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Perspective

Small Peptide, Large Implications: Endotrophin in Heart Failure with Preserved Ejection Fraction

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Heart failure with preserved ejection fraction (HFpEF), the most prevalent type of heart failure (HF), is associated with significant morbidity and mortality and a high rate of hospitalizations. HFpEF incidence is projected to continue to rise over the coming decades, primarily due to the increasing prevalence of multiple risk factors that contribute to the development of this syndrome. Obesity, low cardiorespiratory fitness, hypertension, and chronic kidney disease (CKD) have emerged as crucial elements in the pathogenesis of the syndrome. Obesity, in particular, can trigger and potentiate the systemic inflammatory state that is now recognized as central to the pathogenesis of HFpEF. In HFpEF, a range of cardiovascular changes are observed at the cellular and molecular level, including endothelial dysfunction, cardiomyocyte hypertrophy, abnormal calcium handling, microvascular rarefaction, interstitial fibrosis, and excessive collagen deposition (1).

A biomarker to assist in diagnosis and risk stratification of HFpEF would improve patient evaluation and management and enable clinical and preclinical research to impact the course of this syndrome. The ideal biomarker would reliably aid in the diagnosis of HFpEF at early stages, guide medical management, and predict disease complications. However, the quest to identify a biomarker with these characteristics has proven elusive as most previous biomarker candidates have fallen short of reaching the clinical stage. Of the biomarkers currently used in the clinic, B-type natriuretic peptide and N-terminal pro-B-type natriuretic peptide (NT-proBNP) support the diagnosis of HFpEF in symptomatic individuals and predict adverse outcomes but cannot distinguish HFpEF from heart failure with reduced ejection fraction (HFrEF). Moreover, levels can be normal in up to 30% of patients with HFpEF; are commonly suppressed in patients with high body mass; and are increased substantially in the setting of CKD, pulmonary hypertension, and atrial fibrillation (1). In asymptomatic individuals, small elevations in NT-proBNP and high sensitivity troponin T and I identify individuals at risk for HF but cannot accurately differentiate future risk of HFpEF from HFrEF.

Newer biomarkers, such as soluble suppressor of tumor immunogenicity 2, galectin-3, growth differentiating factor-15, and pentraxin-3, are either not sufficiently specific for HFpEF or have not been validated in large patient cohorts. However, collagen-derived peptides have emerged recently as potential stakeholders in HFpEF. Collagen type I and type III cleavage peptides have previously been associated with the development of HFpEF (1). Now, Chirinos et al. have shown that a collagen type VI derived small peptide, called endotrophin (ETP), is linked to HFpEF (2).

Most of what is known about ETP biology comes from work in the metabolism and cancer fields. ETP is a soluble 77 amino acid peptide derived from the alpha-3 chain carboxyl terminal of collagen type VI (3). In both obesity and cancer, there is active extracellular matrix remodeling, leading to upregulation and processing of many extracellular matrix proteins, including collagen type VI. In obesity, there is massive adipocyte expansion with hypertrophy and poor vascularization leading to a hypoxic microenvironment. Recent work has identified hypoxia-induced matrix metalloproteinases 2, 9, and 16 as upstream cleaving enzymes of ETP, which could explain increased ETP expression in obese adipose tissue (4). Similarly, human breast and colon cancer tissues have increased expression of ETP compared with noncancerous tissue. Moreover, blocking antibodies directed against ETP lead to reduction in tumor size and increased susceptibility to chemotherapy agents in breast cancer models. These effects are partly mediated by inhibition of the fibrotic and angiogenic actions of ETP and a reduction in immune cell infiltration (5). There are, however, no data on cardiac ETP expression either under physiological conditions or in HF. Thus, it remains to be determined if ETP is produced by cardiac fibroblasts or epicardial/pericardial adipocytes. At the clinical level, multiple studies have linked circulating ETP levels to chronic disease, including CKD and HF. However, no clinical studies had previously evaluated plasma ETP in HFpEF. (Fig. 1)

In the recent study published in *NEJM Evidence*, Chirinos et al. performed a multifaceted evaluation of ETP as a HFpEF biomarker. First, they assessed the

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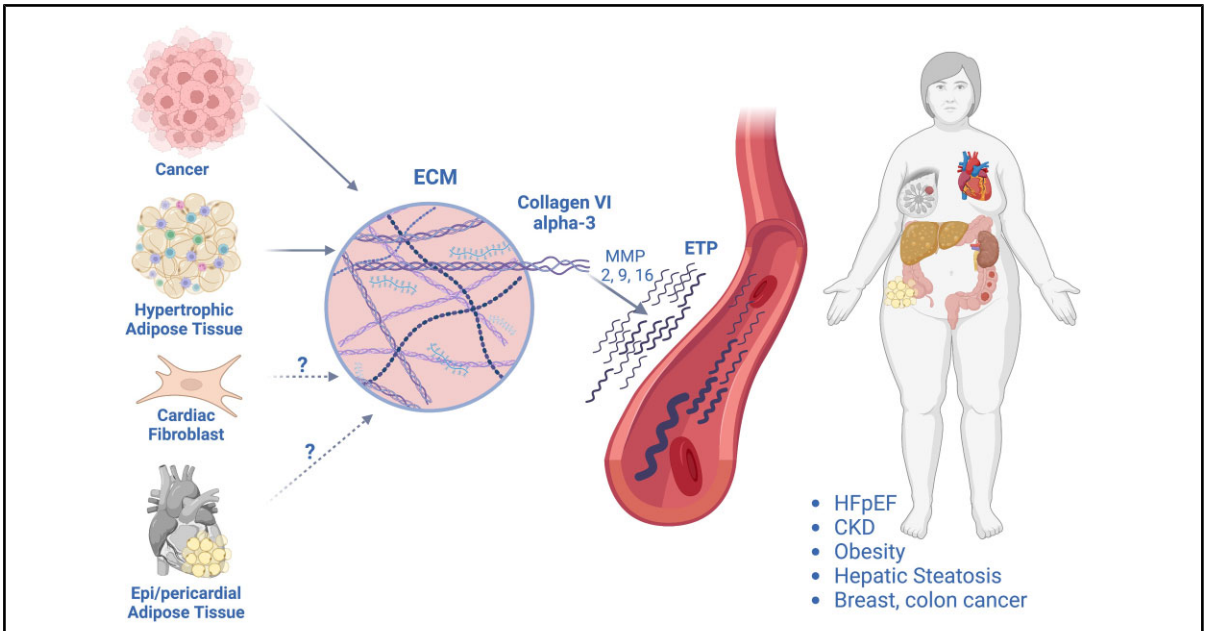

Fig. 1. Endotrophin in disease states. ETP is a cleavage product from the alpha-3 chain of collagen type VI. It is cleaved by MMP 2, 9, and 16 and released into the systemic circulation mainly from obese hypertrophic adipose tissue and cancerous tissue. It is unknown if cardiac tissues (fibroblasts or adipocytes) produce ETP. Plasma ETP levels are elevated in several inflammatory states, including HFpEF, CKD, obesity, hepatic steatosis, and breast and colon cancer. Abbreviations: ECM, extracellular matrix; MMP, matrix metalloprotease.

association of baseline circulating ETP levels with HF outcomes in a series of analyses using biorepositories from 7 clinical trials and observational cohorts that enrolled patients with HFpEF. Blood samples from the Treatment of Preserved Cardiac Function HF with an Aldosterone Antagonist (TOPCAT) trial (n = 205) served as the primary analysis cohort, and samples from 605 individuals from 6 other small prospective HFpEF cohorts were used for replication, with an individual participant meta-analysis performed across these 6 small studies. All but one study measured ETP in plasma with the other using serum. Patients with higher ETP had more advanced CKD and HF symptoms, higher body mass index, and a greater prevalence of diabetes. In multivariable analyses adjusting for the MAGGIC risk score (a prognostic model including 13 clinical variables that has been validated in HFpEF), ETP associated with both death and the composite of death and HF hospitalization in TOPCAT, with findings replicated in the meta-analysis. In TOPCAT, additional adjustment for NT-proBNP resulted in only minimal attenuation of the associations between ETP and adverse HF outcomes.

In another set of analyses, the authors evaluated associations of ETP with outcomes in 1642 patients with HFrEF. Although ETP levels were also associated with risk of death and death or HF-related hospital admission in HFrEF, the magnitude of the association was smaller than had been observed in the HFpEF studies and only marginally significant in models adjusting for the MAGGIC risk score and NT-proBNP. In a final set of analyses including 4 studies, the authors compared ETP levels among patients with HFpEF, HFrEF, and non-HF controls. In general levels were higher in patients with HFpEF vs those with HFrEF and the non-HF controls. However, there was some inconsistency in the findings, with one study of patients with severe HF reporting similar levels between patients with HFpEF and HFrEF. Distributions overlapped between the HFpEF and control groups, and no diagnostic performance metrics were reported. Finally, the influence of comorbidities such as obesity and renal failure on diagnostic performance was not considered. Thus, considerable additional studies are needed regarding the potential diagnostic utility of ETP in HFpEF.

A major strength of the Chirinos et al. study is the inclusion of multiple replication cohorts that show consistent findings and thus increase confidence in the validity of the results. However, it is difficult to know how specific ETP levels are to HFpEF as opposed to "the company HFpEF keeps" such as obesity, decreased renal

clearance, or other inflammatory processes. Such information will be crucial to determine whether ETP has value beyond prognostic assessment and can be used to guide specific therapeutic decisions in a precision-medicine framework. Future studies should aim to characterize ETP levels in HFpEF with and without these inflammatory disease processes, by including stratification within the study or stringent inclusion/exclusion criteria. While a HFpEF specific *diagnostic* biomarker would be of immense clinical value, the road for ETP here will be even more arduous, given the "promiscuity" of the pathophysiological roles and multiple tissue sources of this peptide.

Several additional steps will be needed to better understand the future role of ETP as a prognostic biomarker. Deeper evaluation of preanalytical, analytical, and biological variation in ETP is needed. Studies incorporating serial measurements of ETP should be performed, both to identify determinants of change and to investigate whether changes in ETP are of prognostic significance. Demonstration that changing levels of ETP are associated with HF outcomes would support further investigation of a role for ETP in disease monitoring or as a therapeutic target.

Despite the work left to do, the Chirinos et al. paper is a powerful introduction of a new biomarker for a clinical area of high need. Their studies and results highlight the complexity of HFpEF and the challenges for biomarker development in this syndrome. Like all good research, these studies raise more questions including: How specific is ETP as a biomarker for HFpEF when other comorbidities are accounted for? What is the role of ETP in predicting HFpEF development in healthy or at-risk individuals? Is ETP just a biomarker or does it contribute to the pathogenesis of HFpEF? If properly addressed, the answers to these questions could reveal large implications in HFpEF for this small collagen peptide.

Nonstandard Abbreviations: HFpEF, heart failure with preserved ejection fraction; HF, heart failure; CKD, chronic kidney disease; NT-proBNP, N-terminal pro-B-type natriuretic peptide; HFrEF, heart failure with reduced ejection fraction; ETP, endotrophin.

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