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Editorial

Cardiac biomarkers — A short biography

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Coronary thrombosis was recognized in the 19th century as a cause of death but was considered nothing more than a medical curiosity. Animal experiments showed that sudden ligation of a major coronary artery was immediately fatal. Post-mortem studies where there was demonstration of an occlusive intracoronary thrombus in some cases of sudden death reinforced this concept. Medical opinion at the time was that coronary thrombosis was an immediately fatal event.

It was reported in 1901 that coronary thrombosis does not always result in sudden death. However symptom severity was related to the speed of onset of arterial occlusion. Rapid and acute symptom onset accompanied acute occlusion, whereas gradual occlusion resulted in a more mild illness. James Herrick reported in 1912 the clinical features of acute myocardial infarction (AMI) and characterised it as a distinct clinical entity from angina pectoris [1]. His view was that AMI was not immediately fatal and could be treated with the emphasis on bed rest. Interestingly, he made the remarkably prescient statement at the time: "the hope for the damaged myocardium lies in the direction of securing a supply of blood through friendly neighbouring vessels so as to restore as far as possible its functional integrity". The first diagnostic test was the electrocardiogram (ECG) originally developed by William Einthoven (for which he won the Nobel Prize in 1924) and studied by Thomas Lewis [2]. The primacy of the ECG for AMI diagnosis stood the test of time since 1912, and the ECG remains an essential tool for discriminating non-ST elevation MI from ST elevation MI, and guiding their very different management strategies [3].

Laboratory testing for AMI began with the report by Libman that the number of white blood cells in the peripheral blood was often increased very soon after an event. This finding continues to be replicated in the literature to this day. Sherck reported in 1933 that AMI was associated with a raised ESR which began later but was longer lasting than the white cell count [4].

As displayed in Fig. 1, biochemical biomarkers emerged in the 1950s. The concept that tissue damage resulted in enzyme release that could subsequently be measured was the innovation that began the era of diagnostic enzymology.

The start of biochemical testing for AMI was initiated with the development of an assay for aspartate transaminase (AST), known as glutamic oxaloacetic transaminase, by Karmen and co-workers [5–7]. The initial method utilized an 18-hour incubation of two oxoglutarate with aspartate followed by chromatographic separation of glutamate. The amount of glutamate produced was determined by the ninhydrin reaction [7]. They reported elevation of AST in a number of conditions and in two patients following AMI. This method was not suitable for routine clinical use and a spectrophotometric method was developed [6]. This method was used to determine serial measurements of AST in 16 patients with AMI demonstrating values rising 2 to 20 times normal within 24 h and returning to within the reference interval within 3 to 6 days [5]. This observational study was followed by experimental and further observational work conclusively demonstrating the relationship between AST measurement and the detection of AMI [8]. At the same time that transaminase elevation was being described in AMI, it was also reported that the measurement of C-reactive protein (CRP) and fibrinogen could be used for the diagnosis of AMI [9]. This study was one of the first reports where biochemical testing was found to be more reliable than the ECG.

The next enzyme biomarker to be described was lactate dehydrogenase (LD) by Wroblewski and LaDue who demonstrated that LD could be measured by catalytic reduction of a known amount of a specific ketoacid [10]. Further, they showed that experimental and clinical AMI was associated with a rise in serum LD activity. However, specificity was the main problem with AST raised in liver damage, and LD is found in a wide range of tissues and elevated in a variety of haematological, hepatic, malignant and musculoskeletal disease states. Thus the search was on for a more specific test.

Isoenzymes of LD were demonstrated by Vesell and Bearn in 1957; it was found that the heart was the dominant contributor of LD1, but that red cells were a contributor of LD2 [11]. The early technologies for the separation of LD isoenzymes required time consuming and laborious electrophoresis methods. In 1960 Rosalki showed that alpha oxobutyrate might be an alternative substrate for LD1 [12]. Subsequently a non-electrophoretic means of measuring LD1 and LD2 quantitation by the measurement of hydroxybutyrate dehydrogenase (HBD) activity was developed. This method used alpha oxobutyrate as substrate. It was demonstrated that HBD activity was raised in AMI and reflected heart LD isoenzymes [13–15]. HBD levels were shown to be elevated for longer than either AST or LD and was both more sensitive and specific.

The final addition to the "holy trinity" of cardiac enzymes was the development of creatine kinase (CK) measurement. CK was first described by Ebashi where it is shown that elevated levels were associated with muscle disease, in particular muscular dystrophy. The attraction was the high concentration of CK in muscle tissue and its high degree of tissue specificity. CK was found to be markedly elevated in patients with myocardial injury and to be more useful than the measurement of AST, especially when there was accompanying cardiac failure [16]. The early methodologies in use for the measurement of CK were poor. A new method, based on a modification of the Kornberg ATP assay

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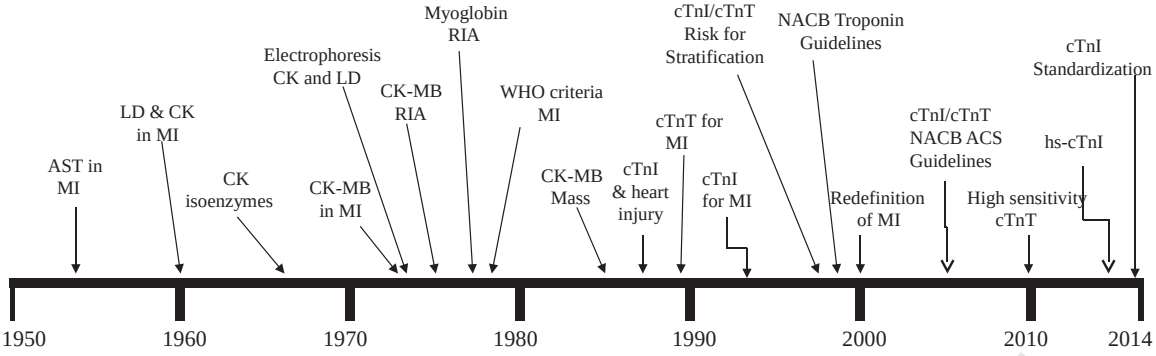

Fig. 1. Cardiac biomarkers timeline.

[17], was developed by Rosalki (sketched out on a restaurant menu card) [18]. This method has become the standard for CK measurement, and serial measurement of CK and observing CK temporal changes allowed an early rule-in and rule-out of AMI in as little as 4 h after presentation [19,20].

The recognition of the clinical value of CK isoenzymes was followed by the development of improved assays for the MB isoenzyme (CK-MB). Although early methods involved electrophoretic separation and were unsuitable for large-scale automation [21], the development of antibodies to the M and B subunits allowed the development of immunoinhibition methods based on binding of antibodies to the M subunit with stearic inhibition of catalytic activity and measurement of residual CK-B. These were widely adopted as they were low cost and available on routine clinical chemistry analysers.

The development and acceptance of biomarker measurement as part of the diagnostic strategies for patients presenting with chest pain and suspected AMI could be said to have reached maturity with the publication of the 1979 WHO criteria for AMI [22]. Diagnosis required at least two of the following three criteria: (i) either a positive clinical history of chest pain, (ii) unequivocal ECG changes or (iii) abnormal serial cardiac enzyme measurements. Directly quoted from the WHO document: "3.1.1 Definite acute myocardial infarction. Definite acute myocardial infarction is diagnosed in the presence of unequivocal ECG changes and/or unequivocal enzyme changes; the history may be typical or atypical."

Development of immunoassays for CK-MB in the mid-1980s marked the beginning of a new era in cardiac biomarker measurement. The shift was away from the measurement of CK-MB enzyme activity and towards the measurement of enzyme as 'protein mass' and to the development of immunoassays as the prime means of cardiac biomarker detection and measurement. Measurement of specific CK-MB 'protein mass' rather than catalytic activity had already begun with the development of immunoassays for myoglobin [23–25]. Of particular interest was the potential for very early diagnosis due to rapid release from necrotic myocardium [26].

The advent of monoclonal antibody technology plus the realization that very small amounts of protein could be detected by immunoassay techniques resulted in two significant developments. First was the development of monoclonal antibodies for CK-MB measurement. The initial description was of a monoclonal antibody to CK-MB (known as Conan MB) which was used as a capture antibody with the measurement of residual CK to give CK-MB activity [27]. The antibody was then combined with an antibody to CK-B and developed into a two-site mass immunoassay that became commercially available in 1988. The second significant change was the development of assays for cardiac structural proteins with the initial studies being performed on myosin light chains [28,29]. However, myosin light chains were found to be non-specific for myocardium.

Assays for cardiac troponins were developed with an assay for cardiac troponin I (cTnI) reported in 1987 [30,31] and for cardiac troponin T (cTnT) in 1989 [32]. The measurement of cTnT and cTnI was truly a paradigm shift in the role of cardiac biomarker measurement in the diagnosis of patients presenting with chest pain. Early clinical studies comparing various biomarkers found that approximately one-third of patients considered to have MI excluded on the basis of either CK-MB [33,34] or CK [35] measurement had an elevated cTnT or cTnI. Further, elevated troponin levels were associated with a significant risk of subsequent major adverse cardiac events (subsequent MI, cardiac death or re-admission with unstable angina). The predictive ability of elevated cTnT and cTnI has subsequently been confirmed in a large number of studies and by meta-analysis [36–38].

The clear diagnostic superiority of measurement of cTnT and cTnI led to a reappraisal of the role of cardiac biomarkers in patients presenting with suspected coronary artery disease. In 1998 the National Academy of Clinical Biochemistry (NACB) arranged a two-day Standards in Laboratory Medicine meeting as part of the American Association for Clinical Chemistry annual meeting devoted to cardiac biomarkers. A set of consensus recommendations and guidance was published, including the recognition of the value of cTnT and cTnI [39]. At that time two diagnostic cut-offs were proposed; the 97.5th percentile of cardiac troponin and a CK-MB WHO AMI equivalent value. Subsequently, proposals were produced by the International Federation of Clinical Chemistry [40,41]. The culmination of this process was the proposed redefinition of MI in 2000 [42], which placed cTnT and cTnI at centre of diagnosis [43], followed by the subsequent Universal Definition, now in its third refinement [44].

Progressive improvements in assay sensitivity have occurred, but it had to be remembered that cardiac troponin is an organ specific biomarker, not a disease specific marker [45]. Thus improved sensitivity has proved to be a two-edged sword, as noted by Robert Jesse's insightful statement "when troponin was lousy assay it was a great test, but now that it's a great assay it's a lousy test" [46]. Clearly, better assay analytical sensitivity has translated into improved precision at low troponin concentrations, but there has been an increase in the number of clinical conditions, other than AMI, where myocardial injury can be detected. These elevations, often misleadingly and incorrectly referred to as "false positives" (only in reference to a diagnosis of AMI but not for the diagnosis of myocardial injury) are not a new phenomenon. Elevation of both cTnT and cTnI outside the AMI population was reported early in the development of cTn assays [47]. The original attraction of cTn measurement was its apparent high sensitivity and specificity for AMI diagnosis, especially in the Emergency Department. The additional clinical value justified the cost of the new test (when first introduced cTn measurement was significantly more expensive than the alternative conventional "cardiac enzyme" strategies). Improved assay sensitivity not only has reduced specificity for a diagnosis of AMI but also has

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