REVIEW ARTICLE

CARDIAC TROPONIN-I: A BIOCHEMICAL MARKER FOR CARDIAC CELL NECROSIS

Kh.H. HAIDER* AND W.H. STIMSON
Department of Immunology, University of Strathclyde, The Todd Centre, 31 Taylor Street, Glasgow, G4 ONR, U.K.
present address: Faculty of Pharmacy, University of the Punjab, Lahore, Pakistan.

Ischaemic heart disease represents the most common of the serious health problems in contemporary society and acute myocardial infarction (AMI) is the major cause of cardiovascular morbidity and death. The accurate determination of infarct size and the volume of myocardium at risk at the time of initial insult is important in the choice of treatment; however, it is probable that it is the ultimate infarct area which determines the longer term outcome. Myocardial ischaemia first appears in the sub-endocardial region. Initially, the ischaemic cells are reversibly injured and if these initial changes are not promptly reversed, it will result in the death of the myocytes. Thereafter, irreversible myocyte necrosis travels transmurally towards the epicardium in the form of a wave-front (Alpert, 1989).

Progress in the field of thrombolytic therapy has significantly affected the management of patients with AMI (Verstraete, 1992). A timely and successful reperfusion during an evolving infarction often prevents immediate mortality, reduces the risk of infarct and thus preserves the left ventricular function.

The familiar triad, clinical history, ECG and serum enzyme analysis, is still of utmost significance in the diagnosis of myocardial infaction (Willerson, 1989). However, none of these criteria reveal quantitative and precise information regarding the extent and location of the infarct. Efforts to relate infarct size to serum level changes of the marker substances released in blood from necrosing myocytes, have met with little success. In addition, a number of specialist techniques such as planar radioisotope imaging, single photon emission computed tomography (SPECT), positron emission tomography (PET), echocardiography, ventriculography and NMR imaging have been employed to substantiate diagnosis in patients showing ambiguous symptoms and ECG findings. However, most of these procedures are not available to patients due to their very high costs.

In recent years, major advances have been made in the methods based on immunological techniques to improve the detection and estimation of infarcts. These

Correspondence to: Prof. W.H. Stimson, Department of Immunology, University of Strathclyde, The Todd Centre, 31 Taylor Street, Glasgow, G4 0NR, U.K.

methods are exclusively based on the production and availability of specific antibodies against intracellular, cardiac-specific substances. The membranes of irreversibly injured and necrosing myocardial cells lose their property of semi-permeability and hence a number of intracellular cytoplasmic components become released into the circulation. The rise in the serum concentration, as well as the activities of these substances, form the basis of many of the presently available biochemical tests for MI. The results of the studies by Serrano *et al.* (1990) and Vannen *et al.* (1990) revealed that although non-specific, serum myoglobin (Mb) levels rise very early in the event of MI. The simultaneous measurement of serum Mb and serum carbonic anhydrase-III (CA-III), a skeletal muscle-specific protein, could be used to assess myocardial damage. Similarly, there is considerable evidence in the literature that there is leakage of mitochondrial and lysosomal enzymes from necrotic myocytes following myocardial infarction (Sylven *et al.*, 1987; Puleo *et al.*, 1988; Pappas, 1989; Doran *et al.*, 1990).

There is an ever growing list of enzymes which have been suggested as being suitable for the detection of myocardial injury. However, only a very small number of enzymes, such as creatine kinase(CK) (Lee *et al.*,1986 & 1987; Apple, 1989; Puleo, 1990 & Prager *et al.*, 1992) and lactate dehydrogenase(LD) (Loughlin *et al.*, 1988; Wolf 1989, Smith *et al.*, 1987, Jensen *et al.*, 1990 & Galbraith *et al.*, 1990) have been singled out as being of particular significance. A serious limitation associated with most of these markers is the lack of cardiac specificity — this often results in a high percentage of false positive results. For this reason, research in the past decade has been focussed on structural as well as functional proteins of the cardiac muscle, that show a very high degree of specificity for the myocardium.

Khaw *et al.*, 1976, undertook a study to visualise damaged myocardium using a radiolabelled polyclonal anti-myosin antibody. Encouraged by the results obtained various groups of research workers then reported producing monoclonal antibodies (McAb) against the light and heavy chains of cardiac myosin (Haber *et al.*, 1981; Khaw *et al.*, 1984; Leger *et al.*, 1985 & 1991; Hoberg *et al.*, 1988 & Jin *et al.*, 1990). Recent reports from Bhattacharya *et al.* (1991), Johnson *et al.* (1992) and Nakata *et al.* (1992) showed that AMA planar and tomographic imaging can detect myocyte necrosis in both Q-wave and non-Q-wave infarctions, with a high degree of sensitivity regardless of infarct age, size and location. The system of cardiac myosin-antimyosin was chosen for the *in vivo* visualisation of myocardial infarction because cardiac myosin is present at a relatively high intracellular concentration. It is also easy to isolate and purify, shows strong immunogenicity and its exposure to the extracellular environment only occurs after cellular damage under pathological conditions (Khaw, *et al.*, 1989). However, there are many problems and questions, associated with anti-myosin monoclonal antibody (AMA) imaging, which still remain unanswered. An important example is the non-specific uptake of AMA Fab in the area of the thorax due to cross reactivity with skeletal myosin (Khaw *et al.*, 1983); more evidence of the non-specific uptake in AMA is provided by the reports of Hoefnagel *et al.*, 1987, Cox *et al.*, 1988 & Planting *et al.*, 1990. Moreover, the very complex situation regarding the specificity of cardiac myosin for myocardial tissue, together with its isoform switching at various developmental stages and under a number of pathological conditions has jeopardised its position as an ideal cardiac-specific marker (Gorza *et al.*, 1984).

The use of cardiac-specific troponin-T (cTn-T) is an interesting new development that promises better diagnostic efficacy and a higher degree of cardiospecificity than the existing markers. Like many other major contractile and regulatory proteins, cTn-T is biochemically and immunologically different from its counterparts in skeletal muscle (Gusev *et al.*, 1983). Its release profile has been reported to be similar to cTn-I and tropomyosin (Tm) after AMI (Cummins *et al.*, 1981 & 1987); however, recent evidence indicates that cTn-I has a significantly different pharmacokinetic profile with a subsequent earlier release of the molecule. Nevertheless cTn-T shows a biphasic release pattern and provides a long diagnostic window of 10.5–140h after the onset of pain (Katus *et al.*, 1991&1992; Mair *et al.*, 1991, Gerhardt *et al.*, 1991). The determination of cTn-T in the serum offers several distinct advantages for the diagnosis of AMI such as very low normal serum levels, cardiospecificity, and as a subcellularly compartmentalised protein, its release into serum lasts longer than many other marker substances.

CARDIAC TROPONIN-I

Troponin plays a key role in the thin filament regulation of both skeletal and cardiac muscle contraction. Depending upon the state of bound Ca^{+2}, it positions tropomyosin along F-actin to either allow or prevent the interaction of myosin with actin (Potter *et al.*, 1982). In other words, it serves as a Ca^{+2} sensitive switch on the thin filament which is involved in regulating muscle contraction.

Troponin(Tn) is a complex of troponin-I (Tn-I), troponin-T (Tn-T) and troponin-C (Tn-C) (Ebashi *et al.*, 1972; Tsukui *et al.*, 1973). The Tn-C component (18.5KD) is a Ca^{2+} binding component which, by an unknown mechanism, conveys a calcium associated signal through Tn-T (38KD), the Tm binding component and Tn-I (23.9KD), the inhibitory component of actomyosin ATPase activity (Greaser *et al.*, 1972). Each of these molecules has been reported to exist in multiple isoforms and is expressed in a tissue-specific and developmentally regulated manner. The troponin complex proteins are single polypeptides and show a relatively simple tissue distribution, compared to myosin or tropomyosin.

In the case of Tn-I, three principle isoforms have been reported — these are associated with cardiac muscle, slow skeletal muscle and fast skeletal muscle, in humans (Cummins *et al.*, 1978). These isoforms may contribute to the functional differences of the various muscle types and separate genes have been found to encode for the three isoforms (Vallins *et al.*, 1990; Hunkler *et al.*, 1991).

The Tn-I isoforms have been found to be tissue specific but species non-specific (Dhoot *et al.*, 1978). They are located in different types of cells in the myofibrils and the cells of the fibres of the type-1 contain only the slow isoform of Tn-I whereas the fast isoform is present in type-2 cells. However, in some developmental and pathological conditions, cells can have both isoforms concommitantly. Studies in a variety of different mammals, including rabbit, cow, baboon, monkey and man have indicated that the structural relationship of cTn-I is strongly conserved in all mammalian species. Its distribution pattern is uniform throughout the atrial and ventricular chambers possibly due to the presence of only one cell type in the heart tissue; moreover, it is uniquely located in the myocardium (Humphreys *et al.*, 1984). A comparative study of cTn-I from different species by Berson *et al.* (1978) showed

that all cTn-I appeared similar with respect to mol.wt but with different overall charges. These different cTn-I molecules have the same inhibitory effect on skeletal actomyosin ATPase activity and the same ability to be phosphorylated. However, a marked variation on the phosphate content was observed between one species and another. This was possibly related to the conditions in which heart was removed as the adrenaline surge before the death of an animal was reported to be capable of phosphorylating cTn-I (Solaro et al., 1976).

cTn-I is a single polypeptide chain of 211 amino acids which exhibits no evidence of heterogeneity in its amino acid sequence. A comparative study of the sequence of cTn-I with that of sTn-I showed that the inhibitory region is almost identical in the two proteins, the former having an additional 26 residues at the N-terminal region not present in skeletal muscle specific troponin-I (sTn-I). cTn-I should, therefore, prove to be a heart-specific biochemical marker which can be detected in blood plasma of a patient with AMI and also can be targetted, in vivo, using radiolabelled cTn-I specific McAbs for localisation and visualisation of the infarct site. Apart from this, there is strong overall homology between the two proteins especially at the C-terminal end, which is the most conserved part of the molecule (Grand et al., 1976). This sequence contains two adjacent serine residues which become phosphorylated in response to ß-adrenergic stimulation of the heart; this is an important feature of cTn-I but it should be noted that the amino acid sequence of this phosphorylation site is conserved between all species. cTn-I, isolated from bovine heart contains two adjacent phosphoserines (Swiderek et al., 1988); absence of the N-terminal serine residues confers reduced responsiveness of the heart to ß-adrenergic stimulation (Mittamann et al., 1990).

The amino acid sequence from 104–115 is the peptide imparting ATPase inhibitory activity to the Tn-I molecule. This region has also been found to be important for Tn-I interaction with Tn-C and Tm and any alteration in this sequence results in loss or reduction in the inhibitory activity of Tn-I (Eyk et al., 1988). Laure et al. (1992) have recently carried out epitope analysis of cTn-I with synthetic peptides using 40 apparently cTn-I specific McAbs. The study revealed the presence of six different epitopes on the cTn-I molecule several of which were highly specific for cTn-I and not found in sTn-I.

Developmental changes in the functional properties of cardiac muscle, particularly Ca^{2+} and pH sensitivity of the contractile system, have been related to Tn-I isoform switching. An embryonic to adult Tn-T switching has been clearly demonstrated in mammalian heart but the results concerning Tn-I are conflicting. Saggin et al. (1989), using a set of McAbs, investigated cTn-I changes in the developing heart. The data showed the presence of two antigenically and electrophoretically distinct isoforms during cardiac development. However, studies on the developmental expression of cTn-I in human heart by Bhavsar et al. (1991) and Hunkler et al. (1991) demonstrated that cTn-I in human fetal heart corresponded to the slow skeletal muscle isoform which lacks the N-terminal serine residues. During development between 20 weeks post-gestation and the 9 month post-natal period, the transition from slow sTn-I to adult cTn-I isoform occurs. However, the molecular mechanism of this transition is unknown although the developmental changes in the hormonal levels, particularly thyroid hormones, may be directly involved in the process (Diekman et al., 1990). The developmental regulation of Tn-I has been reported to

be at the mRNA level in rat hearts. With maturation, the quantity of slow skeletal mRNA decreases and the quantity of cTn-I mRNA increases (Murphy *et al.*, 1991). Recently, production of some McAbs have been reported which can apparently differentiate between the phosphorylated and dephosphorylated cTn-I isoforms (Cummins *et al.*, 1991).

ANTIBODIES TO cTn-I

In myocardial infarction, the pattern of degradation of Tn subunits together with preservation of Tn-T and tropomyosin was reported to be similar to the skeletal muscle in dystrophy. The anaerobic activation of proteolytic enzymes like cathepsin, reduction in tissue ATP, accumulation of lactate, elevation of pH and changes in the intracellular distribution of electrolytes, were all suggested as being responsible for this degradation (Katagiri *et al.*, 1981). These alterations in the intracardiac environment might directly affect the stability of troponin or act by indirectly activating proteolytic enzymes. Although the exact mechanism of this8 is unclear, the degradation in the early stages of myocardial ischaemic necrosis, together with reduction in myosin and a-actinin, is well documented in the literature. Cummins *et al.* (1979) proposed that antibodies specific to cTn-I could be used to assess cardiac damage; based on this assumption, Cummins *et al.* (1983) reported the development of a RIA for cTn-I to diagnose MI. The results of the study showed that the cTn-I release profile resembled that of CK-MB earlier on in the event of MI. The lower limit of assay sensitivity was 10ng/ml which was the upper limit of cTn-I serum level of normal patients. A later study of MI patient blood samples showed cTn-I to exhibit a biphasic release profile with serum levels elevations within 4–6h, reaching a mean peak levels in 112ng/ml (range from 20–550ng/ml) at 18h and remaining above normal for up to 6–8d post-infarction (Cummins et a*l.*, 1987). Moreover, cTn-I levels in patients with chest pain were found to be unchanged or slightly elevated while they were always normal in patients with chest pain of a non-cardiac origin.

The relatively early release of cTn-I could be the result of rapid myofibrillar breakdown but may also reflect the presence of free cTn-I within the sarcoplasm that has not yet been incorporated into the myofibrillar texture; evidence for a free cytoplasmic pool of myosin LC is in existence (Wikman-Coffelt *et al.*, 1973). cTn-I shows a prolonged biphasic release pattern which is probably the result of the loss of the cytoplasmic pool in the first instance. This given an early peak at around 18h post-infarction while a second increase in the serum level is observed at 48h which continues for 8–10d; the later possibly derived from myofibrillar degradation. Hence, rapid loss of cTn-i from the cytoplasmic pool superimposed on the prolonged myofibrillar degradation suggests that cTn-I can be useful for both immediate (6–8h) as well as long term (2–8d) diagnosis of MI when the other clinical diagnostic parameters have returned to normal. Similar prolonged release and biphasic patterns have also been reported for tropomyosin and myosin LC (Nagai et *al.*, 1979).

In order to investigate the possible use of cTn-I as a diagnostic agent, in more detail, Cummins e*t al.* (1987) established an experimental canine model of MI and reported an ELISA for cTn-I with enhanced specificity and sensitivity which used a polyclonal antiserum. A prominent feature to emerge from the study was the prolonged release profile of cTn-I of up to 200h post infarction. The cTn-I serum

levels rose to detectable levels within 4–6h and peaked between 10–16h post ligation. However, the infarct size was found to show a lower correlation with cTn-I values as compared with CK-MB serum concentrations, possibly due to the complex nature of the release pattern from the necrotic tissue.

Hunt *et al.* (1991) investigated the incidence of myocardial cell damage following angioplasty by monitoring CK-MB and cTn-I serum level changes as cardiac specific markers for cell injury. The results supported the idea of no cell damage after successful coronary angioplasty as was evident from results showing normal serum cTn-I levels. However, some discrepency was observed between the results when CK-MB was analysed by immunoassay and an immunoinhibition enzyme assay, due to the false positive results produced by the latter technique; up to 20% of the patient samples were shown to give small but significant elevations of CK-MB. The results supported the idea that cTn-I is the most reliable and specific biochemical marker for myocardial cell necrosis, available at present.

The superiority of cTn-I as a cardiac specific marker was also shown by the results of a comparative study involving measurement of cTn-I, CK and its isoenzymes, Tm, Mb and C-reactive protein in serum samples from marathon runners. While no rise in cTn-I and C-reactive protein was observed in any serum sample, the rest of the markers did register serum level elevations suggestive of cardiac muscle trauma after severe exercise. However, studies using myocardial 'hot spot' scintigraphy and Tl^{201} perfusion imaging suggest that serum CK-MB increases do not necessarily reflect cardiac injury but more likely derive from skeletal muscle damage (Cummins *et al.*, 1987).

Most of the work published regarding the use of cTn-I as a marker for myocyte damage has been carried out *in vitro*, using cTn-I specific polyclonal antisera. Recently, some reports have been published indicating the production of cTn-I specific McAbs. Russell *et al.* (1989) characterised anti-cTn-I McAbs raised against primate cTn-I, and some of the cell lines were reported to show species-cross reactivity as well as tissue non-specificity. Ladenson *et al.* (1990) raised a group of McAbs against human cTn-I and developed a two-site sandwich ELISA for cTn-I measurement in cases of suspected myocardial infarction; this had a minimum detection limit of 1.9μg cTn-I/l (Bodor *et al.*, 1990 & 1992). The results of their studies strongly emphasise the significance of cTn-I as a valuable cardiac-specific diagnostic marker for the assessment of myocardial infarction.

Loss of membrane integrity should allow the entry of radiolabelled antibodies into cells to bind to any remaining myofibrillar cTn-I; Cummins *et al.* (1989) took this point into account and reported the potential use of I^{131}-labelled Fab fragments of cardiac specific IgG for *in vivo* imaging, in a cardiac infarct model. Images of heart muscle slices showed areas of Fab uptake corresponding with areas of histochemically defined necrosis. Images using In^{111}-labelled Fab showed a seven-fold improvement over previous results (Cummins *et al.*, 1990).

A more detailed account of radioiodinated, affinity purified cTn-I antibody uptake in MI was published by Cummins *et al.* (1990). The study showed an inverse relationship between blood flow and antibody uptake in the necrotised myocardium. The study clearly showed that the injected radiolabelled antibodies to cTn-I did localise in the necrotic tissue and that the targeting was mainly due to direct antigen binding. An uptake of up to 0.003% of the injected dose per gram of the infarcted

tissue could be achieved; more important was the uptake ratio of the antibody in infarcted and normal myocardium which ranged from 1.399–3.94. The antibody uptake was reported to be 24 times greater towards the centre of the infarct compared to the peripheral tissue. It has been suggested that the use of Fab fragments in place of the whole antibody molecule and specific high affinity McAbs, in place of polyclonal antibodies, could also improve the uptake significantly. The unique degree of cardiac specificity and both short and long term availability suggest that cTn-I may be a valuable parameter both for in *vitro* serum detection and *in vivo* scintigraphic studies of MI.

In a recently concluded study involving a brain dead model in pigs, the authors have successfully employed radiolabelled anti-cTn-I McAbs to assess the damage to the cardiac tissue resulting from brain death. The results of the study are significant in relation to cardiac transplantation from brain dead donors. The McAbs used in this study were produced in our laboratory against bovine cTn-I and showed a high degree of specificity for the cardiac isoform of Tn-I. However, the antibodies did not show any species specificity; thus allowing their use in this study (Haider & Stimson, in press).

In summary, cTn-I possesses cardiac specificity, a simple tissue distribution pattern in atrial and ventricular myocardium, and a unique location in the myofibrillar organisation; this makes the protein more accessible for antibody targeting. The fact that cTn-I is only exposed to the extracellular environment after myocardial cell necrosis, should ensure that it is a reliable cardiac specific biochemical marker for both in vi*tro* diagnosis as well as in vivo imaging for myocyte necrosis. However, as a number of biochemical markers already exists, it will take extensive experimentation in a clinical setting before cTn-I is finally recognised as the valuable marker molecule we believe it to be.
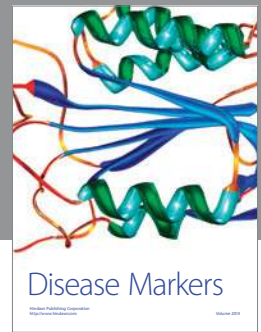
REFERENCES:

Alpert, J.S. (1989). The pathophysiology of acute myocardial infarction. *Cardiology,* **76**, 85–95.

Apple, F.S. (1989). Diagnostic use of CK-MM and CK-MB isoforms for detecting myocardial infarction. *Clin. Lab. Med.*, **9** (4), 643–53.

Berson, G., Samuel, J.L., and Swynghedauw, B. (1978). A comparative study of the cTn-I factor from mammalians. *Pflugers Arch.*, **374**, 277–83.

Bhattacharya, S., and Lahiri, A. (1991). Clinical role of In111⁻antimyosin imaging. *Eur. J. Nucl. Med.*, **18**, 889–95,

Bhavsar, P.K., Dhoot, G.K., Cumming, D.V.E., Butler-Browne, G.S., Yacoub, M.H., and Barton, P.J.R. (1991). Developmental expression of Tn-I isoforms in fetal human heart. *FEBS Letters*, **292** (1, 2), 5–8.

Bodor, G., Porter, S., and Landenson, J.H. (1990). Human cardiac troponin-I measurement in suspected MI with a double monoclonal antibody sandwich ELISA. *Clin. Chem.*, **36** (6), 1103.

Bodor, G. S., Porter, S., Landt, Y., and Ladenson, J.H. (1992). Development of monoclonal antibodies for an assay of cardiac troponin-I and preliminary results in suspected cases of myocardial infarction. *Clin. Chem.*, **38** (11), 2203–14.

Cox, P.H., Planting, A., Verweiji, L., Pillay, M., Stoter, G., and Schonfeld, D.H.W. (1988). Indium111⁻antimyosin for the detection of rhabdo- and leiomyosarcoma. *Eur. J. Nucl. Med.*, **14** (1), 50–52.

Cummins, B., Auckland, M.L., and Cummins, P. (1987). Cardiac specific troponin-I radio-immunoassay in diagnosis of acute myocardial infarction. *Am. Heart J.*, **113**, 1333–44.

Cummins, B., and Cummins, P. (1987). Immunoassay of cardiac specific isoforms of troponin-I in the diagnosis of heart muscle damage. *Biochem. Soc. Trans.*, **15** (6), 1060–61.

Cummins, B., and Cummins, P. (1987). Cardiac specific troponin-I release in experimental canine myocardial infarction: Development of a sensitive ELISA. *J. Mol. Cell. Cardiol.*, **19**, 999–1010.

Cummins, B., Russell, G.J., Chandler, S.T., Pears, D., and Cummins, P. (1989). In *vivo* imaging of damaged cardiac muscle using radiolabelled cardiac troponin-I antibodies. *J. Muscle Res. Cell Mot.*, **10** (2),162.

Cummins, B., Russell, G., Desai, B., Chandler, S., Pears, D., Lanchbury, E., and Cummins, P. (1990). Targetting of damaged cardiac muscle using In^{111} and I^{131}-labelled poly- and monoclonal antibodies to the heart specific isoform of troponin-I. *JMuscle Res.*, **11** (1), 86.

Cummins, B., Russell, G., Chandler, S.T., Pears, D.J., and Cummins, P. (1990). Uptake of the radioiodinated cardiac specific Tn-I antibodies in myocardial infarction. *Cardiovas. Res.*, **24**, 317–27.

Cummins, P., and Perry, S.V. (1973). The subunits and biological activity of polymorphic forms of tropomyosin. *Biochem. J.*, **133**, 765–77.

Cummins, P., and Perry, S.V. (1974). Chemical and immunochemical characteristics of tropomyosins from striated and smooth muscles. *Biochem. J.*, **141**, 43–49.

Cummins, P., and Perry, S.V. (1978). Troponin-I from human skeletal and cardiac muscles. *Biochem. J.*, **171**, 251–59.

Cummins, P., Littler, W.A., and McGurk, B. (1979). Radioimmunoassay of cardiac contractile proteins in the diagnosis of myocardial infarction. *J. Mol. Cell. Cardiol.*, **2** (Suppl. 2), 9.

Cummins, P., McGurk, B., and Littler, W.A. (1979). Possible diagnostic use of cardiac specific contractile proteins in assessing cardiac damage. *Clin. Sci.*, **56**, 30.

Cummins, P., McGurk, B., and Littler, W.A. (1981). Radioimmunoassay of human cardiac tropomyosin in acute myocardial infarction. *Clin. Sci.*, **60**, 251–59.

Cummins, P., and Auckland, M.L. (1983). Cardiac specific radioimmunoassay of troponin-I in the diagnosis of myocardial infarction. *Clin. Sci.*, **64**, 42.

Cummins, P., and Auckland, M.L.(1983). Detection of myocardial cell injury: Evaluation of a novel cardiac specific radioimmunoassay. *Eur. Heart J.*, **4** (Suppl. E), 78.

Cummins, P. (1987). Immunological techniques in the diagnosis and sizing of myocardial infarction. *Clin. Immunol. Allergy*, **1** (3), 503–529.

Cummins, P., Young, A., Auckland, M.L., Michie, C.A., Stone, P.C.W., and Shepstone, B.J. (1987). Comparison of serum cardiac specific troponin-I with creatine kinase, CK-MB isoenzymes, Tropomyosin, myoglobin and C-reactive protein release in marathorn runners: cardiac or skeletal muscle trauma? *Eur. J. Clin. Invest.*, **17**, 317–24.

Cummins, P., Russell, G.J., and Cummins, B. (1991). A monoclonal antibody that distinguishes phospho- and dephosphorylated forms of cardiac troponin-I. *Biochem. Soc. Trans.*, **19**, 161s.

Dhoot, G.K., Gell, P.G.H., and Perry, S.V. (1978). The localisation of the different forms of Tn-I in skeletal and cardiac muscle cells. *Exp. Cell Res.*, **117**, 357–70.

Dhoot, G.K., and Perry, S.V. (1979). Distribution of polymorphic forms of troponin components and tropomyosin in skeletal muscle. *Nature*, **278**, 714–18.

Doran, G.R. and Fleck, A. (1990). Limitations of serum CK-assay in the diagnosis of acute myocardial infarction. *Lancet*, **336**, 697 .

Ebashi, S. (1972). Separation of troponin into three components. *J. Biochem.*, **72**, 787–90.

Eyk, J.E.V., and Hodges, R.S.(1988). The biological importance of each amino acid residue of the Tn-I inhibitory sequence 104–115 in the interaction with troponin-C and tropomyosin. *J. Biol. Chem.*, **263** (4), 1726–32.

Galbraith, L.V., Leun, F.Y., Jablonsky, G., and Henderson, A.R. (1990). Time related changes in the diagnostic utility of total LDH, LDH-1 and two LDH-1 ratios in serum after myocardial infarction. *Clin. Chem.*, **36** (7),1317–22.

Gerhardt, W., Katus, H.A., Ravkilde, J., Hamm, C., Jorgensen, P.J., Peheim, E., Lungdahl, L., and Lofdahl, P. (1991). S-troponin-T in suspected myocardial injury compared with mass and catalytic concentrations of S-CK isoenzyme MB. *Clin. Chem.*, **37** (8), 1405–11.

Gorza, L. Mercardier, J.J., Schwartz, K., Thornell, L.E., Sartore, S., and Shiaffino, S. (1984). Myosin types in the human hearts: An immunofluorescence study of normal and hypertrophied atrial and ventricular myocardium. *Circ. Res.*, **54**, 694–702.

Grand, R.J.A., and Wilkinson, M. (1976). The amino acid sequence of rabbit cardiac troponin-I. *Biochem. J.*, **159**, 633–41.

Greaser, M.L., Yamaghuchi, M., Brekke, C., Potter, J., and Gregley, J. (1972). Troponin subunits and their interactions. *Cold Spring Harbour Sym. Quant. Biol.*, **37**, 235–44.

Gusev, N.B., Barskaya, N.V., Verin, A.D., Duzhenkova, I.V., Khuchua, Z.A. and Zheltova, A.O. (1983). Some properties of cardiac troponin-T structure. *Biochem. J.*, **213**, 123–129.

Haber, E., Katus, H.A., Hurrell, J.G., Matsueda, G.R., Ehrlich, P., Zurawsky, V.R.Jr., and Khaw, B.A. (1982). Detection and quantification of myocardial cell death: Application of monoclonal antibodies specific for cardiac myosin. *J. Mol. Cell. Cardiol.*, **14** (3), 139–46.

Haider, Kh.H. and Stimson, W.H. Monoclonal antibodies to cardiac troponin-I. *J. Clin. Lab. Immunol.*, in press.

Hoberg, E., Eisenhut, M., Hoffman, M., Schuler, G., Brachman, J., Aidonidis, I., Natchkamp, J., Kubler, W., and Katus, H.A. (1988). Monoclonal antibodies specific for human cardiac myosin: selection, characterisation, and experimental myocardial infarction imaging. *Eur. Heart J.*, **9**, 328–36.

Hoefnagel, C.A., Voute, P.A., deKraker, J., and Behrendt, H. (1987). Scintigraphic detection of rhabdomyosarcoma. *Lancet*, **I**, 921.

Humphrey, J.E., and Cummins, P. (1984). Regulatory proteins of the myocardium. Atrial and ventricular tropomyosin and Tn-I in the developing and adult bovine and human hearts. *J. Mol. Cell. Cardiol.*, **16**, 643–57.

Hunkler, N.M., Kullman, J., and Murphy, A.M. (1991). Troponin-I isoform expression in human heart. *Circ. Res.*, **69** (5), 1409–1414.

Hunt, A.C., Chow, S.L., Shiu, M.F., Chilton, D.C., Cummins, B., and Cummins, P. (1991). Release of creatine kinase-MB and cardiac specific troponin-I following percutaneous transluminal coronary angioplasty. *Eur. Heart J.*, **12**, 690–94.

Jensen, A.E., Reikvan, A., and Asberg, A. (1990). Diagnostic efficiency of lactate dehydrogenase isoenzymes in serum after acute myocardial infarction. *Scand. J. Clin. Lab. Ivest.*, **50**, 285–89.

Jin, J-P., Malik, M.L., and Lin, J.J-C. (1990). Monoclonal antibodies against cardiac myosin heavy chains. *Hybridoma*, **9** (6), 597–608.

Johnson, L.L. (1992). Monoclonal Fab antimyosin in cardiac imging. *Clin. Cardiol.*, **15**, 145–153.

Katagiri, T., Kobayashi, Y., Sasai, Y., Toba, K., and Niitani, H. (1981). Alterations in cardiac troponin subunits in myocardial infarction. *Jap. Heart J.*, **22**, 653–64.

Katus, H.A., Remppis, A., Looser, S., Hallermeier, K., Scheffold, T., and Kubler, W. (1989). Enzyme linked immunosorbent assay of cardiac troponin-T for the detection of acute myocardial infarction in patients. *J. Mol. Cell. Cardiol.*, **21**, 1349–53.

Katus, H.A., Remppis, A., Neumann, F.J., Scheffold, T., Diederich, K.W., Vinar, G., Noe, A., Matern, G., and Kubler, W. (1991). Diagnostic efficiency of troponin-T measurements in acute myocardial infarction. *Circulation*, **83** (3), 902–12.

Katus, H.A., Remppis, A., Scheffold, T., Diederich, K.W., and Kubler, W. (1991). Intracellular compartmentation of cardiac troponin-T and its release kinetics in patients with reperfused and non-reperfused myocardial infarction. *Am. J. Cardiol.*, **67**, 1360–67.

Katus, H.A., Looser, S., Hallermayer, K., Remppis, A., Scheffold, T., Borgya, A., Essig, U., and Geub, U. (1992). Development and in vitro characterisation of a new immunoassay of cardiac troponin-T. *Clin. Chem.*, **38** (3), 386–93.

Khaw, B.A., Beller, G.A., Haber, E., and Smith, T.W. (1976). Localisation of cardiac specific antibody in myocardial infarction. *J. Clin. Invest.*, **58**, 439-46.

Khaw, B.A., Mattis, J.A., Mellincoff, G., Strauss, H.W., Gold, H.K., and Haber, E. (1984). Monoclonal antibody to cardiac myosin: Imaging of experimental myocardial infarction. *Hybridoma*, **3** (1), 11–23.

Khaw, B.A., Gold, H.K., Yasuda, T., Fallon, J.T., Leinbach, R.C., Barlai-Kovaich, M., Strauss, H.W., and Haber,E. Imaging with antibodies. In: Fozzard, H.A., Haber, E., Jennings, R.B., Katz, A.M., and Morgan, H.E. (Eds.), *The Heart and Cardiovascular System*, Vol.**I**, 453–68. The Raven Press, New York, 1989.

Kohler, G., and Milstein, C. (1975). Derivation of specific antibody producing tissue culture and tumour lines by cell fusion. *Eur. J. Immunol.*, **6**, 511–19.

Landenson, J.H., Porter, S., Landt, Y., and Bodor, G. (1990). Development and characterisation of monoclonal antibodies specific to troponin-I. *Clin. Chem.*, **36** (6), 1019, .

Laure, C., Defacque-Lacquement, H., Calzolari, C., Nguyen, D.L., and Pau, B. (1992). New monoclonal antibodies as probes for human cardiac Tn-I: Epitopic analysis with synthetic peptides. *Mol. Immunol.*, **29** (2), 271–78.

Lee, T.H., and Goldman, L. (1986). Serum enzyme assays in the diagnosis of acute myocardial infarction. *Ann. Intern. Med.*, **105**, 221–33.

Lee, T.H., Weisberg, M.C., Cook, M.F., Daly, K., Brand, D.A. and Goldman, L. (1987). Evaluation of CK and CK-MB for diagnosis of myocardial infarction. *Arch. Intern. Med.*, **147**, 115–21.

Leger, J.O.C., Bouvagnet, P., Pau, B., Roncucci, R., and Leger, J.J. (1985). Levels of ventricular myosin fragments in human sera after myocardial infarction, determined with monoclonal antibodies to myosin heavy chains. *Eur. J. Clin. Invest.*, **15**, 422–29.

Leger, J., Chenvalier, J., Laure, C., Gautier, P., Planchenault, J., Aumaitre, E., Messner, P., Paech, P., Saccavini, J.C., Pau, B., and Leger, J.J. (199)1. Imaging of myocardial infarction in dogs and humans using monoclonal antibodies specific for human myosin heavy chains. *Am. Coll. Cardiol.*, **18** (2), 473–84.

Loughlin, J.F., Krijnen, P.M.W., Jablonsky, G., Leung, F.Y., and Henderson, A.R. (1988). Diagnostic efficiency of four LD isoenzyme-1 ratios in serum after MI. *Clin. Chem.*, **34** (10), 1960–65.

Mair, J., Artner-Dworzak, E., Lecheitner, P., Smidt, J., Wagner, I., Dienstl, F., and Puschendorf, B. (1991). Cardiac troponin-T in diagnosis of myocardial infarction. *Clin. Chem.*, **37** (6), 845–52.

Mittamann, K., Jaquet, K., and Heilmeyer Jr.,L.M.G. (1990). A common motif of two adjacent phosphoserines in bovine, rabbit and human cardiac troponin-I. *FEBS Letters*, **273** (1, 2), 41–45.

Murphy, A.M., Jones, L., Smis, H.F., and Strauss, A.W. (1991). The molecular cloning of rat cTn-I and analysis of troponin-I isoform expression in developing rat heart. *Biochem.*, **30**, 707–12.

Nagai, R., Ueda, S., and Yazaki, Y. (1979). RIA of cardiac myosin LC-II in the serum following experimental myocardial infarction. *Biochem. Biophys. Res. Commun.*, **86**, 683.

Pappas, N.J. 1989. Theoretical aspects of enzymes in diagnosis. *Clin. Lab. Med.*, **9** (4) 595–625.

Nakata, T., Gohto M., Noto, T., Tanaka, S., Shoji, T., Kubota, M., Tsuda, T., and Iimura, O. (1992). Quantitation of AMA uptake and infarct size at various stages of MI. *Int. J. Cardiol.*, **34**, 85–95.

Planting, A., Vreweij, J., Cox, P., Pillay, M., and Stoter, G. (1990). Radioimmunodetection in rhabdo- and leiomyosarcoma with In111-AMA complex. *Cancer Res.*, **50**, 955s–57s.

Potter, J.D., and Johnson, J.D. Troponin. In: Cheung, W. (Ed.), *Calcium and Cell Function*, Vol.**II**, 145–173, Academic Press, New York, 1982.

Prager, N.A., Suzuki, T., Jaffe, A.S., Sobel, B.E., and Abendschein, D.R. (1992). Nature and time-course of generation of iosforms of CK-MB fractions, *in vivo. J. Am. Coll. Cardiol.*, **20** (2), 414–19.

Puleo, P.R. and Roberts, R. (1988). An update on cardiac enzymes. *Cardiol. Clin.*, **6** (1), 97–109.

Puleo, P.R., Guadagno, P.A., Roberts, R., Schell, M.V., Marion, A.J., Churchill, D. and Perryman, M.B. (1990). Early diagnosis of acute myocardial infarction based on assay of creatine kinase-MB. *Circulation*, **82** (3), 759–64.

Russell, G., Cummins, B., and Cummins, P. (1989). Characterisation of monoclonal antibody to primate cardiac troponin-I. *J. Muscle Res. Cell Motility*, **10** (2), 155.

Saggin, L., Gorza, L., Ausoni, S., and Shiaffino, S. (1989). Troponin-I isoform switching in developing heart. *J. Biol. Chem.*, **264** (7), 16299–302.

Serrano, S., Chueca, P., Carasco, E., Olaz, F., and Zabalegui, A. (1990). Predictive value of myoglobin in early diagnosis of acute myocardial infarction. *Ann. Emerg. Med.*, **19** (8), 953.

Smith, D.A., Leung, F.Y., Jablonsky, G., and Henderson, A. (1987). Determination by radioimmunoassay, of the mass of LDH-1 in human serum and its rate of removal from serum after acute myocardial infarction. *Clin. Chem.*, **33**, 1863–68.

Solaro, R.J., Moir, A.J.G., and Perry, S.V. (1976). Phosphorylation of troponin-I and the ionotropic effect of adrenaline in the perfused rabbit heart. *Nature*, **262**, 615–17.

Swiderek, K., Jaquet, K., Meyer, H.E., and Heilmeyer. M.G.(1988). Cardiac troponin-I from bovine heart contains two adjacent phosphoserines. *Eur. J. Biochem.*, **176**, 335–42.

Sylven, J.R., Kallner, A., and Larsen, F.F. (1987). Release characteristics of enzymes used in diagnosis of myocardial infarction. *Upsala J. Med. Sci.*, **92**, 293–300.

Tsukui, R., and Ebashi, S. (1973). Cardiac troponin. *J. Biochem.*, **73**, 1119–21.

Vallins, W.J., Brand, N.J., Dabhade, N., Bulter-Brown, G., Yacoub, M.H., and Barton, P.J.R. (1990). Molecular cloning of human cTn-I using polymerase chain reaction. *FEBS Letters*, **270** (1,2), 57–61.

Vannanen, H.K., Syrajala, H., Rahkila, P., Vuori, J., Melamies, L.M., Myllyla, V., and Takala, E.S. (1990). Serum carbonic anhydrase-III and myoglobin concentrations in acute myocardial infarction. *Clin. Chem.*, **36** (4), 635–38.

Verstraete, M. (1992). Advances in thrombolytic therapy. *Cardiaovascular Drugs and Therapy*, **6** (2), 111–24.

Wikamn-Coffelt, J., Zelis, R., Fenner, C., and Mason, D.T. (1973). Studies on the synthesis and degradation of light and heavy chains of cardiac myosin. *J. Biol. Chem.*, **248**, 5206.

Willerson, J.T. (1989). Clinical diagnosis of acute myocardial infarction. *Hospital Practice*, **24** (2), 65–77.

Wolf, P.L. (1989). Lactate deydrogenase isoenzymes in myocardial disease. *Clin. in Lab. Med.*, **9** (4), 655–65.

The Scientific
World Journal

Gastroenterology
Research and Practice

MEDIATORS
of
INFLAMMATION

Journal of
Diabetes Research

Disease Markers

Journal of
Immunology Research

International Journal of
Endocrinology

PPAR Research

Hindawi

Submit your manuscripts at
http://www.hindawi.com

BioMed
Research International

Journal of
Ophthalmology

Stem Cells
International

eCAM

Evidence-Based
Complementary and
Alternative Medicine

Journal of
Obesity

Journal of
Oncology

Computational and
Mathematical Methods
in Medicine

Behavioural
Neurology

Parkinson's
Disease

AIDS
Research and Treatment

Oxidative Medicine and
Cellular Longevity