A Review of the Improvement in Diagnostic Accuracy for Acute Heart Failure
(INDICATE-HF)

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Abstract:

The aim of the current study is to unearth a diagnostic model to improve the ruling-in of heart failure, rather than ruling out. The process of heart failure diagnosis is imperative to prevent recurring hospitalization. Through observation of previous approaches, it was determined that an improved clinical procedure to distinguishing heart failure could be achieved by including a multiple circulating biomarker proposal for the different pathophysiological pathways associated with heart failure. This advancement was done through proteomics and blood work assays, which would potentially achieve a multi-marker model to be carried out rapidly in the ED to rule-in heart failure with an increased accuracy. In a study sponsored by Vanderbilt University, the clinical research continues to be carried out in 4 EDs between Nashville, Tennessee and Detroit, Michigan. The title of the study is “Improving Diagnostic Accuracy for Acute Heart Failure” (INDICATE-HF), and is currently conducted at Vanderbilt University Medical Center, Detroit Medical Center (DMC) Sinai-Grace Hospital, DMC Detroit Receiving Hospital, and DMC Harper University Hospital. After approaching patients that fit the inclusion/exclusion criteria, mainly dyspnea (shortness of breath), they were asked for voluntary admission into the study for further proteomic testing. Blood and urine samples were taken, and lab work was carried out for further testing of trends in various biomarkers associated with the pathology of the disease.

Prior studies, although few, have been conducted to deduce a multi-biomarker approach to identify heart failure in patients presenting to the ED with dyspnea. These studies have possessed limitations that include highly correlated markers from known biological pathways\(^1\), which create discrepancies in determination of elevated proteins correlated with heart failure or some other pathology. The current mode of diagnosis revolves around a pair of biomarkers, B-type natriuretic peptide (BNP) and N-terminal pro B-type natriuretic peptide (NT-proBNP), which has a detailed physiology within the body, but is limited in capacity for elucidation of heart failure because of the diversity of the syndrome and presentation in the ED. The current hypothesis of the study is a hope of elucidating a multi-marker panel incorporating novel proteins discovered with plasma proteomics, which has the potential to improve diagnostic accuracy for acute heart failure. There have been many studies carried out, including those on myocardial injury and ischemic stroke, that transitioned from a single biomarker to a multi-marker panel, which allowed for a more accurate diagnosis of these various pathologies. The current review will provide an understanding of the pathology of heart failure, the current mode of diagnosis, and the potential of incorporating a panel of biomarkers for improved accuracy of ruling-in heart failure. These supporting studies argue for a promise of better outcomes in the diagnosis and could potentially reduce adverse outcomes as they pertain to heart failure.
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Introduction:

Heart Failure is defined as a complex clinical syndrome that can result from any structural or functional cardiac disorder that impairs the ability of the ventricle to fill or eject blood. Patients that present to the ED can either possess impairments of contraction (systolic function) or relaxation (diastolic function). Population based echocardiographic studies have demonstrated that more than 50% of participants with left ventricular systolic dysfunction (LVEF of less than 35-40%) have no symptoms or signs of heart failure. The impairment of the ventricle to fill or eject blood results in a form of circulatory failure, and a declining contraction and relaxation function of the heart, if not treated, leads to worsening heart failure. If cells and tissues do not receive an adequate supply of blood, then there is an oxygen deficiency in these metabolizing tissues, as well as a surplus in carbon dioxide and other wastes that result from cellular function that would collect in the extremities of the body. This would ultimately lead to hypertrophy and cell death.

The difficulty in diagnosis heart failure upon presentation to the emergency department revolves around the fact that patients do not express matching signs and symptoms. Often, those that come to the ED have jugular distension, edema, and dyspnea, all of which can be related to inefficiencies of the heart and lungs. A review conducted by Gheorghiade & Pang examined the nature of heart failure syndrome, and they found common presentations to the ED. Approximately 25% of patients are hypertensive (systolic blood pressure > 160 mmHg, <10% are hypotensive, most are taking diuretics, 40% taking angiotensin-converting enzyme (ACE inhibitors, 10% take angiotensin-receptor blockers, 50% take beta-blockers, and 20% to 30% take digoxin. A history of coronary artery disease is present in 60% hypertension in 70%, diabetes in 40%, atrial fibrillation in 30% and moderate to severe renal impairment in 20% to 30%. The diversity in presentation makes the criteria for diagnosis difficult to delineate.

The review of this paper includes current laboratory testing of BNP and NT-proBNP levels, and the shortcomings of the current evaluation approach. BNP and NT-proBNP is a neurohormonal response and is correlated to stretching in the ventricles. These proteins are secreted from the atria and ventricles in response to this dysfunction. Laboratory testing aims to decipher a negative likelihood of heart failure based on the cutoff point of BNP, 95 pg/mL, and NT-proBNP, 643 pg/mL, or a positive correlation to heart failure based on BNP levels being greater than 200 pg/mL and NT-proBNP levels being greater than 5,180 pg/mL. A combination of these factors aims to elucidate ruling-out heart failure, but the variation in population-cohort, and the diversity of signs and symptoms skew diagnostic results.

The study identifies the current evaluation of heart failure as it presents to the ED, but the evaluation reasons that natriuretic peptides are inefficient in being the only biomarker guideline for detection of heart failure (ruling-in vs ruling-out). It has been seen in studies conducted on other diseases (ischemic stroke and myocardial infarction) and syndromes that incorporating a panel of biomarkers has the potential to improve the diagnostic accuracy of heart failure and slash the rate at which treatment can begin. There are currently limited prior studies that explore a multiple biomarker approach, and those that did were limited from known biological pathways, relatively small sample sizes, lack of inclusion of all priori selected biomarkers into a single model, and absence of validation cohorts.
The aim of the work conducted by Vanderbilt University Medical Center, with Wayne State University as a collaborator, includes incorporation of a panel of 21 biomarkers to improve the diagnostic accuracy of heart failure. The authors of the study suspect that incorporating this model would provide an advantage in heart failure diagnosis over the current approach. The study is observational with an estimated enrollment being around 2800 participants, and it began on August 31, 2021. The study population includes adults (18 years or older) presenting with the chief complaint of dyspnea and are being evaluated for heart failure by the physician, which is denoted by the research staff if there was an order for natriuretic peptide (NP) measurement (either BNP or NT-proBNP) or a chest x-ray. Patients whose dyspnea is due to trauma, those who are on chronic hemodialysis, and patients whose primary presentation is consistent with acute coronary syndrome are excluded. The study is guided by very strict inclusion/exclusion criteria:

**Table 1. Inclusion Exclusion Criteria for HF testing.**

<table>
<thead>
<tr>
<th>Inclusion Criteria</th>
<th>Exclusion Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Willing to adhere to the study protocol</td>
<td>History of end-stage renal disease for which dyspnea due to primary presentation of an acute</td>
</tr>
<tr>
<td>Able to provide written consent</td>
<td>English or Spanish speaking</td>
</tr>
<tr>
<td>Adult, defined as 18 years or older</td>
<td>Primary reason for presentation to the ED</td>
</tr>
<tr>
<td>ED physician is considering a diagnosis of HF, defined by by ordering a NP test</td>
<td>ED physician is considering a diagnosis of HF, defined by by ordering a NP test</td>
</tr>
<tr>
<td>and/or a chest x-ray</td>
<td>and/or a chest x-ray</td>
</tr>
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</table>

The study contains three specific aims: 1) discover a multi-marker panel of 21 biomarkers to improve diagnostic accuracy for acute HF, 2) derive a model for diagnosing acute HF incorporating the 21-biomarker panel, and 3) test performance of the multi-marker model in a prospective validation cohort. In aim 1, existing plasma samples from ~900 patients will be used to assay 925 proteins to discover a smaller set of novel biomarkers most strongly associated with an adjudicated acute HF diagnosis. In aim 2, an existing prospective observational cohort, EMROC-AHF, will be utilized to derive the multi-marker model in ~900 patients who presented to the ED with acute dyspnea. In aim 3, from four EDs in Detroit, MI and Nashville, TN a new
sample will prospectively recruit ~1,000 patients presenting with acute dyspnea and adjudicate the presence of acute HF by cardiologist panel review.

It is clear that the heart failure epidemic that is present across the world is led by non-specific guidelines for diagnosing heart failure. There remains to be seen an expedited method of diagnosis. The current method of diagnosis is based on the pathology of heart failure and the neurohormonal response, and the evaluation of heart failure rests in testing of a two sole biomarkers: BNP and NT-proBNP. In understanding the biology of this biomarker, it can be useful for a conceptual and physical diagnosis of heart failure. The potential for greater diagnostic accuracy for ruling-in heart failure provides promise for decreased mortality and morbidity resulting from this syndrome. Examination of studies that have been conducted on various other pathologies for a panel of biomarkers presents a useful foundation for unearthing a distinct multi-biomarker panel for heart failure. In understanding the biological pathways of the body, which relate to heart failure, there is potential for a combination of biomarkers that could provide greater accuracy in heart failure diagnosis, beyond the current standards.
Discussion:

Heart failure is directly correlated to cardiac function abnormalities, and it occurs when the heart is unable to eject blood at a rate suitable for the requirements of metabolizing tissues in the body extremities. The heart compensates for the limited stroke volume with an increased diastolic filling pressure, which can lead to cardiac hypertrophy and an eventual heart failure from the inability to contract adequately. The pathology of heart failure is better understood through examination of the chambers of the heart and the cells that compose the musculature.

Natriuretic Peptide: Normal vs Elevated Levels:

Patients that do not experience congestive heart failure secrete very small amounts of BNP from cardiac musculature. In patients that experience heart failure, the levels of BNP and NT-proBNP are high in the ventricles. The issue in using these peptides in diagnosis of congestive heart failure is the presence of confounding variables that have the capability of altering the levels of BNP and NT-proBNP, which skews evaluation in the ED. These confounding variables include of the pathologies of the heart and lungs that include similar neurohormonal responses as congestive heart failure.

The marquee study in defining BNP and NT-proBNP as useful biomarkers in diagnosis of congestive heart failure was the large Breathing Not Properly multinational study. The study took place at 7 international sites across the world: 5 in the United States, 1 in France, and 1 in Norway. Patients in this defining study had to present to the ED with dyspnea as a prominent symptom. The results of the study included the final diagnosis as congestive heart failure in 744 patients (47 percent), dyspnea due to noncardiac causes in 72 patients with a history of left ventricular dysfunction (5 percent), and no finding of congestive heart failure in 770 patients (49 percent).

In the study, the three cohorts of patients displayed a significantly different concentration of BNP (p<0.001). The figure below displays a box plot diagram of the respective patient cohort and the corresponding levels of BNP. Patients that were diagnosed with congestive heart failure had higher levels of BNP secreted from the cardiac ventricles as denoted by radioimmunoassay. Patients with a diagnosis of acute congestive heart failure had mean (±SD) B-type natriuretic peptide levels of 675±450 pg per milliliter, whereas those without congestive heart failure had B-type natriuretic peptide levels of 110±225 pg per milliliter. The 72 patients who had base-line ventricular dysfunction without an acute exacerbation had a mean B-type natriuretic peptide level of 346±390 pg per milliliter. The levels of BNP are significantly higher in patients that had a confirmed diagnosis of congestive heart failure. The level of BNP in patients that presented to the ED with dyspnea due to non-cardiac causes had levels of BNP that could fit into the class of patients with class I congestive heart failure. That is the reason for some discrepancy in diagnosis of congestive heart failure. If the patient has underlying conditions or has a history of cardiac trauma, then the levels of BNP circulating are higher per milliliter. Patients with other risk factors (obesity, age, impaired renal function, ect.) also can present to the ED with elevated BNP levels, which can alter or delay the diagnosis of heart failure, causing concern or increased mortality as the syndrome goes unnoticed.
Figure 1. Bar graph displaying the class of patients that were diagnosed with congestive heart failure due to dyspneic symptoms compared to the class of patients that presented with dyspneic symptoms due to noncardiac conditions. This was characterized in the landmark Breathing Not Properly study, which evaluated heart failure based on BNP assays for those patients that presented to the ED with dyspnea. It is seen that those that had dyspnea due to heart failure had higher levels of BNP in the blood in comparison to those that had dyspnea due to noncardiac conditions and the control group, however there is overlap in patients that presented to the ED with Dyspnea due to noncardiac causes and those with congestive heart failure.8

The New York Heart Association classifies heart failure based on worsening symptoms into four different classes (Class I, II, III, IV). In the Breathing Not Properly multinational study, the levels of b-type natriuretic peptide based on the 4 classes of patients with worsening heart failure were defined as: 244±286 pg per milliliter among patients in class I, 389±374 pg per milliliter among those in class II, 640±447 pg per milliliter among those in class III, and 817±435 pg per milliliter among those in class IV, and the correlation between BNP levels and worsening symptoms was proven as significantly different (p<0.001).8 The values are displayed in a box plot shown in the figure below. These values are identified as the gold standard in classifying heart failure based on worsening symptoms.
Figure 2. Bar graph depicting the levels of BNP in those that were diagnosed with congestive heart failure. The bar graph is meant to convey the levels of BNP as it relates the class of heart failure that represents the severity of the syndrome. It is seen that those patients that had the greatest advancement of heart failure also had the highest levels of BNP in the blood. This signifies that BNP assays can serve as an absolute in diagnosis of heart failure as long as the patients falls under the class III and class IV advancement of heart failure.  

Another important point addressed in this marquee study was the specificity and sensitivity for cutoff values of BNP in diagnosing heart failure. The study found that the ability of b-type natriuretic peptide to rule-out heart failure had a greater capacity as the cutoff value of BNP levels decreased. The figure below shows the correlation of sensitivity as it relates to specificity in the various cutoff values of BNP in the blood. Using the area under the curve model (positive predictive value is 1.0 and no correlation is 0.5) the level of BNP in diagnosing congestive heart failure from other diseases was 0.91. In the ED, the most common cutoff value is 100 pg/mL, which has a very high accuracy and sensitivity compared to higher values of BNP. The study found that b-type natriuretic peptide cutoff value of 100 pg/mL had a sensitivity of 90%, a specificity of 76%, and an accuracy of 83% in ruling out heart failure for some other condition. The lower the cutoff value the greater the specificity, sensitivity, and accuracy. This is the single greatest drawback from using b-type natriuretic peptide as the sole indicator of heart failure upon presentation with dyspnea.
Figure 3. Line graph that displays the sensitivity and specificity area under the curve in BNP assays. The curve shows that the a BNP level of 100 pg/mL had a sensitivity of 90 percent, a specificity of 76 percent, and an accuracy of 83 percent. This value is the closest to a clinically significant positive predictive diagnosis of heart failure, but the variability among patients leaves room for doubt. The 50 pg/mL is more useful in ruling-out heart failure rather than ruling-in heart failure.

Another pivotal study carried out by Cunningham et al. depicts the role that BNP plays in the diagnosis of heart failure. The study was carried out with 2000 subjects ranging from age 25-74 in north Glasgow, and the aim was to determine the relationship between circulating BNP concentrations in patients with symptomatic or asymptomatic left ventricular dysfunction. In 1252 patients, blood samples were taken, and BNP concentrations were measured. BNP concentrations were corrected for both survivors and non-survivors using the Mann-Whitney U test. It was shown that the cohort with higher mortality rate displayed higher concentrations of BNP circulating in the blood. The median (interquartile range) BNP concentration in those who died, irrespective of the cause, was 16.9 pg/ml (8.8–27) compared with 7.8 pg/ml (3.4–13) in survivors (p < 0.0001). The study shows that those with higher concentrations of BNP in the blood are put at greater risk for mortality correlating with greater LVD and congestive heart disease. The figure below confirms the timeline in mortality for those that had elevated BNP concentrations was shorter and had a greater correlation to death with increased levels of BNP in the blood. This study confirms the correlation of BNP as a biomarker for ruling out heart failure in those patients that had normal or low levels of BNP, yet the study shows no relationship between a certain level of BNP in the blood and a concurrent diagnosis of heart failure.
Regardless of the adjustment, it is seen that BNP concentrations lower than 17.9 pg/mL show a higher survival rate in comparison to those BNP concentrations that are greater than or equal to 17.9 pg/mL. \(^9\)

The study also evaluated the patients with preserved systolic function. Those that did not display a reduced ejection fraction still had a greater mortality rate if the concentration of BNP circulating in the blood was elevated. Patients with preserved systolic function (LVEF > 40%) also had a significantly higher mortality if the BNP concentration was > 17.9 pg/ml at 8.5% (n = 16 of 118) compared with 2% (n = 21 of 891) in those with a BNP concentration < 17.9 pg/ml (p < 0.0001). \(^9\)

In a study conducted by Amirnovin et al, the relationship between a rapid bedside BNP test was evaluated in obtaining levels of circulating BNP and a diagnosis of congestive heart failure. The study took place in San Diego Veteran’s Health Care system and included 250 patients presenting to the ED with dyspnea. Levels of BNP were taken, but to confirm a diagnosis of congestive heart failure, a chest x-ray was taken, and a review of the patient’s history was made available. During evaluation, blood samples were obtained, and bloodwork was conducted to observe the concentration of BNP in the patients that were diagnosed with congestive heart failure.

The difference in BNP levels between patients that had congestive heart failure compared to those without was significantly different (p<0.001). Patients diagnosed with CHF (n=97) had a mean BNP concentration of 1,076±138 pg/ml while the non-CHF group (n=139) had a mean BNP concentration of 38±4 pg/ml. \(^{10}\) The figure below shows a box plot of patients that were diagnosed with congestive heart failure and BNP levels compared to those that were not diagnosed with congestive heart failure and the correlating BNP levels. The study deemed that the most accurate concentration of BNP in the blood for ruling out congestive heart failure was less than 80 pg/ml.
Figure 5. Box plot displaying the levels of BNP in patients diagnosed with congestive heart failure. The difference among patients diagnosed with congestive heart failure was significant, and those patients had a mean BNP concentration $1,076 \pm 138$ pg/ml. The patients that were clinically diagnosed without congestive heart failure had a mean BNP concentration of $38 \pm 4$ pg/ml, and the group that had baseline ventricular dysfunction without acute exacerbation had a mean concentration of $141 \pm 31$ pg/ml.$^{10}$

An interesting point to note, which only further confirms the need for a panel of biomarkers in diagnosis congestive heart failure, was the misdiagnosis of congestive heart failure in the study by physicians that were blind to BNP levels in the blood. In the study, 15 patients were diagnosed as having heart failure by ED physicians when they had other causes for dyspnea upon presentation. The mean BNP level in this group was $46\pm13$ pg/ml. There was yet another 15 patients that were not diagnosed with heart failure by physicians, and the mean BNP concentration in this group was $742\pm337$ pg/ml. With no set cutoff point agreed upon by physicians worldwide, misdiagnosis similar to this still persists.

The study recognized that patient characteristics and comorbidities on NP levels often impairs the functionality of BNP testing. Obesity, age, sex, and renal dysfunction often skew the results of natriuretic peptide testing. The study showed that BNP levels were inversely proportional to body mass index. Obese patients consistently show lower levels of BNP despite a possible heart failure diagnosis in comparison to lean patients. This is an increasing issue because of the increasing prevalence of obesity in America. Not to mention, patients with renal impairment often display altered BNP levels. A review by Baba et al. found that The BNP cut-off point for the diagnosis of HF may need to be raised when the estimated glomerular filtration rate (eGFR) is $<60$ mL/min/1.73 m$^2$, and the BNP level in patients with an eGFR $<60$ mL/min/1.73 m$^2$ was approximately two- to four-fold greater than that in patients with an eGFR $\geq60$ mL/min/1.73 m$^2$.$^{11}$

**Misdiagnosis of Heart Failure**

According to the INDICATE-HF clinical research study, mistreatments that are prescribed to patients that present to the ED with dyspnea have adverse outcomes. The study estimates that
11% of patients hospitalized with acute heart failure receive intravenous fluids during the first two days. The issue with this supplemental fluid is an associated risk of ICU admission, intubation, dialysis, and in-hospital death. Patients that have obstructive lung disease are prescribed corticosteroids, but these lead to fluid retention, tachycardia, and hypertension, which only facilitate the worsening of heart failure signs and symptoms. Not to mention, patients that receive antibiotics as a form of treatment for some other pathology can experience worsened cardiac events, including QT prolongation and sudden cardiac death. Conversely, patients that are misdiagnosed for heart failure are often given diuretics to correct fluid retention, but if the patient has pneumonia or sepsis, it can facilitate volume depletion, hypotension, and renal dysfunction. The study also relays that despite natriuretic peptide testing, there is still uncertainty in heart failure diagnosis. Basing a diagnosis of signs, symptoms, and a chest x-ray was detailed to have median diagnostic accuracy between 50-66%. BNP testing only correlates to a modest increase in accuracy because of low specificity and sensitivity. According to the study protocol, BNP ≥ 100 pg/ml carried a 5% false-negative and 37% false-positive rate, thereby limiting overall diagnostic accuracy for acute HF.

These factors support the need for a model that includes multiple biomarkers in the diagnosis of heart failure. A model like this provides opportunity for improved diagnosis because biomarkers are defined as cellular, biochemical or molecular alterations that are measurable in biological media such as human tissues, cells, or fluids. This indicates that biomarkers signal any pathological disruption in the body. This relates to the proposal of a multiple biomarker model has the potential to unearth disruption in other biological pathways in the body, which may show elevated levels of certain proteins that are not normally hyperactive in conventional pathways. These biomarkers are obtained via the blood and urine samples, and a panel of biomarkers can reduce the heterogeneity of presentation, provide information on the stages of disorder progression, and identify individuals who are at greater risk for contraction.

Other conditions including myocardial infarction, TIA/stroke evaluation, autoimmune disease, endocrine conditions, and hematologic disorders have all included a panel of biomarkers to increase the accuracy in diagnosis. The incorporation of such a model would only provide further confirmation and confidence in congestive heart failure diagnosis for patients that present to the ED with correlating signs and symptoms.

Pathologies That Include a Panel of Biomarkers:

A pathology that has found success with inclusion of a multiple panel of biomarkers for diagnosis is stroke evaluation. Stroke is a multisystem disorder, which involves thrombotic and neurotoxic coupling. In a clinical setting, it can often be difficult to distinguish stroke from some other neurological disorder. Currently, there are biomarkers that are used in stroke evaluation, but there remains room for improvement because many of the biomarkers used now, including LDL-C and HgA1C, are not disease specific. The biomarkers are also associated with other diseases and make diagnosis of stroke unreliable and often unattainable. There have been other biomarkers further evaluated when developing a panel of proteins for stroke evaluation, but when a patient does have a stroke, the blood brain barrier has been found to impede the release of certain biomarkers that could signal ischemic stroke. Similar to heart failure, patients who present with a stroke often have varying signs and symptoms. In relation to stroke, the variance
in infarct size, location, and cause make diagnosis that much more difficult. The heterogeneity of this disease makes a single biomarker far from plausible in diagnosis.

In a study conducted by Brey et al, there were three biomarkers that were tested for in evaluation of stroke. The three biomarkers were glutamate, homocysteine, which both serve as correlates of large and middle artery dysfunction, and the third was NR2A aAb as a criterion of microvascular damage independently associated with neurotoxicity and thrombosis in patients with transient ischemic attack. The study incorporated an interesting way of separating those patients that presented to the ED with varying signs and symptoms. There were 92 patients enrolled into the study, and they were separated according to high blood pressure, pre-stroke, and TIA. There was also a subset of patients that had been diagnosed with a left hemispheric stroke but within 6 hours of presenting to the ED. The concentration of the corresponding biomarkers and the various cohorts of patients are displayed in the table below.

Table 1. Plasma concentrations of glutamate and homocysteine and serum concentrations of NR2A autoantibodies in patients and control subjects.

<table>
<thead>
<tr>
<th>Subjects</th>
<th>n</th>
<th>Age, y</th>
<th>Gender</th>
<th>Concentration</th>
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<tbody>
<tr>
<td></td>
<td>n</td>
<td></td>
<td>M</td>
<td>F</td>
</tr>
<tr>
<td>Control</td>
<td>30</td>
<td>51.6±4.6</td>
<td>12</td>
<td>18</td>
</tr>
<tr>
<td>High blood pressure</td>
<td>25</td>
<td>36.9±2.3</td>
<td>8</td>
<td>17</td>
</tr>
<tr>
<td>Prestroke</td>
<td>12</td>
<td>59.9±4.7</td>
<td>5</td>
<td>7</td>
</tr>
<tr>
<td>TIA</td>
<td>14</td>
<td>58.9±1.7</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>Ischemic stroke</td>
<td>23</td>
<td>54.7±1.4</td>
<td>14</td>
<td>9</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>18</td>
<td>53.0±4.4</td>
<td>12</td>
<td>6</td>
</tr>
</tbody>
</table>

aAb indicates autoantibodies.

The study found that higher concentrations of glutamate was related to patients that presented with TIA, and homocysteine levels were correspondingly elevated in in patients with cerebrovascular abnormalities, pre-stroke, TIA, and ischemic stroke. The levels of homocysteine were directly correlated to stroke severity. Excessive activation of NMDA receptors is the result of glutamate and homocysteine neurotoxicity, and level of NR2A aAb in the blood of healthy controls was 1.4±0.25 ng/mL, whereas for patients with cerebrovascular abnormalities, it began to increase and achieved the highest levels in those with pre-stroke and TIA/stroke.

This study provides a template for developing a panel of biomarkers for diagnosis in heart failure in a bedside manner. These concentrations were obtained from bedside blood samples, and the levels are those of the plasma. Through this study it has become known that the variance in the levels of these biomarkers can be extreme depending on the presentation to the ED for stroke evaluation, yet it is seen that developing a trend for interpretation of concentrations of each of these biomarkers in the blood directly, positively, correlates to diagnosis of a stroke. The goal of this study in particular was to develop a panel of biomarkers to assess the neurotoxicity and thrombosis that is correlated with the severity of cerebral ischemia, and interpretation of concentrations can prove to be effective in understanding the severity of stroke, and likewise heart failure.

Biomarkers have also been evaluated to distinguish the types of myocardial infarction and injury. In a study conducted by Neumann et al, there was a panel of 29 biomarkers that were
investigated in an ED setting among patients with type 1 myocardial infarction, type 2 myocardial infarction, and myocardial injury. Myocardial infarction remains as one of the leading causes of morbidity and mortality across the globe and contributes to over 15 million deaths per year.

There are a few different subsets of myocardial infarction: type 1 myocardial infarction, which is caused by acute plaque disruption or erosion, and type 2 myocardial infarction, which is caused by a myocardial imbalance of oxygen supply or demand. Myocardial injury is understood as increased troponin in an absence of acute myocardial ischemia. Currently, the only biomarkers that are used in understanding the advancement of myocardial infarction or myocardial injury are troponin, N-terminal brain natriuretic hormone, and C-reactive protein. The aim of the study incorporating a panel of 29 biomarkers is to unearth a method to discriminate types of myocardial injury and myocardial infarction.

The study found that only a few of the biomarkers that were tested for had strong positive correlations (>0.7), which were observed from Apo C-1 and Apo A-II, Apo C-1 and Apo C, myoglobin and FABP, TNFR2 and thrombomodulin, TNFR2 and tissue inhibitor metalloproteinases 1, TNFR2 and vascular cell adhesion. A table with the relative concentrations of each biomarker tested for is shown below, and the values have been discriminated among patients with myocardial injury, those with myocardial infarction in general, those with type 1 myocardial infarction, and those with type 2 myocardial infarction.

**Table 2. Table displaying the various log-transformed biomarkers as they relate to a clinical diagnosis of heart failure correlated with myocardial infarction.**

| Table 2. Table displaying the various log-transformed biomarkers as they relate to a clinical diagnosis of heart failure correlated with myocardial infarction. |
There were 4 out of 29 biomarkers that showed statistically significant discrimination between type 1 and type 2 myocardial infarction. The four biomarkers were Apo A-II, NT-proBNP, copeptin, and hs-cTnI. Furthermore, there were 6 out of 29 biomarkers that were selected for that displayed statistically significant discrimination between myocardial infarction and myocardial injury. Those biomarkers were adiponectin, NT-proBNP, pulmonary and activation-regulated chemokine, transthyretin, copeptin, and high sensitivity cardiac troponin I. Use of these biomarkers could allow early intervention and use of invasive treatment for those that have tested positive for a type I or type II myocardial infarction, and also the results of blood samples depicting biomarker concentration could prevent invasive treatment and lead to further monitoring so patients are not subject to unnecessary action.

This study included patients of varying age, sex, body mass index, blood pressure (hypertension), hyperlipoproteinemia, diabetes, current smoking status, history of myocardial infarction, history of coronary artery disease, systolic blood pressure, diastolic blood pressure, glomerular filtration rate, and symptom onset. With all of these variables present in the current study, the importance of a panel of biomarkers not only to distinguish types of myocardial infarction but myocardial infarction in general in comparison to myocardial injury is very important. This relates to those that present to the ED with possible heart failure because the onset of signs and symptoms varies among patients. This study serves as a standard that the current INDICATE heart failure study aims to reproduce in identifying a panel of biomarkers from a larger class of proteins to understand trends in elevated or reduced concentrations of proteins that signal heart failure of any class.

Another study that further supports the advancement of a panel of biomarkers in myocardial infarction is the investigation conducted by Lakhani et al. This particular study investigated the importance of interleukin-10 (IL-10) as a prognostic marker for the cardiovascular outcomes and develop a panel of biomarkers, including circulating microRNAs (miRNAs) for a direct detection of heart failure as a result of myocardial infarction. The advantage of including miRNA in detection is the stability in bodily fluids, as well as the ability for miRNAs to be measured in other species because of the conservation of encoding between species. The study includes the analysis of IL-10, transforming beta growth factor, matrix metalloproteinase-9, tumor necrosis factor, and interleukin-6, which are crucial in the fibrotic and inflammatory cardiovascular damage. Along with these proteins within the blood, the serum levels of five different miRNAs were analyzed: miRNA-24, miRNA-29a, miRNA-34a, miRNA208b, miRNA-126.

The study protocol included 43 patients, which were divided into three groups: a control group of 14 patients that had no incidence of myocardial infarction and a normal ejection fraction, 13 patients with clinical diagnosis of myocardial infarction and a normal ejection fraction, and 16 patients that had a clinical diagnosis of myocardial infarction and a reduced ejection fraction. The study obtained blood samples and ran ELISA quantification for the proteins in the blood and RNA was quantified using miRNeasy serum plasma kit.

The study found that there were elevated levels of metalloproteinase-9 and transforming beta growth factor in patients that had been diagnosed with myocardial infarction and a reduced ejection fraction in comparison to patients that belonged to the other two cohorts (p<0.01).
which is indicative of cardiac remodeling and a facilitation of heart failure. The study was also fortunate in finding that the pro-inflammatory cytokines interleukin-6 and tumor necrosis factor was also higher in patients that had a myocardial infarction with preserved ejection fraction and reduced ejection fraction in comparison to the control group without either of these conditions. There was no correlation between increased levels of IL-10 and myocardial infarction diagnosis with reduced ejection fraction, as the group diagnosed with myocardial infarction and reduced ejection fraction also showed elevated levels of IL-10. The figure below details the levels of the various proteins circulating in the blood and the corresponding cohort that portrayed these concentrations.

**Figure 6.** Panel of figures displaying concentrations of various biomarkers as it relates to myocardial infarction with normal ejection fraction or reduced ejection fraction in comparison to the control. The levels of MMP-9 are elevated in those patients that have a reduced ejection fraction, which can be indicative of heart failure. The levels of trans-beta growth factor are elevated in those with myocardial infarction and reduced left ventricle ejection fraction, which also serves as a positive correlation between myocardial infarction and heart failure. The levels of IL-6 showed an elevated level in those with myocardial infarction and reduced left ventricle ejection fraction, which is indicative of a correlation between myocardial infarction and heart failure. The final two biomarkers showed no positive correlation between elevated levels, myocardial infarction, a reduced ejection fraction, and a positive correlation to heart failure.\(^{16}\)

The study also included detail on the result of miRNA analysis. The figure below details the levels of miRNA that were either elevated or reduced in the cohorts that had myocardial infarction and either normal ejection fraction or reduced ejection fraction as it compares to the control group. The levels of miRNA-126, miRNA-34a, and miRNA-208b were all elevated in the group that had diagnosis of myocardial infarction with normal ejection fraction and showed even greater concentrations in the group that had both myocardial infarction and reduced ejection fraction in comparison to the control group. The levels of miRNA-29a and miRNA-24 were reduced in the group that had myocardial infarction and normal ejection fraction and even lower in the group diagnosed with myocardial infarction and reduced ejection fraction in comparison to the control group.
Figure 7. Panel of bar graphs that show the levels of circulating miRNA in the blood as it relates to patients with myocardial infarction and normal ejection fraction or reduced ejection fraction in comparison to the control. The levels of miRNA-126, mi-RNA34a, and miRNA208b were all elevated in those with myocardial infarction and reduced ejection fraction, which can be a positive indicator of congestive heart failure diagnosis. The levels of miRNA-29a and miRNA-24 showed no elevation in those that had myocardial infarction with normal ejection fraction or reduced ejection fraction, which serves as a negative indicator of congestive heart failure.\textsuperscript{16}

While this study was specific for patients that experienced myocardial infarction and as a result had early onset of heart failure confirmed by ECG, it can be seen that evaluation of various biomarkers associated with these conditions can aid in determination of heart failure. The goal is to find a cost effective and easy to use method to evaluate heart failure for any presentation at the ED. This study was specific to myocardial infarction, but it clearly displayed a diagnostic advantage in developing a panel of biomarkers and miRNA for early detection of heart failure classification. This can be seen as a gold standard in evaluating biomarkers and miRNA in patients that present to the ED with dyspnea resulting from any underlying cardiac condition that may lead to onset of heart failure.

Possible Biomarkers for Use in Heart Failure:

The advantages of including a panel of biomarkers not only aids in understanding the presence or absence of a certain condition but including a multiple marker model allows clinicians to distinguish the advancement of a certain pathology. The final model literature shows potential in various biomarkers that are currently being tested for in the INDICATE heart failure clinical trial, as well as in other clinical settings elsewhere. The following literature details various biomarkers that belong to biological pathways, which pose potential for utility in diagnosis of heart failure for patients that present to the ED with any sign or symptom. This remains to be the optimum outcome of the INDICATE heart failure study. The ability to diagnose onset of heart failure as a result of any sign or symptom presenting to the ED remains to be seen and is continuously evaluated in clinical settings across the globe.

Heart failure includes the interplay of several different adverse outcomes including neurohormonal hyperactivation, inflammation, myocardial stretch, matrix remodeling, and myocyte injury.\textsuperscript{17} This provides the potential to incorporate biomarkers released in irregular amounts from any of the above conditions that are related to onset of heart failure. The importance of understanding the biological pathways that are activated in a different manner...
when patients develop heart failure is imperative in unearthing a panel of biomarkers that can signal irregularities. The current gold standard of heart failure testing is BNP and NT-proBNP, but these biomarkers only provide prognostic information. They are easily measured but are only useful in confirmation of a heart failure diagnosis. There obscure benefit from a BNP or NT-proBNP assay in understanding the patients that are at greater risk of heart failure, the advancement of heart failure, and definitive diagnosis of heart failure in patients.

The idea that both of these peptides can be released as a result of some other disease or syndrome causes elevated levels and a potential to misdiagnose heart failure. The issue with using natriuretic peptides as a clinical signal of heart failure is the fact that levels tend to be relatively normal in those with acute decompensated heart failure in comparison to chronic. The other clinical issue with using these peptides as a biomarker for heart failure relates to patients with kidney impairment. Those with kidney disease are at risk for having elevated BNP or NT-proBNP levels because of altered filtration rate. In addition to issues regarding elevated levels of natriuretic peptides in patients with kidney disease, those that present to the ED with obesity also have skewed concentrations of these peptides in the body, which makes diagnosis unreliable. If a biomarker test was used alone, the concentrations may reflect differently, so an ECG would be needed in addition for confirmation.

In a review written by Ibrahim & Januzzi, the various biomarkers that could be used in a more accurate and precise diagnosis of heart failure were detailed. There were multiple biomarkers detailed corresponding to the various biological pathways. The RAAS biological response plays a role in the neurohormonal response to developing heart failure. As a result of the implications of this system, biomarkers that correspond to the activity of this pathway have been evaluated as possible markers that could be incorporated into a multiple marker model. Arginine vasopressin and copeptin are two possible markers that have been evaluated in detecting onset of heart failure. Arginine vasopressin is an antidiuretic and vasoconstrictive hormone that is released from the hypothalamus, and concentrations of this marker are elevated in patients that present to the ED with worsening heart failure. The only issue with this marker as a possible suitor for a marker model is the fact that is an unstable peptide that is often difficult to quantify. For this reason, there are often terminal ends of hormones that can be obtained for measurement. Copeptin is the C-terminal segment of provasopressin and has been reported as a proper predictor of chronic heart failure. This peptide could be incorporated with BNP or NT-proBNP for thorough diagnosis and prognosis of heart failure. Another related hormone is endothelin-1. This peptide is produced by the endothelium in response to inflammation, stress, or neurohormonal activation. It is responsible for vasoconstriction and proinflammatory actions; moreover, this hormone has been associated with worse left ventricular diastolic performance and worse clinical outcomes. These two hormones associated with the RAAS neurohormonal response to impairment of cardiac homeostasis could serve as potential biomarkers for a multiple marker panel because of the homogeneity of this response in patients. Establishing a near constant that most patients with heart failure can provide opportunity for a more consistent diagnosis and prognosis.

Cardiac remodeling has been a cornerstone in presentation and adverse effects of patients who arrive at the ED with advancing heart failure. A panel of biomarkers would be best suited with markers for diagnosis and prognosis. A marker, ST2, is a protein member of the interleukin (IL-
1) receptor family. This protein is released under conditions that result from myocardial and vascular stress. There are two forms of this protein (isoforms), one is a transmembrane form and the other is a soluble form. The main responsibility of this family of proteins is to mediate the effects of myocardial fibrosis and cardiac remodeling. There is a reduction in programmed cell death and downregulation of profibrotic pathways\textsuperscript{17}. This is not only an important biological factor in reducing the advancement of heart failure, but it can be a marker incorporated into a panel that signals the stage of heart failure a patient is in. In an evaluation called the PRIDE study, the concentrations of soluble ST2 predicted death at 1 year in dyspneic patients (HR, 5.6; 95% CI, 2.2–14.2; \( P<0.001 \)), as well as predicting mortality in patients that present to the ED with acute decompensated heart failure (HR, 9.3; 95% CI, 1.3–17.8; \( P=0.03 \))\textsuperscript{17}. This marker has the potential to be incorporated into a multimarker model that can be taken with serial measurements for a continuous monitoring of the condition of heart failure in patients, which could aid in understanding the pathological outcome.

The importance of microRNA has also been discussed in the prior study regarding myocardial infarction. There has been some introductory research that has been conducted on the uses of these molecules in the field of heart failure. These miRNAs serve as extracellular matrix remodeling biomarkers and can be coupled with matrix metalloproteinases and tissue inhibitors of metalloproteinases. Extracellular matrix remodeling is a form of cardiac remodeling that is present in the progression of heart failure. This remodeling takes place in part through degradation of collagen and other matrix proteins by collagenases, matrix metalloproteinases and mediated by tissue inhibitors of metalloproteinases\textsuperscript{17}. If a patient has an onset of heart failure, then these collagenases will be activated and the blood samples that are taken upon presentation to the ED can evaluate for elevated levels. This will signal cardiac remodeling, which puts patients at greater risk for developing heart failure characterized by weakened heart musculature. Further testing and treatment can then ensue to correct the course of developing heart failure. MicroRNA are responsible for the transcriptional and post-transcriptional regulation of gene expression. There have been many microRNA that have been utilized in pilot studies to demonstrate the correlation to developing heart failure. There has even been research conducted that has displayed the utility in using microRNA to map out the treatment timeline. A study conducted by Rooij et al. found that miR-423-5p was strongly diagnostic of heart failure with an area under the curve 0.91 (with 1.0 being optimal); moreover it has been demonstrated that concentrations of 2 of the identified microRNA, miR-499-5p and miR-423-5p, changed in response to therapy that improved measures of cardiac remodeling and survival\textsuperscript{17}.

There remains potential for many other biomarkers to be utilized in the diagnosis of heart failure, but the structure and tendencies of the biological pathways require further evaluation from pilot studies. The utility of combining these biomarkers labeled above would create opportunities for a culmination of diagnoses of and related to heart failure. If some biomarkers labeled above and the ones that were included in the cited comprehensive review were used, there is potential for evaluating the effectiveness of treatment, the severity of heart failure development, and accuracy in diagnosis in general.
Conclusion:

The purpose of this review was to outline the current evaluation of heart failure that is being studied by Vanderbilt University Medical Center, with Wayne State University as a collaborator. The current hypothesis of the study that is currently carried out in the ED is uncovering the presence of a versatile multiple biomarker panel that incorporates novel proteins discovered with plasma proteomics, which has the potential to improve the diagnostic accuracy for acute heart failure. There is no greater teaching hospital than the DMC Sinai-Grace Hospital and the DMC Detroit Receiving Hospital. These hospitals combined provide opportunities to work with a class of patients that are unique to the Detroit area, and it continues to provide experience to work with those that suffer from a scarcity in medical attention. The evaluation of heart failure is in the EDs located in this area provides versatility in the discovery of a multiple marker model. There are patients that present to the ED with various signs and symptoms that may not be present in other areas of the country. Therefore, the evaluation of a multiple marker model is optimal in this area because the final result has the potential to be equipped with diagnosis of congestive heart failure for any presentation to the ED based off of a blood draw.

The benefits of discovering a multiple marker model for diagnosis of heart failure is the ability to rule-in heart failure based on the concentration of novel proteins instead of ruling-out. The capability of the current form of diagnosis only allows for a ruling out of heart failure, rather than a ruling-in. The current form of diagnosis revolves around the use of natriuretic peptides, namely brain-natriuretic peptide and N-terminal pro brain-natriuretic peptide. This method of detection is scarcely suitable for improvement of diagnostic accuracy of heart failure and a decrease in mortality. The study, Improving Diagnostic Accuracy for Acute Heart Failure, evaluated 21 different biomarkers with an aim to identify the optimal combination of proteins for improved diagnosis, prognosis, and decrease mortality. There were few prior studies conducted that explored a multiple marker approach, and those that did were limited from known biological pathways, relatively small sample sizes, lack of inclusion of all priori selected biomarkers into a single model, and absence of validation cohorts. The single screening requirement for the study is presentation to the ED with dyspnea, but there are further criteria that need to be met by the patient in terms of current health status and historical medical history. The three aims of the study are 1) discover a multi-marker panel of 21 biomarkers to improve diagnostic accuracy for acute HF, 2) derive a model for diagnosing acute HF incorporating the 21-biomarker panel, and 3) test performance of the multi-marker model in a prospective validation cohort. All of this taken together creates the opportunity to fine tune a versatile multiple marker panel that can be used in a multitude of different situations for the diagnosis of heart failure based on differing signs and symptoms.

The elucidation of a panel of biomarkers begins with an understanding of how the syndrome presents to the emergency department. The chief complaint in patients that are diagnosed with heart failure is dyspnea; however, there are other cardiac and non-cardiac conditions that can lead to heart failure. Age is one of the recurring characteristics in patients that present to the ED with heart failure. It is nearly unavoidable, as the heart loses the contractile ability with declining musculature in elderly patients. On top of age, coronary artery disease, history of myocardial infarction, high blood pressure, and cardiomyopathy all induce heart failure if left untreated. These conditions are related to cardiac homeostasis. If these are left untreated, they induce a
neurohormonal response, which leads to cardiac remodeling. This cardiac impairment often leads to impaired systolic and diastolic volumes, which is related to preserved or reduced injection fraction. Increased diastolic filling pressures leads to impairment of the cardiac musculature, characterized by ventricular stiffness and skewed ejection fraction. Systolic dysfunction often occurs as a result of diastolic impairment because of interruption in the signal transduction mechanisms responsible for contraction. The impairment of the filling and contraction of the ventricle often leads to a reduced ejection fraction. Heart failure with left ventricular ejection fraction has the tendency to present in three ways: those with preserved left ventricle ejection fraction (PLVEF), which is often defined as LVEF ≥50%, a normal or mid-range LVEF defined as 40-50%, and heart failure with a reduced LVEF <40%. This is often evaluated via an echocardiogram to confirm the diagnosis of heart failure. The issue with this diagnostic approach revolves around those patients that present to the ED with a preserved ejection fraction, and therefore there is no physical sign of impaired cardiac function. The reduction in ejection fraction is indicative of cardiomyopathy, which is characterized by inability of heart musculature to reproduce and function in a healthy manner. This is often seen as elevated filling pressures or inducing some neurohormonal response that aims to compensate for this reduced contractility or relaxation.

The topics detailed lay the foundation for unearthing a multiple biomarker panel beyond simple evaluation of BNP and NT-proBNP. The current method of diagnosis requires a certain level of BNP and/or NT-proBNP to be circulating in a blood sample. The drawback of this approach is the unending variability in presentation to the ED. The levels of these peptides vary from patient to patient based on their current and past medical condition. The current diagnostic approach revolves around ruling-out of heart failure rather than a ruling-in. This approach, however, requires a patients BNP concentration to be below 100 pg/mL in a blood sample. This is not an absolute in a clinical setting. In the Breathing Not Properly multinational study, the levels of b-type natriuretic peptide based on the 4 classes of patients with worsening heart failure were defined as: 244±286 pg per milliliter among patients in class I, 389±374 pg per milliliter among those in class II, 640±447 pg per milliliter among those in class III, and 817±435 pg per milliliter among those in class IV, and the correlation between BNP levels and worsening symptoms was proven as significantly different (p<0.001)⁸. This data provides information on what the average levels of natriuretic peptides are in those patients that do develop heart failure. Unfortunately, this is not consistent in the mass of people, and therefore pose risk in misdiagnosis. Patients with various characteristics and comorbidities often alter the levels of natriuretic peptides in testing. Obesity, age, sex, and renal dysfunction contain the ability to alter the peptide levels in patients. It has been shown that obese patients consistently display lower levels of BNP despite a possible heart failure diagnosis in comparison to lean patients. Not to mention, patients with renal impairment often display altered BNP levels. A review by Baba et al. found that The BNP cut-off point for the diagnosis of HF may need to be raised when the estimated glomerular filtration rate (eGFR) is <60 mL/min/1.73 m², and the BNP level in patients with an eGFR <60 mL/min/1.73 m² was approximately two- to four-fold greater than that in patients with an eGFR ≥60 mL/min/1.73 m².¹¹

The issue regarding diagnosis of heart failure is an important, developing, condition because of the inability to accurately diagnosis based off current approaches. With an obscure diagnostic approach, the mortality rate of heart failure only increases with time, and according to the
Framingham Heart Study, the mortality rate of heart failure after diagnosis in the US was around 10% after 30 days, 20-30% after 1 year, and 45-60% after 5 years\textsuperscript{18}. This means that with time, the condition of developing heart failure only becomes worse. The research that is being carried out by Vanderbilt University Medical Center and Wayne State University carries great importance in the livelihood of millions not only in the United States but across the world.
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