Biomarkers of Ischemia in Patients With Known Coronary Artery Disease
Do Interleukin-6 and Tissue Factor Measurements During Dobutamine Stress Echocardiography Give Additional Insight?

Robert H. Christenson, PhD; Christopher P. deFilippi, MD; Donald Kreutzer, PhD

A fundamental understanding of the mediators and mechanisms associated with the pathophysiological process of reversible myocardial ischemic injury would be a substantial advancement in our basic understanding of this process and would speed the translation from “bench to bedside.” For example, elucidation of the chemical signals that both indicate the presence and extent of reversible ischemia and have a putative role in cellular dysfunction and injury would clearly have potential as a valuable biomarker for the development of diagnostic tools, in the clinical management of patients, and perhaps as a guide for the development of viable therapeutic targets. Focusing on the inflammatory process would seem to have a high likelihood for success because ischemic events, infection, and mechanical cell injury are known to activate numerous pluripotent pathways, cascades, and networks, including the cytokine and coagulation systems. These systems are highly integrated and redundant because of their critical role in the survival of the organism. Inflammatory cytokines such as interleukin-6 (IL-6) are low-molecular-weight pluripotent glycoproteins with a dynamic range of local and systemic actions and a wide range of target cells and organs. In many instances, individual cytokines have multiple biochemical activities that confer functional redundancy during inflammation and tissue repair; different cytokines can also have the same activity. Inflammatory cytokines and cytokine networks are also intimately associated with other injury response systems, including the coagulation system that controls for fibrinogenesis and fibrinolysis, with all its associated matrices, proteases, and metabolites, including fibrin, thrombin, plasmin, fibrinopeptides, and fibrin metabolites, to name a few. Central to the coagulation system is the transmembrane glycoprotein known as tissue factor, which controls clot formation via the extrinsic pathway. The coordinated integration of cytokine networks and coagulation cascades is essential in controlling inflammation and repair. For example, cytokines such as IL-1, tissue necrosis factor, and IL-6 are known to induce tissue factor expression in a variety of cells. Alternatively, fibrin and fibrin degradation products are known to induce expression of proinflammatory cytokines such as IL-6 and IL-8. Therefore, the concept of “cross regulation” of inflammation, coagulation, and cytokine systems is clearly established.

The study of Ikonomidis et al proposes to add knowledge in the area of myocardial function by hypothesizing that inducible myocardial ischemia increases plasma levels of biomarkers, notably IL-6 and tissue factor, leading to persistent left ventricular dysfunction during recovery from dobutamine stress testing. These authors also propose that an association exists between the extent of ischemia and IL-6 release during heart stress induced by dobutamine. A number of issues with this study appear to moderate the contribution. Administration of dobutamine is intended to cause physiological stress by invoking a mismatch between myocardial demand for oxygen, nutrients, and clearance of metabolites and vascular supply. During dobutamine stress testing, the functional integrity of the myocardium is monitored via wall motion studies by echocardiography. The notion that reversible ischemia induced by dobutamine stress testing can increase circulating levels of IL-6 and other related factors as put forward by Ikonomidis et al is quite reasonable. However, the statistically significant differences between resting and both stress and recovery values of IL-6 demonstrated in this work were very modest, according to data displayed in Figure 1a. In fact, the difference between resting and peak IL-6 measurements was <1 pg/mL; the <1-pg/mL difference documented may well be less than the analytical reliability of the method used in this study. This substantive analytical issue is neither mentioned in discussion nor listed as a limitation in the conclusions. Furthermore, one must ask whether this extremely small IL-6 difference in circulating IL-6 levels translates to mediating or promoting tissue differences in wall motion abnormalities during dobutamine stress, as proposed by the authors. Although a citation is given, the authors offer little evidence that the circulating concentrations of IL-6 are substantial enough at the tissue level to exert a direct, reversible negative inotropic action on myocardium. Furthermore, it is difficult to envision how such minor increases in IL-6 (even if analytically valid) can drive an association between myocardial dysfunction during recovery...
and the extent of ischemia and IL-6 release during dobutamine stress. This underscores the issue of whether IL-6 and tissue factor increases seen in this study are cardiac derived, derived for other tissues and released into the blood stream, or both.

The tissue factor results became somewhat elevated after stress compared with resting values in some patients who demonstrated wall motion abnormalities after dobutamine stress in Figure 1c.2 On the other hand, results of some patients with wall motion abnormalities showed virtually no change in tissue factor levels. Could this be due to a difference in phenotype among the patients (ie, subpopulation)? Conversely, some patients without wall motion abnormalities on dobutamine stress showed dramatic decreases from rest to stress values; however, many patients in this group showed no difference, also suggesting a separate phenotype. The heterogeneity in tissue factor results among the patient groups was neither noted nor commented on by the authors. For example, a substantial number of patients had increased, decreased, or no differences in tissue factor expression (Figure 1c and 1d)2; are these subpopulations of patients with wall motion abnormalities after dobutamine stress compared with resting values in some patients who showed no difference, also suggesting a separate phenotype? Further analysis of these data may answer these questions. These questions require an answer before this could be used as a useful biomarker clinically. Perhaps unmeasured cytokines or other reactive molecules were substantially contributing to the proposed effect of IL-6 and tissue factor observed in this study. For example, fibrin, a known product of tissue factor activity, could have “downstream” local and systemic effects that could have contributed to the observed findings.

The timing of IL-6 and tissue factor release and the physiological effect of wall motion abnormalities also were interesting. Certainly, storage of biologically active signals can cause an immediate physiological effect. However, one must be mindful that it takes some hours for translation from mRNA to protein. Therefore, the notion that an increase in circulating IL-6 enhanced the production of newly synthesized IL-6 by hypoxic myocytes, vascular endothelial cells, or adherent leukocytes as suggested by the authors2 in the time frame of these studies must be thought through carefully.

Biomarkers have a rich history in the context of myocardial cell injury. Development and clinical validation of the biomarker cardiac troponin have changed the diagnostic paradigm for myocardial infarction over the past decade.4 However, it is widely accepted that myocardial cell turnover is necessary before cardiac troponin is released into circulation; therefore, reversible myocardial ischemia occurs without cardiac troponin release. Other tools that are valuable for assessing cardiac ischemia such as the ECG are imperfect in that they are quite diagnostically specific (~95%) but not very sensitive (~50%). For this reason, a biomarker or biomarker strategy that can reliably discriminate myocardial infarction from reversible cardiac ischemia from no cardiac ischemia is considered a holy grail by many scientists and clinicians. An ischemia biomarker “tool” could enhance the care provided (and reduce the associated costs) for literally millions of individuals who present to emergency departments and other healthcare venues each year with a constellation of signs and symptoms that may represent acute cardiac ischemia.

Despite considerable interest in the development of a blood-based biomarker to detect myocardial ischemia in the absence of myocardial injury, the field of candidate markers remains limited and incompletely studied. In part, there is the complexity of isolating cardiac ischemia from the potential upstream processes of vessel plaque rupture, thrombus formation, and subsequent vessel occlusion or distal embolization. Therefore, although markers such as myeloperoxidase5 and pregnancy-associated plasma protein A6 are both potential biomarkers to identify chest pain patients with acute coronary syndromes in the absence of a cardiac troponin elevation, they appear to reflect coronary plaque instability, not myocardial ischemia.

The Table summarizes some currently available biomarkers that identify transient cardiac ischemia by either the temporary interruption of blood supply to a region of the myocardium (typically induced by balloon occlusion of an epicardial vessel during elective percutaneous coronary intervention [supply ischemia]) or myocardial oxygen consumption exceeding supply in the setting of exercise or pharmacological stress (demand ischemia). The mechanism for detectable transient elevation of any of these ischemia biomarkers requires elucidation. Ischemia-modified albumin and unbound free fatty acids are not cardiac-specific molecules.7 It is uncertain whether their response is unique to cardiac ischemia or could be reproduced by ischemia in skeletal muscle or other organs.7 Neither of these biomarkers has yet been shown in published reports to rise in response to demand ischemia. The natriuretic peptide (commercially measured as either the brain natriuretic peptide [BNP] or the N-terminal metabolite [NT-pro BNP]) is a cardiac hormone initially thought to be released almost exclusively as the result of ventricular stretch or pressure. However, it also appears to be upregulated in chronically ischemic myocardium,8 is released in the setting of acute coronary syndromes,9,10 and is a diagnostic marker of both supply-mediated11 and demand ischemia12 in patients with stable coronary disease.

If confirmed, the notion forwarded by Ikonomidis et al2 could expand knowledge of the pathophysiological response during transient demand ischemia and could introduce a third arm of possible candidate biomarkers to pursue for assessment of ischemia. It appears unlikely that the inflammatory cytokine IL-6 will evolve as a clinical marker of choice for reasons outlined elsewhere in this editorial. Many candidates showing statistically significant differences fail to evolve into

### Available Markers That Identify Presence or Absence of Reversible Ischemia

<table>
<thead>
<tr>
<th>Ischemia Marker</th>
<th>Reference</th>
<th>Demand Ischemia</th>
<th>Supply Ischemia</th>
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<tbody>
<tr>
<td>Ischemia modified albumin</td>
<td>16, 17</td>
<td>?</td>
<td>+</td>
</tr>
<tr>
<td>Unbound free fatty acid</td>
<td>18</td>
<td>?</td>
<td>++</td>
</tr>
<tr>
<td>NT-proBNP/BNP</td>
<td>9, 10, 19</td>
<td>+/-</td>
<td>+</td>
</tr>
<tr>
<td>IL-6</td>
<td>2</td>
<td>+</td>
<td>?</td>
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NT-proBNP indicates N-terminal pro-brain natriuretic peptide.
practical biomarkers for clinical use. However, if a local inflammatory response occurs during demand ischemia and is in proportion to the extent and duration of ischemia, then there is potential to add mechanistic insight into rising levels of other proposed ischemia biomarkers and potential substrate for future investigation. Lastly, dobutamine-induced increase in myocardial oxygen consumption is generally less than an equivalent rate-pressure product with exercise.14 The more limited extent of ischemia may be reflected by the fact that BNP levels rise acutely during exercise but not during dobutamine-induced ischemia.11–14 This finding is consistent with that of Ikonomidis et al,2 who show that with larger induced ischemic territories the change in IL-6 level is greater.

Many researchers in the fields of proteomics and metabolomics are actively pursuing biomarker strategies for assessing reversible and irreversible myocardial ischemia. Validation of any proposed ischemia biomarker requires comparing its performance to an independent reference method. Dobutamine stress testing with detection of wall motion abnormalities by echocardiography as was used by Ikonomidis et al2 appears to be an adequate approach. However, dobutamine stress involves therapeutically inducing a supply-demand mismatch. It is noteworthy that the root cause of most clinical cardiac ischemia involves unstable coronary plaque, platelet activation, thrombus formation, and frequently downstream consequences.15 Will ischemia biomarkers validated with dobutamine stress also reflect reversible ischemia caused by a very different etiology?

A more thorough understanding of mechanisms involved in reversible myocardial ischemia is critical in evolving toward biomarkers that will be clinically robust. The study by Ikonomidis et al2 appears to raise many unanswered questions: Are the changes in IL-6 and tissue factor seen in the blood of these patients cardiac related (cause or result) or simply associated? What is the source(s) of IL-6 and tissue factor (cardiac versus noncardiac) detected in the blood of these patients? Do IL-6 or tissue factor response subpopulations exist within these patients? These issues are complicated by the small, albeit statistically significant, increases in IL-6 and tissue factor seen by Ikonomidis et al.2 These small differences may also be difficult to consistently detect in the real clinical world of biomarkers and diagnostics. Thus, the question remains: Are these small but statistically significant differences relevant to the understanding of the pathophysiology of reversible myocardial ischemic injury or useful in developing new biomarkers? Until these questions are answered and these issues are resolved, the significance and utility of these observations remain unclear.16–19

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