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Biochemical Markers of Myocardial Damage for Early Diagnosis and Prognosis in Patients with Acute Coronary Syndromes

BY

BERTIL LINDAHL

ACTA UNIVERSITATIS UPSALIENSIS
UPPSALA 1996

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Abstract

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In 142 patients with chest pain (≤ 12 hours) and nondiagnostic ECG, blood samples for analysis of CK-MB, myoglobin and troponin T (tn-T) were obtained initially every 30 minutes. For diagnosis of AMI a combination of myoglobin and CK-MB had a sensitivity at entry of 59 %, which increased by 5 - 10 % every 30 minutes and reached 92 % after two hours and 98 % after six hours with a specificity of 93 %. Acute MI was safely ruled-out in 64 % of non-AMI patients already after 3 hours using myoglobin.

The ability of using neural networks (NN) together with biochemical monitoring was assessed in 88 patients with chest pain (< 8 h). Fifty patients were used for training of the NN and 38 patients for evaluation and comparison with clinicians. The diagnostic capacity for detection of AMI were high both for the NN and the clinicians, but the NN detected MI earlier. The NN predicted infarct size very early with a high accuracy.

In a placebo controlled study of l.m.w. heparin in unstable CAD, 976 patients were followed for 5 months. The risk of cardiac events increased by increasing tn-T levels. Patients with tn-T < 0.06 , 0.06-0.18 and ≥ 0.18 $\mu\text{g/L}$ had a 4.3, 10.5 and 16.1 % risk of MI or death, respectively. Tn-T level was an independent predictor of death or MI. In the 766 patients with a predischARGE exercise test (ET) both tn-T level and ET response were independent predictors and the combination allowed categorisation into groups with 1, 7 and 20 % death or MI, respectively. In those with tn-T ≥ 0.1 $\mu\text{g/L}$ at inclusion, the event rate after 40 days treatment were 14.2 and 7.4 % ($p < 0.01$) in the placebo and dalteparin group, respectively. In contrast, there was no beneficial effect of dalteparin in those with tn-T < 0.1 $\mu\text{g/L}$.

The results contribute new and extended knowledge regarding the use of biochemical markers for early diagnosis and prognosis, which might have a large impact on the handling and treatment of patients with ACS.

Key words: myocardial infarction, unstable angina, diagnosis, prognosis, CK-MB, myoglobin, troponin T, neural networks, low molecular weight heparin.

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In a placebo controlled study of l.m.w. heparin in unstable CAD, 976 patients were followed for 5 months. The risk of cardiac events increased by increasing tn-T levels. Patients with tn-T < 0.06 , $0.06-0.18$ and ≥ 0.18 $\mu\text{g/L}$ had a 4.3, 10.5 and 16.1 % risk of MI or death, respectively. Tn-T level was an independent predictor of death or MI. In the 766 patients with a predischARGE exercise test (ET) both tn-T level and ET response were independent predictors and the combination allowed categorisation into groups with 1, 7 and 20 % death or MI, respectively. In those with tn-T ≥ 0.1 $\mu\text{g/L}$ at inclusion, the event rate after 40 days treatment were 14.2 and 7.4 % ($p < 0.01$) in the placebo and dalteparin group, respectively. In contrast, there was no beneficial effect of dalteparin in those with tn-T < 0.1 $\mu\text{g/L}$.

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To my colleagues and patients,

Decision-making is . . .

*something which concerns all of us, both as makers of the
choice and as suffers of the consequences.*

D Lindley

LIST OF PAPERS

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

- I** Early diagnosis and exclusion of acute myocardial infarction using biochemical monitoring. Lindahl B, Venge P, Wallentin L, BIOMACS study group. *Coronary Artery Disease* 1995; 6: 321-328.
- II** Early assessment of patients with suspected acute myocardial infarction by biochemical monitoring and neural network analysis. Ellenius J, Groth T, Lindahl B, Wallentin L. Submitted, 1996.
- III** Relation between Troponin T and the risk of subsequent cardiac events in unstable coronary artery disease. Lindahl B, Venge P, Wallentin L, FRISC study group. *Circulation* 1996; 93: 1651-1657.
- IV** Risk stratification in unstable coronary artery disease: Additive value of troponin T determinations and predischARGE exercise tests. Lindahl B, Andrén B, Ohlsson J, Venge P, Wallentin L, FRISC study group. Accepted for publication in *European Heart Journal*, 1996.
- V** Troponin T identifies patients with unstable coronary artery disease who benefit from long term antithrombotic protection. Lindahl B, Venge P, Wallentin L, FRISC study group. Accepted for publication in *Journal of American College of Cardiology*, 1996.

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ABBREVIATIONS

ACS = acute coronary syndromes

IHD = ischemic heart disease

MI = myocardial infarction

AMI = acute myocardial infarction

UA = unstable angina

CAD = coronary artery disease

MMD = minor myocardial damage

CHF = congestive heart failure

CK = creatine kinase

CK-MB = creatine kinase MB

Tn-T = troponin T

Tn-I = troponin I

PTCA = percutaneous transluminal coronary angioplasty

CABG = coronary artery by-pass grafting

ET = exercise test

NN = neural networks

ASA = acetylsalicylic acid

INTRODUCTION

Acute coronary syndromes (ACS), i.e. acute myocardial infarction (AMI) and unstable angina (UA), are among the leading causes of morbidity and mortality in Sweden. Approximately 20 % of all patients visiting the emergency room at the departments of internal medicine have chest pain or other symptoms suggestive of ACS and two thirds of those, amounting to about 100.000 patients/year, will be admitted (1). Among those admitted, two thirds will get a diagnosis of definite or possible ischemic heart disease (IHD), including approximately 20 % with a diagnosis of AMI (1, 2). Apparently, this population is very heterogeneous both regarding cause of symptoms, severity of underlying disease and prognosis. Hence, early diagnosis and prognostic evaluation are essential for application of appropriate acute treatment and further management. This early choice of treatment strategy has large implications both for the individual patient and for the costs of health care.

BACKGROUND

Definition of Acute Coronary Syndromes (ACS)

Ischemic heart disease can be divided into stable coronary artery disease (CAD) and in ACS, respectively. In stable CAD, the ischemia commonly

results from predictable increases in myocardial oxygen demand that outweighs the ability of the stenosed coronary artery to increase its delivery (3). In contrast, the ACS are characterised by abrupt and unpredictable reductions in coronary blood flow causing myocardial ischemia at rest or decreasing levels of exertion. The ACS constitute a continuum and the delimitation between the different forms is somewhat arbitrary in clinical practice, e.g. the distinction between unstable angina (UA) and non-Q-wave MI depends on an arbitrary choice of biochemical markers and their decision limits. Therefore, UA and non-Q-wave MI are often considered together as unstable CAD, emphasising that the common denominator of these entities is the underlying unstable plaque.

Unstable angina is defined clinically, in the absence of ECG or enzymatic changes diagnostic of AMI, in presence of (4): (a) recent onset (<4 weeks) of angina, which is brought on by minimal exertion; (b) crescendo angina, i.e. more severe, prolonged or frequent angina superimposed on a pre-existing stable effort angina or (c) angina at rest. Signs of ischemia such as ST-segment or T-wave deviations in the 12-lead ECG are common and supportive for a diagnosis of UA, but not obligatory.

Myocardial infarction is defined as

necrosis of the myocardium due to interruption of its blood supply and thus a histological diagnosis. However, under clinical conditions AMI is defined as the presence of at least two of the following criteria: a) ischemic chest pain of at least 20 minutes duration, b) diagnostic ECG changes c) significant increases of cardiac enzymes (5). MI might, at autopsy, be subclassified according to the extent of the necrosis, as subendocardial or transmural, and clinically, according to findings in 12-lead ECG, into Q-wave and non-Q-wave MI. Another, clinically valuable classification is into ST-elevation and non-ST-elevation MI, based on findings in the initial ECG.

Sudden ischemic cardiac death is defined as sudden death, of probable or proven ischemic origin, occurring within one hour from onset of symptoms.

Pathogenesis of ACS

Development of acute coronary syndromes

Atherosclerotic plaques in the coronary arteries result from a complex interaction between blood elements, disturbed flow and vessel wall abnormalities involving inflammation, smooth muscle cell migration and proliferation, matrix synthesis, lipid accumulation, necrosis, calcification and thrombosis (6). Key events in the development of ACS, in most cases, are a

disruption of an atherosclerotic plaque and thrombus formation, leading to reduction in coronary blood flow causing myocardial ischemia or infarction (7-9). Although an individual severe stenosis more often becomes occluded than a less severe stenosis (10), MI and UA most frequently evolve from plaques causing only mild to moderate stenosis (11, 12). Mechanical and/or hemodynamic forces may trigger plaque rupture by stressing a vulnerable plaque (9). Several factors influence the vulnerability of the plaque to rupture such as size and consistency of the atheromatous core, thickness and collagen content of the fibrous cap, inflammation within the cap and the phenomenon of cap fatigue (9). The extent and duration of thrombus formation might be influenced by local and systemic factors such as degree of disruption, severity of stenosis, balance between the thrombogenic and fibrinolytic systems and volume of residual thrombus after spontaneous or pharmacological lysis (3, 9, 13). Other mechanisms that alter the balance between myocardial oxygen supply and demand may also be involved in the pathogenesis of ACS, e.g. coronary vasoconstriction (14-16).

The various manifestations of ACS (Fig 1)

Unstable angina: A small disruption of an atherosclerotic plaque may lead to a sudden increase in the size of the

Figure 1. Development of acute coronary syndromes

plaque impeding the coronary blood flow resulting in new or worsening symptoms of angina. A disruption complicated by a thrombus causing temporary and often recurrent occlusion, leads to episodes of angina at rest. These mechanisms probably accounts for two thirds of the episodes of UA. The rest might be caused by transient increase in myocardial oxygen demand or by complete occlusion of an artery with insufficient collateral supply (e.g. in postinfarction angina) (3, 17).

Non-Q-wave myocardial infarction: In non-Q-wave MI the thrombus is often larger, occlusive and less labile than in UA, probably because of worse plaque damage and/or presence of a more unfavourable balance between thrombogenic and fibrinolytic factors (3, 18). However, in about three fourths of patients with non-Q-wave MI the infarct-related artery is patent if coronary angiography is performed soon after the infarction (19). These findings, together with the changes in ECG:s and biochemical markers, suggest a pathophysiology with initial total thrombotic occlusion followed by spontaneous reperfusion before transmural necrosis has developed (3). In the remaining one fourth, the occlusion is permanent with protective collaterals limiting the infarct size (19, 20).

Q-wave myocardial infarction: In Q-wave MI there is typically a fixed, permanent, occlusive thrombus which, in the absence of adequate collaterals, leads to necrosis of the whole thickness of the involved myocardium (3, 21). However, development of Q-waves in the ECG does not always reflect transmural necrosis (22).

Prognosis in acute coronary syndromes

The risk of an unfavourable outcome varies not only between, but also within, the various clinical manifestations of ACS. Patients with UA seem to have a lower risk of death or MI than patients with non-Q wave MI (23). In a pooled analysis of 10 studies with almost 2000 patients with crescendo or rest angina, Betriu et al found a mean mortality of 4 % (range 2-8 %) in-hospital and of 8 % after 6 months. After one year the mortality was 10 % and the rate of death or nonfatal MI 21 % (24). In a more recent large study of patients with UA or non-Q-wave MI the incidence of death and nonfatal MI after one year was 4.3 and 8.8 %, respectively (25). The in-hospital mortality is, in most comparative studies, higher in Q-wave than in non-Q-wave MI, whereas the long-term mortality seems to be equal (26, 27). The risk for development of reinfarction and UA are consistently higher after non-Q wave MI (26-28). In a population based cohort study of prognosis after a first

MI (28), the age and sex-adjusted one-year risk was similar for death, 27.6 vs. 31.7 %; higher for reinfarction, 13.1 vs. 6.4, and lower for congestive heart failure, 10.1 vs. 19.3 % in non-Q-wave compared to Q-wave MI.

Treatment alternatives in acute coronary syndromes

There are several goals of the treatment in ACS: amelioration of symptoms, limitation of myocardial damage, prevention of future cardiac events and progress of the underlying atherosclerotic process. Most patients are treated with a combination of antiischemic drugs, i.e. beta-blockers, organic nitrates, calcium inhibitors, and with thrombolytic and/or antithrombotic drugs and are also evaluated for the need of early revascularisation.

Reperfusion treatment

Reperfusion can be achieved either by lysing the occlusive thrombus by thrombolytic agents or by PTCA. With thrombolytic treatment some 40-80 % of patients will have a patent infarct related vessel within 90 minutes from onset of treatment (29). Effective early reperfusion lowers mortality by reduction of the infarct size (30) and by other positive effects of an open artery, e.g. on the healing process and recovery of function (31). Thrombolytic treatment reduces the mortality by about 20 % in AMI patients pre-

sented with ST-segment elevation or bundle branch block (32). The earlier the treatment is given, the greater the benefit, but some benefit might be obtained up to 12 hours from onset of symptoms (32). Thrombolysis within the first two hours is particularly beneficial (33). Acute PTCA, at least performed at experienced centers, seems superior to thrombolytic treatment in reducing mortality, reinfarctions and stroke in patients with ST-segment elevation (34, 35).

No beneficial effect of thrombolytic treatment was demonstrated in the limited number of patients studied with ST-segment depression or T-wave inversion in the ISIS-2 and GISSI-1 trials (36, 37). However, in a post-hoc analysis of the LATE-study, thrombolytic treatment in AMI patients presenting with >2 mm ST-segment depression was found to lower mortality (38). Thus, in the heterogeneous group of patients presenting with non-diagnostic ECG-changes, there may be subgroups with a developing large MI, who might benefit from thrombolytic treatment. Randomised trials with acute PTCA in AMI patients presenting with non-diagnostic ECG are lacking. Although most patients with UA have an ongoing thrombotic process, no beneficial effect of thrombolytic treatment on the risk of fatal or nonfatal MI has been shown (39, 40).

Antithrombotic treatment

Antithrombotic agents, i.e. platelet and/or coagulation inhibitors, reduce thrombus formation, and might thereby prevent new cardiac events. The effectiveness of platelet inhibition by acetylsalicylic acid (ASA) in ACS is well documented (36, 41-43). Today ASA in doses of 75-325 mg daily, is part of the standard treatment. At present, several ongoing studies in ACS evaluate new platelet inhibitors e.g. inhibitors of glycoprotein IIb/IIIa receptors, ADP receptors etc.

Heparin enhances the antithrombin III activity thereby accelerating its ability to inactivate thrombin, factor Xa and factor IXa. A pooled estimate from three studies (42, 44, 45) in the acute phase of unstable CAD shows a 64 % risk reduction of death or MI during heparin treatment in addition to ASA, compared to ASA alone (44). Low molecular weight heparins inhibit mainly factor Xa. They have a number of advantages over standard heparin, such as longer half-life, complete absorption after subcutaneous administration, more predictable anticoagulant response and probably less bleeding complications (46). In the FRISC trial the addition of subcutaneous l.m.w. heparin (dalteparin) twice daily reduced MI or death by 60 % during the initial 6 days treatment in ASA treated patients with unstable CAD (47). Di-

rect thrombin inhibitors offer a potential advantage over heparin as they are able to inhibit clot-bound thrombin. However, in two recent studies, infusion with direct thrombin inhibitors were not superior to heparin in ACS (48, 49). After cessation of heparin or other thrombin inhibitors a reactivation of thrombotic events has been documented (50, 51), suggesting a need for prolonged antithrombotic treatment in unstable CAD. Warfarin and other agents, inhibiting K-vitamin dependent coagulation factors, have been shown to have at least similar protective effect as ASA during long term treatment (52, 53).

Revascularisation

Revascularisation with either CABG or PTCA is effective in amelioration of symptoms in unstable CAD (54, 55). However, no difference in death or AMI was seen after two years in a study comparing medical and surgical treatment (CABG) for UA, although surgery was associated with a reduced mortality among patients with lower ejection fractions (56). Furthermore, the choice of an early conservative (intense medical treatment) or an early invasive strategy (mainly PTCA) did not influence the occurrence of death or AMI during follow up in the TIMI IIIB trial (25). The incidence of death or MI was fairly high, 12 % after one year, suggesting that there is room for

improvement, e.g. by a more intense long term antithrombotic treatment.

Biochemical markers of myocardial damage

History

Until the first report (1954) on glutamic oxaloacetic transaminase (ASAT) in AMI (57), the clinical diagnosis of MI relied solely on the history and on development of diagnostic Q-waves in the ECG. Since then, a number of enzymatic and non-enzymatic markers have been proposed for diagnosis of MI, e.g. lactic acid dehydrogenase (1956) (58) and creatine kinase (1960) (59). Today, biochemical markers are cornerstones in the diagnosis of MI, and timely obtained, almost a *sine qua non* (60).

The ideal biochemical marker

An ideal marker for diagnosis of MI should (adopted from 61): 1) exist in high concentration in the myocardium; 2) not exist in any other tissue, neither under normal, nor under pathological conditions; 3) not be measurable in plasma under normal conditions; 4) be released rapidly and completely after irreversible damage to the myocardium; 5) be released in direct proportion to the extent of myocardial necrosis; 6) persist in the plasma long enough to allow a convenient diagnostic time window; 7) be suitable for development of rapid, reliable and

inexpensive methods for measurement.

However, an ideal marker for differing chest pain of ischemic origin from other causes and for prognostic use might demand other properties, e.g. be released also after reversible ischemic damage.

Release of biochemical markers

Myocardial ischemia, if not reversed, leads to a chain of cellular events ultimately ending with disruption of the cell membranes and cell death. When the cell membranes have lost their integrity, the cellular marker diffuses into the interstitium and, from there, into the capillaries (70-90 %) and lymphatics (10-30 %) (62, 63). Whether the release of cytosolic markers always indicates cell death is controversial (61). There are several reports indicating that release also may occur from reversibly damaged cells (64-66). That a persistent release of structurally bound markers is due to irreversible damage is less controversial (67).

The appearance of the marker in the circulation depends on several factors: 1) size – smaller generally earlier than larger (62) (exception the large molecule glycogen phosphorylase-BB (68); 2) cellular localisation – cytosolic more rapidly than structurally bound (69); 3) solubility (61); 4) local degra-

dation (70);5) regional lymphatic and blood flow (71,72);6) rate of clearance from the circulation (73).

Specific markers

Creatine kinase / creatine kinase MB

Creatine kinase (CK) is an enzyme that catalyses the reaction Phosphocreatine + ADP $\leftrightarrow$ Creatine + ATP. The CK activity is greatest in striated muscle, brain and heart muscle, but can also be found in other tissues. The enzyme is localised in the cytosol or associated with myofibrillar structures. The active form of CK is a dimer composed of two subunits, B (“brain”) and M (“muscle”), respectively. Hence, three isoenzymes exists, all with a molecular weight of about 86 kD: CK-BB (CK-1), CK-MB (CK-2) and CK-MM (CK-3) (Table 1). Furthermore, CK-MB exists in two and CK-MM in three separate isoforms (Fig 2). A fourth, distinctly different isoenzyme (CK-Mt) located in the mitochondria is rarely observed in plasma. Macro CK, a complex of CK-BB and IgG or aggregates of mitochondrial CK, may be found in up to 4 % of hospitalised patients (74).

The kinetics of CK is reasonably described by a two compartment model (73). There is no excretion of CK in the urine and the CK-level is not influenced by changes in renal or hepatic blood flow (73). CK is inactivated by proteolysis in lymph (71) and seems to be removed by the reticuloendothelial system (73).

In Sweden the catalytic activity of CK is analysed according to the IFCC/ ECCLS standards (75). For the isoenzyme, CK-MB, either the catalytic activity or the mass concentration is measured. For measurement of the catalytic activity, the immunoinhibition technique, in which the enzymatic activity of the M subunit is inhibited and the activity of the B subunit (“CK-B”) is measured (76), is commonly used in Sweden. The mass concentration of CK-MB is determined by specific immunoassays. Numerous different immunoassays exist with variably reference ranges. Assays allowing determination of CK-MB in less than 10 minutes are available (77). The isoforms of CK-MB and CK-MM

Table 1. Isoenzymes of Creatine Kinase

	<u>CK-BB</u>	<u>CK-MB</u>	<u>CK-MM</u>
Tissue distribution			
skeletal muscle	-	<5 % *	>95 %
heart muscle	-	25-45 %	55 - 75 %
other tissues**	>90 %	-	<10 %
Isoforms	none	MB ₂ + MB ₁	MM ₃ + MM ₂ + MM ₁

* a regenerating muscle, e.g. after acute muscle injury, reverts to an embryonic enzyme pattern and CK-MB might in these cases increase >10 %

** brain, prostate, gut, uterus, placenta, bladder

Figure 2. Isoforms of CK-MM and CK-MB. In blood the tissue isoform of CK-MM, MM_3 , is converted to MM_1 via MM_2 , and the tissue isoform of CK-MB, MB_2 , is converted to MB_1 by plasma carboxypeptidase N (CPN), which cleaves C-terminal lysine of tissue-type M subunit. B, B subunit; M_t , tissue-type M subunit; M_s , serum-type M subunit.

might be separated by electrophoresis, isoelectric focusing or chromatofocusing. A rapid high-voltage electrophoresis technique allows determination of CK-MB isoforms in less than 30 minutes (78).

Myoglobin

Myoglobin is a cytosolic oxygen binding protein with a molecular weight of 17.8 kD, found in skeletal and heart muscle, constituting 2 % of the total muscle proteins. Myoglobin is cleared from the circulation by the kidneys (79). The kinetics of myoglobin is adequately described by a two compartment model (80). Under normal conditions the concentration in plasma is lower in females than in males, and increases with age and muscle mass (81). In the last years, immunoassays for rapid measurement (<15 minutes) of Myoglobin have become available (82).

The troponins

The troponin complex, consisting of three subunits, tn-T, tn-I and troponin C, is located in the contractile apparatus of skeletal and heart muscle and regulates muscle contraction (Fig 3). The contractile proteins are sensitive to ischemia and damage to the proteins has been shown to occur already during the reversible phase of injury (83).

Figure 3. The troponin complex (tn-C, tn-I, tn-T) located on the thin filament of the contractile apparatus

Troponin T (tn-T) is the tropomyosin binding subunit with a molecular weight of approximately 39 kD. It exists in three isoforms encoded by different genes in cardiac, slow and fast skeletal muscle (84). However, during fetal development the cardiac tn-T gene is also expressed in skeletal muscle. In experimental animals cardiac tn-T might be reexpressed in response to skeletal muscle injury (85). Tn-T exists in an unbound cytosolic pool of approximately 6 %. The rest is structurally bound (86) explaining the biphasic release pattern and the wide

Table 2. Definition of sensitivity, specificity, positive and negative predictive value

Golden standard	Test	
	<u>Negative</u>	<u>Positive</u>
	<u>Non-disease</u>	<u>Disease</u>
	True negative (TN)	False positive (FP)
	False negative (FN)	True positive (TP)
Sensitivity = $TP / (TP + FN)$		Specificity = $TN / (TN + FP)$
Positive predictive value = $TP / (TP + FP)$		Negative predictive value = $TN / (TN + FN)$

time window after myocardial injury, although the plasma half-life is only two hours (87). The only commercially available quantitative immunoassay for tn-T, which is based on the ELISA principle with two monoclonal antibodies, requires about 90 minutes (88). Recently, a rapid (<20 min) qualitative assay for testing in whole blood has been developed (89).

Troponin I (tn-I) inhibits the actomyosin-ATPase. It also exists in three isoforms encoded by different genes in cardiac, slow and fast skeletal muscle (84). The molecular weight of cardiac tn-I is 22.5 kD. Cardiac tn-I is not expressed in skeletal muscle during fetal development (61). Tn-I also exists in a cytosolic pool, about 3-4 %, beside the structurally bound fraction (90, 91). There are now several different immunoassays commercially available. The detection limit and reference range varies substantially between the different assays (61, 92).

Evaluation of diagnostic tests

The sensitivity, specificity and predictive values (Table 2) of a test depend on several factors:

- 1) The comparator (golden standard) should ideally unequivocally define the disease. However, the golden standard for the diagnosis of MI is in most evaluations the WHO-criteria (5), which are not very rigorously defined and might be inappropriate (60).
- 2) The study population – the size, the prevalence of the disease (Bayes' theorem) and the relevance of the tested population is important for the usefulness of the results. Use of only the "sickest of the sick" and the "weldest of the well" has to be avoided (93).
- 3) The decision threshold (Fig 4) – unless the test completely separates the non-disease and disease populations, one has to choose a decision threshold that necessarily is a compromise. The optimal balance between sensitivity and specificity is a clinical decision depending on what

are the relative costs of the false positive and false negative results (94). Combining several tests, e.g. two different biochemical markers, might be used to improve the diagnostic information. Requiring all tests in a combination to be positive, may increase the specificity, while requiring either test to be positive, may increase the sensitivity (95). Different decision thresholds might also be used for ruling-in and ruling-out the disease. Furthermore, the optimal decision threshold may vary with time, e.g. from onset of AMI (96). Receiver operating characteristic (ROC) curves are useful to elucidate decision thresholds (97).

Figure 4. Relation between sensitivity and specificity

Applications of biochemical markers

Diagnosis of AMI

The usual times to initial elevation, peak value and normalisation of CK, CK-MB, myoglobin, tn-T and tn-I in AMI are summarised in Table 3. Typical examples of time curves for CK-MB (mass), myoglobin and tn-T in patients with MI are shown in Figure 5. Biochemical markers starts to increase earlier in patients with non-Q-

wave MI and after reperfusion compared to non-reperfused Q-wave MI (100). The overall diagnostic sensitivity and specificity in patients with suspected MI is shown in Table 4.

Figure 5. Representative time curves for CK-MB (mass), myoglobin and troponin T in patients with AMI. The y-axis shows relative concentration for each marker (actual conc/upper reference limit), the x-axis time from onset of symptoms in hours. a) without thrombolytic treatment, b) with successful thrombolytic treatment

For retrospective confirmation of classical MI in a typical CCU population, the sensitivity and specificity of CK-MB (mass) is satisfactory and sufficient as a sole marker, unless the patient is admitted very late (107). Despite higher cardiospecificity than CK-MB, the troponins have a lower specificity due to the frequent elevations in patients with UA (87). This emphasises the arbitrary differentiation of MI from UA and the lack of an unequivocal golden standard.

Table 3. Properties of biochemical markers used for diagnosis of acute MI (61, 92, 98, 99)

<u>Marker</u>	<u>Time to initial elevation</u>	<u>Time to peak elevation</u>	<u>Time to return to normal</u>
CK	4 - 8 h	12 - 24 h	3 - 4 d
CK-MB(mass)	3 - 9 h	12 - 20 h	48 - 72 h
Myoglobin	1 - 4 h	6 - 12 h	18 - 36 h
Troponin T	3 - 9 h	12 - 24 h (4 d)	6 - 14 d
Troponin I	3 - 9 h	10 - 20 h	4 - 8 d

Table 4. Summary of overall sensitivity and specificity for MI diagnosis (87, 90, 101-107)

<u>Marker</u>	<u>Sensitivity</u>	<u>Specificity</u>
CK	0.89 - 0.99	0.75 - 0.92
CK-MB (cat. activity)	0.85 - 0.98	0.90 - 0.92
CK-MB (mass)	0.89 - 1.00	0.79* - 1.00
Myoglobin (ref)	0.93 - 1.00	0.82 - 0.89
Troponin T	0.97 - 1.00	0.46* - 0.78
Troponin I	0.97	0.95

*Using very low cut-off levels of CK-MB (5.0 µg/L) and tn-T (0.1 µg/L), respectively

For very early diagnosis and exclusion of AMI, myoglobin, CK-MB isoforms and perhaps in the future, glycogen phosphorylase BB, seem to be the most promising markers. The diagnostic sensitivity and specificity of the markers in blood samples obtained at admission are summarised in Table 5 and the diagnostic sensitivity in relation to time since onset of symptoms in Table 6. However, very little information is available on the use of different decision thresholds or on combinations of several markers for early diagnosis and exclusion of AMI.

Prognosis in unstable angina

Already in the seventies it was observed that many patients with UA had an increase of ASAT within the

normal range (116). Also for total CK elevations in UA-patients were frequently observed (117). However, these transient, minor elevations are not possible to detect with "CK-B" (catalytic activity) probably because of a poor signal to noise ratio with the immunoinhibitor method (117, 118). With the use of more sensitive and cardiospecific markers up to 50 % of patients with UA have minor elevations (87, 90, 118-120). These elevations might either reflect "micro infarctions" or, possibly, reversible ischemic damage. The term "minor myocardial damage" (MMD) has therefore been proposed (121), further categorising the spectrum of acute coronary syndromes (Fig 6). In a few small trials MMD has been associated with an impaired short-term (122-124)

Table 5. Sensitivity and specificity for MI diagnosis on admission in patients with suspected MI (82, 91, 99, 101, 108-111)

Marker	Sensitivity	Specificity
CK	0.15 - 0.46	0.79 - 0.98
CK-MB (cat. activity)	0.20 - 0.50	0.89 - 0.92
CK-MB (mass)	0.25 - 0.76	0.72 - 0.95
Myoglobin (ref)	0.30 - 0.75	0.83 - 1.00
Troponin T (0.5 µg/L)	0.28 - 0.64	0.72 - 0.96
Troponin I	0.23	0.94

Table 6. Diagnostic sensitivity for MI in relation to time since onset of pain (69, 78, 82, 91, 99, 106, 112-115)

	2 h	4 h	6 h
CK	0.08 - 0.46	0.19 - 0.65	0.39 - 0.88
CK-MB (activity)	0.00 - 0.18	0.14 - 0.40	0.19 - 0.88
CK-MB (mass)	0.25 - 0.49	0.42 - 0.71	0.58 - 1.00
CK-MB isoforms	0.08 - 0.13	0.56 - 0.59	0.92 - 0.96
Myoglobin	0.18 - 0.50	0.44 - 0.89	0.55 - 1.00
Tn-T	0.25 - 0.55	0.48 - 0.72	0.58 - 0.92
Tn-I	0.17 - 0.28	0.75	0.77 - 0.95

and long-term prognosis (125, 126), although its importance as an independent predictor has been questioned (127). Hence, the prognostic value of tn-T needs to be confirmed in larger studies and compared with other known prognostic variables.

Figure 6. The spectrum of acute coronary syndromes

Estimation of infarct size

Assessment of infarct size by measurement of cumulative release of cardiac enzymes has been shown to closely correlate with histologic estimates of infarct size (128). However,

the calculation of cumulative release is complicated and based on several assumptions (73), that may be affected by reperfusion therapy (129). In clinical praxis, needing only crude estimation of infarct size, the peak values allow qualitative estimates of infarct size (98). However, the higher peak values for the same infarct volume, in reperfused versus non reperfused patients (130) make the peak values less useful after thrombolysis. The release of structurally bound markers may be an alternative, i.e. the late peak of tn-T day 3-5 correlated well with infarct size measured by scintigraphy regardless of reperfusion status (131).

For early prediction of infarct size the initial slope of the time curve for my-

oglobin and CK, correlates well with the infarct size (132-134). However, no reliable and easily applicable method for early prediction of infarct size has been clinically evaluated.

Detection of reperfusion

The rationale for biochemical monitoring to detect reperfusion is the accelerated release to the blood of the markers after successful reperfusion. The more rapid release may be due to an accelerated cellular leakage caused by the reperfusion, rather than increased regional blood flow (63). Of the markers the early rate of change of myoglobin and the isoforms of CK-MM and CK-MB, have been most promising for detection of reperfusion, with a sensitivity and specificity of >90 and >80 %, respectively (135, 136). However, in a recent prospective study of CK-MB, myoglobin, tn-T, CK-MB isoforms using all previously published cut-offs and algorithms, a considerable lower sensitivity and specificity was found (137). Thus, biochemical markers are at present not considered useful for early identification of reperfusion.

Causes of non-cardiac elevations of biochemical markers

Creatine kinase and CK-MB: After extreme exercise, larger muscle trauma (for total CK even minor muscle trauma), myositis and rhabdomyo-

lysis, CK-MB originating from skeletal muscles might cause elevation in plasma levels (61). ACK-MB/total CK ratio >3-5 % has been proposed to separate CK-MB elevations of cardiac origin from skeletal origin (138), but this has been questioned (139). Other causes of elevation are hypothyroidism (140) and renal failure (in about 5 % (139)), as well as, brain damage, presence of macro-CK, gastrointestinal and prostate surgery, when analysing the catalytic activity with immunoinhibition technique (61).

Myoglobin: The only sources of myoglobin are heart and skeletal muscles. Strenuous exercise, trauma and diseases involving skeletal muscle cause elevation of myoglobin (100). Constant elevation is seen in renal failure (141).

Troponin T: With the development of new generations of the tn-T assay, the previously seen cross-reactivity with skeletal tn-T has diminished (89). Thus, elevations of tn-T in conjunction with acute trauma to healthy skeletal muscle, e.g. accidents or operations, are now rare. However, in patients with diseased and/or regenerating skeletal muscles there may be reexpression of cardiac tn-T (85) giving false positive elevations, e.g. in polymyositis (142). Tn-T elevations of unknown origin

are seen in about 30 % of patients with chronic renal failure (143).

Troponin I: Tn-I seems to be highly specific to myocardial damage, no increase has been seen in patients without evidence of myocardial injury. Multiple trauma, chronic muscle diseases or chronic renal failure do not appear to affect Tn-I levels (61).

Causes of false negative results with biochemical markers

Inappropriate timing of the blood sampling is the principal cause of false negative results. The diagnostic time window is narrowest for myoglobin and widest for tn-T (Table 3). The wide time window might, however, make it difficult to detect early reinfarctions. Inappropriate decision limits might be another cause, e.g. in the case of myoglobin, not having separate decision limits for men and women. High basal plasma concentration and large inter individual differences in concentration of a marker might make it difficult to detect elevations, e.g. for CK small infarctions may cause only elevation within the reference interval (144).

ECG for diagnosis and prognosis in ACS

Diagnosis

ECG-changes occur within seconds after onset of ischemia and make it possible to immediately detect, locate

and estimate the extent of the ischemia/infarction. However, a single ECG recording shows only a snapshot of the very dynamic process of ischemia. In the early phase of ACS the ECG-changes frequently shift from one type to another (145). In some patients the usefulness of the ECG is limited by the occurrence of previous ECG-changes, e.g. bundle branch block.

Development of Q-waves is diagnostic of AMI and is part of the WHO-criteria (5). However, Q-waves do not develop until after several hours after an MI (146). Furthermore transient Q-waves might occur during ischemia (147). Initial ST-elevation is neither a prerequisite for or always associated with the development of Q-waves (148). On admission, ECG:s with ST-elevations, with or without Q-waves, are considered diagnostic of AMI. The specificity of ST-segment elevations is high, >90 % (149), but the sensitivity is poor, <50 % (150). The total sum of ST-elevation might be used for estimation of the extent of myocardium at risk of infarction (151). Other or no ECG-changes are considered as non-diagnostic ECG:s.

Prognosis

The prognostic significance of Q-wave versus non-Q-wave MI has been pointed out above. Increasing number of leads with ST-segment elevation

and bundle branch blocks have been associated with a progressive increase in mortality and also with increasing benefit of early reperfusion (32, 152). In patients with non-Q-wave MI or UA the occurrence of ST-depression indicates an increased risk for adverse cardiac events (153).

Continuous ECG monitoring

In order to more closely follow the dynamic process of ischemia, different techniques for continuous ECG monitoring have been developed, i.e. Holter monitoring and on-line continuous 12-lead ECG or vector cardiographic (VCG) monitoring. These techniques have been used for detection of coronary artery disease (154), identification of reperfusion (155, 156) and prognostic evaluation of patients with IHD (157-159). In patients with ACS repeated episodes of ST-depression indicate an adverse prognosis (158-160).

Stress tests for evaluation of prognosis in ACS

Exercise test

The prognostic value of ET after an episode of unstable CAD has been convincingly demonstrated (161-165). A predischARGE symptom limited ET can be safely done after an episode of unstable CAD (166) and has the same diagnostic value as an ET performed after one month (167). However, an early ET is more useful for prognostic

evaluation, since the risk of new events is greatest the first few weeks after an episode of unstable CAD (167). The diagnostic and prognostic value is considerably greater in symptom limited compared to submaximal ET (168). Not only the occurrence of ST-depression, but also several other parameters such as low work capacity, low rate-pressure product, blood pressure reaction and occurrence of chest pain are associated with an adverse outcome (162-165). A combination of ST-depression and limited work capacity has been shown to increase the prognostic value of the ET (165). A substantial proportion of patients with unstable CAD is unable to perform an ET. However, the inability to perform an ET itself indicates an adverse prognosis (169). No study in patients with unstable CAD has so far compared and combined the prognostic value of predischARGE ET with that of the new sensitive biochemical markers.

Myocardial scintigraphy

Occurrence of perfusion defects on myocardial perfusion scintigraphy has been shown to relate to an adverse outcome in patients with ACS (170), although contradicting studies exist (171). Pharmacological stress is an alternative to dynamic exercise, especially in patients who are unable to exercise. SPECT thallium-201 scintigraphy has been shown to have additional prognostic value to an symp-

tom limited exercise test in patients with unstable CAD (172) and to be superior to exercise echocardiography for prediction of new cardiac events in patients with UA (170).

Stress echocardiography

Stress echocardiography can be performed during or immediately after dynamic exercise or during pharmacological stress (173). Stress induced wall motion abnormalities have been shown to have prognostic significance in patients with ACS in most (174, 175), but not all studies (171).

Coronary angiography for prognosis in ACS

The long term prognosis in patients with ACS depends on the extent of coronary lesions. Complex coronary morphology, multivessel disease, especially with associated left ventricular dysfunction are signs of an unfavourable prognosis (56, 176, 177). However, coronary angiography has obvious limitations. It lacks sensitivity for detection of intracoronary thrombus and plaque rupture, which are important for prognosis (178). Furthermore, the site of a subsequent MI can not reliably be predicted by coronary angiography (12). Because of limited availability and resources coronary angiography is seldom used for routine risk stratification.

Neural networks for diagnosis in ACS

In the early management of patients with acute chest pain the clinician has to sort out a multitude of gradually incoming information in making decisions regarding diagnosis, treatment and prognosis. Therefore, some kind of decision support by a computer system, e.g. based on artificial neural network methodology, might be useful.

Figure 7. Schematic example of a neural network structure

An artificial neural network is a group of interconnected mathematical equations that accept input data and calculate an output conclusion (Fig 7)(179). The input-patterns of measured variables are classified as belonging to one of several pre-defined categories by pattern recognition. This is achieved by "training" of the network with use of a number of representative example cases and their corresponding correct classifications as defined by a 'golden standard'. The network will

learn to correctly classify a major portion of the patterns in this training set by a technique called 'supervised learning'. When exposed to a new, not yet classified case, the network uses its previous training and its capability of generalisation when assigning it to a specific diagnostic category. Artificial neural networks have been applied for AMI diagnosis based on clinical data including ECG (180) and biochemical markers (181). However, so far no study has evaluated the use of neural networks for early diagnosis of AMI and infarct size prediction based on biochemical markers.

AIMS OF THE STUDY

In patients with suspected AMI:

evaluate if early frequent blood sampling ("monitoring") of CK-MB (mass), myoglobin and tn-T alone or in combination could provide a definite positive or negative diagnosis within the first few hours after admission in patients with nondiagnostic ECG (paper I);

evaluate neural network methodology, based on frequent measurement of biochemical markers of myocardial damage, for early diagnosis/exclusion of AMI, early prediction of infarct size, estimation of time from onset of infarction and assessment of minor myocardial damage (paper II).

In patients with unstable CAD:

elucidate the prognostic value of tn-T in a large cohort of patients with unstable CAD, and to compare tn-T with other known early and widely available risk indicators (paper III);

evaluate, compare and combine the prognostic value of tn-T with a predischARGE exercise test and other clinical findings in a large cohort of patients with unstable CAD (paper IV);

evaluate the protective effect of treatment with l.m.w. heparin (dalteparin) in relation to risk stratification by tn-T levels in patients with unstable CAD (paper V).

MATERIAL AND METHODS

Patients with suspected AMI (paper I - II)

In the BIOMACS (BIOchemical Markers in Acute Coronary Syndromes) study, patients admitted to the coronary care units at six hospitals in Sweden were eligible for participation from December 1991 to June 1992. The inclusion criterion was a history of chest pain suggestive of AMI starting within the previous 12 hours. Exclusion criteria were anaemia (Hb <110 g/l), systolic blood pressure below 100 mm Hg on admission or refusal to participate.

The 142 patients with nondiagnostic ECG changes at admission constituted the study population in paper I and all 88 patients with onset of chest pain within the previous 8 hours and, in case of AMI, not given thrombolytic treatment, constituted the study population in paper II.

Patients with unstable coronary artery disease (paper III - V)

The FRISC (FRagmin during InStability in Coronary artery disease) study – a double blind, randomised, placebo controlled trial of low molecular weight heparin (dalteparin sodium, Fragmin[®], Pharmacia AB, Sweden) included patients with unstable CAD at 23 hospitals in Sweden between May 1992 and October 1994 (47). In 15 hospitals the protocol included a special blood sampling schedule and from which the tn-T substudy patients were recruited. Eligible for inclusion were men or women aged over 40 years admitted to hospital because of myocardial ischemia demonstrated both by a) symptoms i.e. either new-onset angina pectoris last two months, increasing angina pectoris last two months or ongoing chest pain suggestive of MI and with the last episode of chest pain within 72 hours, and by b) signs of myocardial ischemia in 12 lead ECG at rest i.e. in two contiguous leads either ST-depression of ≥ 0.1 mV or T-wave inversion, or both. The exclusion criteria

were mainly related to increased risk of bleeding, indication for thrombolytic treatment and serious intercurrent diseases. The patient recruitment and study populations are outlined in Figure 8.

Figure 8. Patient recruitment and study populations in Paper III - V

Definitions of diagnoses and endpoints

1) BIOMACS (paper I - II)

a. AMI was considered present if at least two of three of the following criteria were fulfilled (182): chest pain lasting more than 20 min; evolution of typical changes in at least two leads of the ECG; typical enzyme leakage, in at least one of the blood samples taken at 0, 6, 12, 18 or 24 hours after admission, i.e. a CK-MB ≥ 20 $\mu\text{g/l}$; or a total creatine kinase for men > 6.0 $\mu\text{kat/l}$, for women > 5.0 $\mu\text{kat/l}$ together with a

CK-MB $\geq 10 \mu\text{g/l}$; or in at least two blood samples a creatine kinase $> 3.0 \mu\text{kat/l}$ (men) or $> 2.5 \mu\text{kat/l}$ (women) together with in at least one sample a CK-MB $\geq 10 \mu\text{g/l}$.

b. IHD was considered present if the patient had pathological ST- or T-wave changes in the ECG at rest or a positive exercise test or had a history of prior MI, CABG, PTCA or diagnostic coronary angiography.

c. MMD was considered present if tn-T maximum was $\geq 0.2 \mu\text{g/L}$ and/or if CK-MB maximum was $\geq 8.0 \mu\text{g/L}$.

d. The infarct size (paper II) was arbitrary labelled "major" if peak plasma CK-MB mass conc. was $> 80 \mu\text{g/L}$ and "minor" if peak CK-MB mass conc. was $\leq 80 \mu\text{g/L}$.

e. Onset of infarction (paper II): the stated time for onset of symptoms by the patient was critically examined by an expert clinician and possibly adjusted if found not to correspond with the expected time for onset of infarction based on the pattern of biochemical markers.

2) FRISC (paper III - V)

a. Index event: all patients with elevated maximal levels of creatine kinase-MB (n=666), CK (n=152), CK-B (n=112) and ASAT (n=45), were retro-

spectively classified as having non-Q-MI, all other patients as having UA.

b. End points: all patients were followed while in hospital and thereafter by outpatient visits after 6 weeks and 5-6 months. Occurrence of and time for death, MI and revascularisation was recorded. All deaths and nonfatal MI:s were classified by an independent end point committee. MI was defined by conventional WHO criteria (5). Causes of death were, whenever possible, based on autopsies. Sudden death was considered cardiac.

Laboratory analysis

Biochemical markers

In BIOMACS (paper I - II) venous blood samples were drawn from an indwelling forearm venous catheter according to the time schedule in Figure 9. In FRISC (paper III - V) venous blood samples were obtained at inclusion and after 12, 24, 48 and 120 hours. In both studies the blood was collected in EDTA containing tubes and centrifuged. The plasma was frozen in aliquots and stored for subsequent central analysis.

Analyses of CK, CK-MB, myoglobin and tn-T in BIOMACS, and of tn-T in FRISC were done at one laboratory and without knowledge of the patients diagnosis and outcome. The methods of the analyses are summa-

Table 7. Methods for analyses and upper reference limits of the biochemical markers (75, 88, 183, 184)

Marker	Method	Upper reference limit	Paper
CK	IFCC/ECCLS standards (ref)	men 3.0 µkat/L women 2.5 µkat/L	I - II
CK-MB	NovoClone system (Novo BioLabs)	7 µg/L	I - II
Tn-T	Enzymun-Test system (Boehringer Mannheim)	0.2 µg/L * 0.06 µg/L	I - II III - V
Myoglobin	RIA-method (ref)	men 90 µg/L women 57 µg/L	I - II

*discrimination limit

rised in Table 7. In FRISC, analyses of S-CK-MB (mass), S-CK-B, S-CK or S-ASAT were done locally at each participating hospital in connection with the index event according to local routines and the maximal value were noted in the case record forms.

ECG at rest

Standard 12-lead ECGs were obtained on admission and after 24 and 48 hours in BIOMACS (paper I-II), and at inclusion, after 24 hours and at time for the ET (day 5 - 8) in FRISC (paper III-I).

All ECGs were interpreted centrally according to a modification of the Minnesota code (182) and without knowledge of the patients diagnosis and outcome.

Exercise test

In BIOMACS a symptom limited exercise test was performed before discharge in most patients without verified AMI. In the FRISC trial a predischargesymptom limited ET was performed day 5-8 in all patients without any contraindications. All ET:s

Figure 9. Schedule of blood and ECG sampling in Paper I - II

were performed on electrically braked bicycle ergometers according to a standardised, previously presented, procedure (165, 185) and were interpreted centrally. Computer-aided systems and thus, averaged ECG complexes were used if available (in almost all ET:s in the FRISC study). The ST segment was measured 0.06 seconds after the J-point and related to the PQ segment. In case of ST-depression at rest, the changes were related to the ST-level in the ECG immediately before the start of the test. Contraindications to an ET were: MI less than four days before the ET, appearance of new ECG-changes last two days, ongoing chest pain, recurrent episodes of chest pain at rest or at slight effort, overt heart failure at time of the ET, physical handicap or other conditions precluding an ET.

In BIOMACS a positive exercise test was defined as typical chest pain and/or development of ST-depression of at least 0.1 mV 60 ms after the J-point during or immediately after exercise. In FRISC a combination of a low maximal work load and the number of leads with ST-depression was used to define, according to previous experiences (165, 185), "high risk exercise test response" = low maximal work load in combination with ST-depression of ≥ 0.1 mV in ≥ 3 leads; "intermediate risk exercise test response" = either a low maximal work load or ST-

depression of ≥ 0.1 mV in ≥ 3 leads and "low risk exercise test response" = neither low maximal work load nor ST-depression of ≥ 0.1 mV in ≥ 3 leads. A low maximal work load was defined as a work load below the 33rd percentile of the distribution for men (90 W) and women (70 W), respectively, in the total patient material.

Neural networks (paper II)

The method was implemented in a computer system designed in a modular fashion, where each module performs a specific task of computation (Fig 10). The pre-processed measurement values were first taken as input to computational modules that were used for the detection of AMI. Different methods were used depending on whether the measurement was the first one from that patient or not. If AMI was detected, the system made a prediction of the infarct size, and an esti-

Figure 10. Design of the computer method using different modules for different tasks

Table 8. Neural network structures used in the computations

Type of classification	Neural Network Structure	Input Variables
AMI/non-AMI on first measurement	Multiple single-layer perceptrons	normalised and fuzzified Myo, CKMB, TnT
on repeated measurements	Elman recurrent network	normalised and fuzzified Myo, CKMB, TnT
Major/Minor AMI	Single-layer perceptron	normalised Myo, CKMB, TnT, δ Myo, δ CKMB, δ TnT
MMD/non-MMD	Elman recurrent network	normalised and fuzzified Myo, CKMB, TnT
Time from onset	Multiple single-layer perceptrons	normalised and fuzzified Myo, CKMB

mate of the time that had elapsed since onset of infarction. If, however, AMI was not detected after a predefined optimal period of monitoring (3 hours), AMI was excluded from the list of possible diagnoses. In this case the remaining task for the system was to make an assessment of whether the patient was suffering from an MMD. The types of neural network structures and sets of input variables for the various classification problems are summarised in Table 8.

The 88 patients included in this study were divided into one 'training set' of 50 patients, and one 'test set' of 38 patients. The data in the test set were only used for the evaluation process. The performance of the computer method was compared to the assessment by three experienced clinicians and to the diagnostic rules proposed in paper I. The clinicians, in contrast to the neural networks, had access to data about age, gender, time since

onset of symptoms and ECG on admission, in addition to the results of the biochemical markers.

For assessment of the time since onset of myocardial infarction the training and test sets, were both extended for the training and evaluation of the neural network module. Each of the first 7 measurements (with 30 min intervals) of these AMI patients was counted as a separate case, increasing the number of cases in the test set from 11 to 77. This artificial extension changed the prevalence with regard to arrival times from onset of symptoms. Therefore the predictive values were corrected to the prevalences derived from the Uppsala University Hospital/CCU database (RiksHIA) for the same category of patients admitted to the CCU during 1994.

Statistics

A t-test was used to compare means of numeric data. For non-parametric unrelated data the Mann-Whitney test

was used. Differences in proportions were judged by chi-square analysis or binomial tests as appropriate.

The clinical usefulness of the tested methods regarding diagnosis was evaluated by calculation of diagnostic sensitivity, specificity, positive predictive value and negative predictive value in comparison to the diagnosis according to "golden standard".

The cumulative hazard curves were constructed using the Kaplan-Meier method. Statistical assessment was performed using the log-rank test.

To identify variables of prognostic importance, simple and forward stepwise multiple logistic regression analyses were used.

In paper V all analyses were performed according to the intention-to-treat principle in comparisons between the placebo and dalteparin groups. The relative risk ratio (RR) and the 95%

confidence interval (CI) were calculated as appropriate. Test for interaction between dalteparin/placebo treatment and tn-T level on the risk of death or MI was done by a multiple logistic regression analysis.

A significant difference was considered to exist at the $p < 0.05$ level. All statistical analyses were performed by a computer utilising the SPSS system (Statistical Package for the Social Sciences, 1990 and 1994).

RESULTS

Early diagnosis in patients with suspected AMI (paper I & II)

Frequent analyses and combinations of biochemical markers (paper I)

Ruling in AMI

Clinical characteristics of the patients included are shown in Table 9.

Table 9. Base-line characteristics in patients with and without acute myocardial infarction (AMI) in paper I. Number of patients (% of group)

	No AMI (n=83)	AMI (n=59)	
Age, mean $\pm$ s.d.	67 $\pm$ 9.4	68 $\pm$ 11	
Male	55 (66)	47 (80)	
Previous myocardial infarction	28 (34)	22 (37)	
Hours since onset of chest pain till first blood sample, mean $\pm$ s.d.	6.1 $\pm$ 2.9	6.0 $\pm$ 3.0	
Admission electrocardiogram			
Bundle branch block	9 (11)	15 (25)	$p < 0.05$
ST-depression and T-inversion	24 (29)	29 (49)	$p < 0.05$
Only ST-depression	5 (6)	7 (12)	
Only T-inversion	17 (21)	3 (5)	$p < 0.01$
No sign of ischemia	28 (34)	5 (9)	$p < 0.001$
Thrombolytic therapy	9 (11)	25 (42)	$p < 0.001$
Defibrillation	0 (0)	0 (0)	
Deaths during hospital stay	0 (0)	7 (12)	$p < 0.001$

Table 10. Cumulative sensitivity / specificity for diagnosis of acute MI at different time points after admission for the different markers and for different cut-off levels (*men/women)

Marker	Time from admission		
	0 h	3 h	6 h
<i>Conventional cut-off levels</i>			
CK-MB (20 µg/L)	0.41/1.00	0.90/1.00	0.93/1.00
tn-T (0.5 µg/L)	0.24/0.98	0.80/0.93	0.92/0.88
myoglobin (108/68 µg/L)*	0.83/0.81	0.92/0.72	0.95/0.71
<i>Low cut-off levels</i>			
CK-MB (10 µg/L)	0.59/0.93	0.95/0.81	0.98/0.78
tn-T (0.2 µg/L)	0.59/0.83	0.92/0.69	0.98/0.66
myoglobin (90/57 µg/L)*	0.89/0.73	0.98/0.64	0.98/0.60

Cumulative sensitivity and specificity for different cut-off levels of myoglobin, CK-MB, tn-T and for different combinations of the markers at different time points were evaluated up to six hours after admission. The sensitivity and specificity for detecting AMI using "conventional" and "low" cut-off levels of myoglobin, CK-MB and tn-T, are shown in Table 10. Partly because of our definition of AMI there was no false positive case for CK-MB using "conventional" cut-off levels. For tn-T the false positive cases were found entirely in the IHD-group, while there were also some false positives in the group without evidence of IHD for myoglobin. The increase in sensitivity using "low" cut-off levels was at the expense of a decrease in specificity. However, for CK-MB all and for tn-T all except one, of the "false positive" cases were found in the group with IHD.

A combination of myoglobin and CK-MB (MyCK) and a combination of

myoglobin and tn-T (MyT) were tested (Fig 11). For MyCK the sensitivity was 0.59 in the first sample, 0.92 at two hours and 0.98 at six hours, the corresponding results for MyT were 0.59, 0.82 and 0.98, respectively. Correspondingly the specificities of MyCK and MyT decreased from 0.98 and 0.94, respectively, for the first blood sample to 0.93 and 0.82, respectively, after six hours. For MyCK all, and for MyT all but one, of the false positive cases were found in the group with ischemic heart disease. The sensitivity using the MyCK-combination in patients with ≤ 4 , 4-8 and >8 hours delay from onset of symptoms until admission, are shown in Figure 12. No significant difference could be shown in sensitivity between patients with and without thrombolytic treatment in these three groups.

Ruling out AMI

Based on the fact that the decision threshold for ruling out might be different from that for ruling in a diagno-

Figure 11. Cumulative diagnostic sensitivity (solid line) and specificity (broken line) for MyCK and MyT, respectively, during the first 6 hours after admission. MyCK: (1) CK-MB ≥ 20 $\mu\text{g/L}$ or (2) myoglobin > 90 $\mu\text{g/L}$ / > 57 $\mu\text{g/L}$ (men/women) and CK-MB ≥ 10 μg ; MyT: (1) tn-T ≥ 0.5 $\mu\text{g/L}$ or (2) myoglobin > 90 $\mu\text{g/L}$ / > 57 $\mu\text{g/L}$ and tn-T ≥ 0.2

Figure 12. Cumulative diagnostic sensitivity for MyCK in patients with onset of symptoms ≥ 4 h (solid line), 4-8 h (broken line) and 8-12 h (dotted line). For definition of MyCK, see Figure 11.

sis (compare Fig 5), criteria for ruling-out AMI were developed and tested after three and six hours of monitoring. The criteria and the proportion correctly ruled-out and falsely ruled-out after 3 and 6 hours of monitoring, respectively, are shown in Figure 13.

Neural network methodology and biochemical monitoring (paper II)

Comparison with clinicians

Clinical characteristics of the patients included are shown in Table 11.

The computer method identified AMI earlier than the clinicians (Table 12). Sensitivity, specificity and predictive values for detection of AMI were high and not significantly different for the computer method and the clinicians (Table 13).

The computer method detected MMD faster and with a higher sensitivity and higher positive predictive value

than the clinicians (Table 12 & 13). The computer method correctly assessed MMD in 5 of 8 patients whereas for the clinicians, the best single result was 3 correct assessments.

The clinicians excluded AMI and MMD within 0-540 minutes with an average of 130 minutes compared to 180 minutes in all cases for the compu-

Figure 13. Percentage of correctly and falsely excluded AMI in all patients 3 and 6 hours after admission. Tested criteria for ruling out AMI: 1) no sample of myoglobin ≥ 90 $\mu\text{g/L}$ (≥ 57 for women), 2) no sample of tn-T ≥ 0.2 $\mu\text{g/L}$, 3) no sample of CK-MB ≥ 10 $\mu\text{g/L}$, and 4) no sample of CK-MB ≥ 8 $\mu\text{g/L}$ or tn-T ≥ 0.2 $\mu\text{g/L}$.

Table 11. Clinical characteristics in patients in the training and test set in paper II. Percentage in brackets.

	Training set n=50	Test set n=38
Age (years)	68 ± 8	66 ± 11
Male	41 (82)	28 (74)
Previous myocardial infarction	16 (32)	19 (50)
Previous CHF	12 (24)	7 (18)
Angina pectoris	45 (70)	26 (68)
Diabetes	2 (4)	7 (18)
Hours since onset of chest pain until first blood sample	4.3 ± 1.8	4.5 ± 1.5
ECG on admission		
Q-wave	9 (18)	12 (32)
ST-segment elevation	8 (16)	11 (29)
ST-segment depression	23 (46)	16 (42)
T-inversion	31 (62)	17 (45)
Bundle branch block	6 (12)	5 (13)
No signs of ischemia	6 (12)	8 (21)
Diagnosis		
AMI	18 (36)	11 (29)
with ST-segment elevation	6 (12)	4 (8)
non AMI	32 (64)	27 (71)
with non-MMD	17 (34)	19 (50)
with MMD	15 (30)	8 (21)
Deaths during hospital stay	3 (6)	1 (3)

ter method (Table 12). The specificity and the positive predictive value for the computer method were higher than for the clinicians (Table 13).

Comparison with diagnostic rules

The sensitivity and specificity in the test set were exactly the same for the diagnostic rules for early diagnosis of AMI and the computer system, 1.0 and 0.93, respectively. However, the diagnostic rule required a slightly longer time to diagnosis, the mean time delay to AMI diagnosis was 14 compared to 6 minutes. At 3 hours after admission, the computer system had correctly ruled-out 89 % of the patients in the test set with non-AMI,

none had an uncertain diagnosis while the remaining 11 % were incorrectly classified as AMI in the previous step. The same figures for the diagnostic rules 3 hours after admission were 63 %, 26 % and 11 %, respectively.

Prediction of infarct size

The computer method needed 30 and 38 minutes of monitoring time to make a correct prediction while the clinicians required 119 and 260 minutes on the average, for a correct prediction of major and minor infarctions, respectively. The computer method and the clinicians did not differ significantly in terms of sensitivity (1.0 vs. 0.83), specificity (0.80 vs. 0.93) and positive

Table 12. Time delay, expressed as number of measurements and minutes since start of measurements, to a correct diagnosis for the computer method and the clinicians. Turnaround time for laboratory analysis is not included. The range is given in brackets.

Test for	Computer method		Clinicians' assessment	
	Number of measurements	Time delay	Number of measurements	Time delay
AMI	1 [1, 2]	6 min [0, 30]	2 [1, 5]	20 min [0, 120]
Non-AMI with MMD	7 [7, 7]	180 min [180, 180]	8 [2, 11]	573 min [30, 1080]
Non-AMI without MMD	7 [7, 7]	180 min [180, 180]	5 [1, 10]	130 min [0, 540]

Table 13. Diagnostic performance of the computer method and the clinicians. Results are expressed as average and range (in brackets).

Test for	Measures of diagnostic performance	Computer method	Clinicians' assessment
AMI	sensitivity	1.00 [11/11]	1.00 [11/11, 11/11]
	specificity	0.93 [25/27]	0.95 [25/27, 26/27]
	pos. predictive value	0.85	0.90 [0.85, 0.92]
	neg. predictive value	1.00	1.00 [1.00, 1.00]
Non-AMI with MMD	sensitivity	0.62 [5/8]	0.34 [2/8, 3/8]
	specificity	1.00 [30/30]	0.98 [29/30, 30/30]
	pos. predictive value	1.00	0.83 [0.75, 1.00]
	neg. predictive value	0.91	0.84 [0.83, 0.85]
Non-AMI without MMD	sensitivity	1.00 [19/19]	0.97 [18/19, 19/19]
	specificity	0.95 [18/19]	0.79 [14/19, 16/19]
	pos. predictive value	0.95	0.82 [0.79, 0.86]
	neg. predictive value	1.00	0.96 [0.94, 1.00]

(0.86 vs. 0.95) and negative predictive values (1.0 vs. 0.85) for prediction of major infarct.

Estimation of "time since onset of infarction"

Sensitivity, specificity, positive and negative predictive value for the computer method to identify patients with ≤ 5 hours from "onset of infarction" were 0.51, 0.91, 0.91 and 0.51, respectively. The positive predictive value implied that a positive test result has a high probability of being true and therefore might be considered in the clinical decision making. However, the negative predictive value was low.

Prognosis in unstable CAD (paper III-V)

Clinical characteristics of the patients included in paper III-V are shown in Table 14.

Tn-T for early risk assessment (paper III)

The prognostic value of tn-T was evaluated on the basis of the maximal tn-T value obtained during the first 24 hours. The median tn-T maximum was $0.33 \mu\text{g/l}$ (range: <0.04 - 19.0) and the first, second, third and fourth quintiles were 0.06, 0.18, 0.62 and $2.12 \mu\text{g/L}$, respectively. An increase in the rate of cardiac death or MI was seen from lower to higher quintiles of tn-T (Fig 14). A low risk group (the lowest

quintile), an intermediate risk group (the second quintile) and a high risk group (the three upper quintiles) for cardiac death or MI were thus identified (Table 15). Also the five month risk for cardiac death alone increased from the lowest to the highest quintile, 0, 2.0, 2.1, 6.8 and 8.8 %, respectively (pooled log rank $p<0.0001$). The risk of cardiac death or MI was higher in patients with the index event classified as non-Q compared to UA, 17.6 versus 9.5 % ($p<0.001$). However, even within the UA group there was an increase of cardiac death or MI from lower to higher levels of tn-T (Table 15).

The prognostic value of tn-T was compared to other early and easily available clinical or laboratory variables, using the univariate and multivariate forward stepwise logistic regression analysis. Tn-T level, number of anti anginal drugs at admission, increas-

Figure 14. Cumulative risk and time of occurrence of cardiac death or MI in groups based on quintiles of maximal troponin T levels ($\mu\text{g/L}$). The number of patients in each group (lowest through highest quintile) was 187, 201, 190, 191 and 194, respectively. Pooled log rank $p<0.0001$.

Table 14. Clinical characteristics of the study populations in papers III-V. Statistical comparisons are made between the ET and non-ET group (IV), and between the placebo and dalteparin group in each tn-T subset (V). All figures are given as percentage unless for median age and median delay.

	III	IV		tn-T<0.1		V	
	n=976	ET n=766	non-ET n=197	plac. n=170	dalt. n=157	plac. n=318	dalt. n=326
<u>Background factors</u>							
male sex	65	67	55**	57	55	70	69
median age (years)	70	69	72***	68	69	71	70
previous CHF	9	7	14**	7	12	6	10
previous stroke	4	3	9***	2	5	3	6
hypertension	33	32	38	31	37	33	32
diabetes mellitus	13	11	17*	12	10	11	16
current smoking	19	20	16	18	15	19	21
median delay (hours)†	24	24	19**	22	24	25	24
<u>History of CAD</u>							
previous MI	29	26	39***	29	29	28	28
angina at rest last week	46	45	51	44	41	51	52
<u>Medication on admission</u>							
aspirin	34	32	44**	38	45	31	32
beta-blockade	37	36	39	36	40	38	35
calcium inhibitor	23	21	29*	23	27	22	21
long-term nitrates	27	25	35**	31	29	25	26
diuretics	22	19	33***	24	26	21	19
ACE-inhibitor	9	8	11	10	13	6	9
digoxin	6	6	7	7	12	4	5
<u>ECG at inclusion</u>							
ST-depr. and T-wave-inv.	45	45	49	39	40	49	48
only ST-depression 16	16	19	12	13	19	18	
only T-wave-inversion	38	40	32	49	47	32	34
<u>Inclusion diagnosis</u>							
UA	61	62	56	96	99	43	42
non-Q-wave MI	39	38	44	4	1	57	58

ing age, ST-depression in the 12-lead ECG at inclusion and hypertension were identified as independent prognostic variables for MI or cardiac death at 5 months. The inclusion diagnosis (non-Q-MI or UA) did not add any independent prognostic information. The prognostic value of tn-T was also compared to CKMB. CK-MB (mass) was analysed by the Imx method (Abbott Laboratories) at the local hospital immediately following the index event in 620 patients. The incidence of car-

diac death or MI was 8.7 % in those with CK-MB below the upper reference limit in healthy persons (6 µg/L), 15.8 % in those with CK-MB 6-15 µg/L and 16.9 % in those with CK-MB above the discrimination limit for AMI diagnosis (15 µg/L). In the group of patients with CKMB <6 µg/L there was a higher risk of cardiac death or MI, 13.0 versus 4.6 % (p<0.05), among those with tn-T 0.06-0.18 µg/L (n=108) compared to those with tn-T <0.06 µg/L (n=109).

Comparison and combination of tn-T and predischARGE ET (paper IV)

During the five months follow-up 73 and 23 (10 and 13 %, NS) patients suffered an AMI, 22 and 13 (2.9 and 7.2 %, $p<0.01$) patients died a cardiac death, and 243 and 83 (32 and 42 %, $p<0.01$) had CABG or PTCA among the 766 patients in the ET group and among the 181 patients with contra-indications to the ET in the non-ET group, respectively.

A “low”, “intermediate” and “high risk” ET response was present in 361 (47 %), 325 (42 %) and 80 (10 %) patients, respectively. Tn-T maximum <0.06 , $0.06-0.2$ and ≥ 0.2 $\mu\text{g/L}$ was observed in 154 (20 %), 175 (23 %) and 437 (57 %) patients, respectively. With increasing risk category, both of maximal tn-T and ET response, there was a successive increase in the rates of cardiac death alone and in the combined endpoint MI or cardiac death after five months. However, the rates of CABG or PTCA increased only in relation to the ET response (Table 16).

In the forward stepwise multiple logistic regression analyses regarding cardiac death or MI at 5 months only the categories of exercise test response and maximal tn-T level were identified as variables of independent prognostic value. The incidence of cardiac death or MI after five months in subsets divided according to a combination of the ET response and the maximal tn-T level are shown in Figure 15. The subset with 1 % risk might be considered a definite low risk group, the four subsets with 5-10% risk might be summarised into an intermediate risk group and the four subsets with >15 % risk into a high risk group. The rates of cardiac death or MI were 1, 7 and 20 % after five months in these low, intermediate and high risk groups, respectively. In the non-ET group the incidence of cardiac death or MI were 3 %, 16 % and 27 % after five month (pooled log rank, $p=0.01$), in patients with maximal troponin T level <0.06 , $0.06 - 0.2$ and ≥ 0.2 $\mu\text{g/L}$, respectively.

Table 15. Incidence of cardiac death or MI (%) during 5-months follow up in relation to tn-T levels in the whole study population and in those with UA as index event, respectively.

Index event	Tn-T levels		
	<0.06 $\mu\text{g/L}$	$0.06-0.18$ $\mu\text{g/L}$	≥ 0.18 $\mu\text{g/L}$
UA or MI (n=963)	4.3	10.5 †	16.1 *
UA (n=581)	4.4	11.4 †	14.1 ‡

Log rank * $p=0.05$, † $p<0.05$, each group compared to the group on its left.

Log rank ‡ $p<0.01$, each group compared to the second group on its left.

UA = unstable angina; MI= myocardial infarction.

Table 16. Comparison of tn-T and ET regarding different endpoints after five months in the ET-group (n=766) (%)

	<0.06 µg/L n=154	Troponin		low risk response n=361	Exercise test	
		0.06-0.2 µg/L n=175	≥0.2 µg/L n=437		interm. response n=325	high risk response n=80
Cardiac death	0 (0)	3 (2)	19 (4)++	3 (1)	11 (3)*	8 (10)+++
Cardiac death/MI	7 (5)	16 (9)	58 (13)++	17 (5)	41 (13)***	23 (29)***+++
CABG/PTCA	42 (27)	61 (35)	140 (32)	70 (19)	123 (38)***	50 (63)***+++

* p<0.05, ** p<0.01, *** p<0.001, each group compared to the group on its left
† p<0.05, ++ p<0.01, +++ p<0.001, each group compared to the second group on its left

Effects of dalteparin treatment in relation to troponin T level (paper V)

To study the treatment effect of dalteparin in relation to the tn-T, the patients were first divided into tertiles of the tn-T level at inclusion, i.e. below the 33rd percentile (<0.1 µg/L), between the 33rd and 66th percentile (0.1-0.64 µg/L) and above the 66th percentile (≥0.64 µg/L). At termination of the long-term treatment, day 40, there was no beneficial effect in the lowest tn-T tertile, while there seemed to be a similar decrease in death or MI in the middle and highest tertile. Thus, for the further analyses the patients

were divided according to tn-T level <0.1 and ≥0.1 µg/L.

In patients with tn-T ≥0.1 µg/L, the difference in endpoint rate between the placebo and dalteparin group was significant already after the acute phase, and increased further during the long-term treatment. In contrast, in patients with tn-T<0.1 µg/L, there was no difference between dalteparin and placebo during long-term treatment (Table 17 and Fig 16). The interaction between treatment and tn-T level and the tn-T level alone, but not the treatment alone, remained as statistically significant indicators of the risk of death or MI after 40 days in a multivariate logistic regression analysis. After termination of the randomised treatment, the difference in deaths or MI between the dalteparin and placebo groups of patients with tn-T ≥0.1 µg/L slowly decreased and only a trend of benefit in the dalteparin group remained at the last follow up after 150 days (16.0 vs. 20.4 %; RR 0.78, 95 % CI 0.56-1.09).

Figure 15. Five months risk of cardiac death or MI in relation to exercise test response and maximal troponin T levels. The numbers on the bars denote the number of patients in each group.

Table 17. Endpoint rate (%) in relation to tn-T level at inclusion in patients with placebo (Pl) and dalteparin (Da) treatment, respectively (n=971). RR=relative risk. CI= confidence interval

tn-T	death/MI day 6			death/MI day 40			revascularisation day 40		
	Pl	Da	RR (95 % CI)	Pl	Da	RR (95 % CI)	Pl	Da	RR (95 % CI)
<0.1 µg/L (170/157)	2.4	0	*p=0.12	4.7	5.7	1.22 (0.48-3.1)	20.6	17.8	0.87 (0.55-1.35)
≥0.1 µg/L (318/326)	6.0	2.5	0.41 (0.18-0.92)	14.2	7.4	0.52 (0.32-0.83)	17.9	12.3	0.68 (0.47-0.99)

*p-value is given since RR can not be calculated with zero events in one of the groups

DISCUSSION

Our understanding of the pathophysiology in ACS has improved impressively in the last two decades as outlined in the background. Simultaneously a number of new and effective treatment alternatives have been introduced, e.g. beta blockers, aspirin, heparin, thrombolysis, and early revascularisation by PTCA and CABG. In AMI, these new therapies and the understanding of the critical role of a short time delay for their success, have urged a change in "diagnostic mind-set" (186), away from only retrospective confirmation of the diagnosis to-

wards earliest possible detection of the infarction in all chest pain patients. This new "diagnostic mind-set" is also necessitated by the economic restrictions in the health care systems. Early ruling-out of AMI will ensure a more cost-effective use of critical care resources as a majority (60-70 %) of patients admitted to CCU will not have AMI (187).

A new "prognostic mind-set" is also needed. Unstable CAD encompasses a wide range of patients, with a variable short-term prognosis. The treatment should ideally be tailored according to proper considerations about prognosis and underlying pathophysiology. The number of patients per year with a diagnosis of UA has steadily increased and is today almost as many as those hospitalised with a diagnosis of MI (188). Thus, risk evaluation during the first few days is of great importance for optimal treatment. The role of biochemical markers in these new "diagnostic and prognostic mind-sets" is controversial (189).

Figure 16. Cumulative hazard curves for death or MI in patients with and without dalteparin treatment with and without elevation of troponin T, respectively.

Early diagnosis in patients with suspected AMI (paper I & II)

Frequent analyses and combinations of biochemical markers (paper I)

Methodological considerations

The value of frequent blood sampling and analyses of tn-T, CK-MB (mass) and myoglobin for early rule-in and rule-out of AMI was evaluated in patients admitted without diagnostic ECG-changes. In these patients the current used diagnostic procedure is too time consuming. The clinical characteristics (Table 9) were representative for this large group of patients, except that a rather high proportion of the patients received thrombolytic treatment, despite absence of ST-segment elevations on admission. However, whether or not the patients were treated with thrombolysis, did not influence the results.

The assays used for CK-MB and tn-T, respectively, are commercially widely used assays. The RIA-assay for myoglobin is well described and its precision is comparable with commercially available methods (190). However, the analyses of the markers in the present study were done afterwards and not "on-line". Therefore, the impact of an early diagnosis or exclusion on the handling, treatment of and cost for these patients could not be assessed.

Ruling-in AMI

The sensitivities of tn-T and CK-MB using a single blood sample on admission were low in accordance with other studies (99, 112). However, myoglobin had a relatively high sensitivity already at entry in the present as in some although not all previous studies (101, 112, 191). The present study demonstrated a rapid increase in sensitivity obtained by frequent blood sampling. The rapid rise in diagnostic yield was especially noteworthy in candidates for reperfusion treatment with less than four hours delay. In this group the sensitivities of CK-MB and tn-T increased by approximately 10 - 20 % every half-hour during the first 2,5 - 3 hours reaching ≥ 95 % for all three markers after three hours. Thus, using "biochemical monitoring" almost all patients with ongoing AMI can be identified within three hours from admission.

The specificity of a diagnostic test should be high, especially in patients with a relatively low pre-test likelihood of AMI, such as patients with chest pain and a nondiagnostic ECG. Myoglobin, and low cut-off levels for CK-MB and tn-T, respectively, gave unacceptably low specificities in accordance with most previous studies (99, 112, 192). There was a considerable number of false positive cases even in the group with no evidence of

IHD with myoglobin. In contrast the false positive cases with CK-MB and tn-T were found among patients with IHD. However, using a combination of markers increased the specificity, while maintaining a high sensitivity. The combination of CK-MB and myoglobin (MyCK) was most favourable with 93-98 % sensitivity and 93-93 % specificity after 3-6 hours monitoring which seems considerably better than for any previously suggested combination (111). Since the study was performed the tn-T assay has been improved, both regarding analytical sensitivity and cardiospecificity (89), and therefore, the diagnostic capacity of the combination of tn-T and myoglobin might have improved.

Ruling-out AMI

Based on the present and previous results (112), it is impossible to rule-out AMI with any single or combination of blood tests on admission. The conventional approach to rule-out AMI is to wait until all possible cases of AMI are diagnosed. The present, not previously evaluated, approach was to create a criterion of non-AMI, i.e. if fulfilled, AMI is ruled-out; while if not, the diagnosis will remain uncertain (provided the previous positive criteria for AMI are not fulfilled). With this approach, AMI could be ruled out in a substantial proportion of non-AMI patients after only 3-6 hours of monitoring without falsely

ruling-out any patient with AMI (except one with repeated episodes of chest pain and the first elevation of the markers after 24 hours, indicating start of the infarction >12 hours after admission). For very early rule-out myoglobin was preferable and could safely exclude AMI after three hours (in 64 % of non-AMI patients). After six hours tn-T or CK-MB seemed equally safe and ruled-out a slightly higher proportion.

Clinical implications

Using the currently recommended routine of blood sampling on admission and every sixth hour (193), with analysis of CK-MB (mass) (194) and obtaining the result from the central laboratory after 1.5 - 2 hours, the biochemical diagnosis of AMI in patients subsequently diagnosed as having infarcts will be made in no patient during the first two hours, in approximately 40 % after two hours and in almost all patients after 8 hours from admission. Our suggested alternative approach (Fig 17) of obtaining blood samples for immediate analysis of myoglobin and CK-MB or tn-T every 30 minutes during the first hours will allow a definite diagnosis of AMI in around 60 % on admission, 80 % within one hour and above 90 % within two hours (i. e. earlier than any patient is diagnosed with today's routine). It will also allow on-going AMI to be safely ruled-out in a substantial pro-

portion of patients with non-AMI within 3-6 hours. Provided clinically stable these patients might safely be transferred from the CCU.

Although the assays used in the present study required 60 - 90 minutes, newer methods for analysis of CK-MB, tn-T and myoglobin can deliver the results within 30 minutes. Easy-to-use bedside instruments, with results within 15 minutes, probably soon will be available. Our approach might then be especially valuable for chest pain units, in which rapid assessment of the patients is mandatory (195). Furthermore, it might have a large impact on future studies evaluating treatment alternatives in AMI patients with nondiagnostic ECGs (196). The increased costs for the frequent sampling and rapid analysis

will be compensated for by the shorter stay in CCU and in-hospital of patients without AMI.

Neural network methodology and biochemical monitoring (paper II)

Methodological considerations

We selected neural network structures of minimal complexity in the design of the computational method in order to minimise the risk for the network to lose its capability of generalisation (197). We also incorporated knowledge of the time-distribution of data: the module for detection of AMI on first measurement used the fact that the concentration levels of the biochemical markers for AMI patients have a characteristic development over time. This fact was taken into account by training several networks, making each one of them specialised to recognise patterns of measured biochemical markers at a specific time interval after onset of symptoms. From the second measurement onward, however, a single recurrent neural network structure was used, thus selecting a structure that was suitable for the recognition of temporal patterns.

Although the patients were representative for a CCU population and unique with the early frequent blood sampling, the number of patients was limited in both the test and training

Figure 17. A proposed model for early rule-in and rule-out of AMI by frequent blood sampling and analyses of biochemical markers and using different criteria for ruling-in and ruling-out

sets. Therefore, the results must be interpreted with caution and regarded as indicative of the potential benefit of a neural network-based computer system in the early assessment of patients with acute chest pain. Since the clinicians, in contrast to the computer system, had access to data about age, gender, time since onset of symptoms and ECG on admission, in addition to the results of the biochemical markers, the comparison probably underestimated the advantages of the computer system. Further studies in larger groups are clearly needed.

Detection and exclusion of AMI and MMD

Patients with an AMI were generally correctly classified both by the computer system and the clinicians. However, the mean time was shorter and the variation smaller for the computer. The diagnostic performance of both the computer system and the clinicians was lower for correct classification of patients suffering from MMD than from the other two groups. This is not surprising since MMD is in the grey-zone between non-AMI and minor AMI. However, it was in these difficult cases the computer system proved its potential with a higher diagnostic sensitivity and positive predictive value in the test for MMD. Another consequence was that the clinicians had a lower diagnostic

specificity and positive predictive value than the computer system in the test for non-AMI without MMD. This was due to the fact that most cases of MMD which were incorrectly classified by the clinicians as non-AMI without MMD.

In comparison with the diagnostic rules based on CK-MB and myoglobin the neural networks showed a comparable capacity for detection of AMI. However, for exclusion of AMI the computer method was superior.

Prediction of infarct size

The computer system was designed to perform a crude prediction of infarct size, requiring at most one more measurement after the detection of AMI. The computer system predicted infarct size approximately on the second measurement and 10 out of 11 patients were correctly evaluated by the system. In contrast, the clinicians required a considerably longer time than the computer system. Thus, these results indicate that a crude early estimate of infarct size is feasible with the computer method, but not by clinicians.

Estimation of "time since onset of infarction"

Some patients have difficulties in stating the time for onset of chest pain. Furthermore, the onset of chest pain

might be an uncertain estimate for onset of infarction in the individual patient (198). Therefore, a "second opinion" from the computer, based on the pattern of biochemical markers, might be of value, e.g. for the decision to of thrombolysis. The computer method seemed to provide an estimate of the time from onset of infarction very early and with reasonable reliability.

Clinical implications

The decision-making process in the assessment of chest pain patients may be time consuming and is heavily dependent on the individual clinician's knowledge and experience. Therefore, some kind of decision support might be useful, e.g. by artificial neural networks. A combined approach for early diagnosis, infarct size prediction and estimation of onset of infarction might be of particular clinical relevance in patients without ST-elevation, since the cost-benefit ratio of reperfusion therapy is dependent on a correct diagnosis, time since onset and on the size of myocardium at risk (32, 152). Although thrombolytic treatment is only proven to be beneficial in patients with ST-segment elevation or bundle branch block on admission (32) a recent analysis of the data from the LATE study showed benefits of thrombolytic treatment also in patients presenting with ST-segment depression (38). Thus, thrombolysis might well

be of benefit in certain subgroups of patients with non-diagnostic ECGs, e.g. patients with short delay and an impending large infarction. The possibility to select such subgroups, e.g. by the use of biochemical monitoring and neural network technology, seems to open new opportunities for evaluation of benefit of thrombolytic treatment in new subgroups of AMI patients (196).

Prognosis in unstable CAD (paper III-V)

Methodological considerations

The patients in the present study, with advanced age, history of increasing or rest angina pectoris and ST-depression or T-wave inversion in 12-lead ECG, had a high risk of subsequent cardiac events in accordance with previous experiences of unstable CAD (199, 200). The risk is most pronounced early after the initial event. In the present study approximately one fourth of the events occurred during the first 6 days and more than half during the first six weeks, emphasising the need for early risk assessment in order to properly select further investigations and treatment.

Patients unable to stabilise during the first hours after admission, in spite of adequate medication, might not have been included in the present study because of the need for immediate coronary angiography. However, in

those patients there is hardly any need for additional prognostic information.

Tn-T was chosen as a prognostic indicator because of its wide time window and high sensitivity and specificity for myocardial damage. These properties are especially useful in unstable CAD patients with their often fluctuating symptoms. The discriminator values of tn-T used for detection of myocardial damage have been gradually lowered as the tn-T assay has been improved, from 0.5 g/L (87), to 0.2 µg/L (122) and further to 0.1 µg/L (105). However, even lower discriminator levels for the detection of myocardial damage, as in the present study, might be justified since the upper reference level in healthy blood donors is 0.06 µg/L (184).

Although the patients were admitted to hospital with a median delay of 5 hours from onset of last episode of chest pain, the median delay to inclusion (and first blood sample) was 24 hours. Hence, the inclusion samples of tn-T obtained in the present study might not in clinical practice be translated into admission samples, but rather to samples obtained during the first 24 hours after admission.

The prognostic value of tn-T was primarily evaluated on the basis of the maximal value obtained during the first 24 hours. In order to avoid confu-

sion between the prognostic and diagnostic capacity of tn-T, only patients without events during the first 24 hours and one day thereafter were considered in the prognostic evaluation. However, in the evaluation of the effect of the dalteparin treatment in relation to the tn-T level, only the sample obtained before the start of the randomised treatment (the inclusion sample) was used for two reasons: firstly, to avoid potential interference of the dalteparin treatment on the subsequent tn-T levels and, secondly, in the clinical situation, the decision whether to start therapy with heparin is preferably made as soon as possible.

Prognostic value of tn-T

The present study demonstrated for the first time that the risk of subsequent cardiac events gradually increases with increasing maximal levels of tn-T in patients with unstable CAD. A significant increase in the risk was shown already at very low levels of tn-T, from 4.3 % of cardiac death or MI in patients with tn-T <0.06 µg/L to 10.5 % in patients with tn-T between 0.06-0.18 µg/L, the increase was in fact evident already in patients with tn-T 0.06-0.10 µg/L in whom 11/109 (10.1 %) suffered an event. Tn-T levels of <0.06, 0.06-0.18 and ≥0.18 µg/L, identified groups with low, intermediate and high risk of MI or cardiac death, respectively, both in the total

material and in the UA-group separately. The present study alone included almost as many UA patients as was included in a recently published meta analysis of seven studies regarding the prognostic value of tn-T (107). Using $0.06 \mu\text{g/L}$ as cut-off, the relative risk ratio of 2.7 (CI, 1.3-5.7) for UA patients with elevated tn-T in our study lies just below the lower border of the 95 % confidence interval of the odds ratio of 4.3 (CI, 2.8-6.8) in the metaanalysis. The results from the present study and a number of other published studies (122-126, 201-203) of the prognostic values of tn-T and other biochemical markers in UA patients or chest pain patients without MI, are summarised in Table 18. These studies show a consistent pattern of increased risk of cardiac events in patients with elevation of tn-T. In contrast to Hamm et al (122) we did not find any significant rise of in-hospital risk of cardiac death or MI in those with elevated tn-T in the UA-group. These minor discrepancies from previous studies might be explained by slightly different study populations and our careful separation of index and subsequent events. In addition to previous findings, we also found a higher risk for cardiac death alone with higher levels of tn-T, both in the whole study population and if the analysis was restricted to UA patients. In accordance with most previous

studies (123, 125, 126) a minor increase of CK-MB (mass) also indicated a higher risk of subsequent cardiac events, although it did not reach statistical significance in the present study (Table 18). However, in the group with CK-MB levels below the upper reference limit, it was still possible to demonstrate minor elevations of tn-T in a large proportion of patients. These patients also had a significantly higher risk of cardiac events. Thus, tn-T seems superior to CK-MB (mass) for risk stratification.

In the multivariate analysis the tn-T level was a strong, consistent and independent prognostic variable concerning cardiac death or MI in the present study. This contrasts to a recent report (126) where tn-T level did not add any independent prognostic information when ST-T abnormalities were present at admission. The inclusion diagnosis did not add any independent prognostic information in contrast to the tn-T level. There was no significant difference in the incidence of cardiac events between those with UA and non-Q-wave MI in the same interval of tn-T elevation, suggesting that the tn-T level gives better prognostic information than the crude separation of unstable CAD-patients into UA or non-Q-wave MI according to conventional criteria.

Table 18. Rate of death or MI during follow up in patients with UA or chest pain without MI in subgroups with high and low levels, respectively, of different biochemical markers (122-126, 201-203).

Study	Follow-up	Marker; cut-off (µg/L)	High (%)	Low (%)	p-value
Hamm et al	in-hospital	tn-T; 0.2	10/33 (30)	1/51 (2)	<0.001
Pettersson et al	48 months	CK-MB; *	9/14 (64)†	2/39 (5)†	<0.001
Markenward et al	6 months	CK-MB; 10	8/29 (28)#	3/71 (4)#	<0.001
Ravkilde et al	6 months	tn-T; 0.2	6/44 (14)	3/83 (4)	<0.05
Ravkilde et al	28 months	tn-T; 0.2	6/25 (24)	5/99 (5)	<0.01
		CK-MB; 6	8/35 (23)	3/89 (3)	<0.001
Stubbs et al	34 months	tn-T; 0.2	18/62 (29)	21/121 (17)	<0.05
Wu et al	3 weeks	tn-T; 0.1	8/27 (30)	3/104 (3)	<0.001
		CK-MB; 10	5/8 (63)	6/123 (5)	NS
de Winter et al	6 months	tn-T; 0.1	8/24 (33) □	16/104 (15) □	<0.05
		CK-MB; 7.5	4/13 (31) □	20/115 (17) □	NS
FRISC §	5 months	tn-T; 0.06	48/403 (12)	8/184 (4)	<0.01
		tn-T; 0.2	26/192 (14)	30/395 (8)	<0.05
		CK-MB; 6.0	14/ 80 (15)	25/246 (9)	=0.13

* difference between two adjacent samples >1.5-2 µg/L, depending on which of the three assays was used; † rate of death; # rate of MI; □ rate of MI/cardiac death/revascularisation; § only patients with an index diagnosis of UA

Comparison with exercise test

Apredischarge ET could be performed in a large proportion of patients (80 %). The findings at the ET were comparable to previous findings in similar populations (162, 165, 185). The prognostic value of a combination of ST-depression and/or a low exercise tolerance at an early ET in the present study is in accordance with most (161-163, 165), although not all (169), previous studies. Although the rate of revascularisation was influenced by the ET response (in accordance with the protocol), which might be expected to influence the event rate, the prog-

nostic value of the ET response regarding cardiac death or MI remained impressive (Table 16).

In the multivariate analysis, both ET response and maximal tn-T level were independent risk indicators for cardiac death or MI. The present study demonstrated, for the first time, that the combination of an early symptom limited ET and tn-T determinations further improved the risk stratification compared to either test alone and made it possible to identify subgroups of patients with very low risk and very high risk of death or MI (Fig 15).

The 20% of patients with contraindications against the ET were older and had more severe cardiovascular disease than those in the ET group. Consequently, the incidence of cardiac events was somewhat higher, in accordance with previous experiences (204). However, tn-T determinations, which can be made in all patients, were prognostically useful also in this subset of patients.

In contrast to an ET, the risk assessment by tn-T can be done very early. This is important, since a number of patients will suffer an event (in the present study 29 of 976 patients) before an ET can be performed. Therefore, tests for ischemia that can be performed during the first day, e.g. continuous ECG-monitoring, have a potential to further improve or be complementary in the risk evaluation (158, 159). The prognostic value of continuous ECG monitoring in comparison with tn-T determinations, 12-lead ECG at rest and ET is at present under evaluation.

Effects of dalteparin treatment in relation to troponin T level

The event rate in the total dalteparin group compared to the placebo group was reduced by approximately 65 % during the acute phase treatment. During long term treatment the absolute reduction was maintained, but there was no further reduction (47).

However, in the subgroup with tn-T levels $\geq 0.1 \mu\text{g/L}$ there was an effect of dalteparin treatment not only during the acute, but also an additive effect during the long term treatment. In contrast, there seemed to be no protective effect of long-term dalteparin in patients with very low or no detectable levels of tn-T ($< 0.1 \mu\text{g/L}$). The treatment with dalteparin reduced the risk of events in patients with tn-T ≥ 0.1 almost to the same level as in the "low risk" patients with tn-T $< 0.1 \mu\text{g/L}$ (Fig 14). There were no differences in clinical characteristics between the placebo and dalteparin group within each tn-T subset that could explain these results. The interaction analysis further supported that there was a real difference in effect of dalteparin treatment in patients with and without elevation of tn-T. These findings differ from what was found in the RISC-study comparing aspirin with placebo, in which the absolute risk reduction of aspirin treatment during one year was comparable for death or MI in subgroups with high and low risk ET response (205). The use of tn-T for identifying unstable CAD patients for long term antithrombotic treatment and/or other medical or interventional therapies is at present being evaluated in prospective trials.

Pathophysiological speculations

In patients with stable angina and documented CAD, no elevation of tn-

T could be demonstrated after induction of severe ischemia by atrial pacing (206). Probably, minor elevations of tn-T are due to an, at least transient, embolic or thrombotic occlusion of a coronary artery, with flow interrupted long enough to cause myocardial damage. The gradually increased risk for future myocardial infarctions by increasing tn-T levels, might possibly be explained by tn-T level reflecting the liability of thrombus formation at the unstable plaque (207). Another possible explanation is that the tn-T level reflects the severity of ischemia, which is supported by the more frequent occurrence of left ventricular dysfunction during anginal attacks in UA-patients with, rather than without, tn-T elevation (208). On the other hand, ischemic ECG-changes at the ET indicate a severe stenosis in the coronary vessels impeding the ability to match the raised oxygen demand with increased supply (209), without necessarily implying a thrombotic condition. Furthermore, a low exercise tolerance together with ST-depression during exercise, increases the probability of multivessel disease (185) and diastolic dysfunction (210). Thus, the independent prognostic values of tn-T determinations and ETs might be a reflection of different pathophysiological mechanisms in unstable CAD. Thereby, the substantially increased risk in patients with both elevation of tn-T and a high risk ET response might

be explained by an ongoing thrombotic condition in severely diseased coronary arteries.

An embolising or at least temporary occluding red thrombus is probably the main mechanism, which responds to treatment with l.m.w. heparin in patients with elevation of tn-T (18, 207). On the other hand, in patients without tn-T elevation this process might be less prominent and therefore less responsive to this kind of therapy. In such patients other mechanisms might cause the symptoms, e.g. coronary vasoconstriction, due to impaired endothelial vasoactive function in the atherosclerotic coronary arteries (14, 15). However, it can not be ruled out, that the lack of prophylactic long term effect of dalteparin in the group with tn-T <0.1 µg/L was only a play of chance, since it is necessarily more difficult to show a beneficial effect of any treatment in groups with such a low risk.

Clinical implications

For risk stratification in patients with suspected unstable CAD a strategy based on tn-T determined on admission and after 12-24 hours, findings on ECG at rest (or if available continuous vector cardiography or 12-lead ECG monitoring) and an early ET might be proposed. Patients clinically stabilised and with low or undetectable tn-T levels, have a low risk for cardiac death

or MI both in hospital and during the subsequent five months and might on appropriate medication be discharged early and further evaluated in the out-patient clinic. Clinically stabilised patients with a minor increase in tn-T level and without ST-segment depression (or episodes of ST-changes) might also be further treated and evaluated in the out-patient clinic, while patients with a minor increase in tn-T level and ST-segment depression (or episodes of ST-changes) need further evaluation and treatment while still in hospital. Patients with more definite elevations of tn-T ($>0.2 \mu\text{g/L}$) should be further investigated and have intense medical and/or interventional treatment while still in hospital. In most patients a symptom limited predischARGE ET is preferably done a few days after clinical stabilisation, but in low risk patients as defined above, the ET might be done as an out-patient procedure. Patients with both low risk ET and no tn-T elevation, have a very low risk for cardiac death or MI during the subsequent months and further evaluation and invasive procedures seems unwarranted in the absence of incapacitating symptoms. In contrast, in patients with high risk ET response, or intermediate risk response and tn-T $\geq 0.2 \mu\text{g/L}$, the severe short term prognosis warrants early consideration of revascularisation. The intermediate category often can be handled with intense long term protective medica-

tion and continuous evaluation of the individual risk-benefit ratio of a revascularisation procedure during follow-up. In patients unable to perform an ET, alternative techniques such as dobutamin echocardiography or dypiridamole scintigraphy should be considered, especially in patients with elevated tn-T.

In patients admitted with suspicion of unstable CAD and any objective signs of ischemia, high dose dalteparin or heparin infusion should be initiated. In patients with elevation of tn-T continuation of the dalteparin treatment with low-dose injections, once daily, might be considered, especially in patients scheduled for coronary angiography and revascularisation procedures .

SUMMARY AND CONCLUSIONS

In patients with suspected AMI

Monitoring of a combination of myoglobin and CK-MB or tn-T allowed ruling-in AMI within 2-3 hours and ruling-out AMI within 3-6 hours in almost all patients admitted with chest pain and a nondiagnostic ECG. This might have a large impact on the early handling and treatment of these patients.

The neural network methodology, with monitoring of myoglobin, CK-MB and tn-T allowed, within the first

three hours, reliable diagnosis/exclusion of AMI/MMD, prediction of infarct size and a crude estimate of time since “onset of infarction” in patients admitted with suspicion of AMI. The computer system was faster than both clinicians and clinical rules. Thus, neural network methodology might be a useful support for the early assessment of patients with suspected myocardial infarction.

In patients with unstable CAD

The risk of subsequent cardiac events is increased by increasing maximal levels of tn-T obtained during the initial 24 hours. Thereby a normal, a slightly elevated and a clearly elevated tn-T level identified a low, intermediate and high risk group, respectively, for MI or death. The tn-T level was an independent prognostic variable for MI or death in a multivariate analysis comparing other early available risk indicators. Furthermore, tn-T seemed to be superior to CK-MB (mass) for risk stratification.

In patients able to perform a predischARGE ET both the tn-T level and the ET response were independent prognostic indicators for MI or death. The combination of tn-T and the ET response allowed a further improved risk stratification.

In patients with tn-T elevation at inclusion, prolonged dalteparin treatment was beneficial. However, in patients without tn-T elevation, long term dalteparin treatment had no protective effect.

Thus, tn-T determination provides independent and important prognostic information in unstable CAD. In the selection of treatment strategy for the individual patient, this simple, inexpensive and early available biochemical test might be useful.

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