The Impact of Patients With Cardiac Amyloidosis in HFpEF Trials

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Avoiding the Inclusion of Patients with Cardiac Amyloidosis in Trials in HFpEF

Brief title: Cardiac Amyloidosis in HFpEF trials

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ABSTRACT

Heart failure with preserved ejection fraction (HFpEF) is an increasingly diagnosed condition whose failure to respond to new drugs effective in heart failure with reduced ejection fraction is of great concern. HFpEF is an incompletely understood and markedly heterogeneous syndrome, but cardiac amyloidosis is increasingly recognized as one of its various causes. The specific hemodynamic and pathophysiologic features of cardiac amyloidosis result in poor tolerance of heart failure medications and in worse outcomes compared to other causes. Until recently, patients considered for HFpEF trials were not routinely screened for cardiac amyloidosis. In this review, we discuss how real-world patients with cardiac amyloidosis met inclusion criteria for eight major HFpEF clinical trials, including the recent PARAGON trial. We discuss how the presence in the trial populations of a subset of patients with cardiac amyloidosis might contribute to explain the absence of efficacy of medications for HFpEF in trials so far. We suggest a multistep screening strategy in which patients with red flags for cardiac amyloidosis undergo both a light chain assay and technetium-labeled cardiac scintigraphy (PYP scan), which, when negative, rule out cardiac amyloidosis. Using this strategy would allow the testing of new medications for HFpEF in populations containing no patients with cardiac amyloidosis, thus potentially increasing the likelihood of showing therapeutic efficacy, and finally making some effective treatment available.

KEYWORDS: Cardiac amyloidosis. Heart failure with preserved ejection fraction. Patient selection. Randomized trials. Clinical trial as topic. Echocardiography.

Abbreviations:
AL: light chain amyloidosis
ATTR: transthyretin amyloidosis
CA: cardiac amyloidosis
ATTRv: hereditary transthyretin amyloidosis
HF: heart failure
HFrEF: heart failure with reduced ejection fraction
HFpEF: heart failure with preserved ejection fraction
LVEF: left ventricular ejection fraction
ATTRwt: wild-type transthyretin amyloidosis

HIGHLIGHTS

- The heterogeneity of populations in HFpEF trials partly explains their failure to obtain positive results and, more specifically, the presence of patients with unrecognized cardiac amyloidosis, who are unlikely to respond to trial drugs, may increase the risk of a negative trial result.
- A stepwise screening strategy for cardiac amyloidosis relying on a serum free light-chain assay and PYP scan in patients with red flags is feasible and should prove effective.
- Routinely excluding patients with cardiac amyloidosis would provide more uniform populations and may increase the chances of identifying drugs effective in HFpEF.

INTRODUCTION

Heart failure with preserved ejection fraction (HFpEF) is an increasingly diagnosed condition whose failure to respond to new drugs effective in heart failure with reduced ejection fraction (HFrEF) is of great concern(1–9). Thus, in the latest reported randomized trial (PARAGON), sacubitril-valsartan failed to decrease admissions for heart failure or death from cardiovascular causes in patients with HFpEF(1).

HFpEF is an incompletely understood and markedly heterogeneous condition that may be produced by a variety of causes, including cardiac amyloidosis (CA) (10,11). The specific hemodynamic and pathophysiologic features of CA result in poor tolerance of heart failure medications and in worse outcomes compared to other causes of heart failure(12, 13). Studies have shown CA in up to 29% of patients with HFpEF(14–16). This finding is unsurprising, as criteria used to diagnose HFpEF include the presence by echocardiography of left ventricular hypertrophy with left atrial enlargement and/or diastolic dysfunction, which are typical features of CA(11, 16). The authors of the PARAGON trial suggested that a subset of their study patients may have CA, which may be unresponsive to sacubitril-valsartan and other medications, resulting in failure of the trials to detect a significant mean effect(1).

In this review, we show that real-world patients with CA met inclusion criteria for HFpEF clinical trials (1–8) and we propose a screening strategy that would identify the vast majority of patients with CA, thus allowing their exclusion from clinical trials and referral to appropriate specific care.

FAILURE OF HFpEF CLINICAL TRIALS

Trials of medications for heart failure with preserved ejection fraction (HFpEF)

We selected eight, phase 3, multicenter, randomized controlled trials in patients with HFpEF in which efficacy of a treatment with proven efficacy in HFrEF was studied: CHARM-preserved (candesartan,(2)), PEP-CHF (perindopril(3)), DIG-PEF ancillary trial (digoxin(4)), I-PRESERVE (irbesartan(5)), SENIORS (nebivolol(6)), TOPCAT (spironolactone(8)), EDIFY (ivabradine(7)), and PARAGON (sacubitril-valsartan(1)), in chronological order of publication. In all eight trials, the trial drug failed to significantly improve the composite primary endpoint compared to the control arm. SENIORS was the only trial with a left ventricular ejection fraction (LVEF) cut-off for inclusion below 40% (>35%). The primary endpoint in seven of these trials was a composite of heart failure-related events, including death; EDIFY used three co-primary functional and structural cardiac endpoints.

Why might cardiac amyloidosis (CA) act as a confounder?

HFpEF is a highly heterogeneous syndrome that can be produced by many causes via a range of pathophysiological mechanisms. A clustering analysis identified three significantly different phenotypes(17). CA may be underdiagnosed in HFpEF considering their similar characteristics (increased septum thickness, left atrial enlargement, and diastolic dysfunction)(11–13).

Failure of trials in HFpEF to show therapeutic benefits from the tested drugs has been ascribed to characteristics of the myocardial structure, with concentric left ventricular remodeling and cardiomyocyte hypertrophy, instead of the eccentric left ventricular remodeling and loss of myofilaments seen in HFrEF(18). These structural differences may

translate into differences in responses to various drugs. In addition, the patient selection criteria used in some HFpEF trials included a high NYHA class, high NT-proBNP level, marked left ventricular wall thickening, and abnormal diastolic function, which are common features of CA.

In an effort to improve patient uniformity and to target trials to those patients most likely to benefit, the definition of HFpEF and patient selection criteria for HFpEF trials have changed over time (e.g., LVEF)(19). The populations have remained heterogeneous, however, and the changes may have failed to exclude patients with CA, since many key features of HFpEF are also found in CA.

Just as the inclusion criteria evolved, the number of exclusion criteria in HFpEF trials increased considerably from the CHARM-preserved trial (n=8) to the PARAGON study (n=33). This highlights the efforts made to obtain a uniform population and also reflects the increasing attention paid to CA and infiltrative cardiomyopathies in the field of HFpEF.

Impact of cardiac amyloidosis (CA) on treatment failures

Heart failure due to CA typically shows a poor response to treatment. Its prognosis remains poor, despite the recent introduction of drugs that stop further amyloid deposition, and of patirisan, which is based on a small interfering RNA and can decrease the amyloid burden(13, 20–22). In addition, drugs effective in HFrEF may have deleterious effects in HFpEF due to CA. Tachycardia is the only mechanism that can compensate for the stroke volume reduction induced by restrictive cardiomyopathies such as CA. In addition, bradycardia is common in CA. Therefore, medications with negative dromotropic, inotropic, and/or chronotropic effects, such as beta-blockers and ivabradine, dramatically decrease the cardiac output in patients with CA. Interestingly, a trial of the beta-blocker bucindolol found no effect in the overall population with HFrEF, contrasting with improvements in the non-

black subgroup(23). A pathogenic TTR gene variant (p.Val142Ile) was subsequently found to be highly prevalent in black patients in the trial, notably those older than 60 years(24). This finding raised the hypothesis that failure of the trial intervention was due to the enrolment of patients with hereditary transthyretin-related amyloidosis (ATTRv) CA, whose heart function would have been worsened by the beta-blocker. Furthermore, the autonomic neuropathy seen in amyloidosis often results in an inability to tolerate hypotensive treatments such as angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists(13). Finally, digoxin accumulates in vitro in amyloid deposits, raising concern about a risk of conductive disturbances (25). The inclusion in HFpEF trials of patients with undiagnosed CA would therefore be expected to decrease the likelihood of demonstrating efficacy of drugs effective in HFrEF.

Why is specific screening for cardiac amyloidosis (CA) needed?

CA and/or infiltrative processes, as well as hypertrophic cardiomyopathy, are listed as exclusion criteria in the DIG-PEF, I-PRESERVED, TOP-CAT, EDIFY and PARAGON trials. However, none of these trials used tests specifically designed to screen out patients with CA. The authors of the PARAGON trial themselves hypothesized that the inclusion of patients with CA might have contributed to the absence of efficacy of the test treatment sacubitril-valsartan. In this trial, the two factors associated with less efficacy -- male sex and higher LVEF -- are both associated with wild-type CA (1,13,14). Moreover, some support for this hypothesis can be found in the differences in treatment response across geographic areas, with better efficacy in patients in Western Europe and the United States, where CA was more likely to be identified because of the higher number of CA referral centers compared to other parts of the world (26). This supports the need for specific screening, since CA easily goes unrecognized.

CARDIAC AMYLOIDOSIS PATIENTS AND HFpEF CLINICAL TRIALS

We retrospectively studied a cohort of patients with HFpEF related to CA (n=317) and determined how many of them met the main patient selection criteria used in the eight HFpEF trials (1–8).

Cardiac amyloidosis (CA) patients

We selected CA patients with symptomatic or treated heart failure meeting 2016 European Society of Cardiology (ESC) criteria (11): signs and symptoms of heart failure, NTpro-BNP ≥125 ng/L, and LVEF above a cut-off that varied across trials. The 317 patients had the following amyloidosis types: light-chain amyloidosis (AL, n=98 [31%]), hereditary transthyretin-related amyloidosis (ATTRv, n=87 [27%]), and wild-type transthyretin-related amyloidosis (ATTRwt, n=132 [42%]) The diagnoses were made between September 2008 and June 2017, at a single referral center (Henri Mondor Hospital, Creteil, France). AL diagnosis was based on the presence of monoclonal gammopathy (based on serum ± urine immunoglobulin, immunofixation, and serum immunoglobulin free light chains) combined with myocardial hypertrophy (interventricular septum thickness ≥12 mm) and confirmed by Congo red-positive deposits and immunohistochemistry and/or mass spectrometry findings on endomyocardial or extracardiac biopsy. ATTR diagnosis was based either on 99Tc-bisphosphonate cardiac uptake in the absence of monoclonal gammopathy or on endomyocardial or extracardiac histology, as previously described(27, 28). For some patients enrolled early in the cohort, ATTRv was diagnosed on typical echocardiographic features combined with extracardiac histology and a mutant TTR.

For each patient, we abstracted the following data at diagnosis from the medical files: medical history; body weight, height, and surface area; blood pressure and heart rate; clinical

symptoms of heart failure; electrocardiographic findings (rhythm, low-voltage pattern); and laboratory test results (sodium, potassium, creatinine, troponin, and NT-proBNP). Transthoracic echocardiography (TTE) was performed as recommended (27, 29), using a Vivid 7 system (GE Vingmed, Horten, Norway). LV peak systolic global longitudinal strain (GLS) was computed offline from the standard three LV apical views using 2D speckle tracking analysis through automated function imaging (AFI, EchoPAC version 203, GE healthcare).

All patients gave informed consent to the use of their anonymized data. The study complied with the 1975 Declaration of Helsinki and was approved by our local ethics committee (Créteil) and by the French data protection authority (*Comité National de l'Informatique et des Libertés*, CNIL, #1431858) and regional clinical research authority (DIRC Ile de France, #DC 2009-930). The statistical analysis was performed using STATA software version v14.0 (Lakeway Drive, TX).

Real-world patients with cardiac amyloidosis (CA) met inclusion criteria for HFpEF clinical trials

Figure 1 shows the number of CA patients who met the LVEF criterion for inclusion into each trial. Table 1 reports the numbers (%) of CA patients who met each, and all, of the patient selection criteria used in the eight clinical trials. The proportion of patients potentially eligible for inclusion ranged across trials from 16% to 65%, which is consistent with the increasing recognition of CA as a cause of HFpEF. Among the patients meeting the criteria, the numbers with each type of amyloidosis are indicated. The use in two trials (SENIORS and PEP-CHF) of age older than 70 years for study eligibility was associated with a substantial decrease in the number of CA patients meeting the trial selection criteria, due to a decrease in patients with AL.

Table 2 shows the baseline characteristics of patients included in each trial and those of patients from our cohort who met the selection criteria for the trial. In CA patients meeting trial inclusion criteria and in the trial populations, age ranged from 74 to 81 vs. 67 to 76 years, respectively; the proportion of men from 63% to 74% vs. 38% to 61%; NT-proBNP from 3513 to 4486 vs 335 to 887 ng/L (when reported), IVST from 17 to 19 vs. 11 to 13 mm (when reported), and LAVi from 41 to 54 vs. 12 to 41 ml/m^2 (when reported).

Mortality of real-world patients with cardiac amyloidosis (CA)

All patients received regular follow-up starting at the first visit to our referral center. All-cause mortality was recorded for our cohort and was available for two trials, DIG-PEF and TOPCAT. We plotted Kaplan-Meier curves to superimpose all-cause mortality between the populations of each of these two trials and the patients in the cohort who met the patient selection criteria for the relevant trial (Online Figure 1). Survival was censored at last contact with the patient, and patients with no follow-up data were excluded from this analysis. All-cause mortality was 23% in the DIG-PEF trial after a mean follow-up of 37 months and 15% in the TOP-CAT trial after 40 months vs. 40% and 51% at the same follow-up times in the CA cohort.

Proposed screening strategy to exclude patients with cardiac amyloidosis (CA) from future HFpEF trials

Our results demonstrate that the patient selection criteria used in clinical trials in patients with HFpEF fail to exclude patients with CA. Consequently, there is an argument for routinely screening patients considered for inclusion in clinical trials of heart failure medications and excluding those with the disease. Several red flags for CA are well-known and can be used for a stepwise amyloidosis screening strategy (13, 30–32) based on clinical,

laboratory, and imaging findings. Cardiac biomarker assays (especially natriuretic peptides) are part of the HFpEF diagnostic workup and are elevated in CA, especially the AL type(33, 34). Standard TTE, which is always performed before trial inclusion, becomes a key first-line screening tool if it includes a GLS analysis: a reduction in GLS with apical sparing should lead to CA investigations(35). The presence of any of these red flags requires further tests to rule out CA before the patient can be enrolled in an HFpEF trial. Given that the reference standard investigation, i.e., endomyocardial biopsy, would put trial candidates at unacceptable risk, we propose a non-invasive strategy based on combining a light-chain assay with PYP scanning(28).

To determine whether a serum light-chain assay and PYP scan done as part of the pre-inclusion work-up might help to exclude patients with CA from trials in HFpEF, we plotted receiver operating characteristics (ROC) curves for these tests combined in the cohort of patients with CA and in 174 patients sent to our CA referral center for suspected CA and found to be free of the disease. The diagnostic work-up at our center included TTE, light-chain assay, PYP scan (n=174), cardiac MRI (n=135), salivary gland histology (n=114), and/or myocardial biopsy (n=21). The most common diagnoses were sarcomeric cardiomyopathy (n=70), hypertensive cardiomyopathy (n=30), and dilated cardiomyopathy (n=8). The remaining patients had myocarditis, dialysis-related heart failure, mitochondrial cytopathy, sarcoidosis, non-specific HFpEF, or other conditions. Follow-up was 26 (12;39) months.

The light-chain assay / PYP scan combination sensitivity was 100% to detect patients with CA in our cohort. A normal serum free-light-chain assay combined with absence of cardiac uptake during PYP scan rules out the three major types of CA. Combining PYP and light-chain assay had an area under the ROC curve of 0.94 [95% confidence interval, 0.92-0.96]) for diagnosing CA (Figure 2). These data are consistent with previous reports that PYP

scintigraphy was nearly 100% sensitive for diagnosing TTR amyloidosis and, similarly, given that the pathophysiology of AL is based on the presence of monoclonal gammopathy, the light chain assay is 100% sensitive for AL amyloidosis (20, 36, 37). The performance of the combination of PYP and light chain assay in ruling out amyloidosis (both ATTR and AL) has never been evaluated in HFpEF. The Central Figure depicts a stepwise screening strategy based on red flags and the PYP scan/light-chain assay combination to successfully exclude all patients with CA from future HFpEF trials. We acknowledge that, as recruitment occurred in a tertiary center for CA, the prevalence of the disease in our population was probably higher than in the general population with HFpEF or in clinical trial candidates. However, the prevalence of CA in the general population of patients with HFpEF remains underestimated(13), and the impact of disease prevalence on sensitivity and specificity remains debated(38).

Screening of potential trial participants for CA would have the additional benefit of directing patients with the disease to specialized follow-up and possible treatment with recently introduced medications(21, 39, 40). Pitfalls in interpreting cardiac PYP scans were reviewed very recently(41).

Limitations

The main limitation of our study is the descriptive design. We did not have the raw data from the trials that would have allowed a detailed comparison of patient characteristics between our cohort and the trial populations. Neither did we know the proportion of patients in the HFpEF trials who were excluded based on suspected amyloidosis or the proportion who would have met CA criteria after specific screening. We were not able to perform propensity matching on disease severity and/or prognostic clinical features in our comparison of mortality between our cohort and several HFpEF trials. Furthermore, the retrospective data

collection prevented us from providing detailed prevalence and test characteristic data about each red flag in our population. Our findings can only provide support for a hypothesis, which now deserves more ample investigation. Thus, it would be of major interest to investigate the trial participants for CA. Finally, considering that TTR cardiac amyloidosis might be much more prevalent than previously thought, especially wild-type TTR, its reliability to symptoms of HFpEF could be questioned, and assessed by further studies of HFpEF etiologies.

CONCLUSIONS

Real-world patients with CA sometimes meet inclusion criteria for HFpEF clinical trials. The absence of efficacy of the interventions tested in these trials may be ascribable, at least in part, to the greater refractoriness to treatment and higher mortality that characterize CA compared to other causes of HFpEF. We advocate routine stepwise screening for CA, including a serum free-light-chain assay and PYP scan in patients with red flags, as part of the pre-inclusion work-up in trials of HFpEF medications.

REFERENCES

1. Solomon SD, McMurray JJV, Anand IS, et al. Angiotensin-Neprilysin Inhibition in Heart Failure with Preserved Ejection Fraction. N. Engl. J. Med. 2019;381:1609–1620.

2. Yusuf S, Pfeffer MA, Swedberg K, et al. Effects of candesartan in patients with chronic heart failure and preserved left-ventricular ejection fraction: the CHARM-Preserved Trial. The Lancet 2003;362:777–781.

3. Cleland JGF, Tendera M, Adamus J, Freemantle N, Polonski L, Taylor J. The perindopril in elderly people with chronic heart failure (PEP-CHF) study. Eur. Heart J. 2006;27:2338–2345.

4. Ahmed A, Rich MW, Fleg JL, et al. Effects of Digoxin on Morbidity and Mortality in Diastolic Heart Failure. Circulation 2006;114:397–403.

5. Massie BM, Carson PE, McMurray JJ, et al. Irbesartan in patients with heart failure and preserved ejection fraction. N. Engl. J. Med. 2008;359:2456–2467.

6. van Veldhuisen DJ, Cohen-Solal A, Böhm M, et al. Beta-blockade with nebivolol in elderly heart failure patients with impaired and preserved left ventricular ejection fraction: Data From SENIORS (Study of Effects of Nebivolol Intervention on Outcomes and Rehospitalization in Seniors With Heart Failure). J. Am. Coll. Cardiol. 2009;53:2150–2158.

7. Komajda M, Isnard R, Cohen-Solal A, et al. Effect of ivabradine in patients with heart failure with preserved ejection fraction: the EDIFY randomized placebo-controlled trial. Eur. J. Heart Fail. 2017:n/a-n/a.

8. Pitt B, Pfeffer MA, Assmann SF, et al. Spironolactone for Heart Failure with Preserved Ejection Fraction. N. Engl. J. Med. 2014;370:1383–1392.

9. Shah KS, Xu H, Matsouaka RA, et al. Heart Failure With Preserved, Borderline, and Reduced Ejection Fraction: 5-Year Outcomes. J. Am. Coll. Cardiol. 2017;70:2476–2486.

10. Lam CSP, Voors AA, de Boer RA, Solomon SD, van Veldhuisen DJ. Heart failure with

preserved ejection fraction: from mechanisms to therapies. Eur. Heart J. 2018;39:2780–2792.

11. Ponikowski P, Voors AA, Anker SD, et al. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure. Eur. Heart J. 2016;37:2129–2200.

12. Gertz MA. Immunoglobulin light chain amyloidosis diagnosis and treatment algorithm 2018. Blood Cancer J. 2018;8:44.

13. Ruberg FL, Grogan M, Hanna M, Kelly JW, Maurer MS. Transthyretin Amyloid Cardiomyopathy: JACC State-of-the-Art Review. J. Am. Coll. Cardiol. 2019;73:2872–2891.

14. González-López E, Gallego-Delgado M, Guzzo-Merello G, et al. Wild-type transthyretin amyloidosis as a cause of heart failure with preserved ejection fraction. Eur. Heart J. 2015;36:2585–2594.

15. Bennani Smires Y, Victor G, Ribes D, et al. Pilot study for left ventricular imaging phenotype of patients over 65 years old with heart failure and preserved ejection fraction: the high prevalence of amyloid cardiomyopathy. Int. J. Cardiovasc. Imaging 2016;32:1403–1413.

16. Mohammed SF, Mirzoyev SA, Edwards WD, et al. Left Ventricular Amyloid Deposition in Patients with Heart Failure and Preserved Ejection Fraction. JACC Heart Fail. 2014;2:113–122.

17. Shah SJ, Katz DH, Selvaraj S, et al. Phenomapping for Novel Classification of Heart Failure With Preserved Ejection FractionCLINICAL PERSPECTIVE. Circulation 2015;131:269–279.

18. Paulus WJ, van Ballegoij JJM. Treatment of Heart Failure With Normal Ejection Fraction: An Inconvenient Truth! J. Am. Coll. Cardiol. 2010;55:526–537.

19. Kelly JP, Mentz RJ, Mebazaa A, et al. Patient Selection in Heart Failure With Preserved Ejection Fraction Clinical Trials. J. Am. Coll. Cardiol. 2015;65:1668–1682.

20. Falk RH, Alexander KM, Liao R, Dorbala S. AL (Light-Chain) Cardiac Amyloidosis: A

Review of Diagnosis and Therapy. J. Am. Coll. Cardiol. 2016;68:1323–1341.

21. Solomon SD, Adams D, Kristen A, et al. Effects of Patisiran, an RNA Interference Therapeutic, on Cardiac Parameters in Patients With Hereditary Transthyretin-Mediated Amyloidosis. Circulation 2019;139:431–443.

22. Minamisawa M, Claggett B, Adams D, et al. Association of Patisiran, an RNA Interference Therapeutic, With Regional Left Ventricular Myocardial Strain in Hereditary Transthyretin Amyloidosis: The APOLLO Study. JAMA Cardiol. 2019;4:466–472.

23. Beta-Blocker Evaluation of Survival Trial Investigators, Eichhorn EJ, Domanski MJ, Krause-Steinrauf H, Bristow MR, Lavori PW. A trial of the beta-blocker bucindolol in patients with advanced chronic heart failure. N. Engl. J. Med. 2001;344:1659–1667.

24. Buxbaum J, Jacobson DR, Tagoe C, et al. Transthyretin V122I in African Americans with congestive heart failure. J. Am. Coll. Cardiol. 2006;47:1724–1725.

25. Rubinow A, Skinner M, Cohen AS. Digoxin sensitivity in amyloid cardiomyopathy. Circulation 1981;63:1285–1288.

26. Alexander KM, Orav J, Singh A, et al. Geographic Disparities in Reported US Amyloidosis Mortality From 1979 to 2015: Potential Underdetection of Cardiac Amyloidosis. JAMA Cardiol. 2018;3:865–870.

27. Bodez D, Ternacle J, Guellich A, et al. Prognostic value of right ventricular systolic function in cardiac amyloidosis. Amyloid 2016;23:158–167.

28. Gillmore JD, Maurer MS, Falk RH, et al. Nonbiopsy Diagnosis of Cardiac Transthyretin AmyloidosisCLINICAL PERSPECTIVE. Circulation 2016;133:2404–2412.

29. Nagueh SF, Smiseth OA, Appleton CP, et al. Recommendations for the Evaluation of Left Ventricular Diastolic Function by Echocardiography: An Update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J. Am. Soc. Echocardiogr. Off. Publ. Am. Soc. Echocardiogr. 2016;29:277–314.

30. Maurizi N, Rella V, Fumagalli C, et al. Prevalence of cardiac amyloidosis among adult patients referred to tertiary centres with an initial diagnosis of hypertrophic cardiomyopathy. Int. J. Cardiol. 2020;300:191–195.

31. Vergaro G, Aimo A, Barison A, et al. Keys to early diagnosis of cardiac amyloidosis: red flags from clinical, laboratory and imaging findings. Eur. J. Prev. Cardiol. 2019:2047487319877708.

32. Perfetto F, Bergesio F, Emdin M, Cappelli F. Troponins in cardiac amyloidosis: multipurpose markers. Nat. Rev. Cardiol. 2014;11:179–179.

33. Perfetto F, Bergesio F, Grifoni E, et al. Different NT-proBNP circulating levels for different types of cardiac amyloidosis. J. Cardiovasc. Med. Hagerstown Md 2016;17:810–817.

34. Schelbert EB, Miller CA. Cardiac Amyloidosis as a Potential Confounder in Heart Failure With Preserved Ejection Fraction Trials. JACC Heart Fail. 2017;5:617.

35. Jurcuţ R, Onciul S, Adam R, et al. Multimodality imaging in cardiac amyloidosis: a primer for cardiologists. Eur. Heart J. Cardiovasc. Imaging 2020;21:833–844.

36. Cappelli F, Gallini C, Di Mario C, et al. Accuracy of 99mTc-Hydroxymethylene diphosphonate scintigraphy for diagnosis of transthyretin cardiac amyloidosis. J. Nucl. Cardiol. Off. Publ. Am. Soc. Nucl. Cardiol. 2019;26:497–504.

37. Treglia G, Glaudemans AWJM, Bertagna F, et al. Diagnostic accuracy of bone scintigraphy in the assessment of cardiac transthyretin-related amyloidosis: a bivariate meta-analysis. Eur. J. Nucl. Med. Mol. Imaging 2018;45:1945–1955.

38. Leeflang MMG, Rutjes AWS, Reitsma JB, Hooft L, Bossuyt PMM. Variation of a test's sensitivity and specificity with disease prevalence. CMAJ Can. Med. Assoc. J. J. Assoc. Medicale Can. 2013;185:E537-544.

39. Maurer MS, Schwartz JH, Gundapaneni B, et al. Tafamidis Treatment for Patients with

Transthyretin Amyloid Cardiomyopathy. N. Engl. J. Med. 2018.

40. Liu PP, Smyth D. Wild-Type Transthyretin Amyloid Cardiomyopathy: A Missed Cause of Heart Failure With Preserved Ejection Fraction With Evolving Treatment Implications. Circulation 2016;133:245–247.

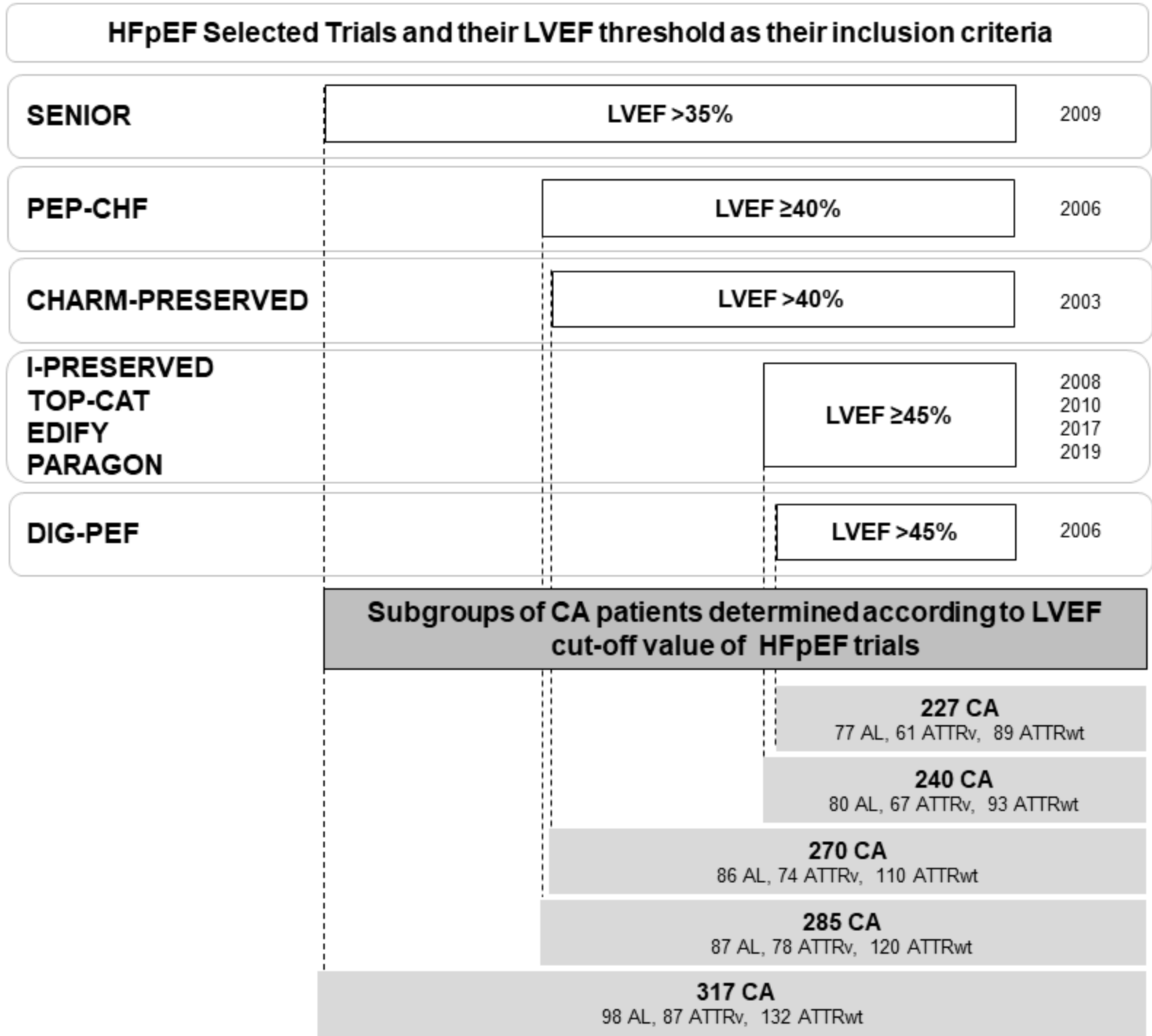
41. Hanna M, Ruberg FL, Maurer MS, et al. Cardiac Scintigraphy With Technetium-99m-Labeled Bone-Seeking Tracers for Suspected Amyloidosis: JACC Review Topic of the Week. J. Am. Coll. Cardiol. 2020;75:2851–2862.

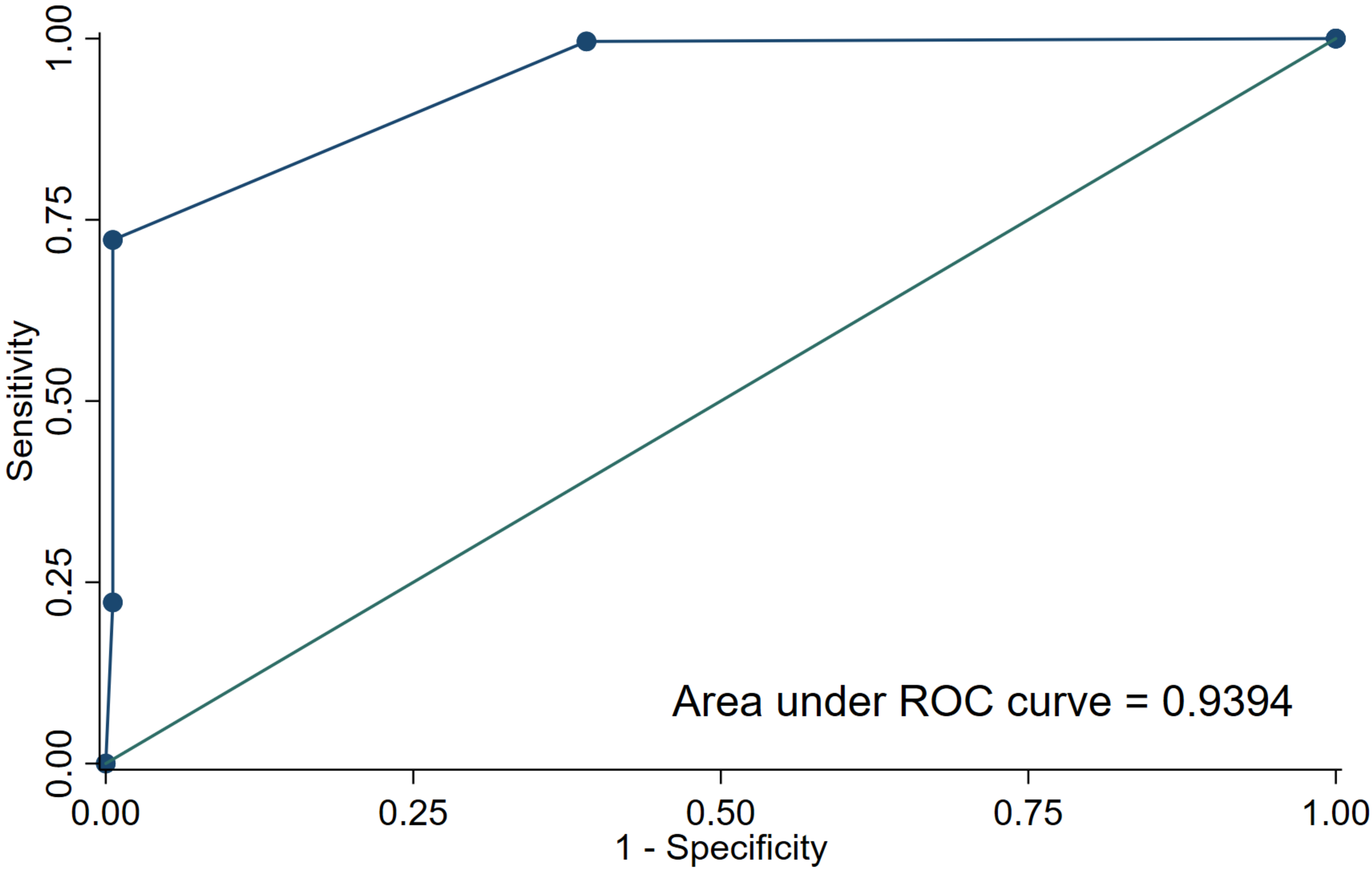
FIGURE LEGENDS

Figure 1: Selected trials in heart failure with preserved ejection fraction, with the left ventricular ejection fraction cut-offs used to select participants.
Of the 317 patients in our cohort with cardiac amyloidosis, an ejection fraction above 35%, symptomatic heart failure, and an NT-proBNP level above 125 pg/mL, 285 had LVEF≥40%, 270>40%, 240≥45%, and 227>45% and could therefore have been enrolled in the corresponding trials.
AL: light chain cardiac amyloidosis; ATTRv: hereditary transthyretin amyloidosis; ATTRwt: wild-type transthyretin amyloidosis; CA: cardiac amyloidosis; HFpEF: heart failure with preserved ejection fraction; LVEF: left ventricular ejection fraction.

Figure 2: Receiver operating characteristic curve for the performance of a negative serum free-light-chain assay and absence of cardiac uptake during technetium-labeled cardiac scintigraphy for ruling out cardiac amyloidosis

Central illustration: Multistep screening strategy to avoid the Inclusion of Patients with Cardiac Amyloidosis in Trials in HFpEF
HFpEF: heart failure with preserved ejection fraction; TTE: transthoracic echocardiography; PYP: technetium-labeled cardiac scintigraphy


HFpEF Selected Trials and their LVEF threshold as their inclusion criteria
SENIOR
LVEF >35%
2009
PEP-CHF
LVEF ≥40%
2006
CHARM-PRESERVED
LVEF >40%
2003
I-PRESERVED
TOP-CAT
EDIFY
PARAGON
LVEF ≥45%
2008
2010
2017
2019
DIG-PEF
LVEF >45%
2006
Subgroups of CA patients determined according to LVEF cut-off value of HFpEF trials
227 CA
77 AL, 61 ATTRv, 89 ATTRwt
240 CA
80 AL, 67 ATTRv, 93 ATTRwt
270 CA
86 AL, 74 ATTRv, 110 ATTRwt
285 CA
87 AL, 78 ATTRv, 120 ATTRwt
317 CA
98 AL, 87 ATTRv, 132 ATTRwt


Sensitivity
0.00
0.25
0.50
0.75
1.00
1 - Specificity
Area under ROC curve = 0.9394

	Number (%) of patients in the cohort with documented cardiac amyloidosis (n=317) who met each of the patient selection criteria in the eight trials							
LVEF, %	**LVEF ≥45% 240**	**LVEF >35% 317**	**LVEF ≥40% 285**	**LVEF >40% 270**	**LVEF ≥45% 240**			**LVEF >45% 227**
Trial name	**PARAGON**	**SENIORS**	**PEP CHF**	**CHARM preserved**	**I-PRESERVE**	**TOP CAT**	**EDIFY**	**DIG PEF**
Drug	**(Sacubirtril-Valsartan)**	**(Nebivolol)**	**(Perindopril)**	**(Candesartan)**	**(Irbesartan)**	**(Spironolactone)**	**(Ivabradine)**	**(Digoxin)**
Other TTE criteria	Structural heart disease	-	≥2/4 (WMI, LVH, LA enlargement, diastolic function)	-	NYHA III-IV + LVH or LA enlargement	-	Diastolic function	-
	240 (100%)	-	251 (88%)[‡§]	-	89 (37%)[‡§]	-	237 (99%)	-
Age, years	≥50	≥70	≥70	> 18	≥ 60	≥50	≥50	-
	232 (97%)	216 (68%)	190 (67%)	270 (100%)	209 (87%)	232 (97%)	232 (97%)	-
NYHA class	II-IV	-	≥3/9 HF signs or symptoms	II-IV	II-IV	HF criteria (study design)	II-III	-
	197 (82%)		222 (78%)[*†]	225 (83%)[*]	197 (82%)[*]	162 (68%)[†]	176 (73%)*	-
Prior hospitalisation for	AHF ≤1 year	AHF <1 year	Cardiac reason <3 months	Cardiac reason	AHF <6 months	AHF <1 year or NT-proBNP ≥360 pg/mL	-	-
	95 (39%)	138 (44%)[‖]	94 (33%)[‖]	204 (76%)[‖]	84 (35%)[‖]	227 (95%)[‖]	-	-
Sinus rhythm	-	-	-	-	-	-	HR ≥70/min in SR	Yes
	-	-	-	-	-	-	125 (52%)	193 (85%)
NT-proBNP	≥300 ng/L in SR Or ≥900 ng/L in AF	-	-	-	-	-	≥220 ng/L	-
	228 (95%)	-	-	-	-	-	236 (98%)	-
Other criteria	-	-	Diuretic treatment	-	-	K <5 mmol/L	-	History of HF
	-	-	184 (65%)	-	-	233 (97%)	-	209 (92%)
All patient selection criteria met	**119 (50%)**	**97 (31%)**	**47 (16%)**	**161 (60%)**	**91 (38%)**	**123 (51%)**	**78 (33%)**	**148 (65%)**
AL/hATTR/wtATTR, n	**39/27/53**	**19/24/54**	**10/7/30**	**50/45/66**	**32/24/35**	**44/36/43**	**39/20/19**	**59/40/49**

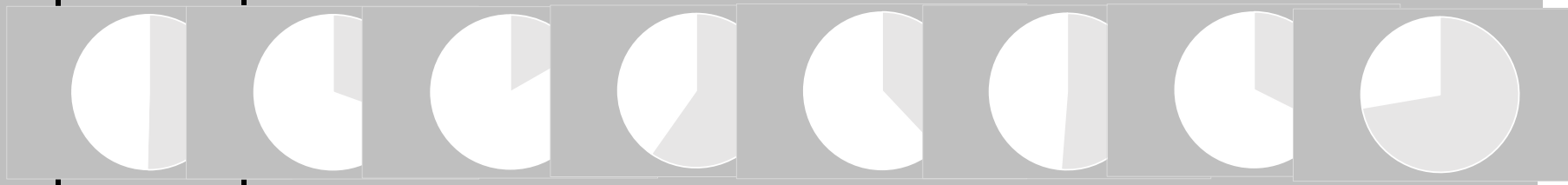


Table 1: Number (%) of patients in the cohort with documented cardiac amyloidosis (n=317) who met each of the patient selection criteria in the eight trials

Values are mean±SD, median (25th-75th percentiles), or n (%).

Colour code: green: less than 50% of CA patients fulfilling the criterion; orange: 50 to 70%; red: more than 70%

AF: atrial fibrillation; AHF: acute heart failure; CA: cardiac amyloidosis; HF: heart failure; HR: heart rate; K: serum potassium; LA: left atrial; LBB: left bundle branch; LVEF: left ventricular ejection fraction; LVH: left ventricular hypertrophy; NYHA: New York Heart Association; SR: sinus rhythm; TTE: transthoracic echocardiography; WMI: wall motion index

*3 missing values for NYHA class; †9 missing values for clinical variables; ‡1 missing value for left atrial volume index (TTE criterion); §in the absence of left atrial anteroposterior linear measurement or left atrial surface, left atrial volume index, according to recommendations, was used (cut-off, 34 mL/m^2); ‖ 5 missing values for previous admission for acute heart failure and 38 missing values for time to previous admission for acute heart failure

HFpEF Trial **Number of patients**	**PARAGON** Sacubitril/Valsartan group N=4822	**CA COHORT** with PARAGON inclusion criteria N=119	**SENIORS** Nebivolol group N=380	**CA COHORT** With SENIORS inclusion criteria N=97	**PEP-CHF** Perindopril group N=424	**CA COHORT** With PEP-CHF inclusion criteria N=47	**CHARM** Candesartan +Placebo N=1514	**CA COHORT** With CHARM inclusion criteria N=161
Age, years	73±8	74±11	76±5	80±6	75 (72;79)	81 (78;85)	67±11	75±10
Men, n(%)	2314 (48)	82 (69)	187 (49)	67 (69)	195 (46)	35 (74)	920 (61)	110 (68)
NYHA class I, n(%)	Excluded	Excluded	15 (4)	8 (8)	327 (77)	0 (0)	Excluded	Excluded
II, n(%)	3471 (72)	56 (47)	236 (62)	35 (36)	931 (62)	20 (23)	931 (62)	73 (45)
III, n(%)	1301 (27)	48 (40)	123 (32)	42 (43)	97 (23)	22 (47)	556 (37)	68 (42)
IV, n(%)	28 (0.6)	15 (13)	6 (1.6)	11 (11)	27 (1.8)	5 (11)	27 (1.8)	20 (12)
LVEF, %	58±8	53±10	49±10	51±10	65 (56;66)	53 (45;60)	54±9	54±9
HR, /min	70±12	77±14	78±13	77±13	74 (66;81)	78 (68;82)	71±12	74±13
SBP, mmHg	136±15	126±21	145±20	125±19	138 (128;150)	126 (113 ;142)	136±19	125±22
DBP, mmHg	77±11	73±11	83±10.5	72±10	80 (74;86)	70 (64;78)	78±11	72±12
Admission for AHF, n(%)	2314 (48)	73 (61)	NA	97 (100)	NA	45 (96)	1038 (69)	117 (73)
AF, n(%)	1543 (32)	27 (23)	133 (35)	26 (27)	79 (19)	12 (26)	439 (29)	32 (20)
Loop diuretics, n(%)	1629 (96)	118 (99)	318 (84)	85 (88)	198 (47)	46 (98)	1138 (75)	128 (80)
ACE or ARB, n(%)	1931 (40)	35 (29)	352 (93)	45 (46)	Excluded	Excluded	296 (20)	70 (43)
Creatinine, µmol/l	NA	105 (86;134)	95.3±36	120±35	95 (81;110)	111 (97;137)	NA	109 (89;133)
GFR by MDRD, ml/min/1.73m^2	63±19	57±20	68.7±22	57±31	NA	55 (43;69)	NA	56 (45;68)
NT-proBNP, ng/l	885 (863; 908)	3513 (1616;6842)	NA	4486 (2325;8431)	335 (160;1014)†	4378 (2267;10684)	NA	3730 (2022;6888)
IVST, mm	NA	17 (15;20)	NA	19 (16;21)	13 (12;15)	18 (16;21)	NA	18±3
LVM, g	NA	306 (240;391)	NA	346 (286;414)	NA	310 (280;402)	NA	338±112
LVMi, g/m^2	NA	168 (133;212)	NA	189 (155;225)	NA	172 (145;213)	111±35†	182±53
LAVi, mL/m^2	NA	47 (38;60)	NA	54 (44;63)	NA	51 (43;60)	36±11†	53±18
E/A	NA	2 (1;3)	0.9±0.6	3±1.5	0.70 (0.60–0.90)	2 (1.2;2.8)	1.15±0.84†	2.0±1.3
E/e' lateral	NA	17 (12;22)	NA	18±10	NA	18 (12;25)	NA	18±9.5

Table 2.

HFpEF	**I PRESERVED** Irbesartan +Placebo	**CA COHORT** I-PRESERVE inclusion criteria	**TOPCAT** Spironolactone group	**CA COHORT** With TOPCAT inclusion criteria	**EDIFY** Ivabradine group	**CA COHORT** With EDIFY inclusion criteria	**DIG-PEF** Digoxin group	**CA COHORT** With DIG PEF inclusion criteria
Number of patients	N=2067	N=91	N=1722	N=123	N=95	N=78	N=492	N=148
Age, years	72±7	77±8	69 (61;76)	76 (68;81)	72 (66;78)	72 (65;79)	67±11	72±11
Men, n(%)	840 (41)	62 (68)	834 (48)	84 (68)	36 (38)	49 (63)	289 (58)	105 (71)
NYHA class I, n(%)	Excluded	Excluded	56 (3.3)	2 (2)	Excluded	Excluded	94 (19)	17 (11)
II, n(%)	426 (21)	27 (30)	1090 (63)	61 (50)	76 (80)	48 (62)	292 (59)	68 (46)
III, n(%)	1582 (77)	49 (54)	568 (33)	46 (37)	19 (20)	30 (38)	102 (21)	50 (38)
IV, n(%)	59 (3)	15 (16)	7 (0.4)	14 (11)	Excluded	Excluded	4 (0.8)	12 (8)
LVEF, %	59±9	56±8	56 (51;61)	56 (49;62)	60 (54;66)	57 (49;63)	55±8	58±8
HR, /min	72±11	77±13	68 (62;76)	75 (68;81)	75 (72;78)	79 (75;85)	NA	76 (68;81)
SBP, mmHg	137±15	127±19	130 (120;139)	121 (108;131)	132 (123;142)	123 (112;133)	NA	123 (112;141)
DBP, mmHg	79±9	74±11	80 (70;80)	70 (64;77)	76 (69;84)	74 (69;80)	NA	71 (66;78)
Admission for AHF, n(%)	< 6 months 906 (44)	< 6 months 55 (60)	< 1 year 1232 (71.5)	< 1 year 64 (52)	NA	33 (47)	NA	80 (55)
AF, n(%)	606 (29)	20 (22)	611 (36)	17 (14)	Excluded	Excluded	Excluded	Excluded
Loop diuretics, n(%)	1078 (52)	67 (74)	1401 (81)	88 (72)	56 (59)	50 (64)	369 (75)	95 (64)
ACE or ARB, n(%)	538 (26)	30 (33)	1452 (84)	51 (41)	84 (88)	25 (34)	425 (86)	62 (42)
Creatinine, µmol/l	88.4±31	109±32	88 (79.2;105.6)	103 (87;125)	NA	102 (86;137)	110±34	109±40
GFR by MDRD, ml/min/1.73m^2	73±23	61±21	65.3 (53.9;79.2)	60 (49;72)	NA	60 (44;76)	62±21	65±26
NT-proBNP, ng/l	360 (139;987)	3549 (2130;6483)	887 (537;1634)	3493 (2000;63)	385 (263;738)	3340 (1546;6092)	NA	3244 (1593;5464)
IVST, mm	NA	18 (16;20)	12 (11;13)†	17 (15;20)	11 (9;15)‡	17 (15;20)‡	NA	17 (15;20)
LVM, g	164±48†	330±108	NA	306 (250;382)	NA	300 (219;387)	NA	301 (243;379)
LVMi, g/m^2	NA	181±58	108 (90;128)†	169 (141;207)	100 (80;132)	167 (127;203)	NA	167 (133;203)
LAVi, mL/m^2	12±3†	52±19	28 (21;35)†	49 (38;60)	41 (34;49)	41 (33;54)	NA	45 (35;59)
E/A	1.05±0.74†	2±1.3	1.03 (0.77;1.49)†	2 (1;2.3.0)	NA	1.8 (1.0;2.7)	NA	2.0 (1.0;2.9)
E/e' lateral	10.0±4.5†	19±8.8	10.5 (7.9;14.3)†	16 (12;22.6)	12.6 (9.7;16.2)	16 (11.2;21.3)	NA	16 (12;21)

Table 2 (continued)

Table 2: Baseline characteristics of the patients included in each HFpEF trial and of the patients in our cardiac amyloidosis cohort who met the selection criteria for the relevant trial

Values are mean±SD, median (25^{th}-75^{th} percentiles), or n (%).

ACE: angiotensin-converting enzyme inhibitor; AF: atrial fibrillation; ARB: angiotensin receptor blocker; CA: cardiac amyloidosis; E/A: mitral early / late diastolic peak-flow velocity; E/e': mitral early diastolic peak-flow velocity/early diastolic velocity by TDI ratio; GFR: glomerular filtration rate; GLS: global longitudinal strain; HF: heart failure; HR: heart rate; IVST: interventricular septum thickness; LAVi: left atrial volume index; LVEF: left ventricular ejection fraction; LVMi: left ventricular mass index; MDRD: Modification of Diet in Renal Disease; NYHA: New York Heart Association; SBP: systolic blood pressure.

† based on sub-group analyses (n=166 for CHARM, n=191 for PEP CHF, n=745 for I Preserved, and n=935 for TOPCAT)

‡ posterior wall thickness used in EDIFY instead of IVST