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(54) Title: USE OF CREATINE KINASE AND ITS ISOZYME CK-MB AND HUMAN VENTRICULAR MYOSIN LIGHT CHAIN 1 IN THE DIAGNOSIS OF HEART FAILURE

(57) Abstract

In accordance with an aspect of the present invention methods for the early detection of a myocardial infarction and for the differentiation between a myocardial infarction and another cardiac event are provided. These methods comprise the sequential or simultaneous testing for creatine kinase and for human ventricular myosin light chain 1 in a sample selected from the group consisting of blood, serum and plasma.

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USE OF CREATINE KINASE AND ITS ISOZYME CK-MB AND HUMAN VENTRICULAR MYOSIN LIGHT CHAIN 1 IN THE DIAGNOSIS OF HEART FAILURE

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FIELD OF THE INVENTION

This invention relates to a method which simultaneously assesses the presence or absence of two independent markers of myocardial damage to improve the
10   accuracy of the early diagnosis of a myocardial infarction and for distinguishing between cardiac ischemic disease, such as angina, and myocardial infarction.

BACKGROUND OF THE INVENTION

15   Myosin is the major protein constituent of cardiac muscle and it consists of two myosin heavy chains and four myosin light chains. Two classes of light chains have been characterized in cardiac myosin: cardiac myosin light chain 1 with a molecular weight of 27,000 daltons
20   and myosin light chain 2 with a molecular weight of 20,000 daltons.

The light chain of myosin is known to be released from cardiac muscle following myocardial infarction and may remain elevated in serum for several days [Khaw et
25   al, CIRCULATION 58: 1130, (1978); Trahern et al., AM. J. CARDIOL 41: 641, (1978)]. Katus et al. [JACC, 2(3): 487] recently reported a correlation between the presence of myosin light chains and signs of ischaemia in the electrocardiogram and extent of coronary artery
30   narrowing. It is also known that myosin light chain is released during ischaemia from other causes such as angina. An elevated plasma light chain concentration has also been observed in patients with congestive cardiomyopathy associated with an inflammatory infiltrate
35   [Haver, E. J. MOL. & CELL CARDIOL., 17(Supplement 2): 53, (1985)].

There are a number of reports describing diagnostic methods for muscle diseases utilizing polyclonal or monoclonal antibodies directed at myosin light chains

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[Trahern et al. supra; Khaw et al. supra; Nagai et al.,
BIOCHEM. BIOPHYS. RES. COMMUN., 86: 683, (1979); Katus et
al., AM. J. CARDIOL., 54: 964, (1984), Katus et al., MOL.
IMMUNO., 19: 451, (1982); and Masahito et al. European
5   Patent Application D205 177 A2). These diagnostic
methods, however, suffer from a number of disadvantages
such as cross-reactivity between cardiac myosin light
chains as well as cross-reactivity with myosin light
chains from other tissues (e.g. skeletal muscle) or other
10   species.

It is known that cardiac myosin light chains have a
distinct primary amino acid sequence which is different
from the equivalent chains from skeletal or smooth muscle
myosin. Patent applications for the DNA sequence of
15   human ventricular myosin light chains 1 (HVMLC1) and 2
(HVMLC2) are pending in the United States [U.S. S.N.
241,672 (HVMLC2); U.S. S.N. 160,892 (HVMLC1)] and
elsewhere. The identification of DNA sequences that code
for these two human myosin light chains enables the
20   preparation of highly specific monoclonal and polyclonal
antibodies useful in diagnostic methods and for various
types of therapy.

It is known that myocardial light chain (MLC)
release follows a byphasic pattern [Hirayama, A. et al,
25   JACC, 13 (2): 95A, (1989) "Byphasic Release of Cardiac
Myosin Light Chains in Patients with Successful
Thrombolysis in Acute Myocardial Infarction"]. According
to Hirayama MLC is released early and rapidly approaches
its peak concentration at 18 - 24 hours after the
30   infarct. A second, subsequent release of MLC reaches its
peak concentration at 3 - 5 days after the onset of the
infarct.

Current serum assays depend on the leakage of
intra-cellular enzymes, such as creatine kinase, or other
35   proteins, from the damaged or necrotic cardiac cells
which result from the myocardial infarction.
Differentiation between myocardial ischaemia and unstable

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angina or the detection of myocardial injury is often
difficult. It is known that the release kinetics of
cardiac myosin light chains differ from the release
kinetics of creatine kinase and its isozymes around the
5   time of acute myocardial infarction (AMI) [Katus, H.A.,
et al, AM. J. CARDIOL., 54: 964-970, (1984) "Diagnosis of
Acute Myocardial Infarction by Detection of Circulating
Cardiac Myosin Light Chains"].
        The method disclosed by the present inventors is a
10 method which surprisingly incorporates two assays, one of
which is known. The second assay is novel in the extent
of its specificity and sensitivity. The first, known,
assay is the creatine kinase test which is the recognized
standard test for the detection of a heart attack. This
15 is the standard assay used by physicians today for the
detection of heart attacks. CK, and its isozymes CK-MB
and CK-MM are proteins that are known to leak out from
the heart during a heart attack. Only the isoenzyme
CK-MB shows unique specificity for the cardiac tissue in
20 that it is not present in significant concentrations in
extracardiac tissues. Unfortunately these proteins are
not first detectable at meaningfully increased levels
until 8 - 10 hours after the heart attack has occurred.
Furthermore, these proteins are not detectable at peak
25 concentrations until around 12 hours and, in some
instances longer than 12 hours. It is currently standard
practice to administer creatine kinase tests at three
different times on a series of samples taken at the
following times: the time of admission, 12 hours after
30 admission, and 24 hours after admission before a final
diagnosis is rendered. At the present time the CK assay
is the best assay available for the detection and
confirmation of the occurrence of a heart attack. Using
this test alone a reliable positive diagnosis of a heart
35 attack cannot be made without waiting for the full 24
hour period to pass.

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There are other problems inherent in the known
standard creatine kinase test assays aside from the
delay, described above, in getting results which are
diagnostically reliable. The most relevant problem, as
5   related to the instant invention, is that the creatine
kinase assay is not specific to the heart. Furthermore
the test is useful only for the detection of a heart
attack and is not diagnostic for other cardiac ischaemic
events, such as angina. At the present time there are no
10   known diagnostic assays which distinguish detection of
heart disease from a heart attack.

DEFINITIONS

The terms creatine kinase (CK) and creatine
phosphokinase are used interchangeably in this
15   application. Both of these terms are intended to include
the known isozymes of this enzyme. One skilled in the
art would know when one or all of the creatine kinase
enzymes would be appropriate.

HVLC1 and HVMLC1 are used interchangeably as
20   abbreviations for Human Ventricular Myosin Light Chain 2.
Similarly HVLC2 and HVMLC2 are used interchangeably as
abbreviations for Human Ventricular Myosin Light Chain 2.

The terms heart attack, myocardial infarct and
myocardial infarction are used interchangeably.
25   The terms myocardial ischaemic event and cardiac
ischaemic event are used interchangeably.

Myocardial infarct is an example of one type of a
myocardia ischaemic event. Myocardial ischaemic events
other than a myocardial infarct include angina and other
30   types of heart failure.

SUMMARY OF THE INVENTION

In accordance with an aspect of the present
invention methods for the early detection of a myocardial
infarction and for the differentiation between a
35   myocardial infarction and another cardiac event are
provided. These methods comprise the sequential or
simultaneous testing for creatine kinase and for myosin

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light chain in a sample selected from a tissue sample, serum sample, or other proteins containing sample.

In accordance with another aspect of the invention a method for the early detection of a myocardial infarction
5   is provided. This method comprises:

(a)   testing for creatine kinase in protein-containing sample;

(b)   testing for myosin light chain in said protein-containing sample; and

10   (c)   determining the presence or absence of creatine kinase and of myosin light chain in said sample.

In accordance with another aspect of the invention a method for differentiating between a myocardial infarction and an cardiac ischaemic event which is not a
15   myocardial infarction is provided. This method comprises:

(a)   testing for creatine kinase in a protein-containing sample;

(b)   testing for myosin light chain in said protein-
20   containing sample; and

(c)   determining the presence or absence of creatine kinase and of myosin light chain in said sample.

In accordance with one aspect of this invention a method is described for the detection of myosin light
25   chains in tissues, serum or other suitable sample of patients suspected of suffering from heart disease, heart failure or myocardial infarct.

In accordance with another aspect of the present invention a method is described for the simultaneous
30   assessment of the presence or absence of two independent markers of myocardial damage, namely creatine kinase (CK) and its isozymes and human ventricular myosin light chain (HVMLC) to improve the accuracy of the detection and diagnosis of a myocardial infarction and, for
35   distinguishing between cardiac ischemic disease, such as angina, and myocardial infarction.

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In accordance with another aspect of the present invention an assay using antibodies prepared from known amino acid sequences of myosin light chain 1 is presented.

In accordance with a further aspect of the invention a highly specific assay is presented for the early detection of a myocardial infarct.

In accordance with another aspect of the invention an assay is presented for the differentiation and distinction between heart disease and a myocardial infarct.

In accordance with yet another aspect of the present invention diagnostic kits are described for the assays taught herein.

BRIEF DESCRIPTION OF THE DRAWINGS

Other and further advantages of the invention will be apparent to those skilled in the art from the following detailed description thereof, taken in conjunction with the accompanying drawings, in which:

Figure 1 is a graphic illustration of the mean serum levels of SGOT, CPK and HVMLC1 (HVLC-1) over time (hours) in 66 patients clinically diagnosed as suffering from an acute myocardial infarct (AMI);

Figure 2 is a graphic illustration of the mean serum levels of CPK and HVMLC1 (HVLC-1) over time (hours) in 86 patients identified as suffering from cardiac chest pain;

Figure 3 is a graphic illustration of the percent positive assays for elevated serum levels of CPK and HVMLC1 (HVLC-1) over time in 66 patients identified as having had an AMI;

Figure 4 is a graphic illustration of the percent positive assays for elevated serum levels of CPK and HVMLCI (HVLC-1) over time in 86 patients identified as having had cardiac chest pain; the cut-off point for a positive result is 0.75 ng;

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Figure 5 is the same as Figure 4 however a greater assay sensitivity is illustrated in Figure 5 in which the cut- off point for a positive result is 0.50 ng.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

5   It is appreciated that the pain associated with angina pectoris which is due to ischaemia of the heart muscle, usually caused by coronary disease, is difficult to differentiate clinically from the pains associated with a myocardial infarct. It is shown in one aspect of

10   the present invention that myosin light chain 1 and myosin light chain 2 are released as early as one hour after an ischaemic event such as acute myocardial infarct (AMI) or angina. In the instance of an AMI these myosin light chains continue to be released and are detectable

15   in the blood stream of the patient for as long as 8 days. In the case of angina the continued release of the myosin light chains would not likely occur for such a length of time after the ischaemic event. CK, and its isozymes, is released only in the instance of an AMI. The kinetics of

20   the release of these enzymes is seen in Figures 1 through 5.

In contrast the known creatine kinase assays do not reach the same level of release as the myosin light chains until 10 - 12 hours after the ischemic event,

25   peaking at 24 hours. CK-MB levels peak rapidly, level off for 2 - 3 days after which they drop sharply. Creatine kinase proteins are not released unless there has been a myocardial infarct and would not be detected in other instances of heart disease such as angina

30   pectoris.

The myosin light chain assay, of the present invention and the known creatine kinase assay are combined in the present invention to create a new assay for the detection of myocardial infarct. This

35   combination assay simultaneously assesses the presence or absence of two independent markers of myocardial damage, namely CK (and its isozymes) and HVMLC. Such an assay is

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not presently available and is particularly needed and
useful for the detection of the early stages of a heart
attack when creatine kinase protein levels have not yet
risen to reliable diagnostic levels.

5   The novel combination assay of the present invention
permits the early detection and recognition of a
myocardial infarct thereby allowing the patient to
receive appropriate treatment as quickly as possible.
The combination assay shows a cumulative sensitivity

10   approaching 100% accuracy in identifying whether or not
an MI has occurred. The occurrence of a positive
creatine kinase test and a positive HVMLC1 between 4 and
12 hours after the onset of a myocardial infarction
permits an earlier and more accurate diagnosis than

15   either test alone. Such a reliable diagnostic test, for
this early phase of a heart attack, is not presently
known.

        While this combination of using the two assays is
particularly useful in recognizing the early stages of a

20   MI, further advantages are also appreciated.

        One such surprising advantage is that by combining
the two tests a novel method for differentiating between
a heart attack and other ischaemic events causing cardiac
pain, such as angina, is also provided. As seen in the

25   data presented in example 1 the HVMLC1 assay of the
preferred embodiment has a sensitivity of 97.6% and a
specificity of 90% for myocardial ischaemia caused by any
event, e.g. angina and myocardial infarctions. HVMLC1 is
only 56% specific for acute myocardial infarctions

30   because it is also released from the myocardium with
reversible ischemic injury, e.g. angina. It has been
shown that the positive biochemical diagnosis of a
myocardial infarction with CK-MB testing is made with a
sensitivity/accuracy of 77% by 12 hours and 96% within 24

35   hours [Lee and Goldman, Ann. Internal. Med., 106:
181-186, (1987)]. In comparison, the data presented in
Example 1 illustrates that the combination of a positive

CK-MM or CK-MB and a positive HVMLC1 is 75%
sensitive/accurate by 4 hours and 100% sensitive/accurate
by 8 and 12 hours after the onset of a myocardial
infarction in the positive identification of a MI. Based

5   on these data the advantage of the combined assays to
form a new methodology for the detection of myocardial
infarction is clear. Surprisingly this data also
illustrates additional advantage of the present
invention. Namely, that the assay is diagnostic of heart

10   disease, other than myocardial infarct, as well 12 to 24
hours a positive HVMLC1 assay and a negative CK, CK-MM or
CK-MB assay (i.e. the combined assay) is indicative of
heart disease other than a myocardial infarction.
Support for these findings can be found in Figures 1

15   through 5.
        Figure 1 illustrates the mean serum levels of the
enzymes SGOT, CPK and of the myosin light chain HVMLC1
(referred to in the figure as HVLC-1) for patients
clinically depicted as having AMI. Time from the initial

20   chest pain is indicated on the x-axis (in hours). The
mean serum level measured in the patient samples was
divided by the normal serum level expected for that
particular protein to generate the factors indicated on
the y-axis of the Figure. For example, if the mean serum

25   level for the protein was twice as high as the expected
normal serum level of that protein then the datum was
plotted on the y-axis at a factor of 2.
If the sample level equalled the expected norm then the
corresponding factor would be 1 and so on. Normal serum

30   levels were always selected from the upper limit of the
normal range.
        It can be seen from the data plotted in Figure 1
that the HVLC-1 curve is consistently elevated from the
onset of the initial chest pain. Two distinct peaks are

35   seen in the HVLC-1 serum levels, an early peak at 8 to 10
hours and a later peak at 2 to 5 days. In contrast

10

the CPK enzyme levels remain at normal levels until six
hours and do not peak until 12 to 24 hours after the
onset of initial chest pain. The surprising elevation of
the HVLC-1 levels at the early stages of the myocardial
5   infarction permits a clear diagnosis of AMI as early as 7
or 8 hours after the initial onset of pain. This early
detection, with nearly 100% certainty, of an AMI is not
presently available through use of the standard CK enzyme
assay alone.
10   The early and late elevated HVLC-1 levels provide
other useful clinical information about the patient's
health. Any elevation of HVLC-1 (and, as one skilled in
the art would appreciate, presumably HVMLC2) is known to
be diagnostic of an ischaemic event be it caused by a
15   heart attack, angina or some other cardiac illness.
Release of these myosin light chains is known to be
associated with a cardiac ischaemic event. However,
until the combined assay of the present invention, one
has been able to appreciate the kinetics of the early
20   release of this protein at the time of the ischaemic
event since no known assay has been sufficiently
sensitive and specific to reliably make such
determinations. The use of known sequences of HVMLC1 and
HVMLC2 now make this possible. The relevant DNA
25   sequences and, by implication, amino acid sequences are
described in the following list of copending patent
applications: U.S. S.N. 160,892 and its c-i-p, U.S. S.N.
239.899, U.S. S.N. 241,672, CA S.N. 576,230 and EP S.N.
88312271.5.
30   Specific antibodies raised against one aspect of the
present invention is its surprising specificity and
sensitivity for human ventricular myosin light chains in
the preferred embodiments described in Examples 1 and 2.
The surprising sensitivity and specificity of the present
35   assay likely results from the use of the known DNA
sequences which can be used to synthetically prepare the
HVMLC1 and HVMLC2 protein molecules. These, in turn, are

used as antigens, to generate the specific antibodies
used in the immunoassay measurements. The use of these
antibodies of unique specificity significantly reduces,
if not eliminates, the high incidence of cross-

5   reactivity one would otherwise expect. This, in turn,
now makes it possible to assay and observe the enzyme
kinetics associated with ischaemic events. This was not
previously possible.

The data presented in Figures 1 and 2 illustrates

10   how a test combining the creatine kinase assay and the
HVMLC assay successfully differentiates between pain
associated with AMI and pain resulting from unstable
angina or subendocardial MI. In Figure 1 data is
presented for those patients who were monitored prior to

15   the onset of the AMI. At time less than zero hours the
angina which the patient is experiencing results in
elevated HVLC-1 levels but CPK levels remain unchanged.
If by 8-10 hours no elevation in CPK occurs then it is
likely that no heart attack has occurred (it is

20   recognized that occasional extremes in variability can
occur between individuals). If, on the other hand, by 8
hours elevated CPK is detected then it can be said with
nearly 100% certainty that the patient has suffered from
an AMI. The peaks of the mean serum levels for CK are

25   noticeably reduced when the non-AMI patients are averaged
into the sample (figure 2). This is because no CK
response is observed in patients suffering from cardiac
chest pain not related to an AMI. As expected, a similar
reduction in mean serum level of HVLC-1 is not observed.

30   Figures 3, 4 and 5 graphically illustrate the
sensitivity of the HVLC-1 assay. The percentage of
patients proven to have had a heart attack who also had
positive results with the relevant test are presented on
a scale of 0% to 100% along the y-axis. In other words

35   the number of true positives as ascertained by the test
data is divided by the number of patients known to have
had the disease and multiplied by 100 to get the "percent

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positive". In Figures 3 and 4 true positives were
assessed as values above 0.75 ng and in Figure 5 the
cut-off value was slightly lower at 0.50 ng. Selection
of the cut-off value will vary depending on the minimum
5   sensitivity achieved during that test run. One skilled
in the art would know how to make this assessment.

In the present invention a method is described to
improve the accuracy of the early diagnosis
of a myocardial infarction by testing simultaneously for
10 two independent markers of myocardial damage. In one
preferred embodiment this method comprises the use of
detecting simultaneously the presence of CK-MM or CK-MB
using known creatine kinase assay techniques and the
presence of human ventricular light chain-1 (HVMLC1)
15 using solid-phase enzyme immunoassays. One skilled in
the art would know other known suitable immunoassay
technologies which could also be used.

One advantage of enzyme immunoassays, such as the
one disclosed for the detection of HVMLC1 in the
20 preferred embodiment of this invention (Example 2), is
the potential use of colour production for the ready
detection of a positive assay. Using different enzyme
substrate combinations the presence or absence of a
positive CK assay and/or a positive HVMLC1 assay will be
25 readily apparent based on the presence or absence of the
colours produced. For example, it is known that the
alkaline phosphatase enzyme, in conjunction with the
para-nitrophenyl substrate, will produce a reaction
product having a yellow colour. In contrast, it is known
30 that substrates used in conjunction with the beta-
galactosidase enzyme will produce reaction products
having a blue colour. The use of distinctive colour
reactions allow the combination of these two assays to
distinguish between the CK assay and the HVMLC1 assay
35 thereby facilitating quick and ready detection of the
presence or absence of myosin light chains and CK-MB or
CK-MM in the patient's blood by merely assessing colour

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change. In one preferred embodiment of the present
invention the two assays would be performed on a
nitrocellulose membrane such that when both of the assays
yield a positive result a green colour (yellow + blue)

5   occurs since both assays can be performed on the same
nitrocellular dish.

    In one preferred embodiment the presence of CK or
CK-MB is detected in one location on a suitable solid
phase membrane such as nitrocellulose and the presence

10 of human ventricular light chain-1 (HVMLC1) is assayed
at another location on the same membrane with the two
assays overlapping in one common area [SHOULD THIS BE
ILLUSTRATED?]. The two separate solid-phase enzyme
immunoassays could be processed simultaneously as one

15 single combined diagnostic test. The use of other
suitable solid-phase supports for such immunoassays would
be known by one skilled in the art.

    The capture antibody for the CK assay could be
either a polyclonal or a monoclonal antibody which

20 recognizes CK-MM and/or CK-MB. These capture antibodies
could be bound, either covalently or non-covalently, to
the solid phase. In similar fashion, the HVMLC1 assay
could utilize sequence specific polyclonal or monoclonal
antibodies which recognize HVMLC1. These too could be

25 either covalently or non-covalently bound to the solid
phase as capture antibody for HVMLC1. Antibodies raised
against either the natural, native proteins or the
synthetic "DNA sequenced" proteins could be used.

    When a blood, plasma or serum sample is brought

30 in contact with the solid phase support and the capture
antibodies bound thereto specific antigens, if these
antigens are present, in the blood, plasma or serum of
the patient being tested will bind to the capture
antibodies. Antigen specific polyclonal or monoclonal

35 detection antibodies bearing suitable enzyme labels, or
suitable control detection antibodies, are then brought
in contact with the solid phase support. Detection

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antibody specific for CK-MM or CK-MB will bind to the
CK-MM or CK-MB antigen if it is present on the solid
phase support. Similarly, the detection antibodies
specific for HVMLC1 would bind to any HVMLC1 antigen
5   which is bound to the capture antibodies.

Unbound detection antibodies are removed with a
washing solution. A colour development solution
containing the appropriate substrate for either or both
enzymes bound to the detection antibodies is then added
10   to the membranes once the detection antibodies have bound
to their appropriate antigens, thereby indicating the
positive presence of these antigens in the patient's
plasma or serum or blood when the substrate is converted
to its coloured end product after reacting with the bound
15   enzyme. Where no such antigens are present then no
detection antibodies will be bound and no enzyme will be
present to react with the substrate in the colour
development solution. The amount of colour formed will
be directly proportional to the amount of antibody-
20   enzyme that is bound to CK-MM, CK-MB or to HVMLC1 on the
membrane thereby indicating the proportion of these
antigens in the sample. If the test is set up such that
substrate reaction products having different colours are
used for the different antigens then ready detection of
25   positive results will be possible. When performed on
nitrocellulose the tests can be overlapped such that a
third colour is produced when both test results are
positive.

Other enzyme immunoassays (enzyme based, radioactive
30   based, fluorescence based, or otherwise) could be used
for detecting the presence or absence of relevant
antigens and these would be known to one skilled in the
art. One skilled in the art would also know that human
ventricular myosin light chain 2 could be used in a
35   similar manner as described herein for HVMLC1. One
skilled in the art would also appreciate that samples

other than blood, serum or plasma could also be tested as described herein.

Further details of the preferred embodiments of the invention will be understood from the following examples
5   which are understood to be non-limiting with respect to the scope of the invention.
EXAMPLE 1 - Combination Testing for Detection and Diagnosis of Heart Attack and Other Cardiac Pain Using CK Assay and HVMLCl Assay
10   116 patients were assessed upon admission to emergency in hospital. All patients were tested using solid-phase enzyme immunoassays (see Example 2) for the presence of CK or CK-MB and for the presence of myosin light chain 1 (HVMLCl) in the serum of patient samples.
15   Patients tested were also assessed based on clinical presentation and were broken down into three groups:

    1.   66 patients judged to have acute transmural
          myocardial infarct (AMI);
20 2.   20 patients with unstable angina or
          subendocardial M.I.; and
    3.   30 patients with no evidence of ischaemia or
          infarction and judged to have non-cardiac pain
          or other diagnosis.
25

Clinical criteria for assessing cardiac chest pain included:
    a)   definite M.I. on electrocardiogram (ECG) as
          measured by ST elevation, QE wave evolution or
30   deep T neg (subendo);
    b)   evidence of ischaemia (ST depression > 1mm, 2
          leads);
    c)   typical hest pain of greater than 30 min.
          duration;
35 d)   angiographic evidence of coronary artery
          disease; and

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     e)   elevation of CK, CK-MB or SGOT with typical
            curve in absence of another cause.

     Patients were assessed as having no cardiac pain by
5 the following criteria:
     a)   no ECG evidence of M.I. or ischaemia;
     b)   no elevation of CK, CK-MB or SGOT enzymes, or
            elevated enzymes explained by documented
            non-cardia cause, or angiography negative for
10   coronary artery disease; and
     c)   no evidence on GXT or thallium scan (if done).

     Table 1 details the findings of the myosin light
chain (MLC) HVMLC1 immunoassays. The sensitivity,
15 specificity and predictive value of the HVMLC1 assay
tests are summarized in Table 2. The data generated in
these studies is presented graphically in Figures 1
through 5.

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TABLE 1

<u>86 PATIENTS WITH "CARDIAC CHEST PAIN"</u>

TIME	POSITIVE MLC[1]	NEGATIVE MLC[1]
By 36 hours	78/84	6/84
By 4 days	84/86	2/86

<u>66 PATIENTS WITH ACUTE M.I.</u>

TIME	POSITIVE MLC[1]	NEGATIVE MLC[1]
by 4 days	65/66	1/66

<u>30 NON CARDIAC PATIENTS</u>

POSITIVE MLC[1]	NEGATIVE MLC[1]
3/30	27/30

<u>50 PATIENTS WITH NO M.I. (Non-Cardiac & Angina)</u>

POSITIVE MLC[1]	NEGATIVE MLC[1]
22/50	28/50

[1]NORMAL MLC < 0.6 ng/ml.

TABLE 2

	ACUTE MI (4 days)	CARDIAC PAIN MI & ANGINA
Sensitivity of MLC immunoassay[1]	98.5%	97.6%
Specificity of MLC immunoassay[1]	56%	90%
Predictive Value Positive Test	75%	96.6%
Predictive Value Negative Test	96%	93%

[1]MLC immunoassay refers to the HVLMC1 immunoassay
described in this specification.

EXAMPLE 2 - <u>Sandwich ELISA for detection of HVMLC1</u>

1.   A 96-well microtiter plate was coated with 0.1 ml of
"159-3" antibody (20 ug/ml). Coating was done in 0.05M
carbonate buffer pH9.6.

2.   The plate was covered with adhesive cover and
incubated overnight at 4.C.

3.   The coating solution was removed by shaking out
contents of the wells into a sink and blocking each well
with 0.2 ml of diluting buffer.

4.   The wells were emptied and patted dry on an
absorbent paper.

5.   0.1 ml of each control (standard curve) and sample
were added into wells. The plate was covered with an
adhesive cover and incubated at 37.C for 1 hour.

6.   The contents were shaken out of the wells. Using a
squeeze bottle the wells were washed 3 times with wash
buffer. The wells were dried on an absorbent paper
between washes.

7.   0.1 ml of "504" antibody (1 ug/ml in diluting
buffer) was added. The plates were covered with an
adhesive cover and incubated at 37.C for 30 min.

8.   Step 6 was repeated.

9.   0.1 ml of goat anti-rabbit horseradish peroxidase-
conjugate was added at 1:2000 dilution in diluting
buffer. The plate was covered with an adhesive cover and
incubated at 37.C for 30 min.

10. Step 6 was repeated.

20

11. 0.1 ml of peroxidase substrate (ortho-
phenylenediamine) solution was added and incubated at
room temperature for 15 min.

5   12. The reaction was stopped by adding 0.1 ml of IN
H_2SO_4.

13. The absorbance was read at 490 nm using a microtiter
plate spectrophotometer.

10

The diluting buffer referred to above was 0.005%
polyoxyethylenesorbitan monolaureate (TWEEN 20), 0.25%
BSA, in phosphate - buffered saline, pH 7.4 (approx.).

15

The wash buffer used was 0.05% TWEEN 20 in phosphate
- buffered saline, pH 7.4 (approx.).

The antibodies identified as "159-3" and "504" were

20   antibodies having specificities for particular sequences
unique to HVMLC1.

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CLAIMS

1.   A method for the early detection of a myocardial
infarction comprising the steps described herein with
5   reference to the accompanying figures.

2.   A mehtod for differentiating between a myocardial
infarction and a cardiac ischaemic event which is not a
myocardial infarction comprising the steps described
10   herein with reference to the accompanying figures.

3.   A method for the early detection of a myocardial
infarction comprising the sequential or simultaneous
testing for creatine kinase and for myosin light chain in
15   a tissue or serum sample.

4.   A method for differentiating between a myocardial
infarction and a cardiac ishaemic event which is not a
myocardial infarction comprising the sequential or
20   simultaneous testing for creatine kinase and for myosin
light chain in a tissue or serum sample.

5.   A method for the early detection of a myocardial
infarction comprising:
25   (a)   testing for creatine kinase in a protein-
containing sample;
          (b)   testing for myosin light chain in said protein-
containing sample; and
          (c)   determining the presence or absence of creatine
30   kinase and of myosin light chain in said sample.

6.   A method for differentiating between a myocardial
infarction and a cardiac ischaemic event which is not a
myocardial infarction, said method comprising:
35   (a)   testing for creatine kinase in a protein-
containing sample;

22

(b)   testing for myosin light chain in said protein-
containing sample; and

(c)   determining the presence or absence of creatine
kinase and of myosin light chain in said sample.

5

7.   A method of claim 5 or 6 wherein said sample is one
of a series of samples taken from a patient at different
times after a cardiac ischaemic event has occurred.

10   8.   A method of claim 7 wherein said sample is taken
between eight to ten hours after a cardiac ischaemic
event has occurred.

9.   A method of claim 8 wherein said sample is a sample
15   selected from the group consisting of human blood, human
serum and human plasma.

10.   A method of claim 9 wherein said myosin light chain
is human ventricular myosin light chain 1.

20

11.   A method of claim 10 wherein said creatine kinase
test and said myosin light chain test are performed
sequentially on said sample.

25   12.   A method of claim 10 wherein said cretin kinase test
and said myosin light chain test are performed
simultaneously on said sample.

13.   A method of claim 11 or 12 wherein said creatine
30   kinase test and said myosin light chain test are enzyme
immunoassays performed on nitrocellulose paper.

14.   A method of claim 13 wherein said creatine kinase
immunoassay and said myosin light chain immunoassay are
35   executed as two spots, on the same nitrocellulose paper
and the enzyme-substrate combination used in the creatine
kinase immunoassay differs from the enzyme-substrate

23

combination used in the myosin light chain immunoassay
such that the coloured reaction products of each
immunoassay are different colours.

5   15.   A method of claim 14 wherein said spots overlap.

16.   A method for the detection of myosin light chains in
blood, serum or plasma comprising the steps of:
        (a)   creating a myosin light chain specific antibody
10 using one or more portions of a synthetically sequenced
myosin light chain protein; and
        (b)   using said antibody in an immunoassay to detect
the presence or absence of myosin light chain antigen in
blood, serum or plasma.
15

17.   A method of claim 16 wherein said myosin light chain
is human ventricular myosin light chain 1.

18.   A method of claim 17 wherein said myosin light chain
20 is human ventricular myosin light chain 2.

19.   A method of claims 16, 17 or 18 wherein said
antibody is a monoclonal antibody.

25 20.   A kit comprising the materials required to perform
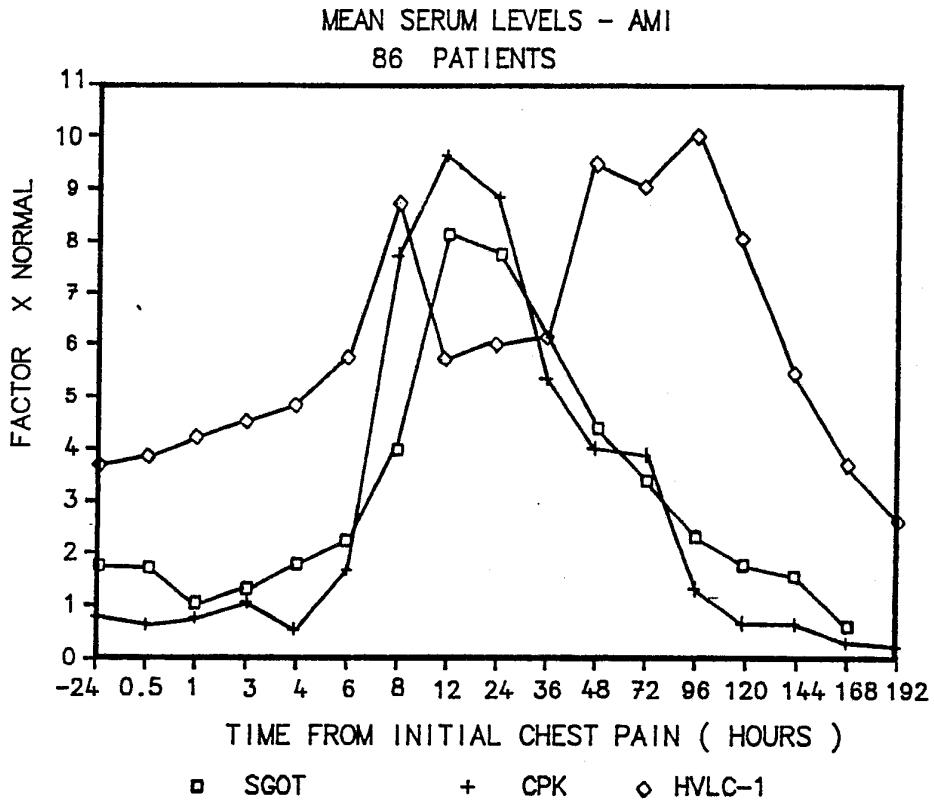the method of claim 14 or 15.

1/3

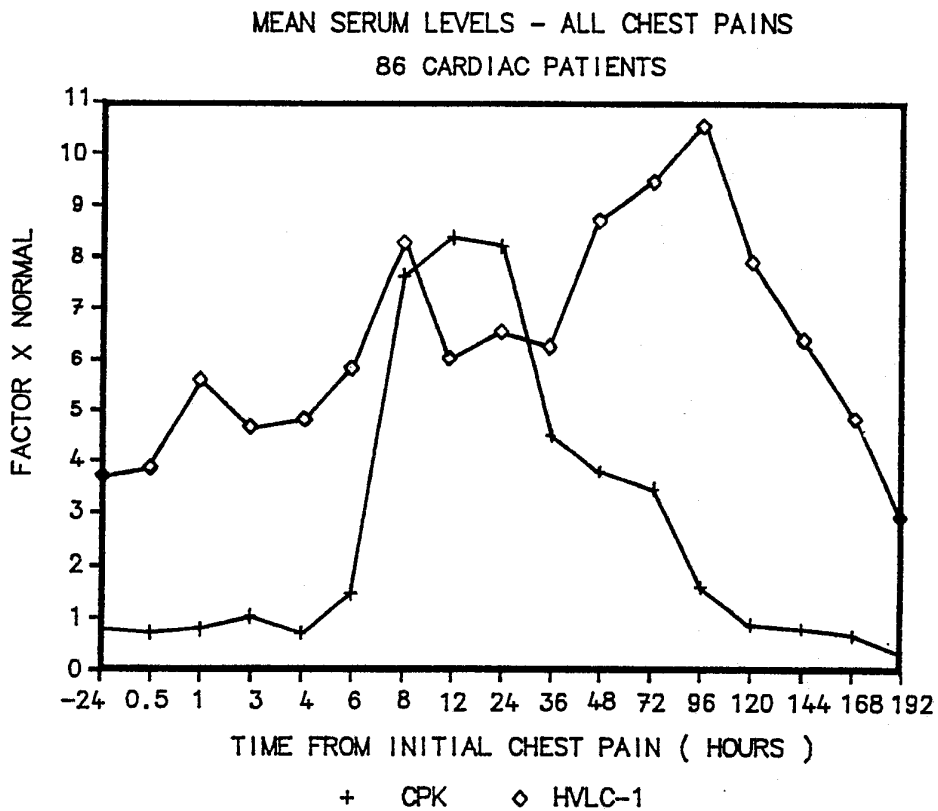MEAN SERUM LEVELS – AMI
86 PATIENTS

TIME FROM INITIAL CHEST PAIN (HOURS)

□ SGOT   + CPK   ◇ HVLC–1

FIG.1.

MEAN SERUM LEVELS – ALL CHEST PAINS
86 CARDIAC PATIENTS

TIME FROM INITIAL CHEST PAIN (HOURS)

+ CPK   ◇ HVLC–1

FIG.2.

SUBSTITUTE SHEET

2/3

SENSIVITY - AMI (ECG or CK POSITIVE)

86 PATIENTS

TIME FROM INITIAL CHEST PAIN (HOURS)

+   CPK   ◊ HVLC-1

FIG.3.

SENSITIVITY - ALL CARDIAC CHEST PAINS
86 PATIENTS

TIME FROM INITIAL CHEST PAIN (HOURS)

+   CPK   ◊ HVLC-1

FIG.4.

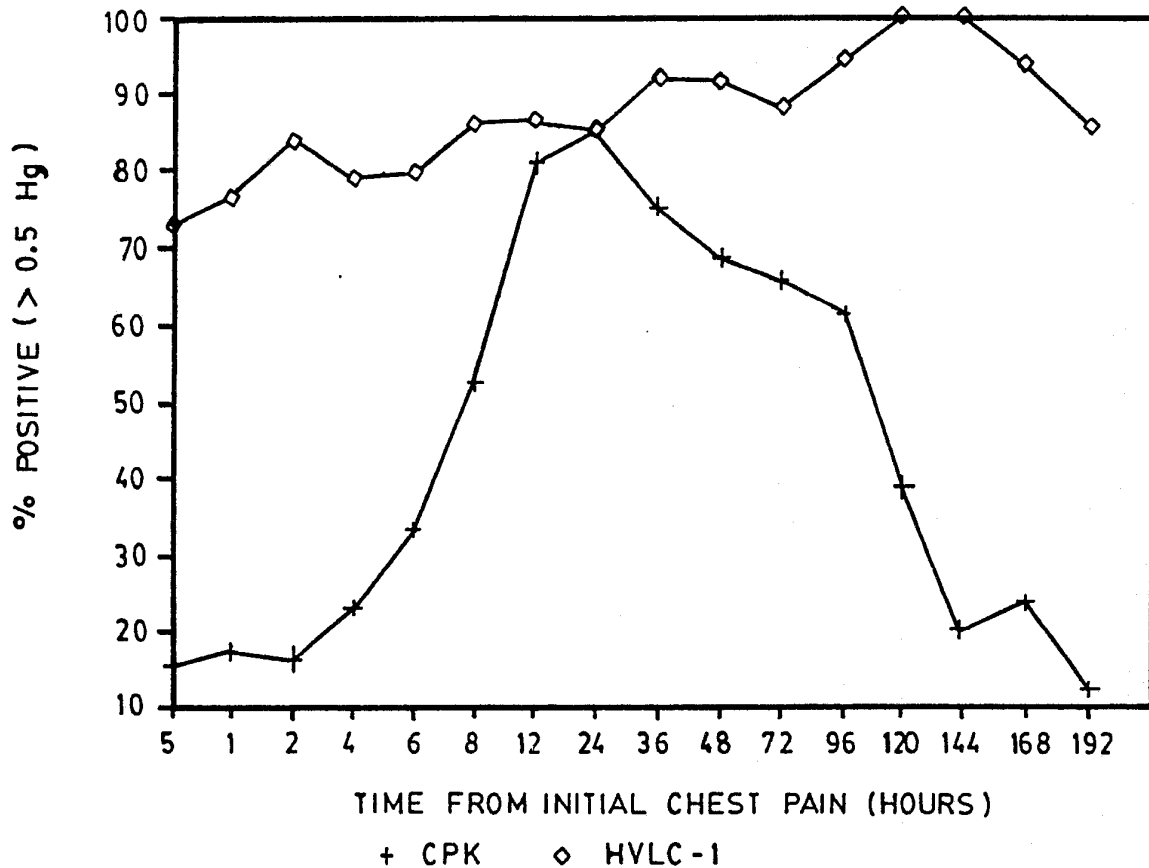**SUBSTITUTE SHEET**
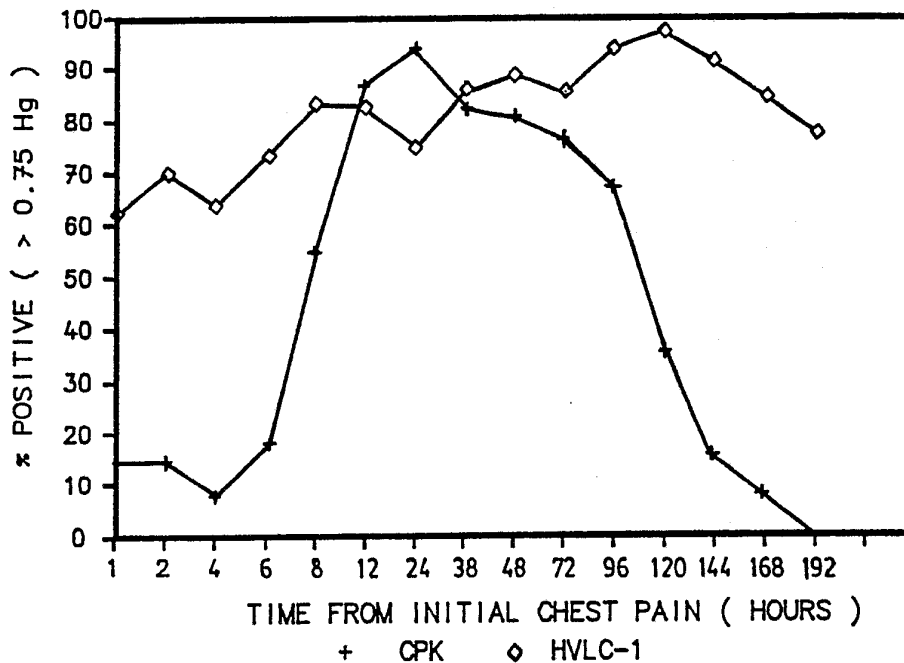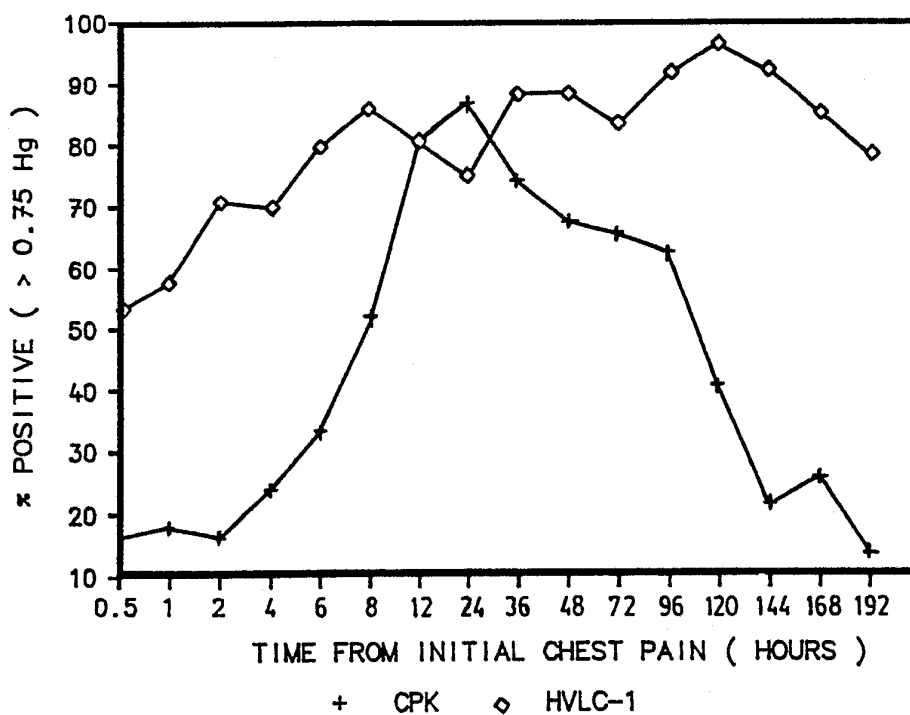
3/3

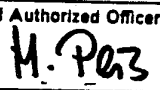SENSITIVITY-ALL CARDIAC CHEST PAINS

86 PATIENTS

FIG.5.

INTERNATIONAL SEARCH REPORT

International Application No | PCT/CA 90/00231

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ⁶

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC⁵: G 01 N 33/50, C 12 Q 1/50, G 01 N 33/68, G 01 N 33/573,
G 01 N 33/548, G 01 N 33/577

II. FIELDS SEARCHED

Minimum Documentation Searched ⁷

Classification System	Classification Symbols
IPC⁵	G 01 N, C 12 Q

Documentation Searched other than Minimum Documentation
to the Extent that such Documents are Included in the Fields Searched ⁸

III. DOCUMENTS CONSIDERED TO BE RELEVANT ⁹

Category °	Citation of Document, ¹¹ with indication, where appropriate, of the relevant passages ¹²	Relevant to Claim No. ¹³
Y	Biological Abstracts, volume 85, no. 1, 1988, (Philadelphia, PA, US), E. Hoberg et al.: "Myoglobin, creatine kinase-B isoenzyme, and myosin light chain release in patients with unstable angina pectoris", see page AB-162, abstract 1493, & Eur Heart J 8(9): 989-994, 1987	1-20
Y	Scand. J. Clin. Lab. Invest., volume 44, no. 8, 1984, O. Baadsgaard et al.: "Myoglobin concentration, creatine kinase, and creatine kinase sub-unit B activity in serum after myocardial ischaemia", pages 679-682 see the whole article	1-20
A	Biological Abstracts; volume 85, no. 5, 1988, (Philadelphia, PA, US), M. Isobe et al.: "Quantitative relationship between left ventricular function and serum cardiac myosin ./.	

° Special categories of cited documents: ¹⁰

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
4th September 1990	- 1. 10. 90

International Searching Authority	Signature of Authorized Officer
EUROPEAN PATENT OFFICE	M. PEIS

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)		
Category *	Citation of Document, " with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	light chain I levels after coronary reperfusion in patients with acute myocardial infarction", see page 167, abstract 44894, & Circulation 76(6): 1251-1261, 1987	
A	Chemical Abstracts, volume 109, no. 25, 19 December 1988, (Columbus, Ohio, US), H.A. Katus et al.: "Acute myocardial infarction: Release of cardiac myosin light chains and left ventricular function", see page 585, abstract 227778c, & Klin. Wochenschr. 1988, 66(17), 784-9	
A	Chemical Abstracts, volume 90, no. 25, 18 June 1979, (Columbus, Ohio, US), H. Kaiser et al.: "Radioimmunologic determination of human myoglobin in the diagnosis of acute myocardial infarction", see page 253, abstract 199809g, & Klin. Wochenschr. 1979, 57(5), 225-35	