

Advances in the detection and quantification of candidate and established biomarkers in heart failure

Progrese în detectarea și cuantificarea biomarkerilor potențiali și uzuali în insuficiența cardiacă

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Abstract

Heart failure (HF) is a common condition, especially in older patients. Considering the increase of life expectancy the total number of HF patients is expected to grow in the future, which makes HF an important focus of research. New high-throughput techniques, such as the “omics” (genomics, transcriptomics, metabolomics) have brought new insight into disease pathophysiology, facilitating the discovery of an impressive number of candidate biomarkers. In this review we consider different perspectives on the evaluation and clinical potential of biomarkers in heart failure as their usefulness in clinical settings is constantly being evaluated in order to bring them closer to the point-of-care. In addition, we examine recent advances in the methods of detection and quantification of biomarkers.

Keywords: heart failure, biomarker, diagnostic, prognostic, multi-marker strategy.

Rezumat

Insuficiența cardiacă este o afecțiune cu o prevalență ridicată, în special în rândul pacienților vârstnici. Pe viitor, se preconizează creșterea numărului de pacienți cu insuficiență cardiacă, luând în considerare creșterea speranței de viață, ceea ce sporește interesul științific pentru această patologie. Noile tehnologii „omice” cum ar fi genomics, proteomics sau metabolomics au contribuit la elucidarea fiziopatologiei insuficienței cardiace prin furnizarea unui număr impresionant de potențiali biomarkeri. În acest review vom prezenta diferenții biomarkeri candidați și uzuali pentru insuficiența cardiacă și utilitatea lor clinică, precum și progresele făcute pentru creșterea sensibilității, selectivității și reproductibilității metodelor lor de detecție și cuantificare.

Cuvinte cheie: insuficiență cardiacă, biomarker, diagnostic, prognostic, strategie multi-marker.

Received: 13th April 2013; **Accepted:** 5th September 2013; **Published:** 9th September 2013.

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Introduction

Heart failure (HF) is the result of a wide range of cardiovascular disorders that influence negatively the heart's property to fill or to pump blood. Due to its multiple etiologies, HF is a common condition, affecting approximately 1-2% of the population in developed countries, with a prevalence of more than 10% within the 70-years-or-older age-group (1). Considering the increase of life expectancy and the reduction of age-adjusted mortality through disease management, the total number of HF patients is expected to grow in the future, which makes HF an important focus of research.

New high-throughput techniques, such as the "omics" (genomics, transcriptomics, metabolomics) have brought new insight into disease pathophysiology. The introduction of mass spectrometry in the analysis of proteins has opened the door to a plethora of candidate biomarkers for HF.

Through the process of validation, performed by multiple reactions monitoring (MRM) or antibody assays, the number of biomarkers decreases. Proteins are then further subjected to MRM or enzyme-linked immunosorbent assays

(ELISA) to test their applicability in clinical trials (5). *Table I* shows some of the important criteria in the development of a biomarker in HF, in order to possess clinical utility.

Biomarkers in HF are classified according to the pathologic processes in which they may be involved, such as inflammation processes, oxidative stress, extracellular matrix remodeling, hypertrophy, fibrosis, neurohormonal activation, myocyte injury, myocyte stress or even apoptosis (4, 7, 8).

HF biomarkers can have cardiac origin and effect, whereas others are the markers of processes that indirectly affect the heart. Many HF biomarkers provide information regarding pathologic processes associated with HF and thus have the potential to be targets of future therapies. *Table II* shows examples of candidate biomarkers in HF.

Biomarkers of myocyte stress and apoptosis in HF

The natriuretic peptides, brain natriuretic peptide (BNP) and its amino-terminal fragment (NT-proBNP), are the most studied HF biomarkers. Currently, they are the "gold standard" biomarkers for HF. They can be used in diagnosis/exclusion, prognosis and management of HF. All the major societies such as the European Society of Cardiology, the American College of Cardiology and the American Heart Association recommend their clinical use in their guidelines (1).

A cutoff value for BNP or NT-proBNP of 100 pg/mL has a high specificity and sensitivity for HF (9). Other pathologies such as obesity, renal failure, anemia, stroke, pulmonary heart disease, cardiotoxic drugs can increase BNP or NT-proBNP values (7). *Table III* shows examples of findings of major studies that evaluated the clinical use of BNP and NT-proBNP in HF. Automated immunoassays have been developed by many companies

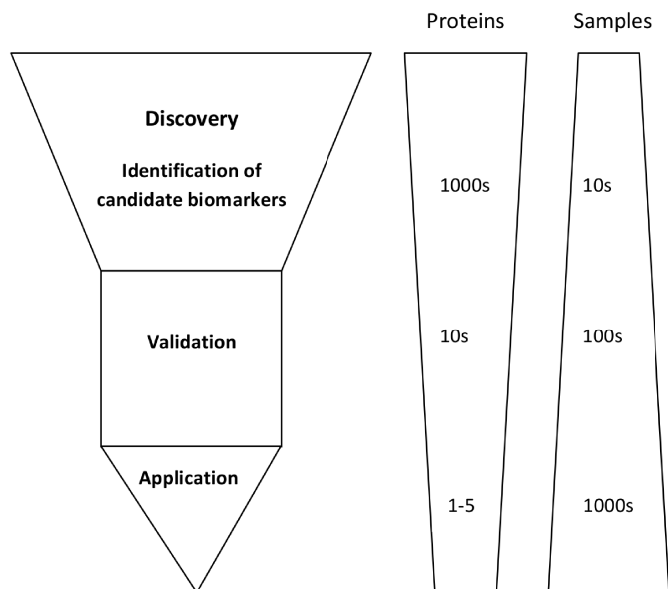


Figure 1. Stages of biomarker development.

Table I. Criteria for new HF biomarkers.

Attributes of a HF biomarker	Reference
1.It is easily and quickly measured, at a reasonable price 2.Has a strong correlation to HF 3.Enables patient management (early detection of disease, diagnosis, risk stratification, therapeutic intervention, monitoring)	Morrow and de Lemos (2).
1.Confirms or excludes the diagnosis of HF and enables risk stratification of patients with HF 2.Can be used for screening for cardiac dysfunction 3.Can guide the management of HF	Tang et al (3).
1.It should be the result of an exhaustive and up-to-date evaluation process 2.It should be easily and shortly quantified, with high accuracy and should have known biological variation 3. Can provide information regarding HF pathophysiology that can help determine diagnosis, prognosis, progression or management of HF 4.It must offer new clinically useful information to enable further decision making	Van Kimmenade and Januzzi (4).

and their utility is constantly tested (10, 11). New, simpler, cost-effective methods for BNP detection such as the immunosensors are being tested as candidates for the detection of NT-proBNP. They detect NT-proBNP in serum such as the regeneration-free immunosensor with novel Fab fragment monoclonal antibodies that detects NT-proBNP from 0.04 to 2.5 ng/ml with a limit of 0.03 ng/ml (12) or in whole blood such as the combination of a microfluidic system with an immunoassay based on an electrochemical immunosensor with magnetic nanoparticles, biotin-avidin system (BAS) and fragment antigen binding (Fab) antibodies (13) or the electrochemical immunosensor based on the nanostructural gold and carbon nanotubes composite, gold nanochains (AuNCs) and horseradish peroxidase (HRP) complex labeled secondary antibodies (AuNCs-HRP-Ab(2)) (14).

An increase of the *A-type natriuretic peptide* (ANP) and *adrenomedullin* (ADM) concentrations was associated with HF and low left ventricular ejection fraction (LVEF) (4, 8). The midregional proADM (MR-proADM) and proANP (MR-proANP) are the more stable fragments of ANP and ADM; they have a longer half

life and thus are easier to measure. The BACH trial (Biomarkers in Acute Heart Failure) has underlined their clinical utility in the diagnosis (MR-proANP ≥ 120 pg/mL is associated with HF) and prognosis (MR-proADM values predict 90-days survival with an accuracy of 73%) of HF (20). It has been suggested that both MR-proADM and MR-proANP may be useful in obese patients or in patients with renal failure, where BNP or NT-proBNP are less accurate (4). Both proADM and proANP can be detected by sandwich immunoluminometric assay using two specific polyclonal antibodies for the amino acids 45-92 of proADM and 1-98 of pro ANP, respectively (21, 22).

ST2 is an inflammatory cytokine, member of the interleukin (IL-1) receptor family. ST2 is thought to be involved in modifying immunologic processes through its soluble (sST2) and membrane-bound (ST2L) forms, produced during myocardial strain (23). The physiologic ligand for ST2 is IL-33, which binds to ST2L and produces beneficial effects such as antihypertrophic, antifibrotic and antiapoptotic effects, but when it binds to sST2, all of its effects are neutralized (24). High levels of sST2 can be correlated to ventricular remodeling and disease

Table II. Biomarkers used in Heart Failure and their clinical relevance (4, 8)

Pathologic processes	Biomarkers	Prognostic value	Diagnostic value	Cardiac origin	Potential targets of therapy
Inflammation	C-reactive protein	+	Risk	-	-
	Tumor necrosis factor α	+	Risk	-	-
	Fas (APO-1)	+	Risk	-	-
	Interleukins 1, 6, 18	+	Risk	-	-
	Pentraxin-3	+	Risk	-	-
	Adiponectin	+	Risk	-	-
	Osteoprotegerin	+	Risk	-	-
Oxidative stress	Oxidized low-density lipoproteins	+	-	-	+
	Myeloperoxidase	+	-	-	+
	Urinary biopyrrins	+	-	-	+
	Urinary and plasma isoprostanes	+	-	-	+
Extracellular matrix remodelling	Plasma malondialdehyde	+	-	-	+
	Matrix metalloproteinases (MMP)	+	-	+	+
Hypertrophy	Tissue inhibitors of metalloproteinases (TIMP)	+	-	+	+
	Collagen propeptides				
	Propeptide procollagen type I	+	-	+	+
Fibrosis	Plasma procollagen type III	+	-	+	+
	Type I collagen telopeptide	+	-	+	+
Neuro-hormonal activation	Galectin 3	+	-	+	-
	Norepinephrine	+	-	-	+
	Renin	+	-	-	+
	Angiotensin II	+	-	-	+
	Aldosterone	+	-	-	+
	Arginine vasopressin	+	-	-	+
	Endothelin	+	-	-	+
Myocyte injury	Chromogranin A	+	-	-	+
	Cardiac-specific troponins I and T	+	+	+	+
	Myosin light-chain kinase I	-	Risk	+	+
	Heart-type fatty acid protein	-	Risk	+	+
Myocyte stress	Creatine kinase MB fraction	-	Risk	+	+
	Brain natriuretic peptide	+	+	+	+
	N-terminal pro-brain natriuretic peptide	+	+	+	+
	Midregion fragment of proadrenomedullin	+	+	+	+
Apoptosis	ST2	+	+	+	+
	Growth differentiation factor-15 (GDF-15)	+	-	+	-

severity in HF. Together with natriuretic peptides and highly sensitive troponins, ST2 has a powerful prognostic value (25). Presage ST2 Assay has been approved by the Food and Drug Administration as a novel high-sensitivity immunoassay for the measurement of soluble ST2 in human plasma and it is established as a linear and stable method of detection (26, 27).

Growth differentiation factor 15 (GDF-15) is a member of the transforming growth fac-

tor- β cytokine superfamily with high hopes of becoming an important biomarker in HF. It is involved in cell differentiation and tissue repair (28). A novel method of detection for GDF-15 is the microparticle-based solid-phase proximity ligation assay which has been tested against the traditional sandwich ELISA and was found to be superior (29). GDF-15 levels are high (>1200 ng/L) in acute myocardial infarction and HF in response to cardiac ischemia and pressure overload. GDF-

Table III. Findings of major studies that evaluated BNP and NT-proBNP

Study	Findings	Year
Breathing Not Properly Study (9).	BNP had a sensitivity of 90% and a specificity of 74% for accurately diagnosing HF at a cutoff value of 100 pg/mL.	1999-2000
ADHERE Study (15).	There was a linear relationship between increasing admission BNP values and increasing in-hospital mortality.	2007
Italian RED Study (16).	Assessment of BNP values at admission, at 24hours after admission and at discharge had a great prognostic value for future cardiovascular events, independent of left ventricular ejection fraction (LVEF).	2010
PRIDE Study (17).	NT-proBNP testing alone was superior to clinical judgment alone for the diagnosis of acute congestive HF.	2005
ICON Study (18).	Clear established correlation between symptom severity and NT-proBNP concentrations. An adjustment of NT-proBNP cutoff values according to age must be made: < 50 years, NT-proBNP \geq 450 pg/mL; 50-75 years, NT-proBNP \geq 900 pg/mL; >75 years, NT-proBNP \geq 1800 pg/mL.	2006
IMPROVE CHF Study (19).	Adding NT-proBNP to clinical judgment reduced the duration of ED visit by 21%, the number of patients rehospitalized over 60 days by 35%, and direct medical costs of all medical services.	2007

15 can be used both as a prognostic and diagnostic biomarker for HF as it can help predict all-cause mortality and identify patients with HF and preserved ejection fraction (30, 31).

Biomarkers of inflammation in HF

The cytokine hypothesis of HF states that cardiac injury, for example ischemia, can trigger stress responses such as liberation of proinflammatory cytokines like tumor necrosis factor (TNF- α), IL-1, IL-6 and IL-18. The expression of such cytokines affects ventricular function and facilitates the installation of HF (32).

TNF- α measurement can have a prognostic value in HF patients but can also predict the development of HF in asymptomatic patients (33). Sandwich ELISA using a well plate is a common procedure, but it is considered time-consuming. A recent study shows the advantages of performing sandwich ELISA on a microchip of cyclic olefin copolymer with 4 straight microchannels for the detection of IL-6 or TNF- α (34). Using a magnetic bead surface coverage assay, Tekin *et al* have detected attomolar quantities of TNF- α (35).

C-reactive protein (CRP) has been associated with HF and cardiovascular risk in many studies, but lacks specificity to heart disease (36). It is believed that CRP may have protective effects but also negative effects in HF, such as up-regulation of TNF- α and IL-6 (37). Recently, a battery of aptamers has been patented for the detection and measurement of CRP. They represent a more accurate and sensitive method of detection than the traditional antibodies (38).

Pentraxin 3 (PTX3) has been shown to have prognostic value in patients with HF (39). PTX3, TNF- α and IL-6 were found to be significantly elevated in patients with HF and preserved ejection fraction (39). Novel detection methods for PTX3 use high sensitive plasma ELISA assay system with monoclonal antibodies, which have a limit of detection of 0,1 ng/mL, a significantly greater sensitivity than the commercially available kits (40).

Osteoprotegerin (OPG) is a member of the tumor necrosis factor receptor superfamily has been associated with ventricular dysfunction. OPG can predict survival after myocardial infarction in HF patients (8). Increased serum

OPG levels have been found in ischemic cardiomyopathy HF patients (41).

Biomarkers of oxidative stress in HF

Markers of oxidative stress, such as *myeloperoxidases* (MPOs) have demonstrated positive correlation with New York Heart Association (NYHA) functional class and diastolic dysfunction (42). MPO values > 99 pmol/L, together with BNP have shown an increased prognostic value than BNP alone (43). MPO can be detected by cytochemistry, flow cytometry and immunohistochemistry, but the results of one study show the importance of using more than one method of detection, for higher accuracy (44).

Biomarkers of extracellular-matrix remodeling in HF

Remodeling of the ventricles is an important process in the progression of HF. An imbalance between *matrix metalloproteinases* (MMPs) and *tissue inhibitors of metalloproteinases* (TIMPs) leads to modifications of the extracellular matrix with further modifications of the size and shape of myocytes (8). There are at least 25 MMPs described in literature. Of all the MMPs so far only MMP2, MMP3, MMP7 and MMP9 have been linked to HF (45). They are zinc-containing endopeptidases. They degrade proteins by cleavage of peptide bonds and can be detected by zymography, while TIMP1 (tissue inhibitor of MMP1) can be detected by reverse zymography (46). TIMP1 has been found to predict all-cause mortality in chronic HF patients (47).

Galectin-3 (GAL-3) is secreted by activated macrophages and stimulates cardiac fibrosis by stimulation of collagen I deposition and irreversible cross-linking in myocytes. GAL-3 may be an indicator of cardiac remodeling and fibrosis in HF (48). Quantitative proteomic analysis employing isobaric labeling (iTRAQ – isobaric tag for relative and absolute quantitation) has been used for the detection of

cardiovascular biomarkers in pregnant women, among which GAL-3 (49). *Park et al* used d-(+)-Galactose-conjugated single-walled carbon nanotubes as biosensors and succeeded in enhancing the sensitivity for GAL-3 (50).

Biomarkers of neurohormonal activation in HF

It is well known that in HF there is an activation of compensatory mechanisms such as the sympathetic nervous system and the renin-angiotensin-aldosterone system (51). The utility of neurohormones as diagnostic or prognostic tools in HF is questionable as therapeutic agents such as beta-blockers, angiotensin converting enzyme inhibitors (ACEI), aldosterone receptors blockers (ARBs) particularly target them (52). Nevertheless, there are some markers of neurohormonal activation that have proven diagnostic or prognostic utility.

Endothelin-1 (ET-1) is considered a marker of sympathetic activation but it is not specific to HF (53). ET-1 has been measured and characterized by *Laricchia-Robbio et al* by using a surface plasmon resonance-based biosensor and ELISA (54). However, ET-1 is difficult to measure because of its instability, and its tendency to bind to receptors and plasma proteins. The stable surrogate of ET-1, C-terminal pro-endothelin-1 (CT-proET1) has also been positively correlated with the risk of cardiac death or heart failure (55). *Papassotiropoulou et al* have reported a robust method of indirectly measuring ET-1 by determining CT-proET-1. CT-proET-1 has been measured using a sandwich immunoluminometric assay with two polyclonal antibodies for amino acids (AA) 168-212 of pre-proET-1 (56).

Arginine vasopressin (AVP) is released from the hypothalamus in states such as hypovolemia or hyponatremia. Its levels are high in HF, but as it has a very short half life, AVP is difficult to analyze. *Thomas et al* developed a liquid chromatography coupled to quadrupole high resolution

Table IV. Examples of multimarkers panels for HF

Markers	Findings	Ref.
NT-proBNP, troponin I, CRP, cystatin C	Improvement of risk stratification for death from cardiovascular causes among elderly men.	Zethelius et al (66).
NT-proBNP, CRP, MPO	Addition of CRP and MPO enhanced the specificity of NT-proBNP in the screening for LVSD.	Ng et al (67).
Hemoglobin, cTnI, BNP	Anemia is associated with elevated BNP and increased mortality in HF. Elevation of BNP and cTnI, in patients with HF and anemia has prognostic value of future events.	Ralli et al (68).
NT-proBNP, CRP, ST2, hemoglobin, blood urea nitrogen (BUN)	Simultaneous measurement of multiple biomarkers in acute dyspnea provides additional prognostic information.	Rehman et al (69).
NT-proBNP, galectin-3	Combination of NT-proBNP and Gal-3 has shown good prediction for prognosis in acute HF.	Van Kimmenade et al (70).
NT-proBNP, MR-proANP, MR-proADM	Combination of the three biomarkers can provide prognostic information regarding cardiovascular and all-cause mortality that could be used in elderly patients presenting with symptoms suggestive of heart failure.	Alehagen et al (71).
hs-cTnT, hs-CRP, and Cys-C along with clinical risk factors	The score identifies patients with low, intermediate and high risk of HF.	Eurlings et al (72).
sST2, GDF-15, hsTn I	The multimarker score adds prognostic value to standard risk factors for predicting death, overall cardiovascular events, and heart failure.	Wang et al (73).

time-of-flight mass spectrometry method of detecting AVP in the urine to probe for doping control purposes. The method has proven high accuracy, robustness and a low limit of detection/quantification (57). C-terminal provasopressin (copeptin) is a more stable propeptide of AVP. Copeptin has been shown to have prognostic value in HF and was correlated with NYHA functional class (58). Copeptin can be measured using a sandwich immunoluminometric assay with two polyclonal antibodies for 132-164 AA of pre-provasopressin (59).

Biomarkers of myocyte injury in HF

Myocardial injury has been shown to be more common than previously thought among HF patients. Loss of cardiomyocytes is associated with ventricle remodeling and in general with a worse outcome (60). With the

emergence of highly sensitive troponin assays, cardiac troponin T (cTnT) was detected in 92% of HF patients in comparison to only 10% in the past (60). Cardiac troponin I (cTnI) at levels ≥ 0.04 ng/mL is considered an independent predictor of death. Levels of cTnT higher than 0.2 ng/mL, in patients with HF, were associated with a high hazard ratio for death (60). There are currently six highly sensitive immunoassays commercially available, five for cTnI and one for cTnT (61). Some of them use the "2+2" concept of combining four antibodies: two for capture and two for detection. These antibodies are sensitive to factors that may influence measurement, such as posttranslational modifications (proteolytic degradation, phosphorylation) or complexing with other molecules (heparin, heterophile or human antimouse antibodies). Recently, *Moreira et al* have tested a novel anti-

ficial antibody for cTnT that showed high selectivity and sensitivity (62). Moreover, *Abad et al* have demonstrated that cTnT can be determined in the range of 0.05-1.0 ng/mL using a cyclo-olefin polymer based microfluidic device for capturing magnetic beads and performing electrochemical detection (63). A detection limit of 2 pg/mL for cTnI was reached by using nanoparticle-based electrochemiluminescence immunosensor labels in a high sensitive sandwich-type immunoassay (64).

Multimarker approach

Even though the field of multimarker evaluation is still at the beginning in HF, many studies, as seen in *Table IV*, have investigated the use of a multimarker strategy in refining diagnosis or risk stratification among patients with HF. Each biomarker is a valuable addition to the multimarker profile, increasing the accuracy of the result (65).

To support this, new multiplex detection methods are being investigated, such as the photonic suspension array for multiplex immunoassay which is a low cost, automated and simultaneous detection method for BNP, cTnI and CRP, used successfully for the investigation of HF patients (74). *Park et al* also developed a "lab-on-a-disc" comprising a multiplex immunoassay for the detection of high sensitivity CRP, cTnI, and NT-proBNP based on a bead-based sandwich type enzyme-linked immunosorbent assay in approximately 20 minutes (75).

Conclusions

Advances in detection methods bring biomarkers closer to point-of-care testing. Although there are currently many candidate biomarkers for HF, only BNP and NT-proBNP are routinely being used in clinical settings for the diagnosis, prognosis and management of HF. More studies are needed to determine the clinical utility of candidate biomarkers and es-

tablish the clinical significance of their values in proximal fluids. Many biomarkers have the potential to be targets of future therapies, this being a starting point for drug design research.

Acknowledgements

This study has been carried out with the support of the POSDRU No.78702 grant.

Abbreviations

HF = heart failure
 MRM = multiple reaction monitoring
 ELISA = enzyme-linked immunosorbent assays
 BNP = brain natriuretic peptide
 NT-proBNP = amino-terminal fragment
 BAS = biotin-avidin system
 Fab = fragment antigen binding
 AuNCs = gold nanochains
 HRP = horseradish peroxidase
 AuNCs-HRP-Ab(2) = gold nanochains and horseradish peroxidase complex labeled secondary antibodies
 ANP = A-type natriuretic peptide
 ADM = adrenomedullin
 LVEF = left ventricular ejection fraction
 MR-proADM = midregional proADM
 MR-proANP = midregional proANP
 BACH = Biomarkers in Acute Heart Failure trial
 IL = interleukin
 GDF-15 = growth differentiation factor 15
 TNF- α = tumor necrosis factor
 CRP = C-reactive protein
 PTX3 = pentraxin 3
 OPG = osteoprotegerin
 MPOs = myeloperoxidases
 NYHA = New York Heart Association
 MMPs = matrix metalloproteinases
 TIMPs = tissue inhibitors of metalloproteinases
 TIMP1 = tissue inhibitor of MMP1
 GAL-3 = Galectin-3
 iTRAQ = isobaric tag for relative and absolute quantitation
 ACEI = angiotensin converting enzyme inhibitors
 ARBs = aldosterone receptors blockers
 ET-1 = Endothelin-1
 CT-proET1 = C-terminal pro-endothelin-1
 AVP = Arginine vasopressin

Copeptin = C-terminal provasopressin
 cTnT = cardiac troponin T
 cTnI = cardiac Troponin I

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