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Review

Multimarker approach for heart failure management: Perspectives and limitations

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ABSTRACT

Heart failure (HF) is a major public health problem and the approach for an accurate individualization of HF risk and care should include a profile of laboratory data, in addition to clinical and imaging data. The possibility of identifying the most vulnerable patients is clinically important, especially considering that many therapeutic interventions are available today. This goal has not been yet reached although many novel biomarkers have been proposed and tested. The complexity of the biochemical network at the basis of HF pathophysiology clearly suggests that a single marker cannot reflect all the features of this syndrome, whereas the combined use of more indices would better characterize HF patients and create new options for their management, helping identify which patients to follow more closely. The multimarker approach, considering various biochemical pathways simultaneously, bases its robustness on a suitable choice of indices known to be individually associated with HF. The choice of biomarker combination is essential to the performance of the multimarker strategy. A major problem in selecting the biomarker profile is the proportional increase in economic burden; thus a "parsimonious" biomarker combination has to be used in a cost-effective evaluation. Statistical analysis and analytical performance of the different elements of the combination, in turn, may heavily influence results.

This review summarizes the results obtained using a multimarker approach for HF risk stratification, for predicting HF incidence in a population, and evaluating the response to therapy. An insight into transcriptomic biomarkers, recently proposed for HF individual risk assessment, is also reported. A reliable selection of biomarkers for the careful management of HF patients is of pivotal importance in reducing healthcare costs without reducing patient care.

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1. Introduction

1.1. The long way of the heart failure biomarkers

Knowledge of the molecular mechanisms at the basis of various diseases plays an important role in biomedical fields such as molecular medicine, thanks to the possibility of finding new biomarkers that can univocally recognize specific disease pathways [1]. As to heart failure (HF), the proposal of new biomarkers follows our understanding of molecular pathways at the basis of this complex disease. The first proposed biomarkers were neurohormones such as catecholamines, in particular norepinephrine. In 1962, elevated levels of norepinephrine in HF patients and their further increase after physical exercise was described, and an activation and a pathogenetic role of the sympathetic nervous system in HF was demonstrated [2]. In 1984, an association between norepinephrine and mortality in HF patients was found [3]. In 1990, the renin–angiotensin–aldosterone system was also shown to be activated in HF [4]. Immediately after its identification, attention was focused on endothelin (ET)-1, a 21 amino acid peptide released by vascular endothelium with potent vasoconstrictive action [5]. Besides inducing fibrosis of ventricles and vasculature, endothelin is a potent modulator of the release of other neuro-hormones, natriuretic peptides included [6]. Natriuretic peptides have a central role in biohumoral characterization of HF, primarily brain natriuretic peptide (BNP), and N-terminal pro-BNP (NT-proBNP), that are indices of myocyte stress, being released during hemodynamic stress [6]. More recently, the C-type natriuretic peptide (CNP) also resulted to be involved in HF pathophysiology [7]; its circulating levels increase as a function of HF severity [8] and cardiac production during HF was shown [9,10]. As to inflammation, in the 1990s a role for Interleukin (IL)-6 and Tumor Necrosis Factor (TNF)α was recognized [11], although attention was focused on inflammation in the pathogenesis and progression of HF long before in 1954, with studies on C reactive protein (CRP) [12]. Very recently, among the novel biomarkers of HF, pentraxin (PTX)-3, an early index of inflammation belonging to the CRP family [13], has been proposed for risk stratification [14]. High circulating levels of PTX-3 are also considered a signal of myocyte damage, as observed in patients with acute myocardial injury [15]. Evaluation of the presence of myocyte injury attracts increasing attention as a factor influencing the HF progression. Cardiac troponins (cTn), sensitive and specific indices of acute myocardial injury, are used to reveal myocyte injury following myocardial stress due to inflammation, oxidative processes and neuro-hormonal activation [16]. Increased cTnI levels, an index of myofibril damage, has been demonstrated to be an independent predictor of mortality in HF [17,18]; more recently, other molecules such as Heart-type Fatty Acid Binding Protein (H-FABP), a marker of cellular membrane damage, resulted relevant in the risk stratification of HF patients [19]. Elevated levels of both cTnT and H-FABP, markers of two different kinds of myocyte damage, are independent predictors of adverse outcome in HF subjects [20].

Table 1 summarizes the main classes of biological molecules to be used as possible biomarkers in HF syndrome. As suggested by Braunwald and Bristow [21] early in the twentieth century, increasing knowledge of the mechanisms leading to the cardiac failure and,

Table 1
Biomarker classes for heart failure.

Biomarker	Suggested clinical applications
Neurohormones	
Catecholamines	Prognosis
Renin–angiotensin–aldosterone system (RAAS)	Prognosis
Natriuretic peptides (*ANP*, *BNP*, *NT-proBNP*, *MR-proANP* and *other related peptides*)	Diagnosis, prognosis, risk stratification, therapy monitoring
Arginine vasopressin and copeptin	Prognosis
Endothelin	Prognosis, therapeutic target
Chromogranin A and B	Diagnosis
Adrenomedullin	Prognosis
Myocyte injury	
Cardiac troponins (*cTnI* and *cTnT*)	Diagnosis, prognosis, risk stratification
Heart-type fatty acid binding protein (H-FABP)	Diagnosis, prognosis, risk stratification
Myosin light-chain kinase I	Prognosis
Fas (APO-1)	Prognosis
Pentraxin (PTX)3	Prognosis, risk stratification
Inflammation	
C-reactive protein	Prognosis, risk stratification
Cytokines and related receptors (*IL-1*, *IL-2*, *IL-6*, *IL-8*, *IL-18*, *TNFα*, *growth differentiation factor 15*, *ST2*)	Prognosis, risk stratification
PTX3	Prognosis, risk stratification
Adipokines (*adiponectin*, *leptin*, *resistin*, *ghrelin*)	Prediction of HF incidence, prognosis, risk stratification
Procalcitonin	Prognosis
Neopterin	Prognosis
Osteoprotegerin	Prognosis, risk stratification
Oxidative stress	
Oxidized low-density lipoproteins	Prognosis
Myeloperoxidase (MPO)	Prognosis
Urinary piopyrrins	Prognosis
Urinary and plasma isoprostanes	Prognosis
Plasma malondialdehyde	Diagnosis
Gamma-glutamyl transferases (GGT)	Prognosis
Uric acid	Prognosis
Matrix and cellular remodelling	
Matrix metalloproteinases (MMPs) and MPP tissue inhibitors (TIMPs)	Prognosis, risk stratification, aid in elucidating the HF pathogenesis
Collagen propeptides	Prognosis
Propeptide procollagen type I and III	Prognosis
Osteopontin (OPN) (*and other matricellular proteins*)	Prognosis, aid in elucidating the HF pathogenesis
Galectin-3	Prognosis, risk stratification
Endothelial dysfunction	
Adhesion molecules (ICAM, selectin-P)	Prognosis
Endothelin	Prognosis, therapeutic target
Adiponectin	Prediction of HF incidence, prognosis, risk stratification
Homocysteine	Prediction of HF incidence
C-type natriuretic peptide (CNP)	Diagnosis, prognosis, aid in elucidating the HF pathogenesis
Other markers (organ failure, cachexia, comorbidity)	
Triiodothyronine	Prognosis, risk stratification
Cystatin C	Prognosis, risk stratification
Plasminogen activator inhibitor (PAI)-1	Prognosis, risk stratification
Cholesterol	Prognosis, risk stratification
Urinary albumin-to-creatinine ratio	Prognosis, risk stratification
Haemoglobin	Prognosis
Creatinine, glomerular filtration rate	Prognosis

in turn, early therapeutic treatments could progressively reduce the incidence of this disease. For this, early diagnosis and management of the HF patient as well as careful risk stratification are needed. In this perspective, biochemical markers, possibly in synergy with cardiovascular imaging markers, could help achieve this [22].

1.2. Novel biomarkers of HF and multimarker strategy

The choice of a new biomarker should be driven by the knowledge of molecular mechanisms associated with HF, obtained following the molecular medicine approach. Subsequently, to be useful in clinical practice, a biomarker should fulfil the main criteria of evidence-based laboratory medicine (EBLM) [23], namely (1) to be accurately determined at a reasonable cost and with rapid response times; (2) to provide information unavailable by clinical evaluation; (3) to be a relevant element of the decision-making process. It is noteworthy that appropriate statistical measures are necessary for drawing meaningful conclusions about the clinical usefulness of the new markers, as recently pointed out [24–27].

Many biochemical processes are known to contribute to HF onset and progression [28,29]. In each of these pathways, the biomarkers whose clinical use has been demonstrated, and the emerging ones that have not yet been fully characterized, are involved. Inflammation, neuro-hormonal modulation, myocyte stress, oxidative stress, myocyte injury and extra-cellular matrix remodelling are the main mechanisms associated with HF considered so far for proposal and evaluation of new biomarkers.

The complexity of the biochemical network at the basis of HF pathophysiology clearly suggests that a single marker cannot reflect all the features of this disease, whereas the combined use of several indices could better characterize HF patients and create new options for their management, helping identify which patients to follow more closely.

The structure of this review is as follows. First, a brief survey of the main mechanisms involved in chronic HF is given. Actually, knowledge of such molecular mechanisms is essential to the choice of any new biomarker. Next, an extensive overview is provided as regards the results obtained using a multimarker approach for HF risk stratification and to predict HF incidence in a population as well as to evaluate the response to therapy. An insight into transcriptomic biomarkers, recently proposed for HF individual risk assessment, is also given. Finally, advantages and limitations of the examined studies are extensively analysed and discussed.

2. Pathophysiology of chronic heart failure

Heart failure, a final common pathway of many cardiovascular diseases, develops when the heart is no longer able to provide adequate blood flow/pressure in response to the body's needs. This in turn induces the activation of several compensatory mechanisms, initially beneficial, which over time contribute to disease progression. These compensatory mechanisms include salt and water retention by the kidneys, activation of neurohormones, and activation of intracellular signalling cascades in the heart and vasculature, resulting in alterations of the cellular and organ morphology and function.

In recent years, better knowledge of the role of hemodynamic and neurohormonal factors in HF and the parallel development of effective treatments shifted HF from an incurable to a chronic disease [30]. For this, new research seeks increased knowledge of the mechanisms of chronic HF in order to develop further therapeutic treatments [30], targeting specific HF mechanisms. Novel types of therapy (intracellular proteins modulation, gene delivery, cell replacement) have been developed starting from knowledge of the intracellular signalling pathways involved in HF molecular mechanisms (see Refs. [1,28,30] for a more exhaustive review).

2.1. Neurohormonal activation

In response to decreased heart function, activation of the sympathetic nervous system (adrenaline and noradrenaline) and of the neurohormonal signalling (angiotensin II, endothelin and natriuretic peptides) is observed as a compensatory mechanism. This activation, whose extent is correlated to heart function, increases the rate and intensity of heart contraction, contributing to preserving cardiac output. Angiotensin II signalling mediated by AT-1 receptor, sympathetic activation, and increased aldosterone production have been reported to have a major role in cardiac remodelling and dysfunction. Some therapies, such as blockade of β-adrenergic receptors (β-AR), angiotensin-converting enzyme (ACE) inhibitors, blockade of the angiotensin II receptor AT-1, and inhibition of aldosterone synthesis, can improve both symptoms and survival in HF patients [31–33]. In particular, additional effects of AR blocker therapy have been observed, mainly related to the kind of AR receptor subtype (β1, β2, α1) targeted, e.g., β1-AR can transactivate epidermal growth factor receptor (EGFR) signalling, which is cardioprotective, and this mechanism is mediated by β-arrestin [34].

The pharmacological blockade of ET, whose role in pathophysiology of HF is well demonstrated, was not effective, probably due to the complexity of its biochemical effects, mediated by two receptor subtypes with opposite actions (ET-A and ET-B). Drugs able to inhibit only ET-A, such as the recently introduced ambrisentan, might have more beneficial effects compared to less selective receptor inhibitors, previously proposed for the clinical setting [5].

Plasma levels of ANP and BNP are increased in HF patients and plasma BNP (or NT-proBNP) is a well-known marker of disease severity [6]. Both ANP and BNP exert cardioprotective effects, and infusion of BNP (nesiritide) was able to relieve symptoms when given within 3 h of the onset of worsening HF in decompensated HF patients (see below for results of clinical trials).

2.2. Cardiac hypertrophy

Myocardial hypertrophy, a major predictor of progressive heart disease, is a response of cardiac muscle to alterations induced by many pathophysiological stress signals. Stress signals include nitric oxide, neurohormones (natriuretic peptides and angiotensin II, the latter binding to G_q/G_{11}-protein-coupled receptors), neurotransmitters (catecholamines that bind to β-adrenergic receptors, β-ARs), cytokines and growth factors, or cardiac injury. The early beneficial effect of cardiac hypertrophy (normalization of wall stress and preservation of contractile performance) may be followed by decompensation and HF.

At the molecular level, hypertrophy is characterized by the activation of gene expression patterns of the fetal stage, such as fetal isoforms of genes whose products regulate cardiac contractility and calcium handling. In parallel, a down-regulation of the respective adult isoforms is observed [35]. Impaired myocardial vascularization, changes in the extracellular matrix composition, and fibrosis are often associated with myocardial hypertrophy [36]. Moreover, alterations of the normal cell turnover in the heart lead to an unfavorable ratio between cardiac apoptosis and regeneration from circulating and cardiac stem cells [37].

Fig. 1 summarizes the cardiomyocyte signalling pathways believed to be involved in HF pathophysiology. The cardiomyocyte signalling pathways are the same ones involved in physiological responses, but in HF hearts there are more stress stimuli, which amplify these molecular mechanisms and produce an imbalance among them. The activation of cell-surface receptors by specific ligands or by a mechanical stimulus induces the activation of stress-response protein kinases and phosphatases, as well as of calcineurin which, in turn, activate transcription factors targeting multiple

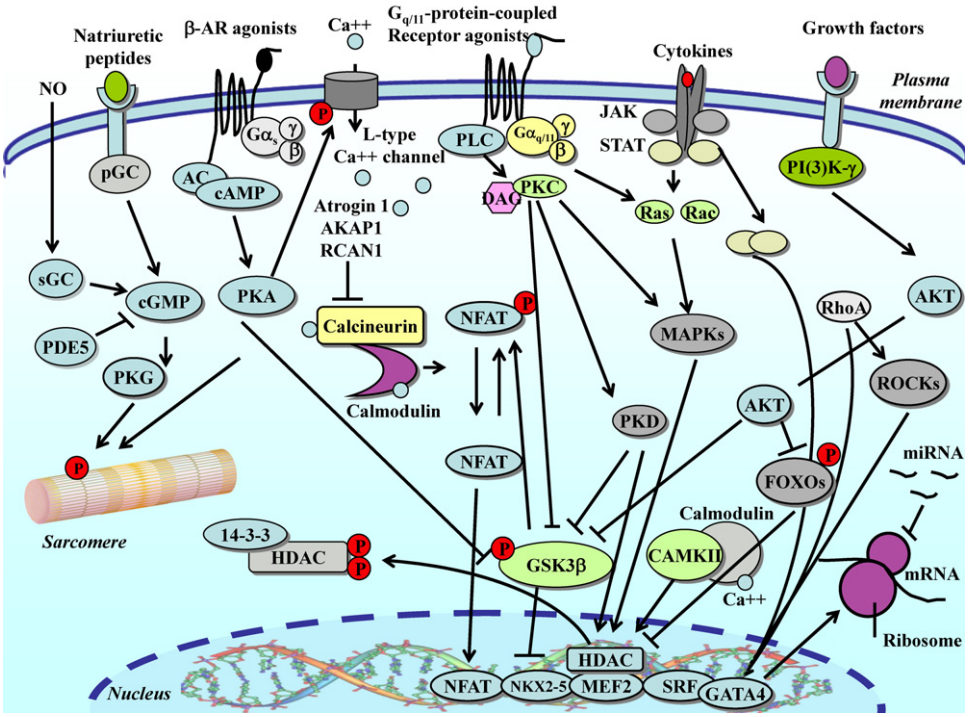

Fig. 1. Cardiomyocyte signalling pathways involved in the pathophysiology of HF. The main intracellular signalling pathways, known to transduce the stress stimuli, are depicted. Stress stimuli include nitric oxide, neurohormones, neurotransmitters, cytokines and growth factors. The signalling nodes (where many pathways converge) include calcium (Ca^{2+})/calmodulin-dependent kinase II (CAMKII), Akt, glycogen synthase kinase 3β (GSK3 β) and cyclic GMP (cGMP)-dependent protein kinase (PKG). AC, adenylyl cyclase; AKAP1, PKA anchor protein 1; cAMP, cyclic AMP; β-ARs, β-adrenergic receptors; DAG, diacylglycerol; FOXO, forkhead-box O proteins; HDAC, histone deacetylase; JAK, Janus kinase; MAPK, mitogen-activated protein kinase; MEF2, myocyte enhancer factor 2; mRNA, messenger RNA; miRNA, microRNA; NFAT, nuclear factor of activated T cells; NKX2-5, NK2 transcription factor related, locus 5; NO, nitric oxide; pGC, particulate guanylyl cyclase; PI(3)K-γ, phosphatidylinositol 3-OH-kinase-γ; PKA, cAMP-dependent protein kinase; PKC, protein kinase C; PDE5, phosphodiesterase 5; PKD, protein kinase D; PLC, phospholipase C; RCAN1, regulator of calcineurin 1; ROCK, Rho-associated, coiled-coil-containing protein kinase; sGC, soluble guanylyl cyclase; SRF, serum response factor; STAT, signal transducer and activator of transcription.
Modified from Mudd and Kass [30].

genes. The result is a change in the cellular structure, size, shape and molecular regulation of the heart (i.e., cardiac remodelling process). In this process, calcineurin has a key role. Calcineurin is a serine/threonine phosphatase able to dephosphorylate the NFAT (nuclear factor of activated T cells) molecules, which trigger a hypertrophic genetic program after translocation to the nucleus [38]. Due to its crucial function in cardiac hypertrophy and remodelling, many therapeutic approaches have been proposed to inhibit the calcineurin–NFAT pathway, including cyclosporin A and other immunosuppressants used in organ transplantation.

Identification of parallel signalling cascades reduces the possibility to modulate individual signalling pathways to inhibit pathological cardiac remodelling. However, it has been suggested that it is possible to modulate the remodelling signals by targeting the signalling "nodes", e.g., points where many pathways converge, such as glycogen synthase kinase 3β (GSK3β) and histone deacetylases (HDACs) (see Fig. 1) [30,39]. A further important therapeutic target is represented by reactive oxygen species (ROS) because many of the signalling pathways reported in Fig. 1 lead to the generation of ROS that heavily contributes to decreased cardiac function and remodelling [40].

2.3. Inflammation

Inflammatory activation is an important pathway in the progression of chronic HF. Increased plasma levels of cytokines are found in HF patients [41,42] and TNFα and other inflammatory interleukins (IL-1, -6, -18) are produced by macrophages and cardiac myocytes in the heart [43]. Following the cytokine hypothesis of HF, cardiac injury induces innate stress responses, including the production of pro-inflammatory cytokines and resulting in deleterious effects on cardiac function [44] and increased HF progression [45]. Pro-inflammatory cytokines can influence myocardial function via effects on both myocyte contractility and extra-cellular matrix, contributing to myocardial dysfunction. In particular, TNFα affects cardiac remodelling through activation of metalloproteases whereas IL-6 induces a hypertrophic response in myocytes [45]. For these reasons, large clinical trials aimed to evaluate the efficacy of anti-inflammatory therapies resulted beneficial in experimental animal models [46,47]. So far, these clinical trials have furnished discouraging results, due to the lack of clear positive effects, or even to a worsening of the disease [48,49].

2.3.1. TNFα-targeting therapy

The results of TNFα-targeting therapy deserve to be described in greater detail. Several randomized placebo-controlled trials testing anti-TNFα treatments have been performed. Two trials, RENAISSANCE and RECOVER, both in the RENEWAL program, evaluated the clinical efficacy of etanercept, a large fusion recombinant molecule with a molecular weight of 150 kDa, that binds to TNFα. These trials, as well as the ATTACH trial that tested infliximab (a monoclonal antibody to TNFα) did not achieve any improvement of disease and higher rates of mortality appeared to be associated with this therapy. A possible explanation for the lack of beneficial effects of this therapy in HF may be the binding of the transmembrane form of TNFα by the TNFα antibody, resulting in apoptosis of TNFα-expressing cardiomyocytes [50]. It is noteworthy that low physiological levels of TNFα exert a cardioprotective role in

the heart against acute myocardial injury, allowing tissue remodelling and repair [44]. Thus, the plasma levels of the anti-TNFα drugs achieved in the abovementioned trials could have reduced the cytokine plasma concentrations below the physiological values necessary to produce these beneficial physiological effects. Moreover, during anti-TNFα therapy, high levels of the drug could be obtained with consequent dose-dependent toxicity [49]. Finally, the selection of patients to test is a crucial issue. Among the patients with HF, elevated circulating levels of TNFα have been generally found in those with more severe disease (NYHA functional class IV) while only 3% in the RENEWAL and 5% of the population in ATTACH were in NYHA class IV, a population too small to obtain reliable data on therapy efficacy. These observations suggest that for evaluation of the efficacy of anti-TNFα therapy, it is necessary to carefully test the optimal pharmacological preparation and dosage in specific patient sub-groups, as indicated by "A Consensus Statement of the Heart Failure Association of the European Society of Cardiology" on this topic [51]. Indeed, inflammatory activation may be different in the different phases of HF (e.g., early stage HF after acute myocardial infarction compared with chronic HF) or in the different forms of HF (ischemic, diabetic, hypertensive, viral, and idiopathic cardiomyopathy). The results of anti-inflammatory trials gave rise to many relevant questions about the role of inflammation in HF pathogenesis and the clinical use of anti-inflammatory therapies. The Consensus Statement [51] highlighted some other critical issues in addition to the abovementioned careful selection of patients. The Consensus Statement strongly indicates that preclinical data in animal experimental models must be obtained in the same conditions as the patients enrolled for anti-inflammatory therapies: indeed, animal models often use relatively acute models whereas clinical trials evaluate chronic HF patients [51]. Finally, novel therapeutic targets to inhibit inflammation in the heart have been proposed, including pentraxin (PTX)3 and matricellular proteins (see Table 1). Regarding anti-TNFα therapy, approaches aimed at blocking its synthesis or release should also be considered [51].

2.4. Apoptosis

In the failing heart, an imbalance between signalling pathways that promote cell survival and those that promote cell death (apoptosis and necrosis) leads to a decrease in the number of cardiomyocytes. The programmed cell death could be triggered in cardiac myocytes by various kinds of stressors, such as cytokines, free radicals, hypoxia and DNA damage. The sustained loss of cardiomyocytes by apoptosis has a critical and important role in pathogenetic progression of cardiomyopathies [52], and in this context the proapoptotic factors could contribute to contractile dysfunction by reducing the myofibrillar turnover into the cardiomyocytes [53,54]. Initiation of apoptosis is associated with activation of the upstream cascade, including the inhibition of Akt phosphorylation [55], the release of Cytochrome C from mitochondria to cytoplasm and the processing of proteolytic caspases (Casp) [56]. Casp expression and activation represents the end-stage of apoptotic signalling, even leading to fragmentation of various cytoplasmic proteins, including contractile proteins [53,57]. Recently, it has been postulated that apoptosis might be one of the meta-stable transition states in HF, which may be reversible with appropriate therapy [54]. Indeed, treatment able to reverse apoptosis could help restore systolic function and reverse remodelling, attenuating the severity of HF [58,59]. It is conceivable that the activation of apoptosis is not homogeneous in the myocardium and does not immediately affect contractile function. In fact, it has been established that not all cardiac cells succumb to apoptotic activation and surviving cells might drive mechanisms to interrupt the process of apoptosis, despite Casp activation or Cytochrome C release [56,60]. The mechanism by which apoptosis is interrupted is still under investigation. Previous data suggest that interruption of the apoptotic process might be due to an adaptive balance between pro- and anti-apoptotic soluble factors to limit cell stress and dysfunction. The presence of this balance has been demonstrated in the cardiac tissue from an animal experimental model of pacing-induced HF [61]. The findings of this study indicate that the apoptotic process is activated in failing hearts, as shown by the significantly increased expression of Casp-3, but this activation is not accompanied by an increase in apoptotic cells or DNA fragmentation. This observation closely agrees with the contemporaneous activation of the apoptosis control systems in HF, B-cell lymphoma 2 (Bcl-2) and heat-shock protein (HSP)72, both overexpressed in HF hearts. It has been hypothesized that cells try to resist apoptotic stimuli over-expressing anti-apoptotic molecules, such as Bcl-2, in order to prevent the complete activation of the cellular death program.

More attention is devoted to promoting cardiac regeneration in failing hearts and many studies used infarcted hearts to attempt to restore cardiomyocytes near the scar. Although it is more difficult to use this strategy with a heart with a global dysfunction, recent data suggest that the efficacy of cell-based therapy may be due to paracrine effects of injected cells on endogenous cells [62], thus indicating the induction of such paracrine effects as a novel therapeutic target.

2.5. Myocardial metabolism

Cardiac energy supply and metabolism are tightly regulated, the heart having a higher and constant workload. A deregulation of the energetic metabolism, as found in HF, results in a state of inefficiency and energy starvation. Due to the low capacity of the heart for storing the substrates, these have to be produced efficiently and rapidly, mainly from free fatty acids and, to a lesser extent, from glucose. In HF the synthesis of adenosine triphosphate (ATP) is reduced due to mitochondrial dysfunction and possible altered substrate utilization (increased catabolism of glucose). Fig. 2a shows the differences in metabolism and energy regulation between normal and failing hearts. In the normal heart, oxidation of fatty acid is preferred to glucose oxidation whereas in the failing heart there is an increased glucose oxidation associated with a reduction in production of the transcriptional coactivator PGC1α (peroxisome-proliferator-activated receptor-γ (PPAR-γ) coactivator 1α). PGC1α regulates the expression of the transcription factors PPAR-α, ERR-α (estrogen-related receptor-α), NRF1 (nuclear respiratory factor 1), and NFR2 that regulate mitochondrial biogenesis and fatty acid oxidation. Another relevant concern in the dysregulation of energy metabolism in HF is the abnormal storage of ATP in this condition. When it needs to produce energy rapidly, ATP is obtained from phosphocreatine and adenosine diphosphate (ADP) by the reversible action of creatine kinase (Fig. 2b); thus phosphocreatine represents a reserve of ATP. The ratio of phosphocreatine and ATP is considered a measure of this energy balance; this ratio is altered in the failing heart, due to the reduced activity of the creatine kinase, with consequent abnormalities in ATP availability [63]. Another important feature influencing energy availability is the density of capillaries in cardiac muscle. In an animal model of hypertrophy, cardiac remodelling resulted associated with an angiogenetic process unable to respond to the muscle growth [64]. The balance between hypertrophy (muscle growth) and capillary density (nutrient supply) is partly regulated by the transcription factor GATA 4, which has an important role in the stimulation of angiogenesis [65]. Thus, modulation of the balance between pro- and anti-angiogenetic factors could be relevant as a novel therapeutic target in some clinical conditions [30].

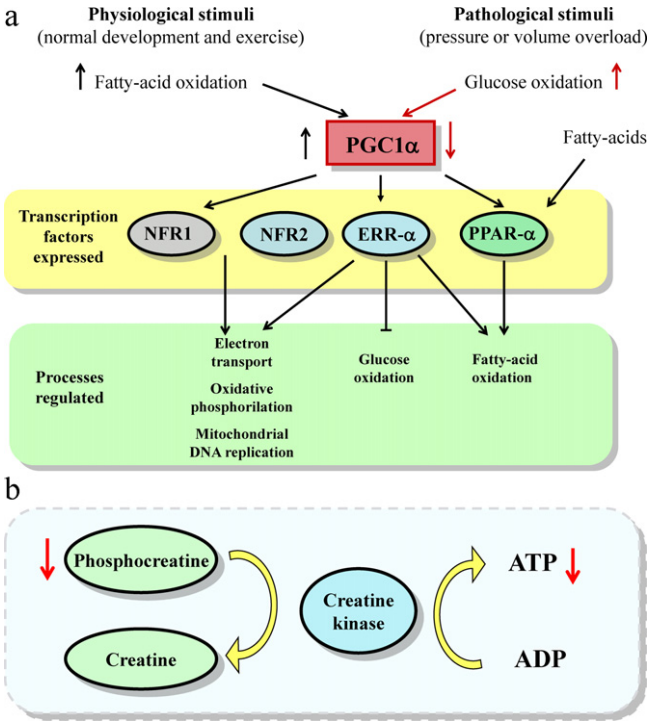

Fig. 2. Metabolism and energy regulation in healthy and failing hearts. (a) Different oxidation patterns and transcription cascades between healthy conditions (left) and HF (right). (b) ATP generation from phosphocreatine and ADP.
Modified from Mudd and Kass [30].

2.6. Contractile machinery

The mechanical function of the heart is associated with the force generated by the sarcomere, through the interaction of myosin thick filaments with actin thin filaments. Two isoforms of myosin heavy chain (α and β) are expressed in the heart and their relative proportions are affected by many pathophysiological stimuli. Normal heart of adult rodents contains α-myosin heavy chain that differs from fetal β-myosin heavy chain for faster cross-bridge kinetics and less tension generated. In HF, the gene encoding for fetal β-myosin heavy chain is re-expressed and represents about 50% of the total myosin heavy chain. In the human heart, β-myosin heavy chain is prevalent in healthy conditions and in failing hearts a further reduction of α-myosin heavy chain is observed [66]. This observation suggests that treatments aimed at increasing the expression of α-myosin heavy chain could prove useful. Many other proteins are found in the sarcomere: troponins and α-tropomyosin (regulatory thin filaments), myosin-binding protein C and titin, also known as connectin (interlinking proteins) and the Z-disc proteins (α-actinin, vinculin, and talin). The Z-disc functions as a physical anchor for myofilament and cytoskeletal proteins and as a coordination center for the reception and transduction of mechanical and biochemical stress signals. Alterations in the proteins of the Z-disc, affecting both the integrity of the Z-disc and the intracellular signalling, induce dilated cardiomyopathy in humans and in animal models [28,30].

Sarcomere proteins, being the connection between mechanical forces and protein kinase and phosphatase signalling, could represent new targets for modulating contractile function [30].

Myofibrillar proteins, the cardiac TnI and T, sensitive and specific marker of myocyte injury in patients with acute coronary syndromes, resulted slightly increased in HF patients, but associated with poor prognosis [17,18]. Other myocyte proteins, namely H-FABP, myosin light chain 1 (see Table 1), are found in the peripheral circulation of HF patients, and their presence, as well as for troponins, reliably predicts the outcome in HF patients [19,67].

3. Multimarker strategy for HF management

In this section, the main results regarding the multimarker approach to HF are reviewed. A summary of the main studies examined in this section can be found in Table 2.

3.1. Risk stratification of HF patients

Assessment of cardiovascular risk is an integral part of clinical decision-making, especially for the rational use of pharmacological therapies or/and device-based procedures. Critical evaluation of new risk markers has become even more relevant, and should be based on a sound study design and a representative population. Because HF is a major public health problem and the severity of disease must be graded, many clinical studies have been devoted to finding suitable markers to improve risk stratification in failing patients. Rates of hospitalization increase steadily both in the Europe [68] and in the United States [69], mortality following hospital discharge reaches one-third of patients within the first year post-hospitalization and about 70% within 5 years of admission [70,71]. New therapeutic treatments have been developed and some of them are quite expensive; thus it is urgent to easily, reliably and cost-effectively stratify severely ill HF patients so the patients at the highest risk will receive the most intensive care.

Among the many biomarkers that have been checked for risk stratification, BNP, an index of cardiac failure and volume overload, is the most well-established and frequently used in all studies that apply a multimarker approach. Although minor myocardial injury seems to be associated with HF, specific markers of cardiac necrosis, such as cTn and H-FABP, are often selected in the combination. Inflammation, due to its fundamental role in pathophysiology and prognosis of HF, is also considered by adding the evaluation of CRP to the multimarker panel, or, more recently, of PTX-3. Other molecules have also been considered to highlight a possible failure of other systems involved in HF pathophysiology. Two studies involving cystatin C, an index of kidney function [72], and free triiodothyronine (T3) [73], respectively, are reported below, as well as a study using a combination of conventional markers beside BNP [74].

The studies described below furnish an overview of the performance of different multimarker combinations employed for HF patient risk stratification. They may help for the critical appraisal of risk assessment strategies to be used clinically.

In the prospective study of Ishino et al. [14], 164 consecutive patients admitted to the cardiac unit for the treatment of worsening HF (NYHA classes I–IV; LVEF 49.6 ± 19.3%) were enrolled. The mean follow-up was 679 ± 438 days and the end-points were cardiac death and readmission for worsening HF. The chosen biomarkers, BNP, H-FABP and PTX-3, were measured at admission. Patients with all three markers higher than the respective cut-off (score 3) presented the highest risk with respect to the other groups with a lesser number of elevated biomarkers (34.6-fold with respect to the score 0) (Fig. 3). A similar indication was obtained by Kaplan–Meier analysis that demonstrated that cardiac events occurred most frequently in patients with three elevated biomarkers. These findings showed that the combination of BNP, H-FABP and PTX-3, reflecting different aspects of the disease, is a highly reliable method for risk stratification of hospitalized failing patients.

Similar results were obtained by a community cohort study, in which BNP, CRP, and cTnT levels were determined in 593 HF patients (NYHA classes III–IV) with preserved ejection fraction (LVEF: 48.7 ± 16.4%) [26]. Higher levels of all three biomarkers

Table 2
Multimarker combinations for HF management.

Reference	Biomarkers	Aim	Study population	HF medication
Ishino et al. [14]	BNP + H-FABP + PTX3	Risk stratification	NYHA classes I–IV	ACEIs/ARBs 64% β-Blockers 37% CCBs 20% Diuretics 71% Statins 18%
Dunlay et al. [26]	BNP + cTnT + CRP	Risk stratification	Community (Olmsted County, MN, US) NYHA classes III–IV (71%)	ACEIs/ARBs 58.5% β-Blockers 65.1% Statins 50.9%
Zairis et al. [75]	BNP + cTnT + CRP	Risk stratification	Decompensated HF NYHA classes III–IV	ACEIs/ARBs 84.1% β-Blockers 60.7% Spironolactone 29.8%
Manzano-Fernández et al. [72]	Nt-proBNP + cTnT + cystatin C	Risk stratification	Acute HF NYHA classes III–IV (30%)	ACEIs/ARBs 80% β-Blockers 51% Statins 49% Spironolactone 32.4% Loop diuretics 84.1%
Passino et al. [73]	BNP + fT3	Risk stratification	NYHA classes I–IV	ACEIs/ARBs 91% β-Blockers 78% Diuretics 77%
Niizeki et al. [74]	BNP + conventional biomarkers	Risk stratification	NYHA classes III–IV	ACEIs/ARBs 70% β-Blockers 31% CCBs 17% Spironolactone 33% Loop diuretics 77% Statins 11%, digoxin 36%
Velagaleti et al. [86]	BNP + PCR + PAI-1 + homocysteine + aldosterone-to-renin ratio + urinary albumin-to-creatinine ratio	Incidence prediction	Community (Framingham offspring study participants)	
Frankel et al. [89]	adiponectin + resistin	Incidence prediction	Community (Framingham offspring study participants)	
Miller et al. [94]	BNP + Nt-proBNP + MR-proANP + copeptin + procalcitonin + neopterin	Therapy response	Decompensated HF NYHA classes III–IV	ACEIs/ARBs 70% β-Blockers 70% Diuretics 85% Aspirin 70% Digoxin 53% Nitrates 30% Hydralazine 10%
Heidecker and Hare [106]	Molecular biomarkers (endomyocardial biopsy)	Risk stratification	New-onset HF NYHA classes I–IV	ACEIs 67% β-Blockers 26% Diuretics 75% Aldosterone antagonist 19%
Lamirault et al. [108]	Molecular biomarkers (endomyocardial biopsy)	Risk stratification	Advanced HF	ACEIs/ARBs 84%[a] β-Blockers 70% Aldosterone blockers 53% Statins 32% Digoxin 26% Adrenergic agonists 42%

BNP: B-type natriuretic peptide; H-FABP: heart-type fatty acid binding protein; PTX3 pentraxin-3; cTnT: cardiac troponin T; fT3: free triiodothyronine; MR-proANP: midregional pro-atrial natriuretic peptide; PCR: C-reactive protein; PAI-1: plasminogen activator inhibitor-1; CCBs: calcium-channel blockers.
[a] Percent values refer to patients of the intermediate HF-severity group.

proved to be strong, independent predictors of mortality and each biomarker provided incremental prognostic value with respect to classic risk factors. An improved 1-year risk prediction was obtained by adding a two-biomarker combination (BNP and CRP) to a model including established risk indicators (age, BMI, NYHA class, creatinine clearance, systolic blood pressure) (integrated discrimination improvement gain of 7.1%, $p < 0.001$). The addition of a third biomarker did not contribute further benefits.

A combination of BNP, CRP, and cTnT was also prospectively investigated in the setting of acutely decompensated low-output HF patients (NYHA classes III–IV; LVEF: $22.7 \pm 5.4\%$; LVEF $\leq$25 in 58.6% of patients) for early risk stratification [75]. Biomarkers were measured at admission in a total of 577 subjects, recruited at five different Greek centers. The relative risk of 31-day cardiac mortality, which was the study endpoint, increased as a function of the number of elevated biomarkers (4.3%, 10%, 20.9% and 53.5% of patients with 0, 1, 2, 3 elevated biomarkers, respectively, reached the study endpoint). Fig. 4 presents the relative risk of 31-day cardiac mortality as a function of the number of the elevated study biomarkers. These findings confirm the robustness of the combination of a marker of myocyte stress with a marker of myocyte injury, and an inflammation marker.

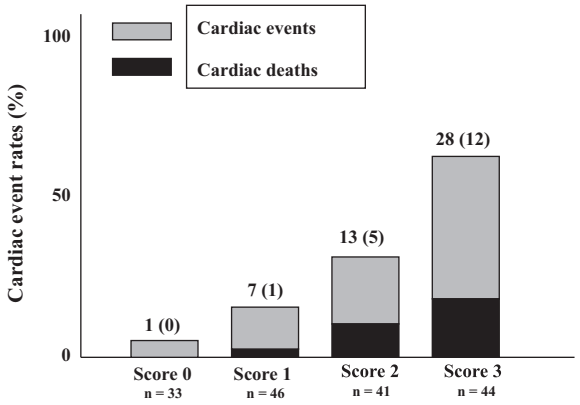

Fig. 3. Cardiac mortality and all cardiac events as a function of biomarker score. Patients with score 3 had significantly higher rates of rehospitalization and cardiac death than those with score 0–2 ($p < 0.0001$ by chi-square test). Cut-off values (200 pg/mL for BNP, 4.1 ng/mL for H-FABP, and 4.0 ng/mL for PTX3, respectively) were determined by ROC curves.
Modified from Ishino et al. [14].

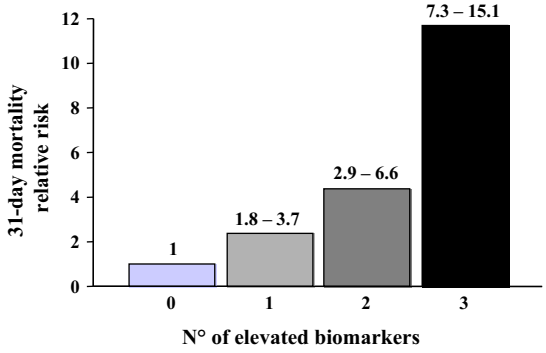

Fig. 4. Bar chart representing the relative risk of 31-day cardiac mortality as a function of the number of the elevated biomarkers.
Modified from Zairis et al. [75].

The study by Manzano-Fernández et al. [72] added the determination of cystatin C, for evaluation of kidney function, to the measurement of NT-pro-BNP and cTnT, whose combination was shown to improve the risk stratification in HF patients [76,77]. A total of 138 patients, admitted with initial diagnoses of acute HF (LVEF: 30–65%), were prospectively studied and the median follow-up was 261 days. Elevated levels of cystatin C resulted in a significant independent risk factor for adverse events (the combination of death and/or HF readmission). Moreover, the multimarker approach improved risk stratification further, showing that patients with 2 or 3 elevated biomarkers had a higher risk for adverse events than patients with no elevated biomarkers (25.8%, 37.1%, 43.6% and 66.7% of patients with 0, 1, 2, 3 elevated biomarkers, respectively, reached the study endpoint). The association between higher levels of cystatin C and increased rate of adverse clinical events, found in this work, strongly indicates that the evaluation of kidney function should have a pivotal role in the risk stratification of these patients in whom an impaired kidney function is related to worse outcomes [78].

The study by Passino et al. [73] is based on the observation that alterations of thyroid function are present in HF patients without primary thyroid disease [79,80]. About 30% of HF patients have low levels of the biologically active T3 and normal values of TSH and T4. This "low-T3 syndrome" is associated with a poor outcome [79], but its negative prognostic power is enhanced in those patients with higher BNP, as shown in Fig. 5, where survival curves as a function of BNP and free T3 values are reported. Data refer to a total of 720 consecutive HF patients of different severities (NYHA classes I–IV; LVEF: $33.5 \pm 9.9\%$) followed-up for 7 years (median value 3 years). The additive prognostic power of BNP and free T3 was demonstrated by the analysis of the survival curve of those patients with high BNP and low free T3, who showed the highest mortality rate. On the contrary, patients with combined low BNP and normal free T3 showed the lowest rate. Moreover, low free T3 was able to identify patients with higher risk of death among those with relatively low BNP.

Many mechanisms have been suggested to explain how free T3, which is not a cardiospecific biomarker, is involved in the prognosis of HF. T3 is mainly produced by 5′ monodeiodination of thyroxine (T4) in liver and free T3 can be considered an index of dysfunction of peripheral tissues, including liver, kidney, and muscles, thus low free T3 serum levels might reflect a condition of advanced disease (pre-cachectic stage). Moreover, low free T3 has important hemodynamic effects on cardiovascular function associated to the reduction of positive inotropic and vasodilatory effects of T3 and of its trophic action on the heart [81,82]. Although the link between low T3 and HF progression is not completely understood, a proof of its pathophysiological role is the restoration of the neuroendocrine condition observed after administration of synthetic T3 in patients with HF [83].

At a time of increasing interest in the proposal and use of newer biomarkers for identification, risk assessment and care of HF patients, as the authors themselves underlined, Niizeki et al. [74] have evaluated the incremental usefulness of multiple conventional biomarkers for risk stratification. They chose a combination of seven biomarkers, each assessing different pathophysiological mechanisms and known to be independently associated with increased risk of cardiac events in HF patients. BNP, uric acid,

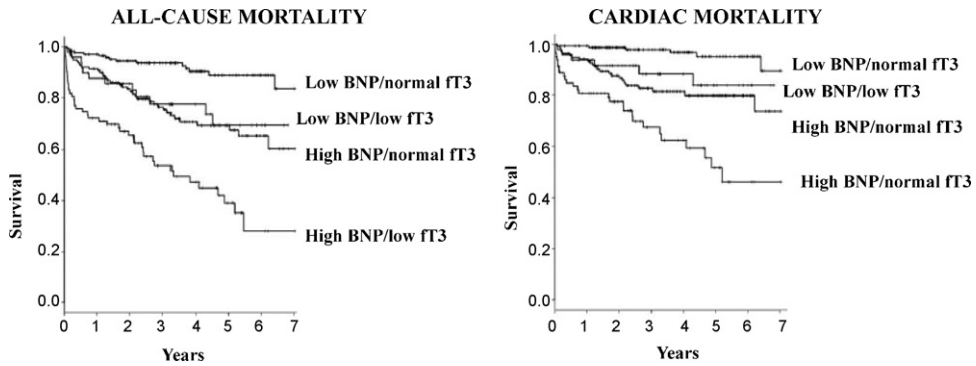

Fig. 5. Kaplan–Meier survival curves for all-cause (left) and cardiac (right) mortality in 4 sub-groups identified according to cut-off values: 2.1 ng/L for fT3, and 165 ng/L for BNP (168 ng/L for cardiac mortality).
Modified from Passino et al. [73].

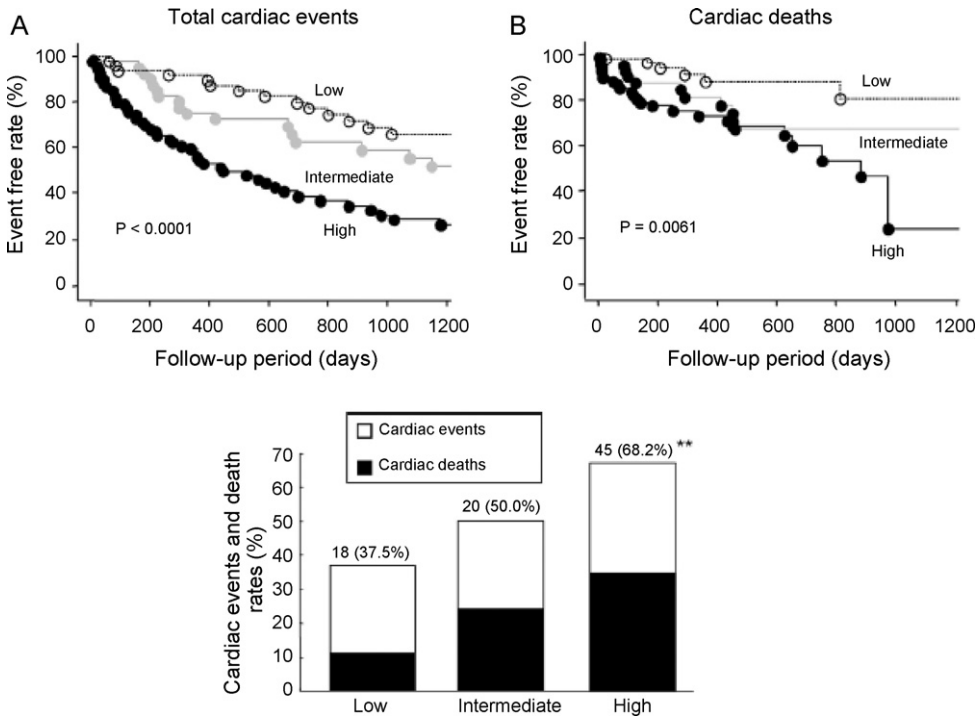

Fig. 6. Kaplan–Meier analysis in HF patients stratified into 3 groups based on multimarker score (top). Cardiac mortality and cardiac events among the 3 groups (bottom). Patients were categorized into 3 groups according to the multimarker score: low stratum (multimarker score 0–3), intermediate stratum (multimarker score 4), high stratum (multimarker score 5–7). Patients of "high" group had significantly higher rates of total cardiac events and cardiac deaths compared with "lower" group. Modified from Niizeki et al. [74].

high sensitive-CRP, sodium, haemoglobin, creatinine, and creatinine clearance were measured at admission in 154 patients (NYHA classes III–IV, LVEF: 42 ± 19%). Patients were divided in three groups following a multimarker score calculated considering the cut-off values of each biomarker. The patients were prospectively followed (mean follow-up 526 ± 313 days), end-points being cardiac death and readmission for worsening HF. Fig. 6 shows that the percentage of total cardiac events increases as a function of the score value in close agreement with the Kaplan–Meier analysis, thus showing that this simple multimarker approach has the potential to assist clinicians in predicting prognosis in HF patients, with low cost and wide availability.

3.2. Prediction of HF incidence

Refining HF risk prediction is a fundamental step in targeting prevention strategies. A HF "risk profile" based on clinical, ECG, and radiological features was described by the investigators of the Framingham Heart Study [84]. These clinical factors, though, do not fully explain the HF risk [85]. For this, many circulating and urinary substances have been proposed to describe the several biochemical pathways involved in the HF evolution; however, at present there are no data on the incremental utility of "a parsimonious set of biomarkers" for predicting HF risk in the community [86]. To obtain reliable indications about the factors involved in the disease onset, longitudinal community based studies are essential. Indeed, the main information about the prediction of HF incidence is derived from the Framingham Offspring study, a longitudinal community-based study initiated in 1971. A sample of 5135 individuals who were children (or spouses of children) of the original Framingham cohort study, were enrolled in the Framingham Offspring study and examined approximately every 4 years (the next exam is scheduled to begin in 2011). The objective of the study was to identify common factors or characteristics that contribute to cardiovascular disease by following its incidence, over a long period of time, in a large group of participants who had not yet developed overt symptoms of cardiovascular disease or suffered a heart attack or stroke [87]. In this context, very recently, a multimarker panel was related to the incidence of a first HF event in a prospective investigation of a large community-based sample of middle-aged whites ($n = 2754$ from the participants to the Framingham Offspring study, at the sixth examination cycle) [86]. A combination of six biomarkers, previously checked as indices of LV remodelling and vascular stiffness [29,88], was used, including CRP, plasminogen activator inhibitor-1, homocysteine, aldosterone-to-renin ratio, BNP and the urinary albumin-to-creatinine ratio. Among these indices, BNP and the urinary albumin-to-creatinine ratio were key biomarkers associated with HF risk. The predictive power of these two indices underlines the importance of natriuretic peptide activation and endothelial dysfunction as marker of disease. However, as declared by the authors themselves, the incremental usefulness of these biomarkers over standard clinical factors is very modest and additional studies are required to assess the applicability of these indices to routine clinical setting.

Although it is not a "true" multimarker approach, it is worth quoting the paper of Frankel et al. [89] where the circulating concentrations of adipokines, resistin [90] and adiponectin [91,92] are associated with the incidence of HF because the results of its study strongly suggest the existence of new molecular pathways leading to HF. A total of 2739 participants in the Framingham Offspring study were followed up for 6 years. The study hypothesis was that greater concentrations of resistin and lower concentrations of adiponectin would be associated with an increased risk of HF. Increased peripheral levels of resistin were associated with incident HF, even after accounting for obesity, inflammation, insulin resistance, and concurrent coronary heart disease, whereas adiponectin was not associated with subsequent development of HF. Although the specific mechanisms whereby resistin, known to promote insulin resistance and inflammation, leads to HF are not fully elucidated, the results of this study underline the importance

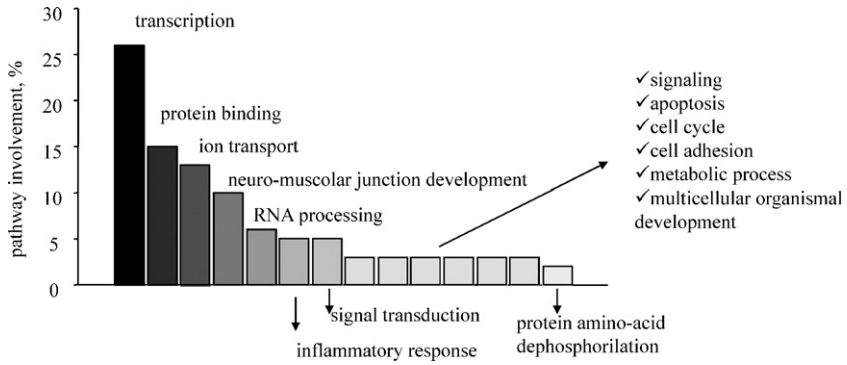

Fig. 7. Bar chart illustrating the pathways involved within the prognostic biomarker. Major pathways overexpressed in patients with good prognosis included transcription (26%), protein binding (15%), ion transport (13%), and neuro-muscular development (10%).
Modified from Heidecker and Hare [106].

of evaluating new biochemical patterns associated with onset of HF.

3.3. Monitoring the response to therapy

Evaluation of the response to therapy in HF patients is a crucial issue, especially in the more vulnerable patients to be followed very carefully, such as patients with decompensated HF. Although many studies evaluated the response to therapy, mainly using neurohormone levels [93], a "true" multimarker platform has been only used to check the response to nesiritide infusion in decompensated HF patients [94].

3.3.1. Monitoring of recombinant human BNP infusion

Nesiritide, a recombinant form of human BNP approved in 2001 to treat decompensated HF, was proposed as a novel and potentially important advance in the care of these patients [95]. This drug might be able to relieve dyspnea when given within 3 h of the onset of worsening HF, but possible harmful side effects, including renal impairment or increased risk of death, have been indicated. The Acute Study of Clinical Effectiveness of Nesiritide in Decompensated Heart failure (ASCEND-HF), a multicenter double-blind randomized trial, which enrolled 7141 patients to receive either intravenous nesiritide or placebo in addition to standard therapy, aimed to evaluate whether this drug is able to improve symptoms and influence the outcome in acute, severe HF [96]. The results of ASCEND-HF indicated that recombinant human BNP is safe in patients with severe HF, but is not associated with significant symptomatic relief or improved outcome [96,97]. As to the biohumoral evaluation of the effects of nesiritide, a multimarker paradigm was proposed for monitoring the response to drug infusion in decompensated HF patients [94] because the BNP and NT-proBNP were not fully able to monitoring the response to the drug. The changes in BNP and NT-proBNP levels after therapy were not rapid nor as large as expected from the clinical response, and further markers would be necessary to evaluate the drug effect [94]. Four novel biomarkers, copeptin, midregional proatrial natriuretic peptide (MR-proANP), neopterin, and procalcitonin, in addition to BNP and NT-proBNP, were checked in a prospective study of 40 patients hospitalized for decompensated HF (LVEF: $25 \pm 1.4\%$) -and treated with BNP as a part of their care. The combination included two inflammatory biomarkers: procalcitonin, a precursor of calcitonin and a marker of systemic infection and inflammation [98], and neopterin, a small peptide produced by macrophages [99]. Circulating levels of the selected biomarkers were elevated in patients with HF and appear to have prognostic value [100].

Before niseritide infusion all biomarkers were higher than normal range, but copeptin, MR-proANP, and NT-proBNP levels significantly decreased in response to the therapy. Copeptin and MR-proANP resulted potentially associated with the acute response to therapy, while higher copeptin levels indicated the non-survivor patients after discharge and were associated with an increased mortality risk. The addition in the statistical analysis of neopterin and procalcitonin did not result in incremental information in response to therapy and risk stratification. Only copeptin and BNP contributed to risk stratification in this group of severe HF patients, although BNP or NT-proBNP does not influence the prognostic value of copeptin alone.

This study highlights the role of copeptin (C-terminal vasopressin) in HF pathophysiology. Copeptin is synthesized and secreted in equimolar amounts to vasopressin, an anti-diuretic and vasoconstricting hormone strongly related to the severity and outcome of HF [101]. Instead of vasopressin, copeptin is highly stable and can be easily and reliably measured in unprocessed plasma or serum; its predictive value resulted superior to benchmark markers, BNP and NT-proBNP, over the entire spectrum of HF in a study of Neuhold of 2008 [102] as well as in patients with HF after acute myocardial infarct (OPTIMAAL study) [103].

3.4. Molecular biomarkers for individual risk assessment

New indications relative to management of failing patients would derive from basic research, in particular from genetic studies. One of the most valuable applications of genomic information has proven to be clinical prediction [104]. Indeed, the pattern of differentially expressed genes, besides providing insight into disease origin, has been also successfully used for the development of biomarkers, such as in neoplastic disease [105]. This approach, now emerging in many other diseases, has been addressed to the possibility of accurately assessing the prognosis in patients with HF [106,107], a major unmet issue in the management of HF. In this context, microarray analysis of cardiac biopsies allows us to identify a transcriptomic biomarker profile able to improve the individual risk assessment and potentially to offer novel therapeutic targets [107]. Using this technique, a transcriptomic signature, generated from a single endomyocardial biopsy, resulted in a novel prognostic biomarker in HF in subjects with new-onset HF (LVEF: $24 \pm 13\%$) [107].

A total of 43 bioptic samples, chosen from among a total of 350 endomyocardial biopsy samples collected for evaluation of cardiomyopathy from 1997 and 2006, were analysed. To avoid possible disease-specific confounding factors, only samples of patients with idiopathic dilated cardiomyopathy were selected. Biopsy samples were further selected in a case–control fashion, based on the phenotypic extremes in survival of the cohort: a group with good prognosis ($n = 25$) was defined as having event-free survival for at

least 5 years after initial presentation with HF symptoms; a group with poor prognosis ($n = 18$) had an event (death, requirement for ventricular assisted device, and cardiac transplant) within the first 2 years of HF onset. The patient group with good prognosis presented 46 over-expressed genes with respect to patients with poor prognosis. Molecular mechanisms with major involvement were transcription (26%), protein binding (15%), ion transport mechanisms (13%), and neuro-muscular development (10%), as can be seen in Fig. 7, which illustrates the pathways involved within the prognostic biomarker.

More recently, a cardiac gene expression profile has been proposed for risk stratification in advanced HF, a crucial issue for the following therapeutic treatments, e.g., heart transplantation and LVAD implantation [108]. Cardiac tissue samples from left (LV) and right (RV) ventricles of 44 patients undergoing heart transplantation or LVAD implantation were checked by a microarray containing 4217 muscular organ-relevant genes. Two gene expression profiles, for LV and RV respectively, were identified, both able to discriminate deteriorating from stable patients with high sensitivity (>88%) and specificity (>96%).

4. Discussion

Most of the multimarker approaches for HF patient management address a major question in HF care, which is the accurate clinical prediction of patient prognosis. It is well-known that patients with very similar conditions at disease onset and undergoing similar therapies, can have very different outcomes: a few patients are able to recover heart function in the subsequent 5 years, whereas in others the disease rapidly progresses and aggressive interventions are necessary. The possibility of identifying these more severe patients is clinically important, especially considering that many therapeutic interventions, including mechanical circulatory assistance or cardiac transplantation, are available today. This goal has not been achieved yet although many novel biomarkers [86] as well as clinical prediction algorithms [26] have been proposed and checked. Many biological markers, reasonably associated with HF pathophysiology, have been considered and their number increases with the increase of knowledge regarding molecular mechanisms of HF. The multimarker approach, considering simultaneously various biochemical pathways, bases its robustness on a suitable choice of indices known to be individually associated with HF. The combination of the biomarkers is the clue for the performance of the multimarker strategy and it generally reflects the main processes of HF. It always includes BNP (or NT-proBNP), a consolidated marker of dysfunction; then other markers, associated with pathways known to be modified in HF, such as inflammation, cardiac injury, renal failure, thyroid function, and metabolism, were added in the combination. However, the increase in the number of biomarkers included in the panel aimed at evaluating as many molecular processes as possible is limited by the observation that the addition of further markers in a combination often does not improve its diagnostic/prognostic power [26]. Moreover, although the combined use of many biomarkers better describes the patient's condition, none of the proposed multimarker strategies resulted univocally able to accurately assess patient prognosis [86].

The limited accuracy and increasing healthcare cost of the multimarker platform suggest the need to develop new tools for HF risk stratification, management, and therapy control. The relevance of gene expression and molecular signature analysis for potential clinical applications of transcriptomics is increasing. Recent technological advances in genomic screening and robust technologies are now available for entire genome screening for expression or single nucleotide polymorphisms. Recently, cardiac gene expression profiles obtained by an endomyocardial biopsy suggest the potential of transcriptomic biomarkers to predict prognosis in patients with HF [107,108]. This exciting technique presents some limitations: firstly, the time of bioptic sampling is critical owing to possible compensatory transcriptomic changes that could have been activated during disease progression [109]; second, although the endomyocardial biopsy is a low-risk procedure it is not easy to transfer to the clinical routine, and methods for obtaining transcriptomic biomarkers, alternative to biopsy, must be proposed. For this purpose, circulating blood cells, often sharing common genes with target tissues, would be the sample of choice for clinical applications. Many studies will need to assess whether the information obtained by biopsy analysis is completely reproduced by transcriptomic analysis of peripheral cells or if a combination of tissue and circulating biomarkers could increase the prediction accuracy. Finally, the combination of transcriptomic biomarkers and classic established biomarkers must be evaluated.

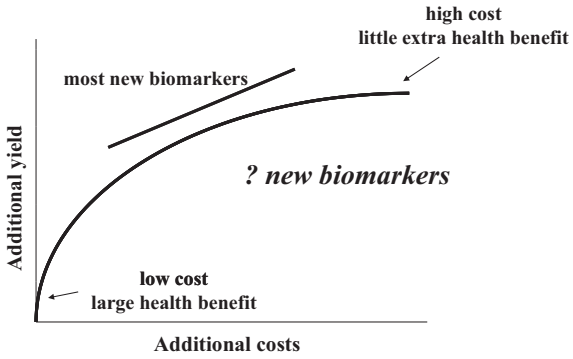

Fig. 8. Health outcome (yield) as a function of the cost. For any new intervention it will need to evaluate where it falls on the curve of additional yield in terms of health benefit versus additional cost.
Modified from Cowie [110].

As to the use of biomarker panels for refining HF prediction, a fundamental phase for preventing the disease, large sample size, standardized measurements of biomarkers and a rigorous definition of HF events are necessary.

A major problem in the choice of the biomarker profile is the proportionally increased economic burden; thus a "parsimonious" biomarker combination should be used in a cost-effectiveness evaluation. The key question for this as well as for all other new interventions is to evaluate whether the new intervention improves outcome sufficiently to justify the additional cost [110]. This is depicted in Fig. 8, reporting the relationship between the additional yield in terms of clinical outcome and the additional cost. Finally, it is noteworthy that a common limitation of all the multimarker panels, besides the need for suitable and robust statistical analysis, is that differences in the analytical performance, especially precision, of the assays of the different markers may influence the results of the analysis.

The final aim of the application of multimarker strategies to a clinical setting is to improve patient prognosis by better describing its conditions and, in turn, by better addressing its care. The robustness of this sequence has been confirmed by the data, recently published, of a population-based study relative to hospitalizations in Scotland from 1997 to 2003 [111] where the observed decrease in the rates of first hospitalization for HF resulted associated with the increase in prescribing rates of evidence-based pharmacological treatments, such as ACE inhibitors, spironolactone and β-blockers.

5. Conclusions

The approach to an accurate individualization of HF incidence risk and care would benefit from a profile of laboratory data, includ-

ing gene expression analysis, in addition to clinical data. However, many intrinsic limitations are associated with the multimarker approaches proposed for evaluation of HF incidence and HF management. The increased information provided by the simultaneous evaluation of different aspects of HF syndrome does not suffice to cover all the mechanisms involved in or influencing HF onset and progression, due to the correspondingly increased economic and organizational burden. Statistical analysis and analytical performance of the different elements of the combination may in turn greatly influence the results. Although fully automated platforms that are able to reliably measure biochemical and transcriptomic indices may soon reduce the economic and technical problems, evaluation of the incremental usefulness of this type of approach remains the central issue, and must be clearly assessed in routine clinical settings.

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