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REVIEW ARTICLE

Proteomic biomarkers for diagnosis in acute myocardial infarction

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Abstract

Acute myocardial infarction (AMI) is considered the leading cause of morbidity and mortality in many industrialized nations. AMI is defined currently by detection of a rise and/or fall of cardiac biomarkers at least above the 99th percentile of the upper limit. Early detection of AMI could conceivably provide important information for understanding the molecular functions of heart disease, and would enable more effective diagnosis and treatment of patients. However, diagnostic approaches currently in use for the evaluation of patients, associated with chest pain or other symptoms suggestive of AMI are acceptable, but they are time-consuming, high-cost and labour-intensive in most cases. Thus, much work is needed in the development of biomarkers for accurate and cost-effective diagnosis of AMI and for effective management of patients. In this article, we give an overview of proteomic biomarkers for rapid and reliable diagnosis of AMI, focusing on biochemical characteristics and their clinical applications for point-of-care of AMI. We also postulate the future directions in the pursuit of integrated multiplex assay systems for multifunctional diagnosis in AMI.

Keywords: *Acute myocardial infarction; biomarker; diagnosis; point-of-care*

Introduction

Acute myocardial infarction (AMI) is considered to be the leading cause of morbidity and mortality in many industrialized nations. In particular, AMI is the result of a sudden occlusion because of coronary plaques in the platelets (Apple et al. 2002, Howie-Esquivel & White 2008, Jaffe 2001, Kost & Tran 2005). According to recent reports from the American Heart Association (AHA) and heart disease statistics in the United States, approximately seven million cases of myocardial infarction (MI) were reported and a considerable proportion among the population were suspected AMI patients (Thom et al. 2006). According to these statistic reports, the total medical costs for coronary heart disease (CHD) including AMI were estimated at 100 billion dollars in the USA, ranking the second among 10 leading diagnostic diseases.

The combined evaluation tools of physical examination together with electrocardiogram and the measurement of goldstandard cardiac biomarkers have been used widely in hospitals. But, this approach gives low sensitivity and specificity for the diagnosis of AMI in some cases (Chu et al. 2002a, Keller et al. 2009, Kost & Tran 2005, Plebani & Zaninotto 1999, Senter & Francis 2009). This conventional method is quite expensive and not widely acceptable in all post-AMI or suspected AMI patients. Early diagnosis of AMI with high accuracy is extremely important because it enablesthe savingof lives and lowers the total medical costs when treating patients. Many efforts have been made to find various cardiac biomarkers for the early diagnosis of AMI (Chu et al. 2002b,Collinson 1998, Howie-Esquivel & White 2008, Jaffe 2001, Keller et al. 2009,Kost&Tran 2005,Plebani & Zaninotto 1999,Senter & Francis 2009). The biomarkers indicating AMI may provide an easy method for

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diagnosis and provide the general diagnostic method for total healthcare of the patients.

This review presents an overview of the molecular basis of several proteomic cardiac biomarkers and their clinical consideration for rapid and reliable diagnosis in point-of-care of AMI.

Gold standard proteomic cardiac biomarkers

An ideal biomarker would have several properties including high sensitivity and specificity, quick release into blood or serum for easy detection, a low detection limit, a reasonable assay time and the ability to be measured quantitatively in a cost-effective manner (Braunwald 2008). These parameters are critical issues in the monitoring of suspected AMI patients and for accessing chemotherapeutic drug treatment. Table 1 summarizes well-characterized cardiac biomarkers for early diagnosis of AMI. Detailed characteristics of several proteomic biomarkers with respect to AMI diagnosis are described below.

Troponin

Since the new definition of the diagnostic criteria of AMI in 2007, cardiac biomarkers (preferably troponins) have been regarded as indicators in the early diagnosis of AMI (Thom et al. 2006,Thygesen et al. 2007a, b). Cardiac troponins (troponin I: ~22 kDa, troponin C: ~17 kDa and troponin T: ~37 kDa) have been widely studied for the diagnosis of AMI (Alaiti et al. 2009, Chapelle 1999, Collinson 1998, Inbar & Shoenfeld 2009, Keller et al. 2009, Mair 1997a,Nageh et al. 2003, Plebani & Zaninotto 1999). Troponins are the regulatory proteins which uniquely exist in striated muscles that regulate

Table 1. The old and new proteomic biomarkers for early diagnosis of acute myocardial infarction (AMI).

Biomarkers	MW (kDa)	Cardiac specific	Initial elevation (h)	Peak elevation(days)	Advantages	Disadvantages	References
Proteomic biomarkers							
CK-MB	86,000	Yes +++	3–12	1	Large clinical experience. Previous 'gold standard'	Low specificity	Apple et al. 2005, Chu et al. 2002b, Gerhardt et al. 1979,Mair et al. 1995,Penttila et al. 1999
Myoglobin	17,800	No	1–4	0.5–0.6	Useful for early detection-but, inadequate for single assay	Low specificity	Apple et al. 2005, Chu et al. 2002b,Gornall&Levinoff Roth 1996,Mair et al. 1995,Penttila et al. 1999
Troponin T	37,000	Yes ++++	3–12	0.5–2	High sensitivity as gold standard	Not clear release kinetics	Apple et al. 2005, Christenson et al. 1997, Chu et al. 2002b,Collinson 1998,Penttila et al. 1999
Troponin I	22,000	Yes ++++	3–12	1	High sensitivity as gold standard	Not enough analytical reference	Apple et al. 2005, Chu et al. 2002b,Collinson 1998,Inbar&Shoenfeld 2009,Penttila et al. 1999
Lactate dehydrogenase	135,000	No	10	1–2	–		Apple et al. 2005,Kost&Tran 2005, Reis et al. 1988
Myeloperoxidase	140,000	Yes +	ND*	ND	Would be helpful. No reference standard	Not clear molecular function	Cheng et al. 2008, Eggers et al., Zhang et al. 2002
sCD40L		Yes +	ND	ND	Would be helpful. No reference standard	Not enough analytical reference	Aukrust et al. 1999, Heeschen et al. 2003, Schonbeck et al. 2001
Choline		Yes +	ND	ND	Would be helpful. No reference standard	Not enough analytical reference	Body et al. 2009,Danne et al. 2003, McDonnell et al. 2009, Yue et al. 2008
Glycogen phosphorylase isoenzyme BB	177,000	Yes +++	2–4	4	Would be helpful. No reference standard	Low reproducibility	Kost&Tran 2005, Mair 1998

MW, molecular weight; ND, not determined.

calcium-mediated interactions of actin and myosin. It is known that most troponin exists in a three-unit complex which consists of troponin I, C and T in myofibrils (Wallace et al. 2004). This subunit complex is located on actin filaments and seems to be essential for calcium-dependent regulation of skeletal and cardiac muscle contraction. It is also known that troponin T can be bound to tropomyosin, while troponin I sequentially binds to actin, tropomyosin, troponin T and troponin C, and it can be suppressed by the interaction of actin and myosin. In particular, troponin C interacts with calcium, inducing a steric configuration change and reversing the inhibitory activity of troponin I (Wallace et al. 2004). It has been reported that troponin assays for diagnosis of AMI are more sensitive and specific than creatine kinase (CK)-MB assays because the expression of CK-MB is not unique to heart tissue (Chapelle 1999, Keller et al. 2009, Peivandi et al. 2001, Penttila et al. 1999, Scirica & Morrow 2004). After the onset of symptoms, cardiac troponin levels begin to rise within 4–6 h and thereafter troponins are released into the blood stream (Wallace et al. 2004). Under these circumstances, the peak values of troponin occur at 18–24 h after symptom onset. As shown in Table 2, the elevation of troponin reflects the myocardial damage, but does not precisely indicate why myocardial injury happens. Like troponin T, troponin I is one of the best-studied proteins controlled by other regulatory mechanisms. Interestingly, the levels of troponin I are measurable in serum as soon as 4–6 h and remain for at least 7 days in the post-AMI period, providing a long window of assay time for identifying AMI (Keller et al. 2009, Zehra et al. 2008). On the basis of these observations, troponin I and T have been widely studied and considered as more specific biomarkers than total CK or its isoform, CK-MB for the diagnosis of AMI (Burlina et al. 1994, Casals et al. 2007, Christenson et al. 1997, Mair 1997a, Nadeau et al. 1997, Nageh et al. 2003, Park et al. 2010). Despite the successful development of troponins, problems remain to be solved. In an attempt to improve the accuracy of the diagnosis of AMI using troponins, questions have been raised as to whether troponins (I, C and T) exist in free or complex forms in blood or serum, depending on the medical history of the patients. In addition, the specificity and sensitivity with troponins need to be considered (Wallace et al. 2004). For example, troponin I is localized predominantly in the heart and is not expressed in skeletal muscle or any other tissue. It was attributed to lack of specificity of the immunoassays currently in use. The release mechanism of cytosolic troponins for considering potential biomarkers is needed, because the first release of troponin I into blood is due to the release of the cytosolic pool, while later or prolonged release of troponins indicates the proteolytic degradation from the troponin I complex with various molecules such as actin, myosin, troponin C or T. Further scientific proof and clinical validation will require thorough preclinical trialsand case studies with biochemical measurements.

Table 2. Some commercially available assays for diagnosis of acute myocardial infarction (AMI).

Company	Assay system	Cardiac biomarker	Specimen	Running time
Abott	*i*-STAT troponin I assay	Troponin I	Whole blood (heparinized)	10 min
Beckman-Coulter	AccuTnI	Troponin I		
BioMerieux	Vidas			
Biosite Inc.	Triage® Cardiac Panel	Myoglobin CK-MB Troponin I BNP	Whole blood, plasma	15 min
Dade Behring	Stratus® CS Opus	Myoglobin CK-MB Troponin I	Whole blood	13–22 min
DiagnosticProducts Corporation	Immulite 1	CK-MB Troponin I Myoglobin	Serum	15 min
Ortho Clinical Diagnostics	VITROS 5600	CKCK-MB Troponin I Myoglobin	Whole blood	16 min
Roche Diagnostics	Cardiac M Test	Myoglobin	Whole blood (heparin)	8 min
	Cardiac T Test	Troponin T		12 min
	Cardiac proBNP test	proBNP		12 min
	TROPT®	Troponin T		15 min
Response Biomedical	RAMP system	Myoglobin CK-MB Troponin I	Whole blood	10–12 min
SIEMENS	ADVIA Centaur®TnI-Ultra™	Troponin I	Serum, plasma (heparin)	18 min
TOSOH	AIA	Troponin I Myoglobin	Serum	10–19 min

Creatine kinase

It is known that CK is an enzyme that can catalyse the transfer of phosphate from creatine phosphate to ADP to produce the formation of ATP. Among three isoenzyme forms (MM, MB and BB), CK-MM exists as a major form in skeletal muscles, while CK-BB presents as the predominant form in brain and kidney (Plebani & Zaninotto 1999,Vasan 2006, Wallace et al. 2004, Yang & Min Zhou 2006). Like myoglobin, CK-MB is also present in skeletal muscles. Once released into the blood stream after AMI onset, CK-MB quickly increases within 5 h and the levels of CK-MB reaches at 12–24 h (Yang & Zhou 2006) (Table 3). Thus, serum CK-MB seems to act as an acceptable biomarker for AMI. However, it has some limitations for diagnosis of AMI, e.g. small quantities and low specificity (Lewandrowski et al. 2002, Malasky & Alpert 2002,Penttil et al. 1999, 2002,Yang & Zhou 2006), as described in Table 1. Therefore, the measurement of CK-MB mass only for AMI diagnosis is not recommended because of the broad distribution in tissue. To improve the specificity of CK-MB, the Joint European Society of Cardiology (ESC) and the American College of Cardiology (ACC) committee have proposed multimarker testing with myoglobin, CK-MB and troponin I for risk stratification of AMI. The upper limit of the CHECKMATE study for each biomarker is the 99th percentile of a normal healthy population (Apple et al. 2001). This multimarker strategy appears to be more powerful than measuring any of individual biomarkers alone. Thus, further evaluation of CK-MB may provide a clue for potential use in AMI diagnosis.

Myoglobin

Unlike CK, myoglobin (17.8 kDa) is a non-enzymatic protein used for the diagnosis of AMI (Gornall & Levinoff Roth 1996, Kost & Tran 2005, Nadeau et al. 1997). Physiologically, myoglobin is found in all muscle tissues, indicating that it lacks specificity for cardiac injury or damage (Yang &Zhou 2006, Zehra et al. 2008). Although myoglobin in serum is rapidly secreted as early as 1–4 h after the onset of AMI, the specificity of myoglobin is low because of its abundant presence in myocardial and skeletal muscle injury and other diseases (Karras & Kane 2001, McDonnell et al. 2009, Wallace et al. 2004) (Table 2). Thus, the use of myoglobin as an early marker of AMI has recently been discouraged mainly because of its poor performance compared with the precise and sensitive biomarkers including the troponin assay. However, previous studies have suggested that a combined approach with myoglobin, troponin I or T as well as CK-MB may enable rapid AMI detection with high sensitivity (Apple et al. 2009).

Lactate dehydrogenase

Lactate dehydrogenase (LDH; ~135 kDa) is an enzyme that catalyses the reversible oxidation of lactate to pyruvate. LDH is found in numerous tissues and it consists of five major isoenzymes. For example, LDH1 is released in the highest concentrations from the heart tissues, indicating it as a potential diagnostic indicator of AMI (Karras & Kane 2001, Mair 1997b, Reis et al. 1988, Wallace et al. 2004, Wu 1999, Yang & Zhou 2006). However, the levels of LDH in

Table 3. Biomarker levels released from patients with or without acute myocardial infarction.

Biomarkers	No acute myocardial infarction (healthy population), mean value	Suspected acute myocardial infarction (patients), mean value	*p*-Value	Reference
BNP (ng l^{-1})	201(80–524)	535 (212–1192)ng l^{-1} for men, 672 (283–1820) ng l^{-1} for women 2008 (707–5944)	<0.001<0.001	(Jernberg et al. 2004) (Jernberg et al. 2002)
CRP(mg l^{-1})	2.5(1.2–70.)	3.5(1.7–11.4)	<0.015	(McCann et al. 2008)
Choline (μmol l^{-1})	20.719.4(6.8)	15.031.1(18.8)	ND<0.05	(McCann et al. 2008) (Danne et al. 2003)
CK-MB (μg l^{-1})	3.09 ± 1.2NS	118.3 ± 14.633 ± 3	<0.001	(McCann et al. 2008) (Chapelle et al. 1986)
GPBB (ng/ml^{-1})	70.(4.1–1.5)77	9.3(5.5–14.8)15–1210(104)55	0.0010.05ND*	(McCann et al. 2008) (Rabitzsch et al. 1995) (Mair 1998)
LDH (U l^{-1})	266.6 ± 12.2165	1108.2 ± 932.2624(276)	<0.001NS	(Majeed et al. 2002)
MPO(ngml^{-1})	248(147–415)	280(153–447)>0.35	0.1780.008	(McCann et al. 2008) (Baldus et al. 2003)
sCD40L (ngml^{-1})	0.9	8.1	<0.05	(Aukrust et al. 1999)
H-FABP (μg l^{-1})	< 5	12.3 ± 9.6	<0.001	(Alhadi&Fox 2004)
Troponin T (ngml^{-1})	<0.010.002 ± 0.007	4.7 ± 2.13.95 ± 5.47	<0.001<0.001	(McCann et al. 2008) (Muller-Bardorff et al. 1995)
Troponin I (ngml–1)	104.80 ± 7.49	20-55047.05 ± 27.63	0.01<0.0001	(McCann et al. 2008) (Simon et al. 2008)

ND, not determined; CRP, C-reactive protein; CK-MB, creatine kinase MB; GPBB, glycogen phosphorylase isoenzyme BB; LDH, lactate dehydrogenase; MPO, myeloperoxidase; sCD40L, soluble CD40 ligand; H-FABP, heart-type fatty acid-binding protein.

serum do not peak until 2 days and may remain up to 2 weeks following AMI progression (Mair 1997b, Reis et al. 1988). Thus, LDH does not appear to be an ideal biomarker for AMI diagnosis because of low specificity and a broad spectrum, but it may also be possible to use it in a diagnosis if the physiological functions are well–characterized and thereafter combined with new analytical tools (Table 2).

Proposed new proteomic biomarkers

As shown in Figure 1, there are a number of biomarkers that may participate in the different phases of AMI associated with inflammation (Eggers et al. 2010, Yu & Rifai 2000), plaque instability, myocardial ischemia (Body et al. 2009,Pelsers et al. 2005) and necrosis processes (Burlina et al. 1994,Mair 1997a). New classes of proposed cardiac biomarkers currently under investigation are described below.

Myeloperoxidase

Myeloperoxidase (MPO) is a hemoprotein (140 kDa) which consist of heavy and light chains. A number of clinical studies have demonstrated the role of MPO as a potential biomarker during inflammatory conditions in acute coronary syndrome (ACS) (Eggers et al. 2010, Mocatta et al. 2007, Zhang et al. 2002). It is known that MPO appears to increase early after AMI and it does identify the patients with ACS earlier than conventional biomarkers such as troponin or CK-MB (Morrow 2007). Most interestingly, it also provides a risk stratification for patients who are troponin negative (Brennan et al. 2003). This is very promising; however, it is not clear yet whether MPO could be useful in practical applications. Another study reported that MPO activity in blood was much higher in patients with ACS than in normal controls, suggesting that MPO activity was significantly associated with the presence of ACS (Zhang et al. 2002).

Although MPO seems to be involved in the inflammatory process in ACS and is considered as a potential biomarker of oxidative stress and heart damage or injury, greater effort for understanding the biochemical properties in the diagnosis of ACS is needed.

sCD40L

Soluble CD40 ligand (sCD40L) has been proposed as another biomarker that indicates platelet activation in AMI. It is known that the CD40 ligand is a transmembrane protein which is expressed on platelets. After platelet activation, it is rapidly released in a soluble circulating form. Both membrane-bound CD40L and sCD40L forms interact with the CD40 receptors, which are present on B cells, monocytes and macrophages (Heeschen et al. 2003, Yang & Zhou 2006). It has also been demonstrated that sCD40L concentration is significantly higher in AMI patients than in normal populations, suggesting that increased sCD40L levels are associated with a high risk in different cardiac symptoms (Schonbeck et al. 2001). According to other reports, sCD40L levels ranged from 0.03 to 4 $\mu g\ l^{-1}$ in control groups; however, the levels of sCD40L were 3–6 $\mu g\ l^{-1}$ in a population of patients with acute chest pain (Schonbeck et al. 2001). The soluble CD40 is not uniformly consistent, but there are some reports that patients with a high level of soluble CD40 do appear to be at increased risk for ischemic events. Therefore, sCD40L concentration may identify AMI patients and be useful as another indicator of ACS patients.

Choline

Choline is the major metabolic product produced by phosphodiesteric cleavage of membrane phospholipids. A number of clinical studies have demonstrated that early ischemic membrane damage and phospholipid breakdown by phospholipase could induce the release of

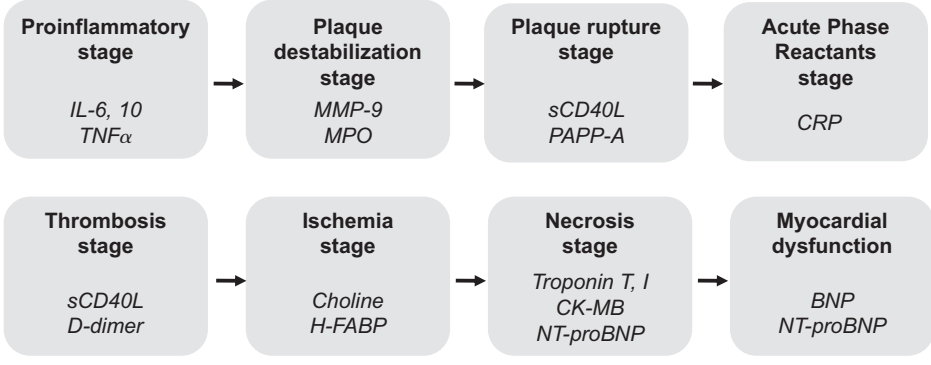

Figure 1. Biomarkers associated with various pathophysiological processes on developing acute myocardial infarction (AMI). Black arrows indicate the progression of AMI. Biomarkers may be released at each phase of AMI. IL, interleukin; TNF, tumour necrosis factor; PAPP, pregnancy-associated plasma protein-A; NT-proBNP, N-terminal proBNP(Vasan 2006).

choline into plasma by choline regulatory systems (Body et al. 2009, Danne et al. 2003).

An increased choline level has been regarded as a significant indicator of cardiac death, cardiac arrest and heart failure (Body et al. 2009). Whole blood choline and plasma choline are quite independent of some factors, such as age, gender, etc.; however both are predictive for cardiac death and heart failure (Danne et al. 2007). The standard measurement of choline in whole blood will be required for identifying high-risk AMI patients in the clinical practice.

ET1/CTproET1

Endothelin-1 (ET1) and C-terminal of pro-endothelin-1 (CTproET1) have been reported to be predictive of heart death or failure after AMI. Several studies have demonstrated that the levels of ET1 and CTproET1 were increased in heart failure and after AMI (Khan et al. 2007a, b). ET1 is a vasoconstrictor peptide which was cloned and isolated from vascular endothelial cells. It is interesting to note that plasma ET1 and CTproET1 levels were highly associated with NTproBNP in 983 AMI patients, indicating the predictive value post-AMI. A suggested multimarker strategy with CTproET1 and NTpro BNP would provide greater accuracy and give more information. However, problems remain. ET1 is very unstable, while CTproET1 is stable. Thus, an optimized sampling method will be needed.

High-sensitivity C-reactive protein

It was known that C-reactive protein (CRP) is elevated post-ACS, indicating a predictive value in MI (Ridker et al. 2005, Shishehbor et al. 2003). One study suggested that CRP measurement predicted the occurrence of heart failure and death (Shishehbor et al. 2003). The elevated CRP levels peak at 2–4 days and can be detected as low as 5 mg l^{-1} using high-sensitivity assays of CRP (hsCRP). However, it still remains to be determined if can provide valuable information.

Glycogen phosphorylase isoenzyme BB

Glycogen phosphorylase is a dimeric enzyme (~97 kDa) composed of two identical subunits. The BB and MM isoenzymes are found in the human heart; however, the BB isoenzyme is the predominant myocardial enzyme. It is known that glycogen phosphorylase isoenzyme BB (GPBB, 177 kDa) is highly sensitive for the diagnosis of AMI within 4 h after the onset of chest pain (Mair 1997b, 1998, Mion et al. 2007, Wu 1998). In several clinical studies, GPBB rapidly increased within 2–4 h after the onset of chest pain, indicating it as a useful indicator for early risk evaluation (Mair 1998). According to these authors, the specificity of GPBB was comparable with that of CK-MB, suggesting that it is a reliable biomarker for the detection of ischemic myocardial damage. One group also reported an elevated rate of GPBB of 93.9% within 3 h compared with myoglobin (66.7%) in 61 patients, including 37 with AMI (Peetz et al. 2005). However, a high-quality GPBB assay will be necessary for use in a clinical point-of-care diagnosis.

Heart-type fatty acid binding protein

Heart-type fatty acid-binding protein(H-FABP) is a small cytosolic protein (~14–15 kDa) that is produced by cardiomyocytes (Gorski et al. 1997, Pelsers et al. 2005). Interestingly, H-FABP content is much higher in the heart than in myoglobin. Elevated levels of H-FABP are measurable as early as 2–3 h and remain up to 12–24 h after myocardial necrosis. In addition, the levels of H-FABP reach a peak earlier than CK-MB in serum (Tanaka et al. 1991). Another study demonstrated that the sensitivity of H-FABP is comparable to initial troponin T within 4 h, but the specificity of H-FABP for AMI is relatively poor (McCann et al. 2008a). A number of reasons for the relatively poor specificity of H-FABP have been pointed out: (1) H-FABP is present in skeletal muscle at lower concentrations;and (2) H-FABP may be released from infarcted myocardium. To improve the specificity of H-FABP for use in AMI diagnosis, a combined approach using H-FABP and troponin T has been suggested. More recently, Kim et al. demonstrated an automated H-FABP assay in serum samples of 117 patients including 64 patients with AMI and 53 other patients (Kim et al. 2010). It was realized that the H-FABP latex turbidimetric immunoassay is highly sensitive to troponin T, CK-MB, myoglobin and H-FABP by enzyme-linked immunosorbent assay (ELISA). However, some questions still remain. H-FABP levels in plasma are regulated by a number of other regulatory systems such as aging, skeletal muscle injury and, probably, kidney function. It is necessary to understand the roles and the complex interactions of H-FABP from different regulatory components.

B-type natriuretic peptide

Another molecule, B-type natriuretic peptide (BNP) has been proposed for use as a biomarker of myocardial injury, for example, myocardial stress and heart failure (Worster et al. 2008, Yang &Zhou 2006). BNP is a cardiac neurohormone which is secreted by myocardium derived from volume expansion and pressure overload. Elevated levels of BNP were associated with a short-term risk in patients with AMI. It has been found that the peak level of BNP is proportionated with myocardial infarction size (Worster et al. 2008). There are number of studies showing that the level of BNP or NT-proBNP was well-correlated with the risk of death, heart failure and AMI, suggesting that they are very powerful tools for predicting the risk of

AMI (de Lemos et al. 2001, Navarro et al. 2006,Omland et al. 2007) Thus, BNP or NP-BNP may provide powerful information for the diagnosis of congestive heart failure. However, further clinical validation of BNP is still required.

ST2

There is evidence that ST2 is a good biomarker for heart failure induced by mechanical stress (Shimpo et al. 2004, Weinberg et al. 2002). ST2 is also known as interleukin-1 receptor-like protein which is expressed by mechanical stress and released into serum. ST2 only appears as a soluble fraction and is increased in the mouse model. In mouse studies, serum levels of ST2 were correlated with NTproBNP, and both markers would be useful to predict heart death or failure (Weinberg et al. 2002). Most interestingly, serum levels of ST2 were correlated with NTproBNP, and both biomarkers can predict heart death or failure post-MI. In human studies (Shimpo et al. 2004), the levels of ST2 were significantly higher in AMI patients. The data suggested that soluble ST2 might be a useful biomarker. However, the specificity of ST2 will need to be determined.

Practical studies: CK-MB, troponin I and troponin T

Table 2 shows biochemical assays currently in use for the detection of AMI. Most cardiac assays are based on immunological methods that utilize highly specific monoclonal antibodies against specific myocardial proteins such as myoglobin, troponin (I, C or T), CK-MB and others.

Several studies have demonstrated the superiority of the CK-MB assay in the diagnosis of AMI (Gibler et al. 1992, Harry et al. 1997, Penttila et al. 1999). CK-MB assays have been extensively used due to improved sensitivity and more rapid assay time. One-step and solid-phase assays have also been developed for improvement of CK-MB detection (Mair et al. 1995). This approach needs small quantities of whole blood and does not require the use of expensive analytical instruments. Panteghini et al. reported a more precise and quantitative assay for multiple detection of myoglobin and CK-MB with cardiac status kits produced by the Spectral Diagnostics Company.

Currently, rapid assays for troponin I have been developed and it is widely used as a gold standard biomarker for AMI (Christenson et al. 1998, Keller et al. 2009). This system can detect troponin I in 10–15 min and showed excellent correlation with existing troponin I and CK-MB in suspected AMI patients (Christenson et al. 1998). Analytical performance and clinical validation of this assay was observed in different patient groups. On the basis of assay results, the STATUS troponin I assay showed comparable sensitivity to CK-MB (Christenson et al. 1997, 1998, 2004, Peter et al. 2006). The Dasgupta group compared the chemiluminescent cardiac troponin I assay with two other immunoassay systems. There was good correlation up to serum troponin I concentrations of 50 $ngml^{-1}$ and the detection limit was 0.1 $ngml^{-1}$ of troponin (Dasgupta et al. 2000). In another recent study, IIva et al. demonstrated the diagnostic performance of troponin I with H-FABP in serum samples of 293 patients by using the Abbott cTnI assay systems. The results demonstrated that troponin I appears to be more sensitive than H-FABP (Ilva et al. 2009).

As discussed earlier, troponin T is a well-studied cardiac biomarker, suggesting another gold standard method for diagnosis of AMI (Burlina et al. 1994, Collinson 1998, Karras & Kane 2001, McDonnell et al. 2009,Scirica& Morrow 2004). The ultrasensitive troponin T assay has a detection limit of 0.08 $ngml^{-1}$ and allows rapid and accurate identification of severe AMI (deFilippi et al. 1998). Many practical studies have demonstrated clinical validation results with commercially available assay kits such as Abbott troponin I assay (Uettwiller-Geiger et al. 2002, Wu et al. 1999), Roche troponin T assay (Giannitsis et al. 2010), Roche troponin I assay (Reichlin et al. 2009), Siemens troponin I assay (Casals et al. 2007),and other Roche troponin T assay systems (Reichlin et al. 2009). In another study, bioenzymatic detection of troponin C by using a microelectro-mechanical system (MEMS) was demonstrated. In this case, an optical beam deflection method was employed to identify the enzymatic products derived from an antigen–antibody interaction on the cantilever surface (Amritsar et al. 2006).

Conclusions and future perspectives

In this paper, we have reviewed recent advances in the development of several proteomic biomarkers for use in the diagnosis of cardiovascular diseases including AMI. Many of these biomarkers will provide new insights into the diagnosis and management of AMI patients. However, problems remain to be solved and improvements to be considered.

First, accuracy is a key issue in determining if a cardiac biomarker assay for the evaluation of AMI is extensively reliable and acceptable in all patients. It has been suggested that the acceptable imprecision (CV) of troponin assays should be <10% (Vasan 2006).

Second, lack of a standardization protocol is also problematic. The ELISA currently in use for detection of cardiac biomarkers may give different results depending on different antibodies, assay imprecision along with multiple enzymatic processes and other experimental conditions. It is reasoned that antibodies can be produced by different

epitopes, resulting in serious clinical problems such as false-positive and false-negative results.

Third, several investigators have focused on the rapid diagnosis of AMI by using multiple markers (McCord et al. 2001, Wang et al. 2006, Zethelius et al. 2008). Owing to large variations in sensitivity and specificity, the use of single biomarkers may be inadequate to make clinical decisions. The Weaver group demonstrated a multimarker strategy with myoglobin, CK-MB and troponin I for several time points and compared with a central laboratory strategy (McCord et al. 2001). Interestingly, the sensitivity and negative predictive value at 90 min were 96.9% and 99.6%, respectively. It was also interesting to note that the whole assay time was about 24 min. Zethelius et al. reported a combined multiple biomarker strategy with troponin I, N-terminal pro-brain natriuretic peptide, cystatin C and CRP to determine myocardial damage, renal failure and inflammation (Zethelius et al. 2008). All biomarkers used in this approach together statistically significantly predicted the risk of myocardial damage compared with single biomarker strategy. Another example of a multibiomarker strategy has been demonstrated by Wang et al. (2006). These authors used 10 biomarkers including CRP, BNP, N-terminal pro-atrial natriuretic peptide, aldosterone, renin, fibrinogen, D-dimer, plasminogen-activator inhibitor type 1 and homocysteine, and the urinary albumin-to-creatinine ratio, for predicting the risk of cardiovascular events. Although there are many examples showing that the combined multibiomarker approach may increase the sensitivity along with the accuracy of the assays, the best combinations of multimarkers for AMI detection need to be defined.

Fourth, several studies have suggested that the optimization of sampling of biomarkers is required for the reliable diagnostic performance (Antman et al. 2000, Yang &Min Zhou 2006). Compared with a serial sampling method, the single sampling of biomarkers gives us low sensitivity for AMI detection in some cases. Because the biomarkers collected from blood or serum have significantly different release kinetics, it can result in wrong diagnostic results. Therefore, the ESC/ACC have recommended that serial blood sampling from AMI patients should be detected before 6–8 h and 24 h after admission (Antman et al. 2000). Concerning this issue, the stability of proposed biomarkers is also an important parameter for performing reliable diagnosis. Many biomarkers currently in use for AMI detection have demonstrated enhanced stability *in vitro*; however, some immunoassays were often affected by interference such as protease activity and instability of analytes (Chan &Ng 2010).

The improved analytical approaches for assessment of early diagnosis are now being rapidly developed with advanced biochemical methods and miniaturized devices that are suitable for a variety of diagnostic applications (Hong et al. 2007, Wang et al. 2009). This miniaturized sensor technology that consists of small devices and components opens up an exciting possibility of integrated multiplex systems for the diagnosis of diseases and for detection of protein biomarkers in blood or serum without any preparation. The Kang laboratory has reported on multiplex devices combined with electromechanical technology and nanoparticle-based molecular probes (Hong et al. 2007, Wang et al. 2009). Indeed, only a few microlitres of plasma sample are needed for 10–15 min during the whole process of the assay. A recent report has also demonstrated the use of saliva associated with AMI for point-of-care diagnosis using multiplex lab-on-a-chip devices (Floriano et al. 2009). A surface plasmon resonance (SPR) immunosensorhas also been developed for the detection of human troponin T in real time with a high sensitivity (Dutra & Kubota 2007). In fact, the detection limit of troponin T was as low as 0.01 ng per ml.

In the near future, early detection of AMI may depend on combining proteomic and genomic biomarkers for the development of powerful assay systems based on capturing and monitoring biochemically measurable components in a single chip device. With all these advances, there is little doubt that integrated multiplex assay systems will revolutionize multifunctional diagnosis of AMI for use in point-of-care.

Declaration of Interest

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