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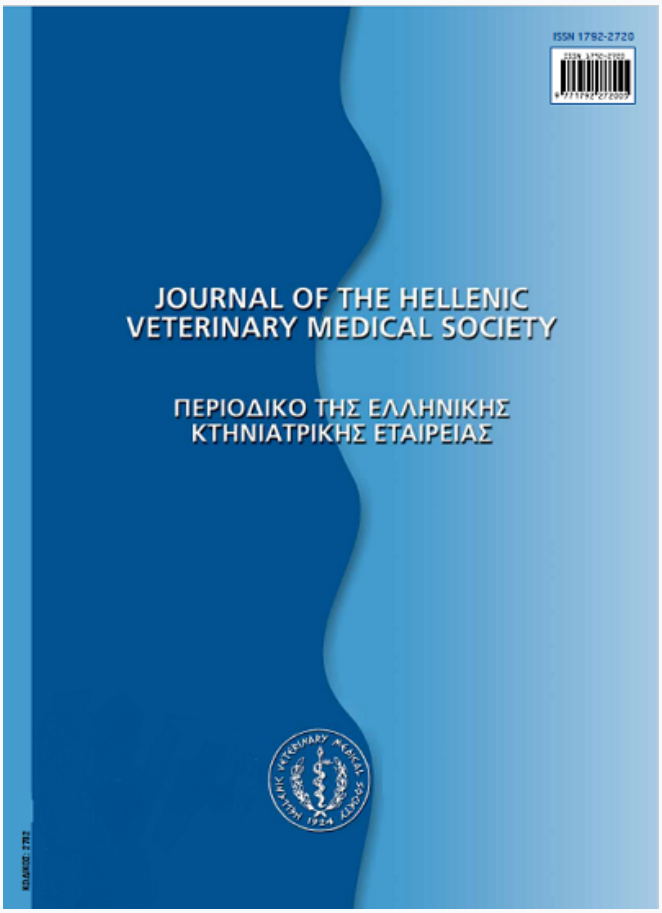

The diagnostic significance of cardiac biomarkers in veterinary medicine

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Review article
Ανασκόπηση

The diagnostic significance of cardiac biomarkers in veterinary medicine

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Η διαγνωστική αξία των καρδιακών βιοχημικών δεικτών στην κτηνιατρική πράξη

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ABSTRACT

Cardiac biomarkers are a group of proteins that have been extensively studied in human and veterinary medicine during the past decades, regarding their diagnostic and prognostic importance in heart disease. Moreover, cardiac biomarkers have been evaluated for their efficacy in the assessment of clinical staging of heart disease, risk stratification of patients, selection of therapeutic regimens and prognosis of cases. Cardiac biomarkers are broadly classified, according to their role in the pathophysiology of heart failure, into markers of myocardial injury (cardiac troponins, creatine kinase MB isoenzyme), myocyte stress (natriuretic peptides), inflammation (acute phase proteins), cardiac remodelling (matrix metalloproteinases), neurohormonal and endothelial dysfunction (endothelin, aldosterone, rennin, norepinephrine). It is unlikely that all of the aforementioned biomarkers will eventually be applied in clinical practice. Nevertheless, some of them (cardiac troponins, natriuretic peptides) have already been available commercially and established as part of the diagnostic investigation in heart disease. This review article focuses on the role of both established and potential cardiac biomarkers in veterinary medicine.

Keywords: Cardiac biomarkers, cat, heart disease, dog

ΠΕΡΙΛΗΨΗ

Η χρήση των καρδιακών βιοχημικών δεικτών εφαρμόζεται σε σημαντικό βαθμό στην κτηνιατρική των ζώων συντροφιάς, εφόσον έχει αποδειχθεί η σημασία τους στην γρήγορη, μη επεμβατική αξιολόγηση του σταδίου της καρδιακής ανεπάρκειας και της κλινικής διαβάθμισης των καρδιοπαθειών. Οι καρδιακοί βιοχημικοί δείκτες ταξινομούνται σε ομάδες με βάση το παθοφυσιολογικό υπόβαθρο των μεταβολών τους (εκφύλιση και νέκρωση καρδιακών μυϊκών ινών, αναδιαμόρφωση μυοκαρδίου, αιμοδυναμι-

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κές μεταβολές, συστηματική φλεγμονώδης αντίδραση, νευροορμονικός έλεγχος). Παρά το σημαντικό αριθμό των ερευνητικών μελετών που αφορούσαν πολλές από τις ουσίες αυτές, τελικά ο αριθμός των καρδιακών βιοχημικών δεικτών που απέκτησε κλινική εφαρμογή στην καθημερινή πράξη είναι μικρός και περιλαμβάνει τις καρδιακές τροπονίνες (I και T) και τα νατριουρητικά πεπτίδια των κόλπων και του εγκεφάλου. Στη βιβλιογραφική αυτή ανασκόπηση αναφέρονται η κλινική σημασία, οι εφαρμογές των καρδιακών βιοχημικών δεικτών στην κτηνιατρική πράξη και η συμβολή τους στη διαγνωστική διερεύνηση των καρδιοπαθειών.

Λέξεις ευρετηρίασης: Βιοχημικοί δείκτες, γάτα, καρδιακή νόσος, σκύλος

INTRODUCTION

According to the current definition standardized by the National Institute of Health (2001), "a biomarker is a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention". Over the last two decades there has been a considerable amount of research in human and veterinary medicine, related to the use of circulating markers of cardiovascular disease in the detection of subclinical disease, the diagnosis of acute or chronic syndromes, the risk stratification of patients, selection of the appropriate treatment and monitoring disease progression and response to therapy (Schober, 2005; Braunwald, 2008; Prošec and Ettinger, 2010). At least 40 substances have been identified up to date and recently classified according to their origin and function as markers of myocyte injury (leakage markers), myocyte stress (functional markers), cardiac remodelling, inflammation, endothelial dysfunction and neurohormonal alterations (Braunwald, 2008; Prošec and Ettinger, 2010). It is, however anticipated that very few of these currently studied measurands will survive the test of long term application in clinical practice (Table 1). A review of all available markers is beyond the scope of this text, since information on newer emerging as well as older established markers is rapidly increasing. Instead, a focus on those already used or currently evaluated in veterinary patients is preferred.

It has been proposed that a candidate cardiac biomarker should fulfil certain criteria in order to have some clinical significance. Desirable features of an ideal biomarker should include low biological variation, absolute cardiospecificity, stability (in vivo and in vitro), adequate analytical sensitivity, accuracy and reproducibility of available diagnostic assays, which should have the potential of automation and international standardization at a reasonable cost and the establishment of reference ranges and cut-off values for each species, breed, age and gender. In addition, the assay should provide information superior to that already obtained from clinical examination and should contribute to the improvement of decision making regarding therapy and prognosis (Morrow and de Lemos, 2008).

The clinical significance of cardiac biomarker evaluation lies in their potential of identifying animals or animal populations at risk of being affected by specific heart diseases, with early asymptomatic/minimally symptomatic congestive heart failure (CHF) or impending deterioration of cardiac function. Furthermore, information about the evolution of cardiac diseases could be useful for monitoring the efficacy of treatment (Schober, 2005)

MARKERS OF MYOCYTE INJURY

Markers of myocardial integrity offer evidence of cell injury in the context that intracellular elements escape into circulation when the cell membrane is severely

Table 1. Classification of cardiac biomarkers evaluated in human and veterinary medicine*

Myocyte stress	Inflammation	Oxidative stress	Extracellular matrix remodelling	Neuro-hormones	Myocyte injury	New biomarkers
B-type natriuretic peptide (BNP)	C-reactive protein (CRP)	Oxidized low density lipoproteins	Matrix metalloproteinases (MMP)	Norepinephrin	Cardiac troponins I and T	Adiponectin
N-terminal pro-BNP	Tumour necrosis factor -a (TNF-a)	Myeloperoxidase	Propeptide procollagen type I	Renin	Creatine kinase MB fraction	Chromogranin
ST2	Interleukins (IL) 1, 6, 18	Urinary biopyrrins	Plasma procollagen type III	Arginine vasopressin	Myosin light-chain kinase I	Osteoprotegerin
Adrenomedullin	Fas	Urinary and plasma isoprostanes	MMP tissue inhibitors	Angiotensin I	Heart-type fatty-acid protein	Galectin 3
		Plasma malondialdehyde		Aldosterone		
				Endothelin		

**Modified from Braunwald, 2008*

damaged. The pathologic changes imposed on the cardiac muscle in heart disease, such as pressure and volume overload, hypoxia, ischemia, neurohormonal alterations and cytokine release, lead to myocardial cell injury and ultimately heart failure. Analysis of cardiac leakage markers has progressed from the determination of specific enzyme activity (creatine kinase isoenzyme MB, lactate dehydrogenase isoenzymes 1 and 2), to the measurement of structural myofibrillary proteins (cardiac troponins I and T).

Creatine kinase (CK) is a dimeric enzyme with M and B subunits, which combine to form the three isoenzymes CK-MM (or CK 3), CK-BB (or CK 1) and CK-MB (or CK 2). A fourth variant form, CK-Mt, is found in mitochondria of several tissues (Stockham and Scott, 2008). Serum CK-MB activity has been used in the past mainly as an indicator of acute myocardial injury, since it is released early after the insult, cleared rapidly and is undetectable in normal dogs and cats (Schober, 2005; Valberg, 2008). Nevertheless, it is not specific for cardiac muscle tissue and can be found in significant amounts in skeletal muscles, the lungs, intestines and spleen (Fredericks et al., 2001; Valberg, 2008; Stockham and Scott, 2008). In addition, CK-MB immunoreactivity is not well conserved across species and there are no commercially available and species-specific assays for use in the dog and cat (O'Brien, 1997; Schober, 2005).

Lactate dehydrogenase (LD) is a cytoplasmic enzyme with H (heart) and M (muscle) subunits forming LD isoenzymes (LD 1-5). LD 1 and 2 also form alpha hydroxybutyrate dehydrogenase (a-HBDH) that has some specificity for the myo-

Table 2. Cardiac and non-cardiac causes of increased cardiac troponins in dogs and cats

Cardiac causes	Non-cardiac causes
Myocardial infarction	Blunt chest trauma
Pericardial effusions	Aortic thromboembolism
Congenital heart diseases	Gastric dilatation-volvulus
Cardiomyopathies (hypertrophic, dilated)	Pyometra/sepsis
Myocarditis	Babesiosis/Ehrlichiosis
Endocardiosis	Renal failure
Drug induced cardiotoxicity	Strenuous exercise
Arrhythmias	Epilepsy
	Neoplasia
	Hypoxia

Table 3. Cardiac and non-cardiac causes of increased natriuretic peptides in dogs and cats

Cardiac causes	Non-cardiac causes
Ventricular hypertrophy	Systemic hypertension
Endocardiosis	Pulmonary hypertension
Congenital heart diseases	Age
Dirofilariosis	Exercise
Arrhythmias	Renal disease
Cardiac hypoxia	

cardium, however both these isoenzymes have several additional sources (erythrocytes, brain, kidneys, pancreas). Moreover, isoenzyme analysis requires special assays and interpretation of results is difficult due to interspecies variation. For these reasons CKMB and LD isoenzymes are no longer recommended as biomarkers for the diagnosis of myocardial injury in the dog and cat (Valberg, 2008; Stockham and Scott, 2008).

Cardiac troponins (cTn) are the most common "leakage" markers applied in veterinary medicine, with several clinical studies demonstrating their value in the detection of myocardial injury, regardless of the primary etiology (Spratt et al., 2005; Wells and Sleeper, 2008). The troponin complex comprises of myofibrillary proteins involved in the regulation of actin-myosin interaction and is composed of 3 subunits, troponin C (cTnC, binding Ca^{+}), troponin I (cTnI, inhibiting tropomyosin) and troponin T (cTnT, binding troponin). In the cardiac myocyte the majority of troponin content is structurally bound, whereas a smaller percentage (3-8% for cTnI and 6-8% for cTnT) is free in the cytoplasm (Sleeper et al., 2001; Oyama and Sisson, 2004). A biphasic cTn release pattern has been suggested to occur in conditions causing loss of cell membrane integrity

and cell necrosis (Prošec and Ettinger, 2010). Slowly progressing damage of the sarcomeres causes initial release of the cytosolic troponin pool, however if the injury continues or increases in severity the structurally bound amounts of cTn are also released, reflecting an irreversible damage to the myocardium (Archan and Fleisher, 2010). Cardiac troponin I is exclusive to the cardiac myocyte, it is immunologically different from its skeletal muscle counterpart and has been shown to increase more frequently and in the earlier stages of many small animal cardiac diseases, when compared to cTnT (Spratt et al., 2005; Wells and Sleeper, 2008). The serum cTnI concentration has been shown to correlate with the evolution of pathophysiologic alterations affecting the myocardium, clinical presentation, histopathologic lesions and outcome of cases (Linklater et al., 2007; Wells and Sleeper, 2008; Serra et al., 2010).

In dogs suffering from cardiac disease of various etiologies and severity there has been a correlation of serum cTnI concentration with the severity of clinical signs. In some of these studies it has been suggested that serial troponin assessment would offer an additional benefit in the management of cardiac patients (Linklater et al., 2007). cTnI was found to be useful in assessing the severity and prognosis in dogs suffering from a variety of congenital and acquired cardiac diseases (Ljungvall et al., 2008; Fonfara et al., 2010). In chronic heart failure, persisting troponin levels have been correlated with clinical and functional worsening and associated with a poorer prognosis and shorter survival time (Tsutamoto et al., 2010, Kawahara et al., 2011). Although an association has been found between the severity of myocardial damage and serum concentration of troponins in acute cardiac problems (e.g. infarction, myocarditis), this is not the case in chronic cardiac disease (eg mitral valve disease, dilated cardiomyopathy). Increases in serum cTn concentration in chronic cardiac diseases are more subtle, although serial monitoring may improve the correlation with the deterioration of cardiac function and the overall prognosis (Fonfara et al., 2010; Tousoulis et al., 2012). Recently, persistently elevated cTnI levels in dogs with endocardiosis were associated with the slowly worsening clinical status, thus indicating the advancement of heart failure. In the latter, increased cTnI concentration is probably attributed to the ongoing myocardial injury and cardiac remodelling lesions which follow chronic hemodynamic abnormalities inflicted by this condition (Tousoulis et al., 2012; Polizopoulou et al., 2014).

Nevertheless, and because troponins have been shown to increase in many cardiac and some non-cardiac diseases (e.g. renal failure, sepsis, babesiosis, thromboembolism, gastric dilataion-volvulus syndrome), it seems than in contrast to human beings their role may be more important in the follow up and prognosis of heart disease in dogs and cats, rather than in its primary diagnosis (Table 2) (Schober et al., 1999; Schober et al., 2002; Prošec et al., 2007; Prošec and Ettinger, 2010, Polizopoulou et al., 2014). Recently, a classification system for the interpretation of cTn results in combination with clinical scoring has been proposed (Prošec and Ettinger, 2010). The development of high sensitivity assays for troponins will improve further their diagnostic significance, as earlier studies had the disadvantage of high detection limits, raising the question on whether patients with mild or moderate disease had undetectable cTn levels and were thus misdiagnosed (Prošec and Ettinger, 2010).

Another issue that has to be taken into consideration when interpreting cTnI results is the assay method and cut-off values used, because lack of standardization inevitably causes significantly variability. This variation is the reason why attention must be paid when data and results of cTnI are interpreted and preferably compared with those from age matched healthy individuals (Schober, 2005; Adin et al., 2006). Since it is impractical in animals to set two cut-off points (one representing the upper reference

limit and one indicating clinically relevant myocardial damage) it appears that the dynamic monitoring of cTnI concentration could be more useful in assessing of myocardial dysfunction (Linklater et al., 2007). In contrast, cTnT assays are relatively uniform regarding cut-off concentrations and precision (Serra et al., 2010).

The diagnostic yield of cTnI may decrease in patients with many co-morbidities, particularly chronic renal failure. The pathogenesis of troponin elevation in chronic renal failure has been considered a multifactorial effect attributed to various pathomechanisms such as hypotension, cardiac microcirculation dysfunction, release of myotoxic substances, but still remains unclear (Buhaescu et al., 2006; Porciello et a., 2008).

MARKERS OF CARDIAC MYOCYTE STRESS

The natriuretic peptides (NPs) are considered to be markers of increased myocyte stress (functional markers). They have been shown to antagonize the renin-angiotensin-aldosterone system (RAAS), protect the cardiovascular system from volume and pressure overload, inhibit the proliferation of smooth muscle cells and induce bronchodilation (Schober, 2005; Braunwald, 2008). Those considered to be more important from a clinical point are the atrial (ANP) and brain or B-type (BNP) natriuretic peptides.

ANP is synthesized in the atria from its precursor proANP and, responding to increased pressure and tension, is released in its two forms, the inactive N-terminal (NT)-proANP and the biologically active C-terminal ANP. BNP is produced in the atria and to a lesser extent in the ventricles and released to the circulation also as the inert NT-proBNP and biologically active BNP forms (Braunwald, 2008). However, in chronic cardiac disease the ventricular myocardium becomes the primary source of its synthesis (Moe, 2006; Prošec and Ettinger, 2010).

As with cTn, ANP and BNP are not specific for a particular heart disease, but their importance may lie in their use as functional markers of ongoing cardiac conditions (Table 3). In human medicine the natriuretic peptides and in particular BNP and NT-proBNP, are currently established parameters in assessing the severity and prognosis of cardiac disease, found also to provide greater sensitivity and specificity than conventional diagnostic methods. Thus, the clinical significance of NT-proBNP monitoring has been associated with improved diagnostic accuracy, shorter hospitalization time, prognostic prediction and risk stratification of patients (Januzzi et al., 2005; Fonarow et al., 2008; Jourdain et al., 2007). It has been suggested that the interpretation of ANP and BNP concentrations could reflect the pathophysiology of a particular cardiac disease since ANP is produced mainly in the atria whereas BNP in the ventricles (Prošek and Ettinger, 2010).

Guidelines for the interpretation of NT-proBNP values in dogs and cats can be retrieved from assay manufacturer websites (www.idexx.com), however as there is active ongoing research regarding the clinical applications of this test, recommendations may change after the evaluation of large numbers of patients. Briefly, in asymptomatic animals NT-proBNP values of less than 900 pmol/l (in dogs) or 100 pmol/l (in cats) indicate a low risk of clinically significant heart disease, whereas values greater than the aforementioned suggest an increased probability of cardiac disease that should be further investigated with other diagnostic means (thoracic radiography, echocardiogram, electrocardiogram).

In veterinary medicine research on the potential significance and utility of NP determination has increased in the past few years. Circulating NP levels, especially NT-proBNP and BNP, have been shown to correlate with the severity of cardiac disease (class of heart failure), the degree of cardiac remodelling (Oyama et al., 2008; Chetboul et al., 2009; Wess et al., 2011), response to therapy, prog-

nosis of outcome (Chetboul et al., 2009; Moonarmart et al., 2011) and even differentiate cardiac from non-cardiac conditions in dogs (DeFrancesco et al., 2007; Prošek et al., 2007; Fox et al., 2009). In cats, serum NP monitoring has proved to be clinically useful as an initial screening test for suspected cardiac disease (Connolly et al., 2008; Wess et al., 2011).

Another potential application of NP determination could be the identification of patients likely to benefit from vasodilator therapy (Clerico et al., 2009). There is still ongoing debate on whether ANP (as indicated by the earlier veterinary studies) or BNP (as suggested by more recent data) is the most appropriate biomarker for clinical use in the dog and cat (Boswood et al., 2008). In addition, there is recent evidence that azotemia may increase circulating NP levels, a factor that should be taken into consideration when interpreting results in a clinical setting (Schmidt et al., 2009).

Other biomarkers of cardiomyocyte stress are adrenomedullin (a potent vasodilator, inotropic and natriuretic substance) and ST2 protein, a member of the interleukin-1 receptor family, however none of them has been evaluated clinically in veterinary medicine (Schober, 2005; Boswood, 2009).

NEUROHORMONAL MARKERS

Endothelin, a potent vasoconstrictor substance, has been recognized as a neurohumoral cardiac biomarker associated with heart failure (Sisson, 2004). The endothelin family is comprised of three related peptides, ET-1, ET-2 and ET-3 that are derived from larger peptides in a sequence similar to that of NP (Sisson, 2004; Braunwald, 2008; Prošek and Ettinger, 2010). Endothelin-1 (ET-1), a product of its precursor big endothelin-1, is the predominant form of endothelin produced mainly by the vascular endothelium. The endothelin system is activated in response to cardiac myocyte stretch, hypoxia, angiotensin II and cytokine effect (Sisson, 2004; Braunwald, 2008; Prošek and Ettinger, 2010). Apart from its vasoconstrictor action, ET-1 is a growth factor for myocardial, endothelial and smooth muscle cells. In chronic heart failure neurohormoral responses aiming in compensating the failing myocardium also increase the production of ET-1 (Braunwald, 2008; Boswood, 2009). Although initially beneficial, ET-1 mediated cardiac effects (activation of the renin-angiotensin-aldosterone axis, vasopressin and the sympathetic nervous system) have been associated with a negative prognosis in human patients, signalling deterioration of myocardial function (Mc Murray et al., 1992; Selvais et al., 2000). Increased ET-1 levels have been reported in canine and feline cases of chronic heart failure (Prošek et al, 2004a; Prošek et al, 2004b; O'Sullivan et al., 2007).

INFLAMMATION MARKERS

Inflammation markers have been identified to coexist with heart failure for many decades, however the stimulus responsible for this systemic inflammatory response has not been identified yet (Boswood, 2009; Prošek and Ettinger, 2010). Various theories for its pathogenesis have been proposed, among them chronic adrenergic stimulation, tissue necrosis or underperfusion, and bacterial dissemination secondary to altered permeability of the intestinal wall (Anker and von Haehling, 2004; Braunwald, 2008). Acute phase proteins (APP) are released from hepatic cells after stimulation by pro-inflammatory cytokines, early in the inflammatory process. In particular, C-reactive protein (CRP), a major positive APP in the dog, has been used as a marker for cardiovascular diseases in humans and animals (Anand et al., 2005; Rush et al., 2006; Saunders, 2009; Tousoulis et al., 2012).

It has been postulated that in chronic heart disease the damaged myocardium triggers the production of proinflammatory cytokines (TNF-α, interleukins 1, 6, 18), which induce apoptosis and necrosis of cardiac

cells and lead to a vicious cycle with further myocardial dysfunction and finally failure. Progressive hemodynamic overload, imbalance between pro- and anti-inflammatory mediators contribute to the establishment of heart failure (Tousoulis et al 2012). There is evidence both in human and veterinary medicine, that the increase in inflammatory markers is associated with clinical staging and prognosis of cases. In people with chronic heart failure neurohormonal mediators and inflammatory cytokines have been shown to increase in the course of cardiac remodeling (Osman et al., 2006).

Furthermore, CRP measurement has been used as a tool to monitor and predict the evolution of clinical signs in cardiac disease (Ridker, 2003). In the dog the half life of CRP is short, thus persistently elevated concentrations could be a warning sign in the management of chronic diseases like MVD (Ceron et al., 2005; Tecles et al., 2005). Increased CRP values have been recorded in dogs with acquired (Rush et al., 2006; Cunningham et al., 2012) and congenital (Saunders et al., 2009) cardiac diseases. However other studies failed to demonstrate that CRP could be a sensitive biomarker in MVD (Ljungvall et al., 2010). Limited information exists however regarding the potential significance of other APP such as haptoglobin (Hp) and ceruloplasmin (Cp) in canine cardiac diseases.

OTHER CARDIAC BIOMARKERS

There are several candidate cardiac biomarkers currently being investigated regarding their potential clinical usefulness in veterinary medicine (Table 1). Their future as objective and significant indicators of cardiac disease remains to be proven.

CONCLUDING REMARKS

The clinical role of cardiac biomarkers remains to be further elucidated, and this applies also to the only two of those discussed in this text that have been established as useful diagnostic indicators in veterinary medicine (cardiac troponins and natriuretic peptides). Lessons from human medicine have pointed out that the combined evaluation of several markers could be more valuable than single ones. Practical issues such as the simplicity of sample handling and the availability of assays should not be overlooked. The potential role of cardiac biomarkers in the early identification of animals at risk of developing heart failure will definitely be explored in the near future and might prove more important than their diagnostic or prognostic one.

CONFLICT OF INTEREST STATEMENT

The author of this paper does not have a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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