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RESEARCH ARTICLE

Risk of bias in studies investigating novel diagnostic biomarkers for heart failure with preserved ejection fraction. A systematic review

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Aim

Diagnosing heart failure with preserved ejection fraction (HFpEF) in the non-acute setting remains challenging. Natriuretic peptides have limited value for this purpose, and a multitude of studies investigating novel diagnostic circulating biomarkers have not resulted in their implementation. This review aims to provide an overview of studies investigating novel circulating biomarkers for the diagnosis of HFpEF and determine their risk of bias (ROB).

Methods and results

A systematic literature search for studies investigating novel diagnostic HFpEF circulating biomarkers in humans was performed up until 21 April 2020. Those without diagnostic performance measures reported, or performed in an acute heart failure population were excluded, leading to a total of 28 studies. For each study, four reviewers determined the ROB within the QUADAS-2 domains: patient selection, index test, reference standard, and flow and timing. At least one domain with a high ROB was present in all studies. Use of case-control/two-gated designs, exclusion of difficult-to-diagnose patients, absence of a pre-specified cut-off value for the index test without the performance of external validation, the use of inappropriate reference standards and unclear timing of the index test and/or reference standard were the main bias determinants. Due to the high ROB and different patient populations, no meta-analysis was performed.

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Conclusion The majority of current diagnostic HFpEF biomarker studies have a high ROB, reducing the reproducibility and the potential for clinical care. Methodological well-designed studies with a uniform reference diagnosis are urgently needed to determine the incremental value of circulating biomarkers for the diagnosis of HFpEF.

Keywords Heart failure with preserved ejection fraction • Diagnosis • Biomarker • Bias • QUADAS-2

Introduction

Heart failure with preserved ejection fraction (HFpEF) is a clinical syndrome that is associated with high mortality rates, poor quality of life and significant healthcare resource utilization.[1,2] Currently, more than 5% of the elderly (>65 years of age) suffer from this debilitating syndrome.[1,2] The prevalence is expected to rise even further in the upcoming years, due to the ageing population and the growing occurrence of other HFpEF risk factors.[1,2]

Unfortunately, diagnosing HFpEF in the non-acute setting remains challenging. Natriuretic peptides (NPs) have limited diagnostic value for this purpose, which is mainly due to the high prevalence of conditions within this syndrome that can lead to higher [e.g. atrial fibrillation (AF), hypertension, pulmonary diseases, renal function disorders] and lower (e.g. obesity) circulating NP levels.[3–11] Moreover, 18% to 30% of patients with haemodynamically proven HFpEF have NP levels below 'diagnostic' threshold.[12–14]

The limited diagnostic accuracy of NPs, and the concept that other circulating biomarkers could help to diagnose this complex syndrome on a molecular level, has resulted in a multitude of studies investigating novel diagnostic HFpEF biomarkers.[3,15] Remarkably, none of the suggested circulating biomarkers have been implemented in the HFpEF clinics. The heterogeneous and systemic nature of the syndrome could contribute to their lack of success,[11] but a comprehensive overview of the literature on this topic is absent. We therefore aimed to provide an overview of studies investigating the diagnostic value of novel biomarkers for non-acute HFpEF and determine their risk of bias (ROB).

Methods

A systematic literature search—based on the PRISMA-DTA statement[16]—of PubMed and EMBASE was performed to find diagnostic papers within the field of HFpEF from its inception until 21 April 2020. A broad search (online supplementary *Appendix S1*) was used for a set of systematic reviews and a meta-analysis for the (early) detection of left ventricular diastolic dysfunction (LVDD) and/or HFpEF. The search strategy and the protocol can be found on PROSPERO (CRD42018065018). Studies that reported the diagnostic value of novel circulating biomarkers for the detection of chronic HFpEF were included in this study.

Study selection

Four reviewers (SR, MLH, AB and JB) screened the titles and abstracts independently. Studies were included if they: (i) reported a diagnostic performance measure (e.g. area under the receiver operating curve, sensitivity, specificity, negative predictive value, positive predictive value) of a novel circulating biomarker for the diagnosis of HFpEF in humans as main or sub-analysis; and (ii) were written in English. Studies were excluded if they: (i) studied the diagnostic value of a biomarker in acute heart failure; (ii) only studied the diagnostic value of NPs; (iii) studied the diagnostic value within a rare patient population (e.g. beta thalassemia); or (iv) were a (systematic) review, meta-analysis, editorial, or conference abstract.

Data extraction

The following data were extracted for each study: publication details (first author, year of publication), study characteristics (patient population description, exclusion criteria), used reference standard, and the biomarker(s) studied (index test).

Risk of bias assessment

The methodological quality of the full-text articles was independently evaluated by four reviewers (SR, ER, RV, MH) by utilising the QUADAS-2 tool.[17] This tool was used to determine the ROB within four domains: patient selection, index test, reference standard, and flow and timing. Based on the information provided in the included studies, the ROB was rated low, intermediate, or high for these domains separately.

For the reference standard domain the ROB was rated low if (exercise) right-sided heart catheterisation was used for the diagnosis of HFpEF, intermediate if signs/symptoms of heart failure with left ventricular ejection fraction $\geq$ 40–50% and structural/functional abnormalities indicative of LVDD was used,[10,18–21] and high for all other reference standards. Within the remaining domains the ROB was rated low, intermediate or high when respectively all, two, and one or none of the supporting questions (online supplementary *Table S1*; three pre-defined questions per domain) were answered in a positive manner. However, certain study characteristics—no avoidance of case-control/two-gated designs, or unclear/inappropriate timing for the index test and/or reference standard—would immediately lead to a high ROB for the respective domain. Inconsistencies in quality assessment between the four reviewers were resolved by discussion until consensus was reached.

Results

Search results

A total of 20 757 studies were derived from the extensive literature search. A total of 28 studies were deemed eligible for this review (online supplementary *Figure S1*). The 28 selected studies included a wide range of potential novel diagnostic HFpEF circulating biomarkers (*Table 1*).[22–49]

Table 1 Overview of the diagnostic heart failure with preserved ejection fraction circulating biomarker studies

Study/country	Biomarkers	Cases (reference standard)	Controls	Cases/controls descriptives			
				Age (years)	Sex (% female)	NT-proBNP[a] (pg/mL)	LVEF (%)[∇] E/e'[◐] LAVI[⊕] LVMI[✿]
Martos, 2009[22] Ireland	CITP; MMP-1,-2,-9; PICP; PINP; PIIINP; TIMP	HFpEF ($n = 32$) • Previous HFH NYHA IV • Continued HF signs/symptoms (≥NYHA II) • LVEF >45% • LVDD	No HFpEF ($n = 53$)	72 ± 11/66 ± 9	47/75	265 ± 182/98 ± 132 BNP	63 ± 14/67 ± 10[∇] _[◐] _[⊕] _[✿]
Stahrenberg, 2010[23] Germany	GDF-15	HFnEFesc ($n = 142$) • Established CHF • LVEF≥50% • LVDD based on ESC, 2007[18]	Healthy controls ($n = 188$)	73 [66–78]/56 [52–63]	64/66	326 [133–634]/64 [39–112]	60 [56–65]/61 [56–66][∇] 12 [9–15]/7 [6–9][◐] _[⊕] _[✿]
		HFnEFnew ($n = 85$) • Established CHF • LVEF >50% • Elevated LV filling pressures ASE, 2009 [21]	"	–/56 [52–63]	–/66	–/64 [39–112]	–/61 [56–66][∇] –/7 [6–9][◐] _[⊕] _[✿]
Zile, 2011[24] America	CITP; CTP; MMP-1,-2,-3,-7,-8,-9; osteopontin; PINP; PIIINP; sRAGE; TIMP-1,-2,-3,-4	LVH with DHF ($n = 61$) • Signs/symptoms of HF • LVEF ≥50% • LVH • LVEDVI <90 • LVDD (measured invasively/non-invasively)	LVH, no DHF ($n = 144$)	66 ± 1/60 ± 1	59/55	214 ± 34/109 ± 12	69 ± 1/69 ± 1[∇] _[◐] _[⊕] 123 ± 3/117 ± 2[✿]
Celik, 2012[25] Turkey	RDW	DHF ($n = 71$) • Symptoms and signs of HF • LVEF ≥50% • LVDD	No signs/symptoms of HF ($n = 50$)	57 ± 7/56 ± 7	63/58	97 [57–264]/57 [26–94]	72 [63–75]/68 [63–73][∇] 9 ± 3/6 ± 2[◐] _[⊕] 103 ± 24/91 ± 20[✿]
Santhanakrishnan, 2012[26] Singapore	GDF-15; sST2; hsTnT	HFpEF ($n = 50$) • Symptomatic • LVEF ≥50%	No history of CAD/HF ($n = 50$)	69 ± 12/63 ± 8	42/54	942 [309–2768]/69 [41–102]	60 ± 7/66 ± 3[∇] 18 ± 9/9 ± 2[◐] _[⊕] _[✿]
		"	HFrEF <50% ($n = 51$)	69 ± 12/59 ± 11	42/14	942 [309–2768]/2562 [1038–6373]	60 ± 7/25 ± 10[∇] 18 ± 9/15 ± 6[◐] _[⊕] _[✿]

Table 1 (Continued)

Study/country	Biomarkers	Cases (reference standard)	Controls	Cases/controls descriptives			
				Age (years)	Sex (% female)	NT-proBNP[a] (pg/mL)	LVEF (%)[∇] E/e′[◐] LAVI[⊕] LVMI[✿]
Baessler, 2012[27] Germany	GDF-15	LVDD with possible HF ($n = 88$) • Symptoms/signs HF • LVEF >50% • LVDD	No LVDD ($n = 119$)	50 ± 7/41 ± 12	55/73	52 [29–96]/42 [25–66]	64 ± 9/64 ± 7[∇] 8 ± 3/5 ± 1[◐] _[⊕] 136 ± 32/102 ± 20
Mason, 2013[28] England	Copeptin; hsCRP; MR-proANP; MR-proADM	HFpEF ($n = 57$) • Clinical features of HF • LVEF >50% • LVDD	No HF ($n = 308$)	87 ± 6/84 ± 7	83/73	1300 ± 1604/764 ± 1280	_[∇] _[◐] _[⊕] _[✿]
Wang, 2013[29] China	sST2	HFnEF ($n = 68$) • NYHA II–III/history of signs and HF symptoms • LVEF ≥50%	No symptoms/signs HF ($n = 39$)	68 ± 10/60 ± 12	54/33	262 ± 470/71 ± 53	68 ± 7/68 ± 7[∇] 12 ± 4/6 ± 1[◐] _[⊕] _[✿]
Jiang, 2014[30] China	Angiogenin	HFpEF ($n = 16$) • NYHA III–IV • LVEF >40% • NT-proBNP >1500 pg/mL	Healthy controls ($n = 16$)	76 ± 4/68 ± 8	62/38	3377 {2178–3995}/55 {27–93}	55 ± 12/70 ± 4[∇] _[◐] _[⊕] _[✿]
Wong, 2015[31] Singapore	Miscellaneous miRNAs	HFpEF ($n = 30$) • Symptomatic • LVEF ≥50%	No history of CAD/HF ($n = 30$)	64 ± 9/66 ± 7	–	1712 (± 2638)/86 (± 83)	59 ± 5/64 ± 4[∇] _[◐] _[⊕] _[✿]
		"	HFrEF ≤ 40% ($n = 30$)	64 ± 9/65 ± 7	–	1712 (± 2638)/6727 (± 6290)	59 ± 5/25 ± 7[∇] _[◐] _[⊕] _[✿]
Zordoky, 2015[32] Canada	Miscellaneous metabolites	HFpEF ($n = 24$) • Symptoms consistent with HF • LVEF >45%	HFrEF <45% ($n = 20$)	68 [58–75]/64 [56–69]	25/30	110 ± 140/238 ± 294	_[∇] _[◐] _[⊕] _[✿]
		"	Healthy controls and patients at risk ($n = 38$)	68 [58–75]/62 [54–69]	25/53	110 ± 140/9 ± 12	_[∇] _[◐] _[⊕] _[✿]

Table 1 (Continued)

Study/country	Biomarkers	Cases (reference standard)	Controls	Cases/controls descriptives			
				Age (years)	Sex (% female)	NT-proBNP[a] (pg/mL)	LVEF (%)$^{\nabla}$ E/e′$^{\bullet}$ LAVI$^{\oplus}$ LVMI*
Watson, 2015[33] Ireland	Miscellaneous miRNAs	HFpEF (n = 75) • Previous HFH NYHA IV • Continued ≥NYHA II • LVEF ≥50% • LVDD	HFrEF <50% (n = 75)	75 ± 7/70 ± 11	39/27	215 [126–353]/139 [71–254] BNP	62 ± 7/36 ± 12$^{\nabla}$ 11 ± 4/10 ± 5$^{\bullet}$ 52 ± 19/46 ± 14$^{\oplus}$ 114 ± 36/126 ± 38
Sanders-van Wijk, 2015[34] Switzerland and Germany	Cys-C; Hb; hsCRP; hsTnT; sST2	HFpEF (n = 112) • Signs/symptoms (NYHA ≥II) of HF • HFH during last year • LVEF ≥50% • NT-proBNP ≥2x ULN	HFrEF ≤40% (n = 458)	80 ± 7/76 ± 7	64/33	2142 [1473–4294]/4202 [2239–7411]	57 ± 6/29 ± 7$^{\nabla}$ _$^{\bullet}$ _$^{\oplus}$ _*
Barroso, 2016[35] Germany	IGFBP-7; IGF-1	HFpEF (n = 77) • With/without HF symptoms/signs • LVEF >50% • LVDD grade II/III[21]	No LVDD, LVEF >50% (n = 55)	73 [68–77]/54 [48–61]	60/47	344 [152–703]/90 [46–129]	_$^{\nabla}$ _$^{\bullet}$ _$^{\oplus}$ _*
Liu, 2016[36] China	sgp130; hsp27; CTSS; DPP4	HFpEF (n = 50) • HF symptoms/signs in last month • LVEF ≥50%	No history of heart disease(s) (n = 50)	64 ± 6/64 ± 6	46/54	982 ± 461/332 ± 327	_$^{\nabla}$ _$^{\bullet}$ _$^{\oplus}$ _*
Polat, 2016[37] Turkey	Gal-3	HFpEF (n = 44) • History of NYHA II–III • LVEF >50% • LVEDVI ≤97 • LVDD	No systolic/diastolic dysfunction (n = 38)	60 ± 7/57 ± 9	46/47	618 ± 271/66 ± 54	59 ± 5/61 ± 4$^{\nabla}$ 16 ± 3/4 ± 2$^{\bullet}$ 71 ± 13/29 ± 4$^{\oplus}$ 166 ± 17/113 ± 10*
Li, 2016[38] China	Adj-Ca	HFpEF (n = 106) • Symptoms and/or signs of HF • LVEF ≥50% • NT-proBNP >125 pg/mL	No HFpEF (n = 701)	76 ± 9/68 ± 12	54/41	645 ± 264/190 ± 70	67 ± 7/66 ± 5$^{\nabla}$ _$^{\bullet}$ _$^{\oplus}$ 118 ± 31/99 ± 21*
Berezin, 2016[39] Ukraine	CD31+/annexin V+ EMPs to CD14+CD309+ cell ratio	HFpEF (N = 79) • Clinical presentation CHF • LVEF >55% • e/e′ >15 • NT-proBNP >220 pg/mL	HFrEF ≤45% (n = 85)	55 ± 7/58 ± 7	53/42	2131 [955–3056]/2774 [1520–3870]	55 [51–58]/37 [31–42]$^{\nabla}$ _$^{\bullet}$ _$^{\oplus}$ _*

Table 1 (Continued)

Study/country	Biomarkers	Cases (reference standard)	Controls	Cases/controls descriptives			
				Age (years)	Sex (% female)	NT-proBNP[a] (pg/mL)	LVEF (%)[▽] E/e'[◐] LAVI[⊕] LVMI[✿]
Toma, 2017[40] Canada	Miscellaneous proteins and transcripts	HFpEF ($n = 21$) • Symptoms consistent with HF • LVEF ≥50%	HFrEF ≤40% ($n = 48$)	70 [63–79]/66 [59–73]	52/27	295 [143–1550]/1174 [401–2516]	60 [56–62]/30 [23–36][▽] _◐ _⊕ _✿
Sinning, 2017[41] Germany	GDF-15; sST2; CRP	HFpEF ($n = 70$) • NYHA II–IV or treatment for HF • LVEF ≥50% • LVDD	HFrEF <50%, NYHA II–IV or treatment for HF ($n = 38$)	67 [62–72]/64 [58–70]	50/21	146 [76–294]/956 [244–1877]	64 [59–70]/43 [36–48][▽] _◐ _⊕ _✿
		"	No HF (N = 4864)	67[62–72]/ 55[46–64]	50/49	146[76–294]/ 60[28–119]	64[59–70]/64[60–68][▽] _◐ _⊕ _✿
Cui, 2018[42] China	Gal-3; sST2	HFpEF ($n = 172$) • HFpEF ESC, 2016[19]	HFrEF ≤ 40% ($n = 45$)	73 ± 9/71 ± 9	56/39	614 [243–1479]/4330 [1747–10013]	60 [56–62]/31 [28–35][▽] 18 [13–23]/14 [12–17][◐] _⊕ _✿
		"	No HF ($n = 30$)	73 ± 9/67 ± 5	56/40	614 [243–1479]/189 [133–214]	60 [56–62]/59 [57–60][▽] 18 [13–23]/7 [5–13][◐] _⊕ _✿
Nikolova, 2018[43] America	cBIN1	HFpEF ($n = 52$) • History of fluid overload, prior HFH, or invasive evidence of elevated cardiac filling pressures • LVEF ≥50%	Healthy controls ($n = 52$)	57 ± 15/52 ± 6	37/37	277 [99–1264]/36 [19–72]	58 ± 7/–[▽] _◐ _⊕ _✿
		"	Controls at risk ($n = 52$)	57 ± 15/52 ± 9	37/37	277 [99–1264]/21 [13–43]	58 ± 7/–[▽] _◐ _⊕ _✿

Table 1 (Continued)

Study/country	Biomarkers	Cases (reference standard)	Controls	Cases/controls descriptives			
				Age (years)	Sex (% female)	NT-proBNP[a] (pg/mL)	LVEF (%)$^{\nabla}$ E/e′$^{\text{◐}}$ LAVI$^{\oplus}$ LVMI$^{\text{✿}}$
Farinacci, 2019[44] Germany	CECs	HFpEF (n = 27) • NYHA I–III • HFH during last year • Cardiac functional/structural abnormalities suggestive for HFpEF or elevated NP levels	Healthy Controls (n = 10)	69 ± 8/56 ± 3	44/55	–	_$^{\nabla}$ _$^{\text{◐}}$ _$^{\oplus}$ _$^{\text{✿}}$
Wong, 2019[45] Singapore and New Zealand	Miscellaneous miRNAs	HFpEF (n = 179) • Symptomatic • LVEF ≥50%	HFrEF ≤40% (n = 145)	77 ± 9/70 ± 14	46/17	2557 ± 2690/4898 ± 7887	62 ± 7/29 ± 7$^{\nabla}$ _$^{\text{◐}}$ _$^{\oplus}$ _$^{\text{✿}}$
Chi, 2019[46] China	CTGF; TGF-β1	DHF (n = 114) • Symptoms or signs of HF • LVEF ≥45% and normal LV size • Structural heart disease such as LVH, left atrial enlargement, previous myocardial infarction and/or diastolic dysfunction	No HF (n = 72)	71 ± 11/69 ± 11	53/43	1224 [499–2472]/70 [25–126]	62 ± 9/67 ± 6$^{\nabla}$ 13 ± 6/-$^{\text{◐}}$ _$^{\oplus}$ 136 ± 52/109 ± 28$^{\text{✿}}$
Berezin, 2019[47] Ukraine	CD31+/annexin V+ MVs; Gal-3; GDF-15	HFpEF (n = 178) • Previously treated primary diagnosis of HF • LVEF ≥50%	HFmrEF/HFrEF (n = 210)	55 ± 7/57 ± 7	57/40	2131 [955–3056]/HFmrEF 2701 [1590–3541]; HFrEF 2775 [1520–3870]	55 [51–58]/HFmrEF 44 [41–48]; HFrEF 37 [31–39]$^{\nabla}$ _$^{\text{◐}}$ _$^{\oplus}$ _$^{\text{✿}}$
Fang, 2019[48] China	RDW	HFpEF (n = 62) • Symptoms or signs of HF • LVEF ≥50% • LAVI ≥34 • NT-proBNP ≥400 ng/L	I. No substantial cardiac dysfunction II. Possible HFpEF (n = 107)	74 ± 9/67 ± 12	45/48	1095 [575–2027] I. 154 [69–286] II. 243 [66–545]	58 ± 7/60 ± 6$^{\nabla}$ 14 ± 5/13 ± 4$^{\text{◐}}$ 46 ± 12/29 ± 7$^{\oplus}$ 114 ± 15/105 ± 16$^{\text{✿}}$

Table 1 (Continued)

Study/country	Biomarkers	Cases (reference standard)	Controls	Cases/controls descriptives			
				Age (years)	Sex (% female)	NT-proBNP[a] (pg/mL)	LVEF (%)▽ E/e'● LAVI⊕ LVMI✿
Merino-Merino, 2020[49] Spain	Urate; CRP; TnT; Fibrinogen; Gal-3; sST-2	Non-reduced HF (n = 87) • Symptoms of HF/AF • LVEF >40% • LVDD	No non-reduced HF (n = 28)	64 ± 9/59 ± 10	33/21	1277 ± 1377/775 ± 561	_▽ _● _⊕ _✿

Only the number of subjects are shown for the validation cohort if multiple cohorts were used in one study; if multiple validation cohorts were used, only the cohort with the most included patients are shown. If only a sub-population in an article was used to determine the diagnostic value of a circulating biomarker, then only the information of this population is provided. To ensure readability, in some cases inclusion criteria were incorporated in the reference standard if they included LVEF, previous HFH, symptoms/signs, or LVDD. More details about the study population—and the used exclusion criteria—can be found in online supplementary *Table S4*. If the mean and SD of one of the 'Cases/controls descriptives' were not directly provided in the article, a pooled mean and SD was calculated if possible. Descriptives are expressed as mean ± SD, median [IQR], mean (±SEM), or mean [95% CI].

Adj-Ca, albumin adjusted calcium; AF, atrial fibrillation; ASE, American Society of Echocardiography; CAD, coronary artery disease; cBIN1, cardiac bridging integrator 1; CD, cluster of differentiation; CEC, circulating endothelial cell; CHF, chronic heart failure; CI, confidence interval; CITP, carboxy-terminal telopeptide of collagen type I; CRP, C-reactive protein; CTGF, connective tissue growth factor; CTP, cardiotropin-1; CTSS, catepsin S; Cys-C, cystatin C; DHF, diastolic heart failure; DPP4, dipeptidyl peptidase 4; E/e', ratio of peak early mitral inflow velocity to early diastolic mitral annular velocity; EMP, endothelial cell-derived microparticle; ESC, European Society of Cardiology; Gal-3, galectin-3; GDF-15, growth differentiation factor-15; Hb, haemoglobin; HF, heart failure; HFH, heart failure hospitalisation; HFmrEF, heart failure with mid-range ejection fraction; HFnEF, heart failure with normal ejection fraction; HFpEF, heart failure with preserved ejection fraction; HFrEF, heart failure with reduced ejection fraction; hsCRP, high-sensitivity C-reactive protein; hsp27, heat shock protein 27; hsTnT, high-sensitivity troponin T; IGF-1, insulin-like growth factor-1; IGFBP-7, insulin-like growth factor binding protein-7; IQR, interquartile range; LAVI, left atrial volume index (mL/m^2); LV, left ventricle; LVDD, left ventricular diastolic dysfunction; LVEDVI, left ventricular end-diastolic volume index (mL/m^2); LVEF, left ventricular ejection fraction; LVH, left ventricular hypertrophy; LVMI, left ventricular mass index (g/m^2); miRNA, microRNA; MMP, matrix metalloproteinase; MR-proADM, mid-regional pro adrenomedullin; MR-proANP, mid-regional pro atrial natriuretic peptide; MV, microvesicle; NP, natriuretic peptide; NT-proBNP, N-terminal pro brain natriuretic peptide; NYHA, New York Heart Association; PICP, carboxy-terminal propeptides of procollagen type I; PIIINP, amino-terminal propeptide of procollagen type III; PINP, amino-terminal propeptide of procollagen type I; RDW, red cell distribution width; SD, standard deviation; SEM, standard error of the mean; sgp130, soluble glycoprotein 130; sRAGE, soluble receptor for advanced glycation end product; sST2, soluble interleukin-1 receptor-like 1; TGF-β1, transforming growth factor β1; TIMP, tissue inhibitor of matrix metalloproteinase; TnT, troponin T; ULN, upper limit of normal.

[a]Or brain natriuretic peptide if stated.

–, not available.

Quality assessment

All papers had at least one domain with a high ROB, and 11 papers (39%) showed a high ROB within all four domains (online supplementary *Table S2*). Main reasons for bias within the QUADAS-2 domains of each individual article are shown in online supplementary *Table S3*.

The ROB within the patient selection domain was high in 24 out of 28 studies (86%; *Figure 1*). This was mainly driven by the use of case-control/two-gated designs. Additionally, in 13 studies inappropriate exclusion criteria were not avoided (online supplementary *Tables S3 and S4*). This was often the result of excluding difficult to diagnose patients—e.g. patients with AF, obesity, and/or pulmonary diseases—or by excluding patient conditions which could possibly influence the outcome of the index test (e.g. kidney function disorders). Only nine studies (32%) did not use a case-control design, in only two of these studies inappropriate exclusion criteria were avoided (online supplementary *Table S3*).

Even though the index tests of all studies were classified as objective, the ROB for the index test domain was rated high in 26 out of 28 studies (93%; *Figure 1*). This was caused by the fact that most studies did not use pre-specified cut-off values and did not perform any external validation. Only one article provided information about the sensitivity and specificity of a pre-specified cut-off value for the index test studied,[23] and one article performed validation of their findings in an external cohort.[45]

All studies suffered from an intermediate or high ROB within the reference standard domain, being rated as intermediate/high in 14 out of 28 studies (50%; *Figure 1*). Different reference standards (and definitions of LVDD) were used, and none of the studies performed (exercise) right-sided heart catheterisation in all study subjects (*Table 1*).

A total of 27 out of 28 studies (96%; *Figure 1*) scored a high ROB within the flow and timing domain. In all these studies this was caused by the fact that the exact timing of the index test and/or reference standard was unclear (online supplementary *Table S3*).

Given the high ROB, combined with limited overlap in investigated biomarkers and different statistical methods used, no areas under the receiver operating curve were reported and no meta-analysis was performed.

Discussion

This is the first study that provides a comprehensive overview of studies that included diagnostic evaluation of novel circulating biomarkers for the detection of HFpEF. All included studies in this review contributed to our current level of knowledge of this complex syndrome. However, this systematic review exposes multiple study limitations that together limit our ability to evaluate the true diagnostic value of circulating biomarkers. The main limitations that we found were: (i) use of case-control/two-gated designs; (ii) exclusion of a relevant/representative subset of the true HFpEF population; (iii) use of optimal rather than pre-specified cut-off points for the index test without the performance of external validation; (iv) inadequate and highly variable reference standards, none including the true gold standard; and (v) unknown

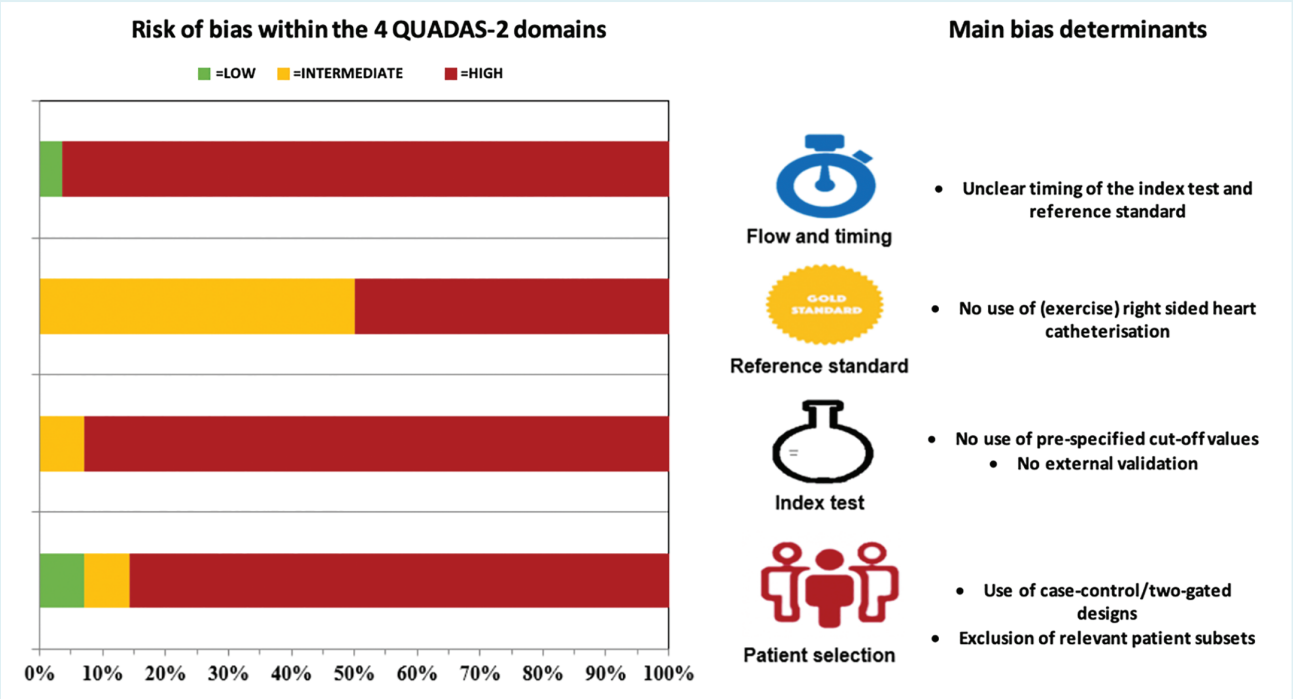

Figure 1 Percentage of studies with low, intermediate or high risk of bias within the four QUADAS-2 domains (patient selection, index test, reference standard, flow and timing) and the main reasons for a high risk of bias within these domains.

timing of the index and/or reference standard. The overall high ROB might play an important role in the limited uptake of these biomarkers in the HFpEF clinics and calls for methodologically well-designed studies.[50,51]

Patient selection

Most studies determined the diagnostic value of the biomarkers in cases with known HFpEF compared to (healthy) controls. During the early stages of novel biomarker discovery, these designs with contrasting populations can be useful to screen whether novel biomarkers might be of any interest for future analysis.[52] Such studies may also reveal mechanistic insights into the syndrome. However, for diagnostic utility these designs induce spectrum bias, which overestimates the diagnostic value of the investigated biomarker(s).[52–55]

Additionally, extensive exclusion criteria including AF, pulmonary diseases, or even chronic kidney function disorders were often used, which are all highly prevalent comorbid conditions in HFpEF.[56–58] For example, over 50% of HFpEF patients have AF.[59–61] Excluding these patients introduces selection bias that could result in a serious misinterpretation of the diagnostic value and reduce external validity of these biomarkers in unselected HFpEF populations.[52,54,62]

Index test

The use of optimal cut-off values for the index test without performing external validation within the majority of previous studies will have resulted in an overestimation of the diagnostic performance of the biomarkers examined.[63] Moreover, a biomarker should have incremental value on top of easy to determine characteristics—e.g. age, sex and body mass index—to really yield potential for clinical use. While this was not part of the ROB assessment within this study, it will partially explain the lack of the implementation of novel diagnostic HFpEF biomarkers.

Reference standard

Test accuracy of a novel biomarker is based on the concept that every inconsistency between the index test and reference standard is due to an incorrect index test.[17,51] Since different reference standards will significantly alter the prevalence of cases within the cohort of interest—as already shown within the field of LVDD[64]—this will significantly affect the diagnostic value of the biomarker(s) studied. None of the included studies used (exercise) right-sided heart catheterisation—the real gold standard for HFpEF—as uniform reference standard. Studies validating the biomarker value against this gold standard are urgently needed.

Recognising the challenges of widespread implementation of gold standard invasive haemodynamic testing, we also examined the use of guideline-recommended reference standards that were published at the moment of publication for the diagnosis of heart failure with normal ejection fraction since 2007[18] or HFpEF since 2016,[19] and found that most studies did not apply these. Also, these reference standards were not in line with the recently published H_2FPEF[59] or HFA-PEFF scores.[10] Nonetheless, even

the recommended reference standards and risk scores differ significantly in included diagnostic criteria, used cut-off values and the role comorbidities play within these standards, highlighting the uncertainty of diagnosing HFpEF.

Flow and timing

Most studies did not provide (detailed) information regarding the timing of the index test and the reference standard. This lack of information is regrettable given that biomarker levels will likely change over time. Moreover, it is highly likely that diuretics are prescribed and/or dosage were changed in patients with signs of congestion. Diuretics will reduce filling pressure and very likely influence the concentration of the circulating biomarker measured. It has already been shown that diuretics affect the urinary proteome in rats,[65] and the pleural protein concentration in patients with congestive heart failure.[66] In the latter also an increase in total serum protein content after the administration of diuretics was observed.[66] Therefore, it is highly desirable that the circulating biomarkers are measured at the same moment as the HFpEF diagnosis is made and before any intervention occurs.

Phenotype specific biomarkers

The question remains to which extent the absence of novel diagnostic HFpEF biomarkers is due to the real lack of diagnostic value of these biomarkers, vs. the heterogeneity of the syndrome itself. In contrast to HFpEF, heart failure with reduced ejection fraction, characterised by cardiomyocyte loss and ventricular dilatation, is diagnostically well-captured by natriuretic peptides that increase in response to wall stress and by troponins indicating cardiomyocyte injury.[3] In the more heterogeneous HFpEF syndrome, biomarkers likely reflect less well the complex, mainly non-cardiac multi-organ nature of the syndrome.[11,56] Therefore, biomarkers reflecting more general pathophysiological processes like inflammation (growth differentiation factor-15), fibrosis (soluble ST2, galectin-3), and metabolic dysfunction (insulin-like growth factor binding protein-7) could have potential; moreover, the search for one single biomarker may not be sufficient.[11] An approach with multiple biomarkers in methodologically well-designed studies may be more appropriate and successful.[11,50,51] One may postulate if it will ever be possible to find a single diagnostic test or panel of biomarkers with adequate diagnostic value for the entire syndrome, and perhaps the optimal approach may be to use specific biomarkers to diagnose distinct subtypes of HFpEF, which could eventually also lead to a more tailored therapy.[67–70]

Future perspectives

There is an urgent need for prospective studies to validate the diagnostic value of the HFA-PEFF score against gold standard invasive exercise haemodynamic testing in unselected symptomatic patients with suspected HFpEF.[10] The inclusion of blood biomarker testing in such a study will enable the evaluation of the possible role of novel biomarkers in the HFA-PEFF algorithm on top of NPs and echocardiographic biomarkers. Possibilities that warrant investigation include implementation of biomarker testing in step 1 (pre-test assessment) or step 2 (diagnostic work-up) of the HFA-PEFF algorithm. Furthermore, promising novel biomarkers may be assessed as potential alternatives to NPs. NP levels should not be used as a selection criterium in these studies since 18% to 30% of patients with haemodynamically proven HFpEF have NP levels below 'diagnostic' threshold.[12–14] Such studies will require close collaboration between basic scientists, clinicians, epidemiologists, industry, and (federal) sponsors.[50,51]

Study limitations

Although all papers were reviewed and discussed by our interdisciplinary team until consensus was reached, the ROB classifications are based on the information provided in the studies, the pre-defined risk of bias criteria, as well as on the interpretation of the reviewers themselves. Therefore, it is possible that analysis of the studies by another group of reviewers results in another level of bias within certain domains of studies. However, we defined clear roles and results are rather uniform and unambiguous, making it highly unlikely that the main conclusion would differ significantly. Our review did not aim for a head to head comparison between these studies, and therefore should not be used for this purpose.

To the best of our knowledge, this review includes all current novel diagnostic circulating biomarker studies to detect chronic HFpEF. However, given the extent of the search performed, it cannot be completely excluded that studies were missed if diagnostic performance measures were not mentioned in the abstract. Additionally, the main aim of some studies was not to study the diagnostic value of circulating biomarkers to detect HFpEF, though since they studied the diagnostic value in sub-analysis, they were still included in this review to provide a complete overview of current circulating diagnostic HFpEF biomarker analysis.

Finally, since some studies included (previous) hospitalised patients and timing of the reference standard and the drawing of blood was often unclear, we may have unintentionally included acute HFpEF populations. Since this does not affect the main conclusion of this review, we decided not to exclude these studies.

Conclusion

The majority of current diagnostic HFpEF biomarker studies have a high ROB, reducing the reproducibility and the potential for clinical care. Methodological well-designed studies with a uniform reference diagnosis are urgently needed to determine the incremental value of circulating biomarkers for the diagnosis of HFpEF.

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Search string for PubMed and EMBASE.

Figure S1. PRISMA flow diagram of study selection.

Table S1. Predefined questions that were used for the risk of bias assessment.
Table S2. Overview risk of bias within the QUADAS-2 domains.
Table S3. Main determinants of level of bias within the QUADAS-2 domains for the articles included in this review.
Table S4. Overview of the patient population and exclusion criteria of the diagnostic HFpEF circulating biomarker studies included in this review.

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