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Doctoral dissertation

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for public examination in Auditorium, Kuopio University Hospital,
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Department of Medicine
Department of Clinical Chemistry
Accident and Emergency Department
University of Kuopio and Kuopio University Hospital

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ABSTRACT

The diagnosis of myocardial infarction (MI) has conventionally been based on symptoms, electrocardiographic findings and elevation of cardiac enzymes. With the introduction of more sensitive and specific biochemical markers of myocardial injury, such as cardiac troponins T (TnT) and I (TnI), smaller myocardial injuries can be recognised. Recently, inflammation has been shown to have a role in pathogenesis and prognosis of coronary heart disease (CHD), and it has become a subject of wide interest. The association of inflammation markers, especially C-reactive protein (CRP), with the risk of future coronary events has been shown not only in patients with stable or unstable angina pectoris (UAP) but also in apparently healthy persons.

The aim of this study was to investigate the role of TnT and creatine kinase isoenzyme MB mass (CK-MBm) in the diagnostic and prognostic evaluation of acute coronary syndromes (ACSs). In the planning phase of this study the first observations on the prognostic value of inflammation markers in patients with chronic stable angina, severe UAP or MI had quite recently been published. Therefore, another aim was to study the prognostic value of CRP, fibrinogen, interleukin (IL)-6 and tumour necrosis factor-α (TNF-α) in patients with ACSs with small or undetectable myocardial injuries. The basic assumption was that the use of cardiac TnT and CK-MBm may lead to a more accurate diagnostic and prognostic evaluation of ACSs, compared with the use of conventional enzyme activities. Moreover, acute-phase proteins and cytokines were assumed to have a prognostic significance beyond myocardial injury in ACSs.

The study population consisted of 559 consecutive patients admitted to the emergency department of Kuopio University Hospital with a suspected ACS. Blood samples for the measurement of conventional enzyme activities, and TnT and CK-MBm concentrations were drawn as soon as possible after the admission, and 2, 4 and 6 h thereafter. During the second and third hospital day blood samples were drawn twice daily, and the last blood sample was drawn in the morning of the fourth day. Inflammation markers were determined from the first blood sample in patients with UAP. Hospital discharge diagnoses (clinical diagnoses) and modified FINMONICA criteria (epidemiological diagnoses) were used for the classification of events. The median follow-up time was 17 months.

In virtually all of the patients with definite MI, TnT and CK-MBm were elevated and conventional enzymes were elevated to above twice the upper reference limit (URL). However, in one-third of the patients with probable MI, TnT and CK-MBm were not elevated and conventional enzymes were above the URL but less than or equal to twice the URL. TnT was elevated in 13% and CK-MBm in 15% of patients with conventional enzymes within normal limits. MI could be ruled out by TnT or CK-MBm within 12 hours from admission to the emergency department in 99% and 96% of the patients, respectively. Among the patients in whom MI was ruled out, a positive history of CHD was an important prognostic factor. The use of TnT as a primary basis of MI diagnosis led to an increase in the number of MI diagnoses. The increase was 33% compared with a clinical and 23% compared with an epidemiological diagnosis. Slightly elevated levels of inflammation markers were associated with an increased risk of CHD death and major CHD events in patients with UAP. Especially CRP and IL-6 were strong predictors of CHD death, most strongly during the first four months. Differing from other inflammation markers, TNF-α was associated with an increased risk of nonfatal CHD events during the first month. In a factor analysis, CRP, fibrinogen and IL-6 clustered on an ‘inflammation factor’. However, TNF-α clustered with TnT and CK-MBm on an ‘injury factor’.

In conclusion, adoption of TnT into the clinical practice as a central element of the diagnosis of MI leads to a substantial increase in the number of patients getting the diagnosis of MI. MI can be ruled out by cardiac troponins or CK-MBm within 12 hours from admission in almost all patients with suspected ACSs. Elevated levels of inflammation markers, particularly CRP and IL-6, are strong predictors of the risk of serious coronary events in patients with UAP.

National Library of Medicine Classification: WG 300
Medical Subject Headings: myocardial infarction; myocardial infarction/diagnosis; myocardial infarction/epidemiology; prognosis; angina, unstable; troponin T; creatine kinase; coronary disease; inflammation; acute-phase proteins; cytokines; biological markers; chest pain; C-reactive protein; interleukin-6; fibrinogen; tumor necrosis factor; evaluation studies; follow-up studies

To my family

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Kuopio, November 2002

Heli Koukkunen

ABBREVIATIONS

AACC	American Association for Clinical Chemistry
ACC	American College of Cardiology
ACS	Acute coronary syndrome
AHA	American Heart Association
ASAT	Aspartate aminotransferase
ATP	Adenosine triphosphate
CABG	Coronary artery bypass grafting
CHD	Coronary heart disease
CI	Confidence interval
CK	Creatine kinase
CK-MB	Creatine kinase isoenzyme MB
CK-MBm	Creatine kinase isoenzyme MB mass (concentration)
CRM	Certified reference material
CRP	C-reactive protein
CV	Coefficient of variation
DNA	Deoxyribonucleic acid
EC	Enzyme Commission's numbering system
ECCLS	European Committee for Clinical Laboratory Standards
ECG	Electrocardiogram
ELISA	Enzyme-linked immunosorbent assay
ESC	European Society of Cardiology
FINMONICA	Finnish contribution to the WHO MONICA project
HDL	High-density lipoprotein
HR	Hazard ratio
ICD	International Classification of Diseases
IFCC	International Federation of Clinical Chemistry
IFN-γ	Interferon-γ
IgX	Immunoglobulin (X=letter)
IL-N	Interleukin (N=number)
IL-1Ra	Interleukin-1 receptor antagonist

ISFC	International Society and Federation of Cardiology
LD	Lactate dehydrogenase
LD-N	Lactate dehydrogenase isoenzyme (N=number)
LDL	Low-density lipoprotein
MI	Myocardial infarction
MONICA	MONItoring Trends and Determinants in CArdiovascular Disease
PCI	Percutaneous coronary intervention
rCK-MB2	Recombinant human CK-MB2
SAA	Serum amyloid A protein
TnC	Troponin C
TNF-α	Tumour necrosis factor-α
TnI	Troponin I
TnT	Troponin T
UAP	Unstable angina pectoris
URL	Upper reference limit
WHO	World Health Organisation

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications, which will be referred to by their Roman numerals:

I Koukkunen H, Penttilä K, Kemppainen A, Halinen M, Penttilä I, Rantanen T, Pyörälä K. Troponin T and creatine kinase MB mass in the diagnosis of myocardial infarction. Ann Med 1998;30:488-496.

II Koukkunen H, Penttilä K, Kemppainen A, Penttilä I, Halinen M, Rantanen T, Pyörälä K. Ruling out myocardial infarction with troponin T and creatine kinase MB mass: diagnostic and prognostic aspects. Scand Cardiovasc J 2001;35:302-306.

III Koukkunen H, Penttilä K, Kemppainen A, Penttilä I, Halinen M, Rantanen T, Pyörälä K. Differences in the diagnosis of myocardial infarction by troponin T compared to clinical and epidemiologic criteria. Am J Cardiol 2001;88:727-731.

IV Koukkunen H, Penttilä K, Kemppainen A, Halinen M, Penttilä I, Rantanen T, Pyörälä K. C-reactive protein, fibrinogen, interleukin-6 and tumour necrosis factor-α in the prognostic classification of unstable angina pectoris. Ann Med 2001;33:37-47.

Additionally, some unpublished data are presented.

CONTENTS

INTRODUCTION

Coronary heart disease (CHD) is a major public health problem in developed countries, and it is becoming more common worldwide. Acute coronary syndromes (ACSs) are attacks in which blood flow in coronary arteries is abruptly reduced or stopped, usually due to fissuring or rupture of atherosclerotic plaques and subsequent thrombosis, leading to myocardial ischaemia. If this process leads to myocardial cell necrosis, the result is a myocardial infarction (MI) (1). In Finland about 44 000 ACSs lead to hospitalisation or death annually (2). Of these events about 23 000 are MIs or coronary deaths (2).

During the past decade the treatment practice of ACSs has become more and more active. A rapid diagnosis or at least a strong suspicion of MI in the acute phase of the event is important in directing the treatment decisions, such as the use of thrombolytic therapies and acute percutaneous coronary interventions (PCIs). The treatment of unstable angina pectoris (UAP) and non-ST-elevation MI has also become more aggressive. It is, however, rational to direct the modern therapies to the patients who are most likely to benefit from them and to avoid unnecessary risks of side effects, such as bleeding complications of potent antithrombotic agents, and also to aim at cost-effectiveness. In the long run, the prognosis of the patients is largely dependent on the severity of the CHD, the presence of residual myocardial ischaemia, the size of the myocardial injury and the residual function of the left ventricle, and the risk of sudden cardiac death (3). With modern therapies it is possible to affect both the short- and long-term prognosis of the patients. Thus, prognosis has become an essential perspective in the evaluation of the patients with MI and other ACSs.

The diagnosis of MI is conventionally based on symptoms, electrocardiographic findings and elevation of cardiac enzymes or other biochemical markers of myocardial injury, and in fatal cases also on autopsy findings. However, the clinical diagnosis of MI has not been coherent due to a lack of standardised diagnostic criteria. The first World Health Organisation (WHO) criteria (4) and the WHO MONItoring Trends and Determinants in CArdiovascular Disease (MONICA) criteria (5) were rather crude tools primarily tailored for the purposes of epidemiological studies, but they have also been applied to clinical use. Nonspecific elevations of conventional enzyme activities, creatine kinase (CK), its MB isoenzyme (CK-MB), lactate dehydrogenase (LD), and its isoenzyme 1 (LD-1), sometimes created problems in the diagnosis of MI, especially in recognising and ruling out probable MI with elevations of conventional enzymes to less than or equal to twice the upper reference limit (URL). In the MONICA project the enzyme criterion of more than twice the URL was agreed for definite MI (5). The high cut-off limit was chosen to assure the specificity of the diagnosis.

With the introduction of new more sensitive and cardiac-specific biochemical markers of myocardial injury, such as cardiac troponins T (TnT) and I (TnI), smaller myocardial injuries could be recognised. These injuries were primarily termed ‘minimal myocardial damage’ (6-8), and they were found to be associated with an adverse prognosis (8-11). Thus, the adoption of these markers in modern clinical practice has led to a need for the redefinition of MI (12). One major goal of this redefinition has been to integrate prognostic aspects with the diagnosis of ACSs. However, the impact of the introduction of these markers instead of the conventional enzymes on the number of MI diagnoses and on the prognostic stratification of ACSs has not been clear.

In recent years, the role of inflammation in the pathogenesis and prognosis of CHD has been a subject of great interest (13-15). The association of inflammation markers, first of all C-reactive protein (CRP), with the risk of future coronary events has been shown not only in patients with stable angina pectoris or UAP (16-19), but also in apparently healthy persons (20-22). However, the mechanisms of these associations are incompletely understood. Inflammation markers such as CRP have been found to give information both on inflammatory activity (the underlying condition) and on myocardial cell injury (the acute event).

This study was designed to compare TnT and creatine kinase isoenzyme MB mass (CK-MBm) with conventional enzyme activities in the diagnosis of ACSs. Another aim was to investigate the diagnostic time-window of TnT and CK-MBm in ruling out MI, and the prognosis of the patients in whom MI was ruled out. Furthermore, the direction and magnitude of the changes in the diagnosis of MI based on TnT was compared with those based on conventional enzyme activities, and the prognosis of patients with discordant diagnoses was examined. In the planning phase of this study the role of inflammation markers in the prognostic assessment of the patients with ACSs was becoming a subject of wide interest. The first observations on the prognostic value of inflammation markers in patients with chronic stable angina, severe UAP or MI, and with or without myocardial injury, had quite recently been published (16). Therefore, the prognostic value of CRP, fibrinogen, interleukin (IL)-6 and tumour necrosis factor-α (TNF-α) was studied in patients with ACSs with small or undetectable myocardial injuries.

The basic assumption of this study was that the use of cardiac TnT and CK-MBm may lead to a more accurate diagnostic and prognostic evaluation of ACSs, compared with the use of conventional enzyme activities. Moreover, acute-phase proteins and cytokines were assumed to have a prognostic significance beyond myocardial injury in ACSs.

REVIEW OF THE LITERATURE

DIAGNOSIS OF MYOCARDIAL INFARCTION

History of the diagnosis of myocardial infarction

MI as a pathologic concept was well recognised already in the beginning of the 20th century (23). However, at that time it was generally believed that MI virtually always leads to death. Hammer was the first to diagnose coronary occlusion during life (23). In 1878 he described a case of a patient in whom thrombotic occlusion of a coronary artery was suspected (24). At autopsy a vegetation due to endocarditis on the aortic valve was found to have blocked the orifice of the right coronary artery.

Obrastzow and Straschenko described in 1910 several cases in which the diagnosis of MI was made during life (25). Post-mortem confirmation was not obtained in all cases, but the clinical picture of 'status anginosus' together with breathlessness was quite consistent. The report of Herrick on the diagnosis of MI during life is, however, better known (26). He described in 1912 six patients with a clinical diagnosis of MI who later died, and in all of whom coronary thrombosis was detected at autopsy (26). He stated that although coronary occlusion caused by thrombosis often led to sudden death, the death could occur several hours or even days later. Furthermore, he stated that sometimes the patient survived, and a functional recovery ensued. However, it was not until in 1918 and 1919 when his later publications, including electrocardiographic findings, gained a wide publicity (23).

In 1901 Einthoven introduced a string galvanometer by which the electric activity of the heart could be registered quantitatively (27). From this discovery, which was awarded by the Nobel prize in 1925, the development of electrocardiography towards a clinical diagnostic tool got its start, although the refinement of electrocardiographic methods to their current status took more than 50 years. Einthoven introduced the three standard limb leads that remained in sole use for almost 40 years (28). In the 1930s Wilson and co-workers developed unipolar limb leads which were modified by Goldberger in 1942 (27). Although chest leads, originally introduced by Wilson, were used by several scientists, their precise standardisation and positions were not defined until 1937 (28,29). It was only after World War II that the unipolar chest leads came into more general use. The use of the current 12-leaded electrocardiogram (ECG) with three unipolar and three bipolar limb leads, and six unipolar chest leads became accepted practice since the 1950s (28).

Pardee published in 1920 his observations on the shape of the ST-segment and T-wave in connection with MI (23,28). At first, his findings did not gain complete acceptance. The situation was clarified when Parkinson and Bedford described in 1928 the serial ECG changes in acute and healing phases of MI (23). Furthermore, Wilson and colleagues had a major role in establishing the evolution of Q or QS waves resulting from transmural MI (27).

The typical symptoms and signs of MI, as well as ECG findings, were well recognised by 1940 (23). Additionally, evidence of tissue damage, such as a febrile reaction, leucocytosis and a raised erythrocyte sedimentation rate, supported the diagnosis of MI (23). Elevation of CRP due to myocardial tissue damage could also be detected in MI patients (30).

The diagnostic value of ECG was not sufficient to detect small infarctions and all reinfarctions. Thus, introduction of enzyme activity measurements was a remarkable advancement in the diagnosis of MI. In 1954 elevated activity of serum aspartate aminotransferase (ASAT, formerly known as glutamate oxaloacetate transaminase or GOT) (31,32) and in 1956 elevated activity of serum LD (33) was reported in patients with MI. However, because the elevated activities of these enzymes were not specific to myocardial injury, LD isoenzymes were introduced. Hydroxybutyrate dehydrogenase representing mostly the activities of LD-1 and LD-2 isoenzymes was introduced in 1961 (34,35), and the determination of LD-1 in 1963 (36). The activities of these LD subfractions were found to be more specific for myocardial injury than the activity of total LD.

Increase of total CK activity in MI was detected in 1960 (37). Six years later CK-MB isoenzyme activity was found to be a more specific indicator of myocardial injury than total CK activity (38). However, its sensitivity and specificity still had their limitations. Progress in the analytic methods led to development of immunoassays that measure enzyme mass (concentration) instead of activity. In the early 1990s determination of CK-MBm was introduced, and it has gradually replaced CK-MB activity in the diagnosis of MI. Another possibility to increase the sensitivity of CK-MB is to determine its isoforms (enzyme molecules which have been modified after translation) (35). The elevation of CK-MB2 (tissue form) and CK-MB2/CK-MB1 (plasma form) ratio can be detected in patients with MI earlier than elevation of CK-MB with conventional assays (39).

Several other markers of myocardial injury have also been proposed for the detection of MI in the early hours. Myoglobin, heart-type fatty acid-binding protein, and glycogen phosphorylase isoenzyme BB are early and sensitive markers of myocardial injury. They are less specific than cardiac enzymes, however, and therefore they have not been adopted into a wide use in the diagnosis of MI.

Since all the markers of myocardial injury reviewed above were not sufficiently specific, research has recently been focused on cardiac contractile and regulatory proteins. Myosin from cardiac muscle consists of two heavy chains and two pairs of light chains. Both myosin heavy chain fragments and light chains are liberated from the myocyte into plasma after MI, and they can be detected by specific assays (40,41). Troponins are structural proteins in myocardium, and they act as a complex regulating muscle contraction (42). Specific immunoassays were developed in the late 1980s to detect cardiac troponin I (TnI) by Cummins *et al.* (43) and troponin T (TnT) by Katus *et al.* (44). Cardiac troponins have proved to be highly sensitive and specific markers of myocardial injury, as will be described in the next subchapter.

Recent developments in biochemical markers of myocardial injury

In the diagnosis of MI, conventional enzymes (LD and its isoenzymes, CK, and CK-MB activity) are in most Western countries being replaced by more sensitive and specific markers, such as TnT, TnI, and CK-MBm. Due to their wide diagnostic time-window, LD and its isoenzymes remained in use until recent years for the cases in which the patient arrived to hospital several days after the acute phase of MI.

Lactate dehydrogenase

LD (EC 1.1.1.27) is responsible for the interconversion of pyruvate and lactate in glycolysis. It is localised in cytoplasm, and the highest activities are found in skeletal muscle, liver, heart, kidney, and red blood cells (45). LD is a tetramer composed of two subunits (M or muscle, and H or heart). The two subunits give rise to five isoenzymes: LD-1 (H_4), LD-2 (H_3M), LD-3 (H_2M_2), LD-4 (HM_3), and LD-5 (M_4) (35,46). LD-1 is the predominant form in heart but it is also found in red blood cells and kidney (45,46). LD-2 is also abundant in heart, whereas LD-5 is predominant in skeletal muscle. Total LD is usually measured from serum as enzymatic activity (46). Techniques used to separate and measure the isoenzymes of LD include electrophoresis, chemical inhibition, ion-exchange chromatography, and immunoprecipitation (35). LD-1 and LD-2 can also be measured by a photometric assay in which 2-oxybutyrate is used as a substrate (35). Hemolysis will markedly increase activities of total LD, as well as LD-1 and LD-2.

LD becomes elevated late after the onset of the MI, but it remains elevated for 1-2 weeks (Table 1). A good correlation has been found between LD (and α-hydroxybutyrate

dehydrogenase) and infarct size, assessed by perfusion scintigraphy or ejection fraction (47). The specificity of LD can be improved by using LD-1 or measuring the ratio of LD-1 and LD-2, which increases to > 1 in MI (46). The specificity may also be improved when LD-1 / total LD or LD-1 / LD-4 is used (48). Recently, however, cardiac troponins, which also have a wide diagnostic time-window, have replaced LD and its isoenzymes in the diagnosis of MI.

Table 1. Characteristics of cardiac markers and time course after onset of MI. Adapted from tables by Adams (46), Donnelly and Millar-Craig (49), and Apple (45).

Marker	Molecular mass (kD)	Time to initial increase (range)	Time to peak* (mean)	Time to return to normal range
LD, LD-1	135	8-12 h	24-48 h	8-14 days
CK	86	3-12 h	24 h	3-4 days
CK-MB	86	3-12 h	24 h	2-3 days
TnI	23	3-12 h	24 h	5-10 days
TnT	42	3-12 h	12-48 h	5-14 days
Myoglobin	18	1-3 h	6-7 h	24 h

* in the absence of thrombolytic therapy

Creatine kinase and its isoenzyme MB

CK (EC 2.7.3.2) is a cytosolic enzyme that catalyses the reversible transfer of a phosphate group from adenosine triphosphate (ATP) to creatine. The activity of CK is dependent on muscle mass. The use of gender-specific cut-off values is therefore currently recommended. It exists as a dimer composed of two subunits (M or muscle, and B or brain, according to the predominant tissue of occurrence). Thus, three different isoenzymes or subforms exist: CK-BB (CK-1), CK-MB (CK-2), and CK-MM (CK-3). The average CK isoenzyme distribution in myocardium is 78.7% CK-MM, 20.0% CK-MB, and 1.3% CK-BB (35). CK-MM is predominant in both heart and skeletal muscle but CK-MB is more specific for the myocardium. However, several tissues and organs, such as skeletal muscle, the gastrointestinal tract and the pregnant uterus, contain CK-MB. Thus, it cannot be regarded as totally cardiac-specific. CK-MB may be elevated in a patient with skeletal muscle trauma,

myopathy, or dystrophy without a concomitant MI. CK activity also may be found in macromolecular form, macro-CK. It is found in up to 6% of hospitalised patients, but only a small proportion of them have abnormal CK activities (35). Macro-CK may lead to false CK-MB activities by interfering with CK-MB assays. Macro-CK exists in two forms. Type 1 is an enzyme-immunoglobulin complex, usually CK-BB-IgG, but other complexes have been described, such as CK-MM-IgA (35). Type 2 is an oligomeric form of mitochondrial CK. It can interfere with the assay of CK-MB by ion-exchange or immunoinhibition methods (35).

Isoenzyme determination methods

The three techniques most commonly used to separate and quantitate CK isoenzymes are electrophoresis, ion exchange chromatography and various immunological methods (35). The immunological methods used to measure the CK isoenzymes require specific antisera against the M and B subunits. The antisera are used to precipitate (immunoprecipitation) or competitively bind specific isoenzymes (immunoinhibition) (35). In contrast to these methods which measure enzyme activity, immunoassays measure enzyme mass. Although not 100% cardiac-specific, CK-MBm assays are more sensitive and specific than activity-based methods (35,50-52). They also detect serum abnormalities earlier, and are not interfered by macro-CK (50-54). It has been suggested that CK-MBm is more suitable for infarct size estimation than measurement of enzyme activity (55). In mass assays two specific antibodies with affinity to different parts of the CK-MB molecule are used sequentially (35). This ensures that only CK-MB is estimated, because neither CK-MM nor CK-BB reacts with both antibodies.

CK-MBm assay standardisation

Henderson *et al.* (56) reported a wide dispersion of results between different analyser systems and also among identical analysers. This is due to the lack of consensus on a primary standard for CK-MBm assays (57). That also makes comparisons of analytical and diagnostic sensitivities and specifities of different methods difficult. An important goal for standardisation is the availability of common reference limits. The American Association for Clinical Chemistry (AACC) committee recommended in 1999 the use of recombinant human CK-MB2 (rCK-MB2) as a reference material for CK-MBm assays (58). Calibration of immunoassay systems with rCK-MB2 reduced the between-manufacturer bias to 13% from the initial 40% (58). Analytical imprecision also is not uniform between commercial assays (59). For CK-MBm the goal for analytical imprecision, expressed as the coefficient of

variation (CV), at the 99th percentile of a reference control group is ≤10%, based on biological variability studies (12,60). Methods of biochemical marker determinations should be as precise and well standardised as possible for a reliable diagnosis of MI in clinical practice as well as in epidemiological and clinical studies.

Detection of myocardial infarction

The ratio of CK-MB and total CK activities was formerly used in the detection of MI with the principle that a greater proportion of CK-MB favours origin from a myocardial source. After CK-MB activity was replaced by CK-MBm assays, the relative index (RI) was introduced (61). RI can be calculated by the following formula:

RI = (CK-MBm [μg/l] / total CK activity [U/l]) x 100

The diagnostic cut-off value of RI for MI is specific to each assay because of lack of standardisation between the assays of different manufacturers. Additionally, MI occurring concurrently with a massive skeletal muscle injury may be hard to detect.

Table 1 shows the molecular masses and time courses after the onset of MI for CK and CK-MB, as well as other markers of myocardial injury. Since CK and CK-MB are cytosolic enzymes, they are released from injured myocardial cells into circulation more rapidly than structural proteins. The half-life of CK-MB in circulation is 10-12 h (45). Since the diagnostic time-window is relatively narrow, CK-MBm is useful in the diagnosis of a reinfarction occurring soon after the initial MI. Conventionally, serial determinations of CK-MBm showing a rise and fall in concentration were used in the detection of MI. Recently, the measurement of slope and the rate of increase has been proposed (62). The time to peak concentration described in Table 1 is not applicable to patients with successful reperfusion by thrombolytic therapy or coronary intervention. Early reperfusion leads to washout phenomenon with an early and high concentration peak (63,64). When reperfusion has occurred it is difficult to assess the actual size of myocardial injury because of the large variability in the amount of enzyme washout (65).

It is somewhat controversial whether elevated levels of CK and CK-MB in circulatory blood are always a sign of irreversible myocardial cell injury (46). Bøtker *et al.* (66) reported in 1991 that 38% of 21 patients with UAP, and with CK-B activity within normal limits, had a CK-MBm concentration curve resembling that of patients with MI, but with markedly lower peak concentrations. There was also an association between the presence of reversible ST-segment depression and CK-MBm fluctuation (66).

Troponins

The troponin complex regulates the calcium-dependent interaction of myosin with actin in muscle contraction. It consists of three subunits, TnT, TnI, and troponin C (TnC), which are located on the thin filament of the contractile apparatus. TnT anchors the troponin complex to tropomyosin, TnC binds calcium ions and initiates the contractile response, and TnI inhibits actin-myosin cross-linking. Separate genes code for the cardiac muscle, fast skeletal muscle and slow skeletal muscle isoforms of TnT and TnI (42). Thus, cardiac TnT and TnI have unique aminoacid sequences that bind to specific monoclonal antibodies (67). On the other hand, identical TnC is expressed in cardiac and slow skeletal muscle in addition to a divergent fast skeletal muscle isoform, which prevents its use in the detection of myocardial injury (67). The regulatory troponin complex does not exist in smooth muscle (42).

TnT assays

Katus *et al.* (44) introduced in 1989 a specific enzyme-linked immunosorbent assay (ELISA) method using two monoclonal antibodies for the detection of cardiac TnT in serum. In the 'first-generation' TnT assay only the capture antibody was completely cardiac-specific. However, the detection antibody was only 78% cardiac-specific (68). This assay had about 1-2% cross-reactivity with skeletal muscle TnT (7). The cross-reactivity was found to be nonimmunologic, and result from unspecific absorption of purified skeletal TnT to the test tubes (7). These molecules were then detected by the unspecific signal-antibody. Thus, the 'first-generation' test could give false-positive results in patients with severe skeletal muscle injury. The first assays of 'premarket generation' had cut-off values as high as 0.5 μg/l (7). The cut-off values for the actual 'first-generation' TnT assays were 0.2 μg/l in the earliest studies and 0.1 μg/l in subsequent studies (69). Subsequently, a cardiac-specific 'second-generation' TnT assay using double monoclonal antibody technique was developed (69). This assay was faster to perform, and the cross-reactivity with human skeletal TnT was reduced to virtually zero. The cut-off value for the 'second-generation' TnT assay was 0.1 μg/l, as recommended by the assay manufacturer (69). However, even lower cut-off limits have been used, such as 0.06 μg/l in the Fragmin During InStability in Coronary Artery Disease (FRISC) study (70). Both the 'first-' and 'second-generation' TnT assays use bovine cardiac TnT as standard material (71). In the 'third-generation' TnT assay, standard material has been changed to a recombinant human cardiac TnT (71). Consequently, the 'third-generation'

assay has a linear calibration curve and high precision, especially at the lower end of measuring range (71). The re-standardisation did not change the cut-off value of 0.1 µg/l.

Cardiac TnT assays have progressively improved, which has lowered their detection limits and functional sensitivities. The background level of cardiac TnT in the circulation, without the presence of myocardial injury, is almost zero or at least currently undetectable. Collinson has even suggested the use of URLs as low as 0.04-0.06 µg/l for TnT (68).

TnI assays

Since cardiac TnI is expressed only in myocardium, it theoretically causes the fewest problems in the detection of MI. Cummins *et al.* (43) introduced in 1987 a radioimmunoassay method to detect cardiac TnI in serum. Subsequently, Bodor *et al.* (72) developed a double monoclonal enzyme immunoassay. Both of the monoclonal antibodies were highly cardiac-specific with cross-reactivity with skeletal muscle TnI of only <0.5% (72). Currently, there are several monoclonal antibody-based assays for cardiac TnI, unlike for TnT. However, there is up to a 20-fold variation in the cut-off values of MI, and even a 60-fold variation in TnI values of patient samples by different assays (68,73).

Cardiac TnI is present in the circulation in three forms: free, as a TnI-TnC complex, and as a TnT-TnI-TnC complex (45). Actually, the predominant part of TnI circulates in the form of a complex (74). Furthermore, these three forms circulate in different degrees of proteolytic degradation (45,75). Katrukha *et al.* showed that in necrotic human cardiac tissue and in serum of MI patients the amino- and carboxyterminal regions of cardiac TnI are vulnerable to degradation (75). The most stable region was found to be located between amino acid residues 30 and 110, possibly due to its protection by TnC (75). In another study by Shi *et al.* (76) the aminoterminal region of cardiac TnI molecule was found to be more stable than the carboxyterminal region. These findings are important in explaining the wide variation of measurements with different TnI assays. Some assays have also been reported to be interfered by rheumatoid factor and heterophilic antibodies, which may lead to false increase of TnI (77-79).

In the majority of commercial TnI assays antibody pairs recognise epitopes in the stable 30-110 region, but in other assays sample instability may be a problem (68). Furthermore, some assays respond to TnI-TnC complex better than to the free TnI molecule (80,81). Thus, a major problem with cardiac TnI is the lack of standardisation of assay systems. Attempts at standardisation are in process by the committees of AACC and International Federation of Clinical Chemistry (IFCC), but a final consensus has not yet been achieved. The TnT-TnI-

TnC complex derived from cardiac tissue has been suggested as one potential calibrator (82,83).

Effect of sample material on troponin values

The optimal sample material for the majority of troponin assay systems seems to be serum. However, problems may arise due to fibrin microclots formed in inadequate clotting, or microparticulates following freeze-thaw cycles (68). Naturally, the use of serum prolongs the turnaround time of the assays. Measurements of cardiac TnT and TnI from heparin plasma tend to give lower concentrations than measurements from serum (84,85), and this may be due to binding of heparin to troponins and reducing their immunoreactivity (84). The current recommendation is not to use heparin for cardiac TnT measurement (68). EDTA plasma can be used in a minority of assay systems. The effect of EDTA is marked and variable in TnI assays due to chelation of calcium and disruption of TnI-TnC and TnT-TnI-TnC complexes (68,81).

Troponins in the detection of myocardial infarction

Cardiac isoforms of TnT and especially TnI are highly specific to myocardium. Their circulating levels are normally low. After onset of MI the early release kinetics of both TnT and TnI are similar to those of CK-MB in patients with no reperfusion (Table 1). Moreover, TnT and TnI levels remain elevated approximately as long as LD and LD-1 activities. Thus, a patient with delayed hospital admission, >48-72 h after the onset of symptoms, can be diagnosed as having had a MI by cardiac troponins. In patients with successful early reperfusion the release kinetics of TnT are biphasic (86). A high initial peak at 12-14 h from the onset of MI is followed by a lower peak and a plateau phase lasting for 48 h, and the concentration subsequently falls by the average 10-14 days (86,87). However, in patients with late (>5.5 h after the onset of symptoms) or no reperfusion, the early TnT peak is absent (87). Early reperfusion causes an earlier increase above the URL and an earlier and higher concentration peak. However, after the initial peak there is no difference in the clearance kinetics of troponins between successful and unsuccessful reperfusion (64).

CK-MB and myoglobin are 100% cytosolic enzymes, which is reflected by their early release into circulation after myocardial injury. In different studies 6-8% of cardiac TnT and 3-8% of cardiac TnI were found as a free cytosolic component (68). The majority of cardiac TnT and TnI is found in the contractile apparatus. The biological half-life of TnT in the

circulation is 120 min (87). Thus, the wide diagnostic time-window is due to the prolonged and continuous release of TnT from the myofibrillar pool as a result of degradation of the contractile apparatus (68). The duration of elevation depends on the size of the infarct and may vary from 7 to 21 days (68).

The release and clearance of cardiac TnI are documented less well than those of TnT. Bertinchant *et al.* reported that in 42 of 48 (87.5%) patients with MI and early reperfusion the release profile of TnI was monophasic with a single peak during the first 24 h after the onset of symptoms (88). The absence of a biphasic release profile has been ascribed to a smaller cytosolic pool of TnI than TnT, although the cytosolic pools are apparently equivalent (68). However, in the study by Bertinchant *et al.*, 6 of the 48 patients with MI and early reperfusion had a biphasic TnI release profile (88).

It is controversial whether the release of troponins into circulation in humans is always a result of irreversible cell necrosis, or does it also happen in connection with reversible ischaemia (89). Some animal studies support the concept of reversible prolonged ischaemia as the cause of troponin release from myocardial cells. Remppis *et al.* (90) showed in isolated perfused rat hearts that cell membrane damage leads to rapid leakage of a functionally unbound cytosolic TnT pool. In this study the membrane damage was induced by the calcium paradox: depletion of calcium and subsequent reperfusion with calcium containing solution (90). In another study on pigs by Feng *et al.* (91), reversible ischaemia induced by sub-occlusion of left anterior descending coronary artery, and without histologic evidence of cell necrosis, was associated with release of TnI but not CK-MB or myoglobin.

The term ´minor myocardial damage´ has been used in the literature to describe an ACS with mildly elevated cardiac troponin level, but with CK-MBm (or CK-MB) within normal limits (6-8). The prognostic significance of the finding will be discussed later in this review section. However, the European Society of Cardiology (ESC) / American College of Cardiology (ACC) Joint Committee for the redefinition of MI no longer recommended the use of the term ´minor myocardial damage´ (12).

Infarct size may be estimated using either of the cardiac troponins but it requires blood sampling several times per day for several days. Wagner *et al.* (92) found a close correlation between scintigraphic estimates of myocardial scar and peak values or areas under the time – concentration curves of TnT. However, in this study 'first-generation' TnT assay was used and all of the patients received thrombolysis. Due to the washout phenomenon of the markers the peak values of cardiac troponins are usually not reliable in estimating the actual size of myocardial injury in patients with early reperfusion. In another study by the same research group (93) significant correlation was found between scintigraphic myocardial defect sizes

and areas under TnI curves in patients who received thrombolysis. Negative correlations were also detected between left ventricular ejection fraction and peak CK-MBm, TnI (94) and TnT (95) concentrations in patients with their first MI.

Troponins in renal failure

It was originally reported that cardiac TnT but not TnI could be elevated in patients with severe renal failure even without myocardial injury (96). This was primarily explained to be due to expression of cardiac TnT in skeletal muscle in end-stage renal disease patients (97). Thus, one potential source of bias was the use of 'first-generation' TnT assays in the earliest studies. However, in subsequent studies the results on cardiac TnT expression in skeletal muscle of patients with end-stage renal disease have been controversial (98-100). Recently, myocardium has been regarded as the source of troponins also in renal failure, but the mechanisms are not totally understood (98,101,102).

In subsequent studies it has been shown that elevation of both cardiac TnT and TnI may occur in end-stage renal disease. In several studies the proportion of patients with elevated TnT (17-75%) has been greater than the proportion of those with elevated TnI (4-19%) (97,103,104). However, the proportions are influenced by the selection of assays (*e.g.*, greater proportions in 'first-' than 'second-generation' assays for TnT), cut-off limits, and patient materials (97,105). The probability of elevation seems to be greatest in acute renal failure (105). Among patients with chronic renal failure the greatest probability is seen in patients on regular haemodialysis (105). In several studies detectable levels of troponins predicted adverse outcome in patients with renal failure (104,106-110). However, in some studies no predictive value of troponins was shown (111-113).

Interpretation of troponin values in other special clinical situations

Cardiac TnT and TnI are highly sensitive indicators of myocardial cell damage. They are also regarded as cardiac-specific. However, they are not specific to MI, but are also elevated as a result of myocardial injury due to mechanisms other than ischaemia. Both TnT (114) and TnI (115) have been reported to be better than CK-MB in detecting myocardial cell damage in patients with myocarditis.

Cardiac troponins may also be elevated in other clinical situations in which myocardial injury may develop through different mechanisms. A large pulmonary embolism often leads to arterial hypoxaemia and this may result in myocardial injury. Thus, it is not a surprise that

Giannitsis *et al.* (116) found that TnT was elevated in 32% of 56 patients with pulmonary embolism. All of them had moderate to large pulmonary embolism, and moreover, TnT was found to be an independent predictor of 30-day mortality (116). In another study by Meyer *et al.* (117) elevated cardiac TnI was found to associate with right ventricular dilatation. Elevated cardiac TnT and TnI levels have also been detected in intensive care unit patients admitted with non-cardiac diseases, *e.g.*, sepsis and septic shock (118-120). Left ventricular dysfunction (120), hypotension and arrhythmias (118,119,121) were associated with troponin elevations in such patients. Elevated troponin levels were also associated with increased morbidity and mortality (118,119). Myocardial injury was often unrecognised clinically, and it is controversial whether elevated troponin is a consequence of myocardial necrosis or reversible myocardial depression (122,123).

Patients with an acute neurological illness often have ECG abnormalities suggestive of myocardial injury. The classic ECG changes of deep T-wave inversion occur most often after subarachnoid haemorrhage. Patients with clinical, ECG or echocardiographic evidence of cardiac injury (19% of all patients) also had elevations of the markers of myocardial injury in a study by Dixit *et al.* (124). However, 29% of the patients with elevated TnI did not have any other evidence of myocardial injury (124). Pre-existing CHD as well as risk factors for CHD were more common in patients with elevated TnT values (124). Thus, such elevations may be related to the presence of underlying CHD exacerbated by the stress of a neurological event (124). But because ECG abnormalities and elevations of markers of myocardial injury may occur also in young patients with subarachnoid haemorrhages, another proposed mechanism is cathecolamine-mediated myocardial injury with microscopic myocardial lesions (125,126).

Detectable levels of cardiac TnT and TnI, but usually not increased to greater than the URL, have been found in patients with chronic severe heart failure (127-131). From one of these studies, by La Vecchia *et al.* (128), heart failure due to myocardial ischaemia was excluded. In another study by Missov *et al.* (130) high troponin levels were detected in patients with idiopathic dilated cardiomyopathy. Detectable troponin levels were found to correlate negatively with ejection fraction (130) and to be associated with adverse prognosis (129,131). Possible mechanisms for the release of cardiac troponins in congestive heart failure are recurrent episodes of ischaemia in patients with CHD and heart failure, ventricular remodelling, abnormalities in coronary microcirculation, and reduced coronary reserve (132). In a study by Anderson *et al.*, myocardial TnT isoform expression was found to be changed in adults with severe heart failure to partially resemble the foetal expression of TnT isoforms (133).

The diagnosis of MI may be difficult in patients with primarily successful resuscitation from out-of-hospital cardiac arrest. Skeletal muscle trauma, prolonged arrhythmia and hypoxaemia confound the interpretation of markers of myocardial injury (134). In a study by Müllner *et al.* (135) minor elevations of TnT were detected within 12 h from the attack also in 12/15 (80%) of patients in whom MI was ruled out. The authors regarded myocardial damage related to cardiac arrest and resuscitation efforts as a possible mechanism. However, in a study with a bigger study population by the same authors (136) the release of cardiac TnT seemed to be only associated with MI, but not with the duration of chest pain compressions, or with the number of defibrillations administered. In these two studies the diagnosis of MI was confirmed or ruled out by means of typical ECG changes, myocardial scintigraphy or autopsy. In a study by Grubb *et al.* (137) TnT was elevated to greater than the URL in 29/34 (85%) of patients in whom MI was ruled out on the basis of ECG only. On the other hand, the data are more consistent on patients with cardioversion for atrial fibrillation or other supraventricular tachyarrhythmias. In most studies direct current transthoracic cardioversion caused no elevation of troponin levels (138-143), but in some studies slight increases were detected (144,145). However, substantial elevation of either of the cardiac troponins after transthoracic cardioversion suggests causes unrelated to electrical cardioversion (134).

The data are scarce on the effect of arrhythmias on troponin levels. In severely ill intensive care unit patients tachycardia (>120 beats / min) or cardiac arrhythmias were associated with higher troponin concentrations (121). High heart rates and arrhythmias shorten the diastolic phase of the cardiac cycle and can precipitate ischaemia. The subendocardium is particularly prone to ischaemic injury under such conditions (121). In a study by Runsiö *et al.* (146) sustained but hemodynamically stable ventricular tachycardia induced in connection with an electrophysiologic study and terminated by overdrive pacing did not cause TnT elevation. In patients who required immediate defibrillation due to ventricular fibrillation or hemodynamically unstable ventricular tachycardia a minor increase of TnT within the URL was detected (146). Intraoperative testing of implantable cardioverter defibrillators with multiple shocks may cause elevations of cardiac troponins and CK-MBm (146-148). The release is usually short and depends on the number and cumulative energy of the shocks (148).

Cardiac troponin measurement has been shown to be a sensitive and specific method for the diagnosis of perioperative MI in connection with noncardiac surgery (149). However, in cardiac surgery patients troponins are detectable in the circulation even in the absence of ischaemic injury (150). Surgical trauma can cause direct cellular damage and lead to release of troponins. Moreover, during the aortic clamping and cardiac arrest a certain degree of

myocardial damage is common (151). It is very difficult to distinguish perioperative MI from myocardial injury due to surgical trauma. Studies on coronary artery bypass grafting (CABG) patients have shown that in patients with perioperative MI peak levels of troponins are on average higher than in patients without MI (150,152-154). However, the surgical technique affects the amount of myocardial injury. After off-pump or minimally invasive direct coronary artery bypass less myocardial damage was detected by TnT and TnI compared with conventional CABG (155). In a study by Greenson *et al.* (154) high TnI levels after cardiac surgery predicted a complicated recovery. However, it is impossible to compare the results of these studies reliably due to the variable definitions of perioperative MI and the problems with TnI assay standardisation. Thus, at the moment there is no consensus on discriminatory values for cardiac troponins in the diagnosis of perioperative MI in connection with cardiac surgery.

Minor elevations of cardiac markers are quite common after PCIs. Ravkilde *et al.* detected elevation of TnT to >0.2 μg/l (by 'first-generation' assay) in 13% of 23 patients with stable angina pectoris after visually successful PCI (156). In more recent studies with larger study populations and by 'second-generation' assays, TnT was elevated to >0.1 μg/l in 18-30% (157-161). In a study by Johansen *et al.* (158) 24% of the patients still had TnT elevated on the fourth day after the PCI, indicating an irreversible myocardial injury. By different assays TnI elevations were detected in 22-34% of patients after PCI (159,160,162). In different studies CK-MBm was elevated to greater than the URL in 11-37% (158-160,163,164), and to greater than three times the URL in 6-18% of patients (163,164). New Q waves developed in only 0.6% of 7147 patients after PCI in a study by Stone *et al.* (164). Thus, ECG is rather insensitive in detecting these minor injuries. Generally, cardiac marker elevations are detected more often after stent placement than after angioplasty alone (157,162,164). In some cases an unambiguous side branch occlusion is detected, but sometimes there is no obvious reason for marker elevation. Possible causes are microembolism, coronary vasospasm, disturbances of microvascular circulation, inflammatory reactions to the stent material, and activation of nociceptive afferent nerves along distended coronary arteries after stenting (165). More marked elevations of TnI to greater than three times the URL, and concordant elevation of TnI and CK-MBm have been found to be associated with increased risk of mortality and other major in-hospital complications but not with adverse medium-term prognosis (160,162,166).

During foetal development the cardiac TnT gene, but not the cardiac TnI gene, has been found to be transiently expressed in skeletal muscle (133). Increased cardiac TnT levels have been reported in polymyositis and dermatomyositis without evidence of cardiac involvement (167). Cardiac TnT has also been detected in samples of regenerating skeletal muscle from

patients with polymyositis and Duchenne muscular dystrophy (168,169), although this has not been confirmed in all studies (170).

Point-of-care assays

Point-of-care or bedside assays have been developed to detect CK-MBm, myoglobin, cardiac TnT and TnI in small specimens of whole blood. They have been recommended for use in emergency departments, chest pain units, and coronary or intensive care units where treatment decisions are made (171). These rapid assays allow shortening the turnaround time of specimen collection, transportation to clinical laboratory, and data reporting (171). Stubbs and Collinson found that the turnaround time could be reduced from the median of 72 min in clinical laboratory testing to the median of 20 min in point-of-care testing (172). Point-of-care testing provides the results more rapidly than the quantitative assays but it does not overcome the biological time courses and diagnostic limitations of the markers (Table 1). Thus, in the early hours after the onset of symptoms negative test results do not exclude MI. Patients with initially negative assays must be retested after an appropriate time has passed. Point-of-care assays are useful in detecting and ruling out MIs in hospitals or health centres that have adequate possibilities to observe and treat patients, but where 24-h quantitative determinations are not available.

Most of the currently available assays require only 150-200 µl whole blood, require little or no instrumentation, and give the result within 10-30 min (171). Most tests are qualitative immunochromatographic assays in which the results are read visually. However, near the detection limit of the assay, interobserver variability may influence interpretation of the results (171,173). In some of the newest assays a strip reader device gives more reliably repeatable quantitative or semiquantitative results (171,173).

Currently, several point-of-care assays are available detecting either a single cardiac marker or their different combinations (171). Detection of myoglobin and CK-MBm have been combined in an assay to compensate the early sensitivity but non-specificity by the better cardiac-specificity. This assay was initially regarded as promising in excluding MI in the early hours, but false negative results when compared to quantitative results limit its use (174). A combination of TnI, myoglobin and CK-MBm point-of-care assays recognised the patients with MI earlier than a single marker (CK-MBm, TnT or TnI) determined in a laboratory (175). The first cardiac TnT rapid assay had the detection limit of 0.30 µg/l (176). Subsequently, the detection limit was improved to 0.18 µg/l (177,178), and finally to the present 0.10 µg/l. Our own experience (179), in accordance with that of others (180,181), is

that concordance between the results of quantitative and point-of care assays for cardiac troponins is good.

Biochemical markers in ruling out myocardial infarction

According to de Winter *et al.* (182), myoglobin was better than TnT and CK-MBm in ruling out MI from 3 to 6 h after the onset of symptoms. However, the maximal negative predictive value (NPV) of myoglobin was only 89%. The NPV of CK-MBm was 95% at 7 h, 98% at 12 h, and reached the maximum of 99% at 16 h after the onset of symptoms. The NPV of TnT was 92% at 12 h and reached the maximum of 97% at 16 h. In another study by de Winter *et al.* (183) on patients admitted to chest pain unit with symptoms of less than 5-h duration, normal CK-MBm on admission and at 7 h correctly ruled out MI in 99% of patients. According to Herren *et al.* (184), MI could be ruled out on the basis of 6-h observation, serial measurements of CK-MBm and continuous ST-segment monitoring at the emergency department. Noble recommended in his editorial article (185) that all patients with chest pain at rest and ST-segment elevation or depression, or conduction defects which are thought to be new (*i.e.*, high-risk patients) should be admitted to the coronary care unit without waiting for results of biochemical marker measurements. Patients with a suggestive history, but normal ST-segments or known established conduction or other ECG abnormalities are an uncertain risk group that should undergo serial ECG and TnT monitoring for 12 h following admissions (185). Low-risk patients on the basis of the history or ECG could be monitored for a shorter time.

The relative roles of symptoms, electrocardiogram and biochemical markers in the diagnosis of myocardial infarction

In the WHO MONICA project, chest pain symptoms with the duration of more than 20 minutes and without definite non-cardiac or cardiac non-atherosclerotic cause were regarded as typical symptoms of MI (5). In recent decades the incidence of ACSs has increased in the elderly age groups (186). It has also been noted that the clinical picture of MI in elderly patients is often atypical (187). Atypical symptoms and silent myocardial ischaemia are frequent in elderly patients, as reviewed by Gregoratos (187), and in diabetics, as reviewed by Airaksinen (188). Goldstein *et al.* (189) found that patients hospitalised with nonpainful MI were more likely to experience late cardiac death or congestive heart failure than patients with typical chest pain, even among diabetics and within elderly age categories.

Symptoms compatible with myocardial ischaemia have conventionally been included in the diagnostic criteria of MI. Even in the era of cardiac troponins symptoms have relevance in differentiating between MI and other diseases with elevated troponins (p 29-33). In clinical practice, continuous or recurrent chest pain is an indication for urgent coronary angiography, as recommended in the ESC and the ACC / American Heart Association (AHA) guidelines for the management of UAP / non-ST-segment elevation ACSs (190,191).

Stubbs and Collinson reported that only 10% of patients seen in a district general hospital in the United Kingdom with suspected ACSs present with ST-segment elevation (172). Thus, biochemical markers were essential in confirming or ruling out the diagnosis of MI in 90% of these patients (172). Of 775 patients admitted to the coronary care unit with symptoms suggestive of MI, 107 (14%) had a normal ECG on admission and 73 (9%) had only minimal nonspecific changes, as reported by Slater *et al.* (192). The final diagnosis was MI in 10% of the patients with normal admission ECG, in 8% with minimal changes, and in 41% of patients with frankly abnormal ECG (192). Rehnberg found a normal ECG on admission in 5% of men with first definite MI and in 21% of men with first probable MI (193). The prevalence rates of normal ECG on admission in women were only 0% and 3%, respectively. However, among the patients with previous MI a normal ECG on admission was noted in only 2% of patients. All of them were men with probable MI or no MI. In a series of 100 patients with suspected MI, Vikenes *et al.* found a diagnostic sensitivity of admission ECG for MI of only 52% compared with the WHO MONICA criteria (194).

In a series of 3123 North Karelian patients with a definite MI by the first WHO criteria, the proportion of definite ECG changes was higher in patients with their first MIs than in those with recurrent MIs, as reported by Mustaniemi *et al.* (195). Moreover, the proportion of definite ECG changes decreased with age in both men and women, even among the patients with recurrent MIs. Approximately 90% of both men and women in all age groups had typical chest pain symptoms, and an equal proportion had serum enzyme elevations. Thus, the contribution of elevated enzymes was particularly important in elderly patients and those with recurrent MIs (195).

Generally, the most severe cases of MI can be detected even by ECG only, or by combining ECG with rather nonspecific enzymes, such as ASAT and LD. The introduction of total CK and CK-MB isoenzyme activities led to recognition of less severe cases, and increase in the number of MIs detected, as demonstrated in the Minnesota Heart Survey in the 1980s (196). In the survey CK and CK-MB activities increased the age-adjusted attack rate of MI by 17% and 24%, respectively, when added to the diagnostic algorithm based on chest

pain symptoms, ECG changes, and activities of ASAT or LD, and autopsy findings in fatal cases (196).

Recommendations for diagnostic criteria of myocardial infarction

There have been several attempts to formulate international standards for the diagnosis of MI for clinical practice, epidemiological studies, and clinical trials. During the last decades, the role of biochemical markers in the diagnosis of MI has been emphasised. This has led to new attempts to define MI.

The first WHO criteria

The first attempt to apply standardised criteria for the diagnosis of MI was performed in 1971 in connection with a study of ischaemic heart disease registers co-ordinated by the WHO (4). According to these criteria the diagnosis of definite MI requires:

(1) unequivocal serial changes in ECG, or
(2) typical or atypical history, together with equivocal ECG and elevated enzymes, or
(3) typical history and elevated enzymes with ECG negative or not available, or
(4) fatal cases with naked-eye appearance of fresh myocardial infarction and/or recent coronary occlusion found at autopsy.

The diagnosis of a possible MI was made

(1) in living patients with typical history, whose ECG and enzyme results do not place them in category of definite MI and in whom there is not good evidence for another diagnosis for the attack, or
(2) in fatal cases (not in category of definite MI) where there is no good evidence for another cause of death, clinically or at autopsy
    (a) with typical or atypical history of pain, or
    (b) without a history of pain, but with evidence of chronic coronary occlusion, or stenosis, or old myocardial scarring at autopsy, or
    (c) with clinical evidence of chronic ischaemic heart disease.

The local ranges of normal, equivocal and abnormal enzymes were used in each of the registers. The registers also defined the enzyme tests in co-operation with their laboratories. It was recommended to use a series of enzyme recordings where transient elevated levels could be observed. However, the occurrence of an abnormal or equivocal level on a single estimation in the period after the acute episode was also accepted for coding.

The 'clinical' International Society and Federation of Cardiology / WHO criteria

Standardised terminology and diagnostic criteria of MI were first applied for epidemiological studies. In 1979 the nomenclature and criteria for diagnosis of ischaemic heart disease were defined by the International Society and Federation of Cardiology (ISFC) and WHO to promote comparability of trials and other studies (197).

According to this recommendation a definite MI could be diagnosed in the presence of unequivocal ECG changes or unequivocal enzyme changes; the history may be typical or atypical. A possible MI could be diagnosed when serial, equivocal ECG changes persisted for more than 24 h, with or without equivocal enzyme changes; the history may be typical or atypical. Unequivocal enzyme change consisted of serial change, or initial rise and subsequent fall of the serum level. The change had to be properly related to the particular enzyme and to the delay time between onset of symptoms and blood sampling. Equivocal enzyme change consisted of an enzyme pattern where an initially elevated level was not accompanied by subsequent fall – the curve of enzyme activity was not obtained.

The WHO MONICA criteria

In 1982 the WHO initiated an international collaborative study, the MONICA project, with the aim to measure the trends in cardiovascular mortality and CHD and cerebrovascular mortality and the relationship of these trends to changes in major cardiovascular risk factors and medical care (5,198,199).

According to the original MONICA criteria (5,198), diagnosis of a definite MI was made with:

(1) definite ECG, or
(2) typical or atypical or inadequately described symptoms, together with probable ECG and abnormal enzymes (> 2 x URL), or
(3) typical symptoms and abnormal enzymes (> 2 x URL) with ischaemic or non-codable ECG or ECG not available, or
(4) fatal cases with naked-eye appearance of fresh myocardial infarction and/or recent coronary occlusion found at autopsy.

The diagnosis of a possible MI was made

(1) in living patients with typical symptoms and whose ECG and enzyme results do not place them in category of definite MI and in whom there is not good evidence for another diagnosis for the attack, or

(2) in fatal cases (not in category of definite MI) where there is no good evidence for another cause of death, clinically or at autopsy

   (a) with typical, atypical or inadequately described symptoms, or

   (b) without typical, atypical or inadequately described symptoms but with evidence of chronic coronary occlusion or stenosis or old myocardial scarring at autopsy, or

   (c) with a good history of chronic ischaemic heart disease such as definite or possible MI, or coronary insufficiency or angina pectoris in the absence of significant valvular disease or cardiomyopathy.

In these criteria, designed for an epidemiological study, a cut-off value of > 2 x URL was chosen for the enzymes. Since there was substantial variation in enzyme determinations in the different MONICA centres, the high cut-off value was chosen to reduce the number of false positive cases.

The FINMONICA (the Finnish contribution to the MONICA project) criteria are a modification of the original MONICA criteria. In the MONICA criteria typical symptoms are sufficient to lead to the diagnosis of possible MI in nonfatal cases. However, the FINMONICA criteria define nonfatal possible MI more strictly (198,200):

(1) typical symptoms and probable ECG or equivocal enzymes (> 1 x, ≤ 2 x URL), or

(2) typical symptoms and normal ECG and abnormal enzymes (> 2 x URL), or

(3) atypical or inadequately described symptoms and probable ECG and equivocal enzymes.

In fatal cases possible MI was defined similarly in both criteria. The reason for making these modifications was that the original MONICA diagnostic category possible MI was defined rather loosely and comprised a large proportion of suspected acute CHD events other than those getting the clinical diagnosis of possible MI (198). Moreover, an attempt was made to reduce the variation in the enzyme determinations between laboratories of the FINMONICA centres by a standardisation project in the late 1980s.

Redefined criteria of the Joint European Society of Cardiology / American College of Cardiology Committee

Modern clinical practice, clinical trials and other studies and the introduction of the new sensitive and specific markers of myocardial injury have led to a need for more precise

definition of MI than defined by the WHO. The redefined criteria of MI by the Joint ESC/ACC Committee were published in 2000 (12). The leading concept in the redefinition is that any amount of myocardial necrosis caused by ischaemia should be regarded as a MI (12). Thus, a patient who was formerly diagnosed as having severe UAP might be diagnosed as having had a small MI, according to the redefined criteria (12).

Either one of the following criteria satisfies the diagnosis for an acute, evolving or recent MI:

(1) typical rise and gradual fall (troponin) or more rapid rise and fall (CK-MB) of biochemical markers of myocardial necrosis with at least one of the following:
   (a) ischaemic symptoms;
   (b) development of pathologic Q waves on the ECG;
   (c) ECG changes indicative of ischaemia (ST-segment elevation or depression); or
   (d) coronary artery intervention (*e.g.*, coronary angioplasty).

(2) pathologic findings of an acute MI at autopsy.

Any one of the following criteria satisfies the diagnosis for established MI:

(1) development of new pathologic Q waves on serial ECGs. The patient may or may not remember previous symptoms. Biochemical markers of myocardial necrosis may have normalised, depending on the length of time that has passed since the infarct developed.

(2) pathologic findings of healed or healing MI at autopsy.

On the other hand, the following are defined as biochemical indicators of myocardial necrosis:

(1) maximal concentration of TnT or TnI exceeding the decision limit (99^{th} percentile of the values for a reference control group) on at least one occasion during the first 24 h after the index clinical event,

(2) maximal value of CK-MB (preferably CK-MBm) exceeding the 99^{th} percentile of the values for a reference control group on two successive samples, or maximal value exceeding twice the URL for the specific institution on one occasion during the first hours after the index clinical event. Values for CK-MB should rise and fall; values that remain elevated without change are almost never due to MI. In the absence of availability of a troponin or CK-MB assay, total CK (greater than two times the URL) or the B fraction of CK may be employed, but these are considerably less satisfactory than CK-MBm.

To harmonise the diagnostic practice throughout Finland, a national recommendation on the diagnostic criteria of MI was also issued in 2000, following the outlines given by the recommendations of the Joint ESC/ACC Committee (201).

The redefined criteria of MI by the Joint ESC/ACC Committee have been criticised (202), especially for not covering early fatal cases (and fatal cases without autopsy) and nonfatal cases in which biochemical tests are partial, delayed, missing or curtailed. The increase in the incidence of MI due to detection of smaller and smaller injuries by cardiac troponins has been thought to cause "labelling" and affect employment and insurance. Therefore, the term "myocardial injury" has been proposed, instead of MI, for events involving only minor damage (202). Furthermore, the differences between the definitions of MI (and UAP) in many clinical trials and the new recommendations may cause confusion in adapting pharmacological therapies for clinical practice (203). This criticism has led to further reconsideration of the definition of MI, but the conclusions of the deliberations are not yet available.

PROGNOSTIC FACTORS IN ACUTE CORONARY SYNDROMES

In acute phase, ACSs may be divided on the basis of initial ECG findings into ST-elevation MI (or imminent Q-wave MI) and non-ST-elevation MI / UAP. On hospital admission and early hours of the hospital care, treatment decisions on thrombolysis and other drug therapies as well as acute revascularisation procedures are based on clinical features and ECG findings. After the acute phase, the final diagnoses of the ACSs, on the basis of ECG changes and biochemical markers of myocardial injury, are Q-wave MI, non-Q-wave MI and UAP.

In stable CHD, angina pectoris usually results from inability of stenosed coronary arteries to increase oxygen delivery to myocardium when oxygen demand is increased. In ACSs blood flow in coronary arteries is abruptly reduced, usually due to a thrombotic process. The presence of local and systemic thrombogenic factors may modify the extent and duration of thrombus deposition (1). ACSs comprise a diagnostic and prognostic continuum from unstable angina pectoris (UAP) with no detectable myocardial injury to non-Q-wave MI and to Q-wave MI.

Fuster *et al.* (1) have presented the common underlying mechanisms of ACSs and the factors leading to different manifestations. In UAP, a relatively small fissuring or disruption of an atherosclerotic plaque may lead to reduction on coronary blood flow, resulting in exacerbation of angina. The thrombus is usually labile, causing temporary vessel occlusion and recurrent episodes of ischaemia during exercise or even at rest. Vasoconstriction due to release of vasoactive substances and endothelial vasodilator dysfunction may further impede coronary flow. In non-Q-wave MI, the plaque damage or thrombogenic risk factors are worse than in UAP, resulting in more persistent thrombotic occlusion. Non-Q-wave MI develops if

occlusion is followed by reperfusion within the first hours, resolution of vasospasm, or perfusion of jeopardised myocardium by collateral vessels. In Q-wave MI, plaque disruption may be associated with deep plaque damage and ulceration, or the overall thrombogenic risk profile may be unfavourable. This results in the formation of a persistent and occlusive thrombus, leading to an abrupt cessation of myocardial perfusion and eventually to myocardial necrosis. Q-wave MI usually develops in cases of incomplete reperfusion after thrombotic occlusion of the vessel. Plaque disruption and abrupt thrombosis may also lead to sudden coronary death due to ischaemia and fatal ventricular arrhythmias. Potential contributing factors are platelet microemboli and absence of collateral blood flow.

Prognostic factors in ST-elevation / Q-wave myocardial infarction

Previously, only the most severe cases of MI were recognised. Due to large myocardial injuries, these patients had a very poor prognosis. The extent of MI as well as the presence and extent of a previous myocardial injury have an effect on prognosis. Both short-term prognosis and long-term survival after MI are known to depend on left ventricular function, remaining ischaemia in the myocardium, and susceptibility to serious ventricular arrhythmias (204).

The essential indicators of an adverse prognosis in patients with ST-elevation / Q-wave MI are presented in Table 2. Left ventricular dysfunction is closely related to infarct size, and even in the thrombolytic era it is a major predictor of mortality after MI (205). Postinfarction angina with recurrent spontaneous or provoked ischaemia indicates the presence of jeopardised myocardium, and thus, a less favourable prognosis (204). Residual myocardial ischaemia may be detected in connection with a predischarge exercise test, or a maximal exercise test or a pharmacological stress test several weeks after the acute event. Perfusion imaging, which detects reversible myocardial perfusion defects, may be helpful for risk stratification after MI in patients with uninterpretable ECGs, but is not recommended for routine use (204).

In addition to playing a central role in the decision making for management of patients in the acute phase of MI, the ECG provides important prognostic information. Q waves in multiple leads are a sign of a large injury and a poor prognosis. Patients with anterior wall MI have worse short- and long-term prognosis than those with inferior MI, even when corrected for infarct size (206).

Table 2. Indicators of increased risk in ST-elevation / Q-wave myocardial infarction, adapted from Antman *et al.* (204), Alexander *et al.* (211) and Michaels *et al.* (3).

History
Advanced age
Diabetes mellitus
Prior angina pectoris
Previous MI
Clinical presentation
Recurrent spontaneous or provoked ischaemia or reinfarction after MI
Decompensated congestive heart failure
Sustained ventricular tachycardia or ventricular fibrillation more than 48 h after the acute event
Electrocardiogram
Multiple leads showing ST-segment elevation or Q-waves
Anterior *vs.* inferior wall MI
Right ventricular infarction complicating inferior infarction (ST-segment elevation in V4R lead)
Persistent advanced heart block (Mobitz type II second-degree or third-degree atrioventricular block)
New intraventricular conduction abnormality (bifascicular or trifascicular)
Persistent horizontal or downsloping ST-segment depression
ST-segment depressions in anterior leads in patients with inferior infarction
Atrial arrhythmias
Depressed heart rate variability
Increased QT-interval dispersion
Abnormal late potentials on a signal-averaged electrocardiogram
Depressed baroreflex sensitivity
Cardiac markers
Magnitude of the increase in troponin T or I or CK-MBm
Increased C-reactive protein
Increased B-type natriuretic peptide (212)
Non-invasive and invasive tests
Presence and severity of left ventricular dysfunction and size of myocardial injury (ejection fraction, ventriculography, perfusion imaging)
Anterior wall motion abnormality
Angina pectoris, development of ST-segment depression, impaired exercise capacity, or insufficient systolic blood pressure response in exercise test or pharmacological stress test
Reversible myocardial perfusion defects
Haemodynamically significant mitral regurgitation
Left main or multivessel CHD in coronary angiogram

In experimental studies the extent of infarction has been found to affect threshold to ventricular fibrillation (207). The incidence of arrhythmias is highest during the first hours after the onset of symptoms of MI. According to the FINMONICA study, about 70% of deaths from MI in men and about 50% in women occur before hospital admission (208,209). Usually these patients die from ventricular fibrillation. Possible mechanisms of arrhythmias in connection with MI are electrical instability, especially micro-reentry due to electrical inhomogeneity of the electrical characteristics of ischaemic myocardium, and increased activity of the autonomic nervous system (204). The susceptibility to serious arrhythmias and increased risk of death are reflected in ventricular ectopic activity and other indicators of electrical instability, such as reduced heart rate variability, depressed baroreflex sensitivity, and abnormal signal-averaged ECG (204,210). After the acute phase the myocardial scar may also act as an arrhythmogenic focus.

Prognostic factors in non-ST-elevation / non-Q-wave myocardial infarction and in unstable angina pectoris

It is sometimes difficult to determine if a patient has myocardial injury or not. Due to low detection limits of sensitive biochemical marker assays, such as cardiac troponins, smaller and smaller injuries may be detected. Thus, the borderline between UAP and non-ST-elevation / non-Q-wave MI is becoming less and less sharp. Despite the attempt of the Joint ESC/ACC Committee to redefine and standardise the diagnosis of MI (12), the consensus is not complete (202). Furthermore, the new definitions of ACSs have been adopted in trials as well as in clinical practice rather slowly.

The incidence of non-Q-wave MI has increased during the past two decades while the incidence of Q-wave MI has declined, as found in the Worcester Heart Study (213). This is partially explained by adoption of thrombolytic therapy into wide use. In non-Q-wave MI the myocardial injury is usually less extensive, and in-hospital mortality somewhat lower than in Q-wave MI (213). However, a larger degree of jeopardised myocardium in non-Q-wave MI leads to a higher incidence of reinfarction and recurrent angina. In Worcester Heart Study, long-term mortality was slightly but not significantly lower in Q-wave MI than in non-Q-wave MI (213). Two-year mortality rates decreased in Q-wave MI, but only non-significantly in non-Q-wave MI within 20 years.

Clinical prognostic factors

The essential indicators of an adverse prognosis in patients with non-ST-elevation / non-Q-wave MI and in UAP are presented in Table 3. Especially, recurrence of chest pain, dynamic ST-segment changes, elevated levels of cardiac troponins, and detection of a thrombus in coronary angiogram are markers of thrombotic risk, and therefore of acute risk (190). Furthermore, age, diabetes, history of prior MI, increased CRP (214), left ventricular dysfunction and the extent of CHD are markers of underlying disease and long-term risk (190). Antman *et al.* (215) developed a scheme, the Thrombolysis in Myocardial Infarction (TIMI) risk score, for patients with UAP or non-ST-elevation MI to predict a patient's risk of death and ischaemic events, and to guide therapeutic decisions. In these patients with generally smaller extent of myocardial injury than in classical Q-wave MI, predictive variables were: (1) age 65 years or older, (2) at least three risk factors for CHD, (3) prior coronary stenosis of 50% or more, (4) ST-segment deviation on ECG at presentation, (5) at least two anginal events in prior 24 h, (6) use of aspirin in the prior seven days ('aspirin failures'), and (7) elevated cardiac markers.

In several studies ST-segment changes but not T-wave changes were found to have an independent prognostic value in ACSs (216-219). However, in a recent study on patients participating in the ThRombin Inhibition in Myocardial ischaemia trial (TRIM), patients with T-wave abnormalities without ST-segment deviations had a higher 30-day risk of death, MI, and refractory angina than patients without T-wave abnormalities (220).

Postinfarction angina and recurrent ischaemia indicate the presence of jeopardised myocardium. Nowadays postinfarction angina often leads to early interventions. Invasive treatment within five weeks after discharge was found to reduce the incidence of reinfarctions, admissions due to unstable angina, and the prevalence of stable angina compared with conservative treatment in the DANish trial in Acute Myocardial Infarction (DANAMI) (221). In the DANAMI study all patients demonstrated postinfarction ischaemia (>24 h after receiving thrombolysis) spontaneously or during a predischarge symptom-limited exercise test (221).

Recently, questions have been raised regarding the improvement in the prognosis of patients by using active invasive treatment strategies. Thus, routine early invasive and conservative (or selectively invasive) strategies in UAP or non-ST-elevation MI have been compared in four randomised, controlled trials: TIMI IIIB (222), Veterans Affairs Non-Q-Wave Infarction Strategies in Hospital (VANQWISH) (223), Fragmin and Fast Revascularization During Instability in Coronary Artery Disease (FRISC II) (224), and Treat

Table 3. Indicators of increased risk in non-ST-elevation / non-Q-wave myocardial infarction and in unstable angina pectoris, adapted from Antman *et al.* (204), Braunwald *et al.* (226), Waters (227), the European Society of Cardiology guideline report (190), and the American College of Cardiology / American Heart Association 2002 guideline update (191).

History
Advanced age
Diabetes mellitus
Prior peripheral vascular disease
Prior cerebrovascular disease
Clinical presentation
Recurrent ischaemia at rest or with low-level activities despite intensive anti-ischaemic therapy
Braunwald Class III (acute pain at rest) (228-230)
Braunwald Class C (post-MI UAP) (228-230)
Signs of serious left ventricular dysfunction
Hypotension
Haemodynamic instability
Major arrhythmias (repetitive ventricular tachycardia or ventricular fibrillation)
Electrocardiogram
New or presumably new ST-segment depression
(New T-wave inversions)
Left bundle branch block
Cardiac markers
Increased cardiac troponin T or I (or CK-MBm)
Increased C-reactive protein
Increased B-type natriuretic peptide (212,231)
Non-invasive and invasive tests
ST-segment depression at low exercise levels in exercise test or reversible perfusion defects in perfusion imaging (quantity of jeopardised myocardium) in initially stabilised patients
Number of vessels with ≥50% diameter stenosis and the presence of left main stenosis in coronary angiogram
Thrombus in coronary angiogram
Presence and severity of left ventricular dysfunction (*e.g.*, ejection fraction less than 40%)

Angina with Aggrastat and Determine Cost of Therapy with an Invasive or Conservative Strategy (TACTICS)-TIMI 18 (225). The most relevant to current clinical practice are the FRISC II (N=2457) and TACTICS-TIMI 18 (N=2220) studies. In intermediate- and high-risk patients in both of these studies, the rates of death or non-fatal MI during six months were significantly reduced in the group of early invasive strategy compared with the group of conservative strategy. However, all four trials support a more conservative treatment for low-risk patients without ECG changes or elevation of biochemical markers. According to the ACC/AHA 2002 guideline update for the management of patients with UAP and non-ST-elevation MI (191) an early invasive strategy is recommended for patients without serious comorbidity and who have any of the defined high-risk indicators which are included in Table 3. In hospitalised patients without any high-risk indicators and without contraindications for revascularisation, either an early conservative or an early invasive strategy may be offered (191).

Markers of myocardial injury as prognostic factors

In early studies, roughly one-third of patients with clinical features of UAP were found to have elevated cardiac troponins (8,9,232). However, in different studies the definitions of UAP have varied, and in some of them actual non-Q-wave MIs have been included. Naturally, this affects the proportion of patients with elevated troponins. Elevated concentrations of cardiac troponins, both TnT and TnI, have been found to be associated with an adverse prognosis in patients with unstable CHD either not fulfilling the conventional diagnostic criteria for frank MI (8,9,11,233-236) or comprising patients with UAP and non-Q-wave (or non-ST-elevation) MI (70,237-241). Moreover, ST-elevation MIs were also included in some studies in which troponins were associated with adverse outcome (242,243). The prognostic value of cardiac troponins was also found in patients with strictly defined UAP (244-247). The study by Hamm *et al.* (8) concentrated on end-points during hospitalisation. In other studies the median follow-up time has varied from three weeks to nearly three years after hospital admission. Most studies have examined the prognostic value of each clinical factor separately rather than in multivariate analyses. However, some studies have shown that cardiac troponins provide independent prognostic value in ACS (235,238,239,242,243,248). As mentioned before, ‘microinfarction’, ‘minimal myocardial damage’, or ‘minimal myocardial injury’ was regarded as an ACS with mildly elevated cardiac troponin level but with CK-MBm or CK-MB activity within normal limits. Cardiac troponins have been shown to predict adverse outcome also in these patients (245,246).

Furthermore, similar findings have been reported on the predictive value of point-of-care assays (181,249-251). In two meta-analyses, by Olatidoye *et al.* (252) and by Heidenreich *et al.* (253), TnT and TnI showed similar prognostic value for death or nonfatal MI.

In the FRISC study, there was an increase in the five-month risk of cardiac death or nonfatal MI from lower to higher quintiles of TnT in unstable CHD (70). Moreover, an additive prognostic value of TnT determinations and pre-discharge exercise tests was found (237). The risk of cardiac death or nonfatal MI during the follow-up of five months was highest (>15%) in patients with intermediate-risk exercise response with maximal TnT ≥0.20 μg/l, and high-risk exercise response. Patients with low-risk exercise response together with maximal TnT <0.06 μg/l constituted the group with the lowest (<1%) risk. Importantly, some recent trials have suggested that elevated troponin levels identify high-risk ACS patients who benefit the most from low-molecular-weight heparin (248,254), glycoprotein IIb/IIIa inhibitor abciximab (255) and tirofiban (256). In the FRISC II (224,257) and TACTICS-TIMI 18 (258) trials, the patients with UAP or non-ST-elevation MI and elevated troponins benefited the most from early invasive treatment strategy. It has also been shown in several studies that patients with unstable CHD and elevated TnT or TnI had more widespread CHD and more often had complex lesions and a visible thrombus in the culprit vessel than those without elevated troponins (259-262).

In several early studies the prognostic value of CK-MBm was more controversial than that of cardiac troponins in ACSs without frank MI (233,236,263,264). In a more recent study by McErlean *et al.* (265), the prognostic values for in-hospital adverse events were equal for TnT and CK-MBm in patients with acute chest pain of suspected cardiac origin. In a study by Newby *et al.* (266), the TnT predicted long-term mortality better than CK-MBm in low- to moderate-risk patients admitted to a chest pain unit with normal or nondiagnostic ECGs.

INFLAMMATION AND CORONARY HEART DISEASE

Virchow published his theory of atherosclerosis as an inflammatory rather than degenerative disease already in 1856 (267). He studied histopathologic features of an atherosclerotic plaque in all its stages and found that particular blood elements were transferred under the intimal lining of the vessel as the initial phase of plaque formation (267). This theory has become revived during the last decades and currently there is no doubt that inflammation plays in several ways a role in the pathogenesis of atherosclerosis. Once initiated, the process is thought to be regulated and sustained by inflammatory mediators,

such as cytokines, adhesion molecules, and growth factors (268). Activated macrophages, lymphocytes, and mast cells are present in coronary atherosclerotic plaques in patients with ACSs (269-275). Increased concentrations of inflammatory markers have also been found in the circulation of these patients (16,17,276-280).

Current concepts on the role of inflammation in the pathogenesis of atherosclerosis

Pathophysiological observations in humans and animals have led to formulation of the response-to-injury hypothesis of atherosclerosis. However, atherosclerosis has also been found to have an important inflammatory component. It was initially proposed that the first step in the process was endothelial denudation (281), but more recently endothelial dysfunction has been emphasised (14). Possible causes of endothelial dysfunction include conventional and non-conventional risk factors of atherosclerosis: elevated and modified low-density lipoprotein (LDL), cigarette smoking, hypertension, diabetes mellitus, genetic alterations, hyperhomocysteinaemia, infectious microorganisms, and combinations of these or other factors (14). The different forms of injury increase the adhesiveness of the endothelium for leukocytes or platelets, as well as its permeability. Monocytes and T lymphocytes are attached on endothelial cells via specific adhesion molecules and migrate across the endothelial barrier (268). The injury also induces the endothelium to have procoagulant properties and to form vasoactive molecules, cytokines, and growth factors (14).

The formation of an atherosclerotic plaque is a slow process, in which monocyte-derived macrophages and T lymphocytes have a central role. The earliest type of lesion, fatty streak, can be seen even in children. The fatty streak is regarded as an inflammatory lesion consisting of macrophages and lipid (282). Some lipid is ingested by macrophages that transform into foam cells. Lipid oxidation leads to an inflammatory process and stimulates migration and proliferation of smooth muscle cells that become mixed with the area of inflammation to form an intermediate lesion (14,282). Macrophages and lymphocytes migrating from the blood and multiplying within the lesion maintain the inflammation. Activation of these inflammatory cells leads to the release of hydrolytic enzymes, cytokines, chemokines, and growth factors (14). These substances can cause further damage and lead to focal necrosis. Further accumulation of mononuclear cells, migration and proliferation of smooth muscle cells, and formation of fibrous tissue lead to enlargement of the plaque (14). An advanced lesion has a core, rich in foam cells and cellular necrotic debris, covered by a fibrous cap, rich in collagen fibres and smooth muscle cells.

Lipid-rich plaques are the most vulnerable to rupture. Fibrotic lesions or those containing abundantly smooth muscle cells are much more stable. Plaque rupture or ulceration usually occurs at sites of thinning of the fibrous cap, often in the shoulder region infiltrated by macrophages (14). Apparently, thinning of the fibrous cap is due to an inflammatory process with activation of macrophages and mast cells, which release proteolytic enzymes (*e.g.*, metalloproteinases) leading to plaque degradation and rupture (14,283). However, the triggers that shift the chronic stable phase of atherosclerotic disease to an ACS remain elusive (268). Inflammation may have a role in this triggering, but plaque instability may also be triggered by factors unrelated to inflammation (284). In these cases, mechanical plaque rupture, hypercoagulable state, or vasospasm may play a central role (268). The consequences of the plaque rupture and pathogenesis of ACSs are described on pages 40-41.

Infections and coronary heart disease

Conventional risk factors, including hyperlipidaemia, hypertension, diabetes mellitus, smoking, and familial history of premature vascular disease, do not explain the presence of atherosclerosis in a large proportion of patients. Infectious theory of atherosclerosis was first proposed in 1891, when Hutchard noted that infectious diseases of childhood were a potential cause of atherosclerosis, as reviewed by Kuvin and Kimmelstiel (285). In 1906, Weisel and Klotz (286) reported a relation between early atherosclerosis and various infectious pathogens, including salomonella (typhoid fever), streptococci (scarlet fever), and measles virus. However, the findings of Osler on a potential link with acute infections and atherosclerosis published in the early 1900s are more widely known (287). A renewed interest in this hypothesis has been aroused during the last decades. In 1978, Fabricant *et al.* (288) found that chickens infected with Marek disease virus, a herpes-type virus, developed vascular lesions resembling human atherosclerosis. Pesonen and Siitonen found in 1981 that MI was often preceded by respiratory and other infections (289). Saikku *et al.* (290) showed in 1988 that patients with MI or chronic CHD had Chlamydia pneumoniae immunoglobulin (Ig)G or IgA antibodies more frequently than population controls. However, it is controversial whether the association of infection and the development of atherosclerosis (as well as plaque disruption) is due to real causality, or whether an infectious agent is an additional factor or just an innocent bystander (291). So far the data on C. pneumoniae has been the most convincing, but the role of other infectious agents, including Cytomegalovirus, Helicobacter pylori, Herpes simplex viruses 1 and 2, and dental pathogens have also been studied (291,292). C. pneumoniae antigen or deoxyribonucleic acid (DNA) has been found in

coronary atherosclerotic lesions (293,294), and the pathogen has actually grown from lesions (295). Inoculation of C. pneumoniae in experimental animals has caused aortic changes resembling atherosclerosis (296). Potential pathophysiological mechanisms by which C. pneumoniae may influence atherosclerosis comprise chronic low-grade inflammation due to persistent infection, endothelial damage and changes in the hemostatic system by bacterial lipopolysaccharides, and interaction with and modification of conventional risk factors, probably in genetically susceptible people or populations (13,291,292). Recently, one cross-sectional study demonstrated that the impact of infection on atherogenesis is related to the total pathogen burden with which an individual is infected, rather than to any single pathogen (297). Moreover, pathogen burden has also been found to independently contribute to the long-term prognosis of patients with CHD in prospective studies (298-300).

The results were promising in two pilot studies on secondary prevention of CHD events by antibiotic treatment of 3-6 days by Gupta *et al.* (301), and of 30 days by Gurfinkel *et al.* (302). However, in 6-month follow-up of the Roxithromycin in Non-Q-wave Coronary Syndromes (ROXIS) trial (303) and in 2-year follow-up of the Azithromycin in Coronary Artery Disease: Elimination of Myocardial Infection with Chlamydia (ACADEMIC) trial (304), no significant reduction in cardiovascular events was seen in patients treated with roxithromycin for 30 days or azithromycin for 3 months compared with placebo groups, respectively. These trials were not adequately powered, but new trials are under way that should give a definitive answer to whether antibiotic treatment can reduce coronary events (305). Interestingly, in the Clarithromycin in the Acute Coronary Syndrome Patients in Finland (CLARIFY) Study on 148 patients treated with clarithromycin or placebo for 3 months, the incidence of MI and all cardiovascular events was lower in the clarithromycin group during an average follow-up time of 555 days (306).

Inflammation markers and their associations with coronary heart disease

Inflammation markers that have been of greatest interest with regard to their association with CHD include proinflammatory cytokines and acute-phase proteins. Cytokines are intercellular signalling polypeptides produced by activated cells (307). Most cytokines have multiple sources, multiple targets, and multiple functions. Actually, cytokines are components of a large, complex signalling network. Systemic or local inflammation, either in the blood vessel itself or elsewhere, triggers the production of proinflammatory cytokines. These cytokines can directly elicit production of adhesion molecules, procoagulants, and other mediators by endothelial cells, leukocytes and other cells (268). Within an atherosclerotic

plaque, lymphocytes produce interferon-γ (IFN-γ), a pleiotropic cytokine that activates macrophages (268). In addition to their other functions, macrophages and circulating monocytes produce proinflammatory cytokines, such as interleukin (IL)-1, IL-6, and tumour necrosis factor-α (TNF-α) (268).

IL-6 is a multifactorial glycoprotein acting as a proinflammatory cytokine, regulated by a variety of signals, including IL-1 and TNF-α (308,309). Thus, IL-6 mediates its own effects and those of TNF-α and IL-1 in inducing the acute-phase response (309). Moreover, IL-6 is the principal stimulator of the production of most acute-phase proteins, such as C-reactive protein (CRP), serum amyloid A protein (SAA), and fibrinogen in hepatic cells, whereas most of the other cytokines influence subgroups of acute-phase proteins (307).

TNF-α is a cytokine with a wide variety of effects, including participation in both acute and chronic inflammatory responses (*e.g.*, rheumatoid arthritis and inflammatory bowel diseases), and inducing fever and acute-phase response (309). TNF-α is also called as "cachectin", since it is related to many of the metabolic abnormalities associated with cachexia, *e.g.*, in severe chronic heart failure (310,311). Actually, elevated TNF-α levels have been detected in failing human myocardium, suggesting that the heart muscle directly produces TNF-α (312). TNF-α has also been shown to induce nitric oxide production, which depresses cardiac function and can induce apoptosis (313,314). Moreover, TNF-α may have a central role in obesity, fat deposition, and insulin resistance (315).

CRP is an acute-phase protein produced by the liver in response to infection, inflammation, and trauma. The biological significance of CRP was obscure for a long time. Recent studies suggest that it plays an essential role in the recognition of self and foreign molecules, leading to activation of the adaptive immune response (316). CRP binds to damaged tissue, activates the classical complement pathway, binds to Fc receptors and acts as an opsonin for various pathogens (316). Moreover, interaction of CRP with Fc receptors leads to stimulation of proinflammatory cytokine formation, including IL-1 and TNF-α (316).

Fibrinogen critically influences platelet aggregation, blood viscosity, and thrombus formation, as well as acts as a substrate of fibrin formation and as an acute-phase protein (317). Fibrinogen associates with several conventional risk factors of CHD. Moreover, it has been regarded as a risk marker and even as a risk factor of atherosclerosis (317,318).

In several studies employing highly sensitive methods, slight elevations in the concentrations of circulating acute-phase proteins CRP and SAA (16,17,276,277), and IL-6 and IL-1 receptor antagonist (IL-1Ra) (278,319), have been detected in patients with ACSs. This suggests that the inflammatory process is not confined to the coronary plaque or even to the arterial wall, but has a systemic component (268). This is further supported by the finding

that differences in inflammatory components observed in patients with ACSs compared with stable patients are quantitative, rather than qualitative (271,273-275). Elevation of the circulatory levels of inflammation markers, even within the "normal" range, has been found to be related to adverse prognosis in patients with UAP (16,17,19,278,319) or UAP / non-Q-wave MI (279). The prognostic value of inflammation markers has also been found in patients with stable angina pectoris (17,320). Interestingly, CRP, IL-6, and fibrinogen have been found to predict the risk of cardiovascular disease (MI, stroke, or peripheral vascular disease) in apparently healthy persons, independently of other risk factors of atherosclerosis (20-22,321-325). In fact, CRP added to the predictive value of total cholesterol and total / high-density lipoprotein (HDL) cholesterol ratio in determining the risk of first MI (326). Moreover, in the Physicians' Health Study, the risk reduction of first MI associated with the use of aspirin was found to be directly related to the CRP level (21). The prognostic value of inflammation markers in ACSs is reviewed in more detail on pages 53-56.

The mechanisms of association of inflammation markers and CHD are not totally understood. In a study by Rifai *et al.* (327), concentrations of CRP, SAA, and IL-6 were elevated in patients with CHD but did not correlate with angiographic severity of the disease. Thus, inflammation markers might reflect the diffuse atherosclerotic process rather than the degree of localised coronary obstruction (327). Moreover, episodic activation of the hemostatic system does not explain the elevation of inflammation markers in UAP, since the elevation of markers of thrombin production was not followed by further elevation of CRP in a study by Biasucci *et al.* (328). The extent of myocardial ischaemia and ischaemia-reperfusion injury are not probable explanations, either, since elevated CRP levels were not detected in patients with variant angina (329). Myocardial cell necrosis leads to elevation of inflammation markers, as noted by de Beer *et al.* already in 1982 (330). However, elevations of CRP and SAA have been detected in UAP patients without elevations of cardiac troponins, and thus, without detectable myocardial injury (16). CRP may be merely a marker of inflammatory response, or a reflection of infectious agents inducing inflammatory reactions. However, CRP has been found to be present in atherosclerotic plaques, where it may colocalise with activated complement (331). According to a hypothesis by Lagrand *et al.* (332), CRP may directly interact with atherosclerotic vessels or ischaemic myocardium by activation of the complement system or by binding to phospholipids. Moreover, CRP has also been found to directly promote inflammation *in vitro* by inducing adhesion molecule expression in endothelial cells (333).

Inflammation markers in relation to prognosis of non-ST-elevation acute coronary syndromes

C-reactive protein

The first study on the prognostic value of CRP and SAA in patients with UAP was published in 1994 by Liuzzo *et al.* (16). In that study, elevated concentrations of CRP (≥3 mg/l) and SAA on admission predicted subsequent in-hospital cardiac events (cardiac death, MI, or urgent need for coronary revascularisation) in 31 patients with severe UAP with TnT within normal limits. However, in another study by Oltrona *et al.* (277) in 140 patients with UAP, increased levels of CRP (>10 mg/l) did not predict adverse in-hospital outcome. In the European Concerted Action on Thrombosis and Disabilities Angina Pectoris Study (ECAT) including 1030 patients with UAP, risk of coronary events during the follow-up of two years increased with increasing CRP concentration measured at study entry (17).

The prognostic value of the combination of an inflammation marker and an injury marker has also been studied. In a substudy of the FRISC trial on patients with UAP or non-Q-wave MI, Toss *et al.* found that increased levels of both CRP and fibrinogen were associated with a poor outcome during the follow-up time of five months (279). The risk of death increased by tertiles of CRP (<2, 2 – 10, and >10 mg/l), whereas the risk of death, and death or MI increased by tertiles of fibrinogen (<3.38, 3.38 – 3.99, and ≥4.00 g/l), respectively (279). Moreover, the prognostic values of both CRP and fibrinogen were independent of, and additive to, the prognostic value of increased TnT. In a TIMI 11A substudy on patients with UAP or non-Q-wave MI, patients with both an early positive rapid cardiac TnT assay and CRP ≥15.5 mg/l at inclusion had the highest 14-day mortality (18). The prognosis was better in patients with either CRP ≥15.5 mg/l or an early positive rapid cardiac TnT assay, whereas patients with both CRP <15.5 mg/l and a negative rapid cardiac TnT assay were at very low risk (18). In a study by de Winter *et al.* on UAP / non-Q-wave MI patients (334), the six-month risk of major cardiac events was higher in patients with CRP >5 mg/l than in patients with CRP ≤5 mg/l, both in patients with normal and elevated TnI. Thus, the independent and additive prognostic value of inflammation and injury markers has been shown in several studies (18,279,334,335) but not in all studies (336). However, in all of the recent studies the study populations and follow-up times have been different, and variable percentile points have been used as cut-off limits for CRP. Thus, no single concentration of CRP has been unambiguously recommended as an optimal cut-off limit for risk stratification.

Early revascularisation may have an effect on the predictive value of these markers. In a substudy of the Chimeric c7E3 Antiplatelet Therapy in Unstable angina REfractory to standard treatment (CAPTURE) trial, 447 patients with refractory UAP (including non-ST-elevation MIs) were treated with conventional pharmacological therapy and coronary intervention (337). Elevated TnT but not CRP (>10 mg/l), measured at inclusion, predicted mortality or MI during the initial 72-h period. On the other hand, during the six-month follow-up both CRP and TnT were independent predictors of mortality and MI. The incidence of coronary restenosis was related to CRP but not to TnT status.

Biasucci *et al.* found that CRP was elevated to >3 mg/l in 42% of 53 UAP patients on admission, and in 49% at discharge (338). Furthermore, CRP remained elevated for at least three months after the waning of symptoms in 42% of patients. The risk of recurrent instability or new MI within one year increased by tertiles of CRP at discharge. The prognostic value of SAA was similar to that of CRP, but that of fibrinogen was not statistically significant. Biasucci *et al.* suggested that elevated CRP levels at discharge identify patients at high risk even after successful revasculatisation who might benefit from more intensive antithrombotic and possibly anti-inflammatory treatment (338). In another study by Ferreirós *et al.* (19), CRP measured both on admission and at discharge predicted an adverse 90-day outcome in UAP patients. However, the prognostic value of the CRP measured at discharge was better than of CRP measured on admission. Thus, the patients in whom CRP remained elevated after the acute event seemed to have worse prognosis than those with low CRP.

In a nested case-control substudy of the Cholesterol And Recurrent Events (CARE) trial (339), concentrations of CRP and SAA measured several months after initial MI were higher in patients with subsequent cardiovascular death or recurrent nonfatal MI during the five-year follow-up, compared with patients who did not have recurrent coronary events. The patients randomised to placebo and with elevations of both CRP and SAA had an almost three-fold risk of recurrent coronary events, compared with the patients randomised to pravastatin and with low concentrations of CRP and SAA. Actually, the association between inflammation and risk of recurrent events was significant among the patients taking placebo, but not among those taking pravastatin. Another substudy of the CARE trial concentrated on a random sample of patients without recurrent coronary events (340). Median CRP levels and the mean change in CRP increased from the baseline to another measurement at five years among the placebo group, and decreased among the pravastatin group. Interestingly, the reductions in CRP levels were not related to the magnitude of lipid alterations observed. The authors suggested non-lipid effects of statins as a potential explanation for these findings, such as

inhibition of endogenous cholesterol synthesis in macrophages, improvement in endothelial function, modulation of immune function, antiproliferative effects on vascular smooth muscle, and antithrombotic properties (339-341).

The reduction of CRP levels was first detected in hyperlipidaemic patients with stable CHD treated with simvastatin or atorvastatin in a study by Strandberg *et al.* (342). Their findings, together with those of Ridker *et al.* suggest that the potential anti-inflammatory effects of statins are a class effect (340,342-344). Strandberg *et al.* also found that the changes of CRP were associated with changes in HDL cholesterol and apolipoprotein A1 but not LDL cholesterol or triglycerides (343).

Recently, the effect of statin therapy on CRP levels was studied in a large prospective trial on 1702 patients randomised to double-blind therapy with placebo or pravastatin (a primary prevention cohort), and on 1182 patients receiving open-label pravastatin (a secondary prevention cohort) (345). Reductions by 13-17% were found in median CRP levels after 12-24 weeks of treatment with pravastatin. Minimal evidence of association was found between the change in CRP concentration and the change in total cholesterol, LDL cholesterol, HDL-cholesterol, or triglyceride levels. In another recent study, a substudy of the Air Force / Texas Coronary Atherosclerosis Prevention Study (AFCAPS/TexCAPS), lovastatin was effective in preventing coronary events among participants with elevated total / HDL cholesterol ratio, regardless of the CRP level (number needed to treat for five years to prevent one event, 47) (346). Interestingly, lovastatin was also effective among participants with relatively low lipid levels but with elevated CRP level (number needed to treat 43).

Fibrinogen, interleukin-6, and tumour necrosis factor-α

With regard to ACSs, data are scarce on the prognostic value of inflammation markers other than CRP. As mentioned on page 53, the risk of death and death or MI during the five-month follow-up increased by tertiles of fibrinogen, independently of TnT in a substudy of the FRISC trial on patients with UAP or non-Q-wave MI (279). In a substudy of the TIMI IIIB trial, elevated fibrinogen concentration, measured at the time of hospital admission, was associated with an increased risk of spontaneous ischaemia, and a combined end-point of death, MI or spontaneous ischaemia during a 10-day and 42-day follow-up in patients with UAP, but not in those with non-Q-wave MI (347). However, there was no association between fibrinogen concentrations measured 96 hours after hospital admission and 42-day clinical events in any patient group. Verheggen *et al.* also found an association between high

(>3.99 g/l) fibrinogen concentration on admission and the occurrence of refractory UAP during the hospital stay among 211 consecutive patients with UAP (348).

Biasucci *et al.* found detectable levels of IL-6 (≥3 ng/l) in 61% of 38 patients with UAP at the time of admission to the coronary care unit, but in only 21% of 29 patients with stable angina (278). Among the UAP patients, 83% of the UAP patients with detectable IL-6, but only 40% with undetectable IL-6, had a complicated in-hospital course. In a more recent study by the same authors on patients with UAP and with normal TnT (319), the patients with complicated in-hospital courses had higher IL-1Ra and IL-6 levels on admission than those with uneventful courses. Moreover, a fall in IL-1Ra and IL-6 48 hours after admission was associated with an uneventful in-hospital course and their increase with a complicated course. In a substudy of the FRISC II trial on patients with UAP / non-Q-wave MI (280), elevated IL-6 concentration (≥5 ng/l) was an independent predictor of increased one-year mortality in noninvasively treated patients, and with increased six-month mortality in patients not administered dalteparin. An early invasive treatment strategy reduced one-year mortality among the patients with elevated IL-6 concentrations, but not among those without elevated IL-6.

There is very little data on the prognostic value of elevated levels of TNF-α in ACSs. However, it has been suggested that the circulatory levels of TNF-α increase in the early stages of MI (349-353). In some of these studies the elevation of TNF-α was associated with the extent of myocardial damage assessed by cardiac enzymes or myocardial scintigraphy (352,353), or with heart failure complicating MI (351-353). Simon *et al.* found that IL-1β and IL-6 levels were elevated in patients with UAP, but not in patients with stable angina or healthy controls (354). However, they found no significant differences in the circulating levels of TNF-α between the groups. In a substudy of the CARE trial (355), plasma concentrations of TNF-α measured several months after initial MI were higher in patients with subsequent cardiovascular death and/or recurrent nonfatal MI within five years, compared with post-MI patients without recurrent coronary events.

AIMS OF THE STUDY

The aim of this study was to investigate the role of biochemical markers of myocardial injury in the diagnostic and prognostic evaluation of ACSs. Moreover, the aim was to evaluate the prognostic value of markers of inflammation in UAP. The more specific aims were:

- To compare TnT and CK-MBm with conventional enzymes in the diagnosis of MI (Study I)

- To investigate the time window for ruling out MI with TnT and CK-MBm, and the prognosis of the patients in whom MI was ruled out (Study II)

- To investigate the difference in the number of MI diagnoses based on TnT compared with clinical and epidemiological diagnoses, and the prognosis of patients with discordant diagnoses (Study III)

- To evaluate the prognostic value of CRP, fibrinogen, IL-6 and TNF-α in patients with UAP (Study IV)

SUBJECTS AND METHODS

Study population at baseline

The present study is a follow-up study of an unselected series of consecutive patients admitted to the emergency department of the Kuopio University Hospital with a suspected ACS. During the study period from 30 August 1995 until 29 February 1996, there were 646 such admissions. Twenty-two admissions were excluded either because drawing a venous blood sample from the patient had been difficult, because the patient had returned home after only one sample had been drawn, because the patient had been transferred to another hospital department or because of missing biochemical marker measurements. However, patients who died during hospitalisation were included in the study. Thus, the final study population comprised 624 patients. The study was approved by the Ethics Committee of the Kuopio University Hospital.

Study population in different substudies

Study I

The study population comprised 624 patients admitted: 351 (56%) men and 273 (44%) women, aged 19 - 96 years (median age 69 years).

Study II

Of the 624 patients, 65 were admitted to the hospital twice during the study period. In this study, the first admission of each patient was regarded as an index event. There were 559 patients with an index event of a suspected ACS. In 482 of them chest pain or equivalent symptoms were relieved within 24 hours from admission. In 399 patients reliable time of onset of the symptoms was also available. Two patients, who died from acute MI at the emergency department soon after the first blood sample had been taken and within less than two hours from the onset of symptoms, were excluded. Both of them had TnT concentrations of 0.08 μg/L. Thus, the study population comprised 397 patients: 227 (57%) men and 170 (43%) women, aged 19 - 96 years (median age 69 years).

Study III

In this study, the first admission of each patient was regarded as an index event and readmissions as follow-up events. Thus, the study population consisted of 559 patients with an index event of a suspected ACS: 315 (56%) men and 244 (44%) women, aged 19-96 years (median age 69 years).

Study IV

Of the 559 patients, 115 patients with the final diagnosis of a noncardiac event, 44 patients with a cardiac event of non-CHD origin, and 137 patients with definite or probable MI were excluded. Thus, in this study the study population consisted of 263 patients with unstable angina pectoris or a prolonged attack of chest pain. The diagnostic classes were combined, and the duration of chest pain during hospitalisation was used as a separate variable. Of these patients, 159 (60%) were men and 104 (40%) women, aged 34-96 years (median age 68 years).

Diagnostic classification of events

Medical records were reviewed by three physicians (H. Koukkunen, K. Penttilä and A. Kemppainen) who were members of the study team and who had agreed on the principles of the review process. The events were classified by modified FINMONICA criteria with regard to symptoms, serial ECG findings, peak values of conventional enzyme activities, autopsy findings and history of previous CHD. The diagnostic classification was based on the data obtained during the first 72 h in hospital. The modified FINMONICA criteria are based on MONICA and FINMONICA diagnostic classifications described in detail in WHO MONICA Manual and by Palomäki *et al.* (5,198). The diagnostic category of possible MI of the FINMONICA classification was slightly tightened to produce the category of probable MI of the modified FINMONICA classification. The diagnostic category of probable MI required that the ECG changes were visible in at least two recordings on successive days. Moreover, gender-specific URLs were used for serum total CK.

The diagnosis of a definite MI was made according to the modified FINMONICA criteria when

(1) a diagnostic Q wave developed or "injury current" ST-segment elevation lasting at least for two days was followed by T-wave inversion in serial ECGs,
(2) symptoms were compatible with MI (chest pain or its equivalent), and any one of the conventional enzymes measured within 72 h from the onset of symptoms was increased to more than two times the URL, and serial ECGs showed ST-segment or T-wave changes, or
(3) in cases of fatal event a recent infarction or an occlusion of a coronary artery was found in autopsy.

The diagnosis of a probable MI required that

(1) in the presence of "typical" symptoms (chest pain lasting for more than 20 min) "probable" ECG changes (either ST-segment depression >1 mm or ST-segment elevation >1 mm in at least two limb leads or >2 mm in at least two chest leads or T-wave inversion >1 mm) were recorded on two subsequent days, or any one of the conventional enzymes increased to more than once the level of URL but remained lower or equal to twice the level of URL.

(2) In case of inadequately described or atypical symptoms (atypical pain, acute pulmonary oedema, or collapse), electrocardiographic and enzyme criteria for probable MI had to be fulfilled.

(3) In fatal cases, the diagnosis of a probable MI was made if

(a) CHD was verified at autopsy but no other cause for death was found; or

(b) when in nonautopsied cases a patient had had typical, atypical or inadequately described symptoms or had had a history of previous CHD before death and no other cause for death was detected.

If the patient had successive attacks of chest pain in the hospital for more than 24 h, the event was classified as unstable angina. If the duration of chest pain period was 24 h or less the event was diagnosed as a prolonged attack of chest pain. Other events were classified either as cardiac events of non-CHD origin or as noncardiac events.

Hospital discharge diagnoses, assigned by physicians treating the patients, were used as clinical diagnoses. The 9th version of the International Classification of Diseases (ICD-9) was used in Finland for hospital discharge diagnoses until 31 December 1995, after which ICD-10 was used.

In Study II, the diagnosis of MI in patients with chest pain attack or other symptoms compatible with an ACS was based on the elevation of serum TnT concentration to >0.10 μg/l within 24 hours from hospital admission. Modified FINMONICA criteria were only used for the description of patient population.

Analysis of biochemical markers

Markers of myocardial injury

The first blood sample for the measurement of conventional enzyme activities (CK, CK-MB and LD-1) and TnT and CK-MBm concentrations was drawn as soon as possible after the

patient arrived at the emergency unit. The following samples were drawn 2, 4 and 6 h after the first sample. During the second and third hospital day blood samples were drawn twice a day: between 07:00 and 08:00 and between 19:00 and 20:00. The last blood sample was taken in the morning of the fourth day. However, the blood sample collection was discontinued if the patient was discharged from the hospital before the end of the sampling schedule. CK, CK-MB and LD-1 activities were measured from serum samples within 4 h after the samples had been drawn and were used in routine diagnostics of MI. Serum samples for CK-MBm were stored frozen at –20 ^{o}C, and the determinations were performed within 72 h after the samples had been drawn. Serum samples for TnT were stored at –70 ^{o}C, and the concentrations were measured at the end of the study. Maximum concentrations of cardiac TnT and CK-MBm obtained within 72 h from hospital admission were used for data analyses in Studies I, III and IV. In Study II, maximum concentrations obtained within 24 h from hospital admission were used. TnT and CK-MBm determinations were used for study purposes only, and they were not available to the physicians treating the patients. The laboratory personnel measuring the TnT and CK-MBm concentrations was blinded with regard to the results of the conventional enzyme determinations and clinical data.

Serum CK was measured by using a commercial assay by Boehringer Mannheim (Mannheim, Germany) with a Hitachi 717 analyser (Tokyo, Japan) according to the recommendations of the European Committee for Clinical Laboratory Standards (ECCLS) (356). The activity of the CK-B isoenzyme was measured after inhibiting the M subunit with anti-CK-M antibody (Boehringer Mannheim) and expressed as the value of CK-MB by doubling the measured CK-B value. The activity of LD-1 after inhibition of the M-containing isoenzymes with urea and quinidine at pH 10 was measured according to the LD method by Boehringer Mannheim by using a Hitachi 717 analyser in accordance with the Scandinavian Recommendation (357). CK-MBm was measured using immunochemical microparticle technique by Abbott IMx® CK-MB assay (Abbott Laboratories, Abbott Park, Chicago, Illinois, USA). This method uses a monoclonal anti-CK-MB antibody bound to latex microparticles and a polyclonal anti-CK-MM antibody coupled with alkaline phosphatase (358). Cardiac TnT was measured photometrically by an immunochemical ELISA method on plates of microtitre using reagents by Boehringer Mannheim (359). The ‘second-generation’ TnT assay with a double monoclonal antibody technique was used to decrease cross-reactivity with skeletal muscle TnT.

Gender-specific URLs were used for CK activity: 270 U/l for men and 170 U/l for women. The URLs for both genders were 130 U/l for LD-1 and 25 U/l for CK-MB activities. The elevations considered to result from macro-MB were excluded. In a series of 95 apparently

healthy persons serum TnT concentration was 0.027 ± 0.025 μg/l (360). Thus, the URL (corresponding to the 99th percentile) for the assay was 0.10 μg/l, also recommended by the manufacturer. In the series of 95 healthy persons CK-MBm concentration was 2.707 ± 1.200 μg/l. The URL corresponding to the 97.5th percentile was 5.1 μg/l and to the 99th percentile 6.3 μg/l. The manufacturer recommended the cut-off value of 5.0 μg/l. In Study I, we evaluated the effect of different cut-off values by using two cut-off values: 0.10 and 0.20 μg/l for TnT, and 5.0 and 10.0 μg/l for CK-MBm. The two cut-off limits were used for CK-MBm also in Study II. In Studies II-IV, the cut-off value of 0.10 μg/l was used for TnT.

The CVs (inter-assay precision) for the conventional enzyme measurements were: for CK <2.0%, for CK-MB activity <3.0% and for LD-1 <3.0%. The CV for CK-MBm was 6.9% at 4.6 μg/L and 5.5% at 15.2 μg/l. The CV for TnT was 8.7% at 0.23 μg/l and 4.7% at 4.35 μg/l. For the conventional enzymes 2-4 different reagent lots were used, but no changes in the levels of measurements were detected during the study. For TnT and CK-MBm measurements the reagent lot remained the same throughout the study.

Markers of inflammation (Study IV)

Blood samples for the measurement of serum CRP, IL-6 and TNF-α, and plasma fibrinogen, were drawn at admission. These determinations were used for study purposes only. The laboratory personnel determining the acute-phase protein and cytokine concentrations was blinded to the conventional enzyme activities and to the clinical data.

Serum and plasma samples for acute-phase proteins and cytokines were stored frozen at –70˚C, and the determinations were performed at the end of the study. CRP was analysed by rate nephelometry using Beckman Array Protein System equipment (Beckman Instruments Inc., Brea, California, USA) and a specific antibody for CRP by Beckman. Calibrators by Orion Diagnostica (Espoo, Finland) were used. The lower limit of the measurement range of the assay was 1 mg/l. For patients in whom the CRP concentration was below this lower limit, a concentration of 0.98 mg/l was assigned in statistical calculations. IL-6 and TNF-α were assayed by quantitative sandwich enzyme immunoassay techniques using specific monoclonal antibodies and microtitre plate method (Quantikine® Human IL-6 and Human TNF-α Immunoassays; R&D Systems Inc., Minneapolis, Minnesota, USA). Plasma fibrinogen was quantitatively determined by a clotting method (Fibri-Prest® Automate; Diagnostica Stago, Asnieres-sur-Seine, France), using Thrombolyzer analyser (Behnk Elektronik, Norderstedt, Germany). The CV (inter-assay precision) for CRP was 4.1-6.2%, for fibrinogen 5.8-7.4%,

for IL-6 6.3% at 17.2 ng/l and 3.3% at 101 ng/l, and for TNF-α 7.4% at 45.8 ng/l and 4.6% at 301 ng/l.

Collection of follow-up data

Study I only used baseline data. In Study II the follow-up time was 1 year for all patients. In Studies III-IV the median follow-up time was 17 months (range 13-19 months). Postal questionnaires about hospitalisations for any coronary events or treatments for CHD were sent to all patients surviving the index event. Hospital records of all the patients who had been readmitted to hospital were checked to confirm end-points. Acute coronary events during the follow-up time were also classified by modified FINMONICA criteria. Hospital records and death certificates of the patients who had died were checked to confirm cause-specific diagnoses. Copies of death certificates of all patients who had died were received from the national Cause-of-Death Register (Statistics Finland). With these procedures complete follow-up data were obtained on all the patients.

The end-points of the study were: 1) coronary death, 2) major coronary event (coronary death or nonfatal MI), and 3) any coronary event (coronary death, nonfatal MI, hospitalisation due to UAP or a prolonged attack of chest pain, or revascularisation procedure – bypass surgery or coronary angioplasty). One patient could have several end-points but only the first one was used in analyses.

Statistical methods

Data analyses were performed with SAS Release 6.12 (SAS Institute, Cary, North Carolina, USA) and SPSS for Windows Releases 9.0 and 10.0 software (SPSS Inc., Chicago, Illinois, USA). Categorical variables were compared using the χ^2 test (Study II). Statistical significance was based on the 0.05 level.

In Study I, the agreement between the modified FINMONICA diagnosis and diagnosis of MI based on TnT and CK-MBm values was assessed by a method which regards neither classification as a golden standard (361). The kappa coefficient and its 95% confidence intervals, the observed proportion of positive agreement (p_{pos}) and the observed proportion of negative agreement (p_{neg}) were calculated by using the following equation:

$p_{pos} = 2a / (f_1 + g_1)$,

where a = number of events classified as positive by both methods;

f_1 = number of positive readings for Method A, and

g_1 = number of positive readings for Method B.

Correspondingly, $p_{neg} = 2d / (f_2 + g_2)$,

where d = number of events classified as negative by both methods;

f_2 = number of negative readings for Method A, and

g_2 = number of negative readings for Method B.

We chose to use a 'cumulative' kappa instead of a 'multilevel' kappa and defined a threshold for each comparison of the agreement between the modified FINMONICA diagnostic classification and TnT measurement. This method is considered to give the most appropriate information on the relative merits of different diagnostic classifications or tests in the diagnosis of MI applying different threshold levels relevant to epidemiological and clinical research (198,361).

In Study III age-standardised incidence rates were calculated by the direct method using the study population of 559 patients stratified into two age groups (< and ≥75 years) as the standard. Kappa coefficient and its 95% confidence interval (CI) were used in estimating the agreement of diagnoses by different criteria. Cox proportional hazards model was used for calculating age-adjusted hazard ratios (HRs) with regard to different coronary end-points.

In Study IV age-standardised incidence rates were calculated by the direct method using the primary study population of 444 patients with suspected ACS by 5-year age groups as a reference. The rates were compared using Mantel-Haenszel test. Cox proportional hazards model was used for calculating HRs. Kaplan-Meier survival analysis was used to estimate event-free survival at each point of time. Non-cardiac deaths were treated as censored observations. The survival curves were compared using the log-rank test.

Factor analysis in Study IV was performed in three steps (362):

1) extraction of the initial factors,
2) rotation of the components, resulting in increased interpretability of the factors, and
3) interpretation of the factors.

Principal component analysis was used for the extraction of the initial factors. It transforms the original correlated variables to uncorrelated linear combinations of variables (the factors), and often a small number of these account for most of the variation or the pattern of correlations (362). Only the components with eigenvalues (which is the sum of the squared factor loadings, representing the variance attributable to each principal component) >1.0 were

retained in the analysis. Factor loadings then describe the correlation between the newly defined component and the original variable component. The components were subjected to Varimax rotation, an orthogonal rotation maintaining the independence of factors, to increase their interpretability (362). The variables with a factor loading >0.40 were used for interpretation. Each factor may then be named according to the variable that loads highest on a particular factor. The percentage of variance from the total variance accounted for each factor describes the relative importance among factors. For each acute-phase protein and cytokine, missing values (<4%) were replaced with the variable median. Scores for the factors obtained in the factor analysis were retained and used as independent variables in Cox model in the prediction of the risk of different coronary end-points.

RESULTS

TnT and CK-MBm compared with conventional enzymes (Study I)

TnT and CK-MBm were compared with conventional enzyme activities (CK, CK-MB and LD-1) in the diagnosis of MI. The distribution of the maximum levels of TnT with two different cut-off limits according to the diagnostic categories of modified FINMONICA classification are shown in Table 4. TnT was elevated (>0.10 μg/l) in 100%, CK-MBm (>5.0 μg/l) in 99%, and both markers in 99% of the 89 patients with the diagnosis of a definite MI. In the 60 patients with the diagnosis of a probable MI, TnT was elevated in 65%, CK-MBm in 67% and both markers in 60%. In the patients with unstable coronary artery disease (UAP or prolonged chest pain attack) and conventional enzymes within normal limits, TnT was elevated in 14%, CK-MBm in 17% and both markers in 9%. When the cut-off limit of 0.20 μg/l was used, TnT was elevated in 100% of the patients with a definite MI and in 63% of the patients with a probable MI. Of the patients with unstable angina 17% had TnT >0.20 μg/l, but only 5% of the patients with a prolonged chest pain attack. When the higher cut-off limit of 10.0 μg/l was used, CK-MBm was elevated in 99% of the patients with a definite MI, in 55% with a probable MI, in 13% with unstable angina, and in 4% with a prolonged attack of chest pain, respectively.

Of the 624 patients, 169 with an event of non-CHD cardiac origin or a noncardiac event were excluded from the following comparisons. In addition, 18 patients with nonspecific elevations of conventional enzymes were excluded. Thus, 145 patients with a definite or a probable MI and 292 patients with an acute coronary event without MI were included.

In Figure 1A the elevations of TnT are compared with the elevations of conventional enzymes. TnT was elevated (>0.10 μg/l) in 100% of those 90 patients in whom conventional enzymes were increased to more than two times the URL. However, TnT was elevated in only 64% of those 55 patients in whom the conventional enzymes were elevated to more than once but equal or less than twice the URL level, suggesting that a substantial proportion of conventional enzyme elevations may have been nonspecific. On the other hand, in the group of 292 patients in whom conventional enzymes remained in the normal range, TnT was elevated in 13%, suggesting a myocardial injury that had remained undetected. When the cut-off limit of 0.20 μg/l was used for TnT, the corresponding proportions in the three conventional enzyme categories were 100%, 62% and 7%, respectively.

The elevations of CK-MBm compared with the elevations of conventional enzymes are shown in Figure 1B. The cut-off limit of 5.0 μg/l was used for CK-MBm. CK-MBm was

Table 4. Maximum levels of CK-MBm and TnT in the diagnostic categories of acute CHD events - based on conventional enzymes (CK, CK-MB or LD-1), symptoms and serial ECGs in 624 patients admitted with suspicion of an acute coronary syndrome.

	CK-MBm, µg/l			
	≤ 5.0	5.1-10.0	> 10.0	Total
Definite MI				
TnT, µg/l				
≤ 0.10	0	0	0	0
0.11-0.20	0	0	0	0
> 0.20	1	0	88	89
Total	1	0	88	89
Probable MI				
TnT, µg/l				
≤ 0.10	17	2	2	21
0.11-0.20	0	1	0	1
> 0.20	3	4	31	38
Total	20	7	33	60
Unstable AP				
TnT, µg/l				
≤ 0.10	35	5	1	41
0.11-0.20	2	1	0	3
> 0.20	2	1	6	9
Total	39	7	7	53
Prolonged chest pain attack				
TnT, µg/l				
≤ 0.10	203	18	2	223
0.11-0.20	9	8	1	18
> 0.20	2	3	7	12
Total	214	29	10	253
Event of cardiac but non-CHD origin				
TnT, µg/l				
≤ 0.10	33	6	2	41
0.11-0.20	0	0	0	0
> 0.20	4	0	2	6
Total	37	6	4	47
Non-cardiac event				
TnT, µg/l				
≤ 0.10	101	8	3	112
0.11-0.20	5	0	0	5
> 0.20	0	2	3	5
Total	106	10	6	122

elevated in 99% of the patients in whom conventional enzymes were increased to more than twice the level of URL, but in only 67% of those patients in whom the conventional enzymes were increased more than once but less than twice the URL. CK-MBm was elevated in 15% of the patients with conventional enzymes within normal limits. When the cut-off limit of 10.0 µg/l was used for CK-MBm, the corresponding proportions in the three categories of conventional enzymes were 98%, 55% and 6%, respectively.

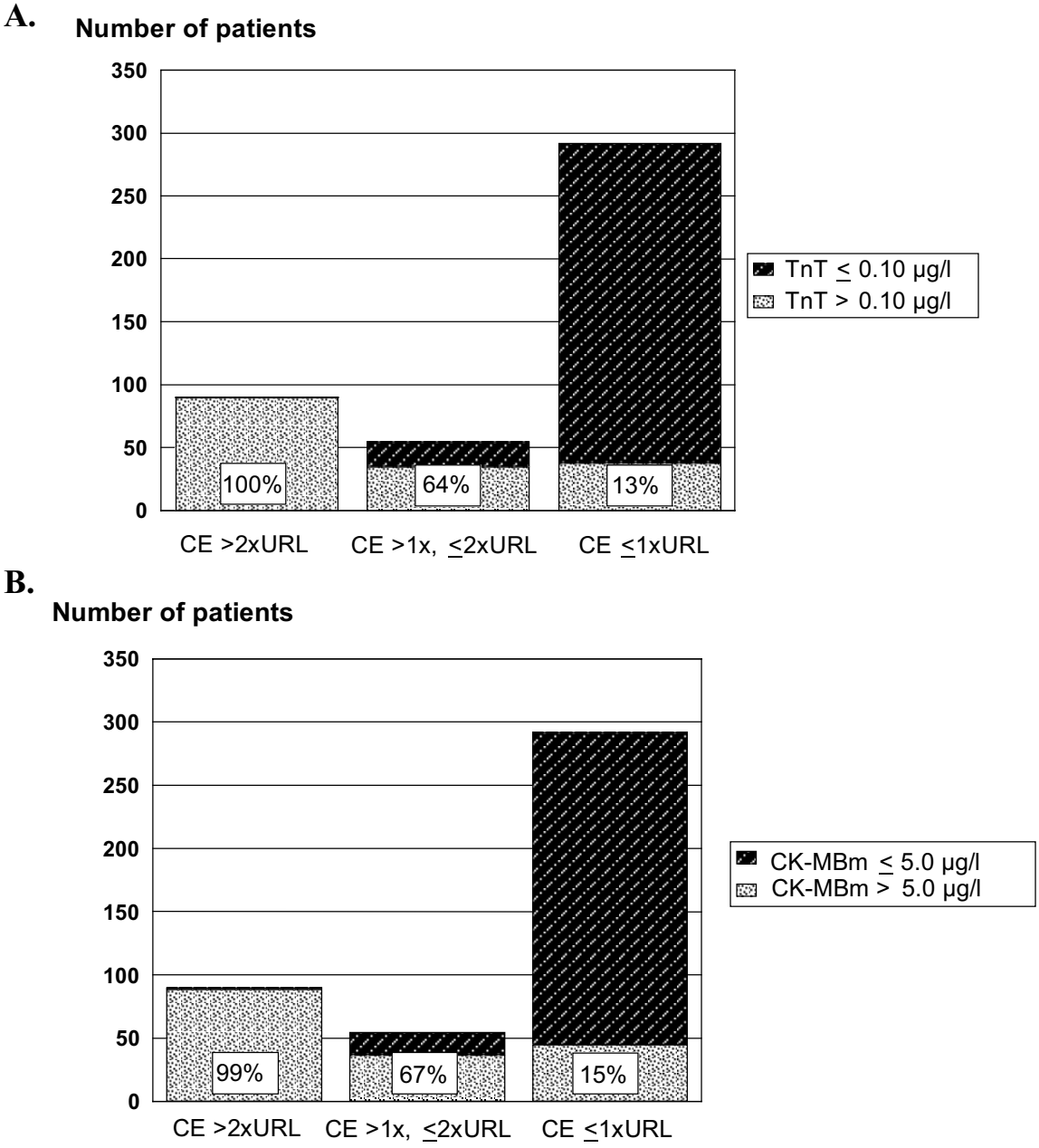

Figure 1. Proportions of patients with TnT maximum values > 0.10 µg/l (**A**) and of patients with CK-MBm maximum values > 5.0 µg/l (**B**) compared with conventional enzyme (CE) activities (145 patients with definite or probable MI and 292 patients with an acute coronary event without MI by modified FINMONICA criteria).

Ruling out MI with TnT and CK-MBm (Study II)

The diagnosis of MI was confirmed with elevated TnT (>0.10 μg/l) in 108 of 397 (27.2%) patients. The patients had been admitted with a suspected ACS but their symptoms had become relieved within 24 h. Of the 108 patients with MI, CK-MBm was ≤5.0 μg/l in 14 (13.0%) patients and ≤10.0 μg/l in 26 (24.1%) patients. On the other hand, of the 289 patients in whom MI was ruled out on the basis of TnT ≤0.10 μg/l, CK-MBm was elevated to >5.0 μg/l in 30 (10.4%) patients and to > 10.0 μg/l in 5 (1.7%) patients.

Figure 2 shows the time from the onset of symptoms to the elevation of TnT and CK-MBm in 108 patients with MI. With TnT the diagnosis of MI was confirmed in 91% of the patients 12-24 h from the onset of symptoms and with CK-MBm (with the cut-off limit of 5.0 μg/l) in 85% during the same time. Among the 94 patients in whom CK-MBm became elevated to >5.0 μg/L, the diagnosis of MI was confirmed 10-12 h from the onset of symptoms in 95%. When cut-off limit of 10.0 μg/l was used for CK-MBm the diagnosis of MI was confirmed in 81% of the 108 patients 24 h from the onset of symptoms. Consequently, MI was ruled out with a high probability if the concentration of TnT remained within normal limits up to 12-24 h or CK-MBm up to 10-12 h from the onset of symptoms.

Figure 3 shows the time from hospital admission to the elevation of TnT and CK-MBm in 108 patients with MI. The diagnosis of MI was confirmed with TnT in 99% of the patients within 12 h and in all of them within 24 h from hospital admission. With CK-MBm and cut-off limit of 5.0 μg/l the diagnosis of MI was confirmed in 88% of the patients within 12 h from hospital admission. However, CK-MBm was elevated to >5.0 μg/l by 6 h from hospital admission in 93 (99%) of the 94 patients in whom CK-MBm became elevated within 72 hours. When cut-off limit of 10.0 μg/l was used for CK-MBm the diagnosis of MI was confirmed in 76% of the 108 patients within 12 h and in 81% of the patients within 24 h from hospital admission. MI was ruled out in 99% of the patients if the concentration of TnT remained ≤0.10 μg/l up to 12 h and in 95% if the concentration of CK-MBm remained ≤5.0 μg/l up to 6 h from hospital admission. If CK-MBm remained ≤5.0 μg/l up to 12h from hospital admission, MI was ruled out in 96% of the patients.

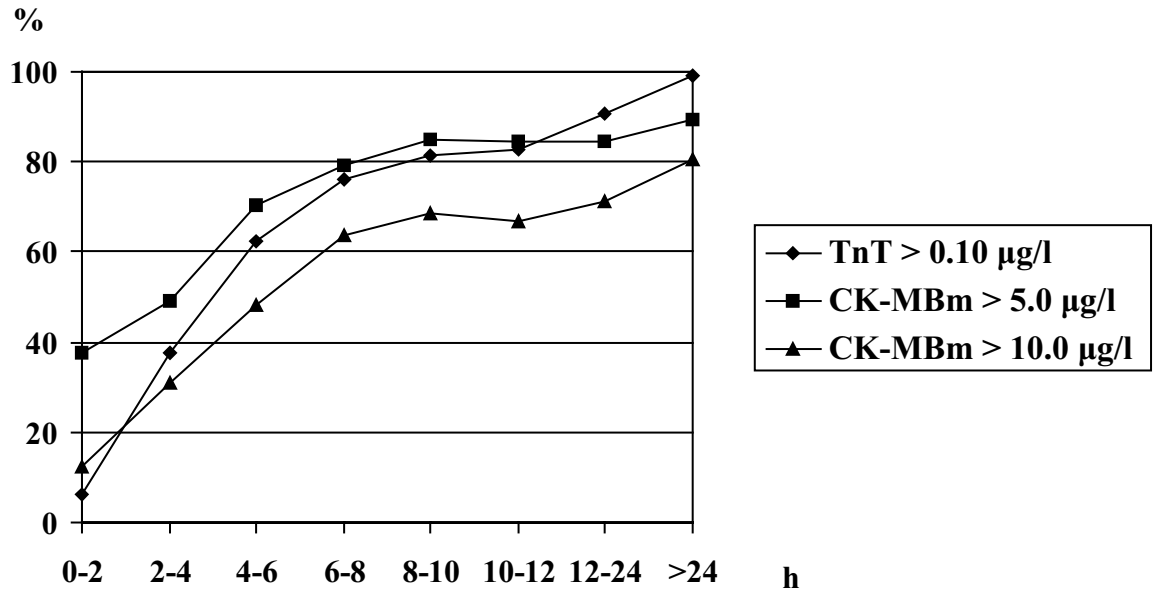

Figure 2. Time for elevation of biochemical markers from the onset of symptoms in 108 patients with MI.

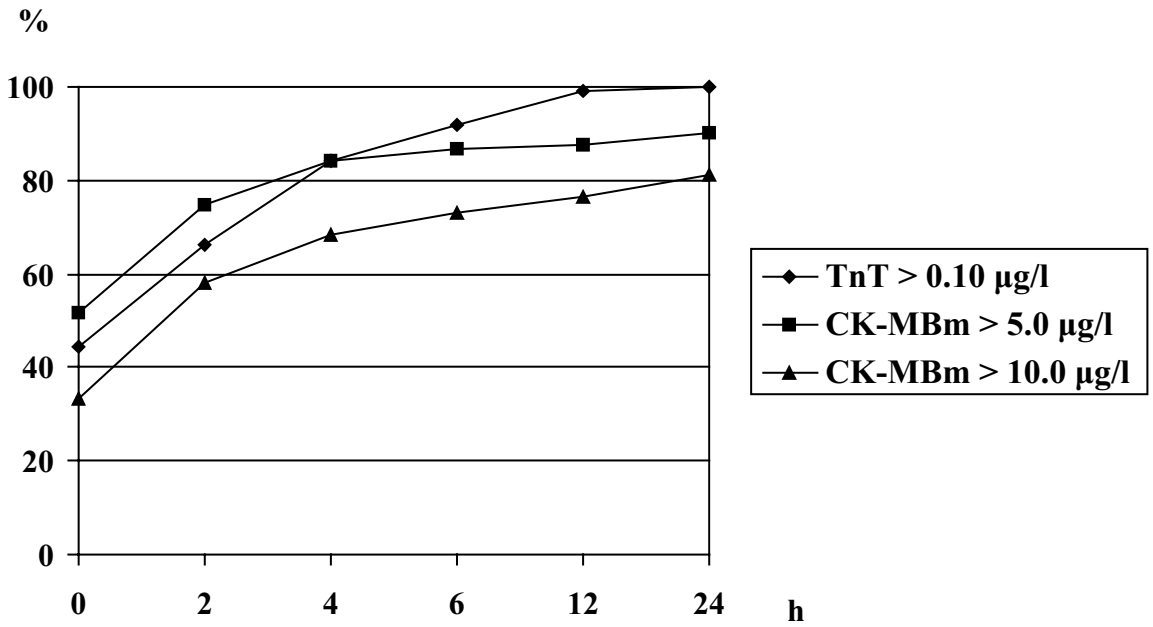

Figure 3. Time for elevation of biochemical markers from hospital admission in 108 patients with MI.

Table 5 shows the incidence of major and all CHD events during the follow-up of 1, 3 and 6 months and 1 year in 289 patients in whom MI was ruled out. Of them 65% had a positive history of CHD, and within 1 year these patients had more major CHD events and all CHD events than patients with no or unknown history of CHD. Within 3 and 6 months, patients with a positive history of CHD had more CHD end-points using any CHD event as a criterion. Only 1 (0.5%) patient with a positive history of CHD died from CHD within 1 month. Within 3 months 5 (2.6%) patients and within 6 months 9 (4.8%) patients with CHD died from CHD, but no deaths occurred among patients without a history of CHD. Within 1 year 11 (5.8%) patients with and 1 (1.0%) patient without a history of CHD died from CHD (p=NS).

Table 5. Incidence of major and all CHD events during the follow-up of 1, 3 and 6 months and 1 year in patients without MI as an index event.

Events	Positive history of CHD (n=189)	No or unknown† history of CHD (n=100)	All patients (n=289)
Major CHD event			
1 month	2 (1.1%)	2 (2.0%)	4 (1.4%)
3 months	7 (3.7%)	2 (2.0%)	9 (3.1%)
6 months	13 (6.9%)	2 (2.0%)	15 (5.2%)
1 year	18 (9.5%)*	3 (3.0%)	21 (7.3%)
Any CHD event			
1 month	11 (5.8%)	3 (3.0%)	14 (4.8%)
3 months	26 (13.8%)*	4 (4.0%)	30 (10.4%)
6 months	42 (22.2%)***	5 (5.0%)	47 (16.3%)
1 year	54 (28.6%)***	7 (7.0%)	61 (21.1%)

† Information on the history of CHD not available.

* $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$ as compared with the incidence in patients with no or unknown history of CHD.

Diagnosis of MI by TnT in comparison with clinical and epidemiological criteria (Study III)

MI was diagnosed clinically in 127 patients (23%) out of all 559 patients. The number of MIs was 169, 33% higher, when MI diagnosis was based on elevated TnT concentration (>0.10 μg/l). Sixteen (13%) of the 127 patients in whom MI was diagnosed clinically had TnT ≤0.10 μg/l. Fifty-nine (14%) of the 431 patients without clinical diagnosis of MI had TnT >0.10 μg/l. Kappa coefficient of 0.65 (95% CI 0.57; 0.73), reflected a relatively good agreement between the clinical and TnT-based MI diagnoses. Of the 169 MI patients with MI diagnosis based on elevated TnT, serial ECGs showed an evolving diagnostic Q wave in 12%, evolving ST-segment or T-wave changes or a constant Q wave in 50%, and non-evolving ST-segment or T-wave findings in 26% (data in addition to original Study III). Serial ECGs were normal in 5% and uncodable in 7% of the MI patients.

Of the 559 patients, 137 (25%) got an epidemiological diagnosis of MI. If diagnosis of MI was based on elevated TnT, the number of MIs increased by 23% compared with epidemiological MI diagnosis. Nineteen (14%) of the patients in whom MI was diagnosed by epidemiological criteria had TnT ≤0.10 μg/l. Fifty-one patients had TnT >0.10 μg/l but no epidemiological diagnosis of MI. The agreement between epidemiological and TnT-based MI diagnoses was relatively good, as assessed by kappa coefficient of 0.69 (95% CI 0.60; 0.77).

Of the 559 patients, 106 (19%) had concordant diagnosis of MI both by clinical and epidemiological criteria, and MI was concordantly excluded in 401 (72%) patients. Twenty-one (4%) patients had only clinical and 31 (6%) only epidemiological diagnosis of MI. The agreement between the clinical and epidemiological criteria was rather good (kappa coefficient 0.74 with 95% CI of 0.66; 0.82).

From the prognostic evaluation we excluded two patients, who died from acute MI at the emergency department soon after the first blood sample had been taken and within two hours after the onset of symptoms. Both of them had TnT concentration of 0.08 μg/l. In-hospital mortality was 9.0% for the patients with definite MI and 7.9% for the patients with definite or probable MI by the modified FINMONICA classification. When the clinical diagnoses were used, in-hospital mortality was 8.3% for the patients with definite MI and 7.4% for the patients with definite or probable MI, respectively. However, in the patients with TnT-based MI diagnosis the in-hospital mortality was lower, 6.5%.

Table 6 shows the number of events, age-standardised incidence rates and age-adjusted Cox hazard ratios for major CHD events during 17-month follow-up in patients with peak values of TnT > or ≤0.10 μg/l, and with or without clinical or epidemiological diagnosis of

MI. As expected, the prognosis was the worst in patients with concordant TnT-based and clinical or epidemiological diagnosis of MI. Incidence rates for major CHD events were 2.6-fold higher in patients with both TnT-based and clinical or epidemiological diagnosis of MI, compared with patients without MI. Among the patients in whom the clinical or epidemiological diagnosis of MI was not made, the prognosis was not significantly worse in patients with TnT >0.10 μg/l than in those with TnT ≤0.10 μg/l. This finding is, however, based on relatively small numbers of patients.

The prognostic values of different markers of myocardial injury were also assessed and compared in patients in whom epidemiological diagnosis of definite MI was excluded. Table 7 shows age-adjusted Cox hazard ratios for different categories of CHD events in these patients, with regard to peak values of CK, CK-MBm and TnT. To make the different markers comparable, hazard ratios were calculated for one standard deviation difference in log 10-based unit for each marker. In these patients TnT and CK-MBm were better than CK in predicting CHD events during 17-month follow-up.

Table 6. Age-standardised rates and age-adjusted Cox hazard ratios (95% confidence intervals) during the median follow-up time of 17 months for major CHD events in patients with and without clinical or epidemiological diagnosis of MI and with peak values of TnT >0.10 or ≤0.10 μg/l, using patients without diagnosis of MI and TnT ≤0.10 μg/l as a reference.

	MI	No MI
	Clinical diagnosis	
TnT >0.10 μg/l	(n=110)	(n=59)
Events (age-standardised rate, %)	27 (24)	11 (17)
HR (95% CI)	1.70 (1.10-2.63)*	1.07 (0.62-1.84)
TnT ≤0.10 μg/l	(n=16)	(n=372)
Events (age-standardised rate, %)	3 (17)	34 (9)
HR (95% CI)	0.93 (0.39-2.22)	1.00
	Epidemiological diagnosis	
TnT >0.10 μg/l	(n=118)	(n=51)
Events (age-standardised rate, %)	29 (24)	9 (16)
HR (95% CI)	1.71 (1.11-2.65)*	1.09 (0.61-1.94)
TnT ≤0.10 μg/l	(n=17)	(n=371)
Events (age-standardised rate, %)	3 (16)	34 (9)
HR (95% CI)	0.87 (0.36-2.10)	1.00

* $p<0.05$.

Table 7. Age-adjusted Cox hazard ratios (95% confidence intervals) for different CHD events in patients in whom epidemiological diagnosis of definite MI was excluded (n=473) with regard to peak values of CK, CK-MBm and TnT.

	HR (95% CI)		
Event	CK*	CK-MBm†	TnT
CHD death	1.56 (1.02-2.37)§	2.09 (1.32-3.32)‡	1.83 (1.23-2.70)‡
Major CHD event	1.35 (0.94-1.94)	1.85 (1.26-2.72)‡	1.74 (1.26-2.40)‡
Any CHD event	1.18 (0.92-1.52)	1.32 (1.01-1.74)§	1.46 (1.17-1.82)‡

Hazard ratios were calculated for one standard deviation difference in log 10-based unit for CK, CK-MBm and TnT.

* nonspecific rises (n=32) excluded

† nonspecific rises (n=18) excluded

‡ $p<0.01$

§ $p<0.05$

Acute-phase proteins and cytokines in relation to prognosis of UAP (Study IV)

In this study the modified FINMONICA classes of UAP and a prolonged attack of chest pain were combined, and the duration of chest pain during hospitalisation was used as a separate variable. In 263 patients with UAP, the conventional enzyme activities were within normal limits. However, TnT was elevated (>0.10 μg/l) in 13% and CK-MBm (>5.0 μg/l) in 14% of them (after exclusion of nonspecific CK-MBm rises in eight patients). An excess risk of coronary events was detected during the follow-up to accumulate into the highest tertiles of acute-phase proteins and cytokines. Thus, the lowest two tertiles for CRP, fibrinogen, IL-6 and TNF-α were combined. The age-standardised coronary mortality rate was 6-fold higher in tertile 3 for CRP and IL-6 and 3.5-fold higher in tertile 3 for fibrinogen and TNF-α than in corresponding combined tertiles 1-2 (data not shown). Incidence of a major coronary event was 2-fold higher in tertile 3 for IL-6 and incidence rate for any coronary event 1.5-fold higher in tertile 3 for TNF-α than in corresponding tertiles 1-2 (data not shown).

Figures 4 and 5 show Kaplan-Meier survival curves for tertiles 1-2 and tertile 3 of CRP, fibrinogen, IL-6 and TNF-α with regard to different coronary end-points. The survival curves for coronary mortality suggest that elevated levels of acute-phase proteins and cytokines on

admission in patients with UAP predicted coronary death during the follow-up time. The curves also show that the risk of a major coronary event was higher in tertile 3 of all acute-phase proteins and cytokines studied, compared to the respective tertiles 1-2. The risk of any coronary event for tertile 3 of TNF-α was also significantly higher than in tertiles 1-2. The association of acute-phase proteins and cytokines with the risk of coronary death was most marked during the first four months of the follow-up. Cox model hazard ratio (tertile 3 *vs*. tertiles 1-2; adjusted for age and gender) for CRP was 4.12 (95% CI 1.49-11.94), for fibrinogen 2.57 (1.17-5.64), for IL-6 4.34 (1.49-12.6), and for TNF-α 2.61 (1.18-5.79). To translate the results into clinical practice, among 100 patients with UAP and CRP >1.49 mg/l on admission, 10 died from CHD within four months, whereas among 100 patients with UAP but CRP ≤1.49 mg/l only one patient died from CHD within four months. The incidence of any coronary event during the first month of the follow-up was significantly increased in the highest tertile of TNF-α (12 events [2 fatal and 10 non-fatal] in 78 patients, 15%) as compared to the combined two lower tertiles (11 events in 172 patients, 6%); Cox model hazard ratio was 1.53 (95% CI 1.01-2.32). Such a significant increase in the incidence of any coronary event was not observed in the highest tertiles of CRP, fibrinogen or IL-6.

Univariate Spearman correlation matrix for acute-phase proteins, cytokines, and markers of myocardial injury showed a relatively strong correlation of CRP with fibrinogen and IL-6 ($r=0.41$ and 0.32, $p<0.001$, respectively). IL-6 and fibrinogen concentrations were correlated ($r=0.30$, $p<0.001$). The CRP concentration correlated weakly with maximum value of CK-MBm ($r=0.14$, $p<0.05$). TNF-α did not correlate with CRP, fibrinogen or IL-6 ($r=0.04$, $r=0.10$, $r=0.08$, respectively) but correlated with TnT ($r=0.22$, $p<0.001$). There was a relatively strong correlation between maximum values of TnT and CK-MBm ($r=0.45$, $p<0.001$).

Factor analysis including acute-phase proteins, cytokines, and markers of myocardial injury yielded two factors, explaining together 54.2% of the total variance (Table 8). Factor 1 comprised CRP, IL-6, and fibrinogen, and it was named ‘inflammation factor’. Both of the markers of myocardial injury loaded strongly, and TNF-α less strongly on Factor 2, named ‘injury factor’. Table 9 shows that in Cox model both the inflammation and injury factor predicted the risk of coronary death and a major coronary event independently of each other.

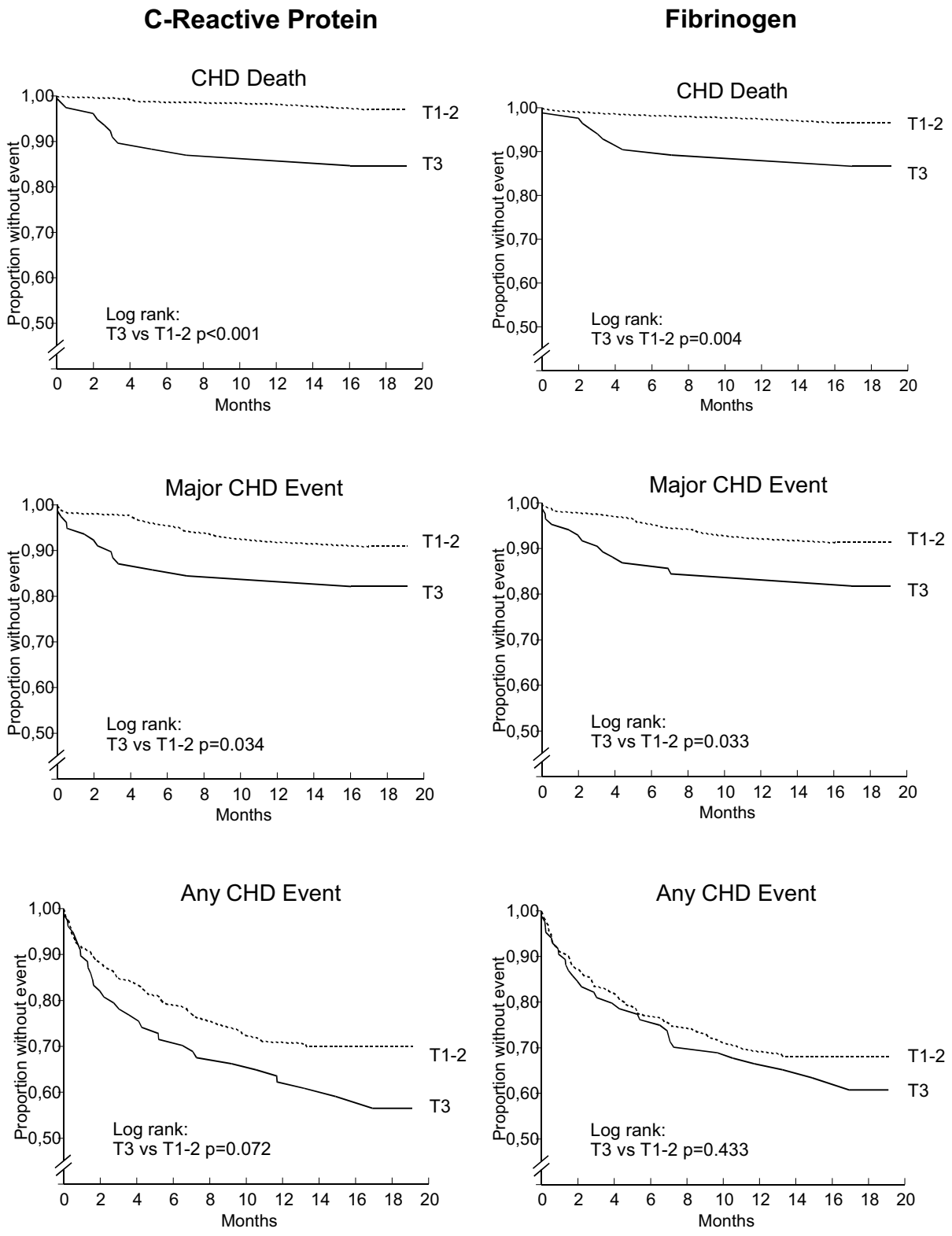

Figure 4. Kaplan-Meier survival curves for tertiles 1-2 (T1-2) and tertiles 3 (T3) of CRP (left column) and fibrinogen (right column) with regard to different CHD end-points.

Any CHD event = CHD death, non-fatal MI, revascularisation procedure or hospitalisation due to an acute CHD event

Major CHD event = CHD death or non-fatal MI

Cut-off value of the highest tertile for CRP was 1.49 mg/l, and for fibrinogen 3.75 g/l.

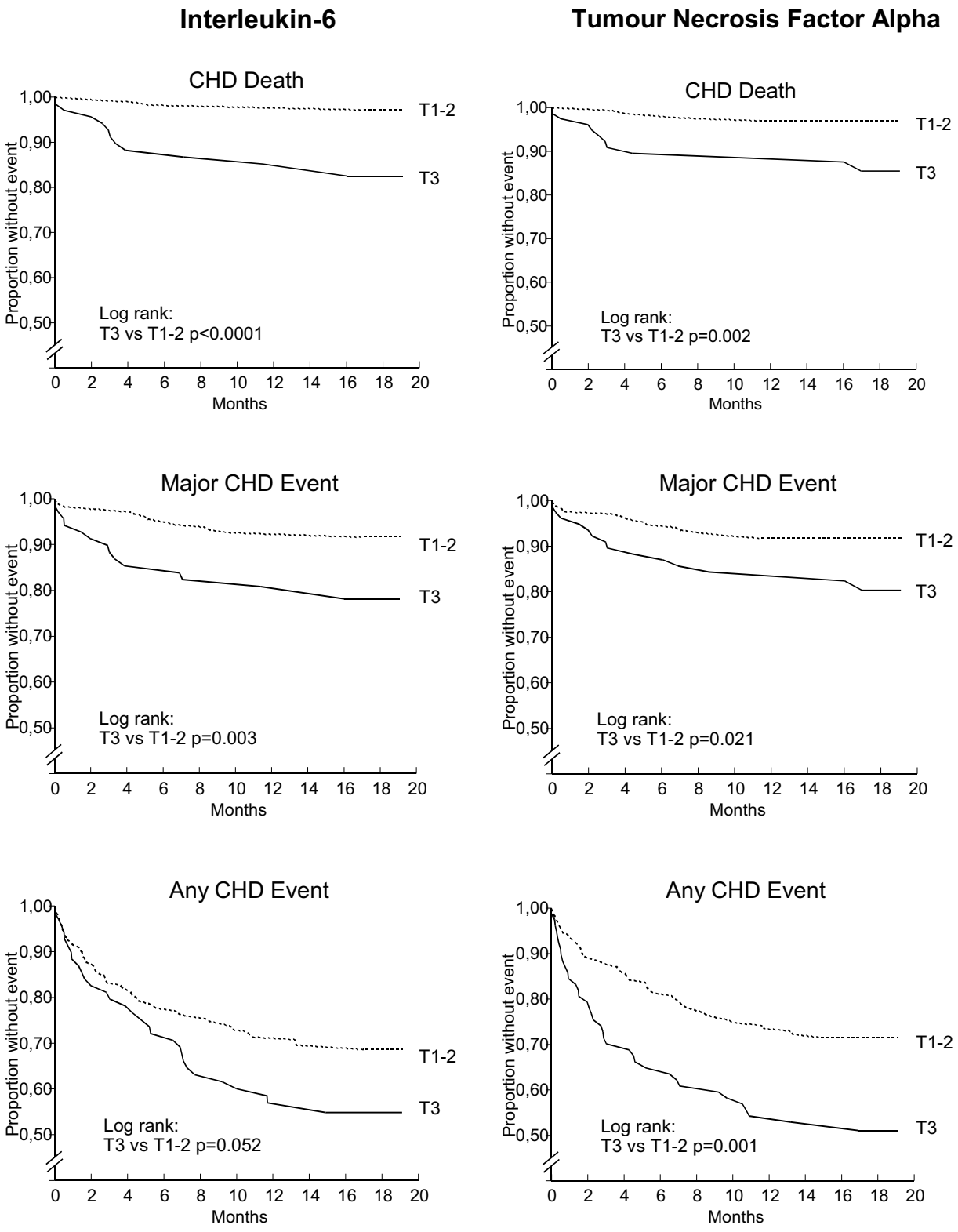

Figure 5. Kaplan-Meier survival curves of tertiles 1-2 (T1-2) and tertiles 3 (T3) for IL-6 (left column) and TNF-α (right column) with regard to different CHD end-points.

Any CHD event = CHD death, non-fatal MI, revascularisation procedure or hospitalisation due to an acute CHD event

Major CHD event = CHD death or non-fatal MI

Cut-off value of the highest tertile for IL-6 was 4.48 ng/l, and for TNF-α 4.91 ng/l.

Table 8. Factor analysis with acute-phase proteins, cytokines, and injury markers (rotated component matrix).

Variable	Factor 1	Factor 2
CRP	**0.77**	0.10
Fibrinogen	**0.78**	0.02
IL-6	**0.69**	0.06
TNF-α	0.09	**0.48**
TnT	-0.01	**0.84**
CK-MBm	0.06	**0.79**
% Variance explained	28.1	26.1

Factor loadings represent the correlation between the individual variable and each factor. Bolding indicates loadings >0.40 (absolute value).
N=255 in factor analyses; eight patients with nonspecific rises of creatine kinase MB mass were excluded.

Table 9. Hazard ratios and 95% confidence intervals for factors determined by factor analyses to predict different CHD end-points (Cox model analysis adjusting for age and gender; n = 255).

	HR (95% CI)		
	CHD death	Major CHD event*	All CHD events†
Factor 1 (inflammation factor)	1.88 (1.44-2.47)‡	1.52 (1.19-1.94)§	1.19 (0.99-1.44)
Factor 2 (injury factor)	1.49 (1.07-2.07)‖	1.31 (1.02-1.69)‖	1.10 (0.92-1.32)

* CHD death or non-fatal MI
† CHD death, non-fatal MI, revascularisation procedure or hospitalisation due to an acute CHD event
‡ $p<0.0001$.
§ $p<0.001$.
‖ $p<0.05$.

DISCUSSION

Study population

The study population consisted of a series of consecutive patients admitted to the emergency room with chest pain or its equivalent, and with a suspected ACS. The study population was unselected, compared with several earlier studies carried out on patients in coronary care units. However, patients who had a clear extracardiac reason for the symptoms, so that clinicians did not request conventional enzyme determinations, were not included in the study. Thus, the study population was slightly selected if it is compared with all patients admitted with chest pain despite the underlying reason. However, in the patients primarily excluded, the probability of an ACS must have been very low. In an unselected series of 3283 patients admitted to the emergency room of Kuopio University Hospital in 1997–99 with chest pain or cardiac arrest, the distribution of clinical diagnoses was somewhat different from this study. The proportion of patients with unspecific chest pain was as high as 36%, and 27% of the patients had MI as the hospital discharge diagnosis (H. Koukkunen *et al.*, unpublished observation).

In Study I, all of the 624 admissions were included to get as high a number of events as possible for the direct comparison of conventional enzymes with TnT and CK-MBm. In a descriptive study of this kind, it is not obligatory that a patient with several events during the study period should be included only once. In Studies II–IV regarding the prognosis of the 559 patients, each patient was included only once, and subsequent attacks were regarded as follow-up events. In Study II, the patients in whom chest pain or equivalent symptoms were relieved within 24 h from hospital admission were included, since these patients could potentially be discharged from the emergency department if MI and UAP could be excluded. In this patient population a reliable time of the onset of the symptoms was available. Thus, it was possible to study the time-window needed for ruling out MI from both the onset of symptoms and the hospital admission. Two patients who died from MI during the early hours were excluded, since it was not even theoretically possible that TnT could have been elevated by that moment. Study III comprised all of the 559 patients with first admission for ACS. In Study IV, we excluded 137 patients with definite or probable MI to ensure that the elevations of acute-phase protein and cytokine levels were not due to myocardial injury. Moreover, we excluded 159 patients with a cardiac event of non-CHD origin or a noncardiac event to diminish the probability of acute-phase protein and cytokine elevations related to other conditions than ACSs. Thus, 263 patients with UAP or a prolonged attack of chest pain were

included in Study IV. The coverage of follow-up in Studies II-IV was 100% with regard to the end-points used in the study.

Methods

Diagnostic classifications

A major problem in the use of diagnostic classifications for ACSs is the lack of a golden standard. In this study, the clinical and epidemiological diagnoses were based on symptoms, serial ECG findings and conventional enzyme determinations, and also autopsy findings in fatal events. The modified FINMONICA classification used for the epidemiological diagnosis is standardised, whereas the clinical diagnoses are more prone to subjective variation by the clinicians. By using noninvasive methods only, it is not always easy to differentiate between chest pain attacks of noncardiac origin and ACSs without detectable myocardial injury. Thus, the possibility of misclassification in some cases cannot be excluded.

Some features of the modified FINMONICA classification have to be taken into account when the results of this study are interpreted. Classification of symptoms was based on the clinicians' descriptions in the medical records. Thus, there may be some variability in the interpretation of the duration and the character of chest pain symptoms, which directly affects the diagnostic class. Serial ECG findings, based on serial Minnesota coding, form another element of the modified FINMONICA classification. Four ECGs, recorded during each hospitalisation on different days, were coded, if available. The ECG recorded on admission was always coded. In addition, another two or three ECGs showing the maximal changes were selected for the coding. Despite the attempt to find the maximal changes, the selection of the ECGs may have an effect on the diagnostic class. In some cases the ECGs were uncodable, *e.g.,* due to left or right bundle branch block, pacemaker rhythm, or third-degree atrioventricular block. In these cases, ECG could not be used in the diagnostic classification, which had to be based on the other elements, if available. The activities of conventional enzymes, CK, CK-MB and LD-1, form the third element of the diagnostic classification by both the clinical and epidemiological criteria. The conventional enzymes are a cornerstone especially in the diagnosis of reinfarctions and non-Q-wave MIs. However, the problem with especially CK and LD-1, but also with CK-MB, is that they are not totally cardiac-specific. Skeletal muscle injury due to trauma, surgery, or defibrillation, liver diseases and infections may be connected with conventional enzyme elevations without myocardial injury. Conventional enzyme elevations were omitted in the patients in whom such a condition was

known and registered in the medical records. The schedule of collecting the blood samples for the conventional enzymes as well as TnT and CK-MBm determinations was designed to detect MI as soon after the admission to the emergency room as possible. However, the schedule was also influenced by laboratory resources and other practical reasons.

Biochemical marker determinations

The 'second-generation' assay was used for measuring cardiac TnT concentrations. This method is very specific to myocardial damage and it has no significant cross-reactivity with skeletal muscle TnT. However, its calibration curve is somewhat non-linear (71). The 'third-generation' assay, introduced in 1999, uses standard material which differs from that used in the 'second-generation' assay. It has a linear calibration curve and a high precision, even at the lower end of the measuring range (71). The recommended CV at the cut-off limit for MI is <10% (12,71). Thus, it is possible that there is some fluctuation in the TnT concentrations near the cut-off limits in the method used in this study. However, the 'second-' and 'third-generation' assays show excellent correlation in the range 0–0.2 μg/l (71) that covers the cut-off limits used in this study.

In Study I, the data were analysed by using two cut-off limits, 0.10 and 0.20 μg/l for TnT. In most of the earlier studies on TnT as a marker of myocardial injury and in the risk stratification of patients with ACSs, the cut-off limit has been 0.20 μg/l (6,8,9,233,235,363-365). In more recent studies the cut-off limit of 0.10 μg/l has been used. The TnT assay manufacturer has recommended 0.10 μg/l as a cut-off limit, and this value was also obtained in our own series of assays in healthy subjects. Thus, the cut-off limit of 0.10 μg/l was used in Studies II-IV.

As mentioned on page 23, a common reference material has been recommended for CK-MBm assays by the AACC committee (58). However, common reference limits for all of the assays have not been achieved yet, and thus, cut-off value for CK-MBm used in previous studies has varied. The manufacturer of the assay used in our study recommended the use of a cut-off value of 5.0 μg/l, which is near to our own estimation of the URL. Since CK-MBm is not totally cardiac-specific, we studied the effect of two cut-off values, 5.0 and 10.0 μg/l, to avoid false MI diagnoses due to CK-MBm elevation in skeletal muscle injury.

In laboratory medicine, the reference interval has been conventionally defined on the basis of values obtained from a healthy population. The most frequently used reference interval is the central 95% interval bounded by the 2.5 and 97.5 percentiles (366). These reference limits are determined as mean value $\pm$ 2 SD if the true distribution is believed to be Gaussian or it

can be transformed to approximately Gaussian shape (366). Therefore, 2.5% of the values in healthy persons remain below and another 2.5% above the reference interval. The distribution curve of enzyme activities and their concentrations is skewed towards the high values even in healthy persons. In contrast, concentrations of cardiac troponins in the circulating blood are extremely low, and their distribution curve is remarkably skewed towards the low values. Therefore, it is appropriate to define the normal range as values below a fixed cut-off limit. In the redefinition of MI by the Joint Committee of the ESC/ACC, it was suggested that the upper limit of the reference interval for TnT or TnI should be defined as the 99th percentile of the value for a reference control group (12). However, 1% of the values in a healthy population are still above this cut-off value. According to the ESC/ACC redefinition, either the 99th percentile of the reference group or a maximal value exceeding twice the URL may be used as the upper limit of the reference interval for CK-MBm. The definition of the upper limit of the reference interval by the use of percentile points has the advantage that the upper limit can be determined directly from the distribution of values in the healthy population without need to consider the shape of the distribution.

TnT and CK-MBm are obviously more sensitive and specific in detecting myocardial injury than the conventional enzymes. However, CK-MBm is not 100% cardiac-specific, and it may be slightly elevated in connection with severe skeletal muscle injury. TnT measured by 'second-' or 'third-generation' assays is highly specific to myocardium, but its elevation is not always due to an ischaemic injury. Thus, as indicated in the review of the literature (pages 29-33) elevated TnT or TnI levels can be detected in patients with myocarditis, arrhythmias, chronic severe heart failure, and after CABG or valvular operations. Moreover, cardiac troponins may be elevated in some patients without conventionally recognised myocardial injury, such as in patients with chronic renal failure, pulmonary embolism or septic shock.

The blood samples for acute-phase protein and cytokine determinations were collected as soon as possible after admission at the emergency room – at the same time as the first samples for other biochemical marker determinations were drawn. In the acute phase of a MI a 'cytokine storm' is elicited. Thus, only the patients with UAP or a prolonged attack of chest pain were included in Study IV to ensure that the elevations of acute-phase protein and cytokine levels were not due to myocardial injury or related to conditions other than ACSs.

A rate nephelometry method with a lower limit of assay of 1 mg/l was used for the measurement of CRP. In modern, highly sensitive or 'ultrasensitive' methods the lower limit of assay may be as low as 0.05 mg/l (367). With the rate nephelometry method it was not possible to examine the association of very low concentrations of CRP with the prognosis of the ACS patients.

TnT and CK-MBm compared with conventional enzymes (Study I)

Study I showed that among patients with ACSs, TnT was elevated to >0.10 μg/l and CK-MBm to >5.0 μg/l, respectively, in virtually all of the patients in whom conventional enzyme activities (CK, CK-MB or LD-1) were elevated to more than twice the URL, corresponding to the diagnosis of definite MI. However, TnT and CK-MBm were not elevated in one-third of the patients with probable MI by conventional enzyme criteria (CK, CK-MB or LD-1 elevated to above the URL but to less or equal to twice the URL). We excluded from this comparison the patients in whom there was an obvious reason for nonspecific elevation in conventional enzyme activities. Thus, the proportion of unrecognised nonspecific conventional enzyme elevations seems to be substantial. Moreover, among the patients in whom the conventional enzyme activities remained within normal limits and the diagnosis was UAP or a prolonged attack of chest pain, TnT was elevated in 13% and CK-MBm in 15%. This finding suggests that the use of TnT and CK-MBm reveals the occurrence of a myocardial injury in a substantial number of patients with ACSs. However, the results were quite sensitive for the cut-off limits used. Moreover, the results for TnT and CK-MBm were not completely consistent with each other, which may at least in part be explained by the different diagnostic time-windows of these markers. Patients who had their MI at home several days before hospitalisation may have only cardiac TnT (and LD-1) elevated at the time of hospital admission.

Comparing our results with those of other studies is somewhat difficult due to various definitions and interpretations of the diagnoses of MI and UAP. The selection of study population and the specimen schedule have also been different. Some studies have concentrated on coronary care unit patients with a high probability of ACS. However, in other studies emergency room patients admitted with chest pain have been included. In some studies actual non-Q-wave MIs were included in the same category as UAP. On the other hand, a strict definition was used for UAP in several studies, as in our study (244-247).

The data on direct comparison of TnT or CK-MBm with conventional enzyme activities are scarce. In a study by Solymoss *et al.* (244) on 115 patients with an ACS, 28 had a diagnosis of MI and elevated CK (>200 U/l) and CK-MB (>30 U/l) activities. Of the 115 patients, 87 had a diagnosis of UAP, and CK-MB activity was within reference range (≤30 U/l) or slightly elevated but stable. Of the UAP patients, 15% had cardiac TnT elevated to >0.10 μg/l, which is approximately the same proportion as in our study. However, in the study by Solymoss *et al.* TnT was elevated in all of the 28 patients with MI. Ottani *et al.* (247) studied a series of 74 patients with clinical UAP, electrocardiographic evidence of myocardial

ischaemia, and total CK activity and CK-MBm within normal limits (<200 U/l and <6.7 μg/l, respectively). They found elevation of cardiac TnT to >0.10 μg/l in 18 patients (24%). They also found that cardiac TnI was elevated to >3.1 μg/l in the same percentage of patients. However, 23 patients had elevations of one or both markers, and in 13 there were elevations of both.

Probable or possible MI has been a controversial diagnostic category. In several studies, it has been placed into the same category as UAP. The cut-off limit of conventional enzymes for definite MI (more than twice the URL) is high, which leads to high specificity for the MI diagnosis. However, this cut-off limit was primarily designed for epidemiological studies, and it was only later adopted into use in clinical practice. To avoid a 'black-or-white' thinking and to increase the sensitivity of the MI diagnosis, the category of probable MI was used in clinical practice internationally until ICD-8 was replaced by ICD-9, and in Finland also thereafter. The diagnostic category "possible MI" of the FINMONICA criteria was specifically created to maintain this practice in epidemiology and to support its use by clinicians. Our study clearly demonstrated the need for new diagnostic criteria for ACSs not defined as Q-wave MIs in the era of sensitive and specific markers of myocardial injury, such as cardiac troponins. This applies to clinical practice, epidemiological studies, and also to the definition of inclusion criteria and end-points of clinical trials. An ACS with a mildly elevated cardiac troponin level but with CK-MBm or CK-MB activity within normal limits has been called in the literature 'microinfarction', 'minimal myocardial damage', or 'minimal myocardial injury' (6-8). However, this term was not included into the redefined criteria of MI by the Joint Committee of the ESC/ACC (12). The leading concept in the redefinition of MI diagnosis was that any amount of myocardial necrosis caused by ischaemia and detected by elevation of cardiac TnT, TnI or CK-MBm should be regarded as MI.

Ruling out MI with TnT and CK-MBm (Study II)

Study II demonstrated that among patients admitted with a suspected ACS and whose symptoms were relieved, MI could be ruled out with a high probability if cardiac TnT remained ≤0.10 μg/l until 12-24 h and CK-MBm ≤5.0 μg/l until 10-12 h from the onset of symptoms. If TnT and CK-MBm remained within normal limits until 12 h from the hospital admission, MI may be ruled out in 99% and 96% of the patients, respectively. Moreover, among patients in whom MI was ruled out the one-year risk of CHD events was related to a positive history of CHD.

‘Rule-in’ is an approach to the diagnosis of MI in which the intent is to recognise patients with MI as soon as possible after arrival to the emergency department by using sensitive indicators of myocardial injury. By using rapid ‘rule-in’ the appropriate treatment procedures can be started without delay. However, we chose to use a ‘rule-out’ protocol in Study II, since the patient triage and the ruling out of the diagnosis of MI in patients admitted with chest pain forms a considerable part of everyday work in emergency departments, and the biochemical markers are important tools in that work. This approach aims to rule out MI and high-risk ACSs and to recognise those patients who do not benefit from hospitalisation and who can be safely referred to outpatient clinics for further assessment.

In some previous studies it has been suggested that the blood sampling schedule for myocardial injury markers should be tailored on the basis of the time of onset of the symptoms (182,183,185,365). However, this is difficult to put into practice in busy emergency departments. Moreover, the patient may have had several successive attacks of chest pain. In these cases the time of onset of the ischaemic myocardial injury may be difficult to define. Even if the patient has had only a single chest pain attack its time of onset cannot always be determined reliably. The time of hospital admission is a more objective moment of time for scheduling the biochemical marker determinations. The ESC/ACC Joint Committee (12) recommended that blood samples for biochemical marker determinations should be routinely taken on admission and 6-9 h after admission. If the previous determinations are negative and MI is still strongly suspected, a third sample should be taken 12-24 h after admission. Our findings agree with this recommendation. However, in cases with a reliable knowledge of the time of onset of the symptoms, it is possible to use that time point as the basis of the blood sampling schedule. Moreover, the ability of biochemical markers to rule out MI is highly dependent on the cut-off values used for them and on the diagnostic criteria used for MI.

The results of Study II are concordant with those of a study by de Winter *et al.* (182), in which the diagnosis of MI was based on symptoms, ECG abnormalities and typical rise and fall in CK-MB activity. They found that MI can be ruled out by CK-MBm with 95% certainty 7 h and with 99% certainty 16 h after the onset of symptoms. By TnT, MI could be ruled out with 92% certainty 12 h and with 97% certainty 16 h after the onset of symptoms. However, CK-MBm and TnT were found to rise later in patients with small infarcts than in patients with large infarcts (182). Thus, the size of the infarcts may influence the sensitivity and specificity for these markers in the first 8 h after the onset of symptoms. In a more recent study de Winter *et al.* (183) found that normal CK-MBm on admission and at 7 h ruled out MI in 99% of patients admitted with symptoms of less than 5-h duration. However, all of the patients

were kept under observation at least until 12 h after the onset of symptoms. Herren *et al.* studied a series of 292 patients admitted to emergency department with chest pain of less than 12-h duration (184). According to their results, MI could be ruled out on the basis of 6-h observation, serial measurements of CK-MBm and continuous ST-segment monitoring at the emergency department. The diagnosis of MI was confirmed or ruled out by measurement of TnT at 48 h. However, the patients had a low to moderate risk of MI, and ultimately, only 36 (12%) of the patients had a diagnosis of MI. In a study by Bakker *et al.* on 290 consecutive patients (365), MI could not be confirmed or ruled out reliably on the basis of any biochemical marker (CK, CK-MB, CK-MBm, TnT or myoglobin) until 10-12 h after the onset of symptoms. Noble (185) recommended that patients with a history suggestive of MI but normal ST-segments or known conduction and other ECG abnormalities should undergo serial ECG and TnT monitoring for 12 h following admissions. Low-risk patients on the basis of the history or ECG could be monitored for a shorter time (185).

Most of the earlier studies have not included data on the prognosis of patients in whom MI was ruled out on the basis of CK-MBm or TnT (182-184,368). Some studies have reported a favourable in-hospital or short-term prognosis in these patients (181,369). In a series of 128 patients without MI by the WHO criteria, de Winter *et al.* reported that although TnT was the only biochemical marker that predicted coronary events within 6 months, the best predictor was a positive history of CHD (236). Our finding in Study II that the one-year risk of CHD events was strongly influenced by a positive history of CHD among the patients in whom MI was ruled out is in accordance with their results.

Diagnosis of MI by TnT in comparison with clinical and epidemiological criteria (Study III)

According to the recommendation for the diagnostic criteria of MI by the Joint Committee of the ESC/ACC, the diagnosis of MI should be primarily based on the elevation of cardiac TnT or TnI above the 99th percentile of the values of a reference control group (12). However, the effect of this fundamental change in the diagnostics of MI on the incidence and case fatality of MI has so far been studied, to our knowledge, in only four published studies. Study III demonstrated that the diagnosis of MI made on the basis of elevated TnT (>0.10 μg/l) increased the total number of MI diagnoses by 33% compared with a clinical diagnosis and by 23% compared with a standardised epidemiological diagnosis of definite or probable MI. On the other hand, TnT was not elevated in 13% of the patients with a clinical or epidemiological MI diagnosis. As in Study I, this finding suggests that in these patients the MI diagnoses may

have been false positive due to nonspecific elevations of conventional enzyme activities. Moreover, in Study III the 17-month prognosis with regard to CHD death or nonfatal MI was worst in patients with concordant TnT-based and clinical or epidemiological diagnosis of MI. Among the patients in whom clinical or epidemiological diagnosis of MI was not made, the prognosis was not significantly worse in patients with TnT >0.10 μg/l than ≤0.10 μg/l. A summary of the studies on the effect of cardiac troponins on the number of MI diagnoses is presented in Table 10.

A Norwegian study by Graven *et al.* (370) was published at about the same time as our study. The study population consisted of 442 patients with a suspected ACS, 172 of whom had a diagnosis of MI on the basis of elevation of total CK activity to more than twice the URL, and 100 of whom had UAP. Thus, their cut-off limit for total CK corresponded with the conventional enzyme criterion of definite MI in Study III. On the basis of the ESC/ACC recommendation, the diagnosis of MI was redefined by using cardiac TnI and CK-MBm. A cut-off value of 1 μg/l, corresponding to the 99th percentile of a reference control group, was used for TnI. Two different cut-off values were used for CK-MBm: 6 μg/l, corresponding to the 99th percentile of a reference control group, and 12 μg/l, corresponding to twice the URL. Graven *et al.* found that by using CK-MBm >6 μg/l or >12 μg/l as the main criterion to distinguish between MI and UAP the number of MIs increased by 29% or 17%, respectively. On the other hand, by using TnI >1 μg/l the number of MIs increased by 37% (370). The percentage increase in the number of MIs was of the same magnitude as in Study III, although the definitions of MI were different, and the redefined diagnoses of MI were based on TnI and CK-MBm instead of TnT. All the patients with Q-wave MI fulfilling the total CK criteria of more than twice the URL had both TnI and CK-MBm elevated (370). This is also in harmony with our finding in Study I that the diagnosis of definite MI with elevation of conventional enzyme activities to more than twice the URL would be virtually unchanged if the diagnosis of MI were based on elevated cardiac TnT or CK-MBm. According to Graven *et al.*, the in-hospital mortality of MI was 14%, 11% or 10%, when the diagnosis was based on total CK activity, CK-MBm or TnI, respectively (370). In Study III, the in-hospital mortality was 8.3-9.0% for definite MI, 7.4-7.9% for definite or probable MI, and 6.5% for TnT-based diagnosis of MI.

Table 10. "Old" and "new" diagnostic criteria of MI, and the percentage increase in the number of MIs by the "new" *vs.* "old" criteria, according to the recent published studies and abstracts.

Study	N	"Old" criteria of MI	"New" criteria of MI	Increase in the number of MIs (by "new" *vs.* "old" criteria, %)
Published studies				
Study III	559	Epidemiological diagnosis	TnT > 0.10 μg/l	23
		Clinical diagnosis (CE > URL in both)	TnT > 0.10 μg/l	33
Graven *et al.* (370)	442	CK > 2 x URL	TnI > 1 μg/l	37
		CK > 2 x URL	CK-MBm > 6 μg/l	29
		CK > 2 x URL	CK-MBm > 12 μg/l	17
Meier *et al.* (371)	493	Elevated CK-MB	Elevated TnI and normal CK-MB	23
Ferguson *et al.* (372)	80	ECG not diagnostic to definite MI and CK > URL or CK-MBm > 2.37 μg/l	ECG not diagnostic to definite MI and TnI ≥ 0.06 μg/l	39
Abstracts				
Meier *et al.* (373)	305	Elevated CK-MB	Elevated TnI and normal CK-MB	16
Goodman *et al.* (374)	3420	CK ≥ URL	Elevated TnT or TnI	15
		CK ≥ 2 x URL	Elevated TnT or TnI	26
		CK-MB ≥ URL	Elevated TnT or TnI	9
Gitt *et al.* (375)	717	Non-ST-elevation MI with elevated CK	Non-ST-elevation MI with elevated TnT or TnI and normal CK	124
Trevelyan *et al.* (376)	401	Elevated CK or ASAT	Elevated TnT	32
			CK-MBm > 5 μg/l	27
Wilson *et al.* (377)	964	CK > 400 U/l	TnT or TnI > 0.1 μg/l	62

Table 10. (continued)

Study	N	"Old" criteria of MI	"New" criteria of MI	Increase in the number of MIs (by "new" *vs*. "old" criteria, %)
Abstracts				
Cassin *et al.* (378)	760	"Traditional criteria"	CK-MBm > 5 μg/l or TnI > 0.13 μg/l	43
Large *et al.* (379)	1348	"Traditional criteria"	ESC/ACC criteria	62
Aguiar *et al.* (380)	315	Non-ST-elevation ACS and elevated CK-MB	Non-ST-elevation ACS and elevated TnI	96

ACS = acute coronary syndrome
CE = conventional enzyme activities
CK = creatine kinase
CK-MB = creatine kinase isoenzyme MB
CK-MBm = creatine kinase isoenzyme MB mass
MI = myocardial infarction
TnI = troponin I
TnT = troponin T
URL = upper reference limit

Ferguson *et al.* (372) studied a series of 80 consecutive patients admitted to the hospital with suspected ischaemic acute chest pain. In contrast to most of the other studies summarised in Table 10, the patients in whom the ECG indicated definite MI were excluded. They found that among patients with ACSs but non-diagnostic ECG changes, 23 patients (29%) fulfilled the conventional diagnostic criteria of MI (total CK > the URL or CK-MBm >2.37 μg/l). However, 32 patients (40%) fulfilled the "new" diagnostic criterion of MI with TnI (Stratus CS assay) elevated to ≥0.06 μg/l, which meets the requirement of 10% CV. Thus, in this selected population the number of patients getting a diagnosis of MI increased by 39%.

The effect of cardiac troponins on the number or prognosis of patients with MI diagnoses has also been studied in at least ten other studies, nine of which have been published so far only as abstracts. Meier *et al.* (373) studied a series of 305 patients with a suspected ACS and elevated biochemical markers. Of those, 264 had an elevated CK-MB regardless of TnI status and 41 had an elevated TnI and normal CK-MB. Thus, the inclusion of TnI in the diagnostic criteria led to an increase of 16% in the number of MIs. In an article published recently by the same group (371), 224 patients had an elevated CK-MB and 51 had an isolated elevation of TnI in a series of 471 patients. The increase in the number of MI diagnoses was therefore 23%. The patients with elevated CK-MB were younger and required more PCIs during hospitalisation. On the other hand, the patients with isolated TnI elevation had a significantly shorter in-hospital stay, and tended to have fewer in-hospital events such as shock, CABG, death or reinfarction than those with elevated CK-MB, although these differences were not statistically significant (371). The 6-month mortality was higher among the patients with isolated TnI elevation than among those with elevated CK-MB, but the finding is based on rather low numbers of deaths. There were no differences in the 6-month incidences of rehospitalisation and the combined end-point of death or rehospitalisation (371). The effect of TnT or TnI on the MI diagnosis rate was also studied in a series of 3420 patients enrolled in the Global Registry of Acute Coronary Events in 94 centres in 15 countries (374). An isolated elevation of troponin concentration as the criterion for MI increased the number of MI diagnoses by 15%, 26%, and 9%, compared to the diagnoses based on CK ≥ the URL, CK > two times the URL, and CK-MB ≥ the URL, respectively. Moreover, among the patients with CK ≤ two times the URL, the troponin positive patients had 3-fold higher in-hospital mortality than those with cardiac troponin within normal limits (374). Among the patients with CK > two times the URL, the in-hospital mortality was 1.5-fold higher in the troponin positive patients compared with those without elevated cardiac troponin. In another substudy of the Global Registry of Acute Coronary Events, the in-hospital risk of death or recurrent MI was 3-fold higher in patients with non-ST-elevation MI based on isolated troponin elevation

than in patients with TnT or TnI within normal limits (381). The risk was 2-fold higher in patients with non-ST-elevation MI with both elevated CK-MB (or CK) and cardiac troponin than in those with isolated troponin elevation, respectively. There was no difference in the likelihood of revascularisation between the two non-ST-elevation MI groups. In a study based on a German Acute Coronary Syndromes registry (375), the new definition increased the number of non-ST-elevation MIs by as much as 124% compared with patients with elevated CK levels and no ST-segment elevation. In-hospital mortality was 2-fold higher in patients with elevated CK levels than in patients with isolated troponin elevation. In a study by Trevelyan *et al.* (382) the 6-month prognosis with regard to major adverse cardiovascular events was similar in patients with elevated TnT, regardless of the diagnosis by the WHO criteria. The patients with TnT within normal limits had a superior prognosis, but they were not event-free. Wilson *et al.* (377) found that in-hospital mortality fell from 7.4% to 4.6% when the diagnosis of MI was based on TnT or TnI >0.1 μg/l instead of CK >400 U/l. Correspondingly, Large *et al.* (379) found that in-hospital case fatality fell from 12.8% to 7.1% when the ESC/ACC criteria were used instead of "traditional criteria". Finally, in the study by Aguiar *et al.* (380) among patients with non-ST-elevation ACSs, 30-day outcome was similar in patients with elevation of CK-MB to those with isolated elevation of TnI.

Due to high sensitivity of cardiac troponins they can show small myocardial injuries not recognised by conventional enzymes. Thus, in Study III, the study by Graven *et al.* (370), and the study published in abstract form by Meier *et al.* (373), the in-hospital mortality of patients with the diagnosis of MI based on cardiac troponins was lower than that of patients with the diagnosis of MI based on conventional enzymes. However, the isolated elevation of cardiac TnT or TnI may be related to an unstable atherosclerotic coronary plaque, and a small myocardial injury may precede a more serious myocardial damage (259-261,383). Among these studies summarised in Table 10, our Study III had the longest follow-up time. Meier (371) and Trevelyan (382) studied the 6-month mortality, and Aguiar (380) studied the 30-day outcome, but the other studies concentrated on in-hospital prognosis.

The inclusion of cardiac troponins in the diagnostic criteria of MI leads to an increase in the number of MI diagnoses. The magnitude of this increase is strongly dependent on which criteria, *e.g.*, enzymes and their cut-off values, are used in the conventional diagnosis of MI, since the definition of MI has varied in different studies. The increase in the number of MIs with the introduction of more sensitive and specific diagnostic methods is not a new phenomenon in the evolution of MI diagnosis. It was demonstrated already in the 1980s in the Minnesota Heart Survey that the attack rate of MI increased by 17% or 24% when CK or CK-

MB activities were added to the diagnostic algorithm (196). A remarkable proportion of patients formerly diagnosed as having UAP will now get a diagnosis of MI.

There is good evidence on the prognostic value of elevated TnT in non-ST elevation ACSs (8,9,11,70,233-241). Among patients with ACSs those with elevated cardiac troponins benefit the most from active revascularisation and antithrombotic treatment strategies (248,254-257). In Study III, in accordance with previous studies, the prognostic value of TnT and CK-MBm was better than of CK activity in predicting the risk of recurrent CHD events in patients without definite MI. Moreover, the prognosis of the patients who did not have MI by clinical or epidemiological criteria but in whom TnT was elevated tended to be worse than the prognosis of the patients in whom MI was concordantly excluded. However, the number of these patients was relatively small, and the prognosis of this patient group needs further examination in larger patient populations.

Acute-phase proteins and cytokines in relation to prognosis of unstable angina pectoris (Study IV)

Study IV demonstrated that slightly elevated levels, even within the reference ranges, of acute-phase proteins (CRP and fibrinogen) and cytokines (IL-6 and TNF-α) measured on admission strongly predicted the risk of CHD death and major CHD events in UAP patients during the median follow-up time of 17 months. Among the patients with UAP, 10% of those with CRP >1.49 μg/l but only 1% of those with CRP ≤1.49 μg/l died from CHD within four months after the index event. As an exception to other inflammation markers, slightly elevated TNF-α was associated with an increased risk of a combined end-point of all CHD events, and also of all nonfatal CHD events. In factor analysis, CRP, fibrinogen and IL-6 clustered on an ‘inflammation factor’. However, TNF-α clustered together with TnT and CK-MBm on an ‘injury factor’. Both of these factors were independent predictors of CHD death or major CHD events. This finding suggests that in patients with UAP, an underlying inflammatory component, which is unrelated to myocardial injury, is associated with the risk of recurrent CHD events. On the other hand, the association of slightly elevated levels of TNF-α with the risk of recurrent CHD events seems to include dimensions other than those reflected by CRP, fibrinogen, and IL-6.

The prognostic value of CRP in ACSs is well documented (16,17,19,214,278,279,319). However, the data are scarce on the relation of other inflammation markers to the prognosis of ACSs (278-280,319,347,348). The prognostic values of inflammation and injury markers are

independent of and additive to each other, which has been shown in several studies (18,279,334,335). In Study IV, a strict definition was used for UAP, and thus, elevated cardiac TnT was detected in only 13% and elevated CK-MBm in only 14% of the patients. The prognostic value of the inflammation markers was independent of the injury markers. Due to the small number of patients with elevated TnT or CK-MBm, these markers were not strong predictors of adverse prognosis in this population. The 'injury factor' nonetheless predicted CHD death and major CHD events, but less strongly than the 'inflammation factor'. Study IV is in agreement with the suggestion by Lagrand *et al.* (332) that subgroups of patients with or without myocardial injury should not be combined when the association of inflammation markers with cardiovascular risk is studied.

Since TNF-α is a part of the same inflammation cascade as CRP, fibrinogen and IL-6, it was surprising why it did not cluster on the 'inflammation factor'. It has been suggested that the heart muscle itself may be the source of TNF-α in severe cardiac failure in humans (312), and also in postischaemic reperfused myocardium of rats (384,385). One possible explanation in our study population is the presence of small ischaemic but reperfused myocardial injuries detected by TnT or CK-MBm but not by conventional enzymes. On the other hand, slightly elevated levels of TNF-α may be observed in connection with heart failure (312,386). However, our clinical data did not allow examination of this hypothesis.

In Study IV, the acute-phase protein and cytokine determinations were based on one blood sample drawn on admission to the hospital. For practical reasons it was not possible to obtain another blood sample for inflammation marker determinations after hospitalisation to study the prognosis of patients in whom inflammation markers remained permanently elevated. In studies by Biasucci *et al.* (319) and Ferreirós *et al.* (19), CRP remained elevated after the acute event in a substantial proportion of the patients with UAP. The permanently elevated CRP was strongly associated with short- and medium-term risk of recurrent CHD events. Thus, in Study IV the use of a single determination of inflammation markers obtained on admission may have underestimated their ability to predict future CHD events.

Production of cytokines, such as IL-6 and TNF-α, and fibrinogen has been found to exhibit diurnal rhythmicity (387-389). In a series of 11 healthy men, Sothern *et al.* (387) found that on average, circulating IL-6 concentrations were greater than the mean throughout the night, with a peak at 01:00 hours and less than the mean throughout the day. In a study by Kanabrocki *et al.* (389) a positive correlation between the points in time of the peak levels of IL-6 and fibrinogen was found. Since IL-6 is known to stimulate fibrinogen production in the liver, Kanabrocki *et al.* suggested that suppression of IL-6 production would lower peak fibrinogen levels occurring in the morning in association with arterial ischaemic events (389).

These circadian characteristics of circulating cytokines and fibrinogen seem to form a confounding factor with regard to studies on the associations of inflammation markers and ACSs.

The diagnosis of myocardial infarction

The diagnosis of MI has traditionally been based on the triad of symptoms, serial ECGs and biochemical markers of myocardial injury. The development of these markers has been reviewed on pages 20-27. Novel biochemical markers introduced into clinical practice have usually been more sensitive and specific than their precursors. As shown in the Minnesota Heart Survey in the 1980s, the insertion of CK and CK-MB activities into the previous diagnostic algorithm led to an increase of 17% and 24% in the attack rate of MI (196). In this thesis, a further transition, *i.e.*, an increase of 23 to 33% in the number of patients getting the diagnosis of MI was found when cardiac troponins were applied as an integral part to the diagnostic algorithm instead of CK, CK-MB and LD-1 activities (Study III), as suggested by the ESC/ACC Joint Committee (12). Actually, Study III was one of the first studies in which this transition was quantified.

An unanswered question is how much myocardium must become necrotic until it is justified to use the term MI. By using more and more sensitive tools, smaller and smaller myocardial injuries can be detected. Thus, when novel biochemical markers are introduced into clinical use, it is important to know their performance in relation to conventionally used methods. In this thesis, the levels of cardiac TnT and CK-MBm were directly compared with conventional enzymes by using several cut-off points (Study I). It was found that in the diagnosis of large MIs the use of TnT or CK-MBm does not bring any additional benefit to the diagnosis formed on the basis of symptoms, ECG and conventional enzyme activities. However, TnT and CK-MBm are helpful in the detection of small myocardial injuries that are undetectable by conventional enzymes. These injuries do not jeopardise the myocardial function. However, their elevation may be a sign of a transient thrombotic vessel occlusion at the site of plaque erosion, or of distal microembolisation from the thrombus in unstable CHD. These small injuries may be related to unstable coronary plaques and predict more serious injuries (259-261,383). Since TnT and CK-MBm are more sensitive than conventional enzymes, they revealed the presence of small myocardial injuries in 13 to 15% of patients diagnosed as having UAP or a prolonged attack of chest pain based on conventional enzymes within normal limits (Study I). Furthermore, in Study I TnT and CK-MBm were found to be within normal limits in one-third of the patients who met the conventional enzyme criterion of

probable MI, indicating nonspecific elevations of conventional enzyme activities in those patients. Accordingly, Study III showed that TnT was not elevated in 13% of the patients with definite or probable MI based on clinical or epidemiological criteria. Thus, modern biochemical markers sharpen the diagnosis of MI by reducing the number of false-positive cases. These findings also suggest that the transition in the number of patients getting a diagnosis of MI by TnT-based criteria is not merely an increase, but rather a crossover. Study I was one of the first studies in which modern biochemical markers were directly compared with conventional enzyme activities, and especially in a patient group with a strictly defined diagnosis of UAP. As mentioned on pages 83-84, the results are in harmony with the studies by Solymoss *et al.* (244) and Ottani *et al.* (247).

Modern biochemical markers are also helpful in detecting or ruling out MI in patients with atypical symptoms or nondiagnostic ECGs (Study II). However, although cardiac troponins are sensitive and specific markers of myocardial injury, their increase is not confined to ischaemic injuries due to coronary artery obstruction. The interpretation of elevated troponins is therefore not always unambiguous. Furthermore, the clinical picture and ECG are still important in the diagnosis of ACSs as well as in clinical decision-making. On admission to the hospital, the presence or absence of ST-elevations on the ECG directly affects the treatment decisions. Correspondingly, recurrent attacks of chest pain and ST-segment depressions in patients hospitalised with ACSs are signs of an adverse prognosis and indicate need for urgent coronary angiography (191).

After the introduction of the modern biochemical markers, their time-window for ruling out MI has been a subject of a wide interest (182-185,365). In this thesis, MI could be ruled out by TnT in 99% or by CK-MBm in 96% of patients within 12 h from admission to the emergency department (Study II). Therefore, MI cannot be diagnosed or ruled out by troponins or CK-MBm essentially earlier than by conventional enzymes. The results are, however, rather sensitive to the cut-off limits used. In Study II, a rather low cut-off limit of 5.0 μg/l was used for CK-MBm to exclude myocardial injury as reliably as possible. Individual timing of blood samples in relation to the onset of symptoms is problematic, and the time of the onset of the symptoms cannot always be defined reliably. In clinical practice, the timing of biochemical marker measurements in relation to hospital admission seems to be sufficient, as suggested in Study II.

The WHO MONICA criteria were initially designed for epidemiological studies, but they were later adopted into clinical use (5). However, there has not been any international consensus on the use of the diagnostic class of probable MI in clinical practice, or in clinical and epidemiological studies. The patients without definite MI (or Q-wave MI) have therefore

in several studies been classified as having UAP. With the adoption of the modern biochemical markers of myocardial injury, the borderline between a detectable MI and an ACS without MI is shifting, and a remarkable proportion of patients formerly diagnosed as having UAP will now get a diagnosis of MI. This development will not only have clinical and epidemiological, but also important psychological, social, and economic consequences for both the individual and society. The redefinition of MI by the ESC/ACC Joint Committee (12) and the Finnish recommendation (201) are steps forward in the long process of standardising the diagnosis of MI. The universal standardisation of the diagnoses would be beneficial not only in everyday clinical practice but also in epidemiological studies and clinical trials, the results of which would therefore be more easily applied to clinical practice. However, several unsolved questions exist, as reviewed on page 40. In the future, uniform definitions for perioperative MIs in connection with CABGs or PCIs are also required. Widespread use of cardiac troponins or CK-MBm, and specific, precise and well-standardised methods for their determination are prerequisites for the use of a diagnostic algorithm based on modern biochemical markers. Overall, it seems that an international consensus will not be easily achieved (202,390).

The prognosis of acute coronary syndromes

Biochemical markers of myocardial injury

As reviewed on pages 46-47, the prognostic value of cardiac TnT and TnI is well established in ACSs. However, the prognostic value of CK-MBm is more controversial (p 47). Elevated levels of cardiac troponins have been regarded as markers of thrombotic risk, and therefore of acute risk in ACSs (190). However, the elevated levels of injury markers may also be reflections from large myocardial injuries, leading to left ventricular dysfunction and worsening the long-term prognosis.

A strict definition of UAP (with conventional enzyme activities within normal limits) was used in Study IV to investigate the prognostic value of the modern injury markers and inflammation markers in patients with minor or undetectable myocardial injuries. Although TnT was elevated to >0.10 μg/l in only 13% and CK-MBm to >5.0 μg/l in only 14% of the patients, the ‘injury factor’ comprising both of the injury markers and TNF-α was an independent predictor of CHD death and major CHD events during the median follow-up of 17 months. In Study III, among the patients without clinical diagnosis of MI TnT was elevated to >0.10 μg/l in 14%, and among the patients without epidemiological diagnosis of

MI in 12%. Among the patients without a clinical or epidemiological diagnosis of MI, the prognosis with regard to major CHD events in patients with TnT >0.10 μg/l was not significantly worse than in those with TnT ≤0.10 μg/l, although there was a trend towards a worse prognosis. This finding was, however, based on relatively small numbers of patients.

Many earlier studies on the prognostic value of cardiac troponins in unstable CHD have included patients with non-Q-wave MI (70,237-241). In this thesis, total CK activity, TnT and CK-MBm predicted the risk of CHD death during the 17-month follow-up among patients with an epidemiological diagnosis of definite MI excluded (Study III). However, TnT and CK-MBm were better than CK activity in predicting major and all CHD events. A question has been raised on the degree to which troponin testing adds to the prognostic information obtained from the ECG and other clinical factors. Most studies have examined the prognostic value of each clinical factor separately rather than in multivariate analyses. However, according to some studies cardiac troponins seem to provide independent prognostic information (215,235,238,239,242,243,248).

Recently, pharmacological treatment and revascularisation practice in patients with ACSs has become more and more active. Patients with elevated cardiac troponins seem to benefit most from these treatment strategies (254-257). However, the treatment strategy for each individual patient has to be chosen on the basis of all-inclusive clinical evaluation rather than just on the elevation of these markers. Especially on admission and during early hours of the hospital care, major clinical decisions, such as the need of thrombolytic treatment or an early coronary angiogram and revascularisation therapy, have to be based on clinical features and findings on the ECG.

Inflammation markers

Inflammation has been found to be associated with CHD, both in the pathogenesis of atherosclerotic coronary lesions and in the triggering of ACSs. Slight elevations of inflammation markers, even within the reference limits, have been found to be associated with adverse prognosis, not only in patients with UAP or non-Q-wave MI (16,279), but also in patients with stable angina pectoris (17,320), and even in apparently healthy persons (21,22).

In Study IV the ‘inflammation factor’ comprising CRP, fibrinogen and IL-6 was an independent predictor of CHD death and major CHD events during the median follow-up of 17 months. In Study IV all of the inflammation markers studied, *i.e.*, CRP, fibrinogen, IL-6 and TNF-α, independently predicted an increased risk of CHD death and IL-6 of major CHD events. Differently from other inflammation markers, TNF-α was an independent predictor of

all CHD events, and it also clustered on the 'injury factor' together with TnT and CK-MBm (Study IV). The potential explanations for this finding are discussed on page 93. CRP reflects the amount and activity of circulating proinflammatory cytokines, such as TNF-α, IL-1 and IL-6. Compared with other inflammation markers, the value of CRP in risk stratification is the most widely documented, and its use has several practical advantages. The 19-hour half-life makes CRP easy to detect in circulation, as reviewed by Futterman and Lemberg (391). On the contrary, the plasma half-lives of proinflammatory cytokines, such as TNF-α, are short (391). There are two widely recognised standards for CRP: the WHO 1st International Reference Preparation for C-Reactive Protein Immunoassay (85/506) (392), and the Certified Reference Material 470 (CRM 470), which is directly traceable to the WHO material (393). However, both of these standards originate from the time before the introduction of the highly sensitive CRP assays. Unlike fibrinogen and proinflammatory cytokines, diurnal variation of CRP levels has not been detected (394). However, among patients with chronic stable angina, higher baseline CRP concentrations have been detected in women than in men (395). Wu *et al.* have also found that mean CRP concentrations increase with age in healthy persons (396).

Based on recent studies, no given concentration of CRP can be unambiguously recommended as an optimal cut-off limit for risk stratification. A suggestion was made by Rifai and Ridker on the use of quintiles of highly sensitive CRP in combination with the quintiles of the ratio of total cholesterol to HDL-cholesterol in cardiovascular risk assessment in previously healthy persons (397). In the suggestion, the quintiles were derived from population-based surveys, whereas cardiovascular risk estimates were obtained from prospective studies. It has also been suggested that in primary prevention the prognosis of the persons with slightly elevated or high-normal levels of CRP improves with the use of statins or aspirin more than the prognosis of those with low levels of these markers (21,339). However, all of the highly sensitive methods for CRP determinations are not yet precise and comparable enough to apply results obtained in population studies to individual patients (398). Therefore, further standardisation efforts are needed. Furthermore, the consideration of confounding factors, such as concomitant trauma or infection, is essential.

CRP has also been suggested as an additional tool for risk stratification in patients with ACSs. Increased CRP is regarded as a marker of underlying disease, and therefore of long-term risk (190). A question has been raised on how much inflammation markers can add to the prognostic value of conventional risk indicators in non-ST-elevation ACSs. In Study IV all of the inflammation markers predicted an increased risk of CHD death, and additionally, IL-6 predicted an increased risk of major CHD events, independently of age, gender and injury markers. Also in other studies the prognostic value of inflammation markers has been

found to be independent of and additive to that of injury markers (214,279,337). Bazzino *et al.* (399) studied a 90-day outcome in a series of 139 medically stabilised patients with UAP. They found that CRP >15 mg/l measured at the discharge predicted the risk of death or MI within 90 days, independently of and additive to the result of stress test performed within the first week after the discharge. The prognostic value of CRP was actually suggested to be better than the prognostic value of the stress test. Rebuzzi *et al.* (335) found that TnT and CRP on admission were better than symptoms, admission ECG, and Holter monitoring in predicting the risk of developing MI during hospitalisation among patients admitted with UAP. On the other hand, among patients with Q-wave MI, high values of CRP were found to predict complications, such as myocardial rupture, in studies by Ueda *et al.* (400) and Anzai *et al.* (401). Pietilä *et al.* (402) found that high CRP levels in the first days after MI in patients treated with thrombolysis predicted increased mortality up to 6 months after MI. However, the highest serum levels of CK or CK-MB were not associated with mortality.

CRP seems to be a promising marker in the risk assessment of patients with ACSs. However, the deficiencies in the highly sensitive methods for CRP determination described by Roberts *et al.* (398) also limit the application of CRP to risk stratification in ACSs. In addition to Study IV, the prognostic value of CRP has been evaluated based on concentrations measured on admission to the hospital in studies by Toss *et al.* (279) and Lindahl *et al.* (214). In Study IV, using a strict definition of UAP the cut-off limit of the second and third tertiles for CRP was 1.49 mg/l. In the studies by Toss and Lindahl with UAP or non-Q-wave MI CRP levels >10 mg/l at all levels of TnT predicted cardiac death within two years. Biasucci *et al.* suggested that among patients with UAP, the CRP levels >3 mg/l determined at hospital discharge were actually better in predicting recurrent ACSs than those measured on admission (319). In contrast, Ferreirós *et al.* suggested a cut-off limit of >15 mg/l for CRP at hospital discharge (19). The differences observed in the cut-off limits for adverse prognosis may be due to differences in the study populations, the points of time of CRP determination, and the end-points used.

CONCLUSIONS

In direct comparison of cardiac TnT and CK-MBm with conventional enzymes, the present study showed that in the diagnosis of large MIs the use of TnT or CK-MBm does not bring any additional benefit to the diagnosis based on symptoms, ECG and conventional enzyme activities. However, the modern biochemical markers are useful in the detection of smaller myocardial injuries that are undetectable by conventional enzymes. Furthermore, MI can be ruled out by TnT or CK-MBm in almost all patients within 12 h from admission to the emergency department, provided that the symptoms are relieved. In clinical practice, the timing of biochemical marker measurements in relation to hospital admission seems to be more practical and less sensitive to misinterpretations than individual timing of blood samples in relation to the onset of symptoms.

According to this study, adoption of TnT into clinical practice as a central element of the diagnosis of MI instead of CK, CK-MB and LD-1 activities, as recommended by the ESC/ACC Joint Committee (12), leads to a substantial increase of 23 to 33% in the number of patients diagnosed with MI. However, the transition in the number of the patients getting a diagnosis of MI by TnT-based criteria is not merely an increase, but rather a crossover, since the use of the modern biochemical markers also reduces the number of false-positive diagnoses of MI. The risk of major CHD events in patients with TnT >0.10 μg/l was not significantly higher than in those with TnT ≤0.10 μg/l among patients without a clinical or epidemiological diagnosis of MI, although there was a trend towards a worse prognosis. Therefore, the prognosis of the patients with an isolated elevation of TnT needs further examination in larger patient populations. However, among patients without epidemiological diagnosis of definite MI, the prognostic value of TnT and CK-MBm was better than of CK activity. These findings suggest that the use of cardiac TnT and CK-MBm may lead to a more accurate diagnostic and prognostic evaluation of ACSs than the use of conventional enzyme activities.

Finally, the present study showed that CRP, fibrinogen and IL-6 have a prognostic significance beyond myocardial injury in ACSs. CRP is a promising marker in the risk assessment of patients with ACSs. However, deficiencies in the highly sensitive methods for CRP determination (398) limit its application to risk stratification. According to this study, the association of elevated serum concentrations of TNF-α with the risk of recurrent coronary events in patients with UAP (with conventional enzyme activities within normal limits) includes dimensions other than those reflected by elevated levels of CRP, fibrinogen and IL-6.

SUMMARY

This study was carried out to compare the role of new biochemical markers of myocardial injury with that of conventional enzymes in the diagnostic and prognostic evaluation of patients with ACSs, and to investigate their time-window in ruling out MI. Moreover, the prognostic value of acute-phase proteins and cytokines (CRP, fibrinogen, IL-6, and TNF-α) in ACSs was investigated.

The study population consisted of 559 consecutive patients admitted with a suspected ACS to the emergency department of Kuopio University Hospital. The first blood samples for the measurement of conventional enzyme activities, and TnT and CK-MBm concentrations were drawn as soon as possible after the admission, and following samples 2, 4 and 6 h thereafter. During the second and third hospital day blood samples were drawn twice daily, and the last blood sample was drawn in the morning of the fourth day, unless the patient had been discharged earlier. Inflammation markers were determined from the first blood sample in patients with the diagnosis of UAP. Hospital discharge diagnoses (clinical diagnoses) and modified FINMONICA criteria (epidemiological diagnoses) were used for the classification of events. The end-points of the study were coronary death (N=53), major coronary event (N=77), and any coronary event (N=175). The median follow-up time was 17 months.

Study I: Among the patients with ACSs, TnT and CK-MBm were elevated in virtually all of the patients with the diagnosis of definite MI and conventional enzyme activities elevated to above twice the URL. However, TnT and CK-MBm were not elevated in one-third of the patients with probable MI and conventional enzymes elevated to above the URL but to less than or equal to twice the URL. TnT was elevated in 13% and CK-MBm in 15% of patients in whom conventional enzymes remained within normal limits.

Study II: MI can be ruled out by TnT within 12 h from admission to the emergency department in 99%, or by CK-MBm in 96% of patients. Among the patients in whom MI was ruled out, a positive history of CHD is an important prognostic factor.

Study III: In patients with a suspected ACS, the use of TnT as a primary basis of MI diagnosis leads to an increase in the number of patients diagnosed with MI. The magnitude of this increase was 33% compared with a clinical and 23% compared with an epidemiological diagnosis of definite or probable MI.

Study IV: Slightly elevated levels, even within the reference limits, of acute-phase proteins (CRP and fibrinogen) and cytokines (IL-6 and TNF-α) were associated with an increased risk of CHD death and major CHD events (CHD death or non-fatal MI) in patients with UAP. Especially CRP and IL-6 were strong predictors of CHD death during the median follow-up time of 17 months, and especially during the first four months. Differing from other inflammation markers, TNF-α was associated with an increased risk of nonfatal CHD events during the first month. In a factor analysis, CRP, fibrinogen and IL-6 clustered on an 'inflammation factor'. However, TNF-α clustered together with TnT and CK-MBm on an 'injury factor'.

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