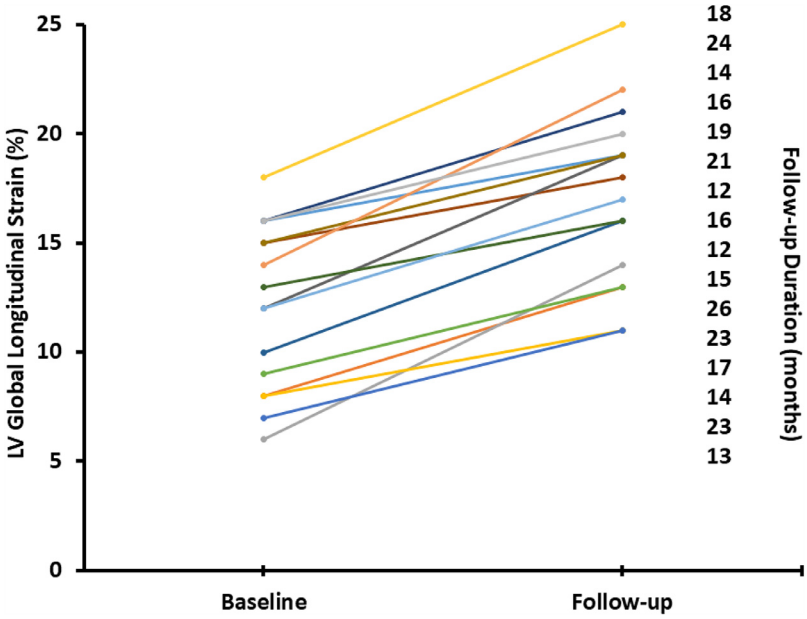

Fig. 1. Change in left ventricular (LV) global longitudinal strain (expressed as an absolute % value) from baseline to follow-up assessment for all 16 patients demonstrating improvement. The follow-up duration (months) for each patient is listed in sequential order in the column on the right.

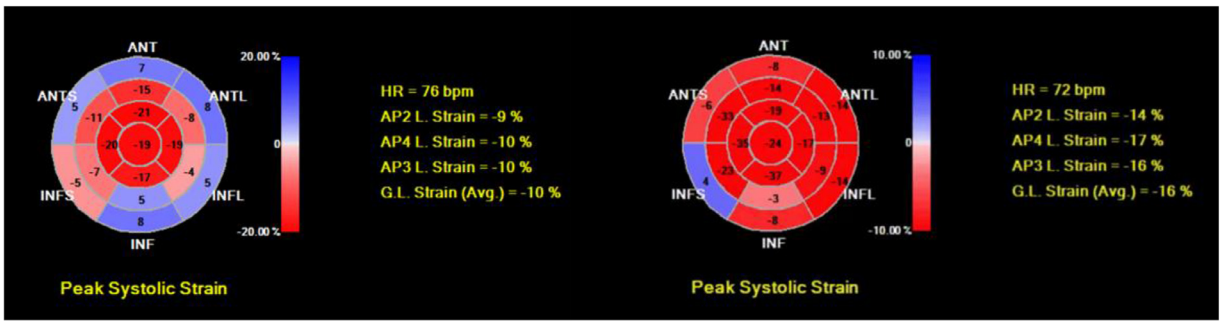

Fig. 2. Left ventricular global longitudinal strain (GLS) results for a patient with transthyretin cardiac amyloidosis treated with doxycycline and ursodiol. Baseline imaging (left) demonstrates GLS of −10%, with subsequent improvement to −16% after 23 months of therapy (right).

Table 3. Baseline Characteristics of Patients Whose LV GLS Improved and Those Whose LV GLS Did Not Improve at Follow-Up

Parameter	Improvement in LV GLS (n = 16)	No improvement in LV GLS (n = 26)	*P* Value
Age (y)	66 ± 7	74 ± 12	.05
Male	14 (88%)	22 (85%)	.59
NYHA functional class III–IV	0 (0%)	21 (81%)	<.01
Troponin-T (ng/L)	35 ± 21	78 ± 16	.04
NT-proBNP (ng/L)	1082 ± 928	1938 ± 1403	.01
eGFR (mL·min^{-1}·1.73 m^{-2})	52 ± 17	50 ± 18	.85
LV mass index (g/m^2)	161 ± 50	157 ± 54	.51
LV EF (%)	52 ± 12	49 ± 13	.26
TAPSE (mm)	13 ± 7	12 ± 6	.93
LV GLS (%)	−12 ± 4	−8 ± 2	.03

EF, ejection fraction; other abbreviations as in Table 1 and 2.
*$P \leq 0.05$.

proBNP and baseline LV GLS were significantly lower among those experiencing an improvement in LV GLS. Although NT-proBNP did not significantly change during follow-up for patients whose LV GLS improved, troponin-T values were lower at follow-up compared with baseline (35 ± 21 to 20 ± 14 ng/L; Fig. 3). Although this difference did not reach statistical significance ($P = .06$), it represents a 43% reduction. There was no significant change in either marker for patients whose LV GLS did not improve. Among patients whose LV GLS improved and those whose LV GLS

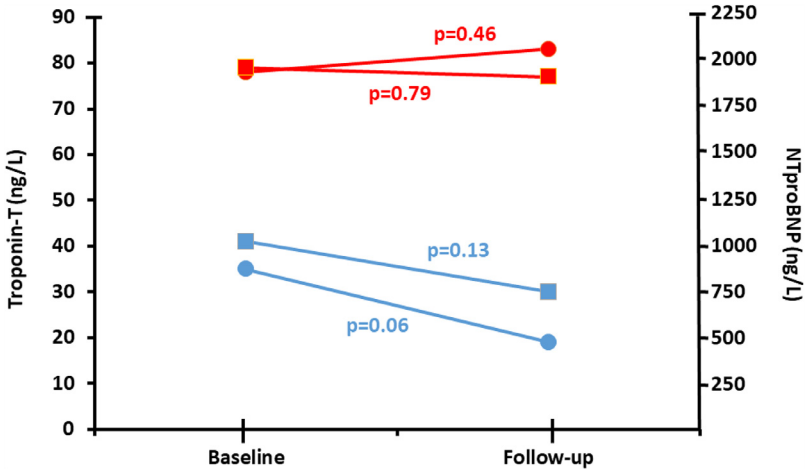

Fig. 3. Change in troponin-T (*circles*) and N-terminal pro−B-type natriuretic peptide (NT-proBNP; *squares*) from baseline to follow-up for transthyretin cardiac amyloidosis patients on doxycycline and ursodiol therapy for those whose left ventricular global longitudinal systolic strain improved (*blue*) and did not improve (*red*) during follow-up.

did not, there were no significant improvements in other echocardiographic or clinical parameters demonstrated.

Discussion

This observational cohort study reports findings from a single-center clinical experience of off-label treatment of ATTR CA with the use of doxycycline and ursodiol. We observed that 89% of patients tolerated the therapy, with a minority discontinuing because of dermatologic effects (namely, increased photosensitivity or rash) or gastrointestinal complaints. During a median follow-up of 22 months there were no significant changes observed in clinical, biochemical, or echocardiographic measures of disease progression. These findings suggest that doxycycline and ursodiol therapy is generally tolerable and may attenuate disease progression, a finding that is consistent with an earlier pilot study.[11] Interestingly, there was improvement in LV systolic mechanical function as measured by GLS among a subgroup of patients who were relatively younger and had NYHA functional class <III, lower baseline troponin-T and NT-proBNP levels and higher baseline LV GLS. Overall, a significant clinical improvement with doxycycline and ursodiol therapy was not demonstrated. However, our findings suggest that with a longer duration of treatment than previously examined, this therapeutic approach may improve subclinical measures of cardiovascular function among patients with less advanced disease. To our knowledge, this is the first report describing clinical experience with the use of combined doxycycline and ursodiol therapy for ATTR CA, and suggests that additional research toward this strategy is warranted.

The chemotherapeutic agent 4′-iodo-4′-doxy-doxorubicin was first found to be associated with inhibition of amyloid fibril formation and to promote resorption, leading to subsequent investigation of using tetracycline antibiotics for this purpose owing to their structural homologies to anthracyclines.[15] In a mouse model of familial amyloid polyneuropathy (FAP, caused by mutant ATTR), doxycyline was found to act as an amyloid fibril disrupter[16] and subsequently to disaggregate amyloid deposits with concurrent reduction of extracellular tissue biomarkers associated with their deposition, such as serum amyloid P component, matrix metalloproteinase 9, neutrophil gelatinase−associated lipocalin, and tissue inhibitor of metalloproteinase 1.[17,18] However, in those studies doxycycline did not reduce nonfibrillar TTR deposits or their associated biomarkers, such as binding immunoglobulin protein (BiP) and 3-nitrotyrosine, among others, which are thought to represent the early stages of amyloid tissue deposition.[10] In a transgenic Val30Met mouse model, TUDCA did reduce nonfibrillar TTR deposits along with associated apoptotic and oxidative biomarkers.[19] Doxycycline and TUDCA were then reported to synergistically lower amyloid fibril tissue deposits and normalize FAP tissue markers in a transgenic Val30Met mouse model by reducing deposition at different stages of the process.[10] Ursodiol has antiapopotiotic and antioxidant properties similar to TUDCA and has been studied in combination with doxycycline to prevent disease progression in ATTR amyloidosis patients.[12]

Initial findings from a small phase II open-label study examining 100 mg doxycycline twice daily and 250 mg TUDCA 3 times daily for ATTR patients (85% with cardiac involvement) reported that 10% discontinued therapy because of poor tolerability. Among 7 patients with cardiac involvement completing 12 months of therapy, NT-proBNP remained stable in 4 and the mean LV wall thickness remained stable in 5 and improved in 2.[11] More recently, a small phase II open-label study of ATTR CA patients receiving doxycycline and ursodiol reported that only 36% of patients completed 12 months of therapy, with 14% discontinuing therapy because of side-effects. The authors reported a high study dropout rate due to treatment failure and voluntary withdrawal, which significantly limited the interpretability of the findings.[12] Our findings demonstrate rates of

therapy intolerance similar to these 2 earlier reports. Incrementally, the present study reported changes in myocardial contractile function according to LV GLS and identified patient characteristics associated with its improvement. LV GLS may represent a useful measure of therapy response for future research and clinical monitoring.

Multiple disease-modifying therapies are now in various stages of development to treat patients with ATTR. TTR tetramer stabilizers diflunisal and tafamidis have recently been reported to be well tolerated and to reduce mortality and the need for cardiac transplantation in patients with ATTR CA.[5] The recently published ATTR-ACT phase III randomized clinical trial demonstrated a reduction in all-cause mortality and cardiovascular hospitalization, in addition to reduced decline in functional capacity and quality of life for patients with ATTR CA receiving tafamidis compared with placebo.[9] Two other phase III randomized clinical trials recently demonstrated improved FAP clinical manifestations with agents that reduce hepatic TTR synthesis—patisiran, a micro-RNA inhibitor, and inotersen, an antisense oligonucleotide inhibitor[6,7]—although the role of these agents for the treatment of ATTR CA remains uncertain at this time. With such developments, the relative and complementary benefit of doxcycycline and ursodiol or TUDCA therapy, particularly among patients with early-stage disease, is an important consideration. The potential synergistic benefit of treatment with multiple classes of anti-ATTR therapy (ie, a combination of TTR suppressors, stabilizers, and amyloid fibril disrupters) is uncertain and warrants further research as more disease-modifying agents for ATTR amyloidosis become available.

Study Limitations

Significant limitations of this study include the retrospective single-center observational design and small sample size, and the presence of bias can not be excluded. The absence of a control group for comparison with the treatment group significantly limits the ability to draw conclusions about the efficacy of this treatment protocol. Importantly, improved symptom control and survival with the use of this therapeutic strategy were not demonstrated. The absence of consensus criteria for defining a clinically significant change in LV GLS may limit its utility as an end point, and similarly there are no established criteria for defining treatment response with the use of other markers of disease severity in this population. Among many factors, the influence of other supportive care treatments used during the follow-up period, such as adjustments to diuretic therapy, reductions or discontinuation of conventional heart failure medical therapies that are often not well tolerated in ATTR CA, and management of atrial fibrillation, could not be controlled for. Results of an ongoing phase III randomized trial evaluating the efficacy of doxycycline and TUDCA plus standard supportive therapy versus standard supportive therapy alone in ATTR CA (NCT03481972) will significantly contribute to our understanding of the efficacy of this therapeutic approach. Our cohort also had a relatively small proportion of patients with mutant ATTC CA, and therefore the findings may be less applicable to that disease type.

Conclusion

In this observational cohort study of patients with ATTR CA treated with a combination of doxycycline and ursodiol, we found that treatment was tolerated by the majority of patients, with stabilization of disease severity parameters in medium-term follow-up. In a smaller subset of patients with less advanced disease, LV contractile function measured as GLS demonstrated improvement. Further research regarding the efficacy of this therapy for the reduction of major adverse cardiovascular outcomes in ATTR CA is warranted.

Disclosures

Dr Fine has received performed consulting for Pfizer, Akcea, and Alynylam and has received research support from Pfizer. The remaining authors report no relevant conflicts of interest.

Supplementary Data

Supplementary data related to this article can be found at doi:10.1016/j.cardfail.2019.01.006.

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