STATE-OF-THE-ART PAPER

Biomarkers in Acute Cardiac Disease
The Present and the Future

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The use of biomarkers to aid diagnosis and treatment is increasing rapidly as genomics and proteomics help us expand the number of markers we can use and as an improved understanding of the pathophysiology of cardiac disease guides their use. However, as with all rapidly expanding fields, there is the risk of excessive enthusiasm unless we are circumspect about the data that guide the clinical use of these new tools. This review focuses first on how to use troponin, which at present is the best validated of the new markers, and will hopefully provide insight into how to use this biomarker more productively by distinguishing subsets of patients and by providing an understanding of the meaning of elevations in various clinical situations. The review then discusses the use as well as the knowledge gaps associated with emerging biomarkers such as B-type natriuretic peptide and C-reactive protein, which are increasingly moving toward more productive clinical use. Finally, it reflects on some of the large number of markers that are still in development.   (J Am Coll Cardiol 2006;48:1–11) © 2006 by the American College of Cardiology Foundation

An improved understanding of pathophysiology and improvements in the ability to identify potentially important biomarkers via genomics and proteomics (1,2) has led to a proliferation in the number of biomarkers available to clinicians. Many of these improve our understanding of pathophysiology (3,4). However, the bias in favor of positive reports combined with the fact that analyses often focus on prognostic value allows for the possibility of not capturing the entire picture when evaluating a new analyte. In addition, there are concerns regarding the analysis of data published about these markers, including the use of inappropriately low cutoff concentrations for standard biomarkers such as cardiac troponin (cTn) and C-reactive protein (CRP), the use of insensitive analytical methods for analytes used for comparison or as covariates, the use of single samples when serial samples would be most useful, the lack of information relating the timing of patient presentation and sampling, the use of specimens without data showing that they have been appropriately preserved, and the technique of using one biomarker at a predetermined concentration and then performing receiver-operating curve analysis to determine the optimal level for the new biomarker being studied (5). At times, populations of patients are admixed to show that a given biomarker does well when compared with another when the marker used as a comparator would never be abnormal in some of the populations included. An example of how much difference these issues can induce is shown in Figure 1 from the FRISC (FRagmin and Fast Revascularization during InStability in coronary artery disease) database (6). Note that even at a value 20 times the upper limit of normal (0.2 ng/ml) for cTnT, only 60% of the values with the less sensitive assay were detected. Rather than troponin identifying 139 of the 154 patients who eventually died, the point of care troponin I assay identified only 98. Thus, a new marker might look very different depending on which troponin assay was used as a comparator. These issues, if not handled carefully, can exaggerate the importance of new markers. Many of the new biomarkers will overlap substantially, and their use likely will not be synergistic but duplicative. It will take some time to sort out which biomarkers are of clinical diagnostic and/or prognostic value. Accordingly, this review will: 1) define the use of the present state-of-the-art biomarkers for the diagnosis of patients with acute cardiovascular disease; 2) touch on markers of historical interest whose use should be diminishing or eliminated; and 3) identify emerging markers, and 4) identify biomarkers in the developmental stage.

ESTABLISHED BIOMARKERS

cTn. There is one biomarker that has been established for diagnosis (7,8) and that also provides robust prognostic information (9–11) and that is cTn. The cTn biomarkers, assuming high-quality analytic methods, whether for cTnI or cTnT, are highly specific and sensitive for cardiac injury (12). With the exception of renal dysfunction (see later text), cTnI and cTnT provide equivalent clinical information.

Abbreviations and Acronyms

ACS = acute coronary syndrome
BNP = B-type natriuretic peptide
CK = creatine kinase
CRP = C-reactive protein
cTn = cardiac troponin
ECG = electrocardiogram
ESRD = end-stage renal disease
PCI = percutaneous coronary intervention

ASSAY-RELATED ISSUES. The cTn are regulatory proteins with both cytosolic and structural pools. Best data suggest that they are released because of necrosis (7,8). Early release is thought to be attributable to the cytosolic pool, and later release to the structural pool (7,8). There are now a multiplicity of assays for cTnI (13), which makes standardization problematic (14). All have different analytical sensitivities. It is essential for clinicians to understand whether they use a highly sensitive assay or one that lacks sensitivity at the cutoff concentrations used for clinical decisions. The International Federation for Clinical Chemistry has provided a comparison of analytical imprecision (sensitivity) for cTnI assays (13). There is substantial heterogeneity of assay sensitivities (Fig. 2). Different assays measure different epitopes and different fragments of cTnI. Thus, there are differences in the responses of specific assays to the various troponin forms (15), and assays probably detect slightly different groups of patients, depending on the nature and timing of cTn release. A list of assays and their cut off values is included in Table 1. Recent data suggest that a subset of patients may have antibodies to parts of cTnI that can result in occasional false-negative results (16). The heterogeneity of assays is not a problem for cTnT because there is only one assay. There is a need to improve the rapidity with which

assay results are available. Clinicians expect values in 30 to 40 min, laboratorians in 60 min, whereas in reality results are often available only after 100 min or more (17).

USE OF THE MARKER. The basic science substrate for use of cTn has been elucidated elsewhere (12,18). For clinical use, several principles are important.

The cTn elevations begin 2 to 4 h after onset of symptoms (Fig. 3). With the use of precise highly sensitive assays and the use of the 99th percentile reference cutoff recommended, cTn provides all the information needed for the evaluation of patients who present with possible acute ischemic heart disease. Other "rapidly increasing biomarkers" have little or no utility if this approach is used (Fig. 4) (19).

Elevations of cTn persist for days (cTn 5 to 10, cTnT 5 to 14). There are emerging data suggesting that re-elevations and an increasing pattern are the best way to determine whether a given event is acute or whether the elevation is one from a previous event or is chronic. This concept is important when there is an elevation in the initial sample, when reinfarction is suspected (20), and in patients with end-stage renal disease (ESRD), who can have chronic elevations.

The number of patients identified with cTn in patients with possible acute ischemic heart disease depends on the subset of patients being evaluated. Patients with ST-segment elevation infarction are different from those with high-risk acute coronary syndrome (ACS) presentations and from those who manifest moderate-risk and/or low-risk presentations (21).

Because of the sensitivity of cTn, elevations are common in patients with a large number of acute and chronic cardiovascular diseases. It is up to the clinician to decide whether the presentation is one of acute ischemia. If so, elevations can make the diagnosis of acute myocardial infarction, as defined by the European Society of Cardiology/
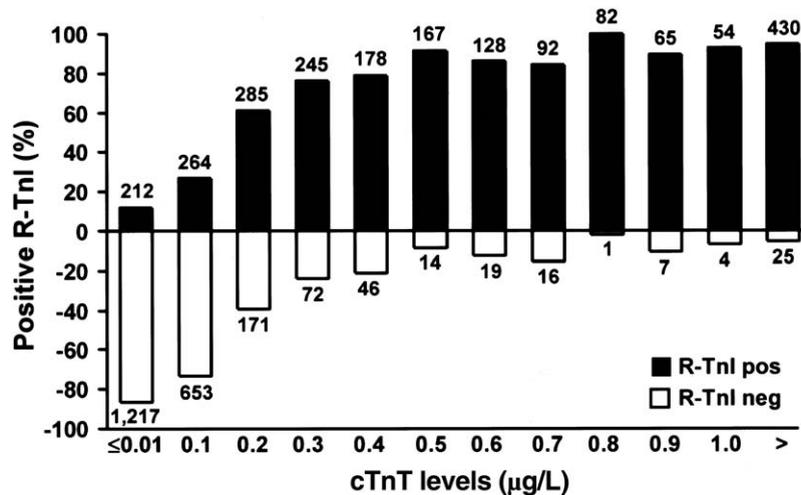

Figure 1. The percentage of values for cardiac troponin (cTn)T collected from the FRISC II study associated with elevated values for a point-of-care assay with less sensitivity and precision. Any cTnT value >0.01 ng/ml is abnormal. Note that even at a value 20 times the upper limit of normal (0.2 ng/ml) for cTnT, only 60% of the values with the less sensitive assay were detected. Thus, rather than troponin identifying 139 of the 154 patients who eventually died, the point-of-care assay identified only 98 patients. Reprinted, with permission, from James et al. (6). R-TnI = rapid troponin I.
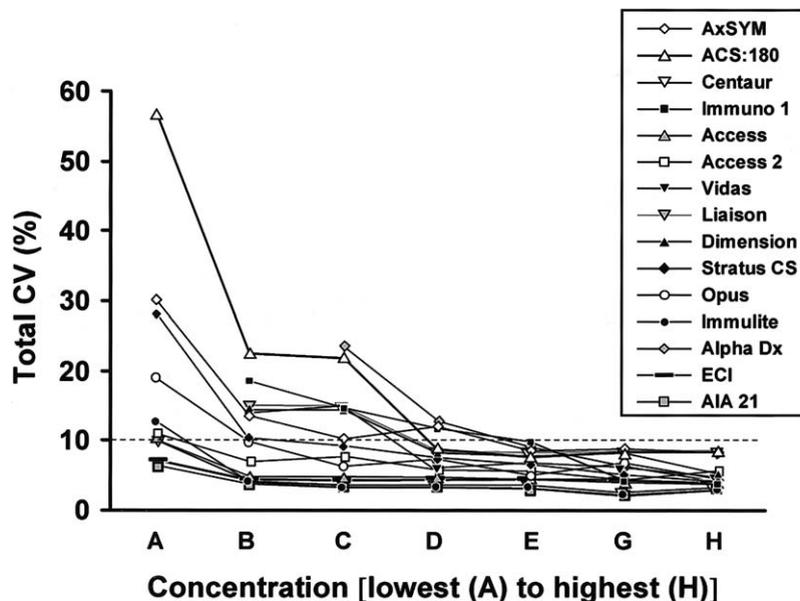
Figure 2. Sensitivity and precision of various cardiac troponin I (cTnI) assays as provided by measurements made by the manufacturers from pools provided by the International Federation for Clinical Chemistry committee. **(A to G)** Increasing concentrations of cTnI. Note that many assays are incapable of detecting the lower values with reasonable precision. Reprinted, with permission, from Panteghini et al. (13). CV = coefficient of variation.

American College of Cardiology (7,8). If acute ischemia is not present, alternative etiologies should be sought and different diagnoses made. A partial listing of situations in which cTn can be elevated in the absence of acute ischemic heart disease is included in Table 2.

Patients with ST-segment elevation myocardial infarction. Patients with acute ST-segment elevation myocardial infarction do not need biomarker measurements before therapy. Patients with elevated cTn on presentation have a lower rate of coronary recanalization, whether with thrombolysis (10) or direct percutaneous coronary intervention (PCI) (22) and an adverse short-term and long-term prognosis (23,24). Part of this effect is related to the fact that it takes time for cTn to become elevated in the blood, and

Table 1. Presently Available Assays for Cardiac Troponin and Key Values

Assay	LLD	99th Percentile	ROC Cutoff
Abbott ARCH	0.009	0.012	0.3
Abbott i-STAT*	0.02	0.08 (WB)	ND
Bayer Centaur	0.02	0.1	1.0
Beckman Accu	0.01	0.04	0.5
Biosite Triage*	0.19	<0.19	0.4
Dade RxL	0.04	0.07	0.6–1.5
Dade CS*†	0.03	0.07	0.6–1.5
DPC Immulite	0.1	0.2	1.0
Orthos Vitros	0.02	0.08	0.4
Response*	0.03	<0.03	ND
Roche Elecsys (cTnT)	0.01	<0.01	0.1
Roche Reader*	0.05	<0.05	0.1
Tosoh AIA	0.06	<0.06	0.31–0.64

*Point-of-care assay. †Cleared by the U.S. Food and Drug Administration as high-sensitivity assay, 2004.
99th = upper limit of normal and the ROC value equates cardiac troponin values in general with creatine kinase-MB elevations; cTnT = cardiac troponin T; LLD = lower limit of detection; ND = not done; ROC = receiver-operating curve; WB = whole blood.

patients who come in late do less well than patients who come in early. However, even when one corrects for time of onset, the same prognostic effect is found (25). In one study of patients with inferior infarctions, stenting seemed to improve the prognosis of this group (26).

Infarct size can be estimated from the 72-h troponin value. The data are stronger for this approach with cTnT than with cTnI (27,28). For cTnI, peak levels work better (29,30), but the data vary depending on whether or not there has been acute reperfusion (29).

High-risk patients with ACS. This group has been extensively studied. Patients often are elderly, and have chest pain at rest, transient electrocardiogram (ECG) changes, increasing symptoms or signs of ischemia, evidence of hemodynamic instability, and/or arrhythmias. They have a high incidence of elevated cTn when one uses the 99th percentile reference range (31,32). They meet criteria for non–ST-segment elevation myocardial infarction (7,8,12). The elevated cTn defines a high-risk subset and provides guidance in regard to therapy. Patients with elevated cTn have more procoagulant activity and an adverse coronary anatomy as judged by the degree and extent of angiographic stenosis, the Thrombolysis In Myocardial Infarction (TIMI) grade of perfusion, and the complexity of the coronary lesions (33). As such, these patients do better with the use of low-molecular-weight heparin (34), IIb/IIIa anti-platelet agents (35), and an early invasive interventional strategy (36). Most of the trials of IIb/IIIa agents were done before the routine use of clopidogrel. Recent data suggest that these agents may be synergistic (37). Clopidogrel is the one agent that accrues benefit to patients whether or not cTn values are elevated (38). These more aggressive therapies not only are not beneficial but in some studies seem to even be detri-
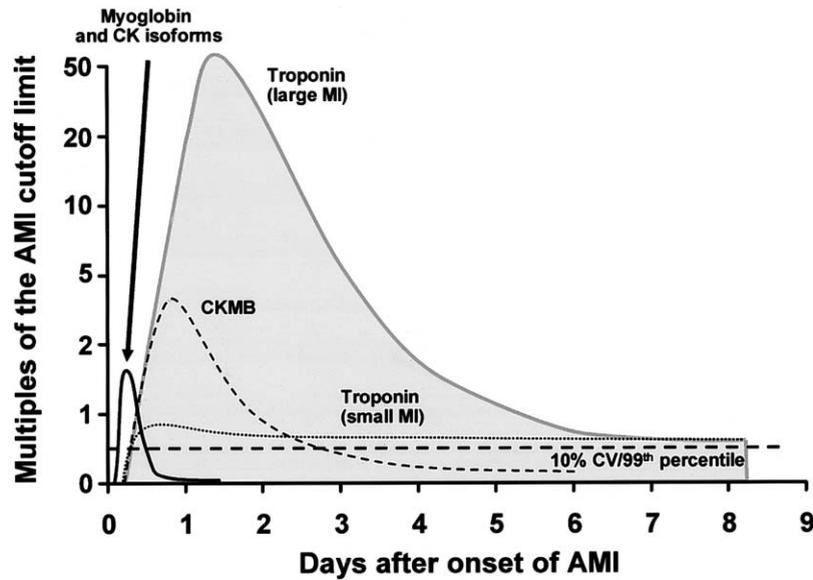
Figure 3. Time course of the appearance of various markers in the blood after acute myocardial infarction (AMI). Shown are the time concentrations/activity curves for myoglobin and creatine kinase (CK) isoforms, troponin after large and small infarctions, and CK-MB. Note that with cardiac troponin some patients have a second peak in addition. CV = coefficient of variation.

mental if applied to patients without cTn elevations. The one area where there is some question comes from the TACTICS–TIMI-18 (Treat Angina with Aggrastat and Determine Cost of Therapy with an Invasive or Conservative Strategy–Thrombolysis in Myocardial Infarction-18) study, in which based on one admission sample for cTn, it was claimed that female patients with elevations in B-type natriuretic peptide (BNP) and/or CRP benefited from an early invasive strategy even if cTn were normal (39).

Indeterminate- and low-risk patients. This patient group is hard to triage clinically. They may not have rest pain or ongoing pain, may have normal ECGs, and depending on the specifics, may be at intermediate or low risk. The frequency of elevated cTn is lower, nonetheless, these

patients benefit optimally from triage with cTn. The most robust trial was one by Hamm et al. (40) in 733 patients. Almost every patient at short-term risk (30 days) was identified by elevations in cTn. Two caveats are important. First, cTn value had to have been obtained at least 6 h after the onset of symptoms. This may be less necessary now with more sensitive assays. The second caveat is related to sensitivity. Detectability was considered abnormal. We now have more sensitive assays, which should make for even greater predictive accuracy. Even for patients at low risk, the finding of an elevated cTn is prognostic. Some studies showing this prognostic effect were not done with contemporary assays, which now allow for lower clinical cutoffs. One would suspect that the data would be even more
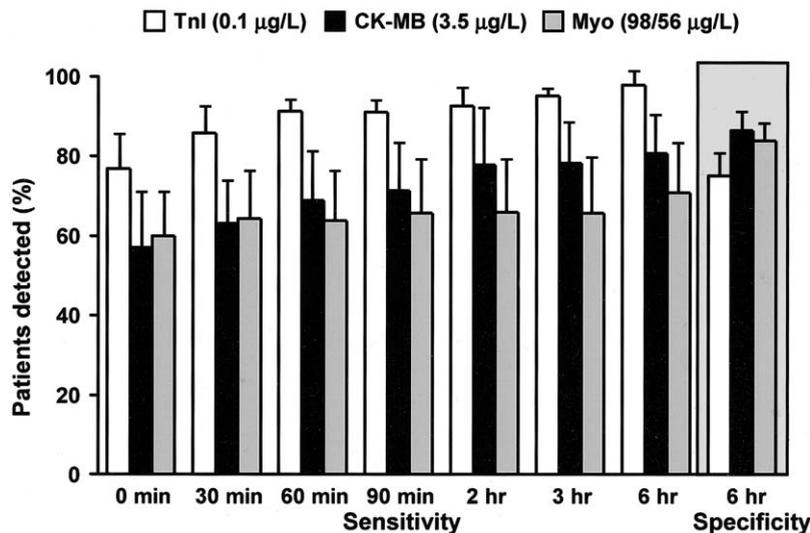

Figure 4. Sensitivity of cardiac troponin (cTn)I compared with myoglobin and creatine kinase (CK)-MB for the detection of myocardial injury. Note the use of the 10% coefficient of variation (0.1 ng/ml) were superior to the use of myoglobin. Reprinted, with permission, from Eggers et al. (19).

Table 2. Elevations of Troponin in the Absence of Overt Ischemic Heart Disease

Trauma (including contusion, ablation, pacing, implantable cardioverter-defibrillator firings including atrial defibrillators, cardioversion, endomyocardial biopsy, cardiac surgery, after interventional closure of atrial septal defects)

Congestive heart failure—acute and chronic

Aortic valve disease and hypertrophic obstructive cardiomyopathy with significant left ventricular hypertrophy

Hypertension

Hypotension, often with arrhythmias

Postoperative noncardiac surgery patients who seem to do well

Renal failure

Critically ill patients, especially with diabetes, respiratory failure

Drug toxicity, e.g., adriamycin, 5-fluorouracil, herceptin, snake venoms

Hypothyroidism

Apical ballooning syndrome

Coronary vasospasm

Inflammatory diseases, e.g., myocarditis, e.g., Parvovirus B19, Kawasaki disease, sarcoid, smallpox vaccination, or myocardial extension of bacterial endocarditis

Post-percutaneous coronary intervention patients who seem to have no complications

Pulmonary embolism, severe pulmonary hypertension

Sepsis

Burns, especially if total body surface area is >30%

Infiltrative diseases including amyloidosis, hemachromatosis, sarcoidosis, and scleroderma

Acute neurological disease, including cerebrovascular accident, subarachnoid bleeds

Rhabdomyolysis with cardiac injury

Transplant vasculopathy

Vital exhaustion

impressive with this approach. Nonetheless, in a cohort at low risk (a 5% to 7% incidence of acute myocardial infarction) whose ECGs are mostly normal, an elevated cTn is associated with nearly a 90% frequency of coronary artery disease by angiography (41). This was compared with a frequency of 23% in the group without elevations. It is likely that the additional patients who had angiographic disease may have been detected had the more sensitive cTn assays and low cutoffs been used. During 1-year follow-up, patients with elevated cTn values, two-thirds of whom had two- or three-vessel disease, had an increased frequency of events. These data are similar to those from emergency room studies of patients who had "minor elevations" of cTn (Fig. 5) (42). Some degree of caution is necessary in all of these situations. A normal troponin value is not an imprimatur against disease, especially if the assay in use is either insensitive or is used at a high cutoff value.

Patients with chronic renal disease. The most common cause of death in chronic renal failure is cardiovascular, and coronary artery disease is common. The cTn values, especially with cTnT, are often abnormal (43,44). Initially, this was presumed to be because cTnT was less tissue specific. However, extensive studies have now been done to show that this is not the case (45). Nonetheless, the frequent cTnT elevations (30% to 70% of ESRD patients compared with <5% in similar patients for cTnI) are problematic to clinicians (43,44). Several suggestions may be helpful.

In patients with an ACS, an elevated cTn, whether cTnI or cTnT, should be treated as if renal failure (chronic or acute) were not present (46). These patients are at increased risk. Although all of the therapeutic caveats cannot be applied from all of the studies, many of which had only limited numbers of patients with ESRD, it seems likely that these individuals also would benefit from an aggressive antithrombotic (47) and interventional approach (48).

For patients with ESRD who do not have ACS but have elevated cTn, several approaches are helpful; cTnT is now approved for prognostication in patients with ESRD. Thus, baseline values can and should be obtained. The prognosis (all-cause death) of patients with ESRD and even minor elevations of cTnT is two to five times higher than those with undetectable values (49,50). C-reactive protein may further improve prognostication (50,51). Having a baseline value allows not only for prognostication but also for an evaluation of whether or not values are changing over time. Increasing values suggest an acute problem. In the absence of increasing values, when a clinician does not find evidence of an acute process known to cause elevations of cTn, one is probably safe to consider these more chronic elevations.
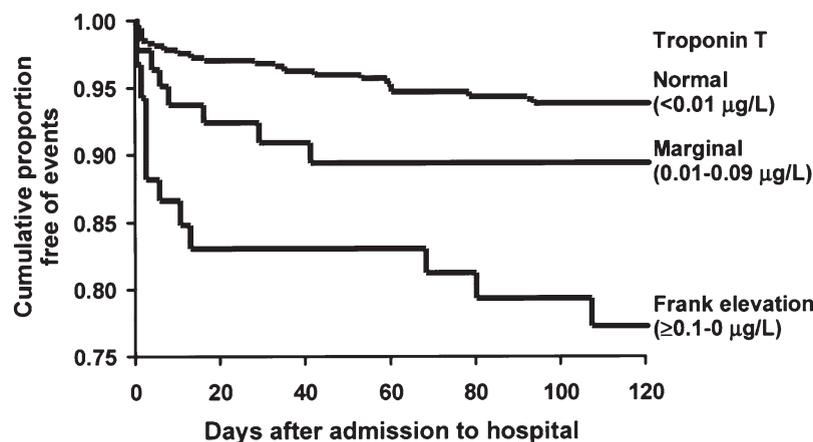

Figure 5. Cardiac events after emergency department discharge based on levels of cardiac troponin T. Reprinted, with permission, from Henrikson et al. (42).

These patients may require alteration in a variety of therapies to improve their prognosis, but may not require acute treatment. The putative mechanisms for these chronic elevations, which are invariably associated with pathologic abnormalities at autopsy (52), include endothelial dysfunction, acute cardiac stretch, intradialysis hypotension and hypertension, the use of iron sucrose, and left ventricular hypertrophy. All are potentially benefited by altering the frequency of dialysis, the weight gain (fluid control), and the blood pressure control. Given the high incidence of coronary disease, some of these patients may have occult underlying coronary artery disease. Elevations of cTn in patients with renal dysfunction but without ESRD are common but likely attributable to the association of other comorbidities and probably cannot be considered the same as those seen in ESRD patients.

Patients after PCI. Elevations of creatine kinase (CK)-MB after PCI presage adverse outcomes (53). The greater the elevation, the more adverse the prognosis. This has not consistently been the case with cTn. However, one would suspect that it should be. There should, for each cTn assay, be values that correlate with the CK-MB values. However, this relationship may need to be confirmed with each assay for each biomarker (CK-MB and troponin) in use locally. Recent data suggest that many of the elevations observed after PCI both for cTn and CK-MB are presaged by elevations in cTn when sensitive assays and lower cutoff values are used (54). Thus, they could reflect ongoing myocardial damage that has little to do with the procedure itself or identify patients with a high likelihood or post-PCI marker elevation. Patients who present with ACS who have cTn elevations manifest worse angiographic disease and an adverse prognosis. It may be that CK-MB not being elevated, because it is less sensitive compared with cTn, is seductive to clinicians who believe it can be used as a baseline; however, the effect may be the same. Thus, it is unclear how to use either biomarker in patients who present acutely or have an elevated cTn pre-procedure. If the baseline cTn is normal pre-PCI and the patient has not presented acutely, cTn and CK-MB values after PCI will likely be informative about the procedure itself and elevations are associated with early major adverse cardiac events (54).

Patients after coronary artery bypass grafting. Patients who undergo cardiac surgery have some amount of cTn and CK-MB release (55). The higher the value, the worse the associated injury, and high values presage subsequent events, including death. Elevations have been related to the surgical approach, the extent of cross-clamp and bypass time, the nature of cardioplegia, and the nature of the procedure (valves and coronaries vs. coronary artery bypass grafting alone). Magnetic resonance imaging studies suggest that most of the injury is subendocardial and apical (56). Graft occlusion (57) as a cause of the injury does not seem to be common.

Patients who present with chest pain who have normal angiograms. One needs to be cautious in evaluating patients with elevated cTn even if an angiogram does not show significant coronary disease. The coronary angiogram is not perfect (58). In the TACTICS–TIMI-18 trial, individuals with an elevated cTn despite angiographically normal coronary arteries had an adverse prognosis (59). This may reflect the inadequacies of the angiogram or may be related to the late angiography in some patients (60). In some data sets, elevated cTn has been associated with asymmetric lesions that tend to be vasoactive (61). Thus, the finding of angiographically "normal" coronary arteries needs to be interpreted within the context of patient care. If the patient does not have an ACS but appears acutely ill, there are a variety of other etiologies, such as pulmonary embolism and congestive heart failure, in which even in the absence of coronary artery disease elevated cTn can be observed (these elevations may be related to wall stress); patients who may have subendocardial ischemia/injury related to left ventricular hypertrophy with diseases such as aortic stenosis or hypertrophic cardiomyopathy or perhaps even severe hypertension; myocarditis; and/or toxic insults such as seen with sepsis, in which tumor necrosis factor alpha and heat shock protein have been impugned as potential cardiac toxins.

POTENTIALLY OUTDATED MARKERS

CK-MB. Creatine kinase-MB (18) is a cytosolic carrier protein for high-energy phosphates. As clinicians become more comfortable with cTn, it will have a diminishing role. Some would argue that it still could be used to define infarct timing or after PCI.

Myoglobin and CK isoforms. Myoglobin, a ubiquitous heme-related protein, and CK isoforms (18) have been used with the hope of shortening the time to a more definitive diagnosis in patients with chest pain. They have been relied on particularly for their negative predictive value. Recent data suggest that with modern contemporary sensitive assays and sensitive cutoffs for cTn, cTn can assume this role (19). In the emergency room, where patients at low risk for cardiovascular disease are still screened with cardiac biomarkers, it has been suggested that myoglobin may identify additional patients at risk. This is likely because of detection of serious noncardiovascular disease (62).

EMERGING MARKERS

For a marker to be useful, it needs to make a diagnosis or to define prognosis in such a way that it guides therapy. In this regard, there are two emerging markers, CRP and the natriuretic peptides BNP and NT-proBNP.

CRP. ASSAY-RELATED ISSUES. C-reactive protein is an acute-phase reactant protein made in the liver. Its most proximate stimulator is interleukin 6. A specific receptor for CRP has recently been reported (63). There is controversy regarding the variability of CRP levels (64,65). Some argue that in the absence of acute illness, including myocardial

injury, levels of CRP are stable. Others argue that levels fluctuate and vary by gender and ethnicity (66). If one has an elevation and is acutely ill or has evolving infarction, the test should be repeated at least 2 weeks later (64). Values above 10 mg/l are likely caused by acute disease (67). Values >3 mg/l are associated with higher risk, and values <1 mg/l are associated with low risk (64). Those between 1 and 3 mg/l are considered intermediate (64). Multiple assays exist for CRP (designated high-sensitive CRP or hsCRP), and most give comparable results.

USE OF THE MARKER. Recent data from the PROVE-IT (Pravastatin or Atorvastatin Evaluation and Infection Therapy) trial (68), which are consistent with those from the REVERSAL (Reversal of Atherosclerosis with Aggressive Lipid-lowering Therapy) trial (69,70), suggest that the titration of statin therapy predicated on CRP values would result in fewer events and regression of atherosclerosis. They probably explain why in prior studies an elevated CRP level was found to be prognostic for late events after presentation with ACS (71). In those studies, the changes in prognosis and extent of disease associated with differences in CRP were not associated with the effects observed with low-density lipoprotein lowering (Fig. 6) (68). Differences in events started early after presentation. It seems that using CRP values as early as 30 days after infarction to avoid the effects of myonecrosis on CRP may be a reasonable approach. These trials did not titrate therapy based on CRP, which make the data provocative and suggestive but not definitive. Elevations of CRP often are related to numerous other clinical issues, such as obesity, diabetes, estrogen therapy, hypertension, smoking, etc. Thus, an argument could be made that one should attempt to control these risk factors before resorting to pharmacology.

The use of CRP for primary prevention is more controversial. There are abundant data concerning its prognostic efficacy, but limited data showing that interventions are of benefit save a suggestion in regard to aspirin (72) and statins (73). Given the large numbers of patients at risk, it is difficult to show the cost effectiveness of this approach. Recent guidelines have suggested the use of CRP in patients who are at intermediate risk for coronary artery disease to assist in defining the level of aggression of risk factor intervention (74).

BNP. ASSAY-RELATED ISSUES. B-type natriuretic peptide is a 32-amino-acid counterregulatory peptide released in response to cardiac stretch. It is synthesized as a pro peptide and then cleaved to the active moiety by a protease called corin (75,76). There are multiple immunoassays (75,76). In general, they have similar diagnostic cutoff values of 100 ng/ml for congestive heart failure, because this has been the value developed in early diagnostic studies. Above and below that value, they correlate less well (76). In addition, there is an immunoassay for NT-proBNP (75,76), which detects the 76 amino acid carrier protein, which with the active 32 amino acid compound is called proBNP. This protein is thought to be cleaved when BNP is secreted. However, it seems that some proBNP circulates, and it is likely that many assays detect it. In addition, for both of these analytes, women and older individuals have higher values (69) and obese individuals have lower values (77) (Fig. 7). Patients with renal failure often have substantially higher values (78). This increase is more accentuated for NT-proBNP. Both BNP and NT-proBNP manifest substantial biological variability (79). If formal values are relied on, values have to either double or half to be sure that a "real" change has occurred.

USE OF THE MARKER. The BNP and/or NT-proBNP values are helpful for the detection of congestive heart failure in patients in whom one is unsure of the cause of dyspnea (80). This is not the same as advocating its routine use (81). For BNP, values <100 ng/l make heart failure unlikely with a negative predictive value of 90%. If the value is >500, heart failure is highly likely with a positive predictive value of 90% (82). For NT-proBNP, levels >450 ng/l for patients <50 years of age and >900 ng/l for patients ≥50 years of age are sensitive and specific for heart failure (83). If the value is
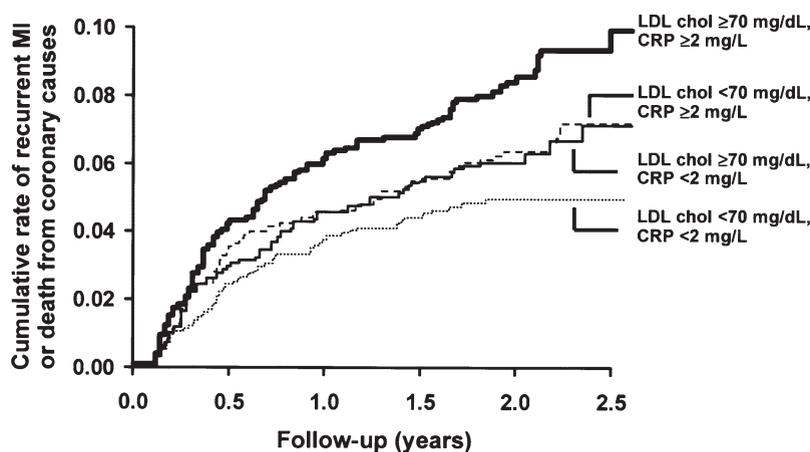

Figure 6. Synergistic prognostic value of C-reactive protein (CRP) and low-density lipoprotein (LDL) in PROVE-IT study. Reprinted, with permission, from Ridker et al. (68). MI = myocardial infarction.
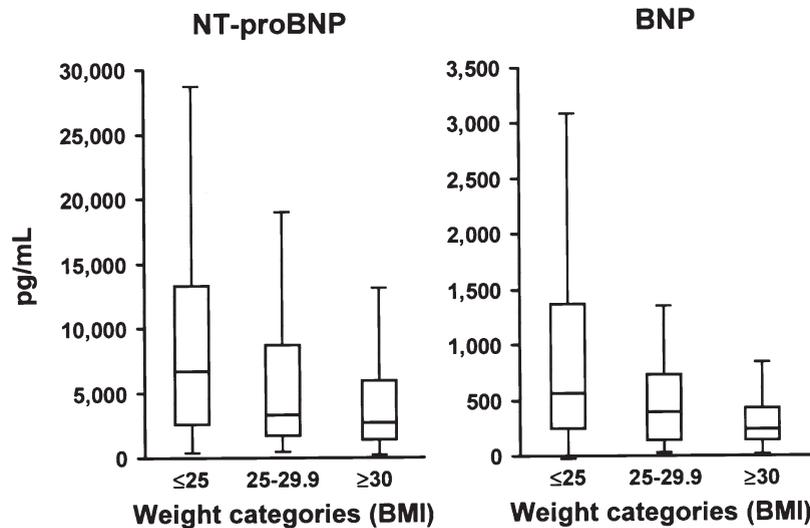
Figure 7. Effect of weight on B-type natriuretic peptide (BNP) levels. Reprinted, with permission, from Krauser et al. (77). BMI = body mass index.

<300 ng/l, heart failure is highly unlikely with a negative predictive value of 99% (83). However, in the middle ranges, the predictive accuracy is less and that is likely where many of the less typical patients' values will reside. Patients with right-sided heart failure, sepsis, volume overload, stroke, and left ventricular hypertrophy also have higher values. In patients with acute pulmonary edema and acute mitral regurgitation, values may not be elevated initially (82).

There is prognostic value of BNP and NT-proBNP as well in patients with heart failure (84,85). Patients with higher values on admission and/or discharge to hospital generally do worse. Those with marked reductions during treatment do better. However, the percentage changes with therapy are less than anticipated and often less than biological variability, suggesting that the natriuretic peptide system may require some time to upregulate and downregulate (86). In addition, in critically ill heart failure patients, low values are predictive of an adverse survival (87).

The BNP and NT-proBNP do not go down nearly as rapidly in response to therapy as one would expect from the short (20 min for BNP and 2 h for NT-proBNP) half-lives (86). This may relate to the time needed for the system itself to autoregulate.

In ACS patients, BNP and NT-proBNP elevations are prognostic for death in all studies, but the data are not consistent for recurrent myocardial infarction. When one has a conjoint end point such as death, one can be confounded in evaluating myocardial infarction, however. Because so many studies have used optimized cutoff values for their own data sets, there is no consensus on the proper cutoff values. In addition, what actions should be predicated on elevated values are unclear (88,89). The only trial in which it seemed that there was therapeutic guidance associated with elevated values was the TACTICS–TIMI-18 study, in which women with elevated BNP or CRP values seemed to benefit from early PCI even if they had normal cTn values (39). If a strategy to treat such cTn-negative patients could be developed, this could prove to be an important adjunct to the evaluation of patients who present with ACS.

Higher BNP values identify patients with ACS who are at higher risk, but what to do in response is unclear. Elevated values also predict an adverse outcome in stroke, obstructive sleep apnea, diabetes, left ventricular hypertrophy, and stable coronary artery disease.

Thus, the questions regarding what are the appropriate cutoffs in the varied situations in which BNP and NT-proBNP might be used and what one does when one exceeds those cutoffs is unclear. Until these issues are better defined, enthusiasm for the routine use of BNP or NT-proBNP will be blunted.

DEVELOPING MARKERS

sCD40 ligand. sCD40 (90) is a signaling protein that reflects both inflammatory and platelet interactions with the plaque. Increased sCD40 ligand has been shown in patients with ACS. Moreover, articles have suggested that is has prognostic significance in these patients. Several of the studies have used high cutoff values for cTn or insensitive assays. A multiplicity of analytic issues remain.

Myeloperoxidase. Myeloperoxidase (90), a degranulation product, comes from white cells. Elevations occur even when values are obtained from the coronary effluent draining a nonculprit coronary vessel. Systemic levels have been said to aid in prognostication in patients presenting to the emergency department with chest pain, but comparisons have often used high cTn cutoff values or only the admission troponin level. Recently, a commercial assay has been made available.

Ischemia-modified albumin. Ischemia-modified albumin (90) has been touted as a way to detect ischemia even if necrosis is not present. The theory is that the metal

binding site on the amino terminus of albumin is damaged by ischemia. It is marketed for its "negative predictive value." It has been difficult to validate ischemia-modified albumin because of the lack of a gold standard for ischemia. In addition, the assay is cumbersome to use.

Pregnancy-associated plasma protein-A. A member of the insulin-like growth factor family of proteins, pregnancy-associated plasma protein-A (90) is thought to be released when neovascularization occurs and thus may be a marker of incipient plaque rupture. It does not correlate with any of the presently available markers of cardiac injury. It has in some studies seemed to identify patients at risk for subsequent events. There is no available standardized assay.

Choline. Choline (90) is a biomarker that is released when phospholipids are cleaved, which suggests that perhaps it could be a marker of ischemia and/or necrosis. Several studies suggest that the marker might improve prognostication in patients with ACS, but high cutoff values for cTn have generally been used.

Placental growth factor. Placental growth factor (90) is a member of the vascular endothelial growth factor family that has been touted by some but not others to be prognostic in patients with ACS. Some of the positive studies have not used appropriate cTn cutoff values. There is no standard assay for placental growth factor.

Cystatin C. This biomarker is a low-molecular-weight basic protein (13 kDa) that is freely filtered and metabolized after tubular reabsorption. It seems that it is less influenced by age, gender, and muscle mass than serum creatinine. There is a U.S. Food and Drug Administration-cleared assay that is analytically robust. Some studies suggest that it is useful for prognostication in heart failure (91,92) and ACS (93), and that would make sense given that it is well accepted that renal function is a critical determinant of prognosis. It seems that there is an influence of inflammation of the marker that may complicate its use.

Fatty acid binding protein. Fatty acid binding protein is rapidly released after infarction and thus has been touted as an alternative to myoglobin. It has been reported to perform better (94), but it still lacks cardiac specificity. There are no U.S. Food and Drug Administration-approved assays.

Conclusions. The present and future for cardiac biomarkers is exciting. In the near future, many of these biomarkers will provide important new insights into pathophysiology and aid in the diagnosis and management of cardiovascular patients. We are likely to be able to multiplex assays, personalizing biomarker strategies and providing large numbers of values quickly and cheaply. There is great enthusiasm for such a multimarker approach. However, before we implement such a strategy, we must overcome poorly done yet enthusiastic limited reports (5), so that what is eventually recommended will work optimally clinically. In the interim, to provide optimal clinical care, clinicians need to learn more about the biomarkers that they rely on in current clinical practice, i.e., cTn, natriuretic peptides, and CRP, both analytically and clinically.

REFERENCES

1. Arrell DK, Neverova I, Van Eyk JE. Cardiovascular proteomics: evolution and potential. Circ Res 2001;88:763–73.
2. Anderson L. Candidate-based proteomics in the search for biomarkers of cardiovascular disease. J Physiol 2005;563:23–60.
3. Szmitko PE, Wang CH, Weisel RD, Jeffries GA, Anderson TJ, Verma S. Biomarkers of vascular disease linking inflammation to endothelial activation: part II. Circulation 2003;108:2041–8.
4. Szmitko PE, Wang CH, Weisel RD, deAlmeida JR, Anderson TJ, Verma S. New markers of inflammation and endothelial cell activation: part I. Circulation 2003;108:1917–23.
5. Jaffe AS, Katus H. Acute coronary syndrome biomarkers: the need for more adequate reporting. Circulation 2004;110:104–6.
6. James SK, Lindahl B, Armstrong P, et al. A rapid troponin I assay is not optimal for determination of troponin status and prediction of subsequent cardiac events at suspicion of unstable coronary syndromes. Int J Cardiol 2004;93:113–20.
7. Myocardial infarction redefined—a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. Eur Heart J 2000;21:1502–13.
8. Alpert JS, Thygesen K, Antman E, Bassand JP. Myocardial infarction redefined—a consensus document of the Joint European Society of Cardiology/American College of Cardiology Committee for the redefinition of myocardial infarction. J Am Coll Cardiol 2000;36:959–69.
9. Antman EM, Tanasijevic MJ, Thompson B, et al. Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. N Engl J Med 1996;335:1342–9.
10. Ohman EM, Armstrong PW, Christenson RH, et al. Cardiac troponin T levels for risk stratification in acute myocardial ischemia. GUSTO IIA Investigators. N Engl J Med 1996;335:1333–41.
11. Galvani M, Ottani F, Ferrini D, et al. Prognostic influence of elevated values of cardiac troponin I in patients with unstable angina. Circulation 1997;95:2053–9.
12. Jaffe AS, Ravkilde J, Roberts R, et al. It's time for a change to a troponin standard. Circulation 2000;102:1216–20.
13. Panteghini M, Pagani F, Yeo KT, et al. Evaluation of imprecision for cardiac troponin assays at low-range concentrations. Clin Chem 2004;50:327–32.
14. Apple FS. Clinical and analytical standardization issues confronting cardiac troponin I. Clin Chem 1999;45:18–20.
15. Panteghini M, Gerhardt W, Apple FS, Dati F, Ravkilde J, Wu AH. Quality specifications for cardiac troponin assays. Clin Chem Lab Med 2001;39:175–9.
16. Eriksson S, Hellman J, Pettersson K. Autoantibodies against cardiac troponins. N Engl J Med 2005;352:98–100.
17. Novis DA, Jones BA, Dale JC, Walsh MK. Biochemical markers of myocardial injury test turnaround time: a College of American Pathologists Q-Probes study of 7020 troponin and 4368 creatine kinase-MB determinations in 159 institutions. Arch Pathol Lab Med 2004;128:158–64.
18. Adams JE 3rd, Abendschein DR, Jaffe AS. Biochemical markers of myocardial injury. Is MB creatine kinase the choice for the 1990s? Circulation 1993;88:750–63.
19. Eggers KM, Oldgren J, Nordenskjold A, Lindahl B. Diagnostic value of serial measurement of cardiac markers in patients with chest pain: limited value of adding myoglobin to troponin I for exclusion of myocardial infarction. Am Heart J 2004;148:574–81.
20. Apple FS, Murakami MM. Cardiac troponin and creatine kinase MB monitoring during in-hospital myocardial reinfarction. Clin Chem 2005;51:460–3.

21. Gibler WB, Cannon CP, Blomkalns AL, et al. Practical implementation of the guidelines for unstable angina/non–ST-segment elevation myocardial infarction in the emergency department: a scientific statement from the American Heart Association Council on Clinical Cardiology (Subcommittee on Acute Cardiac Care), Council on Cardiovascular Nursing, and Quality of Care and Outcomes Research Interdisciplinary Working Group, in Collaboration With the Society of Chest Pain Centers. Circulation 2005;111:2699–710.

22. Matetzky S, Sharir T, Domingo M, et al. Elevated troponin I level on admission is associated with adverse outcome of primary angioplasty in acute myocardial infarction. Circulation 2000;102:1611–6.

23. Giannitsis E, Muller-Bardorff M, Lehrke S, et al. Admission troponin T level predicts clinical outcomes, TIMI flow, and myocardial tissue perfusion after primary percutaneous intervention for acute ST-segment elevation myocardial infarction. Circulation 2001;104:630–5.

24. Stubbs P, Collinson P, Moseley D, Greenwood T, Noble M. Prognostic significance of admission troponin T concentrations in patients with myocardial infarction. Circulation 1996;94:1291–7.

25. Ohman EM, Armstrong PW, White HD, et al. Risk stratification with a point-of-care cardiac troponin T test in acute myocardial infarction. GUSTOIII Investigators. Global Use of Strategies To Open Occluded Coronary Arteries. Am J Cardiol 1999;84:1281–6.

26. Giannitsis E, Lehrke S, Wiegand UK, et al. Risk stratification in patients with inferior acute myocardial infarction treated by percutaneous coronary interventions: the role of admission troponin T. Circulation 2000;102:2038–44.

27. Panteghini M, Cuccia C, Bonetti G, Giubbini R, Pagani F, Bonini E. Single-point cardiac troponin T at coronary care unit discharge after myocardial infarction correlates with infarct size and ejection fraction. Clin Chem 2002;48:1432–6.

28. Licka M, Zimmermann R, Zehelein J, Dengler TJ, Katus HA, Kubler W. Troponin T concentrations 72 hours after myocardial infarction as a serological estimate of infarct size. Heart 2002;87:520–4.

29. Ingkanisorn WP, Rhoads KL, Aletras AH, Kellman P, Arai AE. Gadolinium delayed enhancement cardiovascular magnetic resonance correlates with clinical measures of myocardial infarction. J Am Coll Cardiol 2004;43:2253–9.

30. Tanaka H, Abe S, Yamashita T, et al. Serum levels of cardiac troponin I and troponin T in estimating myocardial infarct size soon after reperfusion. Coron Artery Dis 1997;8:433–9.

31. Luepker RV, Apple FS, Christenson RH, et al. Case definitions for acute coronary heart disease in epidemiology and clinical research studies: a statement from the AHA Council on Epidemiology and Prevention; AHA Statistics Committee; World Heart Federation Council on Epidemiology and Prevention; the European Society of Cardiology Working Group on Epidemiology and Prevention; Centers for Disease Control and Prevention; and the National Heart, Lung, and Blood Institute. Circulation 2003;108:2543–9.

32. James S, Armstrong P, Califf R, et al. Troponin T levels and risk of 30-day outcomes in patients with the acute coronary syndrome: prospective verification in the GUSTO-IV trial. Am J Med 2003;115:178–84.

33. Wong GC, Morrow DA, Murphy S, et al. Elevations in troponin T and I are associated with abnormal tissue level perfusion: a TACTICS-TIMI 18 substudy. Treat Angina with Aggrastat and Determine Cost of Therapy with an Invasive or Conservative Strategy-Thrombolysis In Myocardial Infarction. Circulation 2002;106:202–7.

34. Morrow DA, Antman EM, Tanasijevic M, et al. Cardiac troponin I for stratification of early outcomes and the efficacy of enoxaparin in unstable angina: a TIMI-11B substudy. J Am Coll Cardiol 2000;36:1812–7.

35. Newby LK, Ohman EM, Christenson RH, et al. Benefit of glycoprotein IIb/IIIa inhibition in patients with acute coronary syndromes and troponin t-positive status: the paragon-B troponin T substudy. Circulation 2001;103:2891–6.

36. Morrow DA, Cannon CP, Rifai N, et al. Ability of minor elevations of troponins I and T to predict benefit from an early invasive strategy in patients with unstable angina and non–ST-elevation myocardial infarction: results from a randomized trial. JAMA 2001;286:2405–12.

37. Gurbel PA, Bliden KP, Zaman KA, Yoho JA, Hayes KM, Tantry US. Clopidogrel loading with eptifibatide to arrest the reactivity of platelets: results of the Clopidogrel Loading With Eptifibatide to Arrest the Reactivity of Platelets (CLEAR PLATELETS) study. Circulation 2005;111:1153–9.

38. Yusuf S, Zhao F, Mehta SR, Chrolavicius S, Tognoni G, Fox KK. Effects of clopidogrel in addition to aspirin in patients with acute coronary syndromes without ST-segment elevation. N Engl J Med 2001;345:494–502.

39. Wiviott SD, Cannon CP, Morrow DA, et al. Differential expression of cardiac biomarkers by gender in patients with unstable angina/non–ST-elevation myocardial infarction: a TACTICS-TIMI 18 (Treat Angina with Aggrastat and determine Cost of Therapy with an Invasive or Conservative Strategy-Thrombolysis In Myocardial Infarction 18) substudy. Circulation 2004;109:580–6.

40. Hamm CW, Goldmann BU, Heeschen C, Kreymann G, Berger J, Meinertz T. Emergency room triage of patients with acute chest pain by means of rapid testing for cardiac troponin T or troponin I. N Engl J Med 1997;337:1648–53.

41. deFilippi CR, Tocchi M, Parmar RJ, et al. Cardiac troponin T in chest pain unit patients without ischemic electrocardiographic changes: angiographic correlates and long-term clinical outcomes. J Am Coll Cardiol 2000;35:1827–34.

42. Henrikson CA, Howell EE, Bush DE, et al. Prognostic usefulness of marginal troponin T elevation. Am J Cardiol 2004;93:275–9.

43. Freda BJ, Tang WH, VanLente F, Peacock WF, Francis GS. Cardiac troponins in renal insufficiency: review and clinical implications. J Am Coll Cardiol 2002;40:2065–71.

44. DeZoysa JR. Cardiac troponins and renal disease. Nephrology 2004;9:83–8.

45. Muller-Bardorff M, Hallermayer K, Schroder A, et al. Improved troponin T ELISA specific for cardiac troponin T isoform: assay development and analytical and clinical validation. Clin Chem 1997;43:458–66.

46. Aviles RJ, Askari AT, Lindahl B, et al. Troponin T levels in patients with acute coronary syndromes, with or without renal dysfunction. N Engl J Med 2002;346:2047–52.

47. Januzzi JL Jr., Snapinn SM, DiBattiste PM, Jang IK, Theroux P. Benefits and safety of tirofiban among acute coronary syndrome patients with mild to moderate renal insufficiency: results from the Platelet Receptor Inhibition in Ischemic Syndrome Management in Patients Limited by Unstable Signs and Symptoms (PRISM-PLUS) trial. Circulation 2002;105:2361–6.

48. Januzzi JL, Cannon CP, DiBattiste PM, Murphy S, Weintraub W, Braunwald E. Effects of renal insufficiency on early invasive management in patients with acute coronary syndromes (the TACTICS-TIMI 18 trial). Am J Cardiol 2002;90:1246–9.

49. Apple FS, Murakami MM, Pearce LA, Herzog CA. Predictive value of cardiac troponin I and T for subsequent death in end-stage renal disease. Circulation 2002;106:2941–5.

50. deFilippi C, Wasserman S, Rosanio S, et al. Cardiac troponin T and C-reactive protein for predicting prognosis, coronary atherosclerosis, and cardiomyopathy in patients undergoing long-term hemodialysis. JAMA 2003;290:353–9.

51. Apple FS, Murakami MM, Pearce LA, Herzog CA. Multi-biomarker risk stratification of N-terminal pro-B-type natriuretic peptide, high-sensitivity C-reactive protein, and cardiac troponin T and I in end-stage renal disease for all-cause death. Clin Chem 2004;50:2279–85.

52. Ooi DS, Isotalo PA, Veinot JP. Correlation of antemortem serum creatine kinase, creatine kinase-MB, troponin I, and troponin T with cardiac pathology. Clin Chem 2000;46:338–44.

53. Bhatt DL, Topol EJ. Does creatinine kinase-MB elevation after percutaneous coronary intervention predict outcomes in 2005? Periprocedural cardiac enzyme elevation predicts adverse outcomes. Circulation 2005;112:906–15; discussion 923.

54. Miller WL, Garratt KN, Burritt MF, et al. Baseline troponin level: key to understanding the importance of post-PCI troponin elevations. Eur Heart J 2006;27:1061–9.

55. Adams JE 3rd. The use of biomarkers to provide diagnostic and prognostic information after cardiac surgery. In: Wu A, editors. Cardiac Markers. 2nd edition. Totowa, NJ: Humana Press, 2003:111–21.

56. Selvanayagam JB, Petersen SE, Francis JM, et al. Effects of off-pump versus on-pump coronary surgery on reversible and irreversible myocardial injury: a randomized trial using cardiovascular magnetic resonance imaging and biochemical markers. Circulation 2004;109:345–50.

57. Thielmann M, Massoudy P, Marggraf G, et al. Role of troponin I, myoglobin, and creatine kinase for the detection of early graft failure

following coronary artery bypass grafting. Eur J Cardiothorac Surg 2004;26:102–9.

58. Topol EJ, Nissen SE. Our preoccupation with coronary luminology. The dissociation between clinical and angiographic findings in ischemic heart disease. Circulation 1995;92:2333–42.

59. Dokainish H, Pillai M, Murphy SA, et al. Prognostic implications of elevated troponin in patients with suspected acute coronary syndrome but no critical epicardial coronary disease: a TACTICS-TIMI 18 substudy. J Am Coll Cardiol 2005;45:19–24.

60. Wang CH, Kuo LT, Hung MJ, Cherng WJ. Coronary vasospasm as a possible cause of elevated cardiac troponin I in patients with acute coronary syndrome and insignificant coronary artery disease. Am Heart J 2002;144:275–81.

61. Yamagishi M, Nissen SE, Booth DC, et al. Coronary reactivity to nitroglycerin: intravascular ultrasound evidence for the importance of plaque distribution. J Am Coll Cardiol 1995;25:224–30.

62. McCord J, Nowak RM, Hudson MP, et al. The prognostic significance of serial myoglobin, troponin I, and creatine kinase-MB measurements in patients evaluated in the emergency department for acute coronary syndrome. Ann Emerg Med 2003;42:343–50.

63. Devaraj S, DuClos TW, Jialal I. Binding and internalization of C-reactive protein by Fc-gamma receptors on human aortic endothelial cells mediates biological effects. Arterioscler Thromb Vasc Biol 2005;25:1359–63.

64. Pearson TA, Mensah GA, Alexander RW, et al. Markers of inflammation and cardiovascular disease: application to clinical and public health practice: a statement for healthcare professionals from the Centers for Disease Control and Prevention and the American Heart Association. Circulation 2003;107:499–511.

65. Rifai N, Warnick GR. Quality specifications and the assessment of the biochemical risk of atherosclerosis. Clin Chem Acta 2004;346:55–64.

66. Khera A, McGuire DK, Murphy SA, et al. Race and gender differences in C-reactive protein levels. J Am Coll Cardiol 2005;46:464–9.

67. Rifai N, Ridker PM. Proposed cardiovascular risk assessment algorithm using high-sensitivity C-reactive protein and lipid screening. Clin Chem 2001;47:28–30.

68. Ridker PM, Cannon CP, Morrow D, et al. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005;352:20–8.

69. Nissen SE, Tuzcu EM, Schoenhagen P, et al. Effect of intensive compared with moderate lipid-lowering therapy on progression of coronary atherosclerosis: a randomized controlled trial. JAMA 2004; 291:1071–80.

70. Nissen SE, Tuzcu EM, Schoenhagen P, et al. Statin therapy, LDL cholesterol, C-reactive protein, and coronary artery disease. N Engl J Med 2005;352:29–38.

71. James SK, Armstrong P, Barnathan E, et al. Troponin and C-reactive protein have different relations to subsequent mortality and myocardial infarction after acute coronary syndrome: a GUSTO-IV substudy. J Am Coll Cardiol 2003;41:916–24.

72. Ridker PM, Cushman M, Stampfer MJ, Tracy RP, Hennekens CH. Inflammation, aspirin, and the risk of cardiovascular disease in apparently healthy men. N Engl J Med 1997;336:973–9.

73. Ridker PM, Rifai N, Clearfield M, et al. Measurement of C-reactive protein for the targeting of statin therapy in the primary prevention of acute coronary events. N Engl J Med 2001;344:1959–65.

74. Smith SC Jr., Anderson JL, Cannon RO 3rd, et al. CDC/AHA workshop on markers of inflammation and cardiovascular disease: application to clinical and public health practice: report from the clinical practice discussion group. Circulation 2004;110:e550–3.

75. Clerico A, Emdin M. Diagnostic accuracy and prognostic relevance of the measurement of cardiac natriuretic peptides: a review. Clin Chem 2004;50:33–50.

76. Apple FS, Panteghini M, Ravkilde J, et al. Quality specifications for B-type natriuretic peptide assays. Clin Chem 2005;51:486–93.

77. Krauser DG, Lloyd-Jones DM, Chae CU, et al. Effect of body mass index on natriuretic peptide levels in patients with acute congestive heart failure: a ProBNP Investigation of Dyspnea in the Emergency Department (PRIDE) substudy. Am Heart J 2005;149:744–50.

78. McCullough PA, Duc P, Omland T, et al. B-type natriuretic peptide and renal function in the diagnosis of heart failure: an analysis from the Breathing Not Properly Multinational Study. Am J Kidney Dis 2003;41:571–9.

79. Wu AH, Smith A, Wieczorek S, et al. Biological variation for N-terminal pro- and B-type natriuretic peptides and implications for therapeutic monitoring of patients with congestive heart failure. Am J Cardiol 2003;92:628–31.

80. Maisel AS, Krishnaswamy P, Nowak RM, et al. Rapid measurement of B-type natriuretic peptide in the emergency diagnosis of heart failure. N Engl J Med 2002;347:161–7.

81. Emdin M, Clerico A, Clemenza F, et al. Consensus document: recommendations for the clinical use of cardiac natriuretic peptides. Ital Heart J Suppl 2005;6:308–25.

82. Silver MA, Maisel A, Yancy CW, et al. BNP consensus panel 2004: a clinical approach for the diagnostic, prognostic, screening, treatment monitoring, and therapeutic roles of natriuretic peptides in cardiovascular diseases. Congest Heart Fail 2004;10:1–30.

83. Januzzi JL Jr., Camargo CA, Anwaruddin S, et al. The N-terminal pro-BNP investigation of dyspnea in the emergency department (PRIDE) study. Am J Cardiol 2005;95:948–54.

84. Maisel A, Hollander JE, Guss D, et al. Primary results of the Rapid Emergency Department Heart Failure Outpatient Trial (REDHOT). A multicenter study of B-type natriuretic peptide levels, emergency department decision making, and outcomes in patients presenting with shortness of breath. J Am Coll Cardiol 2004;44:1328–33.

85. Bettencourt P, Azevedo A, Pimenta J, Frioes F, Ferreira S, Ferreira A. N-terminal-pro-brain natriuretic peptide predicts outcome after hospital discharge in heart failure patients. Circulation 2004;110:2168–74.

86. Miller WL, Hartman KA, Burritt MF, Borgeson DD, Burnett JC Jr., Jaffe AS. Biomarker responses during and after treatment with nesiritide infusion in patients with decompensated chronic heart failure. Clin Chem 2005;51:569–77.

87. Miller WL, Burnett JC Jr., Hartman KA, Henle MP, Burritt MF, Jaffe AS. Lower rather than higher levels of B-type natriuretic peptides (BNP and NT-proBNP) predict high short-term mortality in end-stage heart failure patients treated with nesiritide. Am J Cardiol 2005;96:837–41.

88. Jernberg T, Lindahl B, Siegbahn A, et al. N-terminal pro-brain natriuretic peptide in relation to inflammation, myocardial necrosis, and the effect of an invasive strategy in unstable coronary artery disease. J Am Coll Cardiol 2003;42:1909–16.

89. Morrow DA, deLemos JA, Sabatine MS, et al. Evaluation of B-type natriuretic peptide for risk assessment in unstable angina/non-ST-elevation myocardial infarction: B-type natriuretic peptide and prognosis in TACTICS-TIMI 18. J Am Coll Cardiol 2003;41:1264–72.

90. Apple FS, Wu AH, Mair J, et al. Future biomarkers for detection of ischemia and risk stratification in acute coronary syndrome. Clin Chem 2005;51:810–24.

91. Sarnak MJ, Katz R, Stehman-Breen CO, et al. Cystatin C concentration as a risk factor for heart failure in older adults. Ann Intern Med 2005;142:497–505.

92. Shlipak MG, Katz R, Fried LF, et al. Cystatin-C and mortality in elderly persons with heart failure. J Am Coll Cardiol 2005;45:268–71.

93. Jernberg T, Lindahl B, James S, Larsson A, Hansson LO, Wallentin L. Cystatin C: a novel predictor of outcome in suspected or confirmed non–ST-elevation acute coronary syndrome. Circulation 2004;110:2342–8.

94. Pelsers MM, Hermens WT, Glatz JF. Fatty acid-binding proteins as plasma markers of tissue injury. Clin Chem Acta 2005;352:15–35.