ANMCO/ELAS/SIBioC Consensus Document: biomarkers in heart failure

Nadia Aspromonte, FACC, FESC (Coordinator)[1]*, Michele Massimo Gulizia, FACC, FESC (Coordinator)[2], Aldo Clerico (Coordinator)[3], Giuseppe Di Tano (Coordinator)[4], Michele Emdin[5], Mauro Feola[6], Massimo Iacoviello[7], Roberto Latini[8], Andrea Mortara[9], Roberto Valle[10], Gianfranco Misuraca[11], Claudio Passino[5], Serge Masson[8], Alberto Aimo[5], Marcello Ciaccio[12], and Marco Migliardi[13]

[1]*CCU-Cardiology Department, Presidio Ospedaliero San Filippo Neri, Via Martinotti, 20, 00135 Rome, Italy*
[2]*Cardiology Department, Ospedale Garibaldi-Nesima, Azienda di Rilievo Nazionale e Alta Specializzazione "Garibaldi", Catania, Italy*
[3]*Laboratory of Endocrinology and Cardiovascular Cell Biology, Fondazione Toscana G. Monasterio-CNR, Scuola Superiore Sant'Anna, Pisa, Italy*
[4]*Istituti Ospitalieri, Cardiology Unit, Cremona, and Scuola Superiore Sant'Anna, Pisa, Italy*
[5]*Cardiology and Cardiovascular Medicine Department, Fondazione Toscana G. Monasterio, Italy*
[6]*Cardiac Rehabilitation - Congestive Cardiac Unit, Ospedale Maggiore SS. Trinità, Fossano (CN), Italy*
[7]*Cardiology University Unit, Ospedale Policlinico, Bari, Italy*
[8]*Cardiovascular Research Department, Istituto Mario Negri, Milano, Italy*
[9]*Clinical Cardiology and Heart Failure Unit, Policlinico di Monza, Monza (MB), Italy*
[10]*Cardiology Department, Ospedale Civile, Chioggia (Venezia), Italy*
[11]*Cardiology Department, Ospedale SS. Annunziata, Cosenza, Italy*
[12]*Clinical Biochemistry and Molecular Medicine Section, Dipartimento di Pathobiology and Medical Biotechnology Department, Università degli Studi, Palermo, Italy*
[13]*Laboratory of Analysis, A.O. Ordine Mauriziano, Torino, Italy*

Revised by: **Mario Chiatto, Maria Frigerio, Aldo Pietro Maggioni, and Mario Plebani**

Consensus Document Approval Faculty in Appendix

*Corresponding author. Tel: 06 33062429, Fax: 06 33062489, Email: naspromonte@yahoo.it

Biomarkers have dramatically impacted the way heart failure (HF) patients are evaluated and managed. A biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological or pathogenic processes, or pharmacological responses to a therapeutic intervention. Natriuretic peptides [B-type natriuretic peptide (BNP) and N-terminal proBNP] are the gold standard biomarkers in determining the diagnosis and prognosis of HF, and a natriuretic peptide-guided HF management looks promising. In the last few years, an array of additional biomarkers has emerged, each reflecting different pathophysiological processes in the development and progression of HF: myocardial insult, inflammation, fibrosis, and remodelling, but their role in the clinical care of the patient is still partially defined and more studies are needed before to be well validated. Moreover, several new biomarkers have the potential to identify patients with early renal dysfunction and appear to have promise to help the management cardio-renal syndrome. With different biomarkers reflecting HF presence, the various pathways involved in its progression, as well as identifying unique treatment options for HF management, a closer cardiologist-laboratory link, with a multi-biomarker approach to the HF patient, is not far ahead, allowing the unique opportunity for specifically tailoring care to the individual pathological phenotype.

Heart failure (HF) is a complex syndrome involving neuro-humoral and inflammatory changes of different cell types including cardiac myocytes, fibroblasts, endothelial, and vascular smooth muscle cells. A consequence of the complex pathophysiological substrate of HF is the ever increasing number of circulating molecules (i.e. biomarkers) found/discovered to be altered in patients with HF.

Many molecules have been labelled as circulating 'biomarkers' in HF. The present document aims at helping the clinician in (i) appropriately using available biomarkers, and (ii) approaching new biomarkers through the literature with a critical attitude. Of all biomarkers reported to provide original information in diagnosis, prognosis, or management of HF, only cardiac troponins I or T and natriuretic peptides are cardio-specific. This explains why the largest body of evidence supporting their clinical use has been collected on these two families of biomarkers.

Background

The consensus document for Italian clinical cardiologists has been published very recently, where the evidence on the use of troponins and natriuretic peptides in HF are reported in several sections.[1]

The present document does not aim at comprehensively reviewing all established and candidate laboratory biomarkers in HF. Other types of biomarkers, such as imaging or genetic biomarkers, will not be discussed in detail, but will be evaluated in comparison with laboratory biomarkers. Unless otherwise specified, 'biomarkers' will be used for 'circulating biomarkers' or 'laboratory biomarkers' throughout the present document.

Objectives

Aims of the present review are

(1) To summarize the evidence supporting the clinical use of cardiac specific biomarkers, the main issue being how to make a good use of these validated markers in specific clinical/ambulatory settings.
(2) To provide the interested clinician with concise, essential information on the so-called 'new biomarkers' in order to help them in a critical use of the literature.

Premises

Before dealing with individual biomarkers, grouped by 'reasonable' categories, a general agreement on the following two methodological issues should be reached:

(1) Assays of the biomarkers;
(2) Sample size of studies to assess clinical performance of biomarkers.

Analytical issues

The contribution of the laboratory to the clinical management of HF by the biomarkers, represents one of the most important breakthroughs of the last decades. Standardization and validation of diagnostic methods, combined with a close link between the laboratory and the clinic improves the confidence of the cardiologist in the use and in a correct interpretation of analytical results on circulating biomarkers.

Cardiovascular biomarkers are usually measured with non-competitive immunometric assays in clinical laboratory practice.[2] Analytical performance of biomarker laboratory tests has progressively improved in the last 20 years. In particular, the last generation of immunometric assays using automated platforms is able to measure circulating levels of biomarkers [such as B-type natriuretic peptide (BNP), N-terminal proBNP (NT-proBNP), cTnI, and cTnT] with a limit of detection (LoD) of few ng/L (from 1 ng/L to 5 ng/L), a limit of quantification (LoQ) $\leq$ 10 CV% in the range of 5–15 ng/L, and a turn round time (TAT) of less than 30 min.[2] These very good analytical performances allowed

the use of these biomarkers as favourite methods even in the emergency department. Despite this remarkable increase in analytical performances, these immunoassays still suffer significant systematic differences between the biomarkers values measured by commercially available laboratory tests, especially for BNP[2,3] and cTnI[4] methods. Accordingly, clinicians should take great care in comparing results obtained by laboratories using different methods.

More difficulties are to be expected from point-of-care testing (POCT), whose reliability is, at the present time, not always adequately optimized and evaluated.[5] As far as the POCT methods for cardiac troponins are concerned, it is important to consider that the commercial methods so far available, do not satisfy the analytical quality specifications recommended by the international guidelines.[6-8] The POCT methods usually measure the recommended upper limit of normal with an error > 20% CV and so they should be used for *rule-in* and *rule-out* of myocardial infarction only where (or when) the more sensitive immunoassay methods using automated platforms are not available.[8] As far as the POCT methods for the measurement of natriuretic peptides are concerned, these assays are often used in both emergency departments and primary care, although their analytical sensitivity and reproducibility is lower than that of immuno-assay methods using automated platforms.[9-11]

Sample size

It is common belief that sample size calculations, in order to minimize the risk of false-negative results, are the matter of clinical trials testing drugs. This is definitely wrong, since the same risk is inherent in studies assessing predictive value or associations of risk factors, such as biomarkers. Without an adequate power, say above 60%, the risk of a false negative result is too high to reject the null hypothesis. The same is true for positive results obtained in small samples, too susceptible of bias and play of chance to be translated into clinical practice.

An authoritative example of power calculation is provided by an analysis of Framingham on renin. Given the lack of a significant association of renin with cardiovascular risk, the statistical power to detect modest effects was assessed. At an alpha of 0.05, the power was at least 80% for each outcome (hard cardiovascular disease and all-cause mortality), in the full sample and in the hypertensive sub-sample, for the true HR of 1.25 per SD of log-renin.[12]

The data reported in *Table 1*, considering different combinations of hazard ratios and power, provide an idea on the number of patients needed for a reliable assessment of association between biomarkers and risk.

Myocardial stress and function laboratory biomarkers

Pathophysiological and clinical interpretation

HF is considered the fatal finishing line of all cardiovascular disorders. HF is a clinical syndrome characterized by typical symptoms (e.g. breathlessness, ankle swelling, and fatigue) that may be accompanied by signs (e.g. elevated jugular venous pressure, pulmonary crackles, and peripheral oedema) caused by a structural and/or functional

Table 1 Number of patients to be enrolled in studies testing prognostic value of biomarkers. Different calculations are presented according to power (type II error) and impact on outcomes expressed as hazard ratio (HR) of above vs below the median level of the biomarker

HR	Sample size	Power
1.2	4240	80%
1.4	1192	80%
1.6	580	80%
1.2	3334	70%
1.4	937	70%
1.6	457	70%

HR, hazard ratio.

Table 2 Accuracy of history and physical findings in diagnosing acute congestive heart failure (modified from references 13 and 14)

Variable	Sensitivity	Specificity	Accuracy
History of HF	62	94	80
Dyspnea	56	53	54
Orthopnoea	47	88	72
Rales	56	80	70
Third heart sound	20	90	66
Jugular vein distension	39	94	72
Oedema	67	68	68

HR, heart failure.

cardiac abnormality, resulting in a reduced cardiac output and/or elevated intra-cardiac pressures at rest or during stress.[13] Positive history and some physical signs (such as orthopnoea, rales, third heart sound, or jugular vein distension) share a good diagnostic specificity, but also a poor sensitivity in diagnosing acute congestive HF (*Table 2*).[13,14] Therefore, the diagnosis of both acute and chronic HF is based on the clinical judgment including a combination of history, physical examination, and appropriate investigations, as recommended by all international guidelines.[15-19]

The pathophysiological interpretation of plasma BNP/ NT-proBNP variations may be difficult in some clinical settings. The cardiac endocrine function has a pivotal role in the regulation of body fluids, electrolytes, and haemodynamics.[20] The cardiac peptide hormones, ANP and BNP, share diuretic, natriuretic, vasodilating, and anti-hypertrophic activities.[20] A continuous and intense information exchange flows from the endocrine heart system to nervous and immunological systems and to other organs, including kidney, endocrine glands, liver, adipose tissue, immune-competent cells, and *vice versa*. This close link between cardiac natriuretic peptide system and counter-regulatory systems with sodium-retentive, vasoconstrictive and hypertrophic activities (including renin-angiotensin-aldosterone system, endothelins, catecholamines, arginine-vasopressine system, and pro-inflammatory cytokines) may explain the increase in circulating levels of

BNP/NT-proBNP in patients with some extra-cardiac diseases.[20]

A deficiency in biological action of circulating cardiac natriuretic peptide hormones may contribute to explain the altered electrolyte and fluid balance occurring in chronic HF: a phenomenon, defined as the 'endocrine paradox' of HF.[20] Patients with congestive HF show signs of fluid retention and vasoconstriction, despite the extremely high circulating BNP levels, measured by immunoassay methods at present time commercially available.[1,20] Indeed, a blunted natriuretic response after pharmacological doses of ANP and BNP has been observed in experimental models and in patients with chronic HF, suggesting a resistance to the biological effects of natriuretic peptides.[20] An explanation of the paradox of high circulating levels of the hormone, but low plasma natriuretic activity may be the large cross-reactivity of proBNP on the immunoassay methods considered specific for BNP.[1,21] This cross-reaction produces a great overestimation of the measured levels of BNP, because proBNP is the predominant peptide form present in plasma of patients with severe HF.[1,20] Another cause of resistance to biological action of natriuretic peptides is that proBNP is able to bind to the natriuretic peptide receptors, but it stimulates the receptor with lower potency than the active hormones, ANP and BNP, and so it produces a decrease in natriuretic activity by displacing the more biologically active peptide hormones from the receptors.[22]

Another important clinical issue in the interpretation of measurements of natriuretic peptides in HF is that circulating BNP and NT-proBNP levels mirror the effectiveness of the treatment of acute or chronic HF, with lowering of levels over time associated with better clinical outcomes.[23-26]

Use of BNP/NT-proBNP in clinical diagnosis of acute and chronic heart failure

All the most recent national and international guidelines[13,16-20] recommend the natriuretic peptides, and in particular the peptides related to the B-type cardiac peptide hormone (such as BNP and NT-proBNP), as the first line biomarkers for the diagnosis of both acute and chronic HF. In particular, the 2013 ACCF/AHA guidelines recommend the BNP and NT-proBNP measurement for the diagnosis or exclusion of HF in patients with chronic or acute decompensated HF (with the maximum degree of class of recommendation I and level of evidence A).[16] The clinical contribution to the diagnosis of BNP/NT-proBNP assay is particularly significant when the etiology of dyspnoea is unclear. Moreover, the 2013 ACCF/AHA guidelines recommend the measurement of natriuretic peptides with the maximum degree of evidence (class I and level A) also for the prognosis of HF patient.[16]

Usually there are no differences in the diagnostic use of BNP and NT-proBNP immunoassays.[1] However, two recent studies from the PARADIGM-HF (prospective comparison of angiotensin II receptor blocker neprilysin inhibitor (ARNI) with angiotensin-converting-enzyme inhibitor (ACEI) to determine impact on global mortality and morbidity in heart failure (HF)] trial[27,28] reported conflicting results between BNP and NT-proBNP levels. PARADIGM-HF study evaluated the clinical effects of a new drug (denominated LCZ696 or ENTRESTO), including a combination of sacubitril, a neprilysin (NEP) inhibitor, and valsartan, an angiotensin II receptor blocker. The mechanism of of action of this drug is complex, combining the effect of the angiotensin II receptor blocker and that of the neprilysin inhibitor. The enzyme neprilysin causes degradation not only of natriuretic peptides but also of a variety of components affecting the mechanisms of action for several other circulating hormones, including adrenomedullin, bradykinins, angiotensin I, endothelin-1, and substance P.[29] The rationale for the use of a drug containing a neprilysin inhibitor in HF patients is that this proteolytic enzyme can degrade the biologically active natriuretic peptides (ANP, BNP, and CNP).[29] For this reason, a drug, containing a substance inhibiting natriuretic peptide degradation (such as LCZ696), may increase the circulating levels of the biologically active natriuretic hormones and, by doing so, may improve the clinical conditions of HF patients by increasing diuresis and natriuresis and reducing cardiac stress.[29] PARADIGM-HF study found that plasma BNP levels were higher during treatment with LCZ696 than with enalapril, but, on the contrary, circulating levels of NT-proBNP and cardiac troponin T (cTnT) were lower during treatment with LCZ696 than with enalapril in the first week of treatment.[27] Authors explained these conflicting results obtained in the PARADIGM-HF study by considering the combined action of LCZ696 drug. Indeed, BNP (but not NT-proBNP) is a substrate for neprilysin[29,30]; as a result, the increase in BNP levels should reflect the inhibiting action of the drug on the enzyme neprilysin. On the contrary, the decrease in NT-proBNP levels may reflect the beneficial effects of the drug on myocardial function and vascular haemodynamics, especially by inhibiting the renin-angiotensin-aldosterone system activity.[29,30] Indeed, the reduction of cardiac stress during LCZ696 treatment should reduce the production and secretion of natriuretic peptides from cardiomyocytes (and so a fall of the circulating levels of NT-proBNP, too).[1] In conclusion, clinicians should accurately consider the clinical setting in order to correctly interpret the variations of natriuretic peptides measured by commercially available laboratory methods. In particular, clinicians should distinguish the increase in BNP levels, due to the inhibiting effect of LCZ696 from those due to deterioration of clinical conditions.

Several studies, including also several meta-analyses,[31-37] demonstrated the clinical relevance of BNP/NT-proBNP assay in patients with both acute and chronic HF in different clinical settings (including emergency department and primary care). The measurement of BNP and NT-proBNP is useful in supporting clinical judgment for the diagnosis or exclusion of HF in the setting of chronic ambulatory HF or acute decompensated HF (with the maximum degree of class of recommendation I and level of evidence A).[16] Indeed, all international guidelines, starting from the first years of this century, state that lower values of BNP or NT-proBNP actually exclude the presence of HF, while higher values have reasonably high positive predictive value to diagnose HF.[13,16-19,38-41] Therefore, BNP/NT-proBNP assay is recommended for ruling-out HF, but not to establish the diagnosis.[13] Although some international guidelines[13,17-19]

suggest some cut-off values of BNP assay for the ruling-out or ruling-in HF, these values are only indicative, because there are large systematic between BNP immunoassay methods.[2,3] On the contrary, the cut-off values reported by international guidelines for NT-proBNP assay are more reliable, because only one manufacturer distributes the standard and materials for all immunoassay methods, commercially available in Europe for NT-proBNP measurement.[2,3]

BNP/NT-proBNP assay can be also useful for detection of early phases of HF (phase A or B),[16,40] when patients are still asymptomatic or pauci symptomatic (functional NYHA classes I and II).[42] BNP/NT-proBNP assay cannot differentiate the type of cardiac dysfunction (systolic vs. diastolic); however, the BNP/NT-proBNP levels found in HF patients with preserved left ventricular ejection fraction usually are lower than those of HF patients with reduced left ventricular ejection fraction.[13,36] As recommended by national and international guidelines,[13,16-19,38-41] the clinical information obtained from electrocardiographic and echocardiographic examination and from the BNP/NTproBNP assay are not equivalent, but they contribute independently to the HF diagnosis and to the definition of the patient's clinical status.

At present time, with the exception of cardiac natriuretic peptides, no other cardiovascular biomarkers are recommended by international guidelines for the diagnosis of both acute and chronic HF with the maximum degree of class of recommendation and level of evidence.[13,16-19]

Synthesis of evidences
(1) The measurement of cardiac natriuretic peptides is recommended for the diagnosis of HF in patients with dyspnoea by national and international guidelines since year 2005.
(2) Due to the high degree of clinical sensitivity and negative predictive value, the measurement of cardiac natriuretic peptides is useful for excluding the diagnosis of HF, especially using method-specific reference limits and taking into account sex and age of the patient as well as the presence of obesity and kidney failure.
(3) The measurement of cardiac natriuretic peptides cannot differentiate the type of cardiac dysfunction (systolic vs. diastolic).
(4) The clinical information obtained from echocardiographic examination and from BNP/NT-proBNP assay is not equivalent, but they contribute independently to the HF diagnosis and to the definition of the patient's clinical status.

Clinical use of cardiac natriuretic peptides as prognostic biomarkers in heart failure patients
More than 1000 published studies, including several meta-analyses,[43-56] evaluated the prognostic accuracy of natriuretic peptides (in particular BNP and NT-proBNP) in patients with acute or chronic HF. Plasma BNP and NT-proBNP levels usually decrease during treatment of chronic HF, correlating with improved clinical outcomes, including mortality, hospital stay and/or readmission rate.[15,24,25,41,43-47,48,51-54,56-60] According to these experimental and clinical evidences, measurement of natriuretic peptides is recommended with

the maximum score (class I and level A) for prognosis in both ambulatory and acute HF patient by the 2013 AACF/AHA guidelines for the management of HF.[16]

Synthesis of evidences
(1) Cardiac natriuretic peptides measurement is recommended for risk stratification in all patients with acute and chronic HF.
(2) The short- and long-term risk of death and of cardiovascular events increases gradually with the increase of biomarker values, even for BNP and NT-proBNP levels within the normal range.
(3) The prognostic information provided by the cardiac natriuretic peptides is independent of that provided by other known cardiovascular risk factors and is associated additively with that of cardiac troponins and cardiac fibrosis markers, such as galectin-3 and sST2 protein.

Use of cardiac natriuretic peptides in clinical management of HF patients
There is an increasing consensus about the use of natriuretic peptide-guided HF management.[26] Several clinical trials and meta-analyses[23-25,43-56,58-60] demonstrated that either baseline level of BNP and NT-proBNP or its decrease after treatment hold a powerful prognostic value in HF patients. In particular, the decrease in peptide natriuretic levels during treatment is associated with clinical improvement, whereas unchanged or increased levels are associated with disease progression and worse prognosis.[23-26] The rationale for the use of BNP-guided treatment is that the variation in plasma peptide concentrations before and after a period of standard therapy is able to distinguish 'responders' with a better prognosis (i.e. a decrease > 30% in peptide levels after therapy), from 'non-responders' patients with a more severe prognosis (i.e. not decrease or even increase in peptide levels after therapy), who probably need both a re-evaluation of clinical status and a re-assessment of treatment.[16,26,45] Moreover, it is important to note that the rationale for the use of natriuretic peptide-guided HF management is based also on the assumption that laboratory tests are able to accurately assess the true status of the cardiac endocrine function.[2,20]

Despite this large number of experimental and clinical evidences found in the literature,[23-25,43-56,58-60] the international guidelines still report an interlocutory judgement about the natriuretic peptide-guided HF management. It should be recognized that evidence is not based on a single, well designed, adequately sized phase III trial. The ESC 2016 guidelines[13] provide only some general recommendations about the usefulness of the natriuretic peptides as a guide-to therapy. In particular, it is recommended to begin treatment with valsartan-sacubitril only in patients with high levels of natriuretic peptides. The 2013 ACCF/AHA guidelines[16] recommended that the dosage of BNP or NT-proBNP to optimize the therapy should be limited to euvolaemic patients and in the context of a well-structured program of management of chronic HF (class of evidence: IIa, level of evidence: B). As for acute HF, the effectiveness of therapy guided by natriuretic peptides has low levels of recommendation and evidence (class of evidence: class IIb, level of evidence: C

type).[16] The NICE guidelines[19] summarize the evidence in the literature and concluded that the use of natriuretic peptides to guide treatment in chronic HF may lead to potential reduction in mortality in some subgroups, although the overall usefulness in all HF patients remains uncertain.

Synthesis of evidences

(1) Patients who respond to treatment with a significant decrease in circulating levels of BNP or NT-proBNP have a better prognosis, particularly with regard to the decrease of mortality and/or major cardiovascular events.

(2) BNP/NT-proBNP guided therapy significantly reduces mortality in patients younger than 75 years while hospitalization is reduced for all ages and for all causes of hospitalization.

(3) In light of the current scientific evidence, it is appropriate to measure the BNP/NT-proBNP levels in patients hospitalized for acute HF at least at the admission and before the discharge to assess the patient's response to therapy. A reduction in levels greater than 30% should be considered significant.

(4) There is no evidence in the literature regarding the serial evaluation of BNP/NT-proBNP during hospitalization (except for the admission and discharge)

Laboratory biomarkers of myocardial injury

Pathophysiological and clinical interpretation of myocardial release of troponin in heart failure patients

Cardiac troponin I (cTnI) and cTnT are the most widely used biomarkers of myocardial injury in clinical research and in patients with acute coronary syndromes.[1,2] More recently, the availability of highly sensitive assays has markedly increased the analytical sensitivity, thus allowing to monitor also the majority of patients with stable chronic HF, whose concentrations of troponins were often below the LoD of earlier generation assays.[61-64] cTnI and cTnT are essentially equivalent as markers of cardiac injury in HF patients. In virtually all HF patients, highly sensitive methods can measure circulating cTnI and cTnT levels above their respective limits of detection. In particular, patients with HF, aged 70 years or more, often have circulating concentrations of cTnI and cTnT assayed with high sensitivity methods above the decision limit (99th percentile in a reference population of apparently healthy individuals).[61-64]

From an analytical point of view, some important issues should be discussed in detail, regarding the analytical sensitivity and specificity of laboratory tests at present employed for cTnI and cTnT measurement. First, it is important to accurately define the analytical specifications characterizing the highly sensitive methods.[66,67] One should define methods with high sensitivity only the immunoassays that measure the 99th percentile of the distribution of proteins cTnI and cTnT in the reference population (99th ULN) with an error (expressed as coefficient of variation, CV) equal to or lower than 10%,[66,67] as required by all the most international guidelines, including the Third Universal Definition of Myocardial Infarction.[6] The methods showing

an intermediate imprecision (10–20%) should be considered clinically usable (but not highly sensitive methods).[6-8,66,67] The reference population on which the 99th URL value is calculated should consist of at least 300 apparently healthy subjects of both genders with a broad age distribution (usually from 18 to 70 years).[7,66,67] It is important to note that highly sensitive immunoassay methods should also measure the levels of cTnI and cTnT in the majority (i.e. >50%) of apparently healthy adults, who compose the reference population. In particular, the most sensitive, commercially available in Europe, cTnI method is able to measure troponin levels above the detection limit in the great majority (>95%) of healthy subjects,[66,67] also including neonates, children, and adolescents.[68,69] Second, as far as the cardio-specificity of cardiac troponin immunoassay methods is concerned, recent studies suggested that the cTnT may be re-expressed in skeletal myocytes of patients with a wide spectrum of neuromuscular diseases in the absence of clinical and cTnI evidence of myocardial injury.[70-75] Although cTnT and cTnI are both absent in healthy adult skeletal muscle, cTnT (but not cTnI) is present in foetal skeletal muscle. The injured skeletal muscle repairs itself by regeneration; this process recapitulates embryonic myogenesis, and so patients with chronic neuro-muscular diseases can present increased circulating levels of cTnT, but not of cTnI.[70-72] From a clinical point of view, it is important to note that cTnT circulating levels may be increased above the 99th URL value in patients with neuromuscular diseases.[74,75] Furthermore, heart involvement can be excluded by normal values of ECG, echocardiogram, and BNP/NT-proBNP levels. However, to exclude myocardial injury in this group of patients it may be preferable to measure also the cTnI concentration using a high-sensitive method.[74]

Prognostic relevance of cardiac troponins

Several studies demonstrated that increased circulating levels of cTnI and cTnT, especially when these biomarkers are measured with highly sensitive methods, are found in patients with HF, who often do not present obvious myocardial ischemia or underlying coronary artery disease.[61-64] These findings[61-64] suggest that increased cTnI and cTnT in these patients could be caused by cardiomyocyte injury or necrosis. In chronic or acute decompensated HF, elevated cardiac troponin levels are associated with worse clinical outcomes and/or mortality.[61-64,76-83] Indeed, HF patients, showing a significant and lasting decrease in troponin levels after appropriate pharmacological treatment have a better prognosis compared with those who did not show any or only a transient decrease.[61,76-83] Based on these results, 2013 AACF/AHA guidelines for the management of HF recommend that troponin I or T be routinely measured, in addition to natriuretic peptides, in both acute and ambulatory HF patients for improving risk stratification, with the maximum degree of evidence (class I and level A).[16] According to the guidelines by the Heart Failure Section of the Third Universal Definition of Myocardial Infarction Global Task Force, clinicians should be aware of the high frequency of troponin elevation in patients with HF, and for this reason they should keep in mind the possible causes of this phenomenon, and, independent of AMI diagnosis.[84]

Synthesis of evidences

(1) Increasing circulating levels of plasma cardiac troponin, even within a normal range, are associated with a worse prognosis in HF.

(2) The prognostic value of cardiac troponins is independent and incremental compared with other risk factors and cardiovascular biomarkers

Laboratory biomarkers of cardiac remodelling and fibrosis

Cardiac ventricular remodelling occurs progressively in untreated patients after large myocardial infarction and in those with cardiomyopathy. Myocardial remodelling in ischaemic and non-ischaemic cardiomyopathies involves not only the cardiomyocytes, but also non-myocyte cells and the extracellular matrix, which includes fluid, collagen, and glycoproteins.[85,86] Detection of fibrosis and of an ongoing remodelling process holds clinical value and should guide therapeutic strategy in HF patients. For this reason, there is an increasing interest in the development of new biomarkers and a great number of laboratory tests have been recently proposed, whose clinical usefulness, however, is not fully established yet.[86] Fibrosis is an ubiquitous mechanism of tissue repair. An increase in cardiac fibrosis is associated not only to normal ageing but also to arterial hypertension and other less common diseases. An excessive collagen synthesis and deposition and/or a decrease in its degradation cause an increase in collagen content of myocardium and blood vessel wall which can lead to cardiac dysfunction and ultimately cardiac failure. Part of the beneficial effect of recommended therapies for HF may be explained by their anti-fibrotic action. At present, there are no reliable markers with sufficient sensitivity and cardiac-specificity to be used clinically in HF patients.

However, many studies, even including some recent meta-analyses,[86-89] have been published on biomarkers of cardiac remodelling and fibrosis, especially concerning galectin-3 and sST2, in patients with acute and chronic HF. However, the evidence available is not sufficient to support their use in the clinical routine to improve prognostic stratification and diagnosis of individual patients.[16,86] In particular, more studies are needed to document the independent prognostic value of circulating markers of fibrosis, on top of the other established markers, cardiac troponins and natriuretic peptides.[13,16,86] Being non-cardiac-specific biomarkers, the value of galectin-3 and sST2 in HF patients should be established in front of other frequent comorbidities, such as diabetes, systemic chronic inflammatory disorders, renal and liver diseases.[13,86]

The 2016 ESC guidelines[13] mention the increasing interest in 'new' biomarkers of HF, but underscore that the evidence currently available is not sufficient to recommend their use in clinical practice. On the contrary, the 2013 American College of Cardiology Foundation (ACCF)/ American Heart Association (AHA) guidelines[16] contain recommendations on biomarkers of inflammation and fibrosis, suggesting that the most promising biomarkers of this class are galectin-3 and soluble ST2. Even with the low level of recommendation, the 2013 ACCF/AHA guidelines suggest

that the dosage of biomarkers of inflammation and fibrosis, along with the dosage of other biomarkers, may improve the prognostic stratification both in patients with chronic (class of evidence: IIb, level of evidence: B) and acute HF (class of evidence: IIb, level of evidence: A).[16]

Synthesis of evidences

(1) There are still some doubts on efficiency, prognostic role, and cost-effectiveness of biomarkers of cardiac remodelling and myocardial fibrosis, even if many studies have been published recently.

(2) In particular, more studies are needed to confirm their independent and additional prognostic contribution in comparison with other biomarkers, such as natriuretic peptides and cardiac troponins.

(3) Moreover, they are non-cardio-specific biomarkers and their usefulness has to be fully established in the presence of co-morbidities such as diabetes, chronic systemic inflammatory diseases, renal and liver diseases.

(4) Considering the wide range of biomarkers of cardiac remodelling and myocardial fibrosis, the clinician should direct his decision remembering the analytical characteristics of the assay, the efficiency and prognostic effectiveness of the biomarker also in relation to patients' setting, possible confounding variables, co-morbidities and costs.

Novel biomarkers and multi-markers models

In recent decades, several multi-marker models (probably more than 100) have been suggested and evaluated for different populations of HF patients.[13,14,90-92] Criteria to evaluate and compare the prognostic efficacy and efficiency of new cardiovascular risk biomarkers were recently reported and discussed in details.[91,92] Novel risk biomarkers should be evaluated in several phases, including initial proof of concept, prospective validation in independent populations, documentation of incremental information, when added to standard risk markers, assessment of effects on patient management and outcomes, and ultimately, cost-effectiveness.[91,92] Biomarkers that do not change the management of a disease unlikely will significantly affect patient outcome and therefore will not be cost-effective (judged in terms of quality-adjusted life-years gained).[14,91,92] The search is still open for novel biomarkers useful for prognosis and guide therapy in HF patients.[14,26,93] Promising candidate biomarker may be: growth differential factor-15 (GDF-15),[94-96] carbohydrate antigen-125 (CA-125),[97-99] C-terminal pro-vasopressin (copectin),[100-103] mid-regional pro-adrenomedullin (MR-proADM),[100,101,104] NEP,[30,105] and orexin.[93,106] Some of these molecules have been utilized for many years as tumour-markers (CA-125), neuro-hormones (copeptin, MR-proADM) or inflammatory markers (GDF-15) in clinical practice. Therefore, several studies are available on their biological activity and variation, pathophysiological and clinical relevance, reference range values and analytical characteristic of assay methods.[93] Conversely, there are scarce data on neprilysin[30] and orexin,[106-108] which should be considered as biomarkers in the early discovery phase and still under evaluation. A detailed discussion of that

clinical relevance of these promising HF biomarkers is out of the aim of this executive summary and interested readers can consult the references for more information.

The relatively modest performance allowed by individual biomarkers prompted several investigators to evaluate the hypothesis whether multiple biomarkers could be combined to improve prognostic performance.[14,90-92] The 'multimarker approach' has been tested in several studies, primarily with the use of circulating biomarkers.[14,90-92] There are fewer data incorporating imaging into multimarker algorithms and also few data on the use of circulating, genetic, and/or imaging biomarkers in combination.[14,90-92] Multivariable statistical models may be used to calculate some multivariable risk scores.[109] Multivariable risk scores may help predicting death in patients with HF, but they remain less useful for the prediction of subsequent HF hospitalizations.[13] A systematic review examining 64 prognostic models along with a meta-analysis and meta-regression study of 117 prognostic models reported only a moderate accuracy of models predicting mortality, whereas models designed to predict the combined endpoint of death or hospitalization, or only hospitalization, showed a poorer prognostic power.[110]

Synthesis of evidences

(1) There are several novel biomarkers for prognosis and to guide therapy in HF patients, which are in the early discovery phase and still under evaluation.

(2) More studies are needed to evaluate the clinical relevance and especially to confirm the independent and additional prognostic contribution of novel biomarkers in comparison with natriuretic peptides and cardiac troponins.

(3) Multivariable risk scores may help predicting death in patients with HF, but at present time they remain less useful for the prediction of subsequent HF hospitalizations or to guide therapy in the individual patient.

Consensus Document Approval Faculty

Abrignani Maurizio Giuseppe, Alunni Gianfranco, Amico Antonio, Francesco, Amodeo Vincenzo, Angeli Fabio, Battistoni Ilaria, Bianca Innocenzo, Bisceglia Irma, Bongarzoni Amedeo, Cacciavillani, Luisa, Calculli Giacinto, Caldarola Pasquale, Capecchi Alessandro, Caporale Roberto, Caretta Giorgio, Carmina Maria Gabriella, Casazza Franco, Casolo Giancarlo, Cassin Matteo, Casu Gavino, Cemin Roberto, Chiarandà Giacomo, Chiarella Francesco, Chiatto, Mario, Cibinel Gian Alfonso, Colivicchi Furio, De Luca Giovanni, De Maria Renata, Del Sindaco Donatella, Di Fusco Stefania Angela, Di Lenarda Andrea, Egman Sabrina, Enea Iolanda, Fattirolli Francesco, Ferraiuolo Giuseppe, Francese Giuseppina Maura, Gabrielli, Domenico, Geraci Giovanna, Giardina Achille, Gregorio Giovanni, Khoury Georgette, Ledda Antonietta, Lucà Fabiana, Lukic Vjerica, Macera Francesca, Marini Marco, Maseri Attilio, Maurea Nicola, Mazzanti Marco, Mennuni Mauro, Menotti Alberto, Menozzi Alberto, Mininni Nicola, Moreo Antonella, Moretti Luciano, Mureddu, Gian Francesco, Murrone Adriano, Musumeci Giuseppe, Nardi, Federico, Navazio Alessandro, Nicolosi Gian Luigi, Oliva Fabrizio, Parato Vito Maurizio, Parrini Iris, Patanè Leonardo, Pini Daniela, Pino Paolo Giuseppe, Pirelli Salvatore, Pulignano Giovanni, Radini, Donatella, Rao Carmelo Massimiliano, Rasetti Gerardo, Riccio Carmine, Roncon Loris, Rossini Roberta, Ruggieri Maria Pia, Rugolotto, Matteo, Sanna Fabiola, Sauro Rosario, Scherillo Marino, Severi Silva, Sicuro Marco, Sisto Francesco, Tarantini Luigi, Uguccioni Massimo, Urbinati Stefano, Valente Serafina, Vatrano Marco, Vianello, Gabriele, Vinci Eugenio, and Zuin Guerrino.

References

1. Aspromonte N, Gulizia MM, Clerico A, Di Tano G, Emdin M, Feola M, Iacoviello M, Latini R, Mortara A, Valle R, Misuraca G, Passino C, Masson S, Aimo A, Ciaccio M, Migliardi M. [ANMCO/ELAS/SIBioC Consensus document: Recommendations for the use of cardiac biomarkers in heart failure patients]. *G Ital Cardiol* 2016;**17**:615-656.

2. Clerico A, Passino C, Franzini M, Emdin M. Cardiac biomarker testing in the clinical laboratory: where do we stand? General overview of the methodology with special emphasis on natriuretic peptides. *Clin Chim Acta* 2015;**443**:17-24.

3. Prontera C, Zaninotto M, Giovannini S, Zucchelli GC, Pilo A, Sciacovelli L, Plebani M, Clerico A. Proficiency testing project for brain natriuretic peptide (BNP) and the N-terminal part of the propeptide of BNP (NT-proBNP) immunoassays: the CardioOrmocheck study. *Clin Chem Lab Med* 2009;**47**:762-768.

4. Clerico A, Ripoli A, Masotti S, Prontera C, Storti S, Fortunato A, Buzzi P, Casagranda I, Franzini M, Ndreu R, Zucchelli GC, Zaninotto M, Plebani M. Pilot study on harmonization of cardiac troponin I immunoassays using patients and quality control plasma samples. On behalf of the Italian Section of the European Ligand Assay Society (ELAS) and of the Study Group on Cardiovascular Biomarkers of the Società Italiana di Biochimica Clinica (SIBioC). *Clin Chim Acta* 2016;**456**:42-48.

5. Cohen D. Rivaroxaban: can we trust the evidence? *BMJ.* 2016;**352**:i575.

6. Thygesen K, Alpert JS, Jaffe AS, Simoons ML, Chaitman BR, White HD; Joint ESC/ACCF/AHA/WHF Task Force for Universal Definition of Myocardial Infarction.; Authors/Task Force Members Chairpersons.; Thygesen K, Alpert JS, White HD; Biomarker Subcommittee., Jaffe AS, Katus HA, Apple FS, Lindahl B, Morrow DA; ECG Subcommittee., Chaitman BR, Clemmensen PM, Johanson P, Hod H; Imaging Subcommittee., Underwood R, Bax JJ, Bonow JJ, Pinto F, Gibbons RJ; Classification Subcommittee., Fox KA, Atar D, Newby LK, Galvani M, Hamm CW; Intervention Subcommittee., Uretsky BF, Steg PG, Wijns W, Bassand JP, Menasche P, Ravkilde J; Trials & Registries Subcommittee., Ohman EM, Antman EM, Wallentin LC, Armstrong PW, Simoons ML; Trials & Registries Subcommittee., Januzzi JL, Nieminen MS, Gheorghiade M, Filippatos G; Trials & Registries Subcommittee., Luepker RV, Fortmann SP, Rosamond WD, Levy D, Wood D; Trials & Registries Subcommittee., Smith SC, Hu D, Lopez-Sendon JL, Robertson RM, Weaver D, Tendera M, Bove AA, Parkhomenko AN, Vasilieva EJ, Mendis S; ESC Committee for Practice Guidelines (CPG)., Bax JJ, Baumgartner H, Ceconi C, Dean V, Deaton C, Fagard R, Funck-Brentano C, Hasdai D, Hoes A, Kirchhof P, Knuuti J, Kolh P, McDonagh T, Moulin C, Popescu BA, Reiner Z, Sechtem U, Sirnes PA, Tendera M, Torbicki A, Vahanian A, Windecker S; Document Reviewers., Morais J, Aguiar C, Almahmeed W, Arnar DO, Barili F, Bloch KD, Bolger AF, Botker HE, Bozkurt B, Bugiardini R, Cannon C, de Lemos J, Eberli FR, Escobar E, Hlatky M, James S, Kern KB, Moliterno DJ, Mueller C, Neskovic AN, Pieske BM, Schulman SP, Storey RF, Taubert KA, Vranckx P, Wagner DR. Third universal definition of myocardial infarction. *J Am Coll Cardiol* 2012;**60**:1581-1598.

7. Thygesen K, Mair J, Giannitsis E, Mueller C, Lindahl B, Blankenberg S, Huber K, Plebani M, Biasucci LM, Tubaro M, Collinson P, Venge P, Hasin Y, Galvani M, Koenig W, Hamm C, Alpert JS, Katus H, Jaffe AS; Study Group on Biomarkers in Cardiology of ESC Working Group on Acute Cardiac Care. How to use high-sensitivity cardiac troponins in acute cardiac care. *Eur Heart J* 2012;**33**:2252-2257.

8. Jaffe AS, Apple FS, Morrow DA, Lindahl B, Katus HA. Being rational about (im)precision: a statement from the Biochemistry Subcommittee of the Joint European Society of Cardiology/American College of Cardiology Foundation/American Heart Association/World Heart Federation Task Force for the definition of myocardial infarction. *Clin Chem* 2010;**56**:941-943.

9. Lee-Lewandrowski E, Januzzi JL Jr, Grisson R, Mohammed AA, Lewandrowski G, Lewandrowski K. Evaluation of first-draw whole blood, point-of-care cardiac markers in the context of the universal definition of myocardial infarction: a comparison of a multimarker panel to troponin alone and to testing in the central laboratory. *Arch Pathol Lab Med* 2011;**135**:459-463.

10. Tideman P, Simpson P, Trimacco R. Integrating PoCT into clinical care. *Clin Biochem Rev* 2010; **31**:99-104.

11. Prontera C, Masotti S, Franzini M, Emdin M, Passino C, Zucchelli GC, Clerico A. Comparison between BNP values measured in capillary blood samples with a POCT method and those measured in plasma venous samples with an automated platform. *Clin Chem Lab Med* 2015;**53**:e125-e127.

12. Parikh NI, Gona P, Larson MG, Wang TJ, Newton-Cheh C, Levy D, Benjamin EJ, Kannel WB, Vasan RS. Plasma renin and risk of cardiovascular disease and mortality: the Framingham Heart Study. *Eur Heart J* 2007;**28**:2644-2652,

13. Ponikowski P, Voors AA, Anker SD, Bueno H, Cleland JG, Coats AJ, Falk V, González-Juanatey JR, Harjola VP, Jankowska EA, Jessup M, Linde C, Nihoyannopoulos P, Parissis JT, Pieske B, Riley JP, Rosano GM, Ruilope LM, Ruschitzka F, Rutten FH, van der Meer P; Authors/Task Force Members; Authors/Task Force Members; Document Reviewers. 2016 ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure: The Task Force for the diagnosis and treatment of acute and chronic heart failure of the European Society of Cardiology (ESC)Developed with the special contribution of the Heart Failure Association (HFA) of the ESC. *Eur Heart J*. 2016. [Epub ahead of print]

14. Emdin M, Vittorini S, Passino C, Clerico A. Old and new biomarkers of heart failure. *Eur J Heart Fail* 2009;**11**:331-335.

15. Aspromonte N, Ceci V, Chiera A, Coletta C, D'Eri A, Feola M, Giovinazzo P, Milani L, Noventa F, Scardovi AB, Sestili A, Valle R. Rapid brain natriuretic peptide test and Doppler echocardiography for early diagnosis of mild heart failure. *Clin Chem*. 2006;**52**:1802-1808.

16. Yancy CW, Jessup M, Bozkurt B, Butler J, Casey DE Jr, Drazner MH, Fonarow GC, Geraci SA, Horwich T, Januzzi JL, Johnson MR, Kasper EK, Levy WC, Masoudi FA, McBride PE, McMurray JJ, Mitchell JE, Peterson PN, Riegel B, Sam F, Stevenson LW, Tang WH, Tsai EJ, Wilkoff BL. 2013 ACCF/AHA guideline for the management of heart failure: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. *Circulation* 2013;**128**:1810-1852.

17. Heart Failure Society of America., Lindenfeld J, Albert NM, Boehmer JP, Collins SP, Ezekowitz JA, Givertz MM, Katz SD, Klapholz M, Moser DK, Rogers JG, Starling RC, Stevenson WG, Tang WH, Teerlink JR, Walsh MN. Executive Summary: HFSA 2010 Comprehensive Heart Failure Practice Guideline. *J Card Fail* 2010;**16**:e1-194.

18. Emdin M, Clerico A, Clemenza F, Galvani M, Latini R, Masson S, Mulè P, Panteghini M, Valle R, Zaninotto M, Ganau A, Mariotti R, Volpe M, Aspromonte N, Cacciatore G, Cappelletti P, L'Abbate A, Miglio F, Ottani F, Pagani F, Passino C, Plebani M, Sarzani R, Zucchelli G; Italian Association of Hospital Cardiologists; Italian Society of Cardiology; Italian Federation of Cardiology; Italian Society of Clinical Chemistry and Molecular Biology; Italian Society of Laboratory Medicine; Italian Society of Emergency Medicine. Recommendations for the clinical use of cardiac natriuretic peptides. *Ital Heart J* 2005;**6**:430-446.

19. National Clinical Guideline Centre (UK). *Acute Heart Failure: Diagnosing and Managing Acute Heart Failure in Adults*. London: National Institute for Health and Care Excellence (UK); 2014.

20. Clerico A, Giannoni A, Vittorini S, Passino C. Thirty years of the heart as an endocrine organ: physiological role and clinical utility of cardiac natriuretic hormones. *Am J Physiol Heart Circ Physiol* 2011;**301**:H12-H20.

21. Luckenbill KN, Christenson RH, Jaffe AS, Mair J, Ordonez-Llanos J, Pagani F, Tate J, Wu AH, Ler R, Apple FS. Cross-reactivity of BNP, NT-proBNP, and proBNP in commercial BNP and NT-proBNP assays: preliminary observations from the IFCC Committee for Standardization of Markers of Cardiac Damage. *Clin Chem* 2008;**54**:619-621.

22. Dickey DM, Potter LR. ProBNP1-108 is resistant to degradation and activates Guanylyl Cyclase-A with reduced potency. *Clin Chem* 2011;**57**:1272-1278.

23. Bettencourt P, Azevedo A, Pimenta J, Friões F, Ferreira S, Ferreira A. N-terminal-pro-brain natriuretic peptide predicts outcome after hospital discharge in heart patients. *Circulation* 2004; **110**:2168-2174.

24. Latini R, Masson S, Wong M, Barlera S, Carretta E, Staszewsky L, Vago T, Maggioni AP, Anand IS, Tan LB, Tognoni G, Cohn JN; Val-HeFT Investigators. Incremental prognostic value of changes in B-type natriuretic peptide in heart failure. *Am J Med* 2006;**119**:70. e23-e30.

25. Masson S, Latini R, Anand IS, Barlera S, Angelici L, Vago T, Tognoni G, Cohn JN; Val-HeFT Investigators. Prognostic value of changes in N-terminal pro-brain natriuretic peptide in Val-HeFT (Valsartan Heart Failure Trial). *J Am Coll Cardiol* 2008;**52**:997-1003.

26. Troughton R, Felker MG, Januzzi JL Jr. Natriuretic peptide-guided heart failure management. *Eur Heart J* 2014;**35**:16-24.

27. Packer M, McMurray JJ, Desai AS, Gong J, Lefkowitz MP, Rizkala AR, Rouleau JL, Shi VC, Solomon SD, Swedberg K, Zile M, Andersen K, Arango JL, Arnold JM, Bĕlohlávek J, Böhm M, Boytsov S, Burgess LJ, Cabrera W, Calvo C, Chen CH, Dukat A, Duarte YC, Erglis A, Fu M, Gomez E, Gonzàlez-Medina A, Hagège AA, Huang J, Katova T, Kiatchoosakun S, Kim KS, Kozan Ö, Llamas EB, Martinez F, Merkely B, Mendoza I, Mosterd A, Negrusz-Kawecka M, Peuhkurinen K, Ramires FJ, Refsgaard J, Rosenthal A, Senni M, Sibulo AS Jr, Silva-Cardoso J, Squire IB, Starling RC, Teerlink JR, Vanhaecke J, Vinereanu D, Wong RC; PARADIGM-HF Investigators and Coordinators. Angiotensin receptor neprilysin inhibition compared with enalapril on the risk of clinical progression in surviving patients with heart failure. *Circulation* 2015;**131**:54-61.

28. Solomon SD, Zile M, Pieske B, Voors A, Shah A, Kraigher-Krainer E, Shi V, Bransford T, Takeuchi M, Gong J, Lefkowitz M, Packer M, McMurray JJ; Prospective comparison of ARNI with ARB on Management Of heart failUre with preserved ejectioN fracTion (PARAMOUNT) Investigators. The angiotensin receptor neprilysin inhibitor LCZ696 in heart failure with preserved ejection fraction: a phase 2 double-blind randomised controlled trial. *Lancet* 2012; **380**:1387-1395.

29. McCormack. Sacubitril/Valsartan: a review in chronic heart failure with reduced ejection fraction, *Drugs* 2016; **76**:387-396.

30. Bayés-Genis A. Neprelysin in heart failure: from oblivion to center stage. *JACC Heart Fail* 2015;**3**:637-640.

31. Doust JA, Glasziou PP, Pietrzak E, Dobson AJ. A systematic review of the diagnostic accuracy of natriuretic peptides for heart failure. *Arch Intern Med* 2004;**164**:1978-1984.

32. Davenport C, Cheng EY, Kwok YT, Lai AH, Wakabayashi T, Hyde C, Connock M. Assessing the diagnostic test accuracy of natriuretic peptides and ECG in the diagnosis of left ventricular systolic dysfunction: a systematic review and meta-analysis. *Br J Gen Pract* 2006;**56**:48-56.

33. Clerico A, Fontana M, Zyw L, Passino C, Emdin M. Comparison of the diagnostic accuracy of brain natriuretic peptide (BNP) and the N-terminal part of the propeptide of bnp immunoassays in chronic and acute heart failure: a systematic review. *Clin Chem* 2007;**53**:813-822.

34. Mant J, Doust J, Roalfe A, Barton P, Cowie MR, Glasziou P, Mant D, McManus RJ, Holder R, Deeks J, Fletcher K, Qume M, Sohanpal S, Sanders S, Hobbs FD. Systematic review and individual patient data meta-analysis of diagnosis of heart failure, with modelling of implications of different diagnostic strategies in primary care. *Health Technol Assess* 2009;**13**:1-207, iii.

35. Mastandrea P. The diagnostic utility of brain natriuretic peptide in heart failure patients presenting with acute dyspnea: a meta-analysis. *Clin Chem Lab Med* 2013;**51**:1155-1165.

36. Roberts E, Ludman AJ, Dworzynski K, Al-Mohammad A, Cowie MR, McMurray JJ, Mant J; NICE Guideline Development Group for Acute Heart Failure. The diagnostic accuracy of the natriuretic peptides in heart failure: systematic review and diagnostic meta-analysis in the acute care setting. *BMJ* 2015;**350**:h910.

37. Martindale JL, Wakai A, Collins SP, Levy PD, Diercks D, Hiestand BC, Fermann GJ, deSouza I, Sinert R. Diagnosing acute heart failure in the emergency department: a systematic review and meta-analysis. *Acad Emerg Med*. 2016;**23**:223-242.

38. Swedberg K, Cleland J, Dargie H, Drexler H, Follath F, Komajda M, Tavazzi L, Smiseth OA, Gavazzi A, Haverich A, Hoes A, Jaarsma T, Korewicki J, Lévy S, Linde C, Lopez-Sendon JL, Nieminen MS, Piérard L, Remme WJ; Task Force for the Diagnosis and Treatment of Chronic Heart Failure of the European Society of Cardiology.

Guidelines for the diagnosis and treatment of chronic heart failure: executive summary (update 2005): The Task Force for the Diagnosis and Treatment of Chronic Heart Failure of the European Society of Cardiology. *Eur Heart J* 2005;**26**:1115-1140.

39. Nieminen MS, Böhm M, Cowie MR, Drexler H, Filippatos GS, Jondeau G, Hasin Y, Lopez-Sendon J, Mebazaa A, Metra M, Rhodes A, Swedberg K, Priori SG, Garcia MA, Blanc JJ, Budaj A, Cowie MR, Dean V, Deckers J, Burgos EF, Lekakis J, Lindahl B, Mazzotta G, Morais J, Oto A, Smiseth OA, Garcia MA, Dickstein K, Albuquerque A, Conthe P, Crespo-Leiro M, Ferrari R, Follath F, Gavazzi A, Janssens U, Komajda M, Morais J, Moreno R, Singer M, Singh S, Tendera M, Thygesen K; ESC Committe for Practice Guideline (CPG). Executive summary of the guidelines on the diagnosis and treatment of acute heart failure: the Task Force on Acute Heart Failure of the European Society of Cardiology. *Eur Heart J* 2005;**26**:384-416.

40. Hunt SA; American College of Cardiology; American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). ACC/AHA 2005 guideline update for the diagnosis and management of chronic heart failure in the adult: a report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Writing Committee to Update the 2001 Guidelines for the Evaluation and Management of Heart Failure). *J Am Coll Cardiol*. 2005;**46**:e1-e82.

41. Adams KF, Lindenfeld J, Arnold JMO, Baker DW, Barnard DH, Baughman KL. Executive summary: HFSA 2006 comprehensive heart failure practice guideline. *J Cardiac Fail* 2006;**12**:10-38.

42. Emdin M, Passino C, Prontera C, Fontana M, Poletti R, Gabutti A, Mammini C, Giannoni A, Zyw L, Zucchelli G, Clerico A. Comparison of Brain Natriuretic Peptide (BNP) and amino-terminal ProBNP for early diagnosis of heart failure. *Clin Chem* 2007;**53**:1289-1297.

43. Galvani M, Ferrini D, Ottani F. Natriuretic peptides for risk stratification of patients with acute coronary syndromes. *Eur J Heart Fail* 2004;**6**:327-333.

44. Balion C, Santaguida PL, Hill S, Worster A, McQueen M, Oremus M, McKelvie R, Booker L, Fagbemi J, Reichert S, Raina P. Testing for BNP and NT-proBNP in the diagnosis and prognosis of heart failure. *Evid Rep Technol Assess (Full Rep)* 2006;**142**:1-147.

45. Valle R, Aspromonte N, Milani L, Peacock FW, Maisel AS, Santini M, Ronco C. Optimizing fluid management in patients with acute decompensated heart failure (ADHF): the emerging role of combined measurement of body hydration status and brain natriuretic peptide (BNP) levels. *Heart Fail Rev*. 2011;**16**:519-529.

46. Felker GM, Hasselblad V, Hernandez AF, O'Connor CM. Biomarker-guided therapy in chronic heart failure: a meta-analysis of randomized controlled trials. *Am Heart J* 2009;**158**:422-430.

47. Porapakkham P, Porapakkham P, Zimmet H, Billah B, Krum H. B-type natriuretic peptide-guided heart failure therapy: A meta-analysis. *Arch Intern Med* 2010;**170**:507-514.

48. Lam LL, Cameron PA, Schneider HG, Abramson MJ, Müller C, Krum H. Meta-analysis: effect of B-type natriuretic peptide testing on clinical outcomes in patients with acute dyspnea in the emergency setting. *Ann Intern Med* 2010;**153**:728-735.

49. Wessler BS, Kramer DG, Kelly JL, Trikalinos TA, Kent DM, Konstam MA, Udelson JE. Drug and device effects on peak oxygen consumption, 6-minute walk distance, and natriuretic peptides as predictors of therapeutic effects on mortality in patients with heart failure and reduced ejection fraction. *Circ Heart Fail* 2011;**4**:578-588.

50. Smart NA, Meyer T, Butterfield JA, Faddy SC, Passino C, Malfatto G, Jonsdottir S, Sarullo F, Wisloff U, Vigorito C, Giallauria F. Individual patient meta-analysis of exercise training effects on systemic brain natriuretic peptide expression in heart failure. *Eur J Prev Cardiol* 2012;**19**:428-435.

51. Li P, Luo Y, Chen YM. B-type natriuretic peptide-guided chronic heart failure therapy: a meta-analysis of 11 randomised controlled trials. *Heart Lung Circ* 2013;**22**:852-860.

52. Savarese G, Trimarco B, Dellegrottaglie S, Prastaro M, Gambardella F, Rengo G, Leosco D, Perrone-Filardi P. Natriuretic peptide-guided therapy in chronic heart failure: a meta-analysis of 2,686 patients in 12 randomized trials. *PLoS One* 2013;**8**:e58287.

53. Balion C, McKelvie R, Don-Wauchope AC, Santaguida PL, Oremus M, Keshavarz H, Hill SA, Booth RA, Ali U, Brown JA, Bustamam A, Sohel N, Raina P. B-type natriuretic peptide-guided therapy: a systematic review. *Heart Fail Rev* 2014;**19**:553-564.

54. Savarese G, Musella F, D'Amore C, Vassallo E, Losco T, Gambardella F, Cecere M, Petraglia L, Pagano G, Fimiani L, Rengo G, Leosco D, Trimarco B, Perrone-Filardi P. Changes of natriuretic peptides predict hospital admissions in patients with chronic heart failure: a meta-analysis. *JACC Heart Fail* 2014;**2**:148-158.

55. De Vecchis R, Esposito C, Di Biase G, Ariano C, Giasi A, Cioppa C. B-type natriuretic peptide-guided versus symptom-guided therapy in outpatients with chronic heart failure: a systematic review with meta-analysis. *J Cardiovasc Med (Hagerstown)* 2014;**15**:122-134.

56. Xin W, Lin Z, Mi S. Does B-type natriuretic peptide-guided therapy improve outcomes in patients with chronic heart failure? A systematic review and meta-analysis of randomized controlled trials. *Heart Fail Rev* 2015;**20**:69-80.

57. Anand IS, Fisher LD, Chiang YT, Latini R, Masson S, Maggioni AP, Glazer RD, Tognoni G, Cohn JN; Val-HeFT Investigators. Changes in brain natriuretic peptide and norepinephrine over time and mortality and morbidity in the Valsartan Heart Failure Trial (Val-HeFT). *Circulation* 2003;**107**:1278-1283.

58. Frantz RP, Olson LJ, Grill D, Moualla SK, Nelson SM, Nobrega TP, Hanna RD, Backes RJ, Mookadam F, Heublein D, Bailey KR, Burnett JC. Carvedilol therapy is associated with a sustained decline in brain natriuretic peptide levels in patients with congestive heart failure. *Am Heart J* 2005;**149**:541-547.

59. Tsutamoto T, Wada A, Maeda K, Mabuchi N, Hayashi M, Tsutsui T, Ohnishi M, Sawaki M, Fujii M, Matsumoto T, Matsui T, Kinoshita M. Effect of spironolactone on plasma brain natriuretic peptide and left ventricular remodeling in patients with congestive heart failure. *J Am Coll Cardiol* 2001;**37**:1228-33.

60. Fruhwald FM, Fahrleitner-Pammer A, Berger R, Leyva F, Freemantle N, Erdmann E, Gras D, Kappenberger L, Tavazzi L, Daubert JC, Cleland JG. Early and sustained effects of cardiac resynchronization therapy on N-terminal pro- B-type natriuretic peptide in patients with moderate to severe heart failure and cardiac dyssynchrony. *Eur Heart J* 2007;**28**:1592-1597.

61. Latini R, Masson S, Anand IS, Missov E, Carlson M, Vago T, Angelici L, Barlera S, Parrinello G, Maggioni AP, Tognoni G, Cohn JN; Val-HeFT Investigators. Prognostic value of very low plasma concentrations of troponin T in patients with stable heart failure. *Circulation* 2007;**116**:1242-1249.

62. Egstrup M, Schou M, Tuxen CD, Kistorp CN, Hildebrandt PR, Gustafsson F, Faber J, Goetze JP, Gustafsson I. Prediction of outcome by highly sensitive troponin T in outpatients with chronic systolic left ventricular heart failure. *Am J Cardiol* 2012;**110**:552-557.

63. Grodin JL, Neale S, Wu Y, Hazen SL, Tang WH. Prognostic comparison of different sensitivity cardiac troponin assays in stable heart failure. *Am J Med* 2015;**128**:276-282.

64. de Antonio M, Lupón J, Galán A, Vila J, Zamora E, Urrutia A, Díez C, Coll R, Altimir S, Bayes-Genis A. Head-to-head comparison of high-sensitivity troponin T and sensitive-contemporary troponin I regarding heart failure risk stratification. *Clin Chim Acta* 2013;**426**:18-24.

65. Horwich TB, Patel J, MacLellan WR, Fonarow GC. Cardiac troponin I is associated with impaired hemodynamics, progressive left ventricular dysfunction, and increased mortality rates in advanced heart failure. *Circulation* 2003;**108**:833-838.

66. Sandoval Y, Apple FS. The global need to define normality: the 99th percentile value of cardiac troponin. *Clin Chem*. 2014;**60**:455-462.

67. Clerico A, Zaninotto M, Ripoli A, Masotti S, Prontera C, Passino C and Plebani M, on the behalf of the Study Group on Cardiovascular Risk Biomarkers of the Italian Society of Clinical Biochemistry (SIBioC). The 99th percentile of reference population for cTnI and cTnT assay: methodology, pathophysiology, and clinical implications. *Clin Chem Lab Med* 2017; doi: 10.1515/cclm-2016-0933.

68. Koerbin G, Potter JM, Abhayaratna WP, Telford RD, Badrick T, Apple FS, Jaffe AS, Hickman PE. Longitudinal studies of cardiac troponin I in a large cohort of healthy children. *Clin Chem*. 2012;**58**:1665-1672.

69. Caselli C, Cangemi G, Masotti S, Ragusa R, Gennai I, Del Ry S, Prontera C, Clerico A. Plasma cardiac troponin I concentrations in healthy neonates, children and adolescents measured with a high sensitive immunoassay method: high sensitive troponin I in pediatric age. *Clin Chim Acta* 2016;**458**:68-71.

70. Toyota N, Shimada Y. Isoforms of troponin during regeneration of chicken skeletal muscle fibers after cold injury. *Cell Tissue Res* 1984;**236**:549-54.

71. Saggin L, Gorza L, Ausoni S, Schiaffino S. Cardiac troponin T indeveloping, regenerating and denervated rat skeletal muscle. *Development* 1990;**110**:547-54.

72. Bodor GS, Survant L, Voss EM, Smith S, Porterfield D, Apple FS. Cardiac troponin T composition in normal and regenerating human skeletal muscle. *Clin Chem* 1997;**43**:476-84.

73. Ricchiuti V, Apple FS. RNA expression of cardiac troponin T isoforms in diseased human skeletal muscle. *Clin Chem* 1999;**45**:2129-2135.

74. Jaffe AS, Vasile VC, Milone M, Saenger AK, Olson KN, Apple FS. Diseased skeletal muscle: a noncardiac source of increased circulating concentrations of cardiac troponin T. *J Am Coll Cardiol*. 2011;**58**:1819-1824.

75. Rittoo D, Jones A, Lecky B, Neithercut D. Elevation of cardiac troponin T, but not cardiac troponin I, in patients with neuromuscular diseases: implications for the diagnosis of myocardial infarction. *J Am Coll Cardiol* 2014;**63**:2411-2420.

76. Sato Y, Yamada T, Taniguchi R, Nagai K, Makiyama T, Okada H, Kataoka K, Ito H, Matsumori A, Sasayama S, Takatsu Y. Persistently increased serum concentrations of cardiac troponin t in patients with idiopathic dilated cardiomyopathy are predictive of adverse outcomes. *Circulation* 2001;**103**:369-74.

77. Setsuta K, Seino Y, Takahashi N, Ogawa T, Sasaki K, Harada A, Takano T, Kishida H, Hayakawa H. Clinical significance of elevated levels of cardiac troponin T in patients with chronic heart failure. *Am J Cardiol* 1999;**84**:608-11, A9.

78. Hudson MP, O'Connor CM, Gattis WA, Tasissa G, Hasselblad V, Holleman CM, Gaulden LH, Sedor F, Ohman EM. Implications of elevated cardiac troponin T in ambulatory patients with heart failure: a prospective analysis. *Am Heart J* 2004;**147**:546-552.

79. Fonarow GC, Peacock WF, Horwich TB, Phillips CO, Givertz MM, Lopatin M, Wynne J; ADHERE Scientific Advisory Committee and Investigators. Usefulness of B-type natriuretic peptide and cardiac troponin levels to predict in-hospital mortality from ADHERE. *Am J Cardiol* 2008;**101**:231-237.

80. Peacock WF 4th, De Marco T, Fonarow GC, Diercks D, Wynne J, Apple FS, Wu AH; ADHERE Investigators. Cardiac troponin and outcome in acute heart failure. *N Engl J Med* 2008;**358**:2117-2126.

81. Ilva T, Lassus J, Siirilä-Waris K, Melin J, Peuhkurinen K, Pulkki K, Nieminen MS, Mustonen H, Porela P, Harjola VP. Clinical significance of cardiac troponins I and T in acute heart failure. *Eur J Heart Fail* 2008;**10**:772-779.

82. Missov E, Calzolari C, Pau B. Circulating cardiac troponin I in severecongestive heart failure. *Circulation* 1997;**96**:2953-2958.

83. Ather S, Hira RS, Shenoy M, Fatemi O, Deswal A, Aguilar D, Ramasubbu K, Bolos M, Chan W, Bozkurt B. Recurrent low-level troponin I elevation is a worse prognostic indicator than occasional injury pattern in patients hospitalized with heart failure. *Int J Cardiol*. 2011;**166**:394-398.

84. Januzzi JL Jr, Filippatos G, Nieminen M, Gheorghiade M. Troponin elevation in patients with heart failure: on behalf of the third Universal Definition of Myocardial Infarction Global Task Force: Heart Failure Section. *Eur Heart J* 2012;**33**:2265-2271.

85. Fan D, Takawale A, Lee J, Kassiri Z. Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease. *Fibrogenesis Tissue Repair* 2012;**5**:15.

86. Passino C, Barison A, Vergaro G, Gabutti A, Borrelli C, Emdin M, Clerico A. Markers of fibrosis, inflammation, and remodelling pathways in heart failure. *Clin Chim Acta* 2015;**443**:29-38.

87. Chen YS, Gi WT, Liao TY, Lee MT, Lee SH, Hsu WT, Chang SS, Lee CC. Using the galectin-3 test to predict mortality in heart failure patients: a systematic review and meta-analysis. *Biomark Med* 2016;**10**:329-342.

88. Chen A, Hou W, Zhang Y, Chen Y, He B. Prognostic value of serum galectin-3 in patients with heart failure: a meta-analysis. *Int J Cardiol* 2015;**182**:168-170.

89. Huang DH, Sun H, Shi JP. Diagnostic value of Soluble Suppression of Tumorigenicity-2 for heart failure. *Chin Med J (Engl)* 2016;**129**:570-577.

90. Vittorini S, Clerico A. Cardiovascular biomarkers: increasing impact of laboratory medicine in cardiology practice. *Clin Chem Lab Med* 2008;**46**:748-763.

91. Hlatky MA, Greenland P, Arnett DK, Ballantyne CM, Criqui MH, Elkind MS, Go AS, Harrell FE Jr, Hong Y, Howard BV, Howard VJ, Hsue PY, Kramer CM, McConnell JP, Normand SL, O'Donnell CJ, Smith SC Jr, Wilson PW; American Heart Association Expert Panel on Subclinical Atherosclerotic Diseases and Emerging Risk Factors and the Stroke Council. Criteria for evaluation of novel markers of cardiovascular risk: a scientific statement from the American Heart Association. *Circulation* 2009;**119**:2408-2416.

92. Wang TJ. Assessing the role of circulating, genetic, and imaging biomarkers in cardiovascular risk prediction. *Circulation* 2011;**123**:551-565.

93. Jaffe AS, Januzzi JL Jr. Using biomarkers to guide heart failure. *Clin Chem* 2017, doi:10.1373/clinchem.2016. 266106.

94. Gaggin HK, Szymonifka J, Bhardwaj A, Belcher A, De Berardinis B, Motiwala S, Wang TJ, Januzzi JL Jr. Head-to-head comparison of serial soluble ST2, growth differentiation factor-15, and highly-sensitive troponin T measurements in patients with chronic heart failure. *JACC Heart Fail* 2014;**2**:65-72.

95. Mueller T, Leitner I, Egger M, Haltmayer M, Dieplinger B. Association of the biomarkers soluble ST2, galectin-3 and growth-differentiation factor-15 with heart failure and other non-cardiac diseases. *Clin Chim Acta* 2015;**445**:155-160.

96. Cotter G, Voors AA, Prescott MF, Felker GM, Filippatos G, Greenberg BH, Pang PS, Ponikowski P, Milo O, Hua TA, Qian M, Severin TM, Teerlink JR, Metra M, Davison BA. Growth differentiation factor 15 (GDF-15) in patients admitted for acute heart failure: results from the RELAX-AHF study. *Eur J Heart Fail* 2015;**17**:1133-1143.

97. Vizzardi E, D'Aloia A, Pezzali N, Bugatti S, Curnis A, Dei Cas L. Long-term prognostic value of CA 125 serum levels in mild to moderate heart failure patients. *J Card Fail* 2012;**18**:68-73.

98. Zhuang J, Faggiano P, Li Q, Pradelli D, Med V, Peng W, Zuo M, Xu Y. Insights into the clinical implications of carbohydrate antigen 125 as a biomarker of heart failure: a meta-analysis and systematic review of published studies. *J Cardiovasc Med (Hagerstown)* 2014;**15**:864-872.

99. Núñez J, Rabinovich GA, Sandino J, Mainar L, Palau P, Santas E, Villanueva MP, Núñez E, Bodí V, Chorro FJ, Miñana G, Sanchis J. Prognostic value of the interaction between galectin-3 and antigen carbohydrate 125 in acute heart failure. *PLoS One* 2015;**10**:e0122360.

100. Masson S, Latini R, Carbonieri E, Moretti L, Rossi MG, Ciricugno S, Milani V, Marchioli R, Struck J, Bergmann A, Maggioni AP, Tognoni G, Tavazzi L; GISSI-HF Investigators. The predictive value of stable precursor fragments of vasoactive peptides in patients with chronic heart failure: data from the GISSI-heart failure (GISSI-HF) trial. *Eur J Heart Fail* 2010;**12**:338-347.

101. Bahrmann P, Bahrmann A, Hofner B, Christ M, Achenbach S, Sieber CC, Bertsch T. Multiple biomarker strategy for improved diagnosis of acute heart failure in older patients presenting to the emergency department. *Eur Heart J Acute Cardiovasc Care* 2015;**4**:137-147.

102. Balling L, Kistorp C, Schou M, Egstrup M, Gustafsson I, Goetze JP, Hildebrandt P, Gustafsson F. Plasma copeptin levels and prediction of outcome in heart failure outpatients: relation to hyponatremia and loop diuretic doses. *J Card Fail* 2012;**18**:351-358.

103. Pozsonyi Z, Förhécz Z, Gombos T, Karádi I, Jánoskuti L, Prohászka Z. Copeptin (C-terminal pro arginine-vasopressin) is an independent long-term prognostic marker in heart failure with reduced ejection fraction. *Heart Lung Circ* 2015;**24**:359-367.

104. Xue Y, Taub P, Iqbal N, Fard A, Clopton P, Maisel A. Mid-region pro-adrenomedullin adds predictive value to clinical predictors and Framingham risk score for long-term mortality in stable outpatients with heart failure. *Eur J Heart Fail* 2013;**15**:1343-1349.

105. Bayés-Genís A, Barallat J, Galán A, de Antonio M, Domingo M, Zamora E, Urrutia A, Lupón J. Soluble neprilysin is predictive of cardiovascular death and heart failure hospitalization in heart failure patients. *J Am Coll Cardiol* 2015;**65**:657-665.

106. Perez MV, Pavlovic A, Shang C, Wheeler MT, Miller CL, Liu J, Dewey FE, Pan S, Thanaporn PK, Absher D, Brandimarto J, Salisbury H, Chan K, Mukherjee R, Konadhode RP, Myers RM, Sedehi D, Scammell TE, Quertermous T, Cappola T, Ashley EA. Systems Genomics Identifies a Key Role for Hypocretin/Orexin Receptor-2 in Human Heart Failure. *J Am Coll Cardiol* 2015;**66**:2522-2533.

107. Koch WJ, Cannavo A. Eating away at heart failure. *J Am Coll Cardiol* 2015;**66**:2534-2535.

108. Pan S, Cabral CS, Ashley EA, Perez MV. Orexin: a Missing Link Between Sleep Disorders and Heart Failure? *Curr Heart Fail Rep* 2017; doi: 10.1007/s11897-017-0322-3. [Epub ahead of print]

109. Rahimi K, Bennett D, Conrad N et al. Risk prediction in patients with heart failure. *JACC Heart Fail* 2014;**2**:440-446.

110. Ouwerkerk W, Voors AA, Zwinderman AH. Factors influencing the predictive power of models for predicting mortality and/or heart-failure hospitalization in patients with heart failure. *JACC Heart Fail* 2014;**2**:429-36.