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BIOMARKERS OF HEART FAILURE (W H W TANG AND J GRODIN, SECTION EDITORS)

Clinical Phenotyping of Heart Failure with Biomarkers: Current and Future Perspectives

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Abstract

Introduction Heart failure (HF) is a complex clinical syndrome with diverse risk factors and etiologies, differing underlying pathophysiology, and large phenotypic heterogeneity.

Recent Findings Advances in imaging techniques coupled with clinical trials that targeted only in those with impaired left ventricular ejection fraction (LVEF) have largely shaped the current management strategy for HF that focuses predominantly in patients with systolic HF. In contrast, there are no effective treatments for HF with preserved ejection fraction (HFpEF). Instead of this "one-size-fits-all" approach to treatment, better precision to define HF phenotypic classifications may lead to more efficient and effective HF disease management.

Conclusion Integrating variables—including clinical variables, HF biomarkers, imaging, genotypes, metabolomics, and proteomics—can identify different pathophysiologies, lead to more precise phenotypic classification, and warrant investigation in future clinical trials.

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Introduction

The prevalence of heart failure (HF) continues to increase and has been associated with high mortality, recurrent hospitalization, and high economic burden [1, 2]. Despite advances in medical treatment, device therapies, and transplantation, the residual risks of HF remain unacceptably high [1, 2]. Most successful randomized controlled trials in the HF population have been conducted based on left ventricular ejection fraction (LVEF) cutoff points to confirm the presence of HF with reduced ejection fraction (HFrEF) [3, 4]. In fact, there have not been any proven therapies for HF with preserved ejection fraction (HFpEF) [2].

The syndrome of HF is incompletely understood. The 2013 ACCF/AHA guideline for the management of HF defined HF as "a complex clinical syndrome that results from any structural or functional impairment of ventricular filing pressure or ejection of blood" [2]. Clearly summarized from this document is the notion that diagnostic tools and treatment approaches for HF often lack precision, which has limited our advances in both mechanistic understanding and treatment approaches. Results of most clinical trials have been reported as an average treatment effect based on the entire population rather than the specific reduced LVEF population studied. The results of different risk factors and multiple etiologies, in combination with genetic and environmental factors, lead to different clinical presentations and diverse underlying pathophysiologies that are too complex to simply use LVEF cutoff points to define HFpEF or HFrEF. One risk factor, like reduced LVEF, is insufficient to identify a specific phenotype or underlying pathophysiology in a given patient [2, 5].

Successes in the field of oncology identified specific cancer phenotypes (e.g., type of cancer, gene expression of tumor cells, and histologic subtype from tissue biopsy) allowing the disease mechanisms to be reasonably well understood, therapeutic targets to be identified, and targeted treatments to be developed, for example, HER2-targeted therapy in HER2-positive breast cancer. In the field of HF, the role of targeted therapy remains unclear [6]. Therefore, well-defined HF phenotypes are needed to identify specific therapeutic targets and to better understand each patient's underlying biology and pathophysiology instead of the "one-size-fits-all" approach. This review will provide the current and future clinical potential of identifying unique clinical phenotypes (including biomarkers, imaging, genotype, metabolomics, and proteomics) that can ensure the application of precision medicine in HF.

Circulating Biomarkers

Over the last decade, over 6500 studies have been published in the field of HF biomarkers. Unfortunately, many of these reports had scantly described methods and studied heterogeneous HF phenotypes that have substantially limited our clinical translation [7]. Moreover, the majority of HF biomarker studies focused on prognosis prediction and confirmation of a HF diagnosis, a clinically defined entity with relatively subjective interpretation. Many of the studies utilized all-cause mortality as primary endpoint, which is relatively non-specific to the HF syndrome.

The use of biomarkers in HF research has provided a better understanding of the pathophysiology of the disease process, especially in animal models. Clinically however, only the presence of HFrEF provides the most robust biomarker in selecting patients with guideline-directed medical therapies. In contrast, the role of circulating biomarkers in tailoring individual therapy in the field of HF is lagging from other cardiovascular conditions. For example, cardiac troponin has been widely used to define acute coronary syndromes (ACS) and to gauge the benefit of invasive treatment in patients with ACS [8]. There is a need to identify candidate biomarkers that reflect a specific underlying pathophysiology and may have the potential to guide therapeutics. In this section, we will focus on the role of biomarkers in the following specific HF phenotypes: (1) myocardial stress, (2) myocardial injury, (3) myocardial fibrosis, and (4) cardio-renal dysfunction.

Biomarkers of Myocardial stress

The prototype HF biomarker is the natriuretic peptide (NP) family, specifically B-type natriuretic peptide (BNP) and its prohormone fragment, N-terminal pro-B-type natriuretic peptide (NT-proBNP). Discovered over 35 years ago and successfully integrated into clinical practice as HF biomarkers over the past 15 years, NPs are useful in diagnosing and risk stratification for patients with HF [2]. Several clinical trials have examined the hypothesis that NP-guided therapy, informed by serial measurements of NPs, supported clinical outcomes in patients with HF. However, use of this NP-guided therapeutic approach continues to be debated, since previous clinical trials showed inconsistent results [9–15] and were limited by significant heterogeneity in HF phenotypes, poorly defined treatment strategies prescribed to various NP concentrations targeted [16]. The prospective, multi-center, randomized controlled trial, guiding evidence-based therapy using biomarkers intensifies treatment in heart failure (GUIDE-IT, NCT01685840), is ongoing and might address these issues [17••]. Regardless, NPs will never be used alone and requires interpretation in the clinical context since other cardiopulmonary disorders and comorbidities are also associated with elevated BNP or NT-proBNP concentrations (e.g., ACS, arrhythmia, pulmonary hypertension, pulmonary embolism, renal disease, catabolic diseases, or advanced age). Therefore, there is ample room for novel HF biomarkers to identify specific phenotypes, unique pathophysiologies, and potential therapeutic targets.

ST2 is another myocardial stress protein that exists in both soluble form (sST2) and membrane-bound receptor form (ST2L) and is closely associated with left ventricular (LV) strain [18], fibrosis, and remodeling [19]. ST2 gene expression is triggered in the setting of cardiomyocyte or cardiac fibroblast stretch [20], although the cardiac origin of ST2 has not been established. The binding of interleukin-33 (IL-33), the functional ligand of ST2, to ST2L has favorable antifibrotic, antiapoptotic, and antiremodeling effects [19, 21]. These cardioprotective effects are blocked by high levels of sST2 because sST2 also binds IL-33, making it unavailable to bind to the ST2 receptor form [19]. In chronic HF outpatients, sST2 has potent prognostic value, both independently and as an additive to NT-proBNP and clinical risk scores [22]. In a head-to-head comparison of ST2 with galectin-3 (Gal-3), ST2 was shown to be better than Gal-3 in risk stratification and prognosis in patients with chronic HF [23]. Meanwhile in HFpEF, sST2 was more accurate than NPs in identifying the severity of diastolic dysfunction [24]. Because of this predictive power, the role for using serial levels of sST2 to guide therapy has been suggested for both acute and chronic HF [25, 26]. Meanwhile, sST2 levels in chronic HF patients fell after treatment with higher-dose beta-blockers, and that elevated sST2 concentrations (>35 ng/mL) can identify patients who may particularly benefit from higher doses of beta-blockers [27]. However, in the Valsartan HF trial (Val-Heft), treatment with valsartan reduced the rate of increase in sST2 concentration compared with placebo, and they observed that an increase in sST2 was independently associated with patient outcomes [28]. In another study, treatment with a mineralocorticoid receptor antagonist (MRA) had a greater benefit among acute HF patients who had high sST2 concentrations [29]. There is even promise in the acute HF setting, in which

sST2 levels (at a higher cutoff value) was an independent predictor of prognosis regardless of LVEF and was a stronger predictor of prognosis than NT-proBNP [30, 31]. Hence, the role of detecting activation of the IL-33/ST2 system merits further study for additional sources of targeted therapies.

Biomarkers of Myocardial Injury

The principle biomarkers of myocardial injury are two cardiac-specific troponins (cTn), cardiac troponin I, and cardiac troponin T (cTnI and cTnT, respectively). Cardiac troponins are widely used primarily for the diagnosis and management of ACS. Elevated cTn concentrations have also been shown to predict an adverse prognosis in both acute and chronic HF [2], but the mechanism controlling the release of cTn in HF remains unclear, and the release is generally considered to be secondary to myocardial injury (e.g., ischemia, necrosis, or apoptosis) [5]. The prevalence of elevated cTn in patients with HF was non-specific, with differences based on the sample population, clinical setting, and troponin assay used. For example, using a standard cTn assay, abnormally elevated cTn has been detected in about one-quarter of HF patients and was associated with a poorer prognosis [5]. While using a high-sensitivity assay (hs-cTn), abnormally elevated hs-cTn has been detected in virtually all patients with acute HF [32] and in a majority of patients of chronic HF [33]. Elevated cTn (using either a standard or high-sensitivity assay) is independently associated with an adverse prognosis in patients with HF. Importantly, acute HF may be precipitated by ACS; therefore, cTn measurements are recommended in all patients with acute HF to detect ACS and inform treatment [2]. However, specific therapies have not yet been identified to reduce the development or rise of cTn in HF patients, even though cTn can be more sensitive than standard imaging techniques to detect cardiotoxicity.

Biomarkers of Myocardial Fibrosis

Traditional research-based markers of myocardial fibrosis have been plagued with assay variability and lack of specificity. Galectin-3 (Gal-3) is a glycoprotein secreted by activated macrophages. Previous studies have shown that Gal-3 is associated with cell adhesion, inflammation, myocardial hypertrophy, fibroblast proliferation, and tissue fibrosis, all of which play an integral role in myocardial remodeling [33, 34]. Direct Gal-3 infusion results in myocardial fibrosis in HF [35], whereas genetic disruption and pharmacological inhibition of Gal-3 prevents myocardial fibrosis, remodeling, and subsequent HF development [36]. High Gal-3 levels (both systemic and myocardial) has been associated with cardiac remodeling and a poorer prognosis in patients with HF, with a stronger predictive value in HFpEF patients when compared with HFrEF patients [37–39]. Gal-3 was also associated with aldosterone-induced myocardial fibrosis in experimental

models [40], but MRA treatment failed to show a benefit when studied in chronic HFrEF patients [41]. Currently, the ability for Gal-3 to risk stratifies HF patients' rests on the diminishing propensity to recover with increasing levels. Specifically, patients with low plasma Gal-3 levels showed a benefit when treated with rosuvastatin [42] or valsartan [43] for HFrEF, while increasing galectin-3 was associated with worse outcome, independent of treatment, or NT-proBNP [38, 44]. Renal insufficiency is likely a major and often overlooked confounder for these observations, and it may still suffer from lack of specificity [45, 46].

Biomarkers of Cardio-Renal Dysfunction

Renal dysfunction (RD) is commonly associated with a poor prognosis in all phenotypes of HF [47]. Blood urea nitrogen (BUN) and creatinine levels are widely used as renal function markers, but they can vary non-specifically according to sex, age, race, illness, and muscle mass. Moreover, RD in HF patients with cardiorenal syndrome (CRS) is complex and multifactorial. Therefore, there is a need to identify candidate renal biomarkers for the early diagnosis, prognosis, and therapeutic target discovery in CRS.

Cystatin C (CysC) is a cysteine protease inhibitor that is ubiquitously produced at a constant rate, freely filtered, and neither secreted from nor reabsorbed into the bloodstream. CysC has been suggested as a newer more accurate way to calculate the estimated glomerular filtration rate (eGFR) than creatinine-based measures [48]. Moreover, an elevated CysC concentration was associated with poor prognosis in both acute and chronic HF [49]. Unfortunately, the ability of CysC to differentiate between specific mechanisms or phenotypes of CRS, or to serve as a therapeutic target in HF, has been less well established [49]. Furthermore, direct head-to-head comparison across various RD biomarkers in the acute HF setting suggested that BUN remained the strongest prognosticator [50].

Neutrophil gelatinase-associated lipocalin (NGAL) is a small protein found in neutrophil granules. Both urine and plasma NGAL levels are increased in response to tubular kidney injury and predict adverse prognosis in patients with HF, but the differences between urinary and plasma NGAL levels are what may help identify specific CRS phenotypes [49]. In acute decompensated heart failure (ADHF), the majority of patients demonstrated urine NGAL levels lower than acute kidney injury levels [51], while serum NGAL was strongly associated with eGFR and predicted RD development [52]. Clearly, serum and urine NGAL represents different physiologic aspects of renal dysfunction [53].

N-acetyl-β-D-glucosaminidase (NAG) is a lysosomal enzyme originating in proximal tubular cells and is indicative of proximal tubular damage when detected in the urine. Urine NAG predicted worsening renal function and mortality in

patients with chronic HF [49, 54]. However, the ability to use NAG to identify a specific CRS phenotype has been limited since NAG is also increased in diabetes and hypertension [49].

Kidney injury molecule-1 (KIM-1) is a glycoprotein expressed in the proximal renal tubule in kidney injury but not expressed in the normal kidney. KIM-1 levels were inversely associated with LVEF and directly associated with worsening HF symptoms (defined by NYHA class) among chronic HF patients without RD [55, 56]. Furthermore, plasma KIM-1 is associated with poor prognosis and worsening renal function in the acute HF setting, yet its prognostic value diminished following covariate adjustments [57]. Further study may determine whether the changes of KIM-1 with treatment can mirror improved renal function and prognosis in patients with HF.

The candidate biomarkers discussed above may each, with further research, help classify specific patients into distinct subgroups of HF phenotypes. Then, as each of these subgroups may respond differently to treatment, the candidate biomarkers may also aid in the development of tailored therapies. Ahmad et al. recently described three commonly used biomarker study designs, each with their own advantages and disadvantages: biomarker-stratified, enrichment, and biomarker strategy [8]. Importantly, each method can be combined or altered according to the research question. For example, although Gal-3 and ST2 have been approved by the Food and Drug Administration to predict prognosis in HF, the appropriate role of these two biomarkers is still limited because of the small numbers of studies to date. So, a future study could use the enrichment design to test the hypothesis that, in HFpEF patients, Gal-3/ST2 could have a potential role in distinct patient subgroups that might benefit from a specific treatment (e.g., MRA/anti-Gal3).

Multimarker Biomarker Strategies

Recently, there has been an interest in "multiple biomarker strategies" since each biomarker reflects a different pathophysiologic pathway, and the application of a single biomarker is insufficient to evaluate patients with HF. Therefore, multiple biomarker strategies provide the opportunity to evaluate multiple pathophysiologic pathways in patients with HF, such as myocardial stretch, myocardial fibrosis, myocardial injury, and cardiorenal. Combining different biomarkers that reflect different pathophysiologic insights integrate various aspects of the disease and provide a specific HF phenotype. In a study of chronic HFrEF patients, Ky et al.[58] used multiple biomarker panels, consisting of biomarkers that represented different pathophysiology pathways, to generate a multimarker score and found it significantly improved prediction of adverse events and appropriate reclassification of patients. Community-based populations showed that a multimarker score consisting of ST2, hs-cTn, GDF-15, high-sensitivity

C-reactive protein (hsCRP), and BNP provided incremental value in predicting onset of HF [59]. Recently, Sanders-van et al.[60], demonstrated that 15 biomarkers that reflect distinct pathophysiological pathways—such as NT-proBNP, hs-cTn, hemoglobin (a marker of hematopoiesis), ST2, hsCRP, and CysC—were expressed differently between HFrEF and HFpEF. These studies provide keys to the differing pathophysiological pathways in HFrEF and HFpEF. Therefore, biomarker data may help to identify subgroups of patients that respond to a specific drug and using multiple biomarkers to improve pathophysiological insight could lead to personalized HF therapy. Although these strategies are important to risk stratification, further studies are needed to determine the appropriate combinations of biomarkers that will allow for the classification of specific HF phenotypes as molecular "fingerprints" of the disease and the corresponding therapeutic implications.

Genotype

It has been increasingly recognized that subtypes of HF and cardiomyopathies may have a genetic basis, which is largely overlooked in the development of pharmacologic and device therapies for HF. The genetic contribution to cardiac structure and function is complex, which can lead to variable clinical phenotypes [61]. For example, hypertrophic cardiomyopathy (HCM), the most common inherited cardiomyopathy, is the result of one or more of 400+ disease-causing variants in 9 sarcomeric protein-encoding genes. Therefore, there is the potential for substantial phenotypic heterogeneity [61]. Recently, research strategies have focused on scanning the entire genome to search for "candidate genes" that are associated with specific diseases [5] or responses to specific HF therapeutics [62]. As HF is a complex syndrome, new sequencing techniques have the potential to identify patterns of genetic variants that may have an effect on HF development. While these findings can potentially yield mechanistic insights that can further guide therapeutic targets [63–67], discoveries in this area have yet to expand toward treatment decisions.

Micro-RNAs (miRNAs) are small endogenous non-coding RNAs that control gene expression by binding to targeted mRNAs. Recently, miRNAs have been detected in various body fluids, including the bloodstream, and may have potential as novel biomarkers. They have been associated with disordered cardiac structure and function and may have the potential to identify specific HF phenotypes and specific therapeutic targets [68]. Several studies have shown the potential of miRNAs as biomarkers in cardiovascular diseases. Cardiac-enriched miRNAs (e.g., miRNA-1, miRNA-208a/b, and miRNA-499) were significantly increased in plasma early after myocardial infarction (MI) or transcoronary ablation of septal hypertrophy [69], mimicking the kinetics of a

conventional myocardial injury biomarker like cTnT. These miRNAs are likely released from damaged cardiac myocytes into the bloodstream [69, 70]. Tijsen et al. showed that circulating miR423-5p are useful biomarkers for distinguishing HF in dyspnea patients [71]. Moreover, specific miRNAs have the potential to differentiate between HFrEF and HFpEF [72]. Matsumoto et al. found that p53-responsive miRNAs predicted subsequent HF in post-MI patients [73]. The absence of miRNA-22 in genetically altered mice was associated with reduced activity of SERCA2 in their myocytes, LV dilatation, and an impaired response to cardiac overload, all of which enhanced propensity to HF development [74]. Conversely, genetic deletion of miRNA-208a prevents pathological cardiac remodeling [75]. Therefore, the specific treatment-induced changes in the activity of mRNAs could have potentially therapeutic targets in HF.

Metabolomics

Metabolomics is the systematic study of circulating metabolites in order to understand the cellular processes that lead to their production. It has the potential to provide insights into the underlying molecular mechanisms of cardiovascular disease in general, and HF specifically [76]. As discussed previously, traditional HF biomarkers, even BNP and NT-proBNP, did not provide additional therapeutic target possibilities. Therefore, determining specific groups of circulating metabolites, labeling them as metabolic profiles, and using those profiles as biomarkers may lead to better prognostication and tailored treatments in specific subgroups of patients. In fact, several human clinical studies of metabolomics in HF have identified unique circulating metabolic profiles that differ between patients with HF and healthy subjects, as well as between different HF stages and HF phenotypes [77]. Recently, Cheng et al.[78] identified a panel of metabolites, including histidine, phenylalanine, spermidine, and phosphatidylcholine C34:4, which differentiated stage C HF patients from healthy control subjects with a diagnostic value similar to that of BNP. The values of this panel were significantly improved at 6 and 12 months in those patients who recovered from acute HF. In addition, they found that a separate panel of metabolites, consisting of the dimethylarginine/arginine ratio, butyrylcarnitine, spermidine, and total essential amino acid amount, provided significant prognostic value in patients with stages B and C HF. The prognostic value of this second metabolite panel was actually greater than that of BNP in the stage B and C HF patients.

Another strategy is to utilize targeted metabolomics to identify novel pathways relevant to cardio-renal disease progression. For example, our group has identified an obligatory role for gut microbes in trimethylamine N-oxide (TMAO) generation measured by mass spectrometry. Elevated TMAO was associated with more advanced LV diastolic dysfunction and portended poor long-term prognosis in a large cohort of patients with a history of chronic HF independent of cardiorenal indices [79, 80]. To date, evidence supports that HF is associated with metabolic dysfunction [81], yet the clinical utility of metabolomics in HF remains unclear because of differences in study design and statistical methodology and a diverse array of molecular profiles reflecting the heterogeneity of HF phenotypes. Therefore, further studies of metabolomics in HF could have the clinical utility of characterizing unique metabolic phenotypes in HF leading to the identification of specific patient subgroups that may respond better to certain treatment strategies. In addition, the combination of metabolomics with other "omic" biomarkers may offer a robust phenotypic classification system in HF.

Proteomics

Proteomics is the study of the large-scale expression, function, and interaction of all proteins present in the cell [82]. Proteomics analyses have developed from basic protein separation by two-dimensional electrophoresis to mass spectrometry (MS)-based approaches [82]. Proteomics differs from genomics since proteomics has dynamic variability that can reveal the details of functioning within the cell, but genomics is relatively constant. Therefore, proteomic technologies offer potential tools for examining alterations in protein expression in cardiovascular disease and HF that may provide insights into underlying cellular mechanisms. In HF, the proteomics approach may help discover novel biomarkers for diagnosis, prevention, and the identification of specific therapeutic targets. Because of the diverse complexity and highly dynamic range of protein expression, current research in proteomics remains insufficient for a comprehensive understanding of the cellular mechanisms underlying HF and, therefore, the ability to target therapeutics in HF syndrome [83]. There is a clear need for technologic advancements and further well-designed studies robust in proteomic analyses to identify specific proteins unique to specific HF phenotypes.

Advances in Multidimensional Analytical Approaches

Phenotypic heterogeneity is a major problem in contemporary HF patient populations with both HFpEF and HFrEF [84, 85]. Contemporary clinical trials in HF focus on specific populations selected by inclusion/exclusion criteria that do not take into account the heterogeneity of HF phenotypes, which can lead to a mixed phenotypic population and unproductive results. The increasing use of "omics" studies may have the potential to identify unique molecular signatures and targeted

Table 1 Studies investigating the impact of phenotype-based cluster analysis in heart failure

Source	Study population	Phenotypic variables and patient clusters	Clinical impact
Ahmed et al.	1619 of the 2331 patients with chronic HFrEF (LVEF ≤35%) from the HF-ACTION study	Using 45 clinical phenotypic variables included demographics, comorbidities, cardiac variables, exercise testing, and biomarkers. *Cluster 1* (n = 773): Oldest, Caucasian, Ischemic CMP, High comorbidity rate, Advanced disease based on CPET and biomarkers (e.g. lowest peak VO_2 levels and 6MWD; highest NT-proBNP, GAL-3, and ST2 levels). *Cluster 2* (n = 287): Youngest, African Americans, Non-ischemic CMP, Highest BMI, High rates of prior rehospitalization, Lower SES and QOL, Milder disease based on CPET and biomarker assessments. *Cluster 3* (n = 313): Caucasian, Ischemic CMP, High rates of rehospitalization, Lower SES and QOL, Advanced disease based on CPET and biomarkers, and Severe angina symptom. *Cluster 4* (n = 246): Caucasian, Highest percentage of females, Non-ischemic CMP, Highest SES and QOL, and Low rates of comorbidities.	Significant heterogeneity within large HFrEF cohort. *Cluster 1* had highest mortality *Cluster 4* had lowest mortality and hospitalization. *Clusters 2 and 3* had significant improvement in peak VO_2. *Clusters 1 and 2* had risk reduction from exercise training in death and/or HF hospitalization endpoint. *Cluster 4* had increased harm (50 to 62%) from exercise training.
Shah et al.	397 patients with HFpEF (LVEF >50%) from the outpatient clinic of the Northwestern University HFpEF program with internal validation cohort	Using 46 continuous phenotypic variables included demographics, ECG, and detailed structural and functional assessment from echocardiography, and laboratory testing. *Phenogroup 1* (n = 128): Younger patients, moderate diastolic dysfunction, and relatively normal BNP. *Phenogroup 2* (n = 120): Obese patient, more diabetic mellitus (DM), high prevalence of obstructive sleep apnea, worse LV relaxation and had highest fasting glucose. *Phenogroup 3* (n = 149): Oldest, more likely to have CKD, electric and myocardial remodeling, pulmonary hypertension, and RV dysfunction, and highest BNP.	Significant heterogeneity within HFpEF cohort. Step-wise significant increase in adverse events from *phenogroup 1 to 3*.
Kao et al.	4113 patients with HFpEF from I-PRESERVE study with validation usin the CHARM-PRESERVED	Using 11 categorical phenotypic variables included age, gender, BMI, AF, CAD, DM, Dislipidemia (DLD), valvular disease, alcohol use, renal dysfunction (RD), and hematocrit. *Subgroup A* (n = 601): Younger, 100% men, low rates of AF, RD and valvular disease, and more alcohol use, and lowest NT-proBNP. *Subgroup B* (n = 701): Younger, 96% women, low rates of AF, less RD and valvular disease, and more anaemia. *Subgroup C* (n = 322): Obese patients, more likely to have DM, DLD, CAD, anemia, and with more RD. *Subgroup D* (n = 1248):100% women, with average rates of DM, DLD, and obesity and RD *Subgroup E* (n = 760): 100% men, with lower BMI, high rate of AF and CAD. *Subgroup F* (n = 481): Oldest, 76% women with lower BMI and high rates of AF, valvular disease, RD, and anemia, and highest NT-proBNP. Note: Baseline LVEF and systolic blood pressure were similar between all subgroups.	Significant heterogeneity within HFpEF cohort. *Subgroups C and F* had the highest rate of adverse outcomes. *Subgroup B* had the lowest rate of adverse outcomes. *Subgroup C* shown evidence of possible benefit from irbesartan.

Abbreviation: *HF-ACTION* Heart Failure-A Controlled Trial Investigating Outcomes of Exercise Training, *I-PRESERVE* Irbesartan in Heart Failure with Preserved Ejection Fraction Study, *CHARM-Preserved* Candesartan in Heart failure: Assessment of Reduction in Mortality and morbidity, *LVEF* left ventricular ejection fraction, *HF* heart failure, *HFpEF* HF with preserved ejection fraction, *HFrEF* HF with reduced ejection fraction, *CMP* cardiomyopathy, *CPET* cardiopulmonary exercise testing, *6MWD* 6-min walk test, *QOL* quality of life, *SES* socioeconomic status, *VO₂* peak oxygen consumption per unit of time, *NT-proBNP* N-terminal pro-B-type natriuretic peptide, *GAL-3* Galactin-3, *BMI* body mass index, *ECG* electrocardiogram, *CKD* chronic kidney disease, *CAD* coronary artery disease, *AF* atrial fibrillation

treatment approaches and responses in HF. Therefore, the combination of omics biomarkers with other variables and the use of phenotype-based cluster analysis may allow for the characterization of more robust clinical phenotypes on the basis of underlying molecular biology. This will allow us to match more specific treatments with more specific patient subgroups, improving efficacy and cutting costs to patients and hospitals.

The advent of sophisticated phenotyping tools (e.g., cluster analysis) can improve phenotypic characterization in complex and heterogeneous syndromes such as HF. This analysis uses a combination of phenotypic variables to define subgroups based on differences (or similarities) in individually measured characteristics and clusters them, with strong associations between members of the same cluster and weak associations between members of different clusters. Cluster analysis using a big data-driven strategy has the potential to identify complex phenotypes and address the heterogeneity of the HF population. A recent study in HFrEF patients identified four patient clusters using a combination of 45 clinical variables including demographics, comorbidities, cardiac variables, cardiopulmonary exercise testing (CPET), and biomarkers. Each of these four clusters had differing prognoses as well as different responses to exercise training [85]. Similarly, a prospective study of 379 patients with HFpEF used 67 continuous phenotypic variables comprising demographics, and clinical, laboratory, electrocardiographic, and comprehensive echocardiographic findings identified 3 phenotypic classifications an unbiased phenotype mapping algorithm ("phenomapping") [84].

Retrospective analysis of existing datasets from completed HF clinical trials that used phenotype-based cluster analysis may provide insights into the underlying pathophysiology of HF and address phenotypic heterogeneity issues [86]. Unlike the above mentioned cluster analysis that used continuous variables, studies looking at dichotomous variables from large HFpEF trials identified six subgroups that differed significantly in profiles of key characteristics and event-free survival. Interestingly, only those characterized by a high prevalence of obesity, hyperlipidemia, diabetes mellitus, anemia, and renal insufficiency, had a nominally improved outcome with irbesartan (hazard ratio 0.72, $P = 0.046$).

All three of the abovementioned studies used the machine learning algorithm to define clusters of patients based on phenotypic variables (phenotype-based cluster analysis), to classify a heterogeneous HF population into more homogeneous clusters. They were also able to identify subgroups that significantly differed in characteristics and prognoses (Table 1). However, there are several limitations in this approach, especially the fact that these three studies did not include omics, such as genomics biomarkers, as phenotypic variables in the cluster analysis. Since it is unclear whether patients stay within their assigned cluster over time, the clinical applicability of cluster analysis to identify subgroups that will be responsive to specific treatments remains unclear.

Future Outlook

Future advances in therapeutic management of HF will rely heavily on departing from the trajectory of drug and device development over the past four decades: (1) the need to discover (and visualize) novel therapeutic targets that are
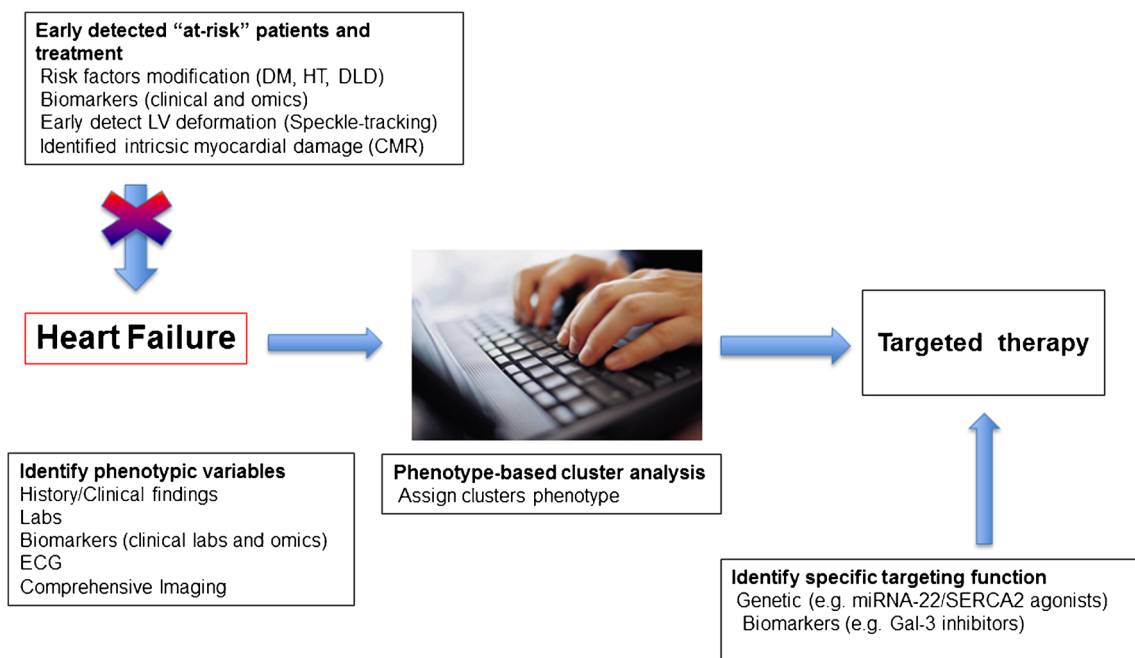
Fig. 1 Future outlook to identify patients with heart failure from phenotype-based cluster analysis or specific targeting function to targeted therapy

currently overlooked; (2) the need to improve precision in diagnostic and prognostic criteria to identify patients that may benefit from prioritizing specific therapeutic options, especially at early stages of the disease; and (3) to tackle heart failure before it develops.

Identify Novel and Specific Therapeutic Targets

Specific targeting of genetic or biomarker functions using specific inhibitors (e.g., direct Gal-3 inhibitors or anti-mRNAs) may be another useful approach to identify individual HF patients who may respond better to targeted treatment. This "proof-of-concept" should be incorporated into prospective studies, such as cluster-based or genotype-based RCTs, to identify individuals or specific subgroups of HF patients who may respond to targeted treatment. Like oncologic therapeutics, future clinical trials should account for the heterogeneity of HF when considering study design and should consider subgroup analysis to identify a specific HF phenotype that may respond to a particular treatment. It is important to recognize that few if any genetic polymorphisms have been clinically indicated to benefit from identifying targeted treatment. Nevertheless, this does not preclude the potential benefit of targeting specific genotypes for drug efficacy or to tailor drugs based on their pharmacokinetic profiling.

Improving Diagnostic Precision by Better Identify Phenotypic Variables

The underlying comorbidities and phenotypic heterogeneity in HFpEF are likely much greater than in HFrEF [2, 87], which may be a key reason for a lack of positive result in many large clinical trials. Improved phenotypic classification of the HF syndrome using a combination of variables, such as demographics, comorbidities, biomarkers, comprehensive imaging modalities, and omics approaches (genomics, proteomics, and metabolomics), is likely to aid in identifying more homogeneous HFpEF phenotype and the matching of appropriate targeted treatment. In fact, such analyses may also reveal that subsets of HFpEF may have absolutely no cardiac relevance at all.

Preventing Heart Failure by Targeting At-Risk Population

There have been major efforts to raise awareness among at-risk patients to prevent HF, including a reclassification of staging to denote a concept of disease progression with antecedent at-risk profiles [2]. Increasing attention has been focused on modifiable risk factors and the development of risk scores to help predict the incidence of HF and provide time to implement prevention strategies [2]. There is an unmet need to predict risk at an earlier time point, and HF risk has yet to be determined by genomic sequencing. There is also a major logistic and knowledge gaps in instituting more timely intervention of promising approaches. For example, the use of CMR (both T1 mapping and LGE) to identify intrinsic myocardial damage and arrhythmogenesis [88, 89]. There is also evidence to support that targeting rise in NP with therapeutic adjustments is associated with better outcomes and lower rates of detecting cardiac dysfunction over time [90].

Conclusions

There is an immediate need to refine the clinical use of current treatment options in order to significantly reduce iatrogenic adverse consequences (i.e., not to assume that current approaches are already optimal). In the future, the identification of robust phenotypic variables, in combination with advanced analytic approaches using computer algorithms, will likely assign HF patients to a unique cluster phenotype—a sort of "cluster fingerprint"—that may respond better to targeted treatment (Fig. 1). In the meantime, important building blocks for such precision medicine needed to be established, with a departure from the current conceptual framework.

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