Session IV. Recommendations for Assay Platforms and Markers of Acute Myocardial Infarction

Introduction to Section IV

The biochemical events that occur following the total occlusion of a coronary artery were summarized by Hearse in 1979, and are still accurate today (109). Figures 7A lists an approximate chronology of these events and the markers that are associated with each step. The initial events occur within the few seconds or minutes after total coronary artery occlusion and are associated with reversible changes. This is characterized by the lack of oxygen delivery, and a reduced production of energy stores (ATP molecules), as the myocyte shifts from aerobic to anaerobic glycolysis and increased glycogenolysis. Enzymes that participate in the breakdown of glycogen such as the phosphorylases are putatively released during this time. In order to conserve energy, there is impairment or failure of the ATP-dependent ion membrane pumps resulting in the release of intracellular electrolytes such as potassium and phosphate (Figure 7A). Concomitant to energy deficits is the inability of the heart to remove waste products. This leads to accumulation and release of metabolites such as lactate and adenosine. Low molecular weight proteins may be able to pass through reversibly injured but repairably membranes.

If the affected artery becomes patent during the early time intervals either spontaneously or by pharmacologic (thrombolytic therapy) or surgical (angioplasty or bypass) means, the jeopardized myocytes can fully recover. Prolonged or permanent occlusion, however, lead to the onset of irreversible damage. The hallmark of irreversible damage is disruption of cellular membranes and release of macromolecules such as enzymes and large molecular weight proteins. The release of mitochondrial proteins in particular, are indicative of cell death and tissue necrosis. Once the marker is released from the myocyte, they must pass through the interstitial space before they can appear in the general circulation. As shown in Figure 7B, ions and low molecular

weight metabolites readily pass through the interstitial space directly into the vascular space. Their appearance into blood (Figure 7C) is rapid. Unfortunately, these ions and metabolites are not specific to myocardial injury, and are not indicative of irreversible damage. Cardiac enzymes and proteins have the advantage of organ specificity, and essentially are only released during irreversible damage. However, they cannot directly pass to the vasculature, and must travel through slow lymphatic drainage. Therefore there is a delay before they appear in blood. The size of the protein and its distribution within the cell dictates the appearance rate. Small intracellular proteins (e.g., myoglobin and fatty acid binding protein) appear first, while large proteins (e.g., CK and LDH) and those that are part of the contractile apparatus (e.g., troponin) have a delayed appearance. Strategies for development of early AMI markers should be focused on proteins that are specific to the heart. In addition, proteins with low molecular weight will appear in blood sooner than large proteins and enzymes.

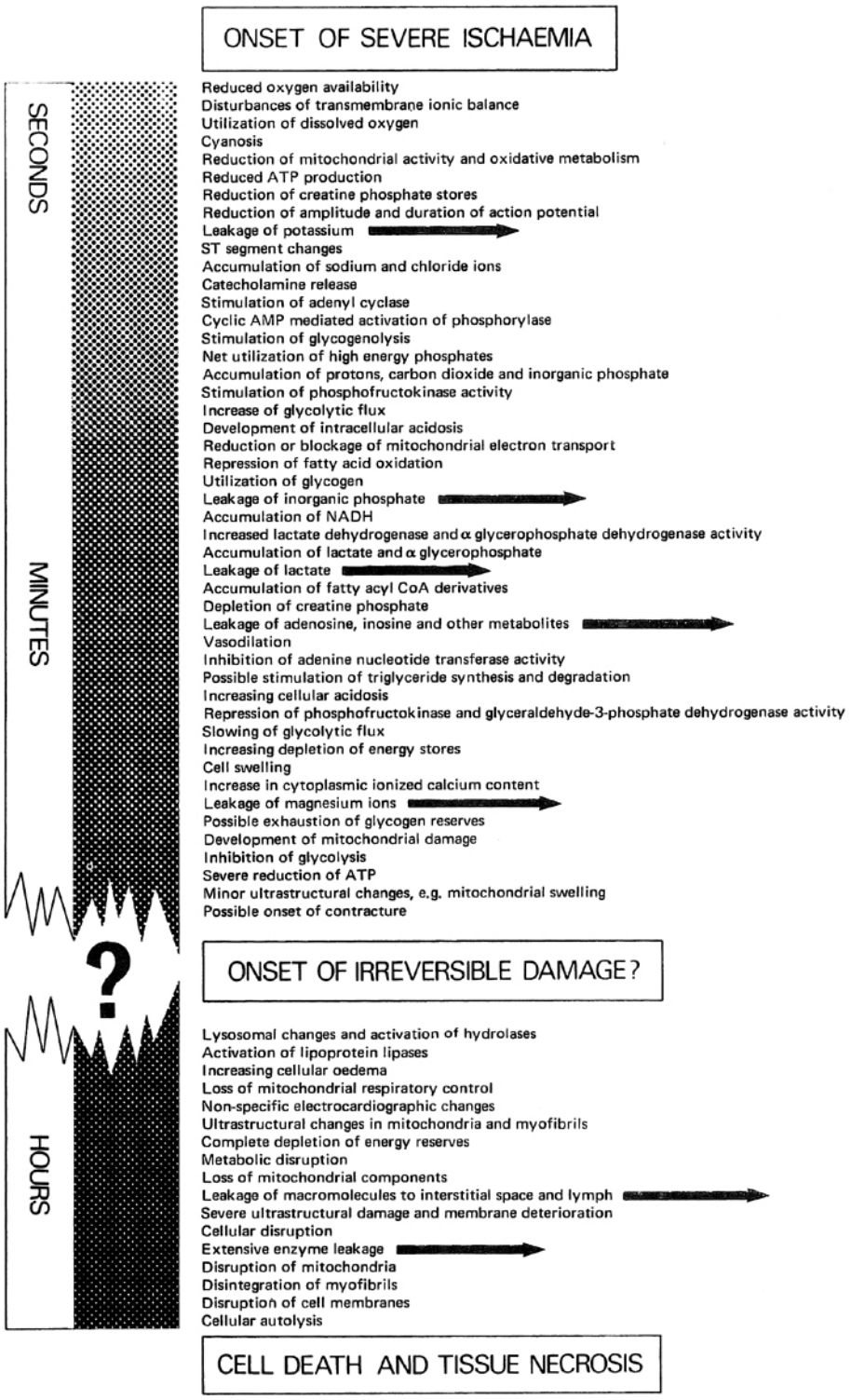
ONSET OF SEVERE ISCHAEMIA
SECONDS
Reduced oxygen availability
Disturbances of transmembrane ionic balance
Utilization of dissolved oxygen
Cyanosis
Reduction of mitochondrial activity and oxidative metabolism
Reduced ATP production
Reduction of creatine phosphate stores
Reduction of amplitude and duration of action potential
Leakage of potassium
ST segment changes
Accumulation of sodium and chloride ions
Catecholamine release
Stimulation of adenyl cyclase
Cyclic AMP mediated activation of phosphorylase
Stimulation of glycogenolysis
Net utilization of high energy phosphates
Accumulation of protons, carbon dioxide and inorganic phosphate
Stimulation of phosphofructokinase activity
Increase of glycolytic flux
Development of intracellular acidosis
Reduction or blockage of mitochondrial electron transport
Repression of fatty acid oxidation
Utilization of glycogen
Leakage of inorganic phosphate
Accumulation of NADH
Increased lactate dehydrogenase and α glycerophosphate dehydrogenase activity
Accumulation of lactate and α glycerophosphate
Leakage of lactate
Accumulation of fatty acyl CoA derivatives
Depletion of creatine phosphate
Leakage of adenosine, inosine and other metabolites
Vasodilation
Inhibition of adenine nucleotide transferase activity
Possible stimulation of triglyceride synthesis and degradation
Increasing cellular acidosis
Repression of phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase activity
Slowing of glycolytic flux
Increasing depletion of energy stores
Cell swelling
Increase in cytoplasmic ionized calcium content
Leakage of magnesium ions
Possible exhaustion of glycogen reserves
Development of mitochondrial damage
Inhibition of glycolysis
Severe reduction of ATP
Minor ultrastructural changes, e.g. mitochondrial swelling
Possible onset of contracture
MINUTES
?
ONSET OF IRREVERSIBLE DAMAGE?
Lysosomal changes and activation of hydrolases
Activation of lipoprotein lipases
Increasing cellular oedema
Loss of mitochondrial respiratory control
Non-specific electrocardiographic changes
Ultrastructural changes in mitochondria and myofibrils
Complete depletion of energy reserves
Metabolic disruption
Loss of mitochondrial components
Leakage of macromolecules to interstitial space and lymph
Severe ultrastructural damage and membrane deterioration
Cellular disruption
Extensive enzyme leakage
Disruption of mitochondria
Disintegration of myofibrils
Disruption of cell membranes
Cellular autolysis
HOURS
CELL DEATH AND TISSUE NECROSIS

Fig. 7A

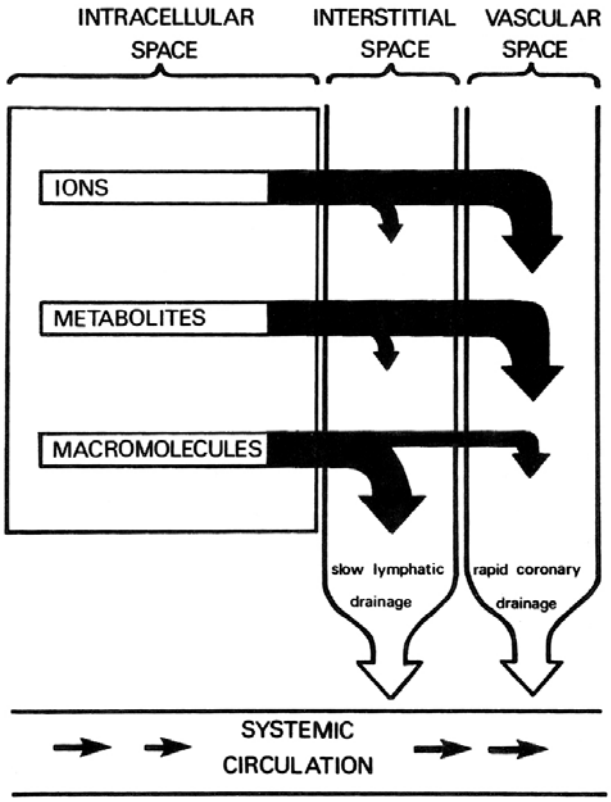

Fig 7B

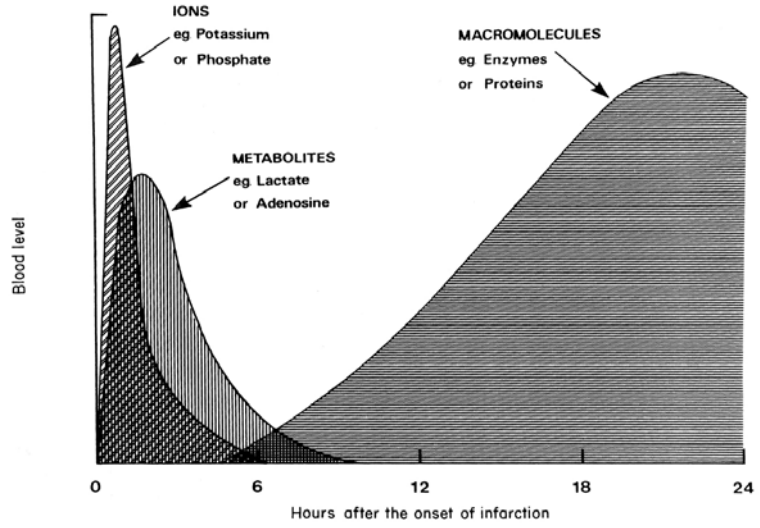

Fig 7C

Fig. 7.
A. List of biochemical events occurring after total occlusion of a coronary artery.
B. Possible routes for release of markers from tissue to blood.
C. Release pattern of marker vs. time after onset of infarction.
Used with permission from Hearse DJ. Cellular damage during myocardial ischaemia: metabolic changes leading to enzyme leakage.
In: Hearse DJ, de Leiris, eds., Enzymes in cardiology. Diagnosis and research. Chichester: Wiley, 1979:4-14.

Recommendation 1

CK-MB has long been considered the biochemical standard for the laboratory diagnosis of AMI (110). The development, characterization, and clinical interpretation of cTnT and cTnI seriously challenge the role of CK-MB. cTnT and cTnI appear in the blood at or near the same time as CK-MB, but remain abnormal for 4-10 days (Fig. 3, peak C).

The use of CK-MB should be phased out over the ensuing years as more cTnT and cTnI assays become available and the cost for such assays becomes competitive with CK-MB mass assays (111). If a hospital is already using cTnT or cTnI, the NACB Committee felt that the measurement of lactate dehydrogenase isoenzymes and β-hydroxybutyric dehydrogenase should be discontinued immediately (23,112). No recommendation is being made as to the discontinuance of assays for total CK. This marker is inexpensive and readily available in clinical laboratories, and it can be very useful for the detection of skeletal muscle injury or disease (113).

Recommendation: Cardiac troponin (T or I) is the new standard for diagnosis of myocardial infarction and detection of myocardial cell damage, replacing CK-MB.

Strength/consensus of recommendation: Class II.

There is no longer a role for lactate dehydrogenase and its isoenzymes for diagnosis of cardiac diseases.

Strength/consensus of recommendation: Class I.

Discussion

There was considerable discussion as to whether cardiac troponins can now replace total CK and/or CK-MB. As summarized in Table 5, there are several ongoing analytical issues that haveslowed laboratories and clinicians towards a more rapid conversion toward cardiac troponin. For cTnT, the first-generation assay had a problem with nonspecific binding of skeletal muscle troponin (corrected with the subsequent generation of assays). For cTnI, a major issue is the lack of standardization. Results from different manufacturers produce cTnI values that differ by a factor of 20 or more (114). Studies have shown that while the predominate form of cTnI in blood of patients after AMI is the binary complex of cTnI-C, there are smaller amounts of the ternary complex of cTnI-T-C and free cTnI (115). In a study conducted by the AACC cTnI Standardization Subcommitte, biases in results for cTnI among commercially available cTnI assays were caused by the lack of standardization to a single reference material, and the lack of standardization of antibodies used in the cTnI assay. (114). On a molar basis, some assays had equal responses to all forms, some were more reactive to the cTnI complex than free forms, while others had a differential response between binary and ternary complexes (Fig. 8). Epitope mapping studies have shown that the specificity of cTnI antibodies is directly dependent on the specific peptides selected from the cTnI amino acid sequence (116). Antibody selection will also determine the ability of an assay to recognize proteolytic degradation products of cTnI (117). Thus after AMI, the apparent clearance rate of cTnI from blood will vary between assays. Given the complexity of cTnI pathophysiology in blood, harmonization of assay results across commercial platforms have proven to be extremely difficult.

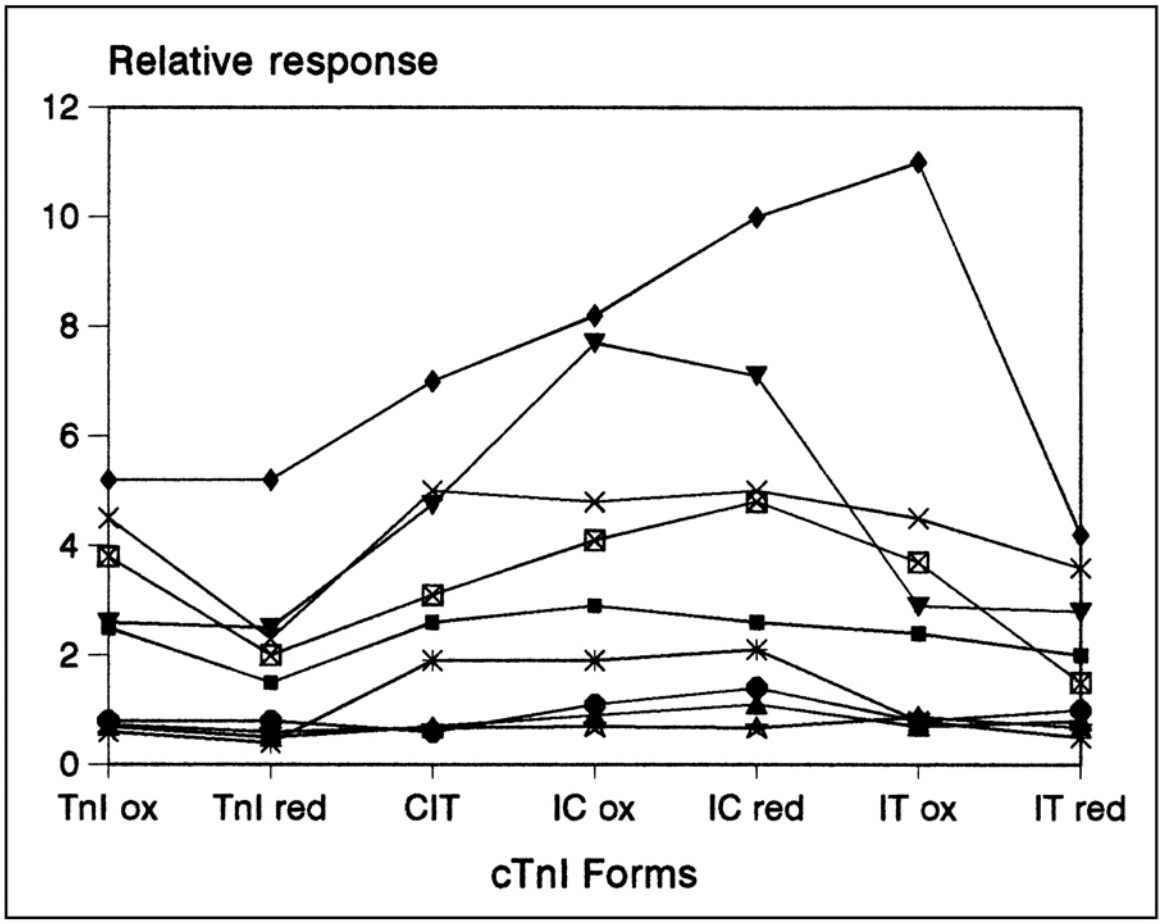

Fig. 8. *Relative response for troponin assays to prepared samples of cTnI.* Used with permission from the American Association for Clinical Chemistry.

Table 5. Continuing analytical issues for implementation of cardiac troponin as an accepted standard for myocardial injury.

lack of assay standardization for cTnI
lack of standardization between laboratory-based and point-of-care testing platforms
lack of good analytical correlation (e.g., r>0.950) among commercial cTnI assays for clinical specimens
variability in imprecision for all cardiac troponin assays
variability in acceptable blood collection tubes
appropriate cutoff concentrations not documented
potential for false positive results due to presence of fibrin and human anti-mouse antibodies.

Within-run and total imprecision also are not uniform between commercial assays (118). In many assays for cardiac troponin, the presence of fibrin clots and heterophile antibodies can produce false-positive results (119). These problems have prompted

manufacturers of troponin assays to produce new generation kits to improve assay sensitivity and specificity.

Cardiologists have also expressed concerns about totally replacing CK-MB. Although quantitative calculations using the area under the CK-MB vs time curve are seldom made, many physicians use peak CK-MB to get a qualitative impression as to the size of a myocardial infarction. Others have questioned whether serial troponin measurements can be used for reinfarction (because of the prolonged release pattern) and suggest a continuing role for CK-MB for this purpose. Still others feel that there has not been enough peer-reviewed publications on various cTnI troponin assays or day-to-day experience by practicing cardiologists to warrant a change at this time. The NACB Committee felt that over the ensuing years, most of these issues will be resolved. Therefore, despite the existence of these limitations, hospitals should begin considering the replacement of CK-MB.

An important issue that must be resolved at each institution is reimbursement for these tests. Recently, the Health Care Finance Administration announced that "it is not necessary to use troponin in addition to creatine kinase (CPT codes 82550-82554) (which includes the MB isoenzyme) in the management of patients with myocardial infarctions", suggesting that reimbursement will not be given when both tests are ordered (120). Private insurance companies may also limit reimbursements for cardiac markers (e.g., Blue Cross/Blue Shield of Michigan does not reimburse for cardiac troponin). Although these Guidelines recommend the use of troponin as the new standard for myocardial injury, the NACB Committee recognizes that it is unrealistic for a hospital or medical center to completely change over to cardiac troponin without a "transition period," during which both CK-MB and cardiac troponin assays are offered. The length of the transition period could be 2-3 months, depending on the acceptance and understanding of the use cardiac troponin results by the medical staff and the degree of continuing education available. After the trial period, the data should be

reviewed and a decision made as to whether to a) continue the trial period, b) keep CK-MB, c) replace it with one of the cardiac troponins, or d) make routine use of both CK-MB and cardiac troponin.

During the presentations, the NACB Committee took a poll as to whether a recommendation can be made now to retire CK-MB. The majority felt that CK-MB still had a role. However, when the conference participants were asked about the future (5 years) use for CK-MB, essentially all felt that CK-MB would eventually be abandoned. The NACB Committee has retained this recommendation because the NACB believes that it should take a leadership role in recommending future clinical laboratory practices. The publication of the recommendation as written may provide documentation and assist laboratory directors and administrators to make changes in testing policies sooner. If laboratories are to retain CK-MB, the NACB Committee recommends the use of mass assays, which have been shown to be superior to activity-based assays (such as immunoinhibition or electrophoresis) (36,121). The calculation of the percent relative index [CK-MB (in ug/L)/total CK (in U/L) x 100] may assist in the differentiation between myocardial and skeletal muscle causes of increased total CK (122,123). Other investigators have concluded that the relative index unacceptably degrades the sensitivity of CK-MB and should be abandoned (124,125).

Recommendation 2

AMI patients with ST-segment elevations on the ECG can be effectively treated with thrombolytic therapy, particularly if therapy is initiated within 12 h after the onset of chest pain. Delays in implementation will reduce the success of this treatment. As such, the National Heart Attack Alert Program has made a recommendation to physicians to treat all AMI patients within 60 min of their arrival in the ED (126) (Figure 9A). Results for serum cardiac markers are not needed in making this therapeutic decision. However, rapid testing and reporting of cardiac marker concentrations may produce other benefits

for cardiac patients. Two outcome studies have shown that testing cardiac markers on a continuous random-access basis decreased the length of stay and overall laboratory costs compared with testing on a batched basis (127,128). It is presumed that providing stat testing will lead to more time-efficient decisions for triage and discharge.

The factors that affect TATs include the delay in the delivery of the sample to the laboratory, the preanalytical steps necessary to prepare the sample, the analysis time itself, and the effort it takes to deliver results to the ordering physician. The NACB Committee understands that the time taken for the delivery of samples to the laboratory is not always under the control of the laboratory. Nevertheless, laboratory personnel should work closely with hospital administrators and nursing staffs to minimize delays. TATs can be improved with the implementation of pneumatic tubes that deliver samples directly and rapidly to the central laboratory. The use of satellite laboratories is another mechanism to reduce delivery and, therefore, reporting turnaround times. Fig. 9B summarizes the steps necessary for reporting a laboratory result for cardiac markers.

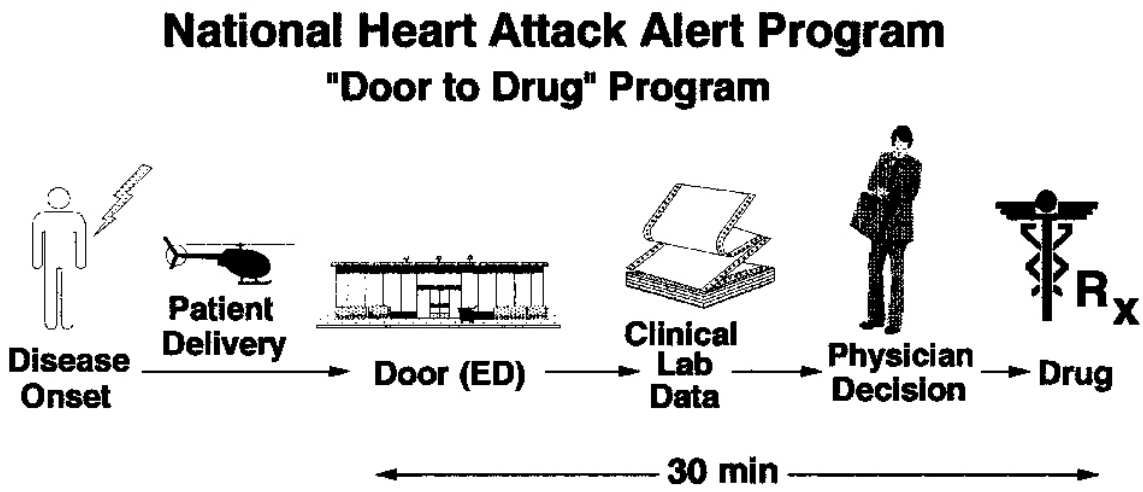

Fig. 9. A. National Heart Attack Alert Program, 60 minutes to Treatment Working Group "Door to Drug" program.

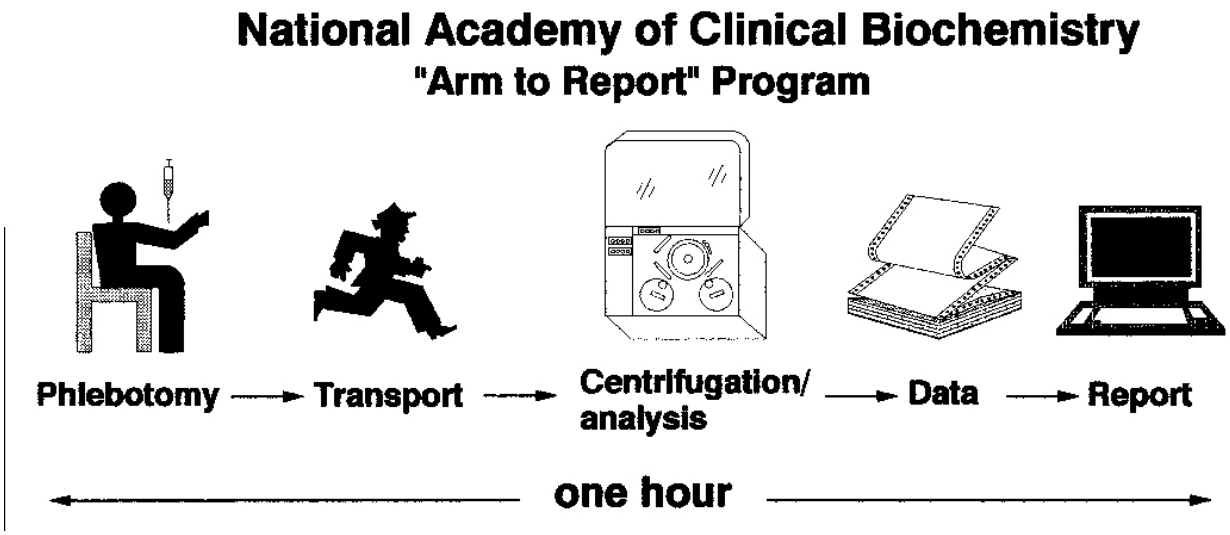

Fig. 9. B. NACB "Arm to Report" recommendation for a 1-hour turnaround time for collection, transportation, analysis, and delivery of results for acute cardiac marker testing.

Recommendation: The laboratory should perform stat cardiac marker testing on a continuous random-access basis, with a target turnaround time (TAT) of 1 h or less. The TAT is defined as the time from blood collection to the reporting of results.

Strength/consensus of recommendation: Class II.

Discussion.

There was considerable discussion on the issue of TAT. There was some support for further reducing TATs. When questioned during the plenary lecture, Dr. Eugene Braunwald responded that 40 min was a target for ED TAT. One reviewer stated that new technologies for sample delivery, bar-coding, and rapid centrifugation will enable laboratories to consistently meet this goal and that the NACB should begin to set very high standards. Decreasing TATs would invariably be received positively by the ED staff if they themselves were not responsible for the testing. On the other hand, other individuals felt that although the technology for rapid TATs exists, many hospitals have limitations in human resources. Thus, if a sample sent from the ED for cardiac markers is accompanied by a request for a complete blood count, blood gases,

electrolyte profile, gram stains, and other tests, the bench technologist must prioritize which test to perform first. When a choice is presented to the ED staff as to which stat analytes should be tested first, a cardiac marker panel might not have the highest priority. Because of the lack of consensus, the NACB Committee has retained the recommendation of a 1-h TAT objective. It is unlikely that a laboratory will be able to consistently (>90%) deliver stat cardiac marker results in 30-40 min, using laboratory-based serum or plasma assays. Results of stat cardiac marker testing are not used to determine the need for thrombolytic therapy. Moreover, rule-out of AMI from the ED does require results of serial sampling, which further diminishes the need for a very rapid TAT on any single sample.

Recommendation 3

Some laboratories do not have automated immunoassay analyzers, rapid tube delivery systems, or staffing to deliver results within 1 h on a continuous basis.

Qualitative as well as quantitative POC testing devices are now available for myoglobin, CK-MB, cTnT, and cTnI (129-132). These assays make use of anticoagulated whole blood, and have analysis times of 20 min. Eliminating the need to deliver samples to the central laboratory and centrifugation enables TATs of 30-40 min. In a recent randomized study, results obtained with POC testing were compared with results obtained in a central laboratory for consecutive admissions to a coronary care unit (133). The POC testing group was associated with a shorter assay TAT (5 min vs 69 min, $p<0.05$) and coronary care unit length of stay (1.94 vs 2.51 days) compared with testing performed in the central laboratory; because of the small number of subjects, the difference in coronary care unit length of stay did not reach statistical significance. Recently, multipanel quantitative POC testing devices have been approved by the Food and Drug Administration for combinations of myoglobin, CK-MB, and cTnI. Quantitative assays may ultimately be more useful than qualitative POC devices. However, because of the newness of quantitative POC assays, there have

been no studies to compare the effectiveness of qualitative vs quantitative POC testing in the ED. Therefore, the NACB Committee was unable to formulate a recommendation at this time. In some qualitative and quantitative POC testing devices, the total number of analytes measured is fixed. Despite this, the NACB Committee endorses the use of only two: an early (myoglobin or CK-MB mass) and a definitive (cardiac troponin T or I).

Although outcome studies have shown that expediated testing and reporting of results for cardiac markers reduces hospital length of stay and laboratory costs for cardiac patients (127,128), there are no outcome studies to validate the specific need for a 1-h TAT. It is clear, however, that early treatment of Q-wave AMI patients with thrombolytic therapy is important for success in terms of reducing mortality and increasing the rate of coronary artery patency. With the development of new therapeutic strategies for unstable angina and non-Q-wave AMI, the NACB Committee anticipates that early detection of any myocardial injury will also be beneficial in the management of these patients. For those patients who are ruled out for acute coronary syndromes, it is expected that fast TATs for laboratory data will lead to faster patient discharges and a reduction in overall hospital costs. The NACB Committee encourages prospective outcome studies to examine the putative advantage of reporting TATs within 1 h.

Recommendations: Institutions that cannot consistently deliver cardiac marker turnaround times of ~1 h should implement POC testing devices. The cardiac troponin cutoff concentration should be set at the 97.5% upper reference limits so that the devices can detect the first presence of true myocardial injury.

Strength/consensus of recommendation: Class I.

Recommendation 4

POC devices are designed for testing to be performed at or near the bedside by the primary caregivers. However, the responsibility for this testing must reside with the laboratory. The success of POC testing programs will depend on cooperation and the acknowledgment of the laboratory's responsibility by hospital administrators, nursing staffs, and the appropriate units within the hospital (e.g., the ED).

When the laboratory staff recognizes a situation of noncompliance, they should have the authority to direct the corrections, and, if necessary, remove POC testing devices and suspend testing from the area of the hospital where the testing was conducted until the deficiencies have been satisfactorily corrected.

Recommendation: Among other tasks, laboratory personnel must be involved in the selection of devices, the training of individuals to perform the analysis, the maintenance of POC equipment, the verification of the proficiency of operators on a regular basis, and the compliance of documentation with requirements by regulatory agencies such as the Health Care Finance Administration and Clinical Laboratory Improvement Act of 1988. In meeting these requirements, a quality-assurance and quality-control program must be instituted and fully documented on a regular basis.

Strength/consensus of recommendation: Class I.

.

Recommendation 5

Assays for cardiac markers for early diagnosis, rule-out, triaging of patients from the ED, or for determination of successful reperfusion require markers that have a short assay TAT. Irrespective of how or where the testing is performed (i.e., laboratory-based or POC testing), assays must meet minimum precision requirements. Imprecise assays at or near cutoff concentrations will adversely affect the clinical performance of the test.

Recommendation: Assays for cardiac markers should have an imprecision (CV) ≤10% at the AMI decision limits and an assay TAT of <30 min. Before launch, assays must be characterized with respect to potentially interfering substances [e.g., other related proteins, human anti-mouse antibodies (134,135), and other interferences].
Strength/consensus of recommendation: Class II.

Discussion

The NACB Committee understands the importance of establishing objective analytical goals for assays for new cardiac markers. This will assist manufacturers in the construction of new assays. The total precision required for a particular assay is dependent on the biological variation of the analyte. The biologic variation has been established at 5.6% for myoglobin (136) and 9.3% for CK-MB (137). The biologic variation for cardiac troponin has not been established. As such, this recommendation for total precision was arbitrarily set at 10% without a prior scientific basis.

Recommendation 6

Most patients with cardiac diseases are heparinized while hospitalized. When serum is collected from these patients, full clot retraction from tubes without preservatives can take 10-15 min or more. Clots can continue to form even after the sample has been centrifuged and the serum placed onto immunoassay analyzers. When this occurs, fibrinous material can interfere with the assays as well as during

sampling to block probes. For automated immunoassay analysis, the use of plasma will eliminate the extra time needed for clotting, thereby reducing the overall preanalytical TATs. Manufacturers should target their assays for use in plasma as well as provide for safety procedures that will detect clots in samples. Results for serum and plasma are not interchangeable for all assays and markers, particularly for cTnI. Therefore, for cardiac troponin, NACB recommends that laboratories do not intermix different types of blood collection tubes at the same facility.

Although whole blood testing is not yet an option for most automated immunoassay analyzers, it is available for POC testing. The use of whole blood can reduce assay and reporting TATs. Currently, the assay TATs for myoglobin, CK-MB, and troponin are 10-20 min. For some samples, dilutions will be necessary to report quantitative results that are outside the limits of the reportable range. Electronic transmission is essential for the efficeint reporting of results.

Recommendation: Plasma or anticoagulated whole blood are the specimens of choice for the stat analysis of cardiac markers.

Strength/consensus of recommendation: Class I.

Discussion.

In the original draft of the Guidelines, the recommendation stated that heparinized plasma is the specimen of choice for troponin measurements. However, some reviewers, particularly those in Europe, suggested that the Guidelines be expanded to included all forms of plasma collection tubes (such as EDTA or citrated collection tubes). Laboratories that choose to use these collection types must proceed with caution. With EDTA tubes, troponin released as a ternary (cTnT-I-C) or binary (cTnI-C) complex will degrade to free subunits because ionized calcium is needed to maintain this complex and is removed by chelation of the metal ions (138). Troponin

assays that do not exhibit an equimolar response between complexed and free subunits will produce significant biases between serum and EDTA plasma (114). Heparin does not disrupt complexes; therefore, no change in results between serum and plasmas are expected. The laboratory must follow the recommendations for acceptable specimen types listed in manufacturers' package inserts and should use a reference interval specific to the specimen type.

General Discussion

One reviewer expressed concern that if these guidelines are enacted, laboratories that choose not to enact one or more of the recommendations may be open to liability if a cardiac patient suffers an unfavorable outcome and a lawsuit is filed. This is an important issue for all clinical practice guidelines committees and expert panels. The Committee believes that thes guidelines provide well accepted goals and objectives for laboratories in the use and application of cardiac markers. They do not set absolute standards of practice, rather, they encourage proper use and establishment of future goals.

The objective of the NACB Committee was not to make recommendations as to how cardiac markers are to be used in conjunction with other diagnostic modalities (e.g., electrocardiography, echocardiography, and nuclear imaging ventriculography) or how results should be used to select specific therapies. Organizations such as the National Heart Attack Alert Program Committee and the Agency for Health Care Policy Research have been developed to address such issues. We hope that the clinical organizations, especially cardiology, will use these guidelines to move forward towards updating the WHO criteria for ruling in and ruling out AMI.

We thank Edward A. Sasse, Medical College of Wisconsin, Milwaukee, WI, for his fund-raising efforts.