

Biomarkers in Cardiology - Part 2: In Coronary Heart Disease, Valve Disease and Special Situations

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Abstract

Cardiovascular diseases are the main causes of mortality and morbidity in Brazil. Their primary and secondary preventions are a priority for the health system and require multiple approaches for increased effectiveness. Biomarkers are tools used to identify with greater accuracy high-risk individuals, establish a faster diagnosis, guide treatment, and determine prognosis. This review aims to highlight the importance of biomarkers in clinical cardiology practice and raise relevant points regarding their application and perspectives for the next few years. This document was divided into two parts. This second part addresses the application of biomarkers in coronary heart disease, valvular diseases, cardio-oncology, pulmonary embolism, and cardiorenal syndrome.

Biomarkers in acute coronary atherosclerotic disease

Diagnostic biomarkers

Advances in the knowledge of the pathophysiology involved in myocardial ischemia, such as instability of the atherosclerotic plaque and mediators of inflammation and coagulation, have been associated with the emergence of new technologies that detect molecules involved at different stages of this process. As a consequence, several biomarkers have been incorporated into the laboratory evaluation of acute coronary syndromes (ACS). However, cost-effectiveness is a relevant aspect in a biomarker's choice since a large proportion of these assays are expensive, and only some have accuracy comparable with traditional diagnostic methods.

Comparison between biomarkers - historical perspective

Measurement of lactic dehydrogenase (LDH) and transaminases (AST), markers that increase in association with extensive myocardial necrosis, is no longer recommended due to their low specificity when compared with that of creatine kinase (CK) isoforms.

Keywords

Coronary Artery Disease; Heart Valve Diseases, Pulmonary Embolism; Cardio-Renal Syndrome; Biological Markers, Pharmacological; Evidence-Based Practice.

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Manuscript received November 10, 2014; revised manuscript January 07, 2015; accepted January, 2015.

DOI: 10.5935/abc.20150061

Myoglobin, a component of the contractile apparatus of cardiac myocytes, has low molecular weight and rapid release kinetics following myonecrosis. However, the diagnostic application of myoglobin has been questioned due to its low specificity to diagnose ACS, even in patients admitted within a few hours of developing symptoms of angina.

Until the 1990s, CK isoforms were the gold standard for laboratory diagnosis of acute myocardial infarction (AMI). Represented by the MB isoenzyme of CK and by the CK-MB mass fraction, they can be detected 4 to 6 hours after the occurrence of necrosis, with a peak release about 24 to 48 hours later. Earlier peaks (12 to 24 hours) are associated with myocardial reperfusion, and their short half-life may justify their use for identification of ischemia as a complication of percutaneous or surgical revascularization strategies in the absence of another biomarker with better accuracy¹.

Troponins

The application of troponin (TPN) has been validated in several clinical trials, and this marker has been incorporated into the main guidelines and international consensuses as an essential element to diagnose AMI. The role of TPN in determining short-and long-term prognoses in ACS has also been confirmed². However, the presence of other high-risk criteria may be associated with worse clinical course, even with normal levels of TPN³.

The persistence of high levels of TPN for up to 14 days after an episode of AMI is helpful to establish a late diagnosis in patients presenting during a subacute phase. However, the detection of myocardial ischemia recurrence during this period may be impaired due to the slow decrease in TPN levels, requiring serial blood samples showing an increase of more than 20% from previously increased TPN levels, or measurement of CK isoforms⁴.

New strategies for evaluation of troponin

Despite the definitive role of TPN in the diagnosis and prognosis of ACS, levels of this marker increase only ~6 hours after symptoms of ischemia have emerged due to characteristics associated with the weight and size of this molecule (21 to 37 kDa). This limitation also applies to the CK isoforms, since they require serial blood sampling after hospital admission, which may delay the diagnosis in patients with atypical symptoms and nonspecific electrocardiogram⁵.

High-sensitivity TPN (hs-TPN) detects TPN levels in the range of ng/L (or pg/mL), which identifies the marker even in healthy individuals⁶. This ability confers higher sensitivity compared with the conventional methodology, in addition to identifying minimal variations in short intervals of time, and allowing early recognition of myocardial injury.

Levels of hs-TPN are often elevated in patients with chronic heart disease. Therefore, elevation of hs-TPN in a single sample should not be considered sufficient to diagnose AMI. In recent guidelines, the European Society of Cardiology recommended confirmation of AMI with a second measurement of hs-TPN 3 hours after the first measurement. The second measurement must show levels more than 20% above baseline values in patients with high hs-TPN (above the 99th percentile) on admission, and more than 50% in those with normal baseline hs-TPN values. In patients with a high probability of ACS in whom AMI is not confirmed with initial hs-TPN measurements, hs-TPN should be repeated after 6 hours⁷.

TPN has better sensitivity and specificity than myoglobin and CK isoforms, and should be used as the biomarker of choice in the evaluation of ACS, preferably in centers able to establish a cutoff value greater than the 99th percentile of the normal population and with a turnaround time (time elapsed from blood collection to delivery of the test result) < 60 minutes⁷.

New biomarkers

Episodes of ischemia not leading to cardiomyocyte necrosis may occur without substantial TPN elevation, even when associated with instability in the atherosclerotic plaque with a risk of coronary occlusion. Biomarkers with the ability to identify this initial phase of ACS are promising. The soluble CD40 ligand is an active fragment of the endothelial CD40 receptor whose levels increase with platelet activation or endothelial inflammation and are associated with increased risk of death or heart failure (HF)⁸. Choline, a by-product of phospholipase D, can signal instability in the atherosclerotic plaque in the presence of endothelial dysfunction. However, based on available evidence, these two biomarkers are unable to add prognostic or therapeutic value when compared with TPN.

The heart-type fatty acid binding protein (h-FABP) is a cytoplasmic protein with low molecular weight (12 to 15 kDa) which is responsible for the intracellular transport of insoluble fatty acids. During myocardial ischemia, h-FABP is released into the plasma due to increased permeability of the cardiomyocyte cell membrane. Levels of h-FABP may be detected in the blood 90 minutes after an ischemic event, with peak elevation after 6 hours, followed by a return to baseline levels after 24 to 30 hours. Recent studies have confirmed these quick release kinetics in patients admitted with chest pain in whom h-FABP was compared with TPN and other biomarkers to diagnose ACS. When compared with myoglobin, concentrations of h-FABP are higher in the cardiac muscle. This renders greater diagnostic accuracy to h-FABP in patients admitted with symptoms lasting less than 6 hours. However, the reduced specificity of h-FABP and lack of comparative randomized trials with modern techniques to detect TPN require additional studies⁹.

Biomarkers in chronic coronary atherosclerotic disease

In order to be incorporated into clinical practice in modern days, a biomarker must provide additional prognostic information to existing variables. The most applied and

complementary techniques for this purpose in prospective studies are the C statistic and the Net Reclassification Index (NRI). C statistic demonstrates a method's ability to identify who will or will not experience an outcome, whereas NRI estimates a marker's ability to reclassify individuals into higher-or-lower-risk categories.

High-sensitivity C-reactive protein

In ST-segment elevation AMI, levels of high-sensitivity C-reactive protein (hs-CRP) are inevitably high and reflect inflammatory activity secondary to myocardial necrosis. According to some studies, levels above 10 mg/L on the first day are associated with an increased risk of death in 30 or more days, regardless of ventricular function¹⁰. In patients with ACS without infarction, increased levels of hs-CRP are more consistently associated with risk of death and render additional independent prognostic value¹¹. High levels of hs-CRP 24 hours after hospital admission for AMI have been independently associated with risk of ventricular remodeling in the following 6 months, although this parameter had little discriminatory ability when assessed by C statistic¹². A study with a large number of patients admitted to the emergency unit with suspected ACS found no advantage in measuring hs-CRP, but demonstrated a five-fold increased risk of death in 2 years associated with elevated brain natriuretic peptide (BNP) levels¹³.

A recently published systematic review found a positive association between elevated hs-CRP during ACS (with cutoff values between 10 and 15 mg/L) and long-term cardiovascular events, with a multivariate odds ratio of 2.5 (95% confidence interval: 1.8-3.4). The predictive power for short-term events was inconsistent, revealing heterogeneity in the results¹⁴. According to this same study, only 2 of the 19 prospective studies included in the analysis assessed the additional discriminatory ability for new events in multivariate models revealing opposite and, therefore, inconsistent results.

Based on the currently available evidence, the conclusion is that assessment of hs-CRP levels in the acute phase of an ACS episode offers little help in identifying patients at risk of additional cardiovascular and cerebrovascular events. Between 4 and 6 weeks after the acute insult, hs-CRP levels return to baseline values, and its prognostic value is similar to that described for primary prevention.

T and I Troponins

These are highly sensitive and specific markers of myocardial necrosis which have also an independent prognostic value after AMI, since their concentrations depend on the amount of infarcted muscle. There are several commercially available assays for TPN T (TnT), which leads to considerable numerical variability in the results. A study with a large number of patients conducted in the era of interventional cardiology showed that TnT levels > 1.18 ng/mL the day after an episode of AMI were associated with a five-fold increased risk of death, three-fold increased risk of recurrent ischemic events, and seven-fold increased risk of HF in 30 days when compared with lower TnT levels. These risks remained increased until the second year after the index event and were independent of clinical, laboratory, and echocardiographic variables¹⁵.

Levels persistently high of TnT 28 days after an episode of AMI have also been independently associated with an increased risk of death, ischemic events, and HF in a large cohort in which 96% of the patients had previously undergone primary angioplasty. In this study, the cutoff value for high TnT was only 0.04 ng/mL¹⁶. High-sensitivity TnT had similar independent prognostic value for mortality in 1 and 4 years in the acute phase¹⁷ and 7 weeks after the index event¹⁸.

In patients with ST-segment elevation AMI undergoing primary percutaneous intervention, TnT levels $\geq 7 \mu\text{g/L}$ demonstrated sensitivity of 73% and specificity of 84% to predict ventricular remodeling 6 months after the index event¹⁹. In another study, increased levels of TnI (TnI) 6 weeks after an episode of ACS was an independent predictor of mortality at 5 years²⁰. These studies, conducted in the modern era, corroborate the results obtained prior to the propagation of percutaneous procedures, demonstrating the predictive value of increased TnI levels for events after an episode of ACS.

Thus, based on the available evidence, determination of serum TnIs levels 24 hours after the index event, which is already incorporated into universally accepted management guidelines, provides relevant prognostic information. Bearing in mind the possibility of percutaneous intervention in the initial hours, the determination of TnIs 24 hours after the procedure is equally desirable and already established as a routine. A new measurement of TnIs days after the event is equally important, but the additional measures to adopt in patients at risk become then unclear, once the ideal therapeutic regimen is delivered according to established guidelines. Even in those individuals reclassified into a lower risk category after measurement of a biomarker longer after the index event, it is worth considering if this information would relax the measures already adopted in secondary prevention: The answer is still probably no.

Brain natriuretic peptide and the N-terminal pro-B-type natriuretic peptide

Both BNP and N-terminal pro-B-type natriuretic peptide (NT-pro-BNP) are easily measurable in a laboratory and have good reproducibility. Most studies with AMI patients, even those without ventricular dysfunction or ST segment elevation, describe a positive association between elevated levels of these peptides with mortality and reinfarction within the first year after the acute episode²¹ and ventricular remodeling 6 months to 1 year later²².

According to a recent study, BNP levels $> 300 \text{ pg/mL}$ determined 96 hours after an episode of non-ST-elevation AMI adds discriminatory value to the Thrombolysis in Myocardial Infarction (TIMI) score to determine the risk of death in 1 year²³. Measurement of NT-pro-BNP 6 weeks after an episode of ACS without ST-segment elevation is predictive of events and adds additional discriminatory value to known prognostic factors²⁴. In another prospective study, levels $\sim 100 \text{ pg/mL}$ were predictive of AMI in patients with ACS without ST elevation²⁵.

Based on available information, it seems appropriate and cost-effective to estimate BNP or NT-pro-BNP close to hospital discharge in patients with ACS, aiming at a more refined risk assessment in combination with the clinical markers.

Biomarkers in valvular disease

Biomarkers of prognosis in mitral stenosis

Brain natriuretic peptide and the N-terminal pro-B-type natriuretic peptide

In patients with mitral stenosis (MS), there is an increase in BNP and NT-pro-BNP that correlates with the largest diameter of the left atrium and right ventricle, and with a higher pulmonary arterial systolic pressure (PASP)²⁶. Studies show a direct association between BNP levels with mitral transvalvular gradient during diastole and an inverse association with the valvular area²⁷. When levels of BNP or NT-pro-BNP were compared in patients with sinus rhythm or atrial fibrillation (AF), the results were controversial²⁸. Studies show a correlation between higher levels of NT-pro-BNP with greater increases in PASP after exercise, and a direct association between decreases in NT-pro-BNP levels after percutaneous mitral commissurotomy and the success rate of the procedure^{29,30}. Data collected from patients who underwent interventional procedures have demonstrated a decrease in BNP and NT-pro-BNP after percutaneous mitral valvuloplasty in patients in sinus rhythm, but not in those with AF.

Levels of these markers vary widely among the studies, and cutoff values have not been standardized to support changes in management based on their isolated analysis. Thus, determination of BNP or NT-pro-BNP levels may be considered for complementation in the assessment of MS patients who are unable to undergo stress testing, when the test is indicated, or in those with limited echocardiographic window.

C-reactive protein

Studies correlating CRP levels with mitral ring calcification have shown controversial results³¹, whereas those analyzing patients with MS have shown increased CRP in this population when compared with controls³². In addition, higher levels of CRP have been associated with a higher degree of valvular stenosis, presence of AF, and atrial spontaneous contrast. Patients with higher CRP levels had lower success rates after percutaneous balloon mitral valvuloplasty³³. Despite these evidences, it is still unclear whether CRP measurement is useful for management guidance in MS patients, and whether measures to reduce CRP levels have any impact on the progression of the disease. Therefore, it is not possible at the moment to recommend for or against the measurement of this marker in MS patients.

Prognostic biomarkers in mitral insufficiency

Brain natriuretic peptide and the N-terminal pro-B-type natriuretic peptide

Similarly to patients with MS, those with mitral insufficiency (MI) also show increase in levels of BNP and NT-pro-BNP, and the correlation of increased levels of these markers with MI severity varies among the studies. In contrast, their correlation

with the occurrence and intensity of HF symptoms is better established. Some studies have shown a direct association of BNP or NT-pro-BNP levels with PASP at rest or after stress echocardiography³⁴. In general, studies examining these markers in patients with AF found a correlation between their levels and the occurrence of this arrhythmia³⁵.

More recently, studies showed a correlation between BNP levels and clinical outcomes. In a study including 269 asymptomatic patients with severe AMI and left ventricular ejection fraction (LVEF) > 60%, BNP levels > 105 pg/mL were associated with a higher risk of progression to the composite outcome of HF, ventricular dysfunction or death³⁶. A study including 135 asymptomatic patients with moderate to severe AMI in sinus rhythm and without ventricular dysfunction demonstrated that those with BNP < 40 pg/mL had higher event-free survival in up to 2 years of follow-up³⁷.

Therefore, despite the variation in cutoff values among studies, BNP measurement may be considered complementary to clinical and echocardiographic evaluations in patients with limited echocardiographic window, or when the cause of the symptoms is questionable, with low levels of this marker suggesting in asymptomatic patients a better outcome.

Prognostic biomarkers in tricuspid valvulopathy

Data on biomarkers in patients with tricuspid valvular disease are very scarce. Yoon et al.³⁸ observed that BNP had a direct correlation with right ventricular volume, and an inverse correlation with LVEF in patients with severe isolated tricuspid insufficiency who had undergone surgical treatment. In addition, patients with BNP < 200 pg/mL had lower postoperative mortality at 1 year. However, it is worth mentioning that only five deaths had occurred (one in the BNP < 200 pg/mL group and four in the BNP ≥ 200 pg/mL group). Therefore, it is not possible at the moment to recommend the use of biomarkers in the evaluation of patients with isolated tricuspid valvulopathy.

Prognostic biomarkers in aortic stenosis

The ideal biomarker in aortic stenosis (AoS) should reflect the severity of the disease, increase with the progression of the disease, reveal subclinical myocardial dysfunction, and identify asymptomatic patients who will develop short- or medium-term symptoms. The ideal biomarker should also identify patients with severe high-risk AoS who would benefit from surgical treatment before the emergence of symptoms, improving their prognosis.

Brain natriuretic peptide and the N-terminal pro-B-type natriuretic peptide

Disease severity and functional status

Patients with AoS have increased serum levels of BNP and NT-pro-BNP, with a direct correlation with pressure gradient, left ventricular mass index, and left ventricular end-systolic pressure, and an inverse correlation with valvular area³⁹. This suggests that plasma levels of natriuretic peptides are associated with disease severity and may be used to monitor the progression of the disease.

Serum levels of NT-pro-BNP show a direct association with AoS severity and symptoms assessed by functional class. In addition, values of NT-pro-BNP < 676 pg/mL and BNP < 130 mg/dL have emerged as predictors of event-free survival in a study with 130 patients with severe AoS⁴⁰.

The consistency of the results in these studies demonstrates that natriuretic peptides are associated with severity and symptoms of AoS. In addition, both peptides are predictors of the appearance of symptoms in asymptomatic patients. Therefore, BNPs are biomarkers that may be incorporated in the evaluation of patients with AoS in association with clinical and echocardiographic assessment, especially when it is unclear whether the patient is indeed asymptomatic.

Prognosis

The prognostic role of BNP has been evaluated in several studies. Lim et al. analyzed 70 patients with AoS and demonstrated that BNP values > 97 pg/mL were associated with worse prognosis. In addition, BNP emerged as the only independent predictor of mortality⁴¹.

In a cohort of 124 patients with clinically-treated moderate to severe AoS, survival was influenced by the presence of symptoms and levels of BNP. One-year mortality was 6%, 34%, and 60% for each tertile increase in BNP. There were no deaths in patients with BNP < 100 pg/mL⁴².

BNP had better accuracy than the logistic EuroSCORE to predict perioperative mortality in 144 patients with severe AoS undergoing surgical treatment, with BNP > 312 pg/mL emerging as the only predictor of mortality⁴³.

A recent Brazilian study assessed the long-term (up to 6 years) prognostic value of BNP and NT-pro-BNP in 64 patients with AoS⁴⁴. A total of 51 patients underwent surgical treatment, whereas 13 were followed up clinically. Mortality rates were 13.7% in the surgical group and 62% in the clinical group ($p < 0.001$). Patients with baseline BNP > 135 pg/mL and NT-pro-BNP > 1150 pg/mL had higher mortality.

Low-flow and low-gradient AoS has been associated with worse prognosis and higher surgical mortality. One-year survival in patients with BNP ≥ 550 pg/mL have been reported as $47 \pm 9\%$ compared with $97 \pm 3\%$ in those with BNP < 550 pg/mL ($p < 0.0001$)⁴⁵.

Although these studies clearly demonstrate the potential of BNPs as prognostic markers in patients with asymptomatic AoS, it is unclear whether surgery would decrease the mortality in patients with high levels of BNP/NT-pro-BNP. However, in patients with symptomatic AoS, natriuretic peptides may be incorporated into clinical practice to improve perioperative risk stratification. When the results of the studies are analyzed in combination, BNP levels of 130 pg/mL and NT-pro-BNP levels of 640 pg/mL may be identified as the best cutoff values to stratify patients with greater severity.

CRP

Some studies have reported high levels of CRP in patients with AoS of degenerative etiology, suggesting that inflammation may have a role in the progression of the disease⁴⁶. Imai et al.⁴⁷ evaluated retrospectively 135 patients with asymptomatic

AoS. Levels of CRP were significantly higher in patients with severe AoS when compared with those in patients with mild or moderate AoS. On multivariate regression analysis, CRP emerged as an independent predictor of AoS severity⁴⁷.

Studies that evaluate the role of CRP in assessing the severity, progression, and prognosis of AoS are scarce and controversial. Therefore, it is not possible at this moment to recommend for or against the use of CRP in these patients.

High-sensitive troponins

New (fifth generation) cardiac TPN T assays are known as high-sensitive TPN (hs-TPN) assays. These assays identify low levels of circulating TPN and, therefore, have the potential to be used for prognostic stratification in patients with stable or subclinical cardiovascular disease.

All 57 patients with AoS and myocardial hypertrophy who were included in a Norwegian study showed detectable TPN levels⁴⁸. Levels of TPN correlated with the diameters of the left ventricle, aortic jet velocity, pressure gradient and left ventricular mass. The discriminatory capacity, *i.e.*, the prognostic accuracy of hs-TPN, was similar to that of NT-pro-BNP. On multivariate regression, both hs-TPN and NT-pro-BNP were independent predictors of mortality.

Hs-TPN appears to be a promising biomarker to evaluate the prognosis of patients with severe AoS. However, prospective studies with a larger number of patients should be conducted before this new biomarker is incorporated into clinical practice.

Prognostic biomarkers in aortic insufficiency

Pressure overload in the myocardium and myocardial fiber distension trigger the release of natriuretic peptides. Serum levels of BNP and NT-pro-BNP are elevated in patients with aortic insufficiency (AoI) and are associated with disease severity⁴⁹. Weber et al.⁵⁰ have demonstrated that levels of NT-pro-BNP are directly associated with AoI severity and functional class.

Another study has also shown that NT-pro-BNP and BNP are associated with symptoms and left ventricular function in AoI. Spina et al.⁵¹ evaluated prospectively 89 young patients with AoI of rheumatic etiology and demonstrated that BNP had the same ability to predict the occurrence of symptoms and requirement of valve replacement when compared with ventricular diameters evaluated by echocardiography during a follow-up of 8 years.

Recently, a study showed a prognostic role for BNP in 294 patients with asymptomatic AoI without ventricular dysfunction. The cutoff value that best discriminated the patients who presented symptoms, ventricular dysfunction, or death was 130 mg/dL. In addition, BNP showed better prognostic ability than echocardiographic parameters, such as end-systolic diameter and effective regurgitant orifice⁵².

Thus, despite the variation in cutoff values among the studies, measurement of BNP/NT-pro-BNP may be considered as a complementary method to the echocardiographic and clinical investigations, or to clarify the cause of the symptoms, with low levels in asymptomatic patients suggesting a better outcome.

Biomarkers in cardio-oncology

Biomarkers of response to therapy and cardiotoxicity

Early diagnosis of cardiotoxicity has a close association with treatment response, which reflects a demand for more suitable markers. In this scenario, biomarkers have been proposed as an effective alternative, with greater sensitivity and specificity.

The value of TPN in evaluating myocardial damage induced by anthracyclines was demonstrated more than a decade ago in animal studies⁵³. This use has been confirmed then in several clinical trials. Measurement of TPN levels in early and late stages has been shown to detect myocardial damage, higher incidence of cardiac events, and subsequent decrease in LVEF, particularly in groups with levels persistently increased during chemotherapy⁵⁴. More recently, increases in TPN levels have been shown to predict substantial increases in the risk of adverse cardiac events during use of trastuzumab, a monoclonal antibody⁵⁶. Natriuretic peptides also have been shown in most studies to have good sensitivity to detect cardiotoxicity, although some of these studies involved small samples and had the assessment of diastolic function as objective⁵⁶.

New biomarkers have been considered and are currently under investigation. Cytochrome C has emerged as an alternative to evaluate mitochondrial damage, with great potential for early detection of cardiotoxicity secondary to chemotherapeutic agents. Genetic investigations may also allow a more customized oncological therapy in the future⁵⁷.

Biomarkers in pulmonary embolism

Pulmonary embolism (PE) remains a challenge in contemporary medicine, despite the improvements in methods for its diagnosis. Due to a low specificity in clinical presentation, predictive rules, such as the Wells score, became necessary in PE⁵⁸ to improve the accuracy of clinical diagnosis and a more rational use of diagnostic tests. Use of biomarkers in PE may be useful in the diagnosis and prognosis of the disease when management is uncertain.

D-dimer

During the acute phase, when patients are stratified based on the likelihood of PE, D-dimer measurement has been widely used to select individuals whose diagnosis can be excluded with an acceptable margin of error. Levels of D-dimer, a by-product of fibrin degradation, are often above normal during thrombus formation and are elevated in most patients with PE. However, since several other circumstances may also affect D-dimer values, the method is characterized as having high sensitivity, but low specificity.

Studies have shown that results of D-dimer within the normal range exclude with a good safety margin the diagnosis of PE in patients with low or moderate clinical probability of the disease⁵⁹. Gingsberg et al.⁶⁰ demonstrated that D-dimer values < 500 ng/dL have broader application in excluding the diagnosis of PE due to its high negative predictive value. Several techniques for measurement of D-dimer have been described with different results. Enzyme-linked immunosorbent

assay (ELISA) is a method with high sensitivity, whereas other techniques, particularly latex and whole blood agglutination methods, show worse results. Therefore, the identification of the technique adopted by the laboratory is fundamental.

Troponin

The biomarker TPN, which has been well studied in ACSs, has been shown useful for risk stratification in patients with PE. The increase in TPN level is independent of the presence of obstructive lesions and occurs due to impaired coronary perfusion caused by myocardial lesion secondary to acute right ventricular overload. TPN is elevated in 30 to 50% of the cases of PE and is associated with a worse prognosis. Giannitsis et al.⁶¹ demonstrated that TPN is an independent predictor of 30-day mortality. TnT has been detected in 50% of the patients and correlated with greater number of in-hospital deaths and complications⁶². Elevations in both TnT and Tnl have been associated with worse prognosis. Despite the association with right ventricular dysfunction, TPN was not helpful in guiding the appropriate therapeutic management in hemodynamically stable patients.

Brain natriuretic peptide

BNP has been used in the emergency room to investigate patients with suspected HF⁶³. The association between elevations in BNP and left ventricular dysfunction in patients with PE has been demonstrated by Kucher et al.⁶⁴, who showed that values < 50 pg/mL, which are below the cutoff value used for the left ventricle, would discriminate better those patients with a good prognosis. A meta-analysis published in 2011 showed that increases in levels of natriuretic peptides correlated with short-term mortality in patients with hemodynamically stable PE⁶⁵. Although the use of BNP in the differential diagnosis of PE in the emergency room showed clinical effectiveness, isolated measurement of BNP, similarly to TPN, was not helpful in guiding the therapeutic approach.

Other biomarkers

In a study published in 2012, Gul et al.⁶⁶ analyzed the association between h-FABP and the pulmonary artery obstruction index determined by chest computed angiotomography, showing this biomarker to be an independent predictor of mortality. Also in 2012, a German study with 126 patients with hemodynamically stable PE evaluated the value of h-FABP to predict death and complications in 30 days, concluding that this biomarker may be useful for risk stratification in normotensive patients.

New biomarkers require more comprehensive studies to be incorporated into clinical practice.

Biomarkers in cardiorenal syndrome

Cardiorenal syndrome (CRS) is highly prevalent in patients with chronic HF and is associated with higher morbidity and mortality at 1 year. The syndrome is present in approximately 30 to 40% of the patients with acute HF and is a marker of worse prognosis, showing association with a higher rate of hospital readmission and mortality in 30 days and 6 months after hospital discharge⁶⁷.

Other situations may present with acute renal failure (ARF), such as cardiac surgery and contrast-induced nephropathy associated with cardiac catheterization. Early diagnosis of renal injury and monitoring of its progression with specific biomarkers are of extreme importance in the prevention and management of CRS. Despite the use of traditional biomarkers of renal function to diagnose ARF, they have low accuracy to diagnose early changes in renal function⁶⁸.

Serum levels of urea and creatinine (S-Cr) remain unchanged until renal function is decreased to about half, and may have an important influence from several extra-renal factors, such as muscle mass, hepatic metabolism, digestive bleeding, and nutritional status. Serum levels of new biomarkers of renal dysfunction increase earlier and are highly specific, emerging as sensitive markers of organic (more than functional) dysfunction in ARF. These biomarkers are associated with the glomerular function (cystatin C – Cys-C) and renal injury (neutrophil gelatinase-associated lipocalin – NGAL; interleukin 18 – IL-18; and kidney injury molecule-1 – KIM-1)⁶⁹.

Cystatin C

Cys-C is an endogenous cysteine proteinase inhibitor produced at constant rates by all nucleated cells. It is freely filtered by the glomerulus and reabsorbed in the proximal convoluted tubule. Serum levels of Cys-C are associated with early detection of glomerular filtration rate (GFR) reduction, are markers of ARF, and are not influenced by body weight, age, or gender⁷⁰.

In patients with AHF, Cys-C demonstrated inferior ability to determine the prognosis of type 1 CRS when compared with NGAL⁷¹. In these patients, Cys-C was also shown to be an independent marker of in-hospital prognosis and death, and hospital readmission at 1 year⁷².

In outpatients with chronic HF, Cys-C has emerged as an independent prognostic marker at 1 year of follow-up⁷³.

Kidney injury molecule-1

KIM-1 is a kidney injury molecule detected in the urine in the presence of ischemic or nephrotoxic injury to the proximal tubule cells. In contrast-induced ARF, peak levels of KIM-1 occur 24 to 48 hours after catheterization, which are later than peak levels observed with other biomarkers (NGAL, IL-18 and Cys-C)⁷⁴.

In patients with chronic HF and apparently normal kidney function, serum levels of KIM-1 correlated with the degree of HF and ventricular dysfunction, indicating possible renal tubular injury not detected by usual biomarkers. In outpatients, KIM-1 levels showed weak correlation with GFR, but the prognostic value of KIM-1 levels on the development of CRS has not been yet evaluated⁷⁵.

Interleukin-18 (IL-18)

IL-18 is a pro-inflammatory cytokine activated in several renal disorders, including ischemia, inflammatory injury (infectious or not), and malignant diseases. In contrast-induced nephropathy, IL-18 diagnosed ARF earlier than

serum creatinine and emerged as an independent predictive marker of cardiovascular events⁷⁶. No clinical studies have been conducted to evaluate the role of IL-18 in the diagnosis and prognosis of CRS in patients with acute or chronic HF.

Neutrophil gelatinase-associated lipocalin (NGAL)

NGAL, a protein associated with neutrophil granules, is secreted by activated immune cells, hepatocytes, colon, lung, myocardium, and renal tubules. It is activated and released after ischemic renal injury, renal and systemic infectious aggression, and during inflammation and myocardial ischemia⁷⁷. Serum NGAL is filtered by the glomerulus and reabsorbed in proximal and (mainly) distal tubules along with renal NGAL.

NGAL may be measured in the plasma and urine. Urinary NGAL has an earlier peak and greater accuracy to detect renal dysfunction, with 100% of sensitivity and 98% of specificity, and is an early biomarker in the diagnosis of ARF, increasing 24 to 48 hours before S-Cr⁷⁸.

Serum NGAL > 140 ng/mL on hospital admission or on the third day after admission has been associated with a 7.4-fold increase in CRS risk, with 86% sensitivity and 54% specificity. NGAL values < 140 ng/mL on admission had a negative predictive value of 86% for development of CRS during admission⁷⁹. Similar results have been observed in patients in the emergency room, in whom serum NGAL > 170 ng/mL predicted the development of type 1 CRS with 100% sensitivity and 86.7% specificity.

Serum levels of NGAL determined on hospital discharge in patients with AHF were shown superior to BNP as a prognostic marker for mortality and hospital readmission within 30 days from discharge⁸⁰.

Among the new biomarkers for CRS detection, NGAL has been the most extensively and carefully studied, with focus on its ability to detect renal dysfunction and on the prognostic value in patients with acute and chronic HF. However, several points need to be clarified for a consistent use of NGAL in early diagnosis of CRS in patients with HF in clinical practice:

(1) definition of the cutoff values of serum and urinary NGAL for the diagnosis of CRS. These values and their "zone of variation" are not well established, since the levels of NGAL are influenced by several clinical variables other than ARF (age, gender, obesity, previous kidney dysfunction, and type of nephrological injury); (2) assessment of the importance of the peak value and the pattern of the curve variation in NGAL levels on the prognostic definition of the degree of ARF.

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Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Sources of Funding

This study was funded by ABBOT Diagnósticos do Brasil.

Study Association

This study is not associated with any thesis or dissertation work.

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