Biomarkers in the clinical management of patients with atrial fibrillation and heart failure

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ABSTRACT   Atrial fibrillation (AF) and heart failure (HF) are two cardiovascular diseases with an increasing prevalence worldwide. These conditions share common pathophysiologiesand frequently co-exit. In fact, the occurrence of either condition can 'cause' the development of the other, creating a new patient group that demands different management strategies to that if they occur in isolation. Regardless of the temproal association of the two conditions, their presence is linked with adverse cardiovascular outcomes, increased rate of hospitalizations, and increased economic burden on healthcare systems. The use of low-cost, easily accessible and applicable biomarkers may hasten the correct diagnosis and the effective treatment of AF and HF. Both AF and HF effect multiple physiological pathways and thus a great number of biomarkers can be measured that potentially give the clinician important diagnostic and prognostic information. These will then guide patient centred therapeutic management. The current biomarkers that offer potential for guiding therapy, focus on the physiological pathways of miRNA, myocardial stretch and injury, oxidative stress, inflammation, fibrosis, coagulation and renal impairment. Each of these has different utility in current clininical practice.

Atrial fibrillation (AF) is the most common type of arrhythmia having an annual prevalence of 33 million patients worldwide, along with a three times higher prevalence in women than in men.[1] There are a number associated risk factors including heart failure, diabetes, hypertension, hyperthyroidism, obesity, structural and ischemic heart disease. However, up to 20% of AF cases cannot be connected with those factors.[2] The development of AF involves a complex interplay between genetic, molecular and environmental factors. Their better identificationcould alter the possible management and treatment of symptomatic and asymptomatic patients, incuding those that are yet diagnosed via the ECG.[3–5] Atrial fibrosis is likely play a key role in the development and prognsosi of AF. The extent of the fibrotic process can predict the response to the use of ablation as a treatment.[6–8] The fibrotic mechanism is not yet fully clarified, but according to some studies, the renin-angiotensin axis[9] and transforming growth factor (TGF) β1, play a key role in the cardiac fibrosis.[10]

Atrial fibrillation is linked with cardiovascular diseases, mortality, central nervous system side effects.[11] Most specifically, AF often precedes or follows the development of HF, both share pathophysiological paths that contribute to cardiac remodelling and the combined presence of the two conditions is connected with an adverse prognosis.[12]

Heart failure (HF) is a clinical syndrome presenting with typical symptoms (breathlessness on exertion, paroxysmal nocturnal dyspnea, orthopnea and fatigue) and signs (elevated jugular venous pressure, pulmonary oedema and peripheral oedema) as a result of a structural and/or functional cardiac abnormalities. These lead to a reduced cardiac output and/or elevated intracardiac pressures at rest or during stress, which result in many physiological changes, including multiple morphological, biochemical and molecular alterations referred to cardiac remodeling.[13,14] The current definition includes stages based on the symptoms observed in the patients requiring medical assistance, however prior to any clinical symptoms patients can present with

asymptomatic structural or functional cardiac abnormalities [systolic or diastolic left ventricular (LV) dysfunction]. The early recognition of these precursors can lead to better outcomes, in terms of both hospitalization and mortality in patients with HF. The prevalence of HF varies according to the definition used, but is approximately 1%–2% of the adult population in developed countries, rising to ≥ 10% among people > 70 years of age worldwide.[15−18] Among people > 65 years old presenting to primary care with breathlessness on exertion, one in six will have undiagnosed HF.[19,20] The lifetime possibility of developing HF at age 55 is 33% for men and 28% for women.[17] The pathophysiology of HF is mediated by a variety of biological mechanisms, with complex interactions between endothelial cells, monocytes, macrophages, cardiomyocytes, fibrocytes and the neuro-endocrine system. On top is the interplay with systemic conditions such as diabetes, advanced age, hypertension, obesity, dyslipidemia and chronic kidney disease. Cardiac troponins and natriuretic peptides are the most widely used diagnostic biomarkers in the management of HF,[21] although there are a number of novel ones are also available, but not widely used in clinical practice.

MICRO RNAS (miRNAS)

MicroRNAs (miRNAs), first reported in 1933 by Lee, et al.[22] are single stranded, non-protein-coding RNAs, of a total length of 22 nucleotides and they participate in the regulation of post-transcriptional gene expression and as a result they have an important role in cell growth, proliferation, differentiation and metabolism.[23,24] The change in miRNA expression has been associated with a variety of pathological conditions: neurological, autoimmune, cardiovascular diseases and malignancies. Modulation of MiRNAs' expression has been frequently reported in models studying circulation and cardiac muscles, as a major factor to cardiac remodeling and susceptibility to cardiovascular diseases.[25] They may act as a both potential prognostic and diagnostic biomarker, as well as a therapeutic target.[26]

Role of miRNA in the Development and Pathophysiology of AF

The levels of miRNA expression are genetically programmed and affect tissue developmental changes.[27] Changes in the regulation of miRNA expression level in the circulating blood and the tissues are associated with myocardial remodeling, which contributes to the development of cardiovascular disease and arrythmiogenic mechanisms.[28,29] Alterations in the miRNA expression are shown to affect the development of AF *in vivo*, inducing electrical, structural and autonomic nerve remodeling,[30−32] causing abnormal calcium handling,[33] and inflammation.[34] Single nucleotide polymorphisms (SNPs) in miRNA genes appear to both initiate and maintain AF.[35]

Electrical Remodeling

Electrical remodelling is perhaps the most common mechanism that results in the development of AF. It is a combination of a decrease in the conductance of L-type Ca2+ current (I_{CaL}) and an increase in the conductance of inward rectifier current (I_{K1}), along with a triggering effect that results in changes in the electrical properties of connexin 40 (Cx_{40}) connexin 43 (Cx_{43}) and ion channels.[30] There are several miRNAs that appear to be associated with electrical remodeling.

miRNA-1. The expression of miRNA-1 (miR-1) in cardiac muscles defines cardiac development and cardiac electrical activity. Abnormalities in the expression of miR-1 result in conditions such as cardiac arrhythmia, cardiac hypertrophy, myocyte proliferation and ischemic heart disease.[36] KCNE1 and KCNB2 are the target genes for miR-1, and it has been proposed that the downregulation of these potassium channel genes intensifies the duration and incidence of AF.[37] The levels of plasma miR-1 have been reported to be higher in the left atrial appendage (LAA) than that in the pulmonary vein.[38] It appears that the expression of miR-1 changes with the age,[39] with decreased levels of miR-1 seen in older patients with AF, compared to younger patients in sinus rhythm. This results in the upregulation of HCN2/HCN4 genes and the following increased conductance of I_{K1} results in slow cardiac conduction and increased risk of AF.[40] Additionally, the levels of miR-1 modulate cardiac electrical remodeling by decreasing intracellular calcium ions that eventually reduce the expression of CACNB2,[41] and the negative regulation of Ca2+ handling pro-

teins -calmodulin, protein phosphatase 2A (PP2A), Na^+/Ca^{2+} exchanger (NCX) and phospholamban. This shortens atrial refractoriness and thus promotes the development of AF.[42] Whereas, the inhibition of miR-1 targets the Bcl-2 gene and reduces the number of apoptotic cardiomyocytes.[43]

miRNA-328. A study revealed that the upregulation of miR-328 in circulating blood causes a reduction in the expression of CACNA1C and CACNB1 genes, the I_{CaL} reduces the L-type calcium channel activity and shortens the action potential duration (APD), leading to a higher prevalence of AF.[44,45] Higher expression of miR-328 were seen in the LAA compared to that in the peripheral and pulmonary vein blood of patients who had AF compared to the control group.[46]

miRNA-499. According to a comparative study on patients with permanent AF and patients with SR, the upregulation of miR-499 occurs due to the remodelling of L-type calcium currents (gene CACNB2)[47] and subsequently downregulates the expression of cardiac SK3 (small conductance calcium-activated potassium channel 3) affecting KCNN3 gene.[48]

Structural Remodeling

This pathophysiological mechanism includes the regulation of genes that are responsible for the eventual formation of extracellular matrix (ECM) and promote atrial fibrosis.[31] Eventually, these miRNAs contribute to the decrease of conduction velocity and increase of reentrant activity interval.[49]

miRNA-21. The upregulation of miR-21 in cardiomyocytes of patients with chronic AF results in downregulation of two voltage-gated calcium channel (VGCC) subunits, CACNA1C (1aC) and CACNB2 (b2) that leads to the reduction in I_{CaL}.[50] Moreover, in the rat model and in the left atrium of patients with AF decreased the expression of Sprouty-1 (SPRY1) target by increasing the mitogen-activated protein kinase/extracellular signal-regulated kinases (MAPK/ERK) signaling pathway, leading eventually to atrial remodeling and fibrosis.[51] Targeting the miR-21-related signaling pathways may be an interesting therapeutic approach of AF. *In vivo* administration of antagomir-21 (anti-miR- 21) silences the miR-21 activity and it could potentially reduce the prevalenceof AF.[52]

miRNA-29. This miRNA targets the COL1A1 (collagen-1A1), COL3A1 (collagen-3A1), and fibrillin genes.[53] The plasma level of miR-29b in patients with AF is reduced by 54% and in patients with both congestive heart failure (CHF) and AF by about 84%. Furthermore, the levels of miR-29b in the atria are reduced to 54% in patients that suffer from chronic AF compared to those with SR. In a mouse model the use of adenovirus in the downregulation of miR-29b resulted in an increase of the atrial COL1A1 mRNA expression and the collagen amount in the cardiac muscle. This observation suggests an association between the miRNA-29 and atrial fibrosis. This raises the possibility of it being used as a diagnostic and therapeutic agent.[54]

miRNA-126. miR-126 is widely expressed in the human heart and contributes to angiogenesis.[55] The levels are significantly lower in patients with AF with or without heart failure than seen in the control group. miRNA-126 blood levels may be an independent marker of disease severity in addition to N-terminal prohormone brain naturetric peptide (NT-proBNP).[56]

miRNA-150. Lower levels of miR-150 show a variable correlation with AF including atrial remodeling, inflammation, platelet function, platelet aggression, and fibrosis. In a prospective study, the plasma and atrial expression levels of miR-150 were calculated in patients with and without AF in patients undergoing cardiac ablation.[57] The plasma miR-150 level in patients with AF was two times lower than that in the control subjects, as well as lower has it been reported in patients with paroxysmal AF than in those with persistent AF. Moreover, the 3-times higher expression levels of miRNA-150 in the one month follow up tests of post AF-ablation patients suggested that higher levels could be beneficial. MiRNA maybe both useful as a diagnostic and therapeutic target.[58]

miRNA-483(-5p). The serum levels of this miRNA could be a possible biomarker for the early prediction of AF. Transcribed by the IGF2 gene, the upregulation of IGF2, induces an overexpression of miR-483 that regulates the pathways of pro-inflammatory mediators such as interleukin-6 (IL-6) and nuclear factor kappa-B (NF-kB). This biomarker is higher in pre-operative patients with AF, or even in those who undergo surgery, making this a potential biomarker.[59]

miRNA-155 and miRNA-24. An increase in both miR-155 and miR-24 are seen in the blood of both human and swine studies with AF, due to an amplification of the expression of endothelial nitric oxide synthase (eNOS)and nitric oxide (NO) production. The levels were significantly lower in AF post-ablation patients compared to those patients who have not undergone catheter ablation, also connected with a reduction in the NO level, associating them with the regulation of the eNOS signaling pathways.[60]

miRNA-409/miRNA-432. Both miR-409-3p and miR-432 are reported to be of lower levels in the plasma samples of patients with AF. They interact with various signaling molecules such as TGF-b signaling molecules, gap junction channels, ECM receptors, rennin-angiotensin system, and MAPK signaling molecules. The expression level of those two molecules varies among patients before and after ablation, which makes them possible biomarkers for AF.[61]

Autonomic nerve remodeling

The autonomic nervous sytem is known to play a role in the development and maintenance of AF. Vagal nerve stimulation and change in the acetylcholine release, results in a shortening of APD (action potential duration) contributing to the development of AF.[62]

miRNA-30. A dysfunction of the cardiac autonomic nervous system increases the G-protein gated potassium channel current (IKACh) accompanied by a shortening in APD.[63] An upregulation of miR-30d is downregulating the acetylcholine-dependent potassium current (IK+) in patients with persistent AF.[64]

miRNA-206. An increase in the expression levels of miR-206 targets the expression of SOD1, increasing the production of ROS (reactive oxygen species) and shortening the AERP.[65] In the canine model a downregulation of miR-206 by targeting the GCH1 is shown to enhance the autonomic nerve remodeling and prevent the expression of AERP via tetrahydrobiopterin (BH4) pathway, which leads to higher susceptibility of AF.[32]

Ca^{2+} handling abnormality

A dysregulation in cardiac miRNA expression affects the calcium handling and plays an interesting role in the pathophysiology of arrythmiogenic mechanisms.[66] Enhanced diastolic Ca2+ release from the sarcoplasmic reticulum via ryanodine receptor 2 (RYR2), as well as a high atrial rate during AF promotes AF by delayed after depolarization (DAD).[67]

miRNA-106b-25. The downregulation of miR-106b-25 results in the enchancement of the protein expression of RYR2, leading in an increase in Ca2+ release through RYR2, which amplifies vulnerability to the development of AF.[68]

miRNA-208. An uprgulation in miR-208b downregulates the protein expression of sarcoplasmic reticulum Ca2+ adenosine tripho-sphates type 2a (SERCA2a) in patients with AF compared to those with SR.[69]

Inflammatory mediators

The role of inflammatory mediators in pathological conditions has been studied thoroughly. The blood serum levels of biomarkers such as C-reactive protein (CRP), interleukins, Tumor Necrosis Factor α (TNF-a), Transforming Growth Factor-b (TGF-b), and Monocyte Chemoattractant Protein-1 (MCP-1) seem to be higher in patients with AF than in those with SR.[70] The levels of inflammatory mediators in the circulation can support the prediction of the prevalence of AF.[34]

miRNA-21. The upregulation of miR-21 induces AF through STAT3 phosphorylation or through inhibitin of TGF-b pathway and by downregulating Smad7, while in the animal model an inhibition in the expression of miR-21 prevents AF and atrial fibrosis.[71,72]

miRNA-150. An interestingly strong association has been described between miR-150 and CRP. Cytokines such as TNF-a, TGF-b, IL-6, and IL-18 produced by macrophages and monocytes in response to inflammation increases the plasma CRP production, which plays an important role in systemic inflammation. In patients with AF, the levels of CRP are higher than in those of the control group. The downregulation in the expression of miR-150 affects the genes that regulate the inflammation and in that way the susceptibility to AF is promoted.[73]

Single nucleotide polymorphisms (SNPs) of miRNA

SNP is the substitution of one nucleotide by another and is associated with phenotypic differences and genetically inherited diseases in humans. SNP

can be occur at any stage of miRNA gene expression, primary, precursor miRNA, target sites (30 UTR of mRNA), and mature miRNA, which affects the expression level and structure of miRNA and regulates the development of various diseases, including cardiovascular diseases and arrhythmias.[74–76] Atrial natriuretic peptide (ANP), cardiac ion channels (Ca^{2+}, K^+, Na^+), nucleoporins, and gap junction proteins genes include polymorphisms that are reported in patients with AF.[77] Moreover, polymorphisms in proteins that regulate the biogenesis of miRNA such as Drosha, DGCR8, Exportin-5, Ran-GTP, AGO2 and the miRNA-RISC complex proteins can regulate theexpression of the mature miRNA and function.[78]

miRNA-125: The rs12976445 SNP of miR-125a affects the normal functioning of mature miRNA by dysregulating the processing of pri-miRNA into pre-miRNA. MiR- 125 has a role in the development of AF by targeting the interleukin-6 receptor (IL-6R) gene, and its expression is reported to be downregulated post-catheter ablation, which enchances AF recurrence.[79]

miRNA-196: Polymorphisms in this molecule affects it's binding in the target mRNA. Patients with CC + TC genotype ("C" carrier) have a higher possibility of developing AF by up to 3-fold compared those with TT genotype.[80]

MicroRNAs as the biomarker for cardiovascular diseases

Heart- and muscle-specific circulating miRNAs (myomirs) were found to be 140-fold inceasedin advanced HF, similar to the increase seen in cardiac troponin I (cTnI) protein levels.[81] These circulating miRNA levels presented with significant reduction 3 months after the application of left ventricular assist device support.[82] In stable HF, there were < 5 fold differences in circulating miRNAs, whereas myomir and cTnI levels were at the detection limit, comparing with the control groups.[83] Additionally, significant changes in circulating muscle-specific miRNA, miR-133b, depict early myocardial injury following heart transplantation. According to these, miR-133b could be a better marker than cTnI in predicting transplanted heart dysfunction and recovery of those patients.[83]

Currently, no significant progress has been made in the suitable biomarkers concerning AF. MiRNAs

can be detected with high specificity and sensitivity in the serum and plasma, as well as in erythrocytes, nucleated blood cells, and platelets. Their levels in plasma can be very stable even in extreme conditions of processing (boiling, altered pH, high or low temperature, multiple freeze–thaw cycles and room temperature).[84,85] MiRNAs can be easily detected, bound to high-density lipoprotein (HDL) or incorporated with micro-vesicles, exosomes, and apoptotic bodies making them resistant to RNase activity. These qualifications make the cardiac-specific miRNAs attractive as possible prognostic, diagnostic and predictive biomarkers for several cardiac diseases: coronary artery disease, AF, acute myocardial infarction, hypertension, and heart failure.[86] The variable miRNA expression level observed in blood and in the left and right atria could indicate the severity and type of cardiac disease, as well as be used for the development of advanced miRNA-based therapies.[27,87] Hence, the mi-RNA expression should be carefully evaluated in correlation with all factors of the cardiac disease, by introducing a reliable, highly sensitive and specific methodology in order to be used as personalized medicine biomarker.

NATRIURETIC PEPTIDES

Natriuretic peptide (NP) levels are amongst the commonest biomarkers to be measured in clinical practice, and cardiovascular research worldwide. B-type natriuretic peptide (BNP) and N-terminal proBNP (NT-proBNP) are used widely in the diagnosis of heart failure (HF).[88–90] NP levels can also contribute to the diagnosis and management of acute coronary syndrome (ACS) [91,92] and AF.[93] Despite their wider use in the clinical care concerning HF and ACS, multiple studies report the utility of NPs in the management of AF.[94–96] In 2016, European Society of Cardiology (ESC) included the use of NPs as a complementary way to estimate the prevalence of strokeand bleeding in AF patients (class IIb recommendation with the level of evidence B).[13]

BNP and ANP: Biosynthesis and Biological Characterictics

Atria Natriuretic Peptide (ANP) and BNP are synthesized as pre-prohormones. The expression of

ANP is released byatrial wall stretch resulting from increased intravascular volume.[97,98] ANP is translated into prepro-ANP, further processed into pro-ANP, which is stored in intracellular granules in the atrial cells. The levels of ANP in the serum of healthy individuals are estimated to be 20 pg/mL, whereas it appears to be 10–100 fold higher in patients with HF.[99] The half-life of ANP is 2 min.[100] The clearance of ANP occurs in the lung (24%), liver (30%) and kidney (35%). In the kidney, there was a good association between creatine clearance and ANP clearance ($r = 0.58$, $P < 0.05$).[101]

BNP is stored in a small percentage in granules in the ventricles and secreted abundantly after stimulation.[90,102] The levels of BNP in the serum of healthy individuals are approximately 3.5 pg/mL and in patients with HF are estimated to be 100-fold higher.[103] The half-life of BNP is 20 min.[104] The peptide is processed into pro-BNP, then into the biologically active molecule BNP and the inactive NT-proBNP. BNP and NT-proBNPare expressed in equal concentrations, and the half-life of NT-proBNP is 120 min. BNP clearance occurs due to a neutral endopeptidase, and NT-proBNP clearance occurs in kidney.[105] Although in healthy individuals the BNP level is much lower than the ANP concentration, the BNP level is increased in patients with HF and the concentration depicts the severity of the disease, and eventually the BNP level is markedly higher than the ANP concentration.[104] ANP is variable and has ashort half-life, therefore BNP is used widely in the clinical practice in the management of HF.[106] Tsutamoto, et al.[107] reported that in the management of patients with chronic HF and with a left ventricular ejection fraction [EF] <45%, only the BNP level ($P < 0.0001$) Was associated with a significant independent association with mortality in patients with HF (by Cox proportional hazard

analysis), however the ANP level was not a significant marker.

ProANP is a polypeptide of 126 amino acids. ANP consists of amino acids 99−126 and the N-terminal portion of proANP (ProANP1-98 or NT-proANP), has a longer half-life than ANP and could be a reliable biomarker for clinical measurement. A fragment of proANP1-98 [mid-regional (MR) proANP (amino acids 53−90)] can be detected through immunoassay in order to measure the proANP level. In 325 healthy individuals, the range of MR-proANP was 9.6−313 pmol/L,[108] whereas the level is increased with age, decreased by a higher BMI, and associated with race and sex (Table 1).[109] In the BACH trial (Biomarkersin Acute Heart Failure), including 1 641 patients worldwide presenting to the ED with dyspnea, the levels of MR-proANP (≥ 120 pmol/L) provided a sensitivity of 97% as well as a negative predictive value of 97.4%.[110] MR-proANP also could be used as a prognostic marker in acute and chronic HF, even with a better prognostic value for mortality than the one that BNP had at five years.[111] In the GISSI-HF trial, 1 237 patients were studied with chronic and stable HF, MR-proANP andNT-pro BNP were measured randomly and after three months. Changes in MR-proANPlevelswere associated with mortality, and not those in NT-proBNP.[112] Therefore, MR-proANP may be useful as a screening tool in community populations. While NT-proBNP and MR-proANP predicted incident HF in the 14 months of follow-up, MR-proANP predicted incident AF.[113]

The use of NPs is specifically mentioned in current guidelines in the diagnosis and management of HF. The ESC guideline and the American Heart Association (ACCF/AHA) state that BNP and NT-proBNP levels are useful (Class I) as a diagnostic and prognostic biomarker or an indicator of the dis-

Table 1 [97-103, 108]. Physiologic characteristics of BNP, NT-proBNP, ANP, and MR-proANP with clinical relevance.

Physiologic Characteristic	BNP	NT-proBNP	ANP	MR-proANP
Cardiac tissue location	Atrial and ventricular	Atrial and ventricular	Atrial	Atrial
Storage levels	Very low levels	Very low levels	In intracellular granules	In intracellular granules
Gene expression in response to stretch	Abundant	Abundant	Slow	Slow
Half-life, min	20	60−120	2	60−120
Biological activity	yes	no	yes	No
Clinical level range	0−5 000 pg/mL	0−35,000 pg/mL	0−2000 pg/mL	0−1 000 pmol/L

ANP: atrial natriuretic peptide; BNP: B-type natriuretic peptide; MR-proANP: mid-regional proANP; NT-proBNP: N-terminal proBNP.

ease severity in chronic HF and acutely decompensated HF (Table 1).[114,115]

Clinical Utility

Screening Biomarker

BNP and NT-proBNP are used as mortality and cardiovascular disease prognostic markers in asymptomatic patients. McDonagh, *et al.*[116] studied the four-year all-cause mortality rate in a random sample of 1 640 men and women, in the age of 25–74. The median BNP in the patients who died was 16.9, in a range 8.8–27 pg/mL, whereas the median in the survivors was 7.8, while the level range was 3.4–13 pg/mL ($P < 0.000 1$). The BNP levels > 17.9 pg/mL ($P = 0.006$) was an independent marker offour-year all-cause mortality. Moreover, in the Framingham Offspring study, of 3 346 people without HF and an average follow-up of 5.2 years, the patients with BNP levels above the 80th percentile (20.0 pg/mL for men and 23.3 pg/mL for women) were presented with hazard ratios (HRs) of 1.62 for death, 1.76 for a first majorcardiovascular event, 1.91 for AF, 1.99 for stroke or transient ischemic attack, and 3.07 for HF. [117]

Diagnosis of HF

Since acute HF if often hard to distinguish in emergency department (ED), with no specific or sensitive symptoms, BNP and NT-proBNPcan be used in diagnosing or excluding the presence of acute HF. The Breathing Not Properly Multinational Study reported for the firsi time the association between BNP and 1 586 patients with acute dyspnea in the ED. The diagnostic accuracyof BNP was calculated to be 83.4% with the cut-off value being 100 pg/mL and the negative predictive value of BNP with a cut-off value of < 50 pg/mL was 96% (area under the curve (AUC) 0.91).[90] The N-terminal Pro-BNP investigation of dyspnea in the emergency department (PRIDE) study measured the NT-proBNP levels in 600 patients with dyspnea in the ED, the cut-off level was set at 300 pg/mL and the estimated results were 90% sensitivity and 85% specificity for the diagnosis of acute HF.[88] BNP is also a useful biomarker for the differential diagnosis between acute HF and acute respiratory deficiency syndrome (ARDS). In 80 ICU patients with acute hypoxemic respiratory failure, at a cut-off level of200 pg/mL, BNP showed a specificity of 91% for

ARDS, while at a cut-off level of 1 200 pg/mL, BNP was presented with aspecificity of 92% for cardiogenic pulmonary edema.[117] Considering the patients with chronic HF, the pathophysiologic mechanisms ofchronic HF are multifactorial and often also accompanied by underlying cardiac and noncardiac diseases. In such an occasion it is often no possible to determine one cut-off value in order to determine the diagnosis of chronic HF. Multiple studies present different cut-off values of BNP and NT-proBNP associated with satisfying percentages of sensitivity and specificity, upon however a variable background of not specific symptoms and different New York Heart Association (NYHA) functional classes. [89,118,119] In the ESC 2016 guideline [114] it is stated that the plasma levels of BNP and NT-proBNPin patients that suffer from HF in a non-acute phase could be used as an initial diagnostic test, distinguishing those who require further cardiac investigation. The cut-off level for BNP is set in 35 pg/mL and for NT-proBNP in 125 pg/mL. Patients with values below those levels, is less likely to be in the non acute phase of HF and they don't require echocardiography. The use of NPs alone cannot establish the diagnosis, especially in the non acute phase. AF, ACS, age, sex, race/ethnicity,[120,121] obesity and renal failure can harden the interpretation of NP measurements. [114] In the Framingham Heart Study, higher NP levels were observed in older age and female sex, while in obese (BMI > 35 kg/m^2) patients the levels were significantly lower than in the non-obese patients (205 ± 22 vs. 335 ± 39 pg/mL, $P = 0.000 7$).[122,123] In 1 103 ambulatory patients with acute dyspnea, the NT-proBNP levels in overweight and obese patients were lower than those in patients with normal BMI, regardless of the presence of acute HF ($P < 0.001$).[124] The reason for the lower BNP in obese patients is not yet clarified, however a cut-off level (< 50 pg/mL) should be used to distinguish HF.[125] Similarly, in a random sample of 2042 community residents > 44 years old, BNP was higher in age patients and in women compared with the levels in male patients.[126] In the Breathing Not Properly Multinational Study patients with acute dyspnea and an eGFR < 60 mL/min per 1.73 m^2, BNP was influenced by renal function and the equivalent levels for BNP were 70.7, 104.3, 201.2, and 225.0 pg/mL for the eGFR categories of < 90,

60−89, 30−59 and < 30 mL/min per 1.73 m^2, respectively.[127] As mentioned above, NT-proBNP clearance depends on direct renal filtration,[105] which makes it more susceptible to renal dysfunction.[128]

Although ventricular wall stretching is stimulating the BNP expression,[129] a LV diastolic wall stress presents an increased BNP, which can be used in the diagnosis between Heart Failure with preserved Ejection Fraction (HFpEF - EF > 50%) or Heart Failure with reduced Ejection Fraction (HFrEF - EF ≤ 50%). In 160 patients with HF, the BNP level was higher in those with HFrEFin comparison with those with HFpEF (267 (136−583) and 105 (64−146) pg/mL, $P < 0.001$).[130] In the Breathing Not Properly Multinational Study, 452 patients were diagnosed with congestive HF. In those with HFpEF (EF > 45%), BNP level was lower compared with the BNP level in those with HFrEF (413 vs. 821 pg/mL, $P < 0.001$).[131] Moreover, 1 670 patients from the Korean Heart Failure registry with HFpEF (EF ≥ 50%) had lowerNT-proBNP levels compared with the patients with HFrEF (median 2 723 vs. 5 644 pg/mL, $P < 0.001$).[132] The use of BNP level along with echocardiographic examination [pulsed-wave Doppler examination of the mitral flow (E/A)] could offer a better approach in the direction of diastolic dysfunction.[133]

Prognosis of HF

The prognostic value of the NPs in the management of HF seems to be of interest, especially the negative prognostic value predicting morbidity and mortality. According to the cut-off points set by the 2016 ESC guideline,[114] in the non-acute and acute setting the negative predictive values are very similar and high (0.94–0.98), while the positive predictive values are lowerin the non-acute setting (0.44–0.57) as well as in the acute setting (0.66–0.67).[134−136] In 452 patients in the ED with reduced EF (< 35%) and three-year follow-up, patients with BNP levels > 130 pg/mL had greater possibilities of sudden cardiac death.[137] The Rapid Emergency Department-Heart Failure Outpatient Trial (REDHOT) study on patients that presented in the ED with shortness of breath, BNP levels > 200 pg/mL were connected with significantly high prevalence of the 90-day combinedevent rate (HF hospitalisation and mortality).[138] In 599 patients presented in the ED withshortness of breath, the NT-proBNPcut-off levelfor

one-year mortalitywas 986 pg/mL and also the strongest one-year death predictor (HR = 2.88, 95% CI: 1.64–5.06, $P < 0.001$).[139]

NP-Guided Therapy

In a STARS-BNP trial including 220 patients with HF NYHA functional class II to III, the received treatment aimed at a goal of BNP levels of < 100 pg/mL for the patients in the BNP group. After the first three months, the mean dosages of angiotensin-converting enzyme inhibitors and beta-blockers werehigher in the BNP group ($P < 0.05$). After fifteen months of follow-up, patients in the BNP-guidedtreatment group had remarkably lower number HF-related events (death or readmission) thanthose treated according to current guidelines (24% vs. 52%, $P < 0.001$).[140] Similarly in the BATTLESCARRED trial, treatment strategies were applied for two years and a follow-up of three years. One-year mortality was less in NT-proBNP (9.1%) and clinically guided (9.1%) groups than the percentage in the usual caregroup (18.9%, $P = 0.03$). Three-year mortality was selectively reduced in patients ≤ 75 years of agereceiving NT-proBNP-guided treatment (15.5%) compared with the patients in the same age group receiving eitherclinicallymanaged treatment (30.9%, $P = 0.048$) or usual care (31.3%, $P = 0.021$).[141] In HFrEF patients, NT-pro or BNP-guided therapy compared with symptom-guided therapy had lower mortality rates (HR = 0.78, 95% CI: 0.62–0.97, $P = 0.03$) and fewer HF-related hospitalization (HR = 0.80, 95% CI: 0.67–0.97, $P = 0.02$). In HFpEF patients, however, renal failure provided the strongest interaction. Should renal failure be present, NT-pro BNP-guided therapy was associated with greater risk ($P < 0.01$), and seemed to be actually beneficial only if none or one of the comorbidities (chronic obstructive pulmonary disease, diabetes, cardiovascular insult, or peripheral vascular disease) was present ($P < 0.01$). Moreover, NT-pro BNP-guided therapy may be harmful in HFpEF patients without hypertension ($P = 0.02$).[142] In the TIME-CHF trial, NT-proBNP-guided therapy was associated with ahigher rate of survival and a lower rate of all-cause hospitalization in patients aged 60 to 70 years, but not in those older than 75 years, after 18 months of examination following the initial admission.[143] In conclusion, elderly and HFpEF patients may not be profited by NP

guided-therapy compared with symptom-guided medication.

The role of NPs in the management of AF

The stimulating effect for the production of ANP is mainly due to atrial dilatation, whereas BNP is produced in response to ventricular stretchand pressure overload and only a very small amount is produced in the atrial myocardium.[144,145] In that case some patients in AF without HF, while the ANP levels were normal those of BNP or NT-proBNP were elevated.[93,146] A dysregulation of atrial contraction causes a tethering effect of atrial myocardial fibers and an elevated atrial pressure stretches the atrial wall (pressure overload), resulting in altered left ventricular filling and elevated BNP levels.[147–149] BNP levels have been shown to decrease dramatically 24 h after the restoration of sinus rhythm (SR) by cardioversion in patients with AF (from 95 to 28 pg/mL inparoxysmal AF and from 75 to 41 pg/mL in persistent AF).[150,151] Bakowski, et al.,[152] investigated 42 patients with AF, with maintenance of SR restored by cardioversion for at least 30 days. The levels of ANP in AF patients with normal diastolic functionwere 167.3 ± 70.1 pg/mL and in those with impaired diastolic function were 298.7 ± 83.6 pg/mL ($P < 0.001$), and those of BNP were 49.5 ± 14.7 and 145.6 ± 49.6 pg/mL, respectively ($P < 0.001$). BNP was a significantly more specific and sensitive marker of impaired Left Ventricular (LV) diastolic function and more valuable in the diagnosis of AF than ANP.[152] In the community-based studies, Atherosclerosis risk in communities (ARIC), Cardiovascular Health Study (CHS), and Framingham Heart Study (FHS), BNP and CRP were positively associated with AF incidence.[153] In the Cardiovascular Health Study, in 5 445 older patients NT-proBNP levels were strongly associated with prevalent AF. However, the cut-off levels of both NPs remain unclear. In the Framingham cohort, the correlation of NT-proBNP with BNP was moderately high at 0.66, and with the presence of both peptides in the study BNP appeared as the strongest biomarker.[154] The structural heart disease between patients with SR and AF can also be indicated by the levels of NT-proBNP. In 793 patients NT-proBNP levels were 960 (IQR: 359–2 625) pg/mL for SR ($n = 591$) and 2 491 (1 443–4 368) pg/mL for AF ($n = 202$) ($P < 0.001$).[155]

NPs as a marker in AF recurrence after cardioversion or pulmonary vein isolation

Baseline BNP and NT-proBNP values seem to predict the recurrence of AF after cardioversion,[156–158] but the cut-off levels are variable among the studies. Solheim, et al.[159] reported that there was no significant differences in NT-proBNP levels (33.5 vs. 29.5 pmol/L, $P = 0.9$) between patients with AF recurrence and nonrecurrence after ablation, whereas after a long-term follow-up, the NT-proBNP level was significantly decreased at 22 ± 5 months after ablation in the cases where the ablation was successful (7.0 vs. 17.5 pmol/L, $P < 0.05$). NT-pro-BNP decreased levels of > 25% from the baseline value could be a useful indicator for the successful ablation. Meta-analysis of studies in electronical databases showed that increased baseline levels of BNP, NT-pro BNP levels and ANP are associated with higher risk AF recurrence after catheter ablation.[160,161] Deng, et al. [162] studied 1 410 patients undergoing AF ablation, with a follow-up of 20.7 ± 8.8 months. The levels of BNP in the group of AF recurrence were 237.45 pg/mL. Similar were the findings among the patients with paroxysmal or nonparoxysmal AF. Additionally, the baseline NT-proBNP level was a marker of independent prediction of AF recurrence ($P < 0.001$) after pulmonary vein isolation with a cut-off level of ≥ 423.2 pg/mL ($P = 0.002$).[146]

NPs in the management of stroke in AF patients

BNP and NT-proBNPcan be used as independent stroke marker predictors in AF patients. High NT-proBNP levels in AF patients receiving anticoagulation are connected with an increased risk of stroke.[163,164] The Apixaban for Reduction in Stroke and other Thromboembolic events in Atrial Fibrillation (ARISTOTLE) trial studying 18,201 patients with AF, co-estimating NT-proBNPlevels in the CHA2DS2-VASc score improved the C-statistic predictive value from 0.62 to 0.65 ($P = 0.000 9$) for stroke or systemic embolism and from 0.59 to 0.69 for cardiac death ($P < 0.000 1$).[165] The biomarker-based ABC stroke score was shown to be better calibrated and have a better predictive value than CHA2DS2-VASc or ATRIA scores in the further management of AF patients.[166,167]

ANP as a biomarker in AF management

BNP is more preferable in the management of AF, while ANP has a short life and is unstable to be

used widely in clinical practice. ANP is basically produced by atrial cardiomyocytes after atrial wall stretching. The MR-proANP is a more stable molecule and it may be more useful for the assessment of AF. In as study of 632 patients with acute dyspnea, the diagnostic accuracy of acute HF in patients with was similar for MR-proANP (HR = 0.90, 95% CI: 0.84–0.95) and NT-proBNP (HR = 0.89, 95% CI: 0.81–0.96). MR-proANP was significantly associated with one-year all-cause mortality (HR = 1.13 (1.09–1.17), per 100 pmol/L increase, $P < 0.001$).[168] However, in the AMIO-CAT trial among patients treated with ablation for AF, the levels of both MR-proANP and NT-proBNP in patients with persistent AF were higher at baseline than in those with paroxysmal AF. While the NT-proBNP level wa connected with AF/AT recurrence within the three-month blanking period after ablation (HR = 1.84, 95% CI: 1.06–3.19, $P = 0.030$), the MR-proANP level was not (HR = 2.87, 95% CI: 0.86–9.50, $P = 0.085$). No significant connection between the baseline levels of MR-proANP and NT-proBNP has been reported with the recurrence of AF at six months after ablation and the utlity of either biomarker is not yet fully clarified.[169] In the atrial wall that has significant fibrosis, ANP production is reduced.[170] A long term AF leads to elimination of cardiomyocytes and their replacement with fibrous tissue.[171,172] In patients that undergo the mazeprocedure, the histological study revealed that preoperative ANP as well as the mRNA level was significantly lower in the AF patients than in the SR patients, whereas the collagen level was higher in the AF group.[173] Yoshida, et al.[174] stated that in patients that suffered from persistent AF and were treated with ablation n an large LA, the success in the reduction of LA volume after ablation was significant in patients with ahigher postoperative ANP level (73 vs. 50 pg/mL, $P = 0.02$), indicating an association between healthy atrial myocardium and preserved ANP secretion. 175. Ogawa, et al.[175] alsoproposed the original index ANP/BNP ratio, which may be moresensitive to the severity of a heart condition and the existence of healthy atrial myocardium than ANP or BNP alone. Patients with less severe HF (lower BNP) andpreserved healthy atrial tissue (higher ANP) have a higher ANP/BNP ratio than those in a worse condition. However, this ratio needs careful validation and calibration, and it has to be studied in association with atrial remodeling in patients with HF and AF.

NPs in the clinical management of AF and HF: coexisting conditions and useful biomarker

Both AF and HF amplify BNP and NT-proBNP levels, but these levels and determine the incidence of HF in patients with AF. In the PRIDE study, 600 dyspneic patientsin the EDhad higher NT-proBNP levels, especially those without acute HF.[176] In the BASEL study of 452 patients with AF and dyspnea, if BNP was < 100 pg/mL, HF was considered unlikely, whereas if BNP was > 500 pg/mL, HF was considered likely. BNP-based management significantly reduced time to discharge (median 8 days in the BNP group vs. 12 days in the control group, $P = 0.046$) and the initiation of sufficient therapy (median 51 min in the BNP group vs. 100 min in the control group, $P = 0.024$).[95] In patients with both conditions the levels that should be used are estimated to be higher. The BACH study on 1 445 patients presented with acute dyspnea, showed that the diagnostic value of BNP and NT-proBNP for acute HF was ineffective due to the presence of AF [177] in a group of 1 431 patients without HF, permanent/paroxysmal AF was reported to be distinguished by higher BNP levels ($P = 0.001$), with a cut-off level of 200 pg/mL that provided specificity and good prognostic value in the diagnosis of HF compared with the conventionally used level of 100 pg/mL,with little loss of sensitivity.[178] On the contrary, BNP cut off levels of100 pg/mL between patients with and without AF had a specificity of 40% and 79% for the diagnosis of acute HF respectively ($P = 0.533$). Another study shows that the BNP level for HF and AF patientsthat was accompanied with significant sensitivity is 150 pg/mL.[179] Additionally, in 1941 elderly community-dwelling residents, NT-proBNP levels of patients with AF were 744 pg/mL in the group with HF and 211 pg/mL in the group without HF.[180] NT-proBNPis associated with negative prognostic value of cardiovascular events, irrespective of AF status. In a large trial of 14,737 patients with HFrEF, NT-proBNP was was used as a marker of cardiovascular death or hospitalization for HF with and without AF. However, when the NT-proBNP level was > 400 pg/mL, the predictive value for adverse cardiovascular events was similar for both AF and SR patients.[181] An association of the cut-off levels of BNP with the clinical conditions presented shows that: (1) 17.9 g/mL is associ-

ated with mortality in asymptomatic patients; (2) 35 pg/mL with a diagnosis of chronic HF, 80 pg/mL wth mortality in ACS; (3) 100 pg/mL with a diagnosis of acute HF; and (4) 150 pg/mL withacute HF with AF.[88−90,116,179,182,183] The complicated pathophysiologic mechanism connecting AF and HF reflects the utility of NPs in the diagnosis and management of the two conditions, when they are coexisting or even the incidence of either when the other is present. The multiple factors that affect the prevalence of each condition mark the importance of correct validation of a biomarker-based combined management. [184]

OXIDATIVE STRESS RELATED BIOMARKERS

Cell interaction, neuro-endocrine system activation and the presence of compounding systemic diseases (diabetes, advanced age, hypertension, obesity, dyslipidemia and renal dysfunction) induce mechanisms of structural and functional remodeling, increasing oxidative stress through reactive oxygen species (ROS), leading to vascular endothelial damage, LV hypertrophy and heart interstitial fibrosis.[185,186] The biochemical events that characterize the onset of HF can be related to three markers indicative of increased level of ROS and possibly useful tools in the clinical management of HF: galectin-3 (GAL-3), α1-antitrypsin (AAT) and lectin-like oxidized low-density- lipoprotein receptor-1 (LOX-1).[187]

ROS is produced in themitochondria of the cardiomyocytes, by an increased activity of NADPH oxidases due to a pathological condition leading to the increase in angiotensin II (Ang II), endothelin-1 and TNF-α,[188,189] xanthine oxidase (higher production in patients with HF)[190] and nitric oxide synthase (NOS), as it is reported that in the damaged heart tissue, NOS becomes structurally unstable, uncoupled and further increased. The oxidants affect subcellular organelles such as the sarcoplasmic reticulum, mitochondria and the nucleus, inducing modifications in the regulation of cardiomyocyte Ca^{2+} homeostasis.[191,192] The contraction is mainly regulated by Ca^{2+} from the type two ryanodine receptors (RyR2) of the sarcoplasmic reticulum and several studies have shown that HF is character-

ized by increased RyR2 activity and diastolic SR Ca^{2+} leak, resulting in arrhythmias and contraction dysfunction.[193−195] Oxidative damage can deregulate the electron transport chain in mitochondria leading to a bio-energetic dysfunction, reduction of ATP production and further accumulation of ROS.[196] There are reports of mitochondrial dysfunction in dilated cardiomyopathy and HF.[197,198] The main antioxidant components [catalase, superoxide dismutase (SOD), glutathione peroxidase (GPx), nicotinamide adenine dinucleotide (NAD+) and glutathione (GSH)] have been shown by some studies to be reduced in HF.[199,200] In HF, increased oxidative stress and the massive production of ROS lead to cardiac fibrosis.[186,201]

Galectin-3 (GAL-3)

GAL-3 is a beta galactoside binding lectin, and is associated with myocardial infarction and fibrosis in HF.[202] It is found basically in the cytoplasm of various types of cell, can easily enter the nucleus and the mitochondria and be exerted in the extracellular space.[203] Intracellular it reacts with anti-apoptotic factors such as Bcl-2,[204] while in the extracellular space regulates cellular adhesion. According to some authors it is involved in pathophysiological paths such as organogenesis, immune system interactions and tumor growth,[205−207] whereas several clinical and experimental studies showed that up-regulation of GAL-3 was associated with HF, AF, dilated cardiomyopathy, fibrogenesis and mortality, implicating GAL-3 as a biomarker of heart disease.[208−213] GAL-3 has been shown to be a significant predictive marker at 18 months if risk factors, such as diabetes and renal insufficiency are present.[214] Additionally, low blood levels (< 11.8 ng/mL) of GAL-3 is a good prognostic factor of the absence of mortality and re-hospitalization at 6 months, compared to higher blood levels (> 17.8 ng/mL) where the risk of readmission is shown to be approximately 2–3 times higher.[215,216] An interesting observation is that a genetic deficiency of GAL-3 or use of its inhibitors (e.g., citrus pectin) reduces cardiac fibrosis and inflammation.[217] In experimental hyperaldosteronism, the increase in GAL-3 expression was connected with cardiac and renal fibrosis, conditions that were prevented by pharmacological inhibition (modified citrus pectin) or GAL-3 gene si-

lencing.[218] Additionally, to this last observation Yu, *et al.*[219] showed the prevention of cardiac remodeling by interfering with myocardial fibrogenesis. However, the facts in this subject are controversial, as other studies in mouse and murine models have not shown the connection between GAL-3 and remodeling and cardiac disfuction.[211,220] There seems also to be a difference between the GAL-3 concentration in endomyocardial biopsies from HF patients and the serum levels,[209] and an unexpected high blood level in patients after heart transplantation, indicating that the cardiac levels may differ from the ones in plasma due to the GAL-3 production by other organs.[221] Another hypothesis is based on the role of GAL-3 after ischaemia reperfusion injury (IRI), where the perturbation of mitochondrial homeostasis and subsequent formation of ROS[222] can affect the early stages of cell death and be a protective mechanism. Overall, those contrasting reduce the value of this molecule as a diagnostic mark of a possible drug target.[223–226]

GAL-3 appears to be marker of atrial fibrosis, with higher levels in patients with more extensive fibrosis.[227] Despite the fibrotic role of GAL-3, the association with AF has not been sufficiently supported by studies. It is reported that there is a connection between higher levels of GAL-3 and increased risk of developing AF in the next 10 years in the age- and sex-adjusted analysis, however that association was not significant after the adjustment on clinical risk factors, while the use of this marker alone is not specific for the cardiovascular system as it can indicate a fibrotic process locating elsewhere.[228] A study in 160 consecutive AF patients (persistent and paroxysmal) treated with pulmonary vein ablation and with a follow-up of 12 months, measured the role of GAL-3 in the recurrence of AF after ablation. Higher levels of GAL-3(≥ 15ng/mL) and larger left atrial diameters (LAD) (≥ 40 ms) independently predict atrial remodeling, recurrences of arrhythmia, whatever the type of AF is, and when combined identify patients at low, intermediate and high risk.[229] Another study of 75 patients with persistent AF with an LVEF ≤ 40%, evaluated the predictive value of GAL-3 in the response after ablation. Higher baseline level of GAL-3 represented the non-responders to ablation, in other words patients at higher risk with arrhythmia recurrence and

worse prognosis. GAL-3 < 26 ng/mL (*n* = 62, 83%) was linked with lower risk of cardiac death, heart transplantation and/or hospitalization for heart failure.[230]

GAL-3 is related with structural heart disease and left atrial remodeling and could be a promising biomarker in the diagnosis of AF-induced cardiomyopathy.[212,231] Galectin-3 may indicate high-risk patients with severe HF, atrial disease, or even both. In the pre- mentioned study patients with GAL-3 ≥ 17 ng/mL had a worse prognosis of LV dysfunction after ablation (20 patients, 51%). Patients with even higher levels (≥ 28 ng/mL) had a risk of cardiac death in the next 6 months (four patients, 33%), or hospitalization in the first year for HF complications (seven patients, 58%). The Catheter Ablation versus standard Conventional Therapy in Patients with LV dysfunction and Atrial Fibrillation (CASTLE-AF) study describes the beneficial effects of ablation upon the cardiovascular events.[232] Dosage of GAL-3 in patients with AF and a reduced LVEF may help in the prognosis and in the conduction of therapeutic process in this population.

A1-Antitrypsin (AAT), A1-protein (A1-Pr)

The increase in ROS is associated with inflammation, the production of cytokines (IL-1β and IL-6) and the induction of proteases.[233,234] Serum AAT protein, the basic product from the α-1 protein (A1-Pr) fraction, is an antiprotease, whose expression is increased in the presence of high protease levels produced in an inflammation.[235] Additionally, to destructive role that the genetic deficiency of AAT has in the lungs causing emphysema,[236] circulating AAT acts protectively in the endothelium, decreasing vascular damage and inflammatory process.[237–241] In the vasculature, a decreased expression of AAT can cause the local degradation of elastin, the increase of collagen deposition and finally the arterial stiffness and atherosclerosis.[237–241] Moreover, AAT is related with an antioxidative stress role, assessed by increased expression of eNOS and vascular endothelial growth factor-1 (VEGFR1) and by decreased expression of matrix metalloproteinase-9 (MMP-9).[242] It is known that AAT undergoes oxidation in HF patients, leading to protein dysfunction loss of its protease activity and against elastin, conditions that can cause myocardial damage.[243] Ac-

cording to this, an increase in the expression of AAT could depict a response to the loss of AAT activity.[244] In a study of 69 individuals presenting with HF, showed that serum a1 protein, evaluated by serum protein electrophoresis (SPE), was positively associated with TNF-a ($P < 0.001$, $r = 0.8$), IL-6 ($P < 0.001$, $r = 0.55$), and CRP ($P < 0.01$, $r = 0.42$) concentrations, and showed a significant increase across the NYHA scales.[245] Data exists that suggest a high concentration of a1 protein in a SPE in patients undiagnosed for HFcan be warning marker.[187] Additionally, the survey in the area of using AAT as a therapy in several diseases, such as diabetes mellitus and organ transplant rejection, indicates that medical applications of AAT can be applied to other pathological conditions.[244] An experimental model on endothelial cell monolayers exposed to ischemic-reperfusion mechanism showed that exogenous AAT alleviated IR injury in a dose- and time-dependent manner. According to this, overexpression of AAT decreased cell apoptosis and enchancedproliferation via inhibition of Rac1/PAK/p38 signalling and ROS production[242] administered 12 h after reperfusion in patients with ST-Segment Elevation Myocardial Infarction (STEMI), led to a major inhibition of CreatineKInase Myocardial Band (CK-MB), so it can be hypothesized that Prolastin C reduces the ischaemia–reperfusion injury time in STEMI patients. Those data suggest that AAT treatment may be used as therapy to reduce IR-induced vascular injury.[246]

Lectin-like oxidized low-density-lipoprotein receptor-1 (LOX-1)

Reperfusion syndrome is associated with huge production of ROS that converts LDL in ox-LDL and an over-expression of LOX-1 receptors in myocardial tissue. LOX-1 is the major receptor for ox-LDL in human endothelial cells, in smooth muscle cells, cardiomyocytes and macrophages, in atherosclerotic lesions and in plaque neovascularization.[247–250] In HF, LOX-1 levels are associatedwith BNP and inversely with the ejection fraction. [251] According to these, mice with LOX-1 gene deletion were treated with doxorubicin and that led to improved cardiac function, reduced myocardial inflammation and fibrosis.[252] LOX-1 pathway in cardiomyocytes is activated by oxidative stress *in vitro*

and by IR injury in vivo and cell apoptosis, while the administration of anti-LOX-1 antibody prevented apoptosis in vitro and reduced the extent of MI *in vivo*.[253,254] The increase in the expression of this receptor amplifies the production of proteases that are involved in HF, which induce collagen fragments such ascollagenases and gelatinases.[255] Hu, et al.[256] studied the regulation of TGFbeta1-mediated collagen formation by interfering in the LOX-1 signalling path in mouse cardiac fibroblasts. Transfection of wild-type mouse cardiac fibroblasts with AAV/TGFbeta1 significantly increased the expression of NADPH oxidases [p22(phox), p47(phox) and gp91(phox) subunits)], LOX-1, ROS and collagen synthesis, along with an increase in the activation of p38 and p44/42 mitogen-activated protein kinases (MAPK). The TGFbeta(1)-mediated increase in collagen synthesis was reduced in cardiac fibroblasts in mice where the LOX-1 was knocked out and in similar wild-type fibroblaststreated with an anti-LOX-1-specific antibody.[256] The expression of LOX-1, MMP-1 and adhesion molecules (P-selectin, VCAM-1 and ICAM-1), together with leukocyte concentration, is increased in IR.[257] The use of LOX-1-specific antibody in IR syndrome as a treatment in rats prevented upregulation ofLOX-1 and reduced MMP-1 and adhesion molecule expression molecules through inhibition of p38 MAPK path, as well as leukocyte concentration. Reduction of the expression of LOX-1 acts protectively against IR cardiomyocytes injury.[257] Additionally, LOX-1 is not only useful inthe diagnosis of left ventricular systolic HF after IR episodes, but also in the diagnosis of HF, for instance in patients with ischaemic cardiomyopathy.[258] The administration of noradrenaline and endothelin in cultured neonatal rat upregulated LOX-1 expressionaccompanied by cardiac cell apoptosis through p38 MAPK path.[253] Moreover, in Dahl salt sensitive rats, with HF, the administration of eplerenone, activated the LOX-1 pathway, inducing the production of endothelial eNOS through AKT andinhibitingthe production of iNOS through nuclear factor kB (NF-kB), suggesting that the suppression ofNF-kB- LOX-1- related activation could improve cardiac functionand remodelling.[259] It has been observed that the oxidation of tropomyosin in cardiomyocyte culture through the inhibition of LOX-1 with antisense RNA can also have an impact

on cardiomyocytecontraction.[258] Theoxidative stress- associated intracellular increment of p38 MAPK via the increased LOX-1 expression, responsible for pro-protein convertasesubtilisin/kexin-9 (PCSK9) production is related to the contraction dysfunction according to some authors.[260] The presence of LOX-1 in fibroblasts of cardiac tissue was related with the increase in angiotensin II (Ang II levels).[261,262] The infusion of Ang II in wild-type mice caused cardiac remodelling, while in mice where LOX-1 was not expressed the effect was less prominent.[261] The incorporation of LOX-1in cardiac fibroblast exposed to ox-LDL amplified the production of adhesion molecules and metalloproteinases.[263]

In conclusion, considering the contradictory aspects of the authors, GAL-3 is not marker of great clinical utility in the diagnosis andcharacterization of HF, while A1-p, AAT and LOX-1 could be effective markers. LOX-1 and AAT have not provided the clinical practice with evidence n discriminating HFpEF and HFrEF, however the throng relation of these markers with the oxidative stress damage on myocardial tissue, it could be expected that discrimination between the two conditions could be supported in the future studies. Being a non-specific marker GAL-3 could not possibly be used as a therapeutic target whereas therapies based on AAT have already gained the attention, whereas LOX-1 activity may be controlled through molecules such asox-LDL, Ang II, ROS and some cytokines, triggering the NF-kB pathway, reducing IR injury as well as LOX-1 antibodies may be a helpful therapy for the HF patients.

INFLAMMATION RELATED BIOMARKERS (TABLE 2)

Ferritin

Iron is atrace element for oxygen binding and transport through red cells [264] but is also a potent generatorof ROS [265] and chronic inflammation.[266] Inflammation and oxidative stress have interrelated and interacting mechanisms and can provoke contractiledysfunction and cardiac remodeling in heart failure and atrial fibrillation.[267] Increased plasma ferritin, with the most commonly used cutoff levels being ≥ 300 µg/L in men and ≥200 µg/L in

women,[268] is often used as a biomarker to depict body iron load, liver disease, and chronic inflammation.[269,270] In large population studies, serum ferritin levels are related with hypertension,[271] QT prolongation,[272] andheart failure,[273,274] but not with left ventricular hypertrophy[275] andthe connection with cardiovasculardisease is still controversial.[276–278] Patients not following a Mediterranean diet presenting with higher iron stores[279] have a higher possibility to developatrial fibrillation.[280] In a meta-analysis of the results of the studies from the Copenhagen City Heart Study (CCHS), the Danish General Suburban Population Study (GESUS), and the Copenhagen General Population Study (CGPS), including 35 799 individuals, the ferritin concentration as biomarker of iron overload or chronic inflammation, was associated with the risk of developing HF or AF in men and women.[281] According to these data increased ferritin concentration increased risk of atrial fibrillationin men and women, with no significant statistical difference between the two populations, although the greater effect size is more prominent in men than in women. Men may be in greater risk of developing iron overload-associated diseases, as they seem to accumulate more iron than women, because of the regular blood loss through menstruation before menopause.[282] The risk of AF was projected in correlation with the levels of ferritin, increasing stepwise, with the highest risk being for ferritin concentrations > 600 µg/L in men and women, regardless of the gender and despite the increased risk described, the population-attributable risk was small at 2.4% in both sexes combined.[281] In animal models, iron overload decreases Cav1.3-dependent L-type Ca^{2+} currents, resulting in bradycardia, modified electrical conduction, and atrial fibrillation, and increases the PR interval, predicting AF in humans. [283–286] In thalassemia major patients and in general population iron overload is associated with prolongedQT interval. [272] Increased ferritin concentration is also linked with higher risk of cardiac death. [277] The severe iron accumulation in heart is located in atrial and ventricularmyocardial tissue, the AV node, perinodal tissue, and the bundle branches,[287] which may be the reason for the developmentof atrial fibrillation,[281] the tachyarrhythmias[288] and conduction dysfunctions.[272] However, moderate increase in ferritin concentra-

tions, is more possibly related to inflammation,[289] as well as to liver disease, chronic kidney disease, cancer, and rheumatic disease,[270] whereas both plasma ferritin and transferrin saturation are acute-phase reactants, and they cannot depict aqurately the iron status especially in inflammation condition or chronic disease.[290]

There was no significnt statistical association between heart failure risk and increased plasma ferritin concentrations.[281] In the AmericanAtherosclerosis Risk in Communities (ARIC) study, both increased and decreased plasma ferritin level compared with normal values were related withincreased risk of heart failure in men and women.[273] In the Prevention of Renal and Vascular Endstage Disease (PREVEND) Dutch study, stepwise increased plasma ferritin level was linkedwith the risk of heart failure in women, but not in men.[274] According to these studies the possibility that increased ferritinconcentration may be linked with the risk of heart failure cannot be excluded, however the results vary across populations. In an international pooled cohort on 1.506 chronic HF patients with reduced or preserved LVEF (pLVEF > 45%), it was shown that iron deficiency: was common in the half of the population studied, was closely related to disease severity, according to NYHA functional class and NT-proBNP levels and was an independent indicator for the patients with an enhanced risk for death.[291] Whereas, the cut-off levels for the studies were set at a serum ferritin level < 100 µg/L or serum ferritin from 100 to 299 µg/L in combination with a transferin saturation (TSAT) < 20%, the association of iron and ferritin with inflammatory conditions and chronic disease, it may be better to use a higher cutoff to define absolute iron deficiency (serum ferritin < 100 µg/L) in chronic HF and distinguish it from functional iron deficiency (an increased ferritin level, usually between 100 and 299 µg/L, with a TSAT <20%, while a reduced TSAT depicts empty iron stores in such situations).[290,292,293]

In some clinical trials, patients with chronic heart failure and iron deficiency (ferritin < 100 µg/L or < 300 µg/L with TSAT< 20%) have shown clinical and functional improvement when treated with iron replacement.[294–296] According to some studies, iron deficiency is connected with exercise capacity and patients with chronic heart failure show clinical

and functional improvementwith iron replacement therapy,[294–296] however,the cause of anemia in those cases wasmost likely associated with chronic renal failure, decreasedplasma erythropoietin, decreased blood hemoglobin, anddecreased plasma iron concentrations,as well as with increased production of cytokines, stimulators ofrelease of hepcidin from the liver, which enchances theiron deficiency.[294] A further study on data from the PREVEND study showed that increased ferritin levels amplify the risk for new-onset HF in women, but not in men as well as the prevalence of HFpEF in women. This relationship was independent of the occurrence of cardiovascular events or all-cause mortality over time.[274]

The studies so far cannot connect the causal connection between increasedferritin concentration and AF or its role as a biomarker, however the regular surveillance of moderate increased iron status with electrocardiogramsis important in order to detect early signs of atrial fibrillationbefore the presence of cardiovascular events. Markers of iron homeostasis may provide useful information to the chronic and new-onset HF, whereas iron administration or elimination could have beneficial effects.

C-reactive protein (CRP)

There is growing evidence on a strong association between inflammation with the pathogenesis of AF and arrhythmias, as well as with the establishment of HF and cardiovascular events. Interleukin-6 and CRP are markers of inflammation and have been most frequently studied in cardiovasculardiseases and AF.[82,289] Despite some contradictions, themajority of studies have shown elevated CRP or other inflammatory markers levelsto be independent risk factors for incidence of AF in subjects with no history of AF. An analysis of the data occurring from CHS, CRP was independently associated with baseline AF and with the future development of AF with a mean follow-up of 6.9 years, as well as CRP remained a significant predictor of AF after adjustment for multiple risk factors for AF and an independent predictor of new AF cases as an increase of CRP levels for 1 standard deviation predicted a 33% greater likelihood of developing AF.[297] This analysis confirms the reported findings of a case control study showing that CRP was >2-

fold higher among patients with AF than among control group participants. Higher CRP levels were reported among patients with persistent compared with those with paroxysmal AF and in patients with AF compared with patients in sinus rhythm.[298] According to a case control study on blood samples taken from individuals during the AF episode, CRP levels were more increased in the left atrium than in the coronary sinus, showing that AF may cause sequestration of inflammatory reactants in the heart.[299] Whether initiation of AF activates provokes inflammatory effects or whether the presence of a systemic inflammatory state leads to AF remains unclear. The progression of the arrhythmia in the presence of systemic inflammation could be compared to other states in hich elevated CRP is associated with a worse outcome, for example patients with acute coronary syndromes and high CRP are in greater risk of mortality and left ventricular dysfunction, reflecting possibly an association with ventricular remodeling.[300] Additionally, high-sensitivity CRP (hs-CRP) levels are positively associated with stroke risk factors (e.g., diabetes and hypertension) in AF patients and are also related to mortality and the development of ischemic stroke, myocardial infarction, andvascular death.[301,302] The association of inflammation with AF may have potential therapeutic implications. Additionally, there are therapeutic strategies targeting the modification of CRP including statins, based on their antiinflammatory actions, the evidence existing including also individuals without overt hyperlipidemia, however the exact beneficial role in AF is not yet clarified.[303]

CRP has also been associated with disease severity and prognosisin patients with HF. The first reported observation was published in 1990, when the levels of serum CRP were higher than normal in 70% ofthe HF group, and the measured levels were directly linked to the severity and stage of HF.[304] Following this observation, more studies in patients with HF, ischemic and nonischemic, elevated CRP levels were connected with the severity of the condition, the hospitalisation, the mortality rate in the follow up period and the NYHA stage comparing to patients with normal levels of CRP.[305–307] Additionally, Cesari et al. reported that in elderly patients, for every one standard deviation increasein CRP levels the risk of HF events is increased by 48%.[308]

Vasan, et al.[309] studied the role ofCRP in the prediction of development of HF. They examined CRP as atriggering factor to HF among elderly people taking part in the Framingham Heart Study. Increased CRP levels ≥ 5 mg/dL was associated with a2.8-fold higher risk of developing HF during amean follow-up period of 5 years compared tosubjects with normal CRP levels.[309] It is also important to note that the use of Angiotensin Converting Enzyme (ACE) inhibitors and beta blockers has been linked with lowerlevels of CRP in HF patients.[310] However, despite the connection with the prognosis and the severity of the condition of the HF patients, it is not clear yet whether CRP is simply a markerof inflammation or it is actually involved in the pathogenesisand progression of HF. Therefore, it is not yet clear if it could have a significant role in the monitoring of administrated therapy for HF (Table 2).

Cytokines

The term cytokine refers to a group of small protein molecules which are secreted by cells in response to a variety of stimuli. Pro-inflammatory mediators are expressed by all the nucleated cell types in the myocardium, including the cardiac myocyte, suggesting that these molecules may have more than just an inflammatory role in the myocardium.[311] The pro-inflammatory cytokine response is controlledby a series of immunoregulatory molecules (anti-inflammatory cytokines) along with specific cytokine inhibitors and soluble cytokine receptors to regulate the immune response.[312]

Interleukin-6

IL-6 is produced by lymphocytes and stimulates inflammatory responses and it also has anti-inflammatory effects, via the inhibition of TNF-αsignaling path and the activation of IL-10, ananti-inflammatory cytokine. A meta-analysis reported that higher IL-6 blood levels were associated with greater AF risk in the general population and also increasedrisks of AF recurrence after electrical cardioversion and catheterablation.[313] Conway, et al.[314] demonstrated that high serumIL-6 levels were an independent marker of stroke and they were releated to adverse events and mortality during a long-term follow-up (> 2 years) in a large cohort of anticoagulated permanent/paroxysmal-AF patients.[315] In a case-control study, it was observed that serum CRP

Table 2   Inflammatory biomarkers in the management of HF, as they are presented in studies and clinical trials.

	TNF-α	IL-6	sTNF-R1	sTNF-R2	CRP
Elevated levels in patients with HF	***	**	**	**	**
High levels and disease severity	***	***	**	**	**
High levels, prognosis and HF outcomes	***	***	**	**	**
High levels and the development of HF in asympomatic patients	**	**	n/d	n/d	**
Level change after HF treatment	***	***	**	**	*
Supporting references	309, 325, 327, 328-332, 335-339, 342-344, 347-350, 356-359, 360, 364, 365, 368, 369, 371, 372, 374, 375, 378	309, 338, 340, 342, 345, 347, 350, 355-359, 364, 367, 373, 378	337, 340, 344, 345, 347, 350, 352-354, 375	340, 344, 345, 347, 350, 353, 354, 368, 371, 374, 375	304, 306-310, 358

***: Based on a large number of studies and more than one large-scale clinical trial, **: Based on several number of studies, small-scale clinical trials and/or one large-scale clinical trial, *:Based on one small study or one small clinical trial, n/d: no data from studies or clinical trials IL-18, IL-10, MCP-1, sST-2, IL-RA and ESR are not included in the table due to the very few supporting data occurring from studies and trials. TNF-α: tumor necrosis factor- alpha, IL-6: interleukin- 6, sTNF-R1: soluble tumor necrosis factor receptor 1, sTNF-R2: soluble tumor necrosis factor receptor 2, CRP: C reactive protein, IL-18: interleukin- 18, IL-10: interleukin-10, MCP-1: monocyte chemoattractant protein 1, sST-2: soluble growth-stimulated expression gene, IL-RA: interleukin 1 receptor antagonists, ESR: eryhtrocyte sedimetation ratio.

and IL-6 levels were similar in patients with or without previous AF episodes, but were significantly increasedin blood samples taken during AF, indicating that AF may be the cause of the production of these acute-phase reactants, rather than the substancesthemselves result in the development of AF (Table 2).[299]

TNF-α

The TNF-α factor conducts an acute immune cell reaction and inflammatory reaction. TNF-α is produced by various immune cells, such as macrophages and lymphocytes. Li, *et al*.[316] demonstrated that serum TNF-αblood levels werehigher in patients with AF compared with those in SR, and in those with persistent and permanent AF compared with the patients with paroxysmal AF. A recent meta-analysis showed that higher TNF-αlevels were connected with greater AF risk. [313] Higher TNF-α levels inpatients with chronic-AF on admission to hospital were also predictive markers of stroke risk during the follow-up period (Table 2). [317]

Interleukin-8 (IL-8)

IL-8 promotes leukocyte migration and induces phagocytosis, enhances endothelial cell activation and modulates theplatelet-platelet and platelet-leukocyte interactions leading to thrombogenesis. Liuba, *et al*. [318] showed that serum IL-8 levels in the right atrium and coronary sinus and not in the pulmonary veinswere higher in patients with perman-

ent AF than in those withparoxysmal AF or SR, while CRP and IL-6 levels showed no difference in the 3 groups.

Interleukin-10 (IL-10)

IL-10 dereases T-cell cytokines, amplifies B-cell-survival, proliferation, and antibody production, and blocksinflammatory signaling via NF-κB. Li, *et al*. [316] reported that serum IL-10 levels were higher inpersistent and permanent AF patients compared with thosehaving paroxysmal AF, as well as the levels of IL-6, IL-8, TNF-α, monocyte chemoattract-ant protein-1 (MCP-1), vascular endothelialgrowth factor and N-terminal pro-brain natriuretic peptide. However, it is unclear whether the high levels are related with acute AF events or underlying inflammation/injury. Before cardioversion treatment, AF patients had higher blood levels of CRP, TNF-α, soluble intercellular adhesion molecule-1 (sICAM-1), malondialdehyde, and nitrotyrosine (NT) compared with theSR control group, and these levels were alsohigher in patients withsubsequent persistent-AF recurrence compared to those without,[319] while successful SR maintenance through cardioversion led to faster decrease in IL-6, sICAM-1 and NT levels.

Soluble growth-stimulated expression gene 2 (sST2)

sST2 is associated with inflammation, fibrosis and cardiac stress. In 2013 it was included in the Amer-

ican College of Cardiology Foundation/American Heart Association (ACCF/AHA) guidelines as an additional marker in the risk stratification of patients with acute and chronic heart failure.[166] sST2 blocks interleukin 33 (IL-33) and inhibits inflammatory gene transcription, inflammatory cytokine release and immunologic response.[320] The higher the levels of IL-33 secretion and activity are, the higher the serum sST2 levels are in response. sST2 may be elevated in any disease where inflammation and fibrosis occur and subsequently sST2 has low specificity for cardiac diseases however, it could be a valuable predictor of severity and prognosis.[321] Rienstra, et al.[322] showed no significant association between fibrosis marker sST2 and incident AF. Differing findings were seen in a 2018 study which was conducted on 100 patients with paroxysmal AF and the role of sST2 in the development of AF. In the first year after the ablation, the rate of patients without AF recurrence was 78% and sST2 was found to be only independent parameter for predicting AF recurrence. sST2 level was not found to be helpful to predict incident AF, however it was found to be independent predictor of AF recurrence after cryoablation treatment. The vast majority of incident AF is associated with pulmonary vein triggers and the recurrent episodes after AF ablation were linked with the reconnection of pulmonary veins and AF originating from larger areas of the atrium, and so sST2 may be a useful indicator for the distinction of the patients belonging to these two groups.[321] Another 2018 study showed that the serum concentration of sST2 in patients with AF was significantly higher than the levels in the healthy control group, and the serum concentration of sST2 in patients with persistent AF was higher than the levels in patients with paroxysmal AF in the AF subgroup, suggesting that the increased serum concentration of sST2 may have a significant role in the pathophysiological development of AF.[323] A study on 60 patients from December 2018 to July 2019 also showed that the serum sST2 level was higher in AF patients and than in those in the control group.[324] sST2 may play a role in supplementing and replacing limitations of BNP such as age or renal function. There was a positive correlation between NT proBNP and sST2 ($r = 0.314$, $P < 0.05$). Left atrial Diameter (LAD) was positively associated with serum sST2 ($r = 0.523$, $P < 0.05$) and there were also statistical differences in the levels of serum sST2, NT-proBNP and LAD among different AF subgroups.[324]

CYTOKINES IN HF MANAGEMENT

Circulating as well as intracardiac levels of thesecytokines are elevated in patients with HF.[325–330] Their physiologic role in inflammation and in the pathology of HF is being increasingly recognized.[331] Not only are inflammatory cytokines levels increasingly raised but also the anti-inflammatory cytokines and inhibitors production is dysregulated. Patientswith severe HF are reported to have decreased levels of transforming growth factor beta-1 (TGF-β1) and inadequately raised levels of IL-10 and TNF, and these abnormalities in the cytokine network are more prominent in patients with the most severe HF.[332] Recently, there has been considerable interest in ST2 and the role of a truncated soluble receptor (sST2) that can be detected in human serum. The trans-membrane form of ST2 is considered to modulate responses of T helper type 2 cells, whereas theexpression of the soluble form of ST2 is upregulated in growth-stimulatedfibroblasts. Infusion of sST2 seems to suppress the production of theinflammatory cytokines IL-6 and IL-12. Elimination of theST2 gene results in advanced myocardial inflammation, while the ST2 gene isupregulated in myocyte stretch, similar to the upregulation of the BNP gene.[333] sST2 acts as a soluble decoy receptor for IL-33, alleviating the effects of excessive IL-33 exposure andreducing the interaction between cardiacmyocytes, fibroblasts, and possibly endothelial cells.[334] Circulating levels of TNF, IL-6, and IL-18 are elevated inpatients with HF[325,327–330,335–347], not only inpatients with end-stage HF[348,349] but also at earlier phases of HF (i.e., NYHA functional class II HF)[327] or asymptomatic leftventricular dysfunction,[309] and continue to increase according to worsening NYHA functional class and this increase is also linked to greater risk of mortality.[327,342,350,351] Additionally, circulating levels of cytokine receptors are elevated in HF, including the soluble TNF receptors (sTNFR1 and sTNFR2),[337,340,352–354] and soluble transmembrane glycoprotein-130 (one of the receptors for IL-6 family), also increased in HF in

close relation to functional class.[309,332,354,355] Circulating levels of TNF,[344,350] IL- 6 [342,350,356−359] and TNF soluble receptors (sTNFR1 and sTNFR2) [344,350] have been reported to predict poorer survival. Levels of soluble ST2 are significantly increased in patients withadvanced chronic HF and with acute decompensated HF compared with control subjects [334,360] and IL-1receptor antagonist levels are also elevated in patients with HF.[332,340,347] Despite the increase ofanti-inflammatory cytokine IL-10 in HF,[340] in patients with severe HF, the levels of TGF-β1 aredecreased and IL-10 levels are inadequately raised in relation to the considering the elevated TNF concentrations.[309] Data on 384 patients withmoderate-to-severe HF in the placebo arm of the VesnarinoneTrial (VEST) have showed that there is a worse prognosis in survival due to the increase of TNF levels,with the worst survival being observed in patients with TNF levels >75thpercentile. [350] When each cytokineand/or cytokine receptor was separately entered into amultivariate Cox proportional hazards model, including age, sex, etiology of HF, NYHA class, ejection fraction, and serum sodium, TNF, IL-6, sTNFR1, and sTNFR2were significant independent predictors of mortality, along with NYHA class and ejection fraction. However, when all the cytokines and receptors were entered into themodel together, only sTNFR2 was significantly a predictor of mortality.[350] Another study of 37patients with HF and 26 age-matched control subjects, thecirculating levels of sTNFR2 was presented again as a powerful predictor of mortality.[337] A recent community-based study reported thathigher TNF levels were independently associated with agreater risk of mortality even in patients with HFpEF (Table 2).[361] A study by Weinberg demonstrated that anincrease in ST2 levels over a 2-week period was asignificant predictor, independent of BNP or proANP, of mortality or transplantation in patients with advancedchronic HF.[360] In addition, Mueller, et al. [362] showed that increased sST2 plasma levels in patients with acute decompensated HF were independently and strongly associated with 1-year mortality. Inflammatory cytokines could be also useful as markers for monitoringresponse to therapy in heart failure. Some of these observations can be attributed to directinteraction of the medications and the neurohormonal antagonists or the pro-

inflammatorycytokines. [363,364] Clinical studies have shown thattreatment with angiotensin receptor antagonists can lead toreductions in circulating levels of TNF and/orcell adhesion molecules in patients with HF.[365] Adrenergic blockade has also been acting protectively in theexpression of inflammatory mediators in post-infarctionanimal models [366] and reduce pro-inflammatory cytokine levels in clinical studies with HF patients. [340,367−372] However, the effect of ACE inhibitors on inflammatory cytokines is not as clear. In a study by Gage, et al., [369] TNF production was lower in patients receiving ACE inhibitors and the serum IL-6 in patients receiving both ACE inhibitors and beta blockers were trending to lower levels. Again, in the same study, the ratios of interferon gamma (INF-γ) to IL-10 levels were lower in patientstreated with a combination of beta-blocker and ACE inhibitor. Contrarily, in a clinical study by Gullestad, et al.,[373] treatment with ACE inhibitors for 34 weeks led to a rise in the serum levels of chemokines, cell adhesion molecules, and pro-inflammatory cytokines, except IL-6. Physical exercise reduces plasma levels of TNF, IL-6, sTNFR1, sTNFR2, and sIL-6R in patients with HF.[374,375] Furthermore, in patients with advanced HF, mechanical circulatory support with ventricular assist deviceled to significantly reduced myocardial expression of TNF after several weeks of support.[376,377] Despite all these studies, there is currently no data from large-scaletrials connecting the changes in inflammatory biomarkers over timewith morbidity and mortality in HFpatients. Furthermore, the sensitivity, specificity, and negative andpositive predictive values of inflammatory biomarkers inpredicting therapeutic responses in HF patients are not known.[343] Elevated levels of IL-6 and TNF have been reported inpatients with left ventricular dysfunction in the absence ofclinical symptoms of HF.[378,379] In a subgroup including 732 elderly subjectswithout prior HF enrolled in Framingham study, Vasanet al. reported that baseline levels of IL-6 and the production of TNF by peripheral blood mononuclear cells (PMBC) were predictive of development of HFin the next 5 years. [309] However, elevatedinflammatory markers in this study may have identified patients with vascular disease at risk for myocardial infarction [380,381] or patients with preexisting subclinical left ventricular dysfunction [310]

and there was no direct relation between the de novo development of cardiomyopathy versus the transition from subclinical left ventricular dysfunction to excessive HF.

Chemokines

Chemokines are pro-inflammatory and immune modula to conducting biological processes such as chemotaxis, activation and migration of leukocytes to areas of inflammation, collagen turnover, angiogenesis, and apoptosis. [331] Macrophage chemo-attractant protein-1 (MCP-1) and macrophage inflammatory protein- 1-alpha (MIP-1a) are chemokines that recruits monocytes into the arterialwall in atherosclerosis and they also modulate other cellular functions e.g., generation of reactive oxygen species. [70,331] Growth differentiation factor 15 (GDF-15) belongs to the subfamily of TGF-β and is associated with oxidativestress and inflammation. TNF and other pro-inflammatory cytokines, such as IL-1b and IL-6 or interferon- c are associated with the production of these chemokines as well as platelets, CD3+ lymphocytes and monocytes. [2,382] Circulating MCP-1 and GDF-15 levelshave been associated with prognosis in patients with AF and HF. [70,82,312] GDF-15 blood levels were independently associated with the risk of stroke, bleeding, and deathin AF patients on anticoagulation therapy,[383] while MCP-1has not been associated with AF risk. [384] Similar to the pro-inflammatory cytokines, the failing human heart also expresses chemokine and chemokine receptors.[385] Increased expression of chemokines, e.g., monocyte chemoattractant protein-1, has been reported in clinical HF.[331] MCP-1 has been reported to be increased in experimental models of HF with pressure or volume-overload.[386,387] Furthermore, transgenic overexpression of MCP-1 in themyocardium has been shown to cause myocarditis and subsequent development of HF in experimental models.[388] Aukrust, et al.,[331] showed that HF patients had significantly elevated levels of all the chemokines, especially those in New York Heart Association functional class IV with MCP-1 and MIP-1a levels being significantly and inversely related with left ventricular ejection fraction. Moreover, GDF-15 plays and important role in the induction of myocardial stress and remodeling and its production is releated with cardiac ischemia (nitric oxide-

dependent) or pressure overload state (angiotensin 2-dependent).[389] Subsequently, high GDF-15 levels have been observed in acute myocardial infarction and HF.[390]

Erythrocyte Sedimetation rate (ESR)

Erythrocyte sedimentation rate (ESR) has been of particular interest in HF due to its easy applicability and low cost repetitibity. However, clinical studies demonstrate controversial results on its role in HF. According to a single report published in 1936, it was long assumed that the ESR is low in patients with HF. [391–393] To reevaluate this concept, Haber et al. measured the ESR in 242 HF patients and showed that the ESR was low (< 5 mm/h) in 10% of the patients, but was higher (above 25 mm/h) in 50%. It was remarkable that patients with low or normal sedimentation rates (≤ 25 mm/h) presented with more severe hemodynamic abnormalities, worse New York Heart Association functional class symptoms, and worse 1-year survival in comparison with the patients with elevated ESR (> 25 mm per hour). [391] Subsequently, in 2001, Sharma et al. studied ESR along with plasma levels of inflammatory cytokines (TNF, sTNFR1, sTNFR2 and IL-6) and mortality in 159 HF patients.[394] The ESR ranged from 1 to 96 mm/h (median 14 mm/h) and only 16% of the patients in this study had an ESR < 5 mm/h, indicating that ESR levels are high in HF as Habe and his collegues have demonstrated.[394] However, in contrast to Haber's study, high ESR levels indicated a poor prognosis, which was independent of age, NYHA class, ejection fraction, and peak oxygen consumption. Patients with ESR above the median (≥ 15 mm/h) compared to patients with ESR < 15 mm/h had a worse prognosis in survival (HR = 2.62).[394] The authors suggested that those differences may be the result of differences in the treatment of HF with ACE inhibitors, as they may improve patients' responsiveness to metabolic and immunological abnormalities. As CRP, ESR may simply indicate systemic inflammation, in a chronic disease and due to the administrated treatment, an "established" marker could completely be changed as far as its clinical information value is concerned, resulting in predicting the opposite outcome. Subsequently, this suggestion could be applicable on other "long-established" survival markers in heart failure.[394]

Biomarkers Associated with Renal Function and Injury

Glomerular filtration rate

Glomerular filtration rate (GFR) is accepted as a useful index of renalfunction. The gold standard measurement for GFR is complicated to be used regularly in clinical practice, therefore, GFR is usually estimated from serum levels of endogenous filtration markers suchas creatinine. Several equations can be used to incorporate demographic variables such as age, gender, body size, and ethnicity along with serum creatinine to estimate GFR.[289] Reduced GFR has been connected with increased risk of death, adverse cardiovascular events, and bleeding events in patients with coronary artery disease and in the general population. The prevalence of AF is higher in patients with end stage renal disease in comparison with the generalpopulation, and the AF prevalence increases when GFR decreases in chronic kidney disease cohorts. An increased risk of short- and long-term AF recurrence after successful electrical cardioversion or AF ablation therapy is also associated with impaired renal function.[395–398]

Renal impairment is a significant factor in heart failure morbidity and mortality. Although not completely understood, the interrelationships between cardiac and renal impairment, termed cardiorenal syndromes, have important clinical impact. The cardiorenal syndromes are characterized by derangements in cardiac and renal function, where injury to one organ results in impaired function of the other.[399] A study on 132 patients observed for 2 years demonstrated the effect of renal dysfunction and its management on HF morbidity. Themanagement of chronic kidney disease (CKD) leads to an improvement in the prognosis of HF as well as improvising the cardiac function leads to improved renal perfusion. It is hypothesized that HF impair renal function by two main mechanisms.[400] A decrease in stroke volume and a lower cardiac output will lead to activation of the renin-angiotensin-aldosterone system (RAAS), leading to a low renal perfusion and a decreased eGFR.[401,402] Another hypothesis suggests that right ventricular dysfunction leads to high central venous congestion and as a result to a decline in eGFR.[401] Worsening renal function dur-

ing HF hospitalizations is related to poorer outcomes [403] and higher rates of readmission compared with those with better renal function.[404,405] The study demonstrates that patients with an average decrease (Admit GFR - Discharge GFR) of 2.46 mL/min per 1.73 m^2 in GFR baseline level had a higher 30-day readmission rate compared to patients who had an average increase in their GFR baseline level by 1.92 mL/min per 1.73 m^2 at the discharge.[400] It is also important to optimize both cardiac and renal functions in patients with HF and CKD to improve the HF outcomes. A worsening in the GFR during hospitalization was negatively associatied with HF morbidity and mortality.[404,406–408] Moreover, patients with increased creatinine level were linked with an increased 30-day readmission rate, patients with an average creatinine level of 2.83 mg/dL had an increased risk of hospitalization compared to those with an average creatinine level of 1.90 mg/dL and patients with preserved kidney function with a GFR less than 60 mL/min per 1.73 m^2 were significantly at risk of hospitalization.[400]

Because urinary biomarkers can detect injury to the kidney before a rise in creatinine, they may offer advantages in the diagnosis and treatment of congestive heart failure. The use of cystatin C, albumin, neutrophil gelatinase-associated lipocalin (NGAL), kidney injury molecule 1 (KIM-1), N-acetyl-β-D-glucosaminidase (NAG), interleukin (IL-18), and is studied in order to estimate their clinical utilty in identifying and measuring kidney injury.[399]

Cystatin C

Cystatin C is a small protein, synthesized in all nucleated cells,[409] it is freely filtered by the glomerulus, does not return to the blood flow, is minimally influenced by disease states (thyroid function, corticosteroids, inflammation [410]), and it is believed to be a better endogenous marker of GFR than creatinine, especially the small reductions in GFR.[411,412] CKD is associated with a prothrombotic state and progressive atherosclerosis and cystatin C is considered to reflect microvascular renal dysfunction, elevated levels of coagulation related markers, raised levels of inflammatory markers and the severity of coronary artery disease.[412–418] The significance of cystatin C in AF population was recently presented by ARISTOTLE and RE-LY bio-

marker substudies.[419] Higher cystatin C levels were independent predictors of increased rates of stroke or systemic embolism, mortality, and major bleedings and were useful markers in the risk stratification and risk prediction. Despite the higher thrombotic risk in patients with renal impairment, the current risk stratification models do not include CKD among the risk evaluation criteria, while renal dysfunction patients might be frequently undertreated with oral anticoagulants due to the associated higher bleeding risk.[420,421] However, according to the ARISTOTLE study, creatinine-based estimates of renal function were better indicators of the risk of bleeding during oral anticoagulant treatment. Additionally, because of the clearance to some point of the novel oral anticoagulants, creatinine-based estimates of GFR have the potential to be more valuable in estimating risk of events and selecting the best dose of the new and old oral anticoagulants.[419] In patients undergoing cardiopulmonary bypass surgery, urinary cystatin C had a sensitivity that varied from 13% to 93%, and specificity between 40% and 97% for the detection of acute kidney injury (AKI)[399] and the resulting ROC ranged from 0.69 to 0.73 according to the level of urinary cystatin C used for detection of AKI. [422] In patients admitted to the intensive care unit, increased urinary cystatin C levels were connected with AKI, sepsis, and 30-day mortality, with ROCs of 0.70, 0.80, 0.64, respectively.[423]

Albuminuria

Albumin is the most abundant protein found in serum and contributes to the maintainance of normal serum oncotic pressure. Urinary albumin is the result of the disruption of the glomerular basement membrane, mostly associated with diabetic nephropathy, but also associated with hypertension, hyperlipidemia, glomerulonephritis, smoking, obesity, metabolic syndrome and previous incidents of myocardial infarction or stroke.[424,425] Microalbuminuria is defined as a urine albumin to creatinine ratio from 30 to 300 mg /g creatinine, while the same ratio being > 300 mg/g creatinine is defined as macroalbuminuria. However, even urine albumin < 30 mg/g creatinine has been associated with an increased risk of cardiovascular disease,[426] left ventricular hypertrophy and heart failure.[427,428] Both microalbuminuria and macroalbuminuria are linked with in-

creased risk for developing HF independent of hypertension or diabetes and their exact association with the pathophysiology of HF is still unclear.[426] In a North American study of 1 349 patients with stable NYHA functional class II to IV HF, the prevalences of microalbuminuria and macroalbuminuria were 30% and 11%, respectively. The risk of death and heart failure hospitalization was stepwise increased with the increase of the levels of urinary albumin, beginning with levels even lower than those required to define microalbuminuria. The backround medical history of the patients included hypertension, diabetes, cardiovascular disease, stroke, atrial fibrillation, CKD stage 3 or higher, and NYHA class III to IV, while angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, and β-blockers were not linked with a reduced prevalence of albuminuria.[429] A second Italian study on 2 131 patients with stable NYHA class II to IV heart failure demonstrated that the incidence of microalbuminuria and macroalbuminuria was less than in the North American study: 19.9% with microalbuminuria and 5.4% with macroalbuminuria. In contrast to the previous study, β-blockers were linked with a reduced prevalence of albuminuria. After 3 years of observation, patients with normoalbuminuria had a mortality rate of 16.8%, patients with microalbuminuria had a mortality rate of 27.9% and for patients with macroalbuminuria the mortality rate was 37.1%.[430] Finally, a multinational trial of 5 010 patients with stable heart failure, left ventricular ejection fraction < 40%, and an average follow up of 23 months, showed that proteinuria was associated with higher systolic and diastolic blood pressure, volume overload on physical exam, orthopnea, paroxysmal nocturnal dyspnea, and higher NYHA class, both with and without CKD, was accompanied with a greater risk of morbidity and hospitalization for HF complications than the groups of nonproteinuric patients with or without CKD. The study also did not show any association between valsartan and improved outcomes.[431] Combining the findings of these three large studies, it is suggested that albuminuria in HF patients is associated with morbidity and mortality.[399] There is also evidence that venous congestion contributes in a greater level to albuminuria than cardiac output.[426] Albuminuria acts as an important prognostic mark-

er for HF outcomes and it remains to be supported by randomized control studies, whether the interventions concerning albuminuria are more beneficial than standard heart failure therapy (e.g., renin-angiotensin-aldosterone blockade).[399]

Neutrophil Gelatinase-Associated Lipocalin (NGAL)

Neutrophil Gelatinase-Associated Lipocalin (NGAL) is a 25-kDa protein found in neutrophil granules, a member of the lipocalin superfamily and involved in iron transport, sequestration and in anemia of chronic disease and serum levels of NGAL are increased in chronic inflammatory conditions.[432] NGAL is expressed throughout embryonic kidney development and within hours of renal injury, NGAL messenger RNA is transcribed in the tubule cells of the thick ascending limb of Henle and the collecting duct. [399,433] NGAL levels are increased serum and urine and reach a peak in about 24 h after injury.[434] Urinary levels of NGAL are increased in a variety of kidney diseases (autosomal dominant polycystic kidney disease, Immunoglobulin A nephropathy, HIV nephropathyand urinary tract infections) and they are significantly elevated in patients with progressive AKI requiring renal replacement therapy.[435–439] Pediatric patients undergoing cardiopulmonary bypass were the first where NGAL was initially studied. Those who developed AKI after surgery, levels of urinary NGAL were significantly elevated up to 48 h after cardiopulmonary bypass compared to patients who did not develop AKI[440] (sensitivity for predicting AKI from 78.8% to 100.0%, specificity from 78.1% and 98.0% [441]). The ROC value was 0.86 for the use of urinary NGALin the prediction of renal replacement in post–cardiopulmonary bypass patients while later studies demonstrated an ROC ranging from 0.59 to 0.93.[442–444] There are few studies in small cohorts examining NGAL in patients with HF. In stable patients with an ejection fraction < 45%, levels of urinary NGAL were significantly increased in comparison withthose of the control groups. The levels of urinary NGAL were correlated with estimated GFR (eGFR), urinary albumin excretion, and N terminal prohormone of BNP (NT-proBNP) indicating the role of venous congestion in cardiorenal syndrome.[445] There was no association between urinary NGAL levels and blood pressure, hemoglobin levels, left ventricular ejection fraction, diuretics, β-blockers, digoxin and aldosterone-receptor antagonists, as was no significant indications that urinary NGAL levels could be used as predictors for later combined death or cardiovascular events.[445] In a study of patients admitted with acute decompensated heart failure, patients who developed worsening renal function had significantly higher levels of serum NGAL on admission, even among patients with normal creatinine.[446] Serum NGAL also was associated with an increased mortality after hospital discharge.[447] Further studies are needed to clarify the role of urinary NGAL in renal and cardiovascular prognosis in HF patients.[399]

Kidney Injury Molecule 1 (KIM-1)

Kidney Injury Molecule 1 (KIM-1) is a transmembrane glycoprotein with a small intracellular and a large extracellular domain with immunoglobulin-like features. KIM-1 is a phosphatidylserine receptor that induces neighboring epithelial cells to phagocytose apoptotic tubular cells, contributing to renal repair and reducing the inflammatory response after kidney injury.[448] In the 24–48 h after kidney injury, KIM-1 expression is increased in proximal tubular epithelial cells. Elevated urinary KIM-1 levels are observed in patients with polycystic disease[435] and renal cell carcinoma.[449] In adult patients who developed AKI within 72 h of cardiopulmonary bypass, urinary KIM-1 levels were elevated immediately postoperatively up to 24 h after surgery, contrasting the patients who did not developed AKI after surgery. The sensitivity immediately postoperatively and at 3 hours varied between 43% to 51% and 32% to 36%, respectively and the specificity varied between 78% to 89% and 90% to 96%, respectively. The ROC was 0.68 immediately postoperative and 0.60 at 3 h.[442] In a study of stable patients with NYHA class II to IV, levels of KIM-1 were significantly increased when compared to healthy control patients without heart failure. Urinary KIM-1 levels were associated with serum NT-proBNP levels, but not with ejection fracture, hemoglobin, or blood pressure. Between patients with heart failure without CKD and those with CKD, there were no statistical differences. Urinary KIM-1 had a significantly better prognostic value than NGAL and NAG in predicting a combined cardiovascular outcome of death, heart transplantation,

myocardial infarction, coronary angioplasty, or heart failure hospitalization.[447] Further studies are required in order to define better the utility of KIM-1 in the management of patients with cardiorenal syndrome.[399]

N-Acetyl-β-D-Glucosaminidase(NAG)

N-Acetyl-β-D-Glucosaminidase (NAG) is a brush-border lysosomal enzyme of the proximal tubule cells. Urinary levels of NAG are increased in the establishment of kidney injury from diabetes, lithium, and hypertension.[399] In patients undergoing cardiopulmonary bypass, the sensitivity of urinary NAG levels for predicting AKI immediately after surgery and at 3 h varied from 74% to 83% and 29% to 54% respectively and the specificity immediately postorerative and in 3 h after the surgery was 44%–50% and 71%–80%, respectively. The ROC immediately postoperative and at 3 hr was 0.61 and 0.63, respectively.[442] In patients admitted to the hospital with AKI, those with the highest urinary NAG levels were associated with higher risk for dialysis and hospital death. The ROC of urinary NAG for predicting the combined outcome was 0.71 and 0.79 when combined with the APACHE II score.[450] In stable patients with NYHA class II to IV, levels of NAG were inceased comparing with the levels in healthy individuals. Urinary NAG was elevated in association with NT-proBNP and eGFR, but not blood pressure, hemoglobin, or left ventricular ejection fraction. Urinary NAG predicted the combined outcomes of death, heart failure hospitalization, and heart transplantation independently of GFR.[447] Additional research in larger studies is needed to determine the role of urinary NAG in cardiorenal syndrome.[399]

Interleukin-18 (IL-18)

Interleukin-18 (IL-18) is a proinflammatory cytokine that is released by the epithelial cells in the proximal tubule shorly after renal injury. IL-18 is significantly increased in AKI compared with urinary tract infection, chronic renal insufficiency, and nephrotic syndrome.[451] In paediatric patients who developed AKI after undergoing cardiopulmonary bypass, urinary levels of IL-18 increased at 4–6 h after surgery and remained increased for 48 h. The sensitivity of IL-18 varied from 20% to 60% and the specificity ranged from 83% to 100% and the ROC was yielding between 0.61 and 0.75.[452] Research is

needed to determine the further utility of urinary IL-18 in cardiorenal syndrome.[399]

Adrenomedullin (ADM)

Adrenomedullin (ADM) was originally discovered in pheochromocytoma cells of adrenal medulla, demonstrating possible vasodilatoryeffects and it has been foundin vascular smooth muscle and endothelial cells [453,454] as well as in the heart, where it increases nitric oxide synthesis in an increased cytokine production and elevated myocardial contractility in a cAMP-independent manner.[455] In patients with HF serum ADM is elevated and it is related with decreased left ventricular ejection fraction, increased pulmonary artery pressures, diastolic dysfunction and restrictive filling patterns.[456] It has been demonstrated that an infusion of ADM in patients with HF causes vasodilation, increase in the cardiac indexand a reduction of pulmonary capillary wedge pressure.[457] Because of its short half-life and instability, its precursor MR-proADM has been suggested and reported to be an independent predictor of mortality in acute decompensated HF (ADHF) [458,459] and of adverse outcomes in chronic HF, including cardiac death and stable coronary artery disease.[460] While this marker has excellent sensitivity in the detection of HF, its specificity has been questioned because of the expression in many tissues resulting in increased levels in sepsis, glomerulonephritis, and chronic renal failure.[461] A study from 2010 to 2013 on 1 107 patients presented in the ED with shortness of breath as the primary complaint showed that MR-proADM was a useful biomarker for the diagnosis of ADHF among patients withunderlying AF who present to the ED with dyspnoea. In dyspnoeicpatients without AF, using a cut-point of 0.81 nmol/L, MR-proADM achieved similar accuracy as NT-proBNP (cut-point of 300 pg/mL) forthe diagnosis of ADHF (69.3% *vs.* 71.3%). Meanwhile, the presenceof AF significantly impaired the accuracy of NT-proBNP (61.6%) compared to MR-proADM (73.3%). The ROC curve presented the substantial degradation of AUC from the absence to presenceof AF for NT-proBNP (0.91 to 0.71), which is less apparentin MR-proADM (0.83 to 0.76).[462] These findings were in correlation with the Biomarkers in ACute Heart Failure (BACH) study that demonstrated the impairing effects that the presence of AF had oncardiac natriuretic pep-

tides in an independent cohort of dyspnoeicpatients.[177] The lack of confounding effects of AF on MR-proADM could posibly enable its use in clinical practice such as in the ED, singly or along with other mainstream markers to improve the diagnostic accuracy of HF in patients previously diagnosed for AF. A possible application of combined biomarkers in clinical practice would be the enrollment of MR-proADM in scenarios where NT-proBNP is elevated in subjects with AF to confirm the diagnosis of HF. According to the findings of this study, a cut-point of 1.1 nmol/L for MR-proADM is presented to be of great diagnostic accurance in the diagnosis of ADHF, regardless of region when NT-proBNP is > 300 pg/mL.[462]

Cardiac Troponins as Markers of Myocardial Injury

Cardiac troponins have been basic biomarkers for the diagnosis of acute myocardial infarction (MI). However, cardiac troponins are elevatedin other cardiac disorders including HF and AF.[289,463] Troponin release in HF can the result of MI types 1 and 2, in the presence or absence of coronaryartery disease respectively, cytotoxicity, apoptosis,and also inflammation and the elevated levels of troponins have a significant prognostic value.[82] The cardiac-specific troponins (cTn) I and T (cTnI and cTnT, respectively) exist in two pools inmyocytes. The larger is located in myofibrillar protein apparatus and is released slowly overseveral days after cell death. The second is a smaller source ofcTnand is located in a cytoplasmic pool and it is released relativelyrapidly in the next 1 to 2 hours of myocyte injury.[453] In 1997, it was reported that cTnI is present in the serum of patients with severe HF withoutischemia,[464] and it was then observed that the levels of cardiac troponins were predictive of adverse clinical outcomes insevere HF patients.[465] Using standard assays, significantly elevated levels of circulatingcTn have been reported in about one-quarter of patientswith HF and were connected with a poor prognosis, including cardiac death or early rehospitalisation for HF complications.[333] The use of high sensitivityassays (hsTn), abnormal cTnelevations have been reported in all patients withADHF,[466–469] in most patients with chronicHF,[470] in some patients with stable CAD and normal EF,[471] as well as in a

minority of asymptomatic elderly populations[472] and middle-aged people.[470] Elevated cTn levels during hospitalization were associated with a poorer outcome thanstable or reduced levels.[473]

The importance of troponin in an AF populationwas first reported in the Randomized Evaluation of Long-Term Anticoagulant Therapy (RE-LY) biomarker substudyincluding 6 189 patients with AF and due to high risk of stroke they were treated with either warfarinor dabigatran.[164] The results showed that: (1) detectable levels of troponin I (≥ 0.01 mg/L) were found in approximately 55% of the patients with AF and atleast one risk factor for stroke, (2) troponin was an independent marker for the increased risk of stroke or systemicembolism. Additionally, concerning CHADS2 andCHA2DS2-VASc scores, theencorporation of information coming from troponin I measurements, the troponinI level was a marker with significant prognostic value,[164] (3) it was shown that both independentlyand when combining troponin I with the CHADS2 and CHA2DS2-VAScrisk scores, the estimation on cardiovascular deathbased on troponin- based information was improved.[164] These results were also confirmed in the ARISTOTLE biomarker study, applied on 14 892 patients with AF, treated with either apixaban or warfarin due to a raised risk of stroke. Bloodsamples were collected and analysed for high-sensitivity troponinT.[474] Using a high sensitivity troponin assay, led to the detection of even lowerlevels of circulating troponin (1.5 ng/L) and as a result a larger proportion of patients (73%), wereidentified to have detectable troponin levels. The results showed that troponin levels were associated withthe risk of stroke and death independentof baseline characteristics or other biomarkers. According to both these studies, patients who present with elevated troponin levels ≥ 99th percentileupper reference limit for healthy individuals (troponin I ≥ 0.04 mg/L, high-sensitivity troponin T ≥ 13 ng/L for the respective assays) were at the greatest risk of thromboembolic events and cardiovasculardeath independent of clinical characteristics andother powerful biomarkers.[164,474] Similarly in a registry cohort of stable AF patients treated with anticoagulants, patients with troponin levels ≥ 50th percentileof the troponin levels in an AF population were a

greater risk of stroke, other ischaemic events and higher mortality regardless of the estimated risk by the CHADS2 andCHA2DS2-VASc risk assessment scores.The exact cause of elevated troponin levels in AF patients needs further explanation, however it could be a result of an increased ventricular rate, causing oxygen demand/availability mismatch and myocardial ischaemia, volume and pressure overload, changes inmicrovascular blood flow, atrial calcium overload, oxidativestress and alterations in tissue structure.[474–479] Nevertheless, theavailability of cardiac troponin measurements for routinecare in most hospitals worldwide makes it an excellent candidatein the management of patients with AF, complmentaryto the currently recommended clinical risk stratification.[289]

Extracellular matrix (ECM)-fibrosis markers

Increased deposition of collagen in the extracellular matrix (ECM) of the heart causes fibrosis and structural remodeling[480] and results in impaired cardiac function and increases the risk of developing adverse cardiac outcomes.[481] Myocardial fibrosis is caused by the disturbed balance between synthesis and degradation of collagen types I and III fibers and the abundancy of free collagen.[289] Serum peptides occurring fromcollagen metabolism reflect both the synthesis and degradationof collagen and they depict the condition of ECM.[482,483] The ratio of serum pro collagen type I aminoterminal-propeptide (PINP), a marker of collagen synthesis, to serum collagen type I cross-linked carboxyterminal telopeptide (CITP), a marker of collagen degradation, shows the collagen accumulation.[484] A multimarker panel consistingof increased levels of matrix metalloproteinase 2 (MMP-2), tissue inhibitor of MMP-4, and collagen III N-terminal propeptide (PIIINP), along with decreased levels of MMP-8, has been reported to characterize HFpEF.[483] Elevated ECM incidence has also been observed in patients with ADHF.[482] Aldosterone stimulates collagen synthesis andprovokes cardiac fibrosis in HF and in ventricular hypertrophycaused by pressure overload. The administration of the aldosterone receptor antagonist spironolactone inpatients with chronic HF in RALES (Randomized Aldactone Evaluation Study) led to a reduction of the elevated

levels ofmarkers of collagen synthesis (PINP and PIIINP) and wasshown to be clinically beneficial.[485] In patients with acuteMI complicated by HF, levels of PINPand PIIINP were elevated.[485] The administration of eplerenone, a specific aldosterone antagonist was presented to lead to the reduction of elevated levels of PINP and PIIINP, accompanied with reductions in mortality and hospitalizationfor HF.[486]

Serum levels of the fibro-inflammatory markers MMP-9, type III procollagen and hs-CRP, were higherin patients with persistent AF than in SR controls and positively releatedwith echocardiographic left atrial volume, an indexof atrial remodeling.[487] After AF ablation, serum levels of somefibro-inflammatory biomarkers (hs-CRP and IL-6) were decreasedin patients without AF recurrence, whereas others (tissueinhibitor of metalloproteinase-2 (TIMP-2), MMP-2, CITP) were increased.[487] Baseline MMP-2 levels were higher inthose with AFrecurrence.[488] In a postmortem analysis, inflammatorymacrophage markers and fibrosis markers were detected in subendocardialatrial tissues of AF patients, indicating an associationbetween inflammation and fibrosis in AF.[489]

Markers of coagulation-D-dimers

The prothrombotic state in AF has been recently described. Among markers of coagulation in AF, plasma D-dimer, a marker of fibrin degradation, is essentially used as an indicator ofthrombogenesis. Levels of D-dimer in AF individualsare elevated compared with controls in sinus rateand seem to remain increased despite successful cardioversion.[490–494] Levels of D-dimer seem to rise further according to the accumulation of clinical risk factors for thromboembolism ordue to the presence of left atrial appendage thrombi.[495–497] According to the resuts of RE-LY biomarker study in 6 216 patients with AF, a significant association between baseline D-dimer levels and therisk of stroke (3.0-fold), cardiovascular death (3.5-fold), and major bleeding outcomes has been demonstrated, independently of established risk factors including the CHADS2 risk scores.[498] These results were confirmed in the ARISTOTLE biomarker substudy, where it was reported that D-dimer levels at baseline, regardless of vitamin-K antagonist treatment are positively associated with stroke, mortality, and major bleeding,[499,500]

suggesting that D-dimermay also be a clinically useful risk marker in AF.

The association of HF with pro-thrombotic state and disturbed blood coagulation regardless of the HF type (systolic or diastolic HF) has been described in previous studies.[501,502] Hypercoagulability in HF could be caused by blood stasis, dilatation of cardiac chambers, reduced myocardial contractility and cardiac output, inflammatory reaction, neuro-hormonal activation, impaired endothelial function and arrhythmias such as AF.[503] Previous studies had shown that elevated D-dimer level predicted the development of incident systolic HF and also adverse outcome in patients with HF.[504] Minami, et al.[505] found that an admission D-dimer level > 3.85 μg/mL was linked with adverse in-hospital and poor medium-term prognosis in patients with ADHF. Zorlu, et al.[506] studied 174 patients with systolic HF and found that D-dimer > 1.43 μg/mL independently predicted a high risk ofcardiovascular mortality. A study on 244 consecutive patients with idiopathic dilated cardiomyopathy (DCM) and end-stage HF between February 2011 and September 2014 demonstrated that: (1) patients with increase admission D-dimer level had a poor outcome, (2) In comparison with the traditional markers, D-dimer levels on admission, had a better predictive value for long-term mortality in patients with end-stage HF, and (3) increased D-dimer levels independently predicted adverse long-term outcome regardless of variables such as left atrial size, age, LVEF inconsistently of the findings of the previous studies,[505,506] indicating the significance of D-dimer as a superior prognostic biomarker in patients with end-stage HF. [496] In this study D-dimer level was significantly increased in the non-survivors, an observation consistent with previous studies[505,506] supporting its potential prognostic value in patients with end-stage HF. In contrast with the traditional markers that only depict the global cardiac structure and function, D-dimers refect the functional status of other organs such as liver, kidney, hemostasis system. [507]

In a prospective cohort study on 4504 consecutive patients presented with acute MI the plasma D-dimer levels on admission were studied in correlation withthe incidence of HF and all-cause mortality. Increased plasma concentrations of D-dimer on admission were independently associated with the subsequent incidence of HF after hospitalisation and all-cause mortality in patients with acute MI. It has also been observedthat elevated D-dimer levels are associated with increased incidence of in-hospital HF. [508] Despite limitations in studies, D-dimers it is important to further support the significance of D-dimer levels in the incidence of HF independently.

CONCLUSION

A vast number of clinically relevant biomarkers are available and constantly new ones are identified and studied. The ideal profile of a biomarker is one that contributes to the understanding of the pathogenesis of the pathological condition, and it is an independent marker in the diagnosis, development, prognosis and the efficacy of the treatment. It should ideally be of low cost and easily performed in routine care, allowing it to be applicable to all healthcare sytems worldwide. Some markers appear to reflect better the pathophysiologic process for the development of AF or HF, while others may simply depict the future risk for cardiovascular events. Further information on the interrelation among the biomarkers could also compile assessment scores increasing their diagnostic value. For example, in the AF population, the importanceof a multimarker approach was highlighted by the simultaneous use of cardiac troponin and natriuretic peptides along with information obtained by each biomarker separately.[289] A combination of 7 biomarkers building a multimarker score led to a significantly better reclassification of HF patients.[509] Biomarkers with limited prognostic value may still offer valuable information in the pathophysiology of AF and HF, when used in a panel of multiple markers. A multi-marker approach for the general management of HF and AF could additionally create a "window" in the multiple associated aspects of the disease process such as renal disease, inflammation, and myocardial stress, injury and fibrosis, something that may offer the clinical practice with an evolving assessment score in every stage of the pathophysiology, development and treatment of Atrial Fibrillation and Heart Failure.

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