Dear Readers,

I have been serving as the Editor-in-Chief of the journal *Heart and Metabolism* since 1999 and I have enjoyed having had the opportunity to contribute to its development. Being Editor-in-Chief of *Heart and Metabolism* has been a very rewarding experience. During this period, the journal has continuously provided you with the newest findings about the role of metabolism in cardiac disease and its clinical and therapeutical implications. I also believe that we have made considerable progress over these past few years in improving the quality of the journal.

The growing interest and understanding of the role of cardiac metabolism has greatly boosted our readership. Today, the journal is distributed in over 30 countries worldwide, and its website has also become increasingly popular. However, due to new horizons in my professional career that require all my attention, I will not have the necessary time to devote to the journal and, as a result, shall be standing down.

Finally, I would like to express my sincere best wishes to the new Editor-in-Chief, Professor M. Marzilli, an experienced, dynamic and talented professional who I am certain will bring vision, passion and energy to the further development of the journal. I wish *Heart and Metabolism* and all its readers continuing success in the future.

Frans Visser
Former Editor-in-Chief

I am honoured to accept the position as Editor-in-Chief of *Heart and Metabolism*. I was a founding Board Member and have been deeply involved in the growth of *Heart and Metabolism* from the beginning.

In assuming this position and on behalf of all the members of the Editorial Board, I pledge that the journal will stay, true to its name, and will continue to give you the very latest evidence on all aspects of fundamental and clinical research on cardiac metabolism, as well as its therapeutical implications.

I will do my best to maintain and further enhance the scientific quality of the journal, so that it becomes recognized as a reference in this field.

At the same time I wish to make it even more attractive and useful for our readers.

A special thank you must go to Frans Visser for the incredible job he has done as Editor-in-Chief of *Heart and Metabolism* and for the kind words. It will not be easy to match the progress the journal has made under his direction, but, with the support of the Editorial Board and of the Editorial staff, we can maintain the pace!

Mario Marzilli
Editor-in-Chief
EDITORIAL
Biomarkers: past, present, and future
Graham Jackson ............................................................... 3

BASIC ARTICLE
The biological basis of troponin in heart disease: possible uses for troponin fragmentology
Vlad C. Vasile and Allan S. Jaffe ........................................... 5

MAIN CLINICAL ARTICLE
The role of biomarkers in clinical practice
Anthony S. Wierzbicki and Adie Viljoen ................................ 9

METABOLIC IMAGING
Troponin compared with late enhancement in the assessment of myocardial injury
Evangelos Giannitsis and Hugo A. Katus ................................. 13

NEW THERAPEUTIC APPROACHES
Trends in genomic biomarkers
Bruce McManus .......................................................... 19

FOCUS ON TRIMETAZIDINE (VASTAREL MR)
Effects of Vastarel MR on brain natriuretic peptide and cardiac troponin concentrations
Pericle Di Napoli ........................................................... 23

CASE REPORT
Serial determination of troponin concentrations in the diagnosis of acute myocardial infarction
Vlad C. Vasile, Lori A. Blauwet and Allan S. Jaffe .................... 27

REFRESHER CORNER
Temporal profile of protein release in myocardial infarction
Piero Montorsi, Marco Villa and Maria Antonietta Dessanai ........ 31

FEATURED RESEARCH
Abstracts and commentaries ............................................ 36

GLOSSARY
Gary D. Lopaschuk ......................................................... 40
Biomarkers: past, present and future

Graham Jackson
Department of Cardiology, Guy’s and St Thomas’ Hospitals NHS Trust, London, UK

Correspondence: Graham Jackson, Cardiology Department, Guy’s & St Thomas’ Hospital NHS Trust, Lambeth Palace Road, London SE1 7EH, UK.
e-mail: graham@jacksonmd.fsnet.co.uk

Coronary artery disease (CAD) continues to be a major cause of morbidity and mortality in both men and women in developed and developing countries. The extent of myocardial damage after an acute myocardial infarction (AMI) determines prognosis. The diagnosis of an AMI is based on a combination of symptoms, electrocardiographic changes, and biomarkers. In this issue of Heart and Metabolism, we focus on established and novel biomarkers and their role in diagnosis, risk stratification, and prognosis.

Drs Wierzbicki and Viljoen provide us with a comprehensive clinical overview, pointing out that, in addition to a diagnostic role in AMI, biomarkers are also used to monitor drug treatments and their potential toxicity. We tend to think in terms of C-reactive protein, troponin (T, I, or C), and brain natriuretic peptide (BNP), but a wider panel of markers is available to aid both diagnosis and risk evaluation.

In the Basic Article, Drs Vasile and Jaffe describe in detail the molecular biology of troponin in cardiac disease, and the potential for using troponin fragments to delineate differing pathological processes, leading to distinct diagnostic and therapeutic potential.

The time course of biomarker release is important, and differences that may occur, and their clinical relevance, are the subject of the Refresher Corner. We know that the release of protein at the time of AMI provides both diagnostic and prognostic information, so it is helpful to have a review of the time courses of individual biomarkers, which in turn provides a framework for evaluating therapeutic intervention (whether mechanical or pharmacological). The Case Report shows how these time courses or increasing trends are a valuable adjunct to management.

In the drug focus article, importantly we are reminded of the role of trimetazidine in reducing left ventricular remodeling and potentially improving prognosis. The significant reductions in troponin T and BNP concentrations after 6 months of treatment (60mg daily) compared with no trimetazidine treatment are associated with preservation of left ventricular function. This signals the need for a large-scale study to evaluate the symptomatic and prognostic role of trimetazidine in patients with reduced left ventricular function.

In the future, genetic biomarkers may transform our understanding and management of patients with CAD, so the New Therapeutic Approaches article by Professor McManus is both timely and enlightening.
The biological basis of troponin in heart disease: possible uses for troponin fragmentology

Vlad C. Vasile and Allan S. Jaffe

Department of Internal Medicine, Division of Cardiovascular Diseases and Department of Laboratory Medicine and Pathology, Mayo Clinic and Mayo Medical School, Rochester, Minnesota, USA

Correspondence: Dr Allan S. Jaffe, Mayo Clinic, 200 First St SW, Division of Cardiovascular Diseases, Gonda 5, Rochester, Minnesota 55905, USA.
Tel: +1 507 284 3680; fax: +1 507 266 0228; e-mail: Jaffe.Allan@mayo.edu

Conflict of interest: Dr Vasile, None; Dr Jaffe is a consultant and has received research support from Siemens and Beckman-Coulter. He is currently a consultant, or has consulted over time, for most of the major diagnostic companies.

Abstract

This article describes the molecular biology of troponin in heart disease. Prospective uses for this biomarker are mentioned, with a particular emphasis on cardiac troponin fragments that could be associated with distinct clinical entities. The article explores the topic of specific cardiac troponin fragments resulting from modification or degradation, linked with different pathological processes, as indications of novel potential uses for this biomarker in heart disease. In addition, it addresses some implications that could have an impact on several circumstances that confront physicians within clinical practice.

Keywords: Troponin fragments, phosphorylation, dephosphorylation

Introduction

Detection of myocardial injury relies on the highly sensitive and specific determination of cardiac troponin (cTn). Thus increased cTn is essential for the diagnosis of acute myocardial infarction (AMI) [1]. However, there may be other specific applications for cTn fragments, and increases in cTn concentrations are observed in conditions other than AMI.

The troponin complex consists of three subunits: troponin C (cTnC), troponin I (cTnI) and troponin T (cTnT); it interacts with components of the thin tropomyosin and actin filament to ensure correct contraction coupling [2]. Several isoforms of cTns C, I, and T assist in this task [3].

Troponin C (TnC) lacks cardiac specificity. In contrast, it is believed that all isoforms of cTnI are expressed exclusively in cardiomyocytes, thus its detection in the blood is synonymous with myocardial injury. The cTnI isoforms are characterized by a 32 amino acid posttranslational tail at the N-terminus [4]. The junction between this sequence and the stable central part of the molecule is the target for monoclonal antibodies that recognize cTnI [5]. Expression of troponin T or I is controlled by three genes [6]. By alternative splicing, several isoforms of cTnT are generated, with sequence variability regions located at the C- or the N-terminus, or both [6,7].

Conditions other than AMI in which cTn concentrations are increased

Physical exercise

Transient increases in cTnT occur after severe physical exercise, early during exertion [8]. Initially, it was
believed that these increases resolved in 24 h, but recent data from sensitive assays do not support this [9]. It is difficult to distinguish whether cTnI is released by reversible or irreversible injury. Similarly, whether there are reparative processes is unclear. Either way, different fragments might be elaborated in this situation.

### Chronic renal failure

Concentrations of cTnT are often abnormal in patients with chronic renal failure [10]. The etiology of these increases is obscure, but probably includes any of: endothelial dysfunction, acute cardiac stretch, intradialysis hypotension and hypertension, left ventricular hypertrophy, and coronary artery disease, occult or overt. Some authors report small cTn fragments [11] but others dispute that [12].

### Myocardial stunning

Myocardial stunning is a reversible myocardial dysfunction seen after ischemia. It also can be induced by arrhythmias or open heart surgery [13]. A specific degradation product occurs in this circumstance from the loss of 17 residues at the C-terminus of cTnI [14]. Transgenic animals overexpressing this truncated form, cTnI1–193, exhibit myocardial dysfunction with properties similar to those of myocyte stunning. A comparable fragment was found after bypass surgery [15]. In in-vitro experiments, transfection of truncated cTnI1–193 produces a diastolic impairment in healthy human cardiomyocytes [16]. This suggests that the degradation of cTnI contributes to diastolic dysfunction in vivo. If these cTn fragments were causative, they could be monitored to identify which patients with impaired function might improve.

### Transient left ventricular apical ballooning syndrome

This syndrome can mimic AMI clinically [17]. It is characterized by more extensive ventricular dysfunction than would be expected from the modest increases observed in cTn values, which are good surrogates for infarct size [18,19]. Phosphorylated or truncated cTn may be the basis for this dysfunction.

### Reperfusion in acute myocardial infarction

Reperfusion in AMI can be induced by thrombolytic therapy or percutaneous coronary angioplasty. It may increase the extent of cardiac injury [20]. Under hypoxia, modifications of cTn may be specifically associated with this “ischemia-reperfusion” process [21]. Patients with larger myocardial infarctions display greater amounts of degraded cTn fragments. It is possible that the proteases involved in clot dissolution or those implicated in induction of inflammation in response to injury could degrade cTns. The biochemical environment of the myocardium might be different than that of blood, leading to different cTn changes. Detectable changes might be useful in distinguishing more from less robust recanalization.

### Acute myocarditis

Acute myocarditis can present similarly to AMI [22]. Increases in cTnI occur in mouse models of autoimmune myocarditis [23] and in patients with heart failure secondary to chronic myocarditis. Presumably, inflammation leads to myocardial injury. It may be that fragments produced secondary to inflammation are different from those triggered by ischemia. Recognition of dissimilarities might permit differentiation of distinct disease states in clinical practice. Moreover, cTn may play a part in inflammation itself [24].

### Basic molecular biology on which fragmentology is based

The regulation of contraction physiology is complex and includes abundant phosphorylation and dephosphorylation steps within troponin and actin-tropomyosin. cTnI is phosphorylated at specific serine/threonine residues by protein kinase A (PKA) [25]. The extent of phosphorylation of cTnI and cTnT depends on these kinases and phosphatases, but also on their spatial conformation, which confers accessibility to phosphorylation sites. Posttranslational phosphorylation of cTnI and cTnT by processes that block or reveal specific sites may also change the status of cTn. Physiologic degradation occurs at the N-terminus: residues 27, 28 or 31 seem to possess key significance.

During ischemia, protein kinase C (PKC) and p21-activated kinase induce cTn phosphorylation [26]. Proteolysis occurs initially at the C-terminus, at amino acid 192, followed by additional cleavages. Truncation of cTnI at amino acid residue 192 is associated with human disease [13,14]. The alteration of cTnI/cTnT by proteolysis, phosphorylation, or both, generates an assortment of fragments (Table I). The majority of cTnI interacts with TnC, and cTn subunits or serum proteins [27]. In patients with AMI, a proportion of the cTnI circulates bound to cTnC along with complexes of cTnT, I and C. During ischemia, truncation of the N-terminus eliminates PKA phosphorylation sites and sites involved in cTnI–TnC interaction. Thus degradation and phosphorylation also influence circulating fragments. The detection of these isoforms could elucidate specific etiologies for cardiac disease.

One pool of cTn corresponds to a “cytosolic” localization. Such localization is based on solubility...
studies; thus a better term might be “early-releasable pool.” The other pool is believed to be structurally bound. Only 4% of cTnI and 5% of cTnT are found in the cytosolic compartment [28]. Presumably, the initial release of troponin derives from the cytosolic pool, whereas the persistent increases are from degradation of the structural compartment. Patients with renal failure demonstrate the same cTn clearance curves [29], suggesting that the persistence of cTn is not as a result of delayed elimination. During reperfusion, the early peak in cTnT occurs at 14 h after the onset of pain [22], probably from the cytosolic pool. This peak is absent in patients reperfused later than 5.5 h after the onset of pain and in patients with AMI who do not undergo reperfusion [28]. The cytosolic compartment of the unbound cTnT and cTnI is responsible for this initial peak in patients with early reperfused AMI, and seems to be composed mostly of free chains [30]. Persistent increases in cTn concentration are attributed to lysosomal degradation of the “structural” pool of cTn during infarct remodeling and collagen deposition [31]. Increases in cTn in pulmonary embolism resolve by 40 h in the absence of current emboli. The short duration of cTn in the bloodstream after acute exertion and pulmonary embolism might be explained by the cTn released from the cytosolic compartment rather than from the structural pool; the latter would be affected only if necrosis occurs, if it is extensive enough for detection, or by changes in the fragments released.

Conclusion

The complex biology of cTns should allow for the development of novel assays able to discriminate various fragments that may be elaborated selectively within distinct pathological processes. These fragments resulting from metabolism or phosphorylation have the potential for both diagnostic and therapeutic importance.

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Heart Metab. 2009; 43:5–8

Table I. Modified forms of cTnI.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Type of modification</th>
<th>Amino-acid position</th>
<th>Enzyme</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physiological</td>
<td>Phosphorylation</td>
<td>N-terminus</td>
<td>Increased concentrations of protein kinase A</td>
</tr>
<tr>
<td>Physiological</td>
<td>Proteolysis</td>
<td>N-terminus (starting at position 27, 28, or 31)</td>
<td>Proteases</td>
</tr>
<tr>
<td>Pathological</td>
<td>Phosphorylation</td>
<td>C-terminus</td>
<td>Reduced concentrations of protein kinase A</td>
</tr>
<tr>
<td>Pathological</td>
<td>Phosphorylation</td>
<td>C-terminus</td>
<td>Protein kinase C</td>
</tr>
<tr>
<td>Pathological</td>
<td>Proteolysis</td>
<td>C-terminus (ends at 192)</td>
<td>Proteases</td>
</tr>
<tr>
<td>Pathological</td>
<td>Proteolysis</td>
<td>N-terminus (ends at 63)</td>
<td>Proteases</td>
</tr>
<tr>
<td>Pathological</td>
<td>Proteolysis</td>
<td>N-terminus (ends at 73)</td>
<td>Proteases</td>
</tr>
</tbody>
</table>


The role of biomarkers in clinical practice

Anthony S. Wierzbicki\textsuperscript{a} and Adie Viljoen\textsuperscript{b}
\textsuperscript{a}Guy’s & St Thomas Hospitals, London, UK, and \textsuperscript{b}Lister Hospital, Stevenage, Hertfordshire, UK

Correspondence: Dr A. S. Wierzbicki, Guy’s & St Thomas Hospitals, London SE1 7EH, UK.
Tel: 00 44 207 1881256; e-mail: Anthony.Wierzbicki@kcl.ac.uk

Conflicts of interest: None.

Abstract

Atherosclerosis is a process that begins intra-arterially and then becomes intraluminal. Imaging is the best method with which to identify and monitor the progression of atherosclerosis; however, until recently, methods have been neither available or practical. Epidemiological studies have shown that biochemical markers, including lipids, glycemia, and renal function, contributed to the risk of cardiovascular events. Later measures of inflammatory processes involved in atherosclerosis and neurohormones (eg, natriuretic peptides) have been added to risk stratification. Biochemical markers are well established, in addition to electrophysiology, for the definition of various grades of myocardial infarction, and biomarker panels now form the core of the diagnostic criteria for definition of acute coronary syndromes. Biomarkers are also used in the development, validation, and safety monitoring of drugs used in the management of atherosclerotic disease. Thus use of biochemical markers is essential to the diagnosis, prognosis, and safety assessment of atherosclerosis.

\textit{Heart Metab.} 2009;43:9–11.

Keywords: Biomarker, asymmetric dimethylarginine, C-reactive protein, troponin, B-type natriuretic peptide, lipoprotein-associated phospholipase A2, cardiovascular risk, myocardial infarction, statin, safety

Introduction

Atherosclerosis is a process that begins in childhood and is responsible for 35% of mortality. The process begins intra-arterially and only at a late stage begins to obstruct the lumen of the vessel [1]. Imaging of atherosclerosis would be the ideal method of diagnosis and monitoring, but, until recently, methods have been either unavailable or impractical because they involved highly invasive procedures (intravascular ultrasound) for routine use in primary prevention. Therefore surrogate markers such as carotid intima media thickness have been used [2,3]. Biomarkers, defined as biochemical analytes measured in plasma or urine, are used in cardiovascular disease for a number of purposes. The three major uses are for risk assessment in cardiovascular prevention, for the diagnosis and staging of ischemic heart disease, and for monitoring of the safety of therapies.

Risk assessment

As there is, as yet, no easy method of assessing the burden of atherosclerosis by imaging methods, so the entire basis of cardiovascular risk assessment relies on epidemiological data for cardiovascular disease [4]. In studies such as the Framingham Heart Study, the risk of events is related to demographic and physiological parameters (body mass index, blood pressure) in addition to a series of biochemical markers for risk assessment in the prevention of cardiovascular disease [5]. The best known are total cholesterol and high-density lipoprotein (HDL) cholesterol, which
form part of the core risk-assessment algorithm. Hyperglycemia and diabetes are also risk predictors, but are usually excluded because diabetes is considered to be a cardiovascular risk equivalent [6]. Hyperglycemia and glycated hemoglobin (HbA1c) can also be used to identify higher-risk groups likely to have insulin resistance/metabolic syndrome [7]. Further information has been added in other epidemiological studies through the addition of both triglycerides as a marker of the atherogenic lipoprotein phenotype, and small dense particles (both low-density lipoprotein [LDL] and HDL) [8]. The combination of lipids, blood pressure, hyperglycemia, and waist circumference is used in definitions of the metabolic syndrome, although other markers such as hyperinsulinemia, hyperuricemia, and increased concentrations of inflammatory markers and plasminogen activator inhibitor-1 (PAI-1) also are associated with this syndrome [9]. Cardiovascular risk is also increased in patients with renal dysfunction identified by measurement of plasma creatinine and increasingly expressed as an estimated glomerular filtration rate (eGFR) [10]. When these are combined with the presence of micro- or macroalbuminuria, further increments of risk can be defined. The presence of microalbuminuria in patients with hypertension defines a subset with target-organ damage and requiring extra treatment. The presence of albuminuria allied to eGFR stratifies patients for renal cardiovascular disease and thus can help determine both risk of cardiovascular disease and how drug doses are adjusted.

The use of such marker panels allows an identification of 70–80% of cardiometabolic risk [11]. Further specificity can be added through the addition of markers of inflammation, including C-reactive protein (CRP), fibrinogen, or sialic acid [12]. Some of these markers (eg, CRP) respond to drug therapies such as statins or fibrates. Recently, the use of CRP as a risk marker has been validated in the JUPITER (Justification for the use of statins in Primary prevention an Intervention Trial Evaluating Rosuvastatin) trial [13]. Another inflammation-associated marker that seems to mark an oxidative phenotype is lipoprotein-associated phospholipase A2 (LpPLA2), which adds information about cardiovascular risk over and above that provided by CRP [14]. The limited relationship of LpPLA2 to markers of oxidation such as oxidized LDL and isoprostanes is unclear, but a possibly stronger association with sialic acid and electronegative LDL species means its role has not been fully clarified. Another indirect marker of oxidative stress involves the nitric oxide system, in which asymmetric dimethylarginine (ADMA) has been shown to mark the degree of established atherosclerosis [15]. ADMA is correlated with endothelial vascular dysfunction, given its close relationship to low concentrations of nitric oxide, but – in contrast to nitric oxide – it is stable. Whether the principal source is vascular or renal is uncertain, but it does mark established atheroma; however, in contrast to many biomarkers, it does not respond to common therapies (eg, antihypertensive agents or statins). Natriuretic peptides are also potential markers of cardiovascular risk, as they may identify patients with left ventricular dysfunction and thus low-grade ischemia, as may other markers of tissue hypoxia such as ischemia-modified albumin [16]. Which risk markers will be added to the basic profile remains, as yet, unclear.

Established cardiovascular disease

Cardiac biomarkers are well established in the diagnosis of acute coronary disease. The release of cardiomyocyte proteins includes (in approximate temporal sequence): myoglobin or fatty acid binding protein 4, glycogen phosphorylase B, creatine kinase MB fraction (CK-MB) and, finally, troponin (T, I or C) [17,18]. The concentrations of troponins are used in conjunction with clinical and electrocardiographic criteria to define the extent of myocardial necrosis and to stratify patients into those with unstable angina, non-Q-wave myocardial infarction, or ST-segment-elevation myocardial infarction, which determine treatment strategies. The availability of newer, more sensitive troponin assays is likely to result in further redefinition between these clinical categories.

None of the above markers is functionally significant. In contrast, the natriuretic peptides are released in response to left ventricular cardiac dysfunction [19]. The most commonly used is brain natriuretic peptide (BNP), which is used as a diagnostic marker of non specific cardiac dysfunction (including pulmonary dysfunction and pericarditis), but is better known as a definitive test to exclude the presence of heart failure [20]. Early studies have not shown a benefit from using BNP to guide management in heart failure, but more sophisticated strategies correcting more accurately for age and sex may prove more useful.

The reliability of cardiac biomarkers for ischemia is such that many are now used in semiquantitative assay formats in point-of-care panels. Typically, these combine hyperacute (myoglobin, fatty acid binding protein) or functional (BNP) markers with intermediate markers (CK-MB), and a late definitive myolytic marker of necrosis (troponin) [17]. There is controversy about the specificity, accuracy and utility of different panels and how they should be integrated with clinical signs and electrocardiographic data, but their convenience is such that usage for triage in emergency rooms is increasing rapidly.
Safety monitoring

Biomarkers are used to monitor drug therapies. The best known are markers of hepatic microsomal induction (γ-glutamyl transferase) and hepatocyte necrosis (transaminases) [21,22]. These are commonly measured to track the toxicity of drug therapies. More specific biomarkers are used to track tissue toxicities. In the severe case of myositis and rhabdomyolysis, the association of statin therapy with myopathy can be tracked by increases in creatine kinase (MM) and myoglobinuria [23]. Nowadays, even subtle toxicities detectable only by changes in transaminases and creatine kinase concentrations, from baseline, allied to genetic biomarkers of statin acid uptake (organic anion transporter), can be used to predict future myositis [24].

Conclusions

Biomarkers are central to the diagnosis and risk stratification of coronary heart disease. Although older biomarkers are well established, newer markers are offering the possibility of further risk stratification, quantification of the underlying atheroma burden, and even marking the correction of cardiac dysfunc-

tion by demonstrating the reappearance of normal physiology.

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Troponin compared with late enhancement in the assessment of myocardial injury

Evangelos Giannitsis and Hugo A. Katus
Medizinische Universitätsklinik Heidelberg, Department of Cardiology, Heidelberg, Germany

Correspondence: Prof. Dr Evangelos Giannitsis, Medizinische Universitätsklinik Heidelberg, Abteilung für Innere Medizin III, Im Neuenheimer Feld 410, 69120 Heidelberg, Germany.
Tel: +49 6221 56 8670; e-mail: evangelos_giannitsis@med.uni-heidelberg.de

Conflicts of interest: None.

Abstract

Cardiac troponins are regarded as the reference standard for detection of myocardial cell necrosis. Contrast-enhanced cardiac magnetic resonance imaging (CE-MRI) represents a modern technology that allows comprehensive evaluation of the heart after myocardial infarction. It enables assessment of global and regional myocardial function, confirmation and quantification of myocardial infarction, identification of viable myocardium suitable for revascularization procedures, and detection of specific complications including microvascular obstruction. Thus CE-MRI represents a useful complementary diagnostic tool after acute coronary syndromes. However, increased concentrations of troponin may also be encountered in symptomatic patients without acute coronary syndrome – ie, in patients with acute pulmonary embolism, myocarditis, cardiac ischemic amyloidosis, and dilated and hypertrophic cardiomyopathies. As increased concentrations of troponin are associated with adverse outcomes in most of these differential diagnoses, the reason for the myocardial damage must be actively pursued. Along with typical morphological and functional findings on cardiac MRI, different patterns of non ischemic late hyperenhancement have proved to be useful in discriminating ischemic from non ischemic myocardial damage, and to establish a correct differential diagnosis.

Heart Metab. 2009;43:13–18.

Keywords: Cardiac MRI, cardiac troponin, differential diagnoses, infarct size, late hyperenhancement, microvascular obstruction

Introduction

Prognosis after acute myocardial infarction is strongly determined by the extent of myocardial injury [1]. Assessment of left ventricular function after myocardial infarction is helpful for risk stratification and selection of patients requiring an implantable cardioverter defibrillator for prevention of sudden cardiac death [2], and the presence and extent of “late hyperenhancement” on contrast-enhanced cardiac magnetic resonance imaging (CE-MRI) has been shown to give information on the ability of the myocardium to resume contractility after revascularization procedures [3,4].

Echocardiography has emerged as the most convenient method for the quantification of left ventricular function, but this technique does not allow direct quantification of infarct size, and gives only limited information on the presence of viable myocardium and the potential for functional recovery after revascularization procedures. Currently, several imaging techniques, including thallium sestamibi and positron emission tomography, are used for quantification of infarct size [5]. Among the novel techniques,
CE-MRI is noninvasive and does not involve exposure of the patient to radiation or potentially toxic contrast material. It has been shown to provide a good approximation of histological infarct size as assessed by triphenyltetrazolium chloride staining [6]. This technique is therefore preferred by some, because it allows noninvasive assessment of myocardial function and viability in vivo. It has a high spatial resolution, and is superior to single-photon emission computed tomography (SPECT) for the identification of subendocardial myocardial infarction [6,7]. Furthermore, CE-MRI is highly sensitive and permits quantification of small areas of myocardial injury attributable to native coronary artery disease or percutaneous coronary interventions, or both [8,9]. However, the use of cardiac MRI for quantification of infarct size is limited by availability and high cost. A convenient alternative, therefore, is to estimate infarct size from concentrations or activities of cardiac proteins in peripheral blood – as has been practiced for some years [5]. Today, cardiac troponins are established as the preferred biochemical markers for the diagnosis of myocardial infarction [10]. Moreover, there is increasing evidence that measurement of cardiac troponins may also allow estimation of infarct size and detection of the presence of microvascular obstruction.

Cardiac troponin and MRI infarct size

The cardiac troponins C, T, and I (cTnC, cTnT, and cTnI) are structural proteins of the myofilament that are exclusively expressed in cardiomyocytes. Upon irreversible cardiac injury, serum concentrations of cTnT show a biphasic curve, with an early peak within 24 h, resulting from the release of a small cytoplasmic pool, and a “plateau phase” 72–96 h after the onset of symptoms, resulting from continuous proteolytic degradation of the contractile apparatus [11,12]. Animal and human studies using thallium SPECT and MRI have demonstrated an excellent correlation between infarct size and cTn concentrations [13–17]. Using cardiac MRI, the pattern of late hyperenhancement is distinctive for myocardial infarction, with a compact area of subendocardial late hyperenhancement visible after the administration of gadolinium [18]. In patients with myocardial infarction, this area typically starts from the endocardial border of the myocardium, with a variable extent towards the epicardial border (Figure 1). The areas of late hyperenhancement correspond to the territory of the infarct-related coronary artery; the extent of late hyperenhancement is usually given as percent transmurality and is reported either semiquantitatively, or quantitatively as absolute or relative infarct size. The transmurality of hyperenhancement has been shown to correlate with the ability of myocardial contractility to recover after revascularization [3,4]. In contrast, several other nonischemic patterns of late hyperenhancement have been reported in patients with myocarditis, cardiomyopathies, cardiac amyloidosis, and other systemic diseases with cardiac involvement [18].

Regarding quantification of infarct size without the need to perform MRI, an increasing amount of evidence coming from CE-MRI studies shows that any single measurement of cTn concentration between 24 and 96 h after the onset of symptoms allows an excellent estimation of infarct size [13–17]. The relationship between cTnT and cTnI is excellent for large ST-segment elevation myocardial infarction (STEMI) and useful – albeit less impressive – for the heterogenous group of non-STEMI episodes [19,20]. Although serial measurements are as effective as single-time-point protocols, the latter may be better accepted in clinical practice, because a simple algorithm is more convenient and more cost-effective than serial measurements [19,20].

The diagnosis of periprocedural myocardial infarction after percutaneous coronary intervention (PCI) is relatively straightforward, as myocardial ischemia is the mechanism underlying PCI-related myocardial necrosis. Johansen et al [21] were able to demonstrate consistently that the majority of increases in cTnT after PCI persisted for at least 96 h, indicating continuing release of cTnT from the contractile apparatus and reflecting irreversible myocardial injury. In cardiac CE-MRI, areas of myocardial infarction have been identified as the source of increased concentrations of minor serum markers [8,9]. Ricciardi et al [8] used CE-MRI and demonstrated that even mild increases in CK-MB concentrations after PCI were attributable to discrete microinfarction [8]. All patients with increased CK-MB concentrations had discrete hyperenhancement in the target-vessel perfusion territory.
Cardiac troponin for prediction of the presence and magnitude of microvascular obstruction

Microvascular obstruction (MVO) is believed to be related to peripheral embolism of platelet microaggregates, intimal edema, vasoconstriction, or leukocyte sticking [22,23]. The findings of experimental and clinical studies clearly demonstrated that, irrespective of cause, MVO is associated with a greater degree of myocardial damage, more severely depressed left ventricular function, and a higher mortality [24–27]. Therefore, identification of patients with MVO would be useful for risk stratification at minimum, and might be helpful also in eventually elucidating both its pathophysiology, and possible therapeutic approaches.

Sophisticated techniques such as contrast echocardiography, delayed radionuclide imaging, and CE-MRI have been shown to be capable of identifying this lack of small-vessel perfusion [28–31]. Data have also confirmed that the presence and maximal extent of MVO are best evaluated by early post-contrast MRI [32]. With CE-MRI, zones of hypoenhancement surrounded by areas of hyperenhancement are believed to represent areas of microvascular obstruction in patients after acute myocardial infarction (Figure 2). These areas correspond well to anatomically defined no-reflow zones determined by histological thioflavin S staining [33]. Several studies have clearly demonstrated that patients with MVO have suffered larger infarcts (as determined by CE-MRI, maximal creatine kinase, and cTnI) than those without MVO [24,34].

The best single value for the prediction of MVO is a cTnT concentration greater than 2.52 μg/L at 24 h after the patient’s admission to hospital, and the ability of cTnT to predict MVO persists after adjustment for potential confounders such as duration of ischemia and success of epicardial reperfusion.

As a general limitation, the rates of MVO reported in studies vary widely. This variation may be explained by the use of different MRI techniques for determination of the obstruction. The two most commonly used methods for assessing no-reflow include first-pass perfusion techniques [35–37] and late-enhancement imaging [24,34,36]. The first-pass perfusion technique has the shortcoming that a perfusion mismatch might be interpreted as an area of no-reflow [38].

Cardiac troponin and nonischemic patterns of late enhancement

Myocarditis

Several cardiac MRI studies have demonstrated that hyperenhancement can be found in at least 85% of patients with acute myocarditis evolving within the first 2 weeks after the onset of symptoms [39]. The two relevant CE-MRI approaches described so far depend on the measurement of myocardial global (early) relative enhancement [39] or the visualization of late gadolinium enhancement. Early enhancement probably reflects myocardial hyperemia and increased capillary permeability as features of present inflammation, whereas late enhancement mostly indicates irreversible myocardial injury (Figure 3). More recently, T2-weighted imaging was found to be useful in a combined imaging approach [40]. The incidence of late hyperenhancement in myocarditis is a controversial issue. Reported incidences vary between 44% and 55% using antimyosin scintigraphy [41,42], and the 88% incidence reported by Mahrholdt et al [43]. The reason for such discrepancies may be related to differences in patient populations or study designs.

Cardiomyopathies

Dilated cardiomyopathy

Increased concentration of cTnT have been reported in dilated cardiomyopathy in the absence of coronary stenoses, and were related to an adverse prognosis [44]. There are substantial differences between...
concentrations of biomarkers in patients who present with acute decompensated chronic heart failure, depending on the underlying etiology of the heart failure syndrome [45]. Hyperenhancement in ischemic cardiomyopathy characteristically spreads from the subendocardium up to the epicardium, and is confined to the perfusion territories of the coronary arteries [46]. In contrast, several patterns of infarct-atypical, nonischemic late hyperenhancement have been reported in dilated cardiomyopathy, including a pattern in the midventricular rim of hyperenhancement that predominantly involves the septum and is found in 9–28% of patients (Figure 4). This hyperenhancement is presumed to reflect patchy areas of replacement fibrosis [47]. The presence of this particular pattern of hyperenhancement has been shown to be an independent predictor of all-cause mortality and the onset of potentially life-threatening ventricular arrhythmias.

**Hypertrophic cardiomyopathy**

Increased concentrations of cTn may be encountered in hypertrophic cardiomyopathy, may be prognostically important, and correlate with worsening of left ventricular function [48]. The exact reason for this is unclear, but it may include relative myocardial ischemia resulting from an imbalance between inappropriate hypertrophy of the myocardium and insufficient coronary arterial supply, and myocyte abnormalities determined by gene mutations causing myocyte injury [48].

Myocardial scarring is a common finding in patients with hypertrophic cardiomyopathy; it occurs mostly in hypertrophied regions and is usually patchy with several foci, predominantly affecting the midventricular wall, the junctions of the interventricular septum, and the right ventricular walls (Figure 5) [49–51]. The extent of hyperenhancement measured by CE-MRI correlates with conventional risk markers, and has also been related to the occurrence of ventricular arrhythmias and an increased number of clinical risk factors for sudden death [51–53]. However, the prognostic value of the presence and extent of hyperenhancement in patients with hypertrophic cardiomyopathy remains unknown, and the results of current studies are awaited [51].

**Amyloidosis**

Cardiac involvement is common in systemic amyloidosis. Usually, it is either diagnosed by a positive heart biopsy, or suspected from left ventricular hypertrophy (interventricular septal thickness 12 mm or more) in

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**Figure 3.** Spotted diffuse non ischemic late hyperenhancement within the lateral wall in a patient with myocarditis.

**Figure 4.** Typical mid-septal rim of non ischemic hyperenhancement in a patient with dilated cardiomyopathy.

**Figure 5.** Patchy non ischemic late hyperenhancement at the junctions of the interventricular septum and the right ventricular walls in a patient with hypertrophic cardiomyopathy.
the absence of hypertension or other potential causes of the hypertrophy [54]. In conjunction with N-terminal pro brain natriuretic peptide, cardiac troponin T has been found to represent a powerful staging system for patients with immunoglobulin light-chain amyloidosis, with the objective of providing an easily available tool allowing comparison between outcomes of therapeutic interventions in the absence of randomized trials [55,56]. Cardiac amyloidosis typically shows an ill-defined global subendocardial pattern of hyperenhancement, matching the distribution of the amyloid deposition. This pattern of hyperenhancement is very characteristic for cardiac involvement of amyloidosis, and can therefore be used to discriminate this disease from other forms of restrictive or hypertrophic cardiomyopathies. Although most profound in the subendocardial layer of myocardium, amyloid deposition occurs throughout the entire myocardium, causing the entire myocardium to have a higher signal on CE-MRI images than normal myocardium. Therefore, “nulling” of myocardium can be extremely difficult when amyloidosis is present. The typical pattern of hyperenhancement, together with the increased T1 signal of myocardium, yields a diagnostic accuracy of 97% in patients with biopsy-proven amyloidosis (Figure 6).

REFERENCES


Trends in genomic biomarkers

Bruce McManus
The James Hogg iCAPTURE Centre, University of British Columbia, St Paul’s Hospital, and The Heart and Lung Institute, Providence Health Care, Vancouver, British Columbia, Canada

Abstract
Cardiovascular disease continues to be a major health concern globally. Developments in genomic discovery have yielded valuable new candidates in the quest for better biomarkers and novel therapeutic targets. This brief perspective focuses on recent trends in this field. DNA microarrays, single nucleotide polymorphism chips, linkage analysis, genome-wide association studies, and other strategies have increased our knowledge of metabolic diseases of the heart. There are many benefits from these approaches, but one must remain cognizant of the importance of patient phenotyping. The integration of new and old tools and technologies promises the discovery and validation of better markers of the presence of cardiovascular disease, its progression, and the response to treatment.

Introduction
Trends in the incidence of cardiovascular disease (CVD) continue upward globally, yielding a significant impact on morbidity and mortality in both developed and developing societies. As the number of patients suffering from CVD mounts, alongside an aging demographic, the economic and social burdens of these diseases will continue to grow, with tremendous consequences for overstretched health-care systems. The search for new and better biomarkers of the presence and progression of disease, risk stratification, and response to treatment has become a matter of urgency. Fortunately, technological advances have facilitated high-throughput assessment and mining of the human genome, proteome, and metabolome. In this context, molecular signatures (biomarkers) will be increasingly useful in the prediction, diagnosis and management of heart disease.

The discovery and development of biomarkers has benefited from the emergence of high-performance genomic and genetic approaches such as DNA microarrays and single nucleotide polymorphism (SNP) chips, respectively. The ability to screen large populations for levels of gene expression, polymorphisms, and genetic linkage has shed light on the complex interplay of genetic and environmental factors involved in CVD. Molecular signatures have been identified that may have utility both in the clinical management of disease and in elucidating the mechanisms involved, thereby providing insights into potentially novel therapies.

Gene expression analysis
With the rise of high-performance technologies such as DNA microarrays, analyses of gene expression have been applied in the setting of various cardiovascular diseases. The level of transcribed genes and the related mRNAs detectable by the microarrays are often examined in an attempt to discover correlations with the presence or absence of disease, clinical outcome, disease progression, and therapeutic responses [1].
Microarray analysis comparing the expression of genes in non-failing hearts and failing hearts (i.e., ischemic or non-ischemic cardiomyopathy) has revealed substantial differences at a molecular level. In a study by Kittleson et al. [2], 288 genes were identified as differentially expressed between groups with non-failing and failing hearts. Although none of the genes is currently used clinically, these genetic biomarkers can still provide therapeutic insights into the metabolic dysregulation underlying the disease conditions. As described by Kittleson and Hare [2] and Tan et al. [3], many of the genes upregulated in failing hearts, relative to those in the non-failing hearts, were associated with fatty acid metabolism, whereas those downregulated were linked to glucose metabolism. This has triggered an investigation into the use of drugs such as trimetazidine and ranolazine, which can shift the lipid and glucose metabolic state in myocardial cells, as a potential treatment for failing hearts [2,4].

In the context of atherosclerosis, gene expression studies have also underscored the importance of regulation of lipid metabolism. Using mouse models, Karr et al. [5] identified numerous genes involved in lipid metabolism that are differentially expressed during early stages of progression of the atherosclerotic lesion. In cardiovascular diseases such as ischemic and non-ischemic cardiomyopathy and atherosclerosis, it is unlikely that a single biomarker can serve sensitively and specifically as the therapeutic or diagnostic biomarker for the disease. Biomarkers of the future are expected to be multi-marker panels characteristic of the complexity of the underlying pathophysiology of the disease process. In fact, only a small portion of familial and sporadic atherosclerosis results from single-gene defects in lipid metabolic pathways [6,7].

**Genome-wide association studies**

Beyond microarray-based expression studies, genome-wide association studies provide an effective approach to discovering genetic biomarkers. Genetic variants, such as those on chromosome 9 (interval 9p21) and chromosome 4 (4q25), have been linked to increased risk for CVD [8]. McPherson et al. [9] applied genome-wide association scanning and discovered a 58 kb interval on chromosome 9p21 that was consistently associated with coronary heart disease in six independent cohorts, containing more than 23,000 participants, from more than four white populations. A similar finding was demonstrated by the Wellcome Trust Case Control Consortium, which found an association between a similar region on chromosome 9p21 and coronary artery disease [10]. In the study by Gudbjartsson et al. [11], two sequence variants on chromosome 4q25 were found to be strongly associated with atrial fibrillation in three populations of European descent and in a Chinese population from Hong Kong.

In certain cases, the risk locus identified via genome-wide association studies contains genes that have yet to be annotated and characterized (e.g., the 58 kb interval on chromosome 9p21). It may also be unclear what cellular and molecular differences are induced by these genetic variants. Certainly, many of the newly identified susceptibility loci or SNPs require further studies to determine their involvement in the pathogenesis of CVD and potential therapeutic targets for testing. It is likely that each SNP may have a modest influence on the concentrations or function of translated protein products, whereas a specific set of SNPs can have a major impact on the pathobiology of a particular CVD [1]. Nonetheless, just as with genes identified in microarray studies, SNPs may also contain valuable information on the mechanisms of CVD and potential new therapeutic targets.

**Linkage analysis**

Linkage analysis is another approach to finding genetic biomarkers of cardiovascular diseases. It allows the identification of disease DNA markers by examining the patterns of heredity in large high-risk families and the occurrence of disease phenotypes among family members [1,12]. Using linkage analysis to look at families with early-onset coronary artery disease, Connelly et al. [13] have demonstrated an association between GATQA2, a transcription factor, and susceptibility for coronary artery disease. Recent studies by the Genetics of Early Onset Coronary Artery Diseases (GENCARD) investigators also revealed novel gene candidates, such as LSAMP, a tumor suppressor gene, and KALRN, a gene involved in the Rho GTPase-signaling pathway, to be associated with coronary artery disease [14,15].

**Discussion**

It is not uncommon, in observing different genomic and genetic biomarkers studies using differing technologies – microarrays, SNP chips, or linkage analysis – that different genes or polymorphic loci are found to be associated with the same cardiovascular pathology. Certainly, an important factor to consider when comparing different genetic biomarkers studies is the selection of patient cohort. Depending on the heterogeneity or the size of the population being analyzed, the genetic biomarkers detected may be significantly different. Ideally, a larger, more
heterogeneous cohort can result in a more widely applicable biomarker discovery. However, this increase in heterogeneity may also compromise the ability to find potentially more specific biomarkers that are associated with particular extreme phenotypes. Ultimately, the key to success in genotypic characterization and expression studies will be the exquisite phenotyping of groups of patients of interest.

The potential genetic basis of cardiomyopathies and therapeutic targets of heart failure have been discussed in detail by Liew and Dzau [16], Kittleson and Hare [2] and Heidecker et al [17]. The genetics with respect to specific types of cardiomyopathies and channelopathies was reviewed in great detail by Bezzina [18], in the previous issue of Heart and Metabolism. In the context of atherosclerosis, relevant genetic biomarkers have also been discussed by Miller and colleagues [6].

Ultimately, the genetic biomarkers identified using various technologies may be complementary to one another. Future systems biology studies may shed light in this regard, and provide a more complete picture of the genetic mechanisms underlying each CVD. It is possible that complex, multifactorial CVDs result from a combination of effects attributable to the presence or absence of specific genetic mutations, polymorphisms, or differential expression [19].

There are probably many effective therapeutic targets that have yet to be discovered. Genetic biomarkers may also help uncover these nuggets of gold. Just to put things into perspective, the human genome contains more than 22,000 genes, but it has been suggested that current medication targets only about 2.3% (approximately 500) of them [6,20].

Developments in the technical capabilities underlying high-throughput genomic approaches, coupled with a focus on patient phenotyping and advanced computational strategies, will continue to add incremental value to our current understanding of heart diseases, and have the potential to revolutionize the management of patients through earlier intervention and more effective interdiction on the processes of disease progression. ■

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Effects of Vastarel MR on brain natriuretic peptide and cardiac troponin concentrations

Pericle Di Napoli
Cardiovascular Section, SMDN-Centre for Cardiovascular Medicine and Cerebrovascular Disease Prevention, Sulmona, and Villa Pini d’Abruzzo Clinic, Department of Cardiology, Heart Failure Unit, Chieti, Italy

Correspondence: Dr Pericle Di Napoli, Via Trento 41, 67039 Sulmona (AQ), Italy. Tel/fax: +39 0864 52716; e-mail: dinapoli@unich.it

Conflicts of interest: None.

Abstract
Metabolic therapy represents an innovative approach to the treatment of coronary artery disease and heart failure. The metabolic modulator, trimetazidine (Vastarel MR), offers particular positive effects in reducing the cell loss and reorganization that characterize the process of left ventricle remodeling, and is useful in improving ischemic or failed cell metabolism. Correction of the metabolic alterations that characterize ischemic cardiomyopathy and heart failure represents a promising novel therapeutic approach useful in reducing left ventricle remodeling and improving the prognosis of patients suffering these conditions.


Keywords: BNP, heart failure, myocardial ischemia, trimetazidine, troponin

Introduction
Coronary artery disease (CAD) and chronic congestive heart failure (CHF) represent major public health concerns that have a poor long-term prognosis [1] and, despite vigorous effort and financial input, few clinical tools have been developed to identify variables that have prognostic significance for patients suffering these conditions. The New York Heart Association (NYHA) functional classification, and several tests – including chest X-ray, electrocardiogram, ultrasound, radionuclide and magnetic resonance imaging, cardiopulmonary exercise, and hemodynamic measurements – are useful for estimating the severity and prognosis of CHF.

The deterioration of cardiac function in chronic CAD and CHF is strictly related to left ventricular remodeling, a pathologic process by which ventricular size, shape, and function are dysregulated by hemodynamic overload, neurohormonal activation (involving adrenergic nervous hyperactivity, the renin–angiotensin system, and inflammatory cytokines), and genetic factors. The pathologic changes in cardiac myocytes and fibroblasts are an important component of cardiac remodeling [2,3], and biochemical markers of the pathophysiology of CHF might be helpful in monitoring its evolution and the development of remodeling, because they are usually easy to measure serially, without inter-observer variability.

Measurement of plasma concentrations of brain natriuretic peptide (BNP), an amino acid peptide secreted by the ventricular myocardium in response to myocardial load, is now increasingly being used as a tool for clinical diagnosis and prognosis in patients with CHF and CAD [4]. Other important biochemical markers are troponins, which are strictly related to the cell loss that characterizes ventricular remodeling after myocardial infarction, chronic ischemia, and pressure or volume overload – factors influencing the progression of left ventricular dysfunction [5]. In addition, norepinephrine (noradrenaline; a marker of adrenergic activity), renin, inflammatory cytokines,
C-reactive protein, and biochemical markers associated with collagen turnover may have significant roles in the pathophysiology [6–8].

**Left ventricle remodeling in ischemic heart disease and heart failure**

Cardiac remodeling is an important determinant of CHF progression. Hemodynamic overload, activation of the renin–angiotensin and sympathetic nervous systems, and inflammatory cytokines are believed to be implicated [1,2,9]. At cellular level, the most important factor influencing ventricular remodeling is progressive cell loss as a result of apoptotic or necrotic processes. This is the first stage in ventricular remodeling, in which the normal architecture of the heart wall is rearranged, with the replacement of contractile mass by noncontractile fibrous tissue. In addition to myocyte injury, the interstitium, fibroblasts, and collagen turnover also have important roles.

Ischemic injury has long been considered to result in necrotic tissue damage; however, in recent decades, studies have focused attention on apoptosis as a significant component of cell loss during reperfusion injury, myocardial infarction, and chronic ischemia [10,11]. Myocardial apoptosis has also been documented in response to a variety of other cardiac stresses, including pressure or volume overload, heart failure, and diabetic cardiomyopathy [12,13]. The most relevant clinical stimulants that initiate the process of apoptosis include pressure or volume overload, heart failure, and diabetic cardiomyopathy [10,11]. Myocardial apoptosis has also been documented in response to a variety of other cardiac stresses, including pressure or volume overload, heart failure, and diabetic cardiomyopathy [12,13]. The most relevant clinical stimulants that initiate the process of apoptosis include pressure or volume overload, heart failure, and diabetic cardiomyopathy [10,11]. Myocardial apoptosis has also been documented in response to a variety of other cardiac stresses, including pressure or volume overload, heart failure, and diabetic cardiomyopathy [12,13]. The most relevant clinical stimulants that initiate the process of apoptosis include pressure or volume overload, heart failure, and diabetic cardiomyopathy [10,11]. Myocardial apoptosis has also been documented in response to a variety of other cardiac stresses, including pressure or volume overload, heart failure, and diabetic cardiomyopathy [12,13].

**The relevance of metabolic therapy in heart failure**

The basic principle of current treatment in patients with heart failure with or without CAD is to modify myocyte dysfunction and to minimize the intensity of the lethal injury acting on the heart. Clinical interventions such as restoration of ischemia by pharmacological or interventional strategies, use of β-blockers, angiotensin-converting enzyme inhibitors, or angiotensin II receptor antagonists may prevent, suppress, or restore cardiac dysfunction. However, the preservation of cells subjected to lethal injury remains an attractive goal, and inhibition of cardiac myocyte apoptosis by metabolic treatments may represent a novel approach for treatment of cardiac disease [9,15]. Available evidence suggests that the failing heart is an engine that is depleted of fuel. In other words, altered energetics plays an important part in the pathophysiology of heart failure. Various groups have pursued this energy-depletion hypothesis over the past 20 years and, today, energy metabolism in the heart is a topic of considerable interest.

Major metabolic changes occurring during the early hours of myocardial infarction include increased secretion of catecholamines and production of circulating free fatty acids (FFAs). Under normal conditions, the myocardium depends on aerobic metabolism, with FFAs as the preferred source of energy. During ischemia-reperfusion, FFA concentrations are greatly increased, and exert a toxic effect on the myocardium. This effect determines increased membrane damage, endothelial dysfunction, tissue inflammation, and decreased cardiac function. Decreasing plasma FFA concentrations and cardiac fatty acid oxidation, together with the stimulation of glucose and lactate uptake, might reduce these detrimental effects [9,15,16]. This might be achieved by the use of glucose–insulin–potassium solutions at the time of reperfusion [17], and by inhibiting fatty acid oxidation with 3-ketoacyl coenzyme A thiolase inhibitors, such as trimetazidine [9,15]. Currently, we have many remarkable experimental and clinical findings regarding the beneficial effects of metabolic treatment in CAD and heart failure. The drug studied most is trimetazidine. A variety of clinical studies of this drug have demonstrated that it offers a significant cardioprotection that is achieved through several mechanisms (*Table* I).

**Plasma brain natriuretic peptide and troponin concentrations**

In contrast to other neurohormones that exhibit increased concentrations in heart failure, natriuretic
peptides have an adaptive counter-regulatory role [18]. Plasma BNP concentrations are currently used as diagnostic and prognostic markers in patients with CHF [18]. It is particularly noteworthy that, in multivariate analyses, BNP and N-terminal proBNP (NT-proBNP) are stronger predictors of mortality than NYHA functional class, norepinephrine (adrenaline), left ventricular ejection fraction (LVEF), or age [19,20]. Serum BNP is also a valid marker in assessing the risk of ventricular tachycardia and, probably, sudden death, in patients with ischemic or non ischemic cardiomyopathy [21].

Various studies have provided evidence that metabolic treatment with trimetazidine could positively influence the prognosis and quality of life of patients with CAD and CHF [9] and reduce left ventricular remodeling and plasma concentrations of BNP and TnT [27]. In 50 patients with stable ischemic cardiomyopathy after 6 months of trimetazidine treatment, we showed a significant improvement in functional capacity (6 min walking test) associated with a significant reduction in plasma BNP concentration (the latter was significantly increased in controls); TnT concentrations also reduced significantly during trimetazidine treatment, whereas they were un-

REFERENCES


Serial determination of troponin concentrations in the diagnosis of acute myocardial infarction

Vlad C. Vasile, Lori A. Blauwet and Allan S. Jaffe
Department of Internal Medicine, Division of Cardiovascular Diseases and Department of Laboratory Medicine and Pathology, Mayo Clinic and Mayo Medical School, Rochester, Minnesota, USA

Correspondence: Dr Allan S. Jaffe, Mayo Clinic, 200 First St SW, Division of Cardiovascular Diseases, Gonda 5, Rochester, Minnesota 55905, USA.
Tel: +1 507 284 3680; fax: +1 507 266 0228; e-mail: Jaffe.Allan@mayo.edu

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Abstract

Contemporary guidelines for the definition of acute myocardial infarction recommend the presence of an increase or decrease in troponin concentration in patients presenting to the hospital with chest pain and electrocardiogram changes. Here we report the case of a 49-year-old woman with recurring chest pain, nausea, and diaphoresis with initially undetectable troponin concentrations that subsequently increased. This case demonstrates the importance of serial determination of troponin concentrations and changing biomarker patterns in the management of patients presenting with possible cardiac ischemic injury.


Keywords: Acute myocardial infarction, chest pain, troponin changes

Case report

In April 2008, a 49-year-old woman presented to the emergency department after three episodes of chest pain, the first of which had occurred 6 h before presentation. The chest pain was described as a sensation of tightness associated with shortness of breath, which awoke her during the night. The second and third episodes were accompanied by mild nausea and diaphoresis. The patient was free of pain at the time of presentation. Her personal medical history included essential hypertension, under medical control. She had been told that her cholesterol was high but, given her high high-density lipoprotein concentrations, she did not require treatment. She had recently returned from a long vacation during which she and her spouse had driven across the country. The patient’s electrocardiogram (ECG) in the emergency department showed normal sinus rhythm with minor anterior T-wave abnormalities. Blood tests were unremarkable, including a cardiac troponin T (cTnT) value less than 0.01 ng/mL. Chest X-ray showed clear lungs, normal heart size, and a tortuous aorta. The d-dimer concentration was mildly increased.

The patient was placed in the observation room. At approximately 4 h after presentation, her ECG was unchanged, but her cTnT concentration had increased to 0.02 ng/mL. At 6 h after the patient’s admission, the third cTnT value was 0.03 ng/mL, indicative of a pattern of increasing values. Because of this increasing pattern, the patient was admitted from the emergency department to a cardiology service. Aspirin had been given in the emergency department, but, because of the modest cTnT concentrations, the
Case report
Vlad C. Vasile, Lori A. Blauwet, and Allan S. Jaffe

Figure 1. Computed tomography contrast-enhanced coronary angiogram of the left anterior descending artery. Arrows indicate noncalcified lesions.

The patient was not treated with heparin or a GpIIb/IIIa inhibitor. She did, however, receive a β-blocker. A computed tomography (CT) angiogram was obtained to evaluate for possible pulmonary embolism and to assess her coronary arteries noninvasively. Computed tomography excluded pulmonary emboli, but revealed discrete noncalcified lesions in her proximal and mid left anterior descending (LAD) coronary artery, estimated at 75% each (Figure 1). The distal LAD was normal. Shortly thereafter, the patient developed severe chest pain, diaphoresis, and nausea. The ECG showed changes suggestive of an acute anteroseptal myocardial infarction, so she was taken expeditiously to the cardiac catheterization laboratory. Coronary angiogram revealed that the proximal LAD was 100% occluded and the mid LAD had a discrete 70% obstruction lesion; the distal segment was of normal size. The proximal circumflex artery was 30% obstructed by a single discrete lesion, and the proximal right coronary artery was 20% obstructed by a single discrete lesion. Each of the two LAD lesions was treated with a bare metal stent. The patient continued to take aspirin 325 mg daily and was discharged from hospital 3 days later, receiving clopidogrel 75 mg, lisinopril 5 mg, metoprolol 200 mg, and simvastatin 40 mg, all daily.

Comment

This case report emphasizes the importance of a pattern of increasing cTn values. The concentrations of cTn currently measurable with contemporary assays are far greater than those we now consider to be normal values [1], therefore increases in cTn are usually important as a marker of a cardiac abnormality [2]. There can be analytic false-positives, but they are uncommon and rarely manifest a changing pattern of values. Thus an increasing pattern should prompt additional investigation.

In this patient, because of the antecedent history of a long car journey and a mildly increased D-dimer value, the diagnosis of pulmonary embolism was considered. Pulmonary embolism can cause increases in cTn but, when they occur, they usually mark quite large pulmonary emboli. In this case, the patient appeared very stable, so a large pulmonary embolism was unlikely. However, given the ability of CT angiography to evaluate both the pulmonary circulation and the coronary arteries, the decision was made to obtain a CT angiogram, which excluded pulmonary embolism but documented significant coronary artery disease. This is perhaps not unexpected, because coronary artery disease is common in our society.

However, we now understand that many other diseases can also be associated with increases in cTn concentrations. In this context, myocarditis appears to be a common mimicker of coronary artery disease [3] in patients who present acutely. In addition, toxic metabolic insults such as carbon monoxide poisoning can cause cardiac injury. Critically ill patients [4,5] often have a pattern of increasing values of cTn. Some of these increases may be the result of occult coronary artery disease with or without supply–demand abnormalities, but they can also be result from hypotension, or medications such as catecholamines that are used to treat these patients. Regardless of the etiology, increases in troponin concentration appear to be highly prognostic, in both the short and longer terms [4,5].

On occasion, no etiology is apparent, and that reflects the fact that there are causes for cardiac injury of which we are unaware or for which we lack knowledge as to their assessment. A word of caution is necessary, however. At times, we overestimate the accuracy of some of our tests. We [6] and others have reported a pattern of acute myocardial infarction (AMI) that was revealed by magnetic resonance imaging in patients, mostly women, with what appeared to be normal coronary arteries on coronary angiography. Therefore, in our view, an apparently normal coronary angiogram does not always exclude AMI, especially in women.

This case report illustrates the importance, as recommended by the recent guidelines for the diagnosis of AMI [7], of serial determination of troponin concentrations, and the significance of a changing pattern of troponin concentrations, in the management of patients presenting with possible cardiac disease.
Case report
Troponin in the diagnosis of AMI


Temporal profile of protein release in myocardial infarction

Piero Montorsi, Marco Villa and Maria Antonietta Dessanai
Institute of Cardiology, University of Milan, Centro Cardiologico Monzino, IRCCS, Milan, Italy

Correspondence: Dr Piero Montorsi, Institute of Cardiology, University of Milan, Centro Cardiologico Monzino, IRCCS, Via Parea 4 – 20138 Milan, Italy.
Tel: +39 02 58002576; fax: +39 02 58002398; e-mail: piero.montorsi@unimi.it

Conflicts of interest: None.

Abstract

Acute myocardial infarction (AMI) is one of the most important cardiovascular diseases in developed countries, where it represents the major cause of death and disability. The diagnosis of “classic” AMI is based on the triad of typical symptoms and changes in the electrocardiogram and in biomarkers. Biomarkers are proteins that are released into the circulation by the damaged myocardial cells. The rate of appearance of these proteins depends on several factors, mainly the rate of their elimination from the blood. Each protein has a specific time course of release in terms of first detection in the blood at concentrations above the upper reference limit, peak plasma concentration, and normalization (Table I). The ideal biomarker should have high sensitivity and specificity, appear early during the course of the disease, remain abnormal for several days to allow a late diagnosis, and be easily assessed by user-friendly assay. This ideal marker has not yet been discovered; however, cardiac troponins and creatine kinase MB mass are the biomarkers most used for the diagnosis of AMI. Emerging markers include C-reactive protein, pentraxin-3, and brain natriuretic peptide.

Keywords: Acute myocardial infarction, biomarkers, protein release

Introduction

Acute myocardial infarction (AMI) is one of the most important diseases in developed countries, where it represents a major cause of death and disability. In the majority of cases, AMI is caused by an abrupt coronary thrombosis as a result of erosion of, or development of a fissure in a “vulnerable” non flow-limiting coronary atherosclerotic plaque. The diagnosis of “classic” AMI is based on the triad of typical symptoms and changes in the electrocardiogram and in biomarkers. As acute coronary artery occlusion occurs, myocardial cell necrosis develops after 20 min and can be identified by the appearance in the blood of different proteins released into the circulation by the damaged cardiac myocytes [1]. The rate of appearance of biomarkers depends on several factors, mainly the rate of their elimination from the blood (greatly influenced by the patency of the culprit vessel). Release of each marker is characterized by a specific time course, including the first detection in the blood at concentrations above the upper reference limits, the peak concentration, and normalization (Table I). The ideal biomarker should have high sensitivity and specificity, appear early during the course of the disease to allow a rapid diagnosis, remain abnormal for several days to allow a late diagnosis, and be easily assessed by user-friendly assay. This ideal marker has not yet been discovered; however, cardiac troponin is an established marker for both diagnosis and prognosis in acute coronary syndromes. Emerging markers – such as high-sensitivity C-reactive protein (CRP) and brain natriuretic peptide (BNP) – are used as inflammatory indexes to evaluate the extent of the necrosis and to determine prognosis [2,3].
Refresher corner
Piero Montorsi, Marco Villa, and Maria Antonietta Dessanai

Table I. First detection, peak, and normalization of protein plasma concentration during acute myocardial infarction.

<table>
<thead>
<tr>
<th>Marker</th>
<th>First detection</th>
<th>Peak</th>
<th>Normalization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myoglobin</td>
<td>2 h</td>
<td>4–12 h</td>
<td>24 h</td>
</tr>
<tr>
<td>MB-CK mass</td>
<td>3–12 h</td>
<td>24 h</td>
<td>48–72 h</td>
</tr>
<tr>
<td>Troponin</td>
<td>3–6 h</td>
<td>24–48 h</td>
<td>5–10 days (cTnI)</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>4–6 h</td>
<td>50 h</td>
<td>NA</td>
</tr>
<tr>
<td>Pentraxin TX3</td>
<td>2–4 h</td>
<td>20–24 h</td>
<td>NA</td>
</tr>
<tr>
<td>Brain natriuretic peptide</td>
<td>early</td>
<td>20 h to 5 days</td>
<td>&lt;30 days to NA</td>
</tr>
<tr>
<td>NA, not available.</td>
<td></td>
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</table>

Myoglobin

Myoglobin is a small, nonenzymatic heme protein, rapidly released into the circulation from injured myocardial cells. It is detectable in blood as early as 1 h after myocardial injury. The peak of its concentration is reached earlier (approximately 12 h) than that of any other biomarker and tends to normalize within 1 day (Figure 1). Because of its high sensitivity and low specificity for AMI, this marker is particularly suited to excluding AMI at the earliest phase of the disease. As it is not cardiac specific, false-positives may be found in patients with acute or chronic skeletal muscle damage or in patients with chronic renal failure. As for many other biomarkers, a more rapid increase in the plasma concentration of myoglobin is found in patients who achieve a full reperfusion. The early normalization of myoglobin makes this marker particularly indicated for the diagnosis of re-infarction [4,5]; in this event, a “double-peaked” curve is usually detected.

Creatine kinase MB isoenzyme

Creatine kinase MB (CK-MB) is a cytosolic carrier protein for high-energy phosphatase. For several years, the isoenzyme MB has been the marker of AMI used most. The plasma concentration of CK-MB mass starts to increase above the cutoff value between 3 and 12 h after the onset of chest pain. It reaches a peak at 24 h and returns to normal values within 48–72 h (Figure 1). Although it is found in the greatest concentration in cardiac tissue, increased concentrations of CK-MB may be found in renal failure, hypertension, skeletal muscle injury, and other conditions. However, a high concentration of CK-MB may be considered diagnostic for myocardial infarction, especially if combined with the typical increase and decrease in values [6,7].

Troponin

Cardiac troponin (cTn) is one of the biomarkers that have been found to have both diagnostic and prognostic importance in AMI. It is a protein complex that regulates the calcium-modulated interaction between actin and myosin in striated muscle, and is constituted of three subunits: T, C, and I. Troponins I (cTnI) and T (cTnT) are highly specific and sensitive for cardiac injury [8]. A significant increase in cardiac troponin value is defined as a plasma concentration exceeding the 99th percentile of that of a normal reference population [1].

Generally, in AMI the plasma concentration of troponin begins to increase above the cutoff value by 2–4 h after the onset of symptoms, reaching a peak after 24–48 h; concentrations may remain increased for up to 5–10 days (cTnI) or 5–14 days (cTnT) (Figure 1). This long-lasting increase, probably resulting from the continuous release of troponin from the degenerating contractile apparatus of necrotic myocytes, helps in the correct diagnosis of AMI late in the course of the disease. Detection of the typical increase and decrease in concentrations is essential to the diagnosis of AMI, especially if there are coexisting extracardiac conditions that may be associated with high plasma concentrations of troponin (eg, chronic renal failure, trauma, extreme exertion) [9–11].

Measurement of troponins in AMI should be made when the patient is first seen and again 6–9 h later. If AMI is highly suspected but the first measurement does not reveal increased concentrations of troponin, a third

Figure 1. Profile of release of troponin I (TnI), creatine kinase MB (CK-MB) and myoglobin in acute myocardial infarction (AMI), upper reference limit (URL), data from De Groot et al [5] and Larue et al [8].
blood sample for measurement of troponin should be
drawn after an interval of 12–24 h. Myocardial re-
infarction may alter the standard time course of release
of troponin, accounting for a renewed increase in, or a
progressively increasing, plasma concentration. In
such an event, two additional blood sample measure-
ments, 3–6 h apart, are recommended. Recurrent myo-
cardial infarction is diagnosed if there is an increase of
at least 20% in the second measurement.

Myocardial reperfusion, whether spontaneous, or
achieved pharmacologically with lytic agents or
mechanically with percutaneous coronary interven-
tion, may affect the kinetics of release of biomarkers in
AMI, including troponin. Patients with ST-segment
elevation myocardial infarction who achieve an effec-
tive reperfusion have a greater and earlier peak
plasma concentration of troponin, followed by a faster
return to normal – the so-called ‘‘wash-out phenom-
omenon’’ – compared with those patients having no
significant reperfusion (Figure 2) [12,13]. In this event,
two blood samples should be collected – at the time of
the patient’s admission to hospital, and 90 min later –
and the enzyme plasma concentrations compared.
The ratio between the concentrations at these two
points can be used to discriminate between successful
and unsuccessful reperfusion. In general, the greater
the ratio (at least 5), the more likely it is that reperfu-
sion has occurred. If reperfusion has indeed occurred,
estimation of infarct size using peak biomarker con-
centration may be not reliable.

C-reactive protein

Inflammation is a factor in all stages of the ather-
sclerotic disease process, and represents a pathophy-
siologic link between the formation of plaque and its
rupture, which are responsible for AMI. To date, CRP
(an acute-phase reactant protein made in the liver) has
been the preferred marker for the detection of inflam-
mation and to define prognosis during acute coronary
syndromes [14,15]. When AMI occurs, an exponen-
tial increase in CRP is observed, starting 4–6 h after
the onset of symptoms and reaching a peak about 50 h
later (Figure 3). The increase in plasma concentrations
of CRP is strictly related to the degree of cardiac
damage [14]. High concentrations of CRP, in patients
with persistent ST-segment elevation after AMI, is an
important negative prognostic factor, in both the short
and the long term [16,17].

The specificity of CRP as a marker is very low,
because increased concentrations of CRP are detected
in several conditions, such as diabetes, obesity, estro-
gen therapy, hypertension, and smoking.

Pentraxin-3

Pentraxin-3 (PTX3) is related to classic pentraxins (like
C-reactive protein CRP or serum amyloid P SAP) but is
structurally different. It is made in the liver in response
to inflammatory mediators, mainly interleukin-6. It is
also produced in large amounts by the heart. PTX3 is
detected inside both normal and hypertrophic cardi-
omyocytes, and is increased in AMI. Its plasma con-
centration increases rapidly after the onset of symp-
toms, preceding the increase in CRP concentration,
and reaching a peak at 20–24 h after onset of symp-
toms (Figure 3) [18,19]. In patients with unstable
angina, the PTX3 concentration increases to a lower
value than in AMI. Accumulating evidence suggests
that PTX3, binding with C1q in the same way that CRP
and SAP bind to C1q, contributes to the mechanism of
increase in tissue damage [20].

Brain natriuretic peptide

Brain natriuretic peptide is 32-amino-acid peptide
released in response to ventricular stretch. Its functions
are similar to those of atrial natriuretic peptide: mainly, reducing systemic vascular resistances and central venous pressure, and increasing natriuresis. Two types of time course of change in BNP concentrations have been reported in association with AMI. The first is a single-peak curve reaching a maximal plasma concentration approximately 20 h after symptom onset and returning slowly to normal within 4 weeks. The second type of curve is characterized by a late peak at 5 days, with values remaining increased at 4 weeks (Figure 4). Reasons for the increase in BNP concentrations during AMI are not easily understood. In a study by Morita et al [21], BNP concentration did not correlate significantly with hemodynamic parameters in the early phase of AMI, suggesting that the increased concentration may be the result of myocardial necrosis or local mechanical stress, or both. Interestingly, the biphasic curve was observed more frequently in patients with anterior AMI, signs or symptoms of heart failure, lower ejection fraction, and higher plasma concentrations of CK-MB than was the single-peak curve. These data suggest that increased concentrations of BNP in the late phase of AMI may be related to infarct expansion and ventricular remodeling [21,22]. Kaya et al [22] reported greater plasma concentrations of BNP (single sample) in inferior AMI with right ventricular involvement, compared with those in isolated inferior AMI. This finding reinforces the concept that increased ventricular stretch and filling pressure are potential stimuli for the secretion of BNP during the early phase of AMI in certain patient populations.

Conclusions

Proteins released during acute myocardial infarction can provide important diagnostic and prognostic information. Each possesses a specific time course of release. Because of the rapid increase and decrease in its concentration, and its negative predictive value, myoglobin has a role in the exclusion of AMI. Troponin, with its high cardiac specificity, is the preferred marker for myocardial injury and AMI; several blood sample should be obtained at different times along the course of the disease, to enable diagnosis of cases of late AMI, CRP, PTX3 and BNP are emerging biomarkers that have specific indications in the setting of AMI.

REFERENCES


Prognostic value of troponin I levels for predicting adverse cardiovascular outcomes in postmenopausal women undergoing cardiac surgery


Adverse cardiac events that follow cardiac surgery are an important source of perioperative morbidity and mortality for women. Troponin I provides a sensitive measure of cardiac injury, but its concentrations after cardiac surgery may vary between the sexes. Our purpose in this study was to evaluate the prognostic value of troponin I concentrations for predicting cardiovascular complications in postmenopausal women undergoing cardiac surgery. The cohort of this study were women enrolled in a previously reported clinical trial evaluating the neuroprotective potential of 17β-estradiol in elderly women. In that study, 175 postmenopausal women not receiving estrogen replacement therapy and scheduled to undergo coronary artery bypass graft (with or without valve surgery) were prospectively randomly allocated to groups to receive 17β-estradiol or placebo in a double-blind manner, beginning the day before surgery and continuing for 5 days postoperatively. Serial 12-lead electrocardiograms were performed and serum troponin I concentrations were measured before surgery, after surgery on arrival of the patient in the intensive care unit, and for the first 4 days after operation. The primary endpoint of the present study was major adverse cardiovascular events (MACE), defined as a Q-wave myocardial infarction, low cardiac output state or death within 30 days of surgery. The diagnosis of Q-wave myocardial infarction was made independently by two physicians blinded to the treatment and patient outcomes, with the final diagnosis requiring consensus. A low cardiac output state was defined as cardiac index <2.0 L/min per m² for >8 h, regardless of treatment. Troponin I concentrations on postoperative day 1 were predictive of MACE (area under the receiver operator curve = 0.862). A cutoff point for troponin I of >7.6 ng/mL (95% confidence interval 6.4 to 10.8) provided the optimal sensitivity and specificity for identifying patients at risk for MACE. The negative predictive value of a troponin I concentration for identifying a patient with a composite cardiovascular outcome was high (96%) and the positive predictive value moderate (40%). Postoperative troponin I concentrations were not different between women receiving perioperative 17β-estradiol treatment and those receiving placebo, and the frequency of MACE was not influenced by 17β-estradiol treatment. We conclude that, in postmenopausal women, increased troponin I concentrations on postoperative day 1 are predictive of MACE. Monitoring of perioperative troponin I concentrations might provide a means for stratifying patients at risk for adverse cardiovascular events.

Commentary

A small increase in troponin concentration may occur in clinically stable populations, and is frequently observed after successful percutaneous coronary intervention (PCI) or bypass surgery.

With the new universal definition of myocardial infarction, routine dosage of troponin I after revascularization procedures could result in an overdiagnosis of myocardial infarction. A recent American Heart Association/American College of Cardiology statement defined an increase of troponin I greater than 3 times the 99th percentile after PCI as “periprocedural myocardial infarction”. In patients with stable coronary disease, troponin increases significantly after PCI in 31% of patients and is independently and significantly associated with an increase in major adverse cardiac events at 1 and 18 months [1]. Conversely, in a more recent study [2], such an increase occurred in 23.4% of patients who underwent PCI, but was not associated with a greater rate of adverse cardiac events at 1 year. The investigators concluded that the definition of myocardial infarction may be too strict, and that measurement of troponin I after PCI is of questionable use. A meta-analysis of 20 studies involving 15,581 patients undergoing PCI [3] led its authors to conclude that an increase in troponin I is significantly associated with increased mortality and non-fatal myocardial infarction.

In this study by Stearns et al in postmenopausal women, increased postoperative troponin I concentrations were, again, predictive of major adverse cardiac events.

It may be concluded that monitoring of periprocedural troponin I in patients undergoing percutaneous or surgical coronary revascularization is helpful in stratifying those at risk, and in predicting adverse events.
Diabetes increases the risk of cardiovascular disease, with diabetic individuals having a two- to fourfold greater risk of death from myocardial infarction than non-diabetic individuals \([1,2]\). Multiple abnormalities associated with diabetes mellitus – such as hyperglycemia, hyperlipidemia, and insulin resistance – have been postulated to contribute to adverse outcomes in diabetes following myocardial ischemia \([3]\). However, the specific contribution of impaired myocardial insulin action \(\text{per se}\) in the response of the heart to myocardial ischemia is incompletely understood. Sena et al sought to determine the impact of impaired myocardial insulin signaling, in the absence of diabetes, on the development of left ventricular dysfunction after myocardial infarction.

To examine the role of altered cardiomyocyte insulin signaling in the adaptation to myocardial infarction, independently of confounding systemic factors, the authors subjected CIRKO mice and wild-type mice to proximal left coronary artery ligation (myocardial infarction) and followed them up for 14 days. The goal was to assess potential mechanisms of left ventricular dysfunction during the period of greatest risk. Echocardiographic data, mitochondrial respiration, and gene expression data collected 14 days after infarction in both groups showed that, despite equivalent infarct size, mortality was increased in CIRKO mice compared with wildtype mice, whereas, in surviving mice, left ventricular ejection fraction and \(\text{dP/dt}\) were reduced by more than 40% in the CIRKO group.

To elucidate the mechanisms for the accelerated left ventricular dysfunction in CIRKO mice, the authors examined mitochondrial function by determining oxygen consumption in cardiac fibers that were exposed to fatty acid. They found that, whereas the maximal \(V_{\text{ADP}}\)-stimulated mitochondrial rate of \(O_2\) consumption \((V_{\text{ADP}})\) with palmitoyl carnitine as substrate were unchanged in WT-MI mice relative to sham-operated animals, \(V_{\text{ADP}}\) was significantly reduced in CIRKO-MI mice \((13.17 \pm 0.94 \text{ mmol } O_2/\text{min per mg dry weight}; P < 0.05)\). Relative to those in WT-MI, in CIRKO-MI the levels of expression of glucose transporter 4 \((\text{GLUT}4)\), peroxisome proliferator activated receptor-alpha \((\text{PPAR} \alpha)\), sarcoplasmic reticulum \(\text{Ca}^{2+}\)-ATPase \((\text{SERCA}2)\), the fatty acid oxidation genes, \(\text{MCAD}, \text{LCAD}, \text{CPT}2\), and the electron transfer flavoprotein, \(\text{ETF} \text{DH}\), were repressed. Thus reduced insulin action in cardiac myocytes accelerates after myocardial infarction left ventricular dysfunction, in part because of a rapid decline in mitochondrial fatty acid oxidative capacity, combined with limited glucose transport capacity that may reduce substrate utilization and availability.

**Commentary**

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Mitochondrial \( \beta \)-oxidation capacity, and a decrease in levels expression of the gene for GLUT4 (an important mediator of glucose uptake). These findings indicate that insulin signaling might play an important part in sustaining left ventricular metabolic capacity post myocardial infarction.

This study has some limitations. CIRKO mice have total deletion of insulin signaling, rather than insulin resistance. It is therefore possible that, under such conditions, the absence, not only of insulin-dependent metabolic pathways, but also of insulin signaling – particularly the absence of pro-survival mechanisms mediated by insulin signaling [4] – could have contributed to the accelerated rate of left ventricular dysfunction. Genetic deletion of insulin signaling is obviously more drastic than the more partial degrees of cardiac insulin resistance that might occur in individuals with insulin resistance or diabetes. Although this study provides an indication of some major metabolic and functional consequences of defects in insulin signaling, additional work in models with partial impairment in myocardial insulin action will be required to determine the consequences of lesser degrees of insulin resistance on the myocardial adaptations that occur after myocardial infarction.

REFERENCES


Danielle Feuvray

**Featured research**

**Abstracts and commentaries**

**Body-mass index and cause-specific mortality in 900 000 adults: collaborative analyses of 57 prospective studies**


The main associations of body-mass index (BMI) with overall and cause-specific mortality can best be assessed by long-term prospective follow-up of large numbers of people. The Prospective Studies Collaboration aimed to investigate these associations by sharing data from many studies. Collaborative analyses were undertaken of baseline BMI versus mortality in 57 prospective studies with 894 576 participants (mean recruitment age 46 [SD 11] years; mean BMI 25 [SD 4] kg/m\(^2\); 541 452 men, 61% in western Europe and North America. The median recruitment year was 1979 (interquartile range 1975–1985). The analyses were adjusted for age, sex, smoking status, and study. To limit reverse causality, the first 5 years of follow-up were excluded, leaving 66 552 deaths of known cause during a mean of 8 (SD 6) further years of follow-up (mean age at death 67 [SD 10] years: 30 416 vascular; 2070 diabetic, renal, or hepatic; 22 592 neoplastic; 3770 respiratory; 7704 other. In both sexes, mortality was lowest at BMI about 22.5–25 kg/m\(^2\). Above this range, positive associations were recorded for several specific causes and inverse associations for none, the absolute excess risks for greater BMI and smoking were roughly additive, and each 5 kg/m\(^2\) greater BMI was, on average, associated with about 30% greater overall mortality (hazard ratio per 5 kg/m\(^2\) 1.29, 95% confidence interval [CI] 1.27 to 1.32): 40% for vascular mortality (hazard ratio 1.41, 95% CI 1.37 to 1.45); 60–120% for diabetic (hazard ratio 2.16, 95% CI 1.89 to 2.46), renal (hazard ratio 1.59, 95% CI 1.27 to 1.99), and hepatic (hazard ratio 1.82, 95% CI 1.59 to 2.09) mortality; 10% for neoplastic mortality (hazard ratio 1.10, 95% CI 1.06 to 1.15); 20% for respiratory (hazard ratio 1.20, 95% CI 1.07 to 1.34) and all other mortality (hazard ratio 1.20, 95% CI 1.16 to 1.25). Below the range 22.5–25 kg/m\(^2\), BMI was associated inversely with overall mortality, mainly because of strong inverse associations with respiratory disease and lung cancer. These inverse associations were much stronger for smokers than for non-smokers, despite cigarette consumption per smoker varying little with BMI. Although other anthropometric measures (eg, waist circumference, waist-to-hip ratio) could well add extra information to BMI, and BMI to them, BMI is in itself a strong predictor of overall mortality both above and below the apparent optimum of about 22.5–25 kg/m\(^2\). The progressive excess mortality above this range is mainly attributable to vascular disease and is probably largely causal. At 30–35 kg/m\(^2\), median survival is reduced by 2–4 years; at 40–45 kg/m\(^2\), it is reduced by 8–10 years (which is comparable to the effects of smoking). The definite excess mortality below 22.5 kg/m\(^2\) is mainly attributable to smoking-related diseases, and is not fully explained.

**Commentary**

This Herculean analysis leaves little room for doubt. It is best to have a BMI around 22.5–25 kg/m\(^2\). Either
side of this narrow range, mortality increases. However above 25 kg/m$^2$ this increase follows a log-linear relationship and is therefore most obvious for BMI values greater than 30 kg/m$^2$. In fact, it seems it is safer to be slightly above the ideal range (BMI 27.5–30 kg/m$^2$) than below it (18.5–22.5 kg/m$^2$).

The power of this study lies in its size and in its design. Events were collected prospectively, but 16,000 deaths occurring within 5 years of first enrolment were excluded to remove bias from reverse causality – established disease affecting baseline BMI, rather than BMI affecting occurrence of subsequent disease. Potential participants with overt pre-existing heart or cerebrovascular disease were not enrolled. Despite the exclusion of the first 5 years of follow-up, the study still included 6.5 million person-years of observation during which 72,749 deaths were identified. Approximately 60% of these deaths occurred in patients aged 35–69 years, reflecting the demographics of the populations recruited to the constituent studies. The average duration of follow-up beyond the 5th year was 8 years. The sheer number of events allowed separate analyses of death by cause. The positive correlation between BMI above 22.5 kg/m$^2$ and death was seen most clearly for ischemic heart disease, stroke, other vascular diseases, diabetes-related death, and deaths related to non-neoplastic kidney and liver disease. The determination of cause of death varied by constituent study, but was most commonly by death certificate.

Finally, the excess mortality seen in individuals weighing less than the ideal BMI was primarily related to respiratory disease, and this was most marked in smokers. It is possible that this was the result of pre-existing chronic respiratory disease with a natural history that exceeded the arbitrary 5 year cutoff, and thus the result of reverse causality.

The main difficulty in translating this powerful study into changes in practice is knowing which component of the excess mortality seen with BMI >25 kg/m$^2$ is a direct result of BMI and which is the result of established risk factors that are associated with BMI. The authors demonstrate a clear positive correlation between low-density lipoprotein (LDL) cholesterol and systolic and diastolic blood pressure and BMI, and a clear negative correlation between high-density lipoprotein (HDL) cholesterol and BMI. Thus the authors estimate that much, if not all, the increased cardiovascular mortality can be explained by these risk factors, up to a BMI of approximately 30 kg/m$^2$. Beyond this, mortality increases more steeply than blood pressure, LDL cholesterol or LDL:HDL ratio. Thus the excess mortality must be explained by other factors, such as insulin resistance, which were not systematically assessed. The other problem is that, although some of the constituent studies performed more sophisticated analyses of fat distribution and lean body mass, these were not performed sufficiently frequently to be included in the analyses. It is possible, therefore, that individuals with similar BMI values may have different risks according to the presence and distribution of fat. There was no analysis of “normal weight obesity”, although most participants were from Western countries. The lack of these refinements makes it difficult to translate the findings of the study to an individual patient, although the findings send a very clear message regarding population risk and the increasing prevalence of obesity (BMI >30 kg/m$^2$) in most countries.

The clear and unambiguous findings of this study suggest that it is best to have a BMI of 22.5–25 kg/m$^2$ – a target that is very difficult to achieve for most overweight and obese individuals. They provide some reassurance that, up to a BMI of 30 kg/m$^2$, risk seems largely to accrue through established risk factors that are more easily modified than BMI itself. Beyond a BMI of 30 kg/m$^2$, risk increases sharply, and it is even more unclear how this risk can be reduced independently of tackling BMI directly.
**Asymmetric dimethylarginine (ADMA)**

ADMA is a guanidino-substituted analogue of L-arginine that acts as an endogenous, inhibitor of nitric oxide synthase (NOS) by competitively displacing L-arginine from the NOS active site. The concentration of ADMA is increased in the plasma of human patients with hypercholesterolemia, atherosclerosis, hypertension, chronic renal failure, and chronic heart failure. Moreover, ADMA concentrations are elevated in proportion to the severity of carotid, coronary, and peripheral atherosclerosis, leading to the suggestion of ADMA as a novel marker of cardiovascular risk.

**B-type natriuretic peptide**

Also known as brain natriuretic peptide, is a 32 amino acid polypeptide that is produced and secreted by the ventricles of the heart during excessive stretching of cardiac myocytes. Although it is produced primarily in the ventricles of the heart in humans, its name originates from it initially being discovered in porcine brain extracts.

**Genomics**

Genomics is the study of an organism’s genome (full DNA sequence). Genomics includes an intensive effort to determine the complete DNA sequence of organisms and fine-scale genetic mapping efforts. Also included in this field are studies of intragenomic phenomena such as epistasis, heterosis, pleiotropy, and other interactions between alleles and loci within the genome.

**Lipoprotein-associated phospholipase A2 (Lp-PLA_2)**

A protein predominantly synthesized by macrophages that belongs to the calcium-independent family of phospholipase A_2_ proteins. Lp-PLA_2_ is considered a vascular-specific inflammatory marker. In plasma Lp-PLA_2_ is bound to low density lipoproteins (LDL) and high density lipoproteins (HDL), with higher affinity for LDL molecules that have been minimally oxidatively modified. Elevated Lp-PLA_2_ (mass concentration and/or elevated activity) is recommended as a novel risk factor and risk marker involved in the causal pathway of atherosclerotic plaque inflammation and the formation of rupture-prone plaque, as such is an emerging candidate for future cardiovascular disease (CVD) risk.

**Protein kinase C**

Is a family of ~10 isoforms divided into 3 classes (classical, novel, and atypical) capable of controlling the function of other proteins via phosphorylation of hydroxyl groups on serine and threonine amino acid residues of these proteins. Protein kinase C isoforms in general are activated by signals that elevate the concentration of Ca^{2+} and diacylglycerol. Protein kinase C isoforms play important roles in the regulation of several signal transduction cascades.

**Proteolysis**

Is the degradation (digestion) of proteins by a family of cellular enzymes known as the “proteases”.

**Phosphorylation**

Is the addition/introduction of a phosphate group (PO_{4}) onto a protein or other organic molecule by the action of a wide class of enzymes known as the “kinases”. Protein phosphorylation by kinases plays a major role in many signalling cascades, such as insulin regulation of glycogen mobilization.

**Troponin**

A regulatory protein present in striated muscle (skeletal and cardiac muscle) that in conjunction with tropomyosin forms a regulatory complex that controls the interaction of actin and myosin. The binding of Ca^{2+} to troponin permits muscle contraction. Cardiac troponins are released from cardiac myocytes following myocardial damage and loss of membrane integrity, and serve as highly sensitive and specific biomarkers for establishing the diagnosis of myocardial infarction.