Methodologies for Measurement of Cardiac Markers

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KEYWORDS

• Laboratory medicine • Cardiac markers • Biomarkers • Immunoassay
• Assay principles • Assay design • CKMB • Troponins

KEY POINTS

- Understanding of the methodological basis of biomarker measurement supports the interpretation of clinical results.
- Clinical cardiac biomarker measurements obtained using automated analyzers in the central laboratory or near patient/point-of-care devices are antibody-based methods.
- Inherent limitations associated with the use of antibodies and surrogate calibrators underlie method-specific and sometimes specimen-specific results.
- It is critical to appreciate the limitations and recognize the often-cited caveat that results from antibody-based measurements must always be interpreted within the context of the full clinical picture.

INTRODUCTION

A biomarker, as defined by the National Institutes of Health working group in an attempt to standardize the term, is "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to a therapeutic intervention".[1] This rather broad definition encompasses biomarkers as surrogate endpoints for clinical trials[2,3] and risk factors (such as lipid profiles) for the development of disease in addition to clinically actionable indices of disease burden or adverse events.

For the purposes of this article a cardiac biomarker is a substance produced by diseased or healthy cardiac tissue, whose concentration in peripheral circulation reflects the presence of disease and is thus of clinical diagnostic and/or prognostic value. Specifically, the clinical goals of biomarker measurement include the following:

- Identifying the presence of disease (diagnosis)
- Predicting outcomes (prognosis)
- Monitoring response to intervention (monitoring)

The author has nothing to disclose.
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Clin Lab Med 34 (2014) 167–185
http://dx.doi.org/10.1016/j.cll.2013.11.012 **labmed.theclinics.com**
0272-2712/14/$ – see front matter

Historically, clinical cardiac markers were restricted to proteins associated with tissue damage and included myoglobin, creatine kinase, and its isoezyme (CKMB). These proteins were measured in serum or plasma for the diagnosis of myocardial infarctions using a wide range of antibody-based assay methods. Today many new cardiac biomarkers are measured in routine practice and additional ones are in development. These markers encompass more protein types and physiology than previously. They are useful prognostically as well as diagnostically for a wide range of cardiac diseases in addition to improved diagnosis and management of myocardial infarctions. The assay methods currently used are essentially all modern double-antibody, nonisotopic systems that offer major advantages in achieving rapid testing at points of care as well as in the central laboratory. Future methods include innovative assay designs including enhanced signaling systems and multiplex assays to combine biomarkers for improved clinical utility.

CARDIAC BIOMARKERS

The most common clinical cardiac biomarkers in use currently include the following:

- CKMB
- Cardiac troponins I and T
- B-type natriuretic peptide (BNP): mature BNP and N-terminal proBNP (NTproBNP)

Newer markers whose clinical use is rapidly developing include the following:

- Galectin 3
- ST-2
- Myosin binding protein C

Table 1 summarizes the biochemical characteristics of these cardiac biomarkers. Shown also are the typical concentrations of each in serum or plasma that influence diagnostic or prognostic clinical decisions. The purpose of this article is to illustrate how modern antibody-based assays are designed to measure these concentrations as precisely and accurately as possible. Serious challenges remain in achieving this goal. Significant limitations of current antibody-based assays are discussed.

ASSAY FORMATS AND DESIGNS

Principles of Immunometric Assay Design

Circulating cardiac biomarkers are all proteins characterized by multiple antigenic epitopes. The presence of multiple epitopes on the protein biomarker allows the use of immunometric assays that are the current method of choice for achieving specific, quantitative measurements as quickly as possible.

The essential components of an immunometric assay are as follows: (1) 2 antibodies with noncompeting epitope specificity, allowing them to simultaneously bind the biomarker, (2) a signal generating system that is proportional to the amount of biomarker in the specimen being measured, and (3) the biomarker exists in a form that can be used to calibrate the signal generated. Most assay designs in clinical use are heterogeneous (multiple steps) and require a separation of the unbound reactants from the antibody-antigen complex, which generates the signal measured. Separation is usually achieved by attaching one antibody to a solid surface (plastic microtiter well, microparticle, membrane strip, and so on), allowing unreacted reagents to simply be "washed" away after the biomarker is "captured." A second antibody/detection system is then bound to form a complex of the 2 antibodies, the biomarker and the signaling system. Having the complex immobilized on a solid surface is also the basis for the