

## REVIEW ARTICLE

## MEDICAL PROGRESS

## Biomarkers in Heart Failure

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**H**EART FAILURE, A MAJOR AND GROWING PUBLIC HEALTH PROBLEM, APPEARS to result not only from cardiac overload or injury but also from a complex interplay among genetic, neurohormonal, inflammatory, and biochemical changes acting on cardiac myocytes, the cardiac interstitium, or both. An increasing number of enzymes, hormones, biologic substances, and other markers of cardiac stress and malfunction, as well as myocyte injury — collectively referred to as biomarkers — appear to have growing clinical importance. Although biomarkers include genetic variants, clinical images, physiological tests, and tissue-specimen biopsies, this review focuses on biomarkers derived from the blood or urine other than serum levels of hemoglobin, electrolytes, liver enzymes, and creatinine, which are routinely determined as part of clinical care.

Morrow and de Lemos<sup>1</sup> have set out three criteria a biomarker should fulfill to be useful clinically. First, accurate, repeated measurements must be available to the clinician at a reasonable cost and with short turnaround times; second, the biomarker must provide information that is not already available from a careful clinical assessment; and finally, knowing the measured level should aid in medical decision making.

Although relatively few of the biomarkers discussed in this review satisfy all three criteria, many appear to provide important information regarding the pathogenesis of heart failure or the identification of subjects at risk for heart failure or appear to be useful in risk stratification, in the diagnosis of heart failure, or in monitoring therapy. Many biomarkers may be risk factors themselves and therefore may be potential targets of therapy. Although no specific classes for biomarkers are accepted, I propose that they could be divided into six categories, as well as a seventh category of new biomarkers that have not yet been fully characterized (Table 1).

## INFLAMMATION

Inflammation is important in the pathogenesis and progression of many forms of heart failure, and biomarkers of inflammation have become the subject of intense inquiry (Table 2).<sup>3</sup> Interest in the presence of inflammatory mediators in patients with heart failure began in 1954, when a crude assay for C-reactive protein, a protein that appears in the serum in a variety of inflammatory conditions, became available. A study published in 1956 reported that C-reactive protein was detectable in 30 of 40 patients with chronic heart failure and that heart failure was more severe in those with higher levels of C-reactive protein.<sup>4</sup> Subsequently, C-reactive protein was described as an acute-phase reactant synthesized by hepatocytes in response to the proinflammatory cytokine interleukin-6.<sup>5</sup> The use of C-reactive protein as a biomarker became more common when a low-cost, high-sensitivity test for C-reactive protein was developed.<sup>6</sup> Multivariate analysis indicated that increased C-reactive protein level is an independent predictor of adverse outcomes in patients with acute or

**Table 1. Biomarkers in Heart Failure.**

<b>Inflammation</b> *†‡
C-reactive protein
Tumor necrosis factor $\alpha$
Fas (APO-1)
Interleukins 1, 6, and 18
<b>Oxidative stress</b> *†§
Oxidized low-density lipoproteins
Myeloperoxidase
Urinary biopyrrins
Urinary and plasma isoprostanes
Plasma malondialdehyde
<b>Extracellular-matrix remodeling</b> *†§
Matrix metalloproteinases
Tissue inhibitors of metalloproteinases
Collagen propeptides
Propeptide procollagen type I
Plasma procollagen type III
<b>Neurohormones</b> *†§
Norepinephrine
Renin
Angiotensin II
Aldosterone
Arginine vasopressin
Endothelin
<b>Myocyte injury</b> *†§
Cardiac-specific troponins I and T
Myosin light-chain kinase I
Heart-type fatty-acid protein
Creatine kinase MB fraction
<b>Myocyte stress</b> †‡§¶
Brain natriuretic peptide
N-terminal pro-brain natriuretic peptide
Midregional fragment of proadrenomedullin
ST2
<b>New biomarkers</b> †
Chromogranin
Galectin 3
Osteoprotegerin
Adiponectin
Growth differentiation factor 15

\* Biomarkers in this category aid in elucidating the pathogenesis of heart failure.

† Biomarkers in this category provide prognostic information and enhance risk stratification.

‡ Biomarkers in this category can be used to identify subjects at risk for heart failure.

§ Biomarkers in this category are potential targets of therapy.

¶ Biomarkers in this category are useful in the diagnosis of heart failure and in monitoring therapy.

**Table 2. Deleterious Effects of Biomarkers of Inflammation in Heart Failure.\***

<b>Known</b>
Left ventricular dysfunction
Pulmonary edema
Cardiomyopathy
Decreased skeletal-muscle blood flow
Endothelial dysfunction
Anorexia and cachexia
<b>Potential</b> †
Receptor uncoupling from adenylate cyclase
Activation of the fetal-gene program
Apoptosis of cardiac myocytes

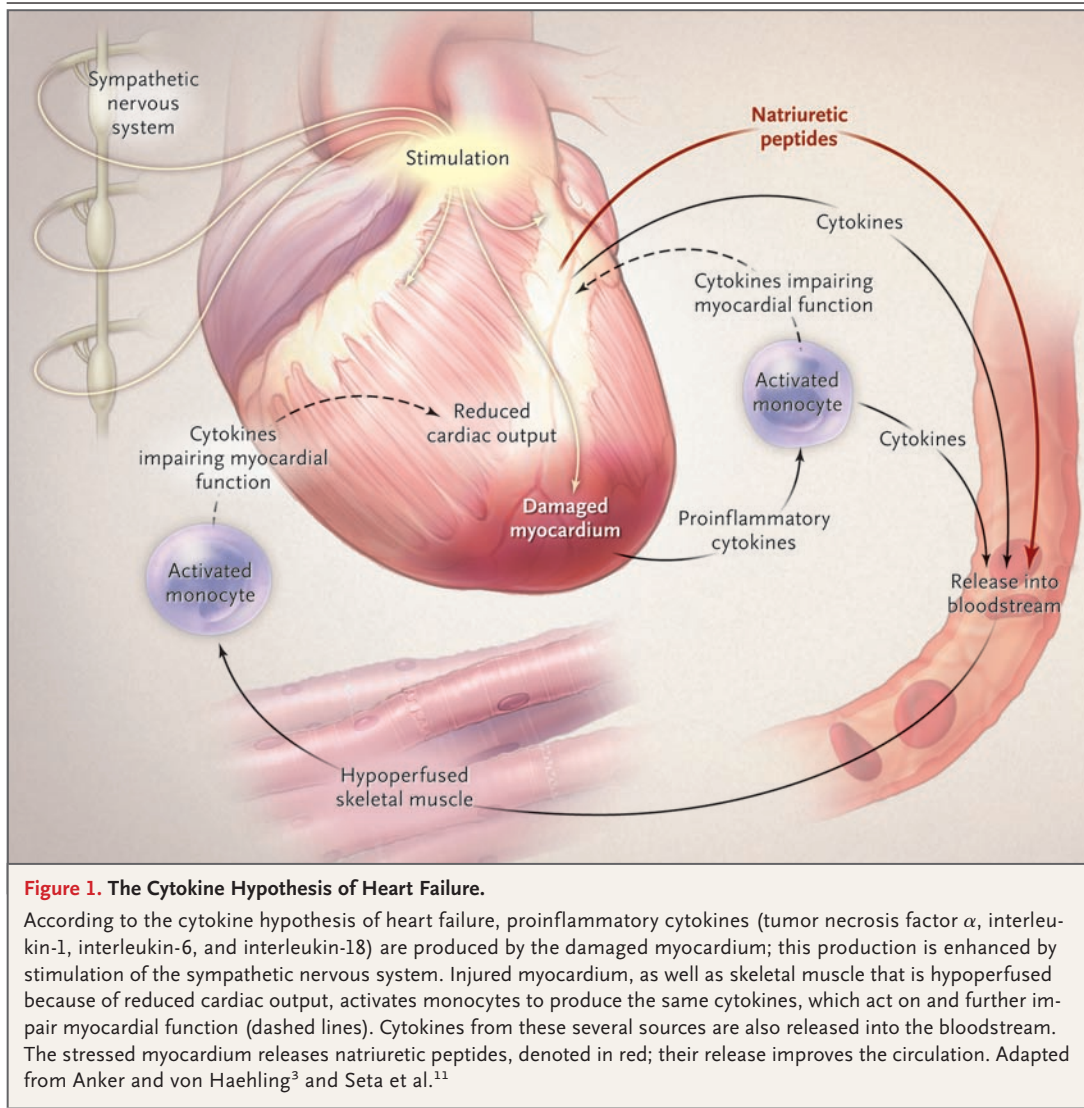
\* Adapted from Mann.<sup>2</sup>

† Effects shown in animals but not yet in humans.

chronic heart failure.<sup>7</sup> In the Framingham Heart Study, for example, C-reactive protein (as well as the inflammatory cytokines interleukin-6 and tumor necrosis factor  $\alpha$  [TNF- $\alpha$ ]) was noted to identify asymptomatic older subjects in the community who were at high risk of the future development of heart failure.<sup>8</sup>

Further, C-reactive protein has been shown to exert direct adverse effects on the vascular endothelium by reducing nitric-oxide release and increasing endothelin-1 production, as well as by inducing expression of endothelial adhesion molecules.<sup>9</sup> These findings suggest that C-reactive protein may also play a causal role in vascular disease and could therefore be a target of therapy. However, elevated levels of C-reactive protein lack specificity; for example, acute and chronic infection, cigarette smoking, acute coronary syndromes, and active inflammatory states are frequently associated with elevated levels of C-reactive protein.

In 1990, Levine et al. described elevated levels of circulating TNF- $\alpha$  in patients with heart failure.<sup>10</sup> TNF- $\alpha$  and at least three interleukins (interleukins 1, 6, and 18) are considered to be proinflammatory cytokines and are produced by nucleated cells in the heart.<sup>3</sup> The cytokine hypothesis of heart failure proposes that a precipitating event — such as ischemic cardiac injury — triggers innate stress responses, including elaboration of proinflammatory cytokines, and that the expression of these cytokines is associated with deleterious effects on left ventricular function and accelerates the progression of heart failure<sup>11</sup> (Fig. 1). Proinflammatory cytokines appear to



cause myocyte apoptosis and necrosis; interleukin-6 induces a hypertrophic response in myocytes,<sup>11</sup> whereas TNF- $\alpha$  causes left ventricular dilatation, apparently through activation of matrix metalloproteinases. Interleukin-6 and TNF- $\alpha$  levels could be used to predict the future development of heart failure in asymptomatic elderly subjects in one study,<sup>12</sup> though blockade of TNF- $\alpha$  has not resulted in clinical benefit in patients with heart failure.<sup>3,13</sup>

Fas (also termed APO-1) is a member of the TNF- $\alpha$  receptor family that is expressed on a variety of cells, including myocytes. When Fas is activated by the Fas ligand it mediates apoptosis and plays an important role in the development and progression of heart failure. Elevated serum

levels of a soluble form of Fas have been reported in patients with heart failure, and high levels are associated with severe disease.<sup>14</sup> The inhibition of soluble Fas in animals reduces postinfarction ventricular remodeling and improves survival.<sup>15</sup> Pharmacologic efforts to reduce Fas levels are still in their infancy but may represent a new direction in the treatment or prevention of heart failure. Indeed, the administration of a nonspecific immunomodulating agent — pentoxifylline<sup>16</sup> or intravenous immunoglobulin<sup>17</sup> — reduces plasma levels of Fas as well as C-reactive protein and is reported to improve left ventricular function in patients with ischemic or dilated cardiomyopathy.

Thus, measurements of C-reactive protein, in-

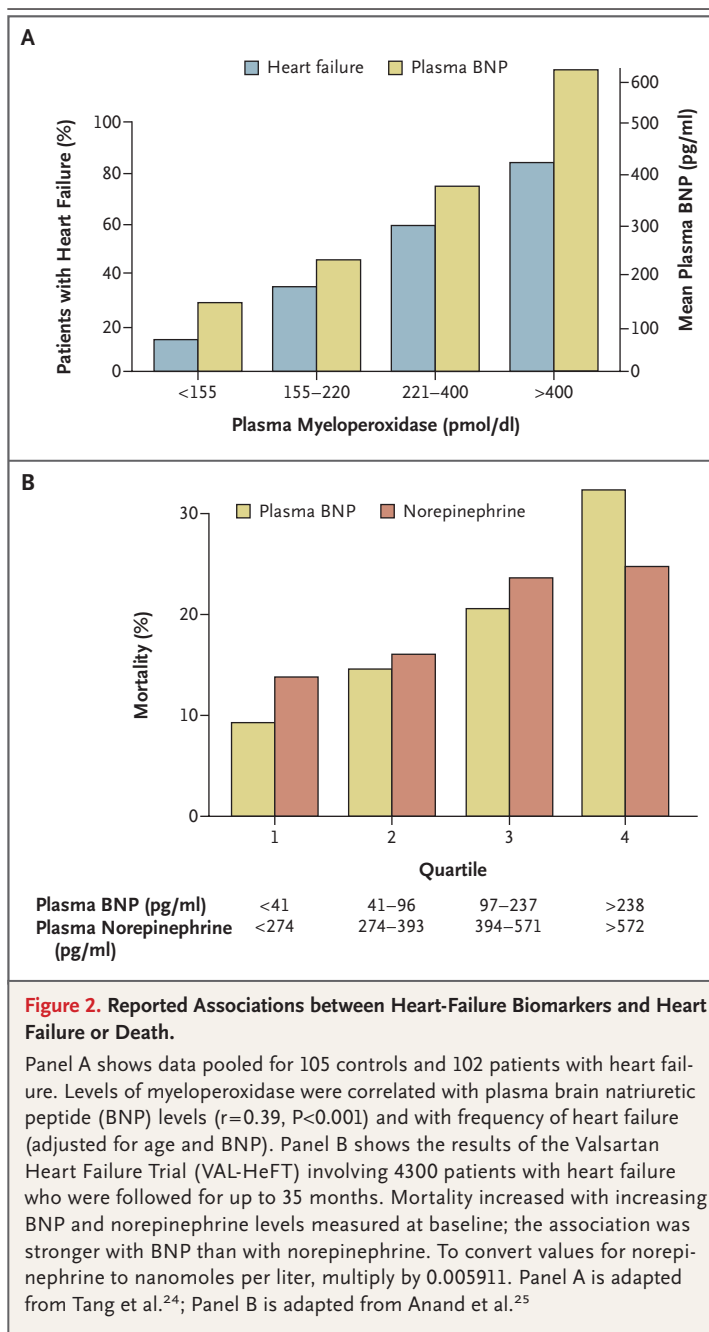
flammatory cytokines, Fas, and their soluble receptors appear to be useful in risk stratification of patients with heart failure and in screening to identify asymptomatic subjects at risk for heart failure. In the future, the profile of changes in inflammatory biomarkers might help to identify the specific inflammatory disturbances in any given patient and thereby might aid the selection of appropriate therapy.

OXIDATIVE STRESS

Increased oxidative stress results from an imbalance between reactive oxygen species (including the superoxide anion, hydrogen peroxide, and the hydroxyl radical) and endogenous antioxidant defense mechanisms. The imbalance can exert profoundly deleterious effects on endothelial function<sup>18</sup> as well as on the pathogenesis and progression of heart failure.<sup>19</sup> Oxidative stress may damage cellular proteins and cause myocyte apoptosis and necrosis. It is associated with arrhythmias and endothelial dysfunction, with the dysfunction occurring through reduction of nitric oxide synthase activity as well as the inactivation of nitric oxide.<sup>20</sup> Inflammation and immune activation, activation of the renin-angiotensin-aldosterone system and the sympathetic nervous system, and increases in circulating catecholamine levels and peroxynitrite formed from the interaction of the superoxide anion and nitric oxide all may increase oxidative stress.<sup>21</sup>

Since it is difficult to measure reactive oxygen species directly in humans, indirect markers of oxidative stress have been sought. These include plasma-oxidized low-density lipoproteins, malondialdehyde and myeloperoxidase (an index of leukocyte activation), urinary levels of biopyrrins (oxidative metabolites of bilirubin),<sup>22</sup> and isoprostane levels in plasma and urine.<sup>23</sup> The levels of plasma myeloperoxidase<sup>24</sup> (Fig. 2A) and isoprostane excretion correlate with the severity of heart failure and are independent predictors of death from heart failure, even after adjustment for baseline variables.<sup>26</sup> The urinary excretion of 8-isoprostane correlates with the plasma levels of matrix metalloproteinases, which at high levels can accelerate adverse ventricular remodeling and increase the severity of heart failure.<sup>26</sup>

There is increasing evidence that xanthine oxidase, which catalyzes the production of two oxidants, hypoxanthine and xanthine, plays a



**Figure 2. Reported Associations between Heart-Failure Biomarkers and Heart Failure or Death.**

Panel A shows data pooled for 105 controls and 102 patients with heart failure. Levels of myeloperoxidase were correlated with plasma brain natriuretic peptide (BNP) levels ( $r=0.39$ ,  $P<0.001$ ) and with frequency of heart failure (adjusted for age and BNP). Panel B shows the results of the Valsartan Heart Failure Trial (VAL-HeFT) involving 4300 patients with heart failure who were followed for up to 35 months. Mortality increased with increasing BNP and norepinephrine levels measured at baseline; the association was stronger with BNP than with norepinephrine. To convert values for norepinephrine to nanomoles per liter, multiply by 0.005911. Panel A is adapted from Tang et al.<sup>24</sup>; Panel B is adapted from Anand et al.<sup>25</sup>

pathologic role in heart failure.<sup>27</sup> Uric acid production is elevated in association with increased xanthine oxidase activity. Elevated levels of uric acid correlate with impaired hemodynamics<sup>28</sup> and independently predict an adverse prognosis in heart failure.<sup>29</sup> Although more studies are required, uric acid may prove to be a simple, useful, albeit nonspecific, clinical indicator of excess oxidative stress.

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 EXTRACELLULAR-MATRIX  
REMODELING
 

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Remodeling of the ventricles plays an important role in the progression of heart failure.<sup>30</sup> The extracellular matrix provides a “skeleton” for myocytes and determines their size and shape. Normally, there is a balance between matrix metalloproteinases (proteolytic enzymes that degrade fibrillar collagen) and tissue inhibitors of metalloproteinases. An imbalance, with dominance of matrix metalloproteinases over tissue inhibitors of metalloproteinases, is associated with ventricular dilatation and remodeling. An abnormal increase in collagen synthesis may also be deleterious to cardiac function because the resultant excessive fibrosis can impair ventricular function. The propeptide procollagen type I is a serum biomarker of collagen biosynthesis. Querejeta et al.<sup>31</sup> observed a positive correlation between the serum level of propeptide procollagen type I and the fractional volume of fibrous tissue determined from cardiac biopsies in patients with essential hypertension. Cicoira et al.<sup>32</sup> reported that the level of plasma procollagen type III in patients with heart failure is an independent predictor of adverse outcomes.

Thus, elevated markers of increased extracellular-matrix breakdown on the one hand and of excessive collagen synthesis on the other are associated with impaired left ventricular function and adverse clinical outcomes in patients with heart failure. Markers of these processes appear to be important targets of therapy. However, at least 15 matrix metalloproteinases and several forms of procollagen and of tissue inhibitors of metalloproteinases have been identified. Which of these are the most informative and appropriate for routine measurement requires clarification.<sup>33</sup>

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 NEUROHORMONES
 

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In the early 1960s it was reported that patients with heart failure had abnormally elevated levels of plasma norepinephrine at rest and that further elevations occurred during exercise.<sup>34</sup> The urinary excretion of norepinephrine was also increased.<sup>35</sup> These findings suggested that the sympathetic nervous system is activated in patients with heart failure and that a neurohormonal disturbance might play a pathogenetic role in heart failure. Cohn et al.<sup>36</sup> subsequently demonstrated that plas-

ma norepinephrine level was an independent predictor of mortality (Fig. 2B). Swedberg et al.<sup>37</sup> made the important observation that the renin-angiotensin-aldosterone system becomes activated in patients with heart failure as well.

Subsequently, after its discovery, attention focused on big endothelin-1, a 39-amino-acid prohormone secreted by vascular endothelial cells that is converted in the circulation into the active neurohormone endothelin-1, a peptide hormone 21 amino acids in length. Endothelin-1 is a powerful stimulant of vascular smooth-muscle contraction and proliferation and ventricular and vessel fibrosis and is a potentiator of other neurohormones.<sup>38</sup> The plasma levels of both endothelin-1 and big endothelin-1 are increased in patients with heart failure and correlate directly with pulmonary artery pressure,<sup>39</sup> disease severity, and mortality.<sup>40</sup> The Valsartan Heart Failure Trial (Val-HeFT) investigators compared the prognostic values of plasma neurohormones (norepinephrine, plasma renin activity, aldosterone, endothelin-1, big endothelin-1, and brain natriuretic peptide [BNP]) among 4300 patients.<sup>41</sup> The most powerful predictors of mortality and hospitalization for heart failure, after BNP, were big endothelin-1, followed by norepinephrine, endothelin-1, plasma renin activity, and aldosterone. However, trials involving several endothelin-1-receptor antagonists have failed to show any beneficial effects on clinical outcomes.<sup>38</sup>

In the Randomized Aldactone Evaluation Study (RALES) of patients with severe heart failure, Zannad et al.<sup>42</sup> found that administration of the aldosterone blocker spironolactone was associated with a reduction of plasma procollagen type III and clinical benefit, but only in patients whose baseline levels of the procollagen were above the median. Administration of spironolactone in patients with acute myocardial infarction reduced myocardial collagen synthesis, as reflected by plasma procollagen type III, as well as postinfarct adverse left ventricular remodeling.<sup>43</sup> Taken together, these findings suggest that limiting the synthesis of the extracellular matrix might be an important component of the beneficial action of spironolactone in patients with severe heart failure.

Arginine vasopressin is a nonapeptide that is synthesized in the hypothalamus and stored in the posterior pituitary gland and that has anti-diuretic and vasoconstrictor properties. Excess re-

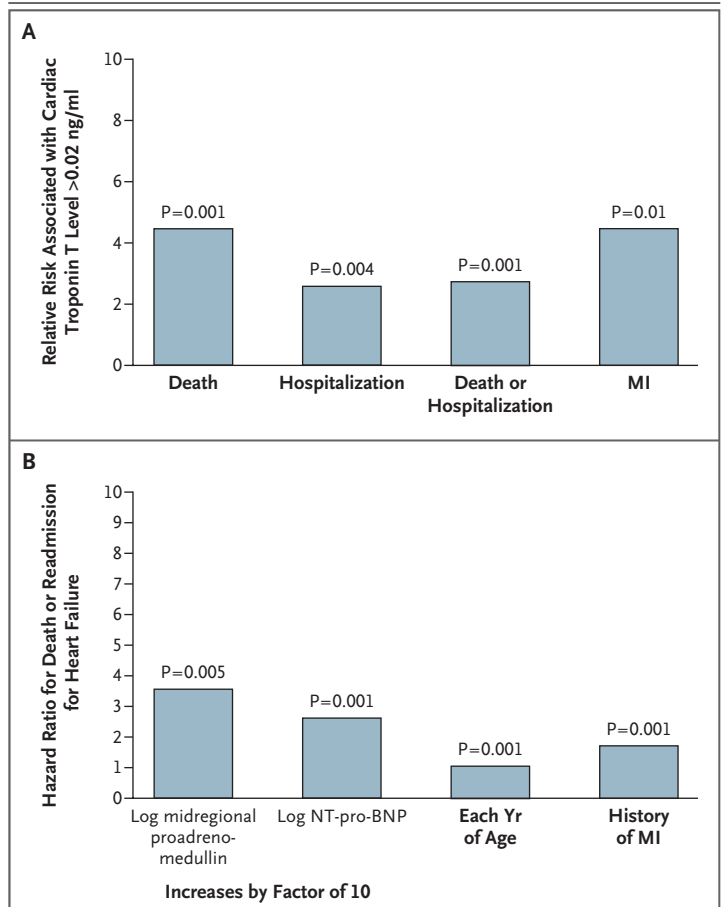
lease of arginine vasopressin intensifies heart failure associated with dilutional hyponatremia, fluid accumulation, and systemic vasoconstriction. Whereas plasma levels of arginine vasopressin are elevated in patients with acute or chronic heart failure<sup>44</sup> and are associated with poor clinical outcomes, blockade of the vasopressin 2 receptor relieves acute symptoms but does not appear to alter the natural history of severe heart failure.<sup>45</sup> Thus, it is not yet clear whether the vasopressin 2 receptor should be considered to be a therapeutic target.

Although elevated levels of several neurohormones can be used to predict adverse outcomes in patients with heart failure, they are relatively unstable in plasma and may be difficult to measure on a routine basis. However, it is likely that these neurohormones are important contributors to the pathophysiological changes that occur in heart failure. Although the various neurohormones are distinct, they have common features. Norepinephrine, angiotensin II, aldosterone, endothelin-1, and arginine vasopressin are vasoconstrictors, thereby increasing ventricular afterload. The fact that blockade of the sympathetic nervous system and of the renin-angiotensin-aldosterone system are cornerstones of current pharmacologic treatment of heart failure supports the concept that several of these biomarkers are probably part of the direct causal pathway for heart failure.

MYOCYTE INJURY

Myocyte injury results from severe ischemia, but it is also a consequence of stresses on the myocardium such as inflammation, oxidative stress, and neurohormonal activation. During the past two decades, the myofibrillar proteins — the cardiac troponins T and I — have emerged as sensitive and specific markers of myocyte injury and have improved greatly the diagnosis, risk stratification, and care of patients with acute coronary syndromes.

Modest elevations of cardiac troponin levels are also found in patients with heart failure without ischemia.<sup>46</sup> Horwich et al.<sup>47</sup> reported that cardiac troponin I was detectable ( $\geq 0.04$  ng per milliliter) in approximately half of 240 patients with advanced, chronic heart failure without ischemia. After adjustment for other variables associated with poor prognosis, the presence of cardiac troponin I remained an independent pre-



**Figure 3. Reported Risks of Adverse Events Associated with Heart-Failure Biomarkers and Other Risk Factors.**

Panel A shows the relative risks of various adverse outcomes associated with an elevated cardiac troponin T level ( $>0.02$  ng per milliliter) at baseline among 136 patients with heart failure followed for a mean ( $\pm$ SD) of  $14.0 \pm 4.3$  months. A total of 33 patients had an elevated troponin T level at baseline. The relative risks for death, hospitalization for heart failure, and myocardial infarction (MI) were all significantly elevated in these 33 patients as compared with those without elevated troponin T levels. Panel B shows the hazard ratios for death or readmission for heart failure among 983 patients with acute MI, according to risk factor. Hazard ratios were calculated with the use of multiple Cox proportional-regression analysis. History of MI refers to a myocardial infarction occurring before the acute event. NT-pro-BNP denotes N-terminal pro-brain natriuretic peptide. P values are for the comparison of the relative risks or hazard ratios among patients with the risk factor and among those without. Panel A is adapted from Hudson et al.<sup>48</sup>; Panel B is adapted from Khan et al.<sup>49</sup>

dictor of death. Cardiac troponin T levels greater than 0.02 ng per milliliter in patients with chronic heart failure were associated with a hazard ratio for death of more than 4 (Fig. 3A).<sup>48</sup> In this issue of the *Journal*, Peacock et al.<sup>50</sup> report that troponin measurements are a predictor of outcome in hospitalized patients with acute de-

compensated heart failure. Latini et al.<sup>51</sup> found that, with a standard assay, cardiac troponin T was detectable in 10% of patients with chronic heart failure, but with a new high-sensitivity assay, it was detectable in 92% of these patients. After adjustment for baseline variables and BNP level, the detection of troponin T by means of the high-sensitivity assay was associated with an increased risk of death. This study showed that previously nondetectable levels of cardiac troponin T can provide important additional prognostic information. As the sensitivity of cardiac troponin analysis increases further, the biomarker will probably be detectable in the entire population, and along with the natriuretic peptides it will be used routinely to assess the prognosis and response to treatment of patients with heart failure.

Other myocardial proteins — including myosin light chain 1, heart fatty-acid binding protein, and creatine kinase MB fraction — also circulate in stable patients with severe heart failure. Like cardiac troponin T, the presence of these myocardial proteins in the serum is an accurate predictor of death or hospitalization for heart failure.<sup>52</sup> Future studies should compare the predictive accuracy of troponin measured with a high-sensitivity assay and the predictive accuracy of these other biomarkers of myocyte injury to determine whether the latter add information.

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#### MYOCYTE STRESS

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##### NATRIURETIC PEPTIDES

The precursor of BNP and N-terminal pro-brain natriuretic peptide (NT-pro-BNP) is a pre-prohormone BNP, a 134-amino-acid peptide that is synthesized in the myocytes and cleaved to the prohormone BNP of 108 amino acids. The prohormone is released during hemodynamic stress — that is, when the ventricles are dilated, hypertrophic, or subject to increased wall tension.<sup>53</sup> Prohormone BNP is cleaved by a circulating endoprotease, termed corin, into two polypeptides: the inactive NT-pro-BNP, 76 amino acids in length, and BNP, a bioactive peptide 32 amino acids in length. BNP causes arterial vasodilation, diuresis, and natriuresis, and reduces the activities of the renin-angiotensin-aldosterone system and the sympathetic nervous system. Thus, when considered together, the actions of BNP oppose the physiological abnormalities in heart failure.

The natriuretic peptides are cleared by the kidneys, and the hypervolemia and hypertension characteristic of renal failure enhance the secretion and elevate the levels of BNP, especially the NT-pro-BNP.<sup>54</sup> There is also a moderate increase in the level of circulating BNP with increasing age, presumably in relation to myocardial fibrosis or renal dysfunction, which are common in the elderly.<sup>55</sup> Pulmonary hypertension from a variety of causes may increase the plasma level of BNP.<sup>52</sup> The level varies inversely with the body-mass index.<sup>55</sup> All of these physiological conditions and disease states must be taken into consideration in the interpretation of natriuretic peptides in individual patients. Assays for BNP and NT-pro-BNP are commercially available, and these biomarkers of heart failure are the most widely tested; such testing is recommended in current guidelines.<sup>55,56</sup>

Measuring levels of BNP is most useful in the evaluation of patients with dyspnea presenting to the emergency department, where point-of-care testing may provide the advantages of convenience and rapid turnaround times, thereby facilitating clinical management. Maisel et al.<sup>57</sup> showed in the Breathing Not Properly study that BNP levels greatly increased the accuracy of the diagnosis of heart failure in patients presenting to emergency departments with dyspnea; in these patients, a level of more than 100 pg per milliliter renders the diagnosis of heart failure unlikely, whereas a level of more than 400 pg per milliliter makes the diagnosis likely. Similar findings, albeit with different cutoff values, were reported from the ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) study.<sup>58</sup> The cutoff values may differ in patients with chronic heart failure as compared with patients with acute heart failure. For example, in one series of patients with established chronic symptomatic heart failure, one fifth had plasma BNP levels below 100 pg per milliliter.<sup>59</sup>

As compared with standard care, a strategy using a single measurement of BNP or NT-pro-BNP in patients presenting with acute dyspnea was associated with a shorter hospital stay and lower cost of hospitalization.<sup>60,61</sup> Thus, in patients presenting to the emergency department with possible heart failure, decisions regarding hospital admission or referral to an outpatient clinic are facilitated by knowledge of natriuretic peptide levels. BNP level is also an accurate pre-

dictor of survival in patients with acute decompensated heart failure, irrespective of left ventricular ejection fraction. Fonarow et al.<sup>62</sup> measured the level at hospital admission in 48,629 patients with acute decompensated heart failure in the Acute Decompensated Heart Failure (ADHERE) registry (ClinicalTrials.gov number, NCT00366639). After adjustment for baseline variables, an almost linear relation between BNP level and in-hospital mortality was found.

Measurements of BNP appear to be useful in the diagnosis and risk stratification of patients with chronic heart failure<sup>52,54</sup> and are a better predictor of death than is plasma norepinephrine<sup>25</sup> (Fig. 2B) or endothelin-1.<sup>63</sup> In the Systolic Heart Failure Treatment Supported by BNP (STARS-BNP) trial, Jourdain et al.<sup>64</sup> randomly assigned outpatients with New York Heart Association class II or III heart failure to receive therapy either according to current clinical guidelines (control group) or with a goal of decreasing BNP levels to less than 100 pg per milliliter. The primary end point (death from heart failure or hospital admission for heart failure) occurred in 24% of patients in whom the BNP level was lowered, as compared with 52% of the control group ( $P < 0.001$ ), suggesting that therapy directed by BNP level is superior to guideline-directed therapy. Logeart et al.<sup>65</sup> reported that, in patients hospitalized for decompensated heart failure, the predischarge level of BNP was a strong, independent predictor of postdischarge outcomes and proposed that patients with heart failure whose BNP level does not decline to below approximately 600 pg per milliliter should receive intensified treatment before discharge.

Natriuretic peptides also appear to be useful in screening asymptomatic subjects at risk of developing heart failure, such as the elderly and those with hypertension, diabetes, or asymptomatic coronary artery disease.<sup>53,54</sup> Measurement of natriuretic peptides may also be used to screen for acute or late cardiotoxic effects associated with cancer chemotherapy.<sup>66</sup>

Two studies have directly compared BNP and NT-pro-BNP.<sup>67,68</sup> Both found that the N-terminal prohormone was slightly superior to BNP for predicting death or rehospitalization for heart failure. The longer half-life of NT-pro-BNP may make it a more accurate index of ventricular stress and therefore a better predictor of prognosis.

#### ADRENOMEDULLIN

Adrenomedullin is a peptide of 52 amino acids and a component of a precursor, pre-proadrenomedullin, which is synthesized and present in the heart, adrenal medulla, lungs, and kidneys.<sup>69</sup> It is a potent vasodilator, with inotropic and natriuretic properties, the production of which has been shown to be stimulated by both cardiac pressure and volume overload.<sup>70</sup> The level of circulating adrenomedullin is elevated in patients with heart failure and is higher in patients with more severe heart failure.<sup>71</sup> The midregional fragment of the proadrenomedullin molecule, consisting of amino acids 45 to 92, is more stable than adrenomedullin itself and easier to measure. Khan et al.<sup>49</sup> compared midregional proadrenomedullin and NT-pro-BNP levels in patients after acute myocardial infarction. Both biomarkers were equally strong predictors of cardiovascular death or heart failure (Fig. 3B). Measurements of midregional proadrenomedullin provided additional prognostic value when combined with those of NT-pro-BNP.

#### ST2

ST2, a member of the interleukin-1 receptor family, is a protein secreted by cultured monocytes subjected to mechanical strain.<sup>72</sup> The ligand for this receptor appears to be interleukin-33, which — like BNP and adrenomedullin — is induced and released by stretched myocytes. Infusion of soluble ST2 appears to dampen inflammatory responses by suppressing the production of the inflammatory cytokines interleukin-6 and interleukin-12.<sup>73</sup> Elevated levels of ST2 occur in patients with severe heart failure. In patients presenting to the emergency department with myocardial infarction with ST elevation and dyspnea, ST2 levels were strongly predictive of mortality.<sup>74</sup> In patients with heart failure, an increase of ST2 during a 2-week period was an independent predictor of subsequent death or the need for cardiac transplantation.<sup>72,75</sup>

Thus, there are now three biomarkers that appear to reflect ventricular stress and may be powerful predictors of risk. There is substantial experience with measuring the natriuretic peptides BNP and NT-pro-BNP, for which excellent assays are available. Less information is available for the two newer markers, adrenomedullin and ST2, and analytic methods for determining them have not yet been standardized. However, adrenomedullin and ST2 appear to yield information

independent of, and thus supplementary to, that provided by the natriuretic peptides.

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#### NEW BIOMARKERS

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Biomarkers other than those already discussed are under investigation. These include chromogranin A, a polypeptide hormone produced by the myocardium, which has potent negative inotropic properties and elevated plasma levels in patients with heart failure.<sup>76</sup> A second is galectin-3, a protein produced by activated macrophages, for which plasma levels have been reported to predict adverse outcomes in patients with heart failure.<sup>77</sup> A third is osteoprotegerin, a member of the tumor necrosis factor receptor superfamily that has been implicated in the development of left ventricular dysfunction<sup>78</sup> and in predicting survival in patients with heart failure after myocardial infarction.<sup>79</sup>

Biomarkers well known in other pathological states may also be helpful in diagnosing heart failure. Levels of adiponectin, a 244-amino-acid peptide, are inversely related to body-mass index, are elevated in patients with advanced heart failure<sup>80</sup> (especially those with cardiac cachexia), and are a predictor of death in patients with heart failure.<sup>81</sup> Growth differentiation factor 15, a stress-response member of the transforming growth factor  $\beta$  superfamily, also predicts the risk of death in patients with heart failure and deserves further study.<sup>82</sup>

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#### FUTURE DIRECTIONS

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The traditional approach to the classification of heart failure has focused on the pathological cause of failure of the cardiac pump (e.g., chronic coronary artery disease), the pathophysiological characteristics (e.g., systolic heart failure), and the acuity and severity of the heart failure. A biomarker profile may be a valuable addition to this approach.<sup>83</sup> The groups of biomarkers for heart failure discussed in this review are usually considered individually. A multimarker strategy has been reported to be useful in refining risk stratification among patients with acute coronary syndromes,<sup>84</sup> and there is a growing interest in this approach for categorizing heart failure, as suggested by Lee and Vasan.<sup>12</sup> For example, the use

of data on BNP together with troponin has been shown to achieve better risk stratification than that obtained with either biomarker alone.<sup>47,51</sup> The accuracy of risk prediction was enhanced when a natriuretic peptide was coupled with other biomarkers of myocardial stress: adrenomedullin,<sup>49</sup> ST2,<sup>74</sup> or the inflammatory biomarkers C-reactive protein and myeloperoxidase.<sup>85</sup> Although the biomarkers discussed in this review each provide prognostic information, examination of an extensive set of markers in a large cohort of patients with heart failure followed prospectively could identify those that are independently predictive of outcome. Indeed, in this issue of the *Journal*, Zethelius et al.<sup>86</sup> have shown that the combination of four biomarkers (troponin I, NT-pro-BNP, C-reactive protein, and cystatin C) improved risk stratification for death from cardiovascular causes among elderly men.

Proteomics, the evaluation of proteins using mass spectrometric analysis coupled with high-pressure liquid chromatography, is likely to yield totally new classes of biomarkers for heart failure.<sup>87</sup> Large platforms that would facilitate the study of hundreds of proteins are likely to become available, which may provide a greatly expanded approach to the early detection of ventricular dysfunction, elucidating its pathogenesis and making it possible to monitor the therapy of heart failure in new ways.

A logical next step might be to obtain a profile by measuring representatives of distinct classes of biomarkers, as described in this review. In addition to the use of biomarkers in risk classification, their use for monitoring therapy and for targeting therapy require overcoming additional hurdles. Doing so would likely enhance their clinical value. At present only the natriuretic peptides appear to be useful for these purposes. New approaches in bioinformatics, including the use of artificial neural networks, will probably be needed to assist in data analysis and its clinical application.

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