Biomarkers in Heart Failure
Clinical Insights

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KEYWORDS

• Biomarkers • Heart failure • Prognosis • Risk stratification • Outcomes • Diagnosis

KEY POINTS

- Biomarkers have a preponderant role in Heart Failure general management.
- A novel categorisation of HF biomarkers, grouping them into five groups according to their function and suitability (i.e. community-based screening, diagnosis, risk stratification, phenotyping, and management/tailoring treatment), would allow a more useful clinical approach.
- Natriuretic peptides (i.e. BNP and NT-proBNP) represent, to date, the gold standard biomarkers in HF.
- Emerging biomarkers (e.g. source of tumorigenicity 2, galectin-3, Trimethylamine N-oxide, hormones) showing their importance particularly in risk stratification, phenotyping, and HF management, are currently under investigation with promising results.

Heart failure (HF) is a clinical syndrome caused by structural and/or functional cardiac abnormalities and resulting from impaired cardiac output or an increase of intracardiac pressures at rest and/or during stress. Typical signs and symptoms of HF include ankle swelling, fatigue, dyspnea and peripheral edema, pulmonary crackles, or increased jugular venous pressure. Usually, patients with ejection fraction (EF) greater than or equal to 50% are defined as HF with preserved EF, whereas those with EF less than 40% have HF with reduced EF. Patients with EF between 40% and 49% are now classified as HF with midrange EF.

INTRODUCTION

Heart failure (HF) is a clinical syndrome caused by structural and/or functional cardiac abnormalities and resulting from impaired cardiac output or an increase of intracardiac pressures at rest and/or during stress.[1–3] Typical signs and symptoms of HF include ankle swelling, fatigue, dyspnea and peripheral edema, pulmonary crackles, or increased jugular venous pressure.[1–3] Because of different underlying causes, demographics, comorbidities, and treatment response, the classic classification used to describe HF is based on left ventricle ejection fraction (EF) measurement. Usually, patients with normal EF (accepted as $\geq$50%) are defined as HF with preserved EF (HFpEF), whereas those with EF less than 40% have HF with reduced EF (HFrEF). In the latest European Society of Cardiology (ESC) guidelines, patients with EF between 40% and 49%, previously considered as a gray area, are now classified as HF with midrange EF (HFmrEF).[1]

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Despite the reduction in total mortality from cardiovascular disease over recent years, HF still maintains high levels of mortality.[4] This high mortality associated with a slow but steady increase in prevalence represents negative HF characteristics[5] and results in a huge economic and social burden.[1,4] Thus, the scientific community is trying to change this tendency, with great efforts in the research and development of biomarkers for the early detection, diagnosis, prognosis, and management of HF to reduce the associated mortality, morbidity, and economic burden. Over the past few years, the level of interest in the discovery of new biomarkers has progressively grown.[6,7] Biomarkers are now routinely used in clinical practice for diagnosis, monitoring, and risk stratification.[8] However, none of the novel biomarkers have shown sufficient prognostic impact when used alone and therefore it has been suggested that, rather than a single biomarker, the integration of multiple biomarkers would represent the best strategy, although further studies are needed to test this hypothesis.[9]

Nowadays, the term biomarker is routinely used, and in some cases misused, to describe several emerging tools, technologies, and strategies intended to progress knowledge about diseases.[10,11] In 2001, the Biomarkers and Surrogate End Point Working Group suggested the following definition: "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention."[12] Therefore, biomarkers may be derived from the blood, urine, genetic samples, imaging, physiologic tests, and tissue-specimen biopsies.[13]

Biomarkers have been grouped into 3 different categories[14]:

- Type 0: specific disease biomarkers, correlated with clinical indices that can be longitudinally monitored.
- Type I: biomarkers used to evaluate pharmacologic intervention effects together with the mechanism of action of a therapeutic drug.
- Type II: surrogate end points able to predict clinical benefits, representative of the patient's clinical status, disease progression, and outcomes. Surrogate end points are in need of fulfilling at least 4 major criteria.[15,16]:
  - Correlation between the interventional impact on the biomarker and the interventional impact on clinically meaningful end points
  - Ability of therapeutic intervention to modify the outcome of interest
  - Should reflect both benefits and risks related to the intervention
  - Clear knowledge of the sampling strategies, the related relative risk, and the time course between the outcome and the change in the biomarker

Research for the ideal biomarker is focused on the improvement in disease management, and in the reduction of health care costs.[17,18] As a result, a biomarker should be disease specific, easy to detect, cost-effective, and able to provide precise results.[19] New disease biomarkers are identified and reported in several studies; however, many of them do not fulfill the analytical validation process, which requires meeting specific sensitivity and specificity criteria, thus rendering them inapplicable in clinical practice.[20] For this reason, it is necessary to identify appropriate protocols and standards when developing and validating clinical biomarker assays (eg, accurate selection of patients, sample preparation, improvement of laboratory assays, proper statistical/analytical validation).[19]

To expand, Morrow and de Lemos[21] identified 3 criteria a biomarker should fulfill to be useful in the clinic context:

1. The biomarker needs to be accurate, and repeated measurement must be possible with a reasonable cost and time.
2. The biomarker must offer additional information that is not already available from clinical assessment.
3. The biomarker should support clinical decision making.

Outline of the Review

The present article provides clinical insight with straightforward key points and critical commentary on HF biomarkers based on the most recent international recommendations (ie, ESC/Heart Failure Association [HFA] and American College of Cardiology [ACC]/American Heart Association [AHA]/Heart Failure Society of America [HFSA]) and evidence from the literature.

With the aim of offering a more useful clinical approach, biomarkers are categorized here into 5 groups according to their function and suitability: community-based screening, diagnosis, risk stratification, phenotyping, and management/tailoring treatment (**Fig. 1**).

HEART FAILURE SCREENING IN THE COMMUNITY

The use of biomarkers to screen asymptomatic patients with HF in the community (ie, community-based screening) is an emerging

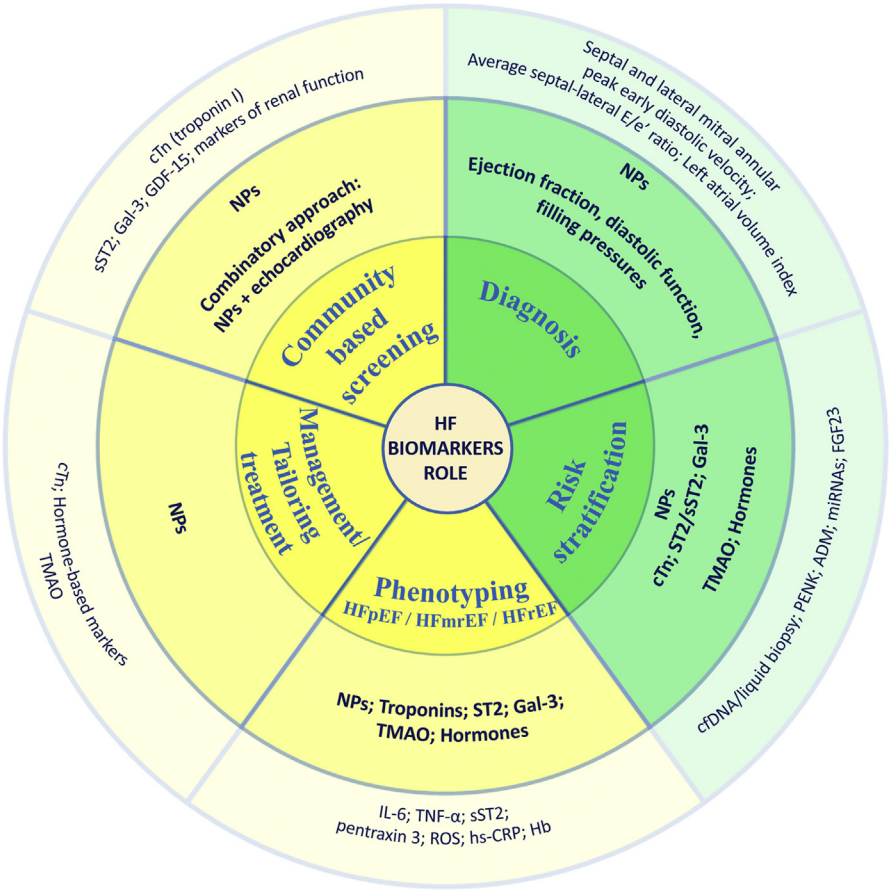

Fig. 1. Biomarkers and Heart Failure: A New Biomarker Classification Proposal. A new biomarker classification proposal based on identified clinical roles for biomarkers in Heart Failure. Green boxes: well-established and supported by data; recommended or suggested by consensus or guidelines. Yellow boxes: promising but not yet recommended in any consensus or guidelines. HF, heart failure; EF, ejection fraction; HFpEF, HF with preserved EF; HFrEF, HF with reduced EF; HFmrEF, HF with mid-range EF; NPs, Natriuretic peptides; cTN, cardia troponins; ST2, source of tumoregenicity 2; Gal-3, Galectin-3; TMAO, Trimethylamine N-oxide; IL-6, Interleukin-6; TNF, Tumor necrosis factor; hs-CRP, High sensitivity C-reactive protein; GDF-15, Growth differentiation factor-15.

issue. This approach is based on the concept that the identification of patients with left ventricular (LV) dysfunction before HF symptoms develop would lead to a delay or a reduction of clinical HF and its related morbidity and mortality. However, apart from the high-risk conditions, currently there are no guideline-based recommended strategies.

Of the different strategies, the most evaluated and promising approach is the evaluation of cardiac-specific biomarkers (mostly natriuretic peptides [NPs] and troponins) in the general population. This approach starts from the concept that B-type natriuretic peptide (BNP) was able to identify patients with AHA/ACC stage A and B HF at higher risk of worse outcome.[3] Furthermore, the St Vincent's Screening to Prevent Heart Failure (STOP-HF) study showed that, between patients at risk of HF, BNP-based screening and collaborative care were able to reduce the rates of systolic dysfunction, diastolic dysfunction, and HF,[22] estimating a good probability of being cost-effective.[23] In the Screening Evaluation of the Evolution of New Heart Failure (SCREEN-HF) study, performed in subjects at high risk of HF (defined as age >60 years plus at least 1 HF risk factor), significant ventricular dysfunction was documented in about 25% of asymptomatic elderly subjects displaying at least 1 HF risk factors and a very high N-terminal proBNP (NT-proBNP) level.[24] Recently, it has been shown that the use of single or serial NT-proBNP measurements for predicting the risk of HF at 5 years has a sensitivity exceeding 75% and specificity in the range of 47% to 69% when specific age cut-offs were applied,[25] confirming previous findings from the literature.[26] An individual-participant-data meta-analysis performed by the Natriuretic

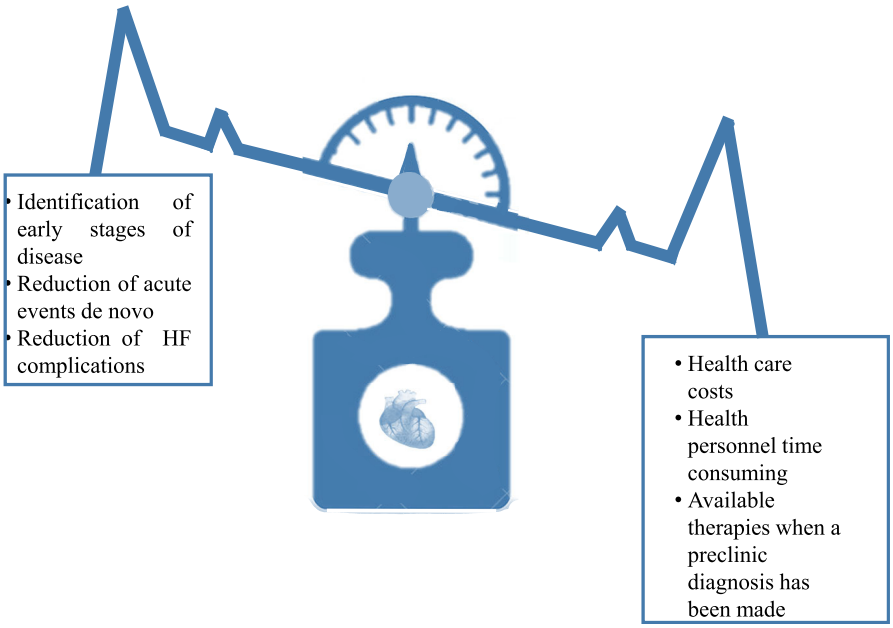

Fig. 2. Advantages and disadvantages of HF community-based screening.

Peptides Studies Collaboration group showed that NT-proBNP concentration predicted first-onset HF and augmented coronary heart disease and stroke prediction in people without cardiovascular disease.[27] As a result, the investigators suggested that NT-proBNP is potentially suitable for general population-level risk assessment.

However, some concerns have been raised around this approach, particularly regarding cost-effectiveness and the possible therapeutic options, particularly for patients with preserved EF, for which treatment options are not yet satisfactory.[28,29]

With regard to cardiac troponins (cTn), recent evidence supports the idea that clinicians would be able to identify individuals at highest risk of developing symptomatic HF, resulting in early diagnosis and improved prognosis.[30,31] The Biomarkers for Cardiovascular Risk assessment in Europe (BiomarCaRE) consortium, a European consortium including 31 institutions, combining data from 4 large populations of healthy people, has shown that high-sensitivity troponin I could independently predict incident HF, with a better predictive value when combined with NT-proBNP.[32]

As for other emerging biomarkers, galectin-3 (Gal-3) and soluble source of tumorigenicity 2 (sST2) have been tested (respectively, in the PREVEND[33] and in the Finnish study[34]), with contrasting results. Multimarker strategy (ie, the addition of multiple biomarkers to clinical variables) could potentially lead to an improvement in discrimination and reclassification abilities of predictive models.[35]

With regard to the use of imaging biomarkers, the Echocardiographic Heart of England Screening study showed that, in a large representative adult population in England, LV dysfunction (defined as LV EF<40%) was detectable in about 1.8% of subjects whereas EF of 40% to 50% was detectable in 3.5% of them.[36] Again, the cost-effectiveness of this approach represents a cause for concern.

Going forward with these concepts, it has been shown that the use of BNP, added to other simple clinical parameters/measurements(eg, electrocardiographic abnormalities) was able to reduce the number of patients needed to perform an echocardiographic study to identify LV systolic dysfunction.[37] Therefore, the combinatory approach seems to be the most effective. Despite the reported effectiveness of this approach in community screening, inadequate access to primary care physicians and its expense have limited the application of echocardiography to screening healthy people (**Fig. 2**).[38,39] Additional strategies to select patients for referral to echocardiography (eg, combine blood and urinary NPs[40] or the addition of other biomarkers such as C-reactive protein and myloperixodases[41]), although fascinating, remain without current clinical application. Results from The Screening of adult Urban Population to Diagnose Heart Failure (SOBOTA-HF) study, currently ongoing (all patients aged >55 years in the large Monska Sobota region are being testing for NT-proBNP and, if increased levels are detected, echocardiographic assessment is performed),[42] will add information

about the feasibility of the use of NP screening in the general population.

Community-Based Heart Failure Screening: Should it be Done?

In summary, population screening with NPs followed by echocardiography is a promising approach; however, there is still no evidence of cost-effectiveness to justify its adoption at a large scale[28] (see **Fig. 2**). The AHA, in a recent statement on biomarkers, concluded that the measurement of NPs or cTn alone adds prognostic information to standard risk factors for predicting new-onset HF, with the measurement of emerging biomarkers (ie, sST2, Gal-3, growth differentiation factor 15 [GDF-15], and markers of renal function) alone or in a multimarker strategy, providing additional risk stratification.[11] In contrast, the most recent position paper from European Society of Preventive Cardiology[43] does not recommend HF screening in the community.

DIAGNOSIS

In the latest version of HF guidelines,[1,3] biomarkers (circulating and imaging) have an essential role in HF diagnosis. NPs can be considered the gold standard circulating biomarkers, with increased levels helping to identify patients in need of a further evaluation and lower levels helping to exclude HF diagnosis, regardless of phenotype.[44,45] In parallel, among imaging techniques, echocardiography is considered the most valuable tool owing to its wide availability, easy accessibility, and low cost. EF, ventricle volumes, systolic and diastolic function, valvular diseases, and right ventricle impairment represent fundamental information, changing the clinical management of patients suspected of HF in order to confirm or exclude an HF diagnosis.

Circulating Biomarkers

Among circulating biomarkers, only NPs have shown a clear role in HF diagnosis and have been widely approved with this purpose.[1–3]

Natriuretic peptides

NPs (mainly BNP and NT-proBNP) are strongly linked to the presence and the severity of hemodynamic stress, intracardiac volumes, and filling pressures. Considering this strict correspondence, it is not surprising that other biomarkers

Table 1
Suggested natriuretic peptide cutoffs

	HFrEF		HFpEF	
	Chronic Setting	**Acute Setting**	**Major Criteria**	**Minor Criteria**
BNP	• <35 pg/mL HF unlikely • 35–150 pg/mL gray zone • >150 pg/mL HF likely	• <100 pg/mL HF unlikely • 100–400 pg/mL gray zone • >400 pg/mL HF likely	• >80 pg/mL if sinus rhythm • >240 pg/mL if atrial fibrillation	• 35–80 pg/mL if sinus rhythm • 105–240 pg/mL if atrial fibrillation
NT-proBNP	• <125 pg/mL unlikely • 125–600 pg/mL gray zone • >600 pg/mL HF likely	• <300 pg/mL unlikely • 300–450 pg/mL (<50 y) gray zone • 300–900 pg/mL (50–75 y) gray zone • 300–1800 pg/mL (>75 y) gray zone • >450 pg/mL or >900 or >1800 (>75 y) HF likely	• >220 pg/mL if sinus rhythm • >660 pg/mL if atrial fibrillation	• 125–220 pg/mL if sinus rhythm • 365–660 pg/mL if atrial fibrillation
MR-proANP	—	• 120 pmol/L	—	—

Current guidelines suggested cutoffs for NPs in HF, with regard to phenotype (preserved vs reduced), setting (acute vs chronic), and presence of relevant comorbidities (ie, atrial fibrillation).

Abbreviation: MR-proANP, midregional pro–A-type natriuretic peptide.

have not shown a better performance in HF diagnosis. To date, NPs represent the only circulating biomarkers with an undeniable application, and their use in clinical practice represents the first step for the diagnosis of HF.

What are the cutoffs?

Different cutoffs have been suggested for HFrEF and HFpEF (**Table 1**).

Heart failure with reduced ejection fraction Most recent ESC guidelines[1] and focused NPs practical guidance[46] identified specific cutoffs for chronic and acute setting. These cutoffs, despite being limited by several caveats, should be used in clinical practice in all patients suspected to have HF. Notably, a single-cutoff strategy or a multiple-cut-point strategy can be applied, with higher levels in the acute setting. In the first case, cutoffs have the role of ruling out HF. In the latter case, they also have a good performance in supporting HF diagnosis.

Considering the relationship between age, renal function, and NPs, in the multistep approach, NT-proBNP levels need to be age adjusted, whereas BNP levels do not, because NT-proBNP has a greater degree of correlation with age.

- Chronic setting
  - BNP: less than 35 pg/mL, HF unlikely; 35 to 150 pg/mL, gray zone; greater than 150 pg/mL, HF likely
  - NT-proBNP: less than 125 pg/mL, HF unlikely; 125 to 600 pg/mL, gray zone; greater than 600 pg/mL, HF likely
- Acute setting
  - BNP: less than 100 pg/mL, HF unlikely; 100 to 400 pg/mL, gray zone; greater than 400 pg/mL, HF likely
  - NT-proBNP: less than 300 pg/mL, HF unlikely; 300 to 450 pg/mL if <50 years old, 300 to 900 pg/mL if 50 to 75 years old, and 300 to 1800 pg/mL if >75 years old, gray zone; greater than 450 pg/mL or greater than 900 pg/mL, or greater than 1800 pg/mL if age greater than 75 years, HF likely
  - Midregional pro–A-type natriuretic peptide (MR-proANP): 120 pmol/L

Other cutoffs have been suggested by other experts.[15] However, all the investigators agreed that NPs should be used as continuous variables rather than as singular cutoffs.[1,15,46]

However, there are some categories of patients in whom these cutoffs need to be reconsidered. For instance, patients with altered kidney function (ie, when estimated glomerular filtration [eGFR] is <60 mL/min) are in need of different cutoffs. Therefore, although NT-pro-BNP, when corrected by age, does not need additional changes, for BNP, when eGFR is less than 60 mL/min, it is necessary to increase the rule-out cutoff to 200 pg/mL rather than 100 pg/mL.

Furthermore, there are some groups of patients (eg, obese patients or patients with fatigue as main symptoms) in whom levels are lower than expected. Therefore, in these patients, a lower cutoff (ie, <50 pg/mL BNP in presence of obesity) should be used. With regard to gender differences,[47–50] lower NP levels have been described in men compared with women.[51]

Further, in patients with HF with pre–left ventricle causes (eg, mitral stenosis and acute mitral regurgitation) or pericardial abnormalities, NP levels can be lower than expected or not increased at all, in particular at an early stage, reflecting the lack of the left ventricle overload. In addition, patients of African/African Caribbean origin, and those taking renin angiotensin aldosterone modulators and biotin supplements, also have lower-than-expected NP levels.[46]

Heart failure with preserved ejection fraction For the diagnosis of HFpEF, the ESC suggests the use of a new score (HFA-PEFF score), in which different biomarkers (circulating and imaging) are split into major and minor criteria and combined to strongly suggest or to exclude HFpEF diagnosis.[52] With regard to NPs, different cutoffs are applied depending on the presence or absence of atrial fibrillation (resulting in NP levels about 3 times higher). Notably, normal NP levels do not exclude HFpEF, especially in the presence of obesity.

- BNP:
  - Major criteria: greater than 80 pg/mL if sinus rhythm, greater than 240 pg/mL if atrial fibrillation
  - Minor criteria: 35 to 80 pg/mL if sinus rhythm, 105 to 240 pg/mL if atrial fibrillation
- NT-proBNP:
  - Major criteria: greater than 220 pg/mL if sinus rhythm, greater than 660 pg/mL if atrial fibrillation
  - Minor criteria: 125 to 220 pg/mL if sinus rhythm, 365 to 660 pg/mL if atrial fibrillation

Which is the clinical utility of natriuretic peptides?

Suggested cutoff levels for NPs have very high negative predictive values but very low positive predictive values (in particular in the acute setting). Therefore, they should be used to rule out HF (ie, patients with low levels are unlikely to have an

Box 1
Factors influencing natriuretic peptide levels

Factor that increase NP concentrations

Left ventricular dysfunction

Hypertrophic heart muscle diseases

- Infiltrative myocardiopathies
- Acute cardiomyopathies
- Inflammatory
- Valvular heart disease

Arrhythmias

- Atrial fibrillation
- Atrial flutter

Ischemic heart disease

- Acute coronary syndromes
- Coronary artery ischemia

Cardiotoxic drugs

- Anthracyclines and related compounds

Significant pulmonary or cardiopulmonary diseases

- Acute respiratory distress syndrome
- Pulmonary diseases with right-sided HF
- Obstructive sleep apnea
- Pulmonary hypertension
- Pulmonary embolism

Advanced age

Renal dysfunction

Critical illness

- Burns
- Stroke

High-output states

- Hyperthyroidism
- Cirrhosis
- Sepsis
- Anemia

Myocardial dysfunction

- Systolic dysfunction
- Diastolic dysfunction
- Fibrosis/scar
- Hypertrophy
- Infiltrative disease

Valvular abnormalities

- Mitral stenosis, regurgitation
- Aortic stenosis, regurgitation
- Tricuspid regurgitation
- Pulmonary stenosis

Increased cardiac chamber size

- Ventricular enlargement
- Atrial enlargement

Increased filling pressures

- Atrial
- Ventricular
- Pulmonary

Congenital abnormalities

- Shunts
- Stenotic lesions

Factors that do not show increased NP concentrations as expected

Pericardial constriction

Obesity

Cardiac tamponade

Flash pulmonary edema

HF diagnosis), whereas higher values support but do not make a diagnosis of HF per se (ie, not all patients with high NP levels are have HF). Indeed, several cardiac and noncardiac conditions cause increased NP levels (eg, atrial fibrillation and renal impairment) (**Box 1**).

However, NPs show good diagnostic accuracy in discerning HF from other causes of dyspnea: the higher the NP levels, the higher the likelihood that dyspnea is caused by HF. For example, in patients with preexisting chronic obstructive pulmonary disease, NPs can be valuable in diagnosing masked coexisting HF.

Therefore, in all patients presenting with dyspnea, the American guidelines recommend the measurement of NP levels to support the diagnosis or the exclusion of HF (class I, level of evidence [LOE] A),[2,3,11] with ESC guidelines on the same page.

Are natriuretic peptides interchangeable?

Both NPs (BNP and NT-proBNP) can be used in patient care settings. Overall, they have comparable diagnostic accurateness. However, it is important to consider that their values are not interchangeable. Although some conversion formulas have been suggested, these have no role in clinical practice.[53]

Notably, in patients having angiotensin receptor neprilysin inhibition (ARNI) treatment, BNP assessment is not reliable, and NT-proBNP levels should be considered.[46] In addition, even if both NPs are

reported to be predictive of outcome, BNP levels are confusing, particularly in the early phase of switching to and uptitrating ARNI.[54]

Imaging Biomarkers

Among imaging biomarkers, transthoracic echocardiography (TTE) is an indispensable tool in the diagnosis of patients with HF. American guidelines recommend that all patients with suspected, acute, or new-onset HF should have a TTE performed (class I, LOE C).[2,3] European guidelines recommend TTE for the assessment of myocardial structure and function in all patients with suspected HF in order to establish a diagnosis of HFrEF, HFmrEF, or HFpEF (class I, LOE C).[1]

Ejection fraction

EF is the most important parameter to evaluate, and the modified biplane Simpson rule is recommended by international guidelines. LV end-diastolic volume (LVEDV) and LV end-systolic volume (LVESV) should be obtained from apical 4-chamber and 2-chamber views. Based on EF, at least 2 different HF phenotypes can be identified:

- HFrEF: EF less than 40%
- HFpEF: EF greater than 50%

Patients with EF between 40% and 50% are defined as HFpEF borderline in American guidelines, whereas they are defined as a new entity in the latest European guidelines: HF-mrEF.[1]

In this context, the latest British Society of Echocardiography guidelines suggests that an EF of 50% to 54% should be considered as borderline low rather than normal LV EF.[55]

Diastolic function

Evaluation of diastolic function with TTE represents another fundamental imaging biomarker. The evaluation of diastolic function together with evaluation of the filling pressures, through the measurement of parameters such as mitral inflow during early diastole (E) recorded by pulsed Doppler between the tips of the mitral leaflets, the average of septal and lateral mitral annular early diastolic peak velocities (e') recorded by tissue Doppler, and its ratio (E/e'), are now considered mandatory for a basic TTE in patients with HF. Further, the end-systolic maximal volume of the left atrium indexed for body surface area (left atrial volume index) represents a surrogate marker of LV filling pressures.

Full description of these parameters is available[56]; however, for the purpose of the present article, the latest cutoff is reported as suggested by the ESC in the HFA-PEFF diagnostic algorithm for the diagnosis of HFpEF[52]:

- Septal and lateral mitral annular peak early diastolic velocity (e'; the main determinant of e' is LV relaxation)
  - Major criteria:
    - Septal e' less than 7 cm/s; or lateral e' less than 10 cm/s in patients aged less than 75 years
    - Septal e' less than 5 cm/; or lateral e' less than 7 cm/s in patients aged greater than 75 years
- Average septal-lateral E/e' ratio (reflecting the mean pulmonary capillary wedge pressure):
  - Major criterion: average septal-lateral E/e' ratio greater than or equal to 15
  - Minor criterion: average septal-lateral E/e' ratio 9 to 14
- Left atrial volume index
  - Major criteria:
    - Greater than 34 mL/m^2 in sinus rhythm
    - Greater than 40 mL/m^2 in atrial fibrillation
  - Minor criteria:
    - From 29 to 34 mL/m^2 in sinus rhythm
    - From 34 to 40 mL/m^2 in atrial fibrillation

With regard to left atrial volume index, the latest British Society of Echocardiography guidelines introduced a new borderline LA volume range of 34 to 38 mL/m^2.[55]

RISK STRATIFICATION

The most active research topic in biomarkers is the search for biomarkers able to identify patients with HF with worse outcomes. Again, NPs are the most validated, with current guidelines recommending measurement of NPs on admission (class of recommendation (COR) I, LOE A) and predischarge (COR IIa, LOE B) to assess HF prognosis.[3] In addition, it has been suggested that NPs alone have at least the same reclassification capability of HF clinical scores in predicting HF prognosis.[57] Therefore, this article does not discuss these biomarkers here, to avoid redundancy with other publications. However, other biomarkers showed their importance in risk stratification,[58] and, even if they have not yet entered into clinical practice, they warrant further discussion.

Therefore, a quick overview is provided here of the best-validated biomarkers (ie, troponins, ST-2, galectin 3) and the emerging biomarkers with most robust evidence investigated (ie, trimethylamine N-oxide [TMAO] and hormones) are briefly described.

Troponins

cTn are biomarkers of myocyte injury and are the cornerstone of the diagnosis of myocardial

infarction.[59] However, in patients with HF, circulating cTn can be detected regardless of the presence of coronary artery disease.[60] For instance, in different cohorts, it has been shown that high-sensitivity assays may detect cTn at greater than the 99th upper reference limit in about 50% (ASCEND-HF trial) or 90% (Relaxin in Acute Heart Failure [RELAX-AHF]) of patients presenting with acute HF.[61–63] cTn was shown to have prognostic value in acute HF. A retrospective analysis from the Acute Decompensated Heart Failure National Registry (ADHERE) suggested that detection of cTn was associated with an increased risk of in-hospital mortality, and the higher the increase, the higher the risk[64]; further, increased cTn level was associated with an increased risk of intensive care admission, mechanical circulatory support, and mechanical ventilation.[64] In the RELAX-AHF study, cTn levels were higher in patients with lower blood pressure, EF, and incidence of atrial fibrillation, and increased baseline cTn levels were associated with adverse cardiovascular events; with regard long-term outcomes (>6 months), inconclusive results were derived from trials. Although in RELAX-AHF they were associated, in the ASCEND-HF, cTn predicted in-hospital outcome but was not an independent predictor of long-term outcomes.[63]

With regard to the chronic setting, it is common to detect circulating cTn in CHF (CHF) and the likelihood increases depending on the sensitivity of the assay used.[65] Increased high-sensitivity cTn level was an independent risk factor for mortality and adverse outcome in patients with CHF in several studies. Troponin level increase may be useful in predicting mortality in patients with CHF and may have a complementary role to NP measurement in this context.[66–68]

In summary, cTn levels are associated with an increased risk of morbidity and mortality in both acute HF and CHF, providing incremental prognostic information. Therefore, it is recommended to assess (COR I, LOE A) baseline levels of cTn on admission to the hospital because of their utility to establish a prognosis in acutely decompensated HF.[3]

Source of Tumorigenicity 2

Source of tumorigenicity 2 (ST2), first described in 1989, is a member of the interleukin (IL)-1 receptor family, secreted in response to mechanical strain, therefore reflecting myocardial stretch.[69,70] The Pro-Brain Natriuretic Peptide Investigation of Dyspnea in the Emergency Department study showed that concentrations of sST2 were higher in patients who were dead at 1 year compared with survivors, with a cutoff of 20 pg/mL predicting mortality.[71] Recently, it has been shown that sST2 has a diurnal rhythm, with lower values in the morning.[72] Notably, sST2 was less influenced by age than NT-proBNP and cTn; the addition of age-specific and outcome-specific cutoffs of sST2 to NT-proBNP and cTn allowed a more accurate risk stratification than NT-proBNP alone or the combination of NT-proBNP and troponins.[73] No ethnicity differences have been shown,[74] whereas men showed higher levels than women.[49] A score based on ST has been proposed as a predictor of sudden cardiac death (ST2-SCD score).[75] Several findings suggest that sST2 granted a strong and independent predictive value for poor outcome in CHF.[76] As a cutoff, a level of 35 pg/mL in the outpatient setting seems to define low-risk patients.[77]

In summary, sST2 seems to be the most promising of the novel biomarkers, with the advantage of not being affected by age, renal function, body mass index, atrial fibrillation, and other comorbidities.[78] On one hand, ESC guidelines concluded that there is no definite evidence to recommend sST2 for clinical practice[1]; however, the American guidelines recommend the use of sST2 in addition to NPs in risk stratification (class IIB, LOE B).[3]

Galectin-3

Gal-3, also known as carbohydrate-binding protein 35, is a protein acting on fibroblasts and macrophages activation[79,80] with profibrotic actions. Women showed higher levels compared with men,[49] possibly related to fat mass.[81] It has been shown among patients with dyspnea referred to emergency departments that Gal-3 concentrations were associated with echocardiographic markers of ventricular dysfunction, with the ability to predict mortality in acute HF.[82]

As a prognostic biomarker, Gal-3 is an independent marker for outcome in HF, and seems to be particularly useful in patients with HFpEF.[83] Further it has been shown that higher Gal-3 levels at discharge after an acute decompensation predict later events[84] and early HF rehospitalization. However, a head-to-head comparison of fibrosis biomarkers ST2 and Gal-3 in CHF revealed superiority of ST2 to Gal-3 in risk stratification.[85]

In summary, similar to sST2, ESC guidelines concluded that there is no definite evidence to recommend Gal-3 for clinical practice, whereas the American guidelines recommend the use of Gal-3 in addition to NPs in risk stratification (class IIB, LOE B).[3]

Trimethylamine N-oxide

A novel pathophysiologic model of interest is the association between HF and the gastrointestinal (GI) system,[86,87] the so-called gut hypothesis.[88]

In recent years, TMAO, a gut microbiota–mediated metabolite originating from the metabolism of choline and carnitine, has been thought to be the missing link between the Western diet and cardiovascular disease risk,[89,90] shown to be a strong prognostic biomarker in both acute[91,92] and stable CHF,[93–98] with some intriguing geographic[99] and ethnicity differences.[100] Further, it seems to be a promising biomarker when combined with NPs in patients with HFpEF.[101]

To date, despite growing evidence, no guideline recommendations have been provided with regard to TMAO's role in HF.

Hormones

A growing body of evidence suggests that, in addition to neurohormonal hyperactivity, the imbalance between catabolic and anabolic hormonal axes depicts HF progression.[102–106] In this context, it has been shown that HF could be considered as a multiple hormone deficiency syndrome (MHDS).[107,108] Each component of MHDS (eg, growth hormone [GH]/insulinlike growth factor [IGF]-1 axes, thyroid hormones, androgens, insulin resistance) is associated with impaired functional capacity and poor clinical outcome.[102,105–113] Furthermore, HF prognosis has been shown to be strictly related to the number of coexistent deficiencies,[105] in line with the important relationship between hormones and the cardiovascular system shown in other clinical settings.[114–117] Notably, the prevalence of hormonal deficiency is increased in HF and consequently is related to poor cardiovascular performance and prognosis[107,108]; further, targeted hormone replacement therapy leads to an improvement in cardiovascular performance and outcomes.[118–122] These data provide strong evidence to suggest that the reversal of MHDS should be considered as an exciting and novel strategy in HF management.[123,124]

In this context, the latest version of the AHA guidelines on dilated cardiomyopathies suggest testing for thyroid disorders (LOE C) and GH/IGF-1 disorders (ie, acromegaly and GH deficiency) (LOE C) in patients with dilated cardiomyopathy and that appropriate therapy should be performed to correct a thyroid disorder (LOE C), acromegaly, or GH deficiency when present (LOE C).[125] Furthermore, thyroid hormone assessment should be considered in the initial HF assessment (LOE C).[2]

With regard to ESC guidelines, thyroid disorders have been confirmed and included as possible causes of dilated cardiomyopathies,[126] with thyroid-stimulating hormone recommended in the initial assessment of patients with HF (class I, LOE C).[1] For GH/IGF-1, acromegaly but not GH deficiency has been indicated as a possible causal factor of dilated cardiomyopathies,[126] whereas GH deficiency has been indicated as a possible cause of HF, both with reduced[1] and preserved EF.[52]

Although the American guidelines do not recommend the use of testosterone,[2] European guidelines cautiously suggest a possible role in the treatment of sarcopenia and cachexia.[1]

Emerging Biomarkers

Cell-free DNA

Circulating cell-free DNA (cfDNA) is genomic DNA released as result of cell death mechanisms.[127] Mostly investigated in oncology, it has been shown to be associated with cardiovascular risk factors,[128–131] cardiovascular diseases (eg, acute myocardial infarction and atrial fibrillation),[130] and with the early diagnosis of heart transplant rejection.[132–134] Recently, in 71 consecutive patients with chronic stable HFrEF and HFmrEF, cfDNA levels showed association with morbidity and mortality in HF; as a result, liquid biopsy may be considered an additional tool in risk stratification.[135]

Proenkephalin

Proenkephalin, a 243-amino-acid precursor for endogenous opioid peptides interacting with delta-receptors for morphine (ie, enkephalins), has been shown to have a role in HF because of its relationship with sympathetic activation.[136] Because of their short-half life, enkephalins are difficult to assess, whereas the product of the proenkephalin cleavage, the proenkephalin molecular form 119-159 (PENK) is stable in plasma and cerebrospinal fluid for at least 48 hours.[136] For this reason, together with the notion that proenkephalin is the predominant source of mature enkephalin, this peptide fragment could serve as a surrogate measurement of systemic enkephalin synthesis. With regard to HF, it has been suggested that higher PENK levels are associated with lower EF and kidney function, and poor outcome in both acute and chronic HFrEF.[137–140] Further, it has been shown that PENK levels are related to body mass index, diastolic dysfunction, and prognosis in HFpEF.[141]

Therefore, PENK represents an interesting emerging biomarker reflecting cardiac, glomerular, and tubular dysfunction, giving remarkable information about the intricate relationship among the opioid systems and HF.

Adrenomedullin

It has been suggested that adrenomedullin (ADM), a vasodilatory peptide originally described in

pheochromocytoma tissues and expressed in the heart, vasculature, and the kidneys, has positive inotropic, antiapoptotic, angiogenic, antiinflammatory, and antioxidant effects, acting as a protective factor in HF.[142] Several reports showed that plasma ADM levels are increased in HF, strictly related to HF severity.[143–145] Further, the midregional pro-ADM showed a good role in risk stratification.[146–148] In addition, it has been shown that bioactive ADM in plasma is associated with increased biventricular filling pressures in patients with advanced HF.[149] It has been shown that beneficial vasodilatory, diuretic, natriuretic, and positive inotropic effects are exerted by its acute administration in HF.[142]

MicroRNA

Recent evidence suggests that there are genome portions, previously known as junk DNA, that are transcribed and give rise to a large and heterogeneous family of RNA molecules with a variety of functions. These regulatory molecules seem to be a promising tool in diagnostics, therapeutics, and personalized medicine in HF.[150] For instance, angiogenic early-outgrowth cells from patients with CHF show reduced miR-126 and miR-130a expression levels, with subsequent impaired ability to improve cardiac function,[151] whereas expression levels of miR-126 and miR-508-5p in endothelial progenitor cells have been shown to be prognostic for CHF in patients with cardiomyopathy.[152] Moreover, several studies have shown that circulating microRNAs can react in a dynamic way in response to therapy, indicating potential efficacy of treatment.[153]

Fibroblast growth factor-23

High levels of fibroblast growth factor-23, a phosphaturic hormone regulating phosphate metabolism acting on kidney phosphate absorption and vitamin D production, have been shown to be related to clinical severity and adverse outcomes in HFrEF,[154] with a strict link with volume overload, worse renal function, and an independent association with less successful uptitration of guideline-recommended angiotensin-converting-enzyme inhibitors (ACEi)/angiotensin receptor blocker (ARB) therapy.[155] Notably, it also seems a promising biomarker for patients with HFpEF,[156] showing association with reduced exercise capacity and increased risk of death.[157]

PHENOTYPING

A recent research field of interest, fueled by the unknown HFmrEF group of patients previously considered to be in the gray zone by the ESC,[1] is focused on the possibility of identifying specific biomarkers for specific HF phenotypes.[158] Identifying the pathophysiologic pathways most involved in the different HF phenotypes would allow a better tailoring of management and treatment.

It has been shown that the 3 different HF phenotypes (ie, HFpEF, HFmrEF, and HFrEF), despite sharing some common features, are characterized by different pathophysiologic indices, different clinical comorbidities, different response to treatment, and different prognosis.[159] Therefore, the possibility of distinguishing peculiar biomarker pathways and different pathophysiologic routes involved, with the aim of identifying possible specific therapeutic targets, represents one of the possible uses of biomarkers.

General Features

It has been proved that HFpEF is characterized by myocardial dysfunction driven by a very high proinflammatory systemic state also affecting microvascular endothelium, with increased plasma levels of IL-6, tumor necrosis factor-α, sST2, and pentraxin 3 and resulting in an increase in reactive oxygen species (ROS) production.[160] In contrast, myocardial dysfunction, typical in HFrEF, is characterized by ROS production caused by cardiac ischemia, infections, or toxic agents.[160] As a result, these differences are documented by the different expression of biomarkers, particularly those related to myocardial stress (eg, NPs), myocardial injury (eg, troponins), fibrosis (eg, ST2), inflammation (eg, high-sensitivity C-reactive protein), and hematopoiesis (eg, hemoglobin). In particular, myocardial stress/injury is more pronounced in HFrEF, whereas HFpEF is more characterized by inflammation and fibrosis, after adjustment for several confounders (ie, comorbidities).[161,162]

Recently, a network analysis confirmed that HFrEF showed a peculiar biomarker profile related to cellular proliferation and metabolism, whereas HFpEF biomarker profiles are related to inflammation and extracellular matrix reorganization.[163]

Specific Biomarkers

- NPs: because NPs are released in the blood as a response to LV end-diastolic stress in patients with HFpEF, being wall stress counteracted by the typical hypertrophy of HFpEF, NP levels are less increased or normal in HFpEF.[52]
- Troponins: it has been shown that troponin levels are more increased in HFrEF than in HFpEF.[161,162] To date, no different cutoffs have been suggested in clinic practice.

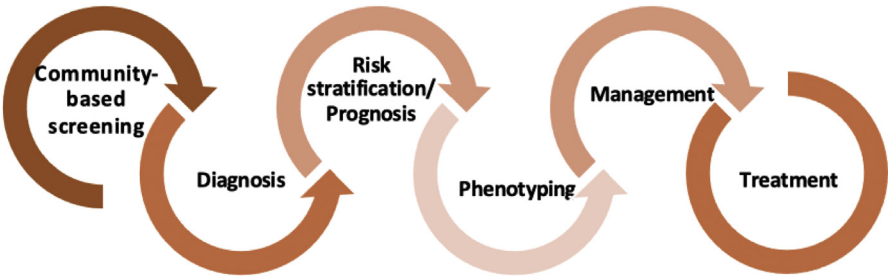

Fig. 3. The 'Biomarkers Continuum' in Heart Failure.

- ST2, galectin: ST2 and Gal-3are more increased in HFpEF.
- TMAO: several studies have shown the role of TMAO in HFrEF.[87,93–98] With regard to HFpEF, results are less consistent[95]; however, it has been shown that the combination of TMAO with NPs is the most valuable tool in this group of patients.[101]
- Hormones: the presence of a catabolic/anabolic imbalance in HF has been clearly shown in HFrEF (discussed next). Patients with HFpEF seem to have a lower prevalence of hormone deficiencies, however, a strong association with outcomes has been showed in HFpEF too.[108]

MANAGEMENT AND TAILORED TREATMENT

An emerging body of evidence suggests that serial measurement of biomarkers could have a positive role in HF management, allowing a tailored management approach. However, despite BNP measurements at discharge/follow-up (ie, serial measurements) showing prognostic association,[164] and BNP reduction in response to guideline-recommended HF treatment being reported to add value to tailoring risk, the proper role of serial NP measurements in clinical practice still remains a matter of debate.[164,165]

It has been clearly shown that HF treatment directly or indirectly affects processes related to increase of NP levels.[164,165] β-Blockers, ACEi/ARBs, magnetic resonance angiography, and cardiac resynchronization therapy lead to a reduction in NP levels.[166–170] Data from several studies showed that NP concentration 2 to 4 weeks after a therapy change gives the best prognostic value, suggesting that this can be considered an appropriate window to reassess NP values after medical titration.[171]

With regard to serial NP measurements, it has been shown that follow-up levels after treatment showed better association with adverse outcomes compared with baseline levels.[172–175] Further, it has been suggested that personalized scheduling of NP measurements performs similarly with respect to the prediction of recurrent events but requires fewer total measurements than fixed scheduling.[176,177]

Given these premises, several trials investigated the role of NP-guided HF management, with conflicting results. Some trials showed a clear benefit (eg, the Use of NT-proBNP Testing to Guide Heart Failure Therapy in the Outpatient Setting [PROTECT] trial,[178] the Systolic Heart Failure Treatment Supported by BNP [STARS-BNP][179] trial, and others[180,181]) or a positive trend (eg, the NT-proBNP-Assisted Treatment to Lessen Serial Cardiac Readmissions and Death [BATTLESCARRED] and the Trial of Intensified vs Standard Medical Therapy in Elderly Patients With Congestive Heart Failure [TIME-CHF] study) when a biomarker-guided HF treatment approach was used. However, other trials have shown neutral results (eg, the Swedish Intervention Study–Guidelines and NT-ProBNP Analysis in Heart Failure [SIGNAL-HF],[171] the Can Pro-brain-natriuretic Peptide Guided Therapy of Chronic Heart Failure Improve Heart Failure Morbidity and Mortality [PRIMA] study,[182] the Use of Peptides in Tailoring Heart Failure Project [UPSTEP],[183] and the Strategies for Tailoring Advanced Heart Failure Regimens in the Outpatient Setting: Brain Natriuretic Peptide Versus the Clinical Congestion Score [STARBITE] study[184]). In this context, although preliminary results of the GUIDE-IT trial showed guideline-directed medical therapy guided by NT-proBNP levels was not superior to guideline-directed medical treatment alone,[185] a following analysis showed that patients whose levels at follow-up decreased (NT-proBNP levels $\leq$1000 pg/mL) had better outcomes.[186] However, when results from several randomized clinical trials were combined, different metanalyses reported that HF management and treatment titration incorporating serial NPs levels was associated with a significant reduction of outcomes compared with usual care.[187–192]

Recently, an interesting study performed by the BIOSTAT-CHF consortium showed that uptitrating patients with HF based on biomarker values might

have resulted in fewer deaths or hospitalizations compared with a hypothetical scenario in which patients were uptitrated without considering biomarkers levels.[193]

With regard to cTn, it has been shown that serial measurements of cTn may have a value in the risk stratification of patients with CHF and perhaps intensification of treatment.[194,195]

Hormone-based markers, testifying to hormone deficiencies, can be directly considered as therapeutic targets, with replacement treatment showing benefit in HF cardiovascular performance and outcome.[118,120,122] Therefore, their serial measurements, after correction of specific hormone deficit, can be specifically used to guide treatment on top of HF guidelines. Second, in the BIOSTAT-CHF consortium, TMAO was not directly affected by medication uptitration, suggesting the need to search for other complimentary treatments to decrease its levels.[97] Neither of these 2 biomarkers has clinical approval; however, given the promising supporting literature, their status as promising emerging biomarkers to clinical translation is well deserved.

SUMMARY

In conclusion, beyond NPs, other well-known or emerging biomarkers are under investigation to provide useful clinical information in HF management, which is becoming ever more personalized and tailored. The most valuable approach seems to be the multiparameter approach, specifically when different types of biomarkers (eg, circulating and imaging) and biomarkers from different pathophysiologic pathways are combined. Further, in the present review, the authors have spotlighted that several biomarkers have different potential roles, identifying the concept of a biomarker continuum in HF (**Fig. 3**). However, further robust randomized trials are needed before novel biomarkers can enter clinical practice.

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CLINICS CARE POINTS

- Population screening with NPs followed by echocardiography is a promising approach; specifically, the measurement of NPs or cardiac troponins alone adds prognostic information to standard risk factors for predicting new-onset HF, with the measurement of emerging biomarkers (i.e. sST2, Gal-3, GDF-15, and markers of renal function) alone or in a multi-marker strategy, providing additional risk stratification; however there is still no evidence of cost-effectiveness to justify its adoption at a large scale.
- Biomarkers (circulating and imaging) have an essential role in HF diagnosis. Without doubt, NPs can be considered the gold standard circulating biomarkers with elevated levels helping to identify subjects in need of a further evaluation and lower levels helping to exclude HF diagnosis, regardless of phenotype.
- The most active research topic in the biomarker field is the search for biomarkers able to identify HF patients with a worse outcome. Again, NPs are the most validated, with current guidelines recommending measurement of NPs on admission and predischarge to assess HF prognosis. However, other biomarkers showed their importance in risk stratification (e.g. troponins, source of tumorigenicity 2, galectin-3, Trimethylamine N-oxide, hormones) and even if they have not yet entered into clinical practice, they warrant further discussion.
- A recent research field of interest is focused on the possibility of identifying specific biomarkers for specific HF phenotypes. Indeed, the possibility to identify pathophysiological pathways mostly involved in the different HF phenotypes would allow a better tailoring of management and treatment.

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