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Coagulation, inflammation and myocardial dysfunction in unstable coronary artery disease and the influence of glycoprotein IIb/IIIa inhibition and low molecular weight heparin

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ABSTRACT

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Patients with unstable coronary artery disease (CAD) have an increased risk of subsequent myocardial infarction and death. This study evaluated the safety and efficacy of treatment with glycoprotein IIb/IIIa inhibition in addition to aspirin, low molecular-weight heparin and its influence on coagulation and inflammation. Also, early and differentiated risk assessment utilising markers of inflammation, myocardial damage and dysfunction were evaluated.

The Global Utilisation of Strategies To open Occluded arteries-IV (GUSTO-IV) trial randomised 7800 patients with unstable CAD to 24 or 48 hours infusion of abciximab or placebo in addition to routine treatment with aspirin and heparin or dalteparin. Baseline levels of creatinine, C-reactive protein (CRP), troponin T (TnT) and N-terminal pro-brain natriuretic peptide (NT-proBNP) were analysed. At selected sites, all patients received subcutaneous dalteparin (n=974), in stead of heparin infusion (n=6826). In a sub-population of dalteparin treated patients (n=404), serial measurements of markers of coagulation, fibrinolysis and inflammation were also performed.

Addition of abciximab to dalteparin as the primary treatment of unstable CAD was not associated with any significant reduction in cardiac events but a doubled risk of bleedings. The combination of abciximab with dalteparin seemed as safe when

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Abbreviations: Acute coronary syndrome (ACS), Brain natriuretic peptide (BNP), Coefficient of variance (CV), Coronary artery disease (CAD), Coronary artery bypass grafting (CABG), Creatine kinase-MB (CK-MB), C-reactive protein (CRP), Electrocardiogram (ECG), Glycoprotein IIb/IIIa (GP IIb/IIIa), Global Utilisation of Strategies To Open Occluded Arteries IV (GUSTO-IV), Interleukin-6 (Il-6), Low-molecular weight heparin (LMWH), N-terminal pro-Brain natriuretic peptide (NT-proBNP), Myocardial infarction (MI), Percutaneous coronary intervention (PCI), Plasminogen activator (tPA), Plasminogen activator inhibitor (PAI-1), Soluble fibrin (SF), Thrombin-antithrombin complex (TAT), Troponin T (TnT), Unfractionated heparin (UFH).

used with heparin. Despite full dose dalteparin and aspirin there was a simultaneous activation of the inflammation, coagulation and fibrinolysis systems without any influence of the abciximab treatment. Elevated levels of CRP, TnT, and NT-proBNP and reduced creatinine clearance were independently related to short and long-term mortality. The best prediction of high and low risk was provided by a combination of NT-proBNP and creatinine clearance. Any detectable elevation of TnT and reduced creatinine clearance, but neither elevation of CRP nor NT-proBNP, were also independently associated to a raised risk of subsequent myocardial infarction.

INTRODUCTION

Cardiovascular disease is the most common cause of death in the industrialised world, comprising 46 % of all deaths in Sweden (1). In spite of the steady decline in the incidence of myocardial infarction in recent years, the incidence of unstable coronary artery disease (CAD) has increased considerably(1). Chest pain, or other symptoms suggestive of unstable CAD, is furthermore one of the most common reasons for admission to the hospitals emergency rooms. These patients constitute a very heterogeneous population regarding the clinical history and the underlying cause of the symptoms. Subsequently, also the prognosis varies considerably. Early risk prediction is essential for identification of patients at high risk and selection of the most appropriate treatment strategy. Moreover, with early risk-prediction, patients at low risk can be identified and costly and potentially hazardous treatments and prolonged hospital stays can be avoided.

Unstable CAD is caused by a complex interaction between the endothelium, platelets and cascades systems of inflammation, coagulation and fibrinolysis, leading to thrombus formation and compromised coronary blood flow. Subsequent myocardial damage, caused by downstream embolization of thrombotic material, as well as inflammation and myocardial dysfunction, contributes to an increased risk of serious complications. Treatment of patients with unstable CAD aims to symptom relief and to limitation of myocardial damage and prevention of recurrent ischemic events at a tolerable level of side effects. Despite substantial improvements in the treatment there is still an approximately 7–10% risk of death or myocardial infarction the initial month after the index episode(2–4). Therefore, further improvements by new anti-thrombotic and anti-inflammatory agents and by better and more differentiated risk assessment to tailor the treatment to individual patients, is highly warranted.

In the present study, the safety and clinical efficacy of the glycoprotein IIb/IIIa inhibitor abciximab in combination with either the low molecular-weight heparin, dalteparin or unfractionated heparin in patients with unstable CAD was investigated. Also, the influence of treatment with abciximab on the activation of systems of inflammation, coagulation and fibrinolysis was elucidated. Finally, the utility of biochemical markers of inflammation, myocardial damage and myocardial dysfunction for prognostication of the risk for death and myocardial infarction was elucidated.

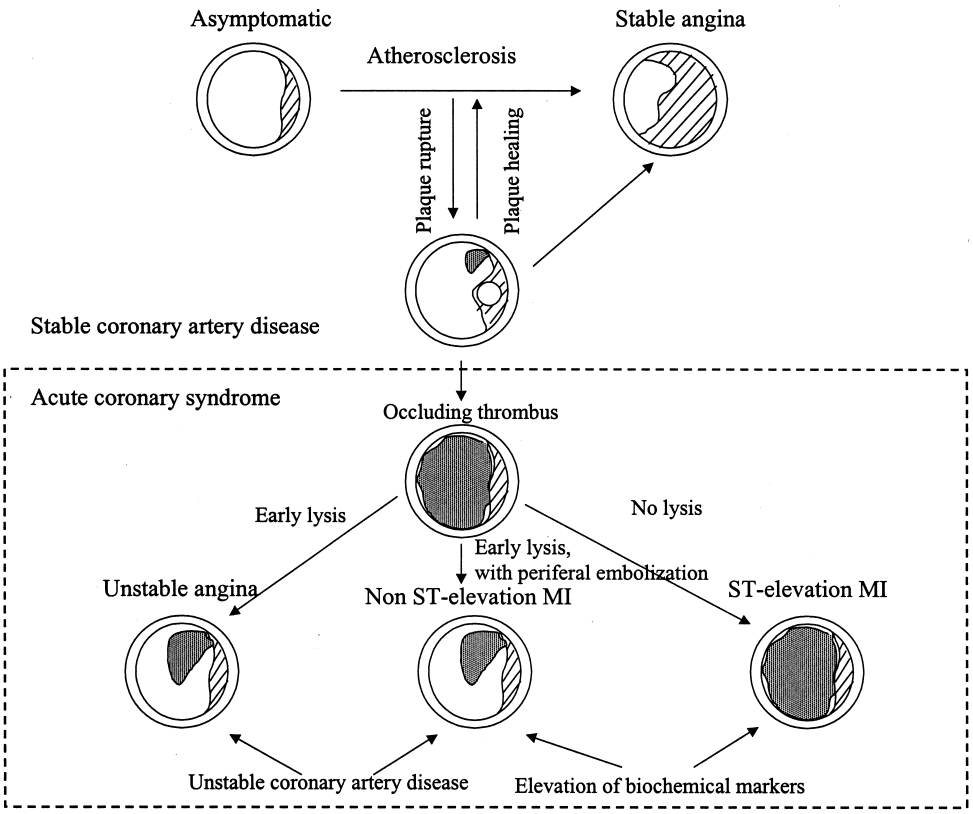


Fig. 1. Atherosclerosis, plaque rupture and thrombus formation. Definitions of various types of coronary heart disease. MI = Myocardial infarction.

BACKGROUND

Clinical manifestations and definition of coronary syndromes

The coronary arteries continuously supply blood for the myocardium's demands of oxygen and nutrients. Interruption of the blood flow creates a state of ischemia with accumulation of acid waste products and disintegration of cell membranes resulting in ischemic chest pain. The induced changes in electric potentials can be detected with an electrocardiogram (ECG) and biochemical substances released from the injured myocardial cells can be detected in the peripheral blood.

The classification of coronary syndromes is based on the acuteness by which they develop; stable coronary artery disease (CAD), acute coronary syndromes (ACS) and sudden cardiac death. Stable CAD is often a chronic disease with a deciduous onset and associated with a relatively low risk (figure 1). In contrast, ACS starts acutely and has a considerably worse prognosis. ACS is further divided into myocardial infarction (MI) and unstable angina (UA). Typically, MI is characterised by a sudden onset of severe chest pain in combination with a shortness of breath,

nausea and circulatory instability and defined as symptoms or signs of myocardial ischemia associated with a certain degree of elevation of biochemical markers(5). Unstable angina on the other hand is defined as a condition of coronary ischemia without elevation of biochemical markers and is clinically characterised by; a) recent onset (<4 weeks) of angina pectoris on minimal exertion, b) worsening of a stable angina with prolonged, more severe or more frequent episodes of chest-pain c) angina at rest or d) angina after a myocardial infarction (6). Transient ischemic ECG changes such as ST-depression or T-wave inversion are not obligatory but strongly support the diagnosis.

Based on the patients ECG findings on arrival, MI are further classified as ST-elevation or non-ST elevation MI. ST-elevation MI is a special entity associated with the most dramatic symptoms and the strongest need for urgent treatment in order to restore the obstructed blood flow in the diseased coronary artery. Patients with a complete bundle branch block on ECG belong to the same high-risk category.

Considering both the underlying pathophysiology and symptom evolution, non ST-elevation MI and unstable angina are parts of a continuum of unstable CAD, only separated from each other by the detection of biochemical markers of myocardial damage, above a somewhat arbitrary and variable limit. The focus of interest in the present study is non ST-elevation MI and UA.

Atherosclerosis and plaque formation

The predominating underlying cause of ACS is atherosclerosis, which starts in early adulthood in the innermost layer of the vessel wall, the intima. Several factors accelerate this process, among them smoking, hereditary factors, hypertension, elevated levels of serum lipids and diabetes mellitus. The first visible signs of atherosclerosis are the so called “fatty streaks” consisting of pools of enlarged macrophages containing engulfed oxidised LDL particles that usually develop in response to mechanical stress (7). Over time, smooth muscle cells proliferate and tissue matrix is synthesised in the intima driven by a chronic inflammatory process creating a fibromuscular atherosclerotic plaque (8)(Figure 1). Late in the process, collagen and calcium is often deposited in the plaque as suggested by the term “athero*sclerosis*”(9). As the coronary plaque grows larger, the blood flow will be obstructed and may not be able to meet to current metabolic demands of the myocardium distal to the coronary stenosis. The latter situation is the pathophysiological background to the clinical entity called “chronic stable angina pectoris”.

Plaque disruption and thrombus initiation

The atheromatous core of a plaque is a soft, gruel-like hypo-cellular material mainly consisting of lipids. The overlying fibrous cap separates the plaque content from the blood. Disruption of a plaque is not an uncommon event but still a complex pathological process that is central to the initiation of the acute coronary syndrome (10). Importantly, not only the large plaques or tight stenoses create the acute coronary

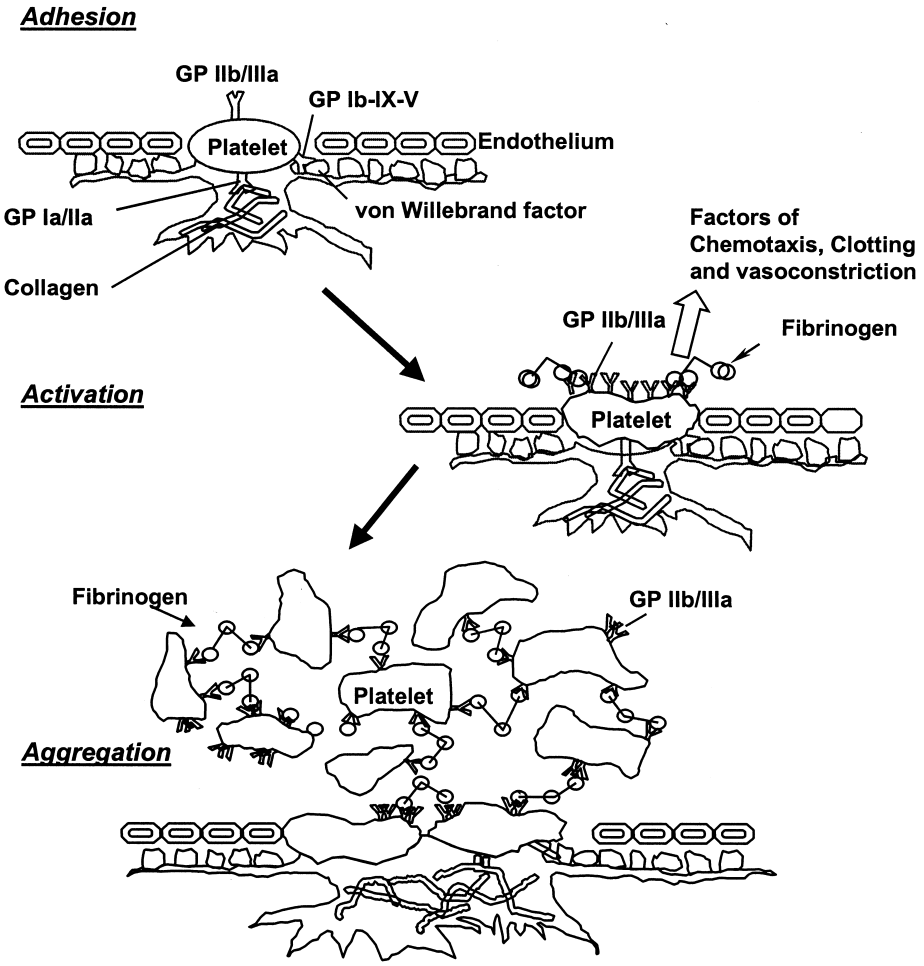


Fig. 2. Platelet activation.

syndrome. Two thirds of arteries with a plaque-rupture and an occlusive thrombus have stenoses of 50 percent or less (11). The major determinants of plaque rupture are the size and consistency of the atheromatous core, the thickness of the fibrous cap, the inflammation and repair within the plaque and the phenomenon of plaque fatigue (12). However, also sudden changes in blood pressure and increased shear stress are important contributors. The plaque rupture leads to exposition of highly thrombogenic factors to the coagulation system and platelets, initiating the thrombus formation, which further exaggerate the narrowing of the coronary artery.

Platelet activation

Platelets are small cells found in large numbers in the blood and play a pivotal role in the thrombotic process. At the site of arterial injury, subendothelial tissue is

exposed to the blood constituents (figure 2). Platelets adhere to the exposed collagen, von Willebrand factor (13) and fibrinogen by specific cell receptors i.e. glycoprotein (GP) Ia/IIa and Ib-IX-V complex (14). Platelet adhesion results in outside-in signalling resulting in a rapid change of shape. Thereby, the platelets increase their surface in order to cover the lesion. In addition to adhesion, several independent mechanisms are important platelet activators, such as thromboxane, serotonin, epinephrine, adenosine diphosphate and thrombin (15). Simultaneously, they also release vasoconstrictors, chemotactic factors, clotting factors and receptors (i.e. p-selectin and CD40 ligand) from their granules, promoting vasospasm, additional platelet aggregation and thrombin generation. Release of adenosine diphosphate and thromboxane from δ-granules amplifies the process by autocrine feedback loops.

Activated platelets undergo structural changes for activation of the most numerous receptor found on the platelet surface, the GP IIb/IIIa receptor which exists at a number of 40.000–80.000 per platelet (16). The activated GP IIb/IIIa receptor crosslinks platelets by binding to fibrinogen, creating a platelet- fibrinogen net over the site of tissue injury.

Coagulation cascade

(Proteins indicated with bold text were evaluated in the study)
At the site of a plaque rupture, the glycoprotein tissue factor (TF) will be exposed to the blood. TF is the initiator of the coagulation cascade in vivo and is expressed on smooth muscle cells, fibroblasts and macrophages in the vicinity of the vessel wall(17) (Figure 3). The coagulation cascade (18) (19) is complex and includes a series of interactions between a large number of pro-coagulant and anti-coagulant proteins interacting with platelets and endothelial cells. TF binds to small amounts of factor VII, which become activated by proteolytic cleavage into its active form, VIIa. An auto-amplifying system called the “extrinsic system” thereby becomes activated, ending up with the conversion of prothrombin to thrombin. Thrombin is considered a key protein in the cascade by its ability to activate other coagulation factors i.e. factor V, VIII and XI, and by controlling the final step in the clot formation; conversion of **fibrinogen** to soluble fibrin.

Soluble fibrin (SF) is formed when fibrinopeptide A and B are cleaved from fibrinogen by the action of thrombin. SF circulates in plasma with a half-life of 4–6 hours. The level of SF reflects thrombin activity as the basis for fibrin formation and is therefore a sensitive marker of activation if the coagulation cascade (20). Monomers of fibrin rapidly polymerise into cross-linked strands which are finally stabilised by factor VIII (activated by thrombin) to form the skeleton in the arterial clot together with the platelets.

Several anti coagulant proteins are involved in the counter regulation of the coagulation system. One of the most important inhibitors is antithrombin, which acts by forming inactivated complexes with thrombin, **Thrombin- antithrombin complex (TAT).** The inhibition of antithrombin is markedly accelerated in the presence of heparin. Thus, TAT can be detected in plasma after thrombus formation and is con-

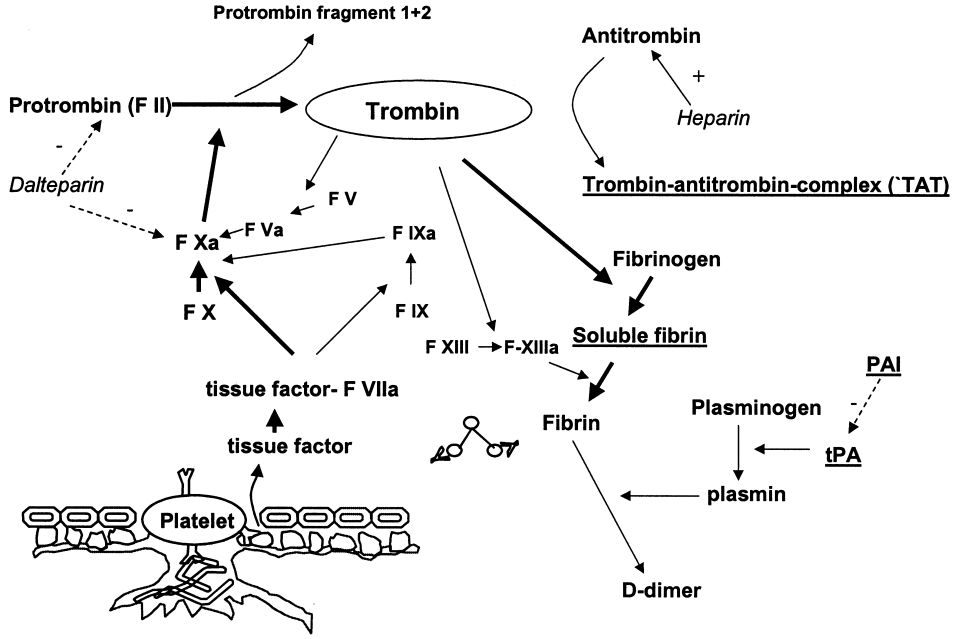


Fig. 3. Coagulation system.

sidered to reflect thrombin production and activity. The half-life of the complexes is thought to be approximately 5 minutes (20).

In the present study fibrinogen, TAT and SF were used as markers of coagulation activity for the investigation of influences of abciximab on the coagulation system.

Fibrinolysis

(Proteins indicated with bold text were used in the study)

The fibrinolytic system has a very important role in balancing the coagulation cascade by inhibiting formation of thrombi and dissolving existing clots (21) (figure 3). The key protein, plasmin, acts by degrading fibrin into its soluble degradation components, one of which is D-Dimer. Plasmin is produced on the fibrin surface in the blood, by cleavage of the inactive pro-enzyme plasminogen. Plasminogen, in turn, is activated by plasminogen-activators. The most important plasminogen activator in the blood is **tissue plasminogen activator (tPA),** which is produced and released into the blood by endothelial cells as a response to various stimuli, for example ischemia. tPA has short half-life of about 5 minutes due a rapid removal from plasma by the liver and by complex formation with plasminogen activator inhibitor 1 (PAI-1). tPA antigen concentration therefore reflect the free tPA level as well as the tPA/ PAI-1 complex concentration (22). Free plasmin in plasma is extremely rapidly inactivated by α2-antiplasmin in order to reduce the potent effects of plasmin in the circulation. Thus, PAI-1 activity in plasma attenuates endogenous fibrinolysis and

increase fibrin concentration (22). PAI-1 is produced by endothelial cells and smooth muscle cells in the vessel wall and by macrophages in the liver and spleen as well as by adipose tissue. While the biosynthesis rate of PAI-1 is high, its short biological half-life (8–10 minutes) causes low plasma concentrations (23). Since PAI-1 belongs to a group of acute phase reactants, its activity in plasma can be increased by inflammatory stimuli as well as by high fibrinolytic activity. In the present study tPA and PAI-1 were used as markers of fibrinolytic activity.

Inflammation

Inflammation plays an essential role in the pathogenesis of atherosclerosis (7). The earliest evidence of the influence of inflammatory mediators are the "fatty streaks", which are pure inflammatory lesions, containing monocyte-derived macrophages and T-lymphocytes. Continued inflammation at every stage of the disease is indicated by an increased number of macrophages and lymphocytes, (24) secreting cytokines and hydrolytic enzymes. Sero-epidemiological and pathophysiological studies suggest that infectious organisms such as herpesvirus, Chlamydia pneumoniae and Helicobacter pylori may play a role in the initiation and progression of atherosclerosis (25). The exact mechanisms for the regulation of the atheroma progression are still however, largely unknown but contains a complex interaction between different cell-types and inflammatory mediators.

Furthermore, the rupture of a fibrous cap and thus the initiation of the acute coronary syndrome involves inflammatory action (26) as reflected by an increased concentration of activated macrophages and T-lymphocytes promoting degradation of extracellular matrix in the unstable plaque (27, 28). Increased concentrations of monocytes (29), lymphocytes (30), inflammatory mediators such as C-reactive protein (CRP), (31) interleukin-6 (IL-6), serum amyloid protein A (32) as well as cytokines (33, 34) have been reported in patients with UA and myocardial infarctions at the time of hospital admission.

Interleukin-6 (Il-6) is a cytokine with pro-inflammatory and pro-coagulant properties affecting many different cell-types. It also affects endothelial function and platelet production and is the only cytokine known to induce the synthesis of all the acute phase proteins by the liver. The production of Il-6 is stimulated by tumour necrosis factor and interleukin-1 (35, 36). **C-reactive protein (CRP)** is an acute phase protein synthesised by, and released from, the liver in response to circulating interleukin-6. CRP is a sensitive marker of infection, tissue damage and inflammation (37). Its plasma half-life is rapid (approximately 19 hours) and identical under all circumstances. Thus, in contrast to other cytokines the concentration of CRP in plasma is mainly dependent on its production.

The increased inflammatory activity in patients with unstable CAD may also, at least partly, reflect an acute phase reaction resulting from myocardial necrosis (38). In unstable CAD there is also evidence of a widespread inflammation in the coronary arteries but the actual source of inflammatory mediators is still unknown (39). However, ischemic episodes themselves, as reflected by ST-depressions, do not

seem to illicit CRP elevation in patients with unstable angina (40). Furthermore, recent evidence suggests that CRP itself might be involved in the pathogenic mechanisms of the myocardial damage (41). CRP has been shown to display pro-inflammatory properties (42) by co-localisation with and ligand binding to, complement in the myocardium (43). Activated complement may in turn mediate further vascular and myocardial destruction and induce arrythmias (41). In addition, CRP induces monocytes to express tissue factor, which may contribute to the development of disseminated intravascular coagulation and thrombosis in inflammatory states (44).

Fibrinogen is an acute phase protein produced in the liver and exists in the circulation in increased concentrations in various inflammatory conditions. There is a high degree of correlation between levels of fibrinogen and several well-established clinical risk factors for cardiovascular disease (45). Since fibrinogen is both an inflammatory and coagulation mediator it is an unspecific marker reflecting increased coagulation as well as inflammatory activity.

In the present study the above mentioned inflammatory markers were used for evaluation of a potential influence of abciximab on the inflammation system as well as regarding CRP, also for prediction of coronary events.

Myocardial damage

The **troponin** complex is formed by three different forms of structural proteins (troponin C, I and T) located in the thin filament of the contractile apparatus of both skeletal and myocardial myocytes regulating the calcium dependent interaction between actin and myosin. Cardiac isoforms of troponin I and T are expressed solely on myocardial cells and released from the cytoplasma after disintegration of the cell membrane caused by myocardial necrosis. Accordingly, measurable levels of troponin I or T are highly specific for myocardial damage indicating even microscopic areas of necrosis irrespective of the cause (46). The initial rise of troponin concentration occurs 3 to 4 hours after the ischemic injury with a persistent elevation up to two weeks after the event. The MB isoform of Creatine kinase (CK-MB) is a cardiac specific enzyme also useful for detection and exclusion of myocardial damage. However, the specificity as well as the sensitivity is lower than for troponins. Thus, CK-MB is regarded as the second best alternative for diagnostic and prognostic purposes.

Myocardial dysfunction

Brain natriuretic peptide (BNP) is a neurohormone synthesised and released from the cardiac ventricles in response to increased wall tension (47). By affecting the nephrons resulting in natriuresis, BNP and other natriuretic peptides are involved in the tight regulation of extracellular volume. The plasma level of BNP is increased in patients with heart failure and increases in proportion to the degree of left ventricular dysfunction (48). BNP levels also increase after MI and in unstable angina pectoris (49). BNP is produced as a pro-hormone, pro-BNP, which is enzymatically cleaved into BNP and the amino terminal portion of the pro-hormone, N-terminal

proBNP (NT-proBNP) (50). It has been shown that mean levels of NT-proBNP are similar to mean levels of BNP in healthy people whereas in response to cardiac impairment the absolute and relative increase of NT-proBNP exceeds that of BNP up to fourfold (50).

RISK ASSESSMENT IN PATIENTS WITH ACUTE CORONARY SYNDROMES

Clinical factors

Patients with ACS are heterogeneous both regarding the clinical background, extent and severity of the underlying coronary disease. Several clinical (51) as well as biochemical and ECG indicators have been described by multivariable analyses (52). Age and male gender are among the most important clinical predictors associated with an unfavourable outcome. Various indicators of a history of cardiovascular disease, such as diabetes mellitus, heart failure, hypertension and renal dysfunction are also important factors for risk assessment (52). Due to the heterogeneity of ACS-patients and the variable risk and alternative treatment strategies, a better and a more individualised risk assessment than clinical factors can offer, is needed.

Electrocardiogram

ST segment depression > 0.1 mV on the ECG on admission has consistently been found to indicate an increased risk of subsequent death or myocardial infarction (53) with a further increased risk in relation to the magnitude of ST-depression (54). Even ST-depressions of 0.05–0.1 mV provide prognostic information regarding future coronary events (55). T-wave inversion is a less specific ECG finding, concerning the diagnosis as well as the prognosis (56, 57).

Continuous ST monitoring with 12-lead ECG or vectorcardiography better reflects the dynamic nature of myocardial ischemia than the occasional ECG recordings and adds to risk assessment(58) and identification of patients who benefit from extended anti-thrombotic treatment (59).

Coronary angiography

Coronary angiography provides an anatomic outlining of the coronary arteries and is considered the golden standard for the assessment of the existence, location and severity of the CAD. The number of diseased vessels (>50% stenosis) as well as the complexity of the lesions (60) and existence of visible thrombi (61) contribute to an increased risk. Furthermore, coronary angiography is a prerequisite for the decision on interventional procedures. However, still coronary angiography is an invasive procedure with an inherent, although low, risk of adverse events with a not negligible cost. Therefore, mainly patients with a high likelihood of unstable CAD and a moderate-high risk of subsequent events, based on other predictors, are suitable for an invasive assessment.

Markers of coagulation and fibrinolysis activity

Activation of the coagulation and fibrinolytic systems, as demonstrated by elevated markers of thrombin generation, thrombin activity and fibrin turnover, have been demonstrated in the acute phase of unstable CAD and are associated with an adverse outcome (62–64). Also reduced fibrinolytic capacity has been associated with an increased risk of coronary events in community based populations (65) as well as in unstable angina (66). In addition, increased levels of PAI have been associated with an increased rate of events in survivors of MI (67). None of these hemostatic markers are however recommended for use as risk predictors in a clinical setting (68).

Inflammatory markers

Elevated markers of inflammatory activity are associated with an increased risk for future cardiovascular events in healthy individuals (69,70), as well as in patients with stable (71), and unstable CAD (72–75). In patients with unstable CAD the erythrocyte sedimentation rate as well as CRP and fibrinogen levels were found to be higher in patients developing refractory unstable angina than in patients with an uneventful clinical course (76). Elevation of IL-6 on admission for ACS seems to be associated with an increased risk of long-term mortality (77, 78) and also identifies those who derive the greatest benefit from an early invasive treatment (78).

In large epidemiological studies, an increased fibrinogen concentration has been shown to predict future coronary events (79). Also in unstable CAD, fibrinogen levels have been shown to contribute to prediction of future risk of death and/or MI in short and long term, independent of troponin elevation and ECG changes (72).

In unstable CAD, CRP elevation on hospital admission has been shown to be an independent predictor of mortality (72) (73–75). However, the association between the CRP level and the early risk of MI in unstable CAD has not been established, as most studies have presented small patient numbers and combined endpoints (table 3) (31, 71, 74, 80).

Markers of myocardial damage

Numerous previous studies have shown that troponin elevation is associated with an impaired outcome in patients with unstable CAD (81–83). It has been convincingly shown that elevation of troponin T raises the probability of significant coronary stenoses and advanced coronary artery disease i.e. three vessel disease and left main disease (84). Also, thrombus formation is more frequent in patients with troponin elevation (84). Recently it has been shown that even very low troponin levels just above the detection limit of the assay is associated with an increased risk of MI and death (84). However, few studies have had a sample size and event rate allowing the separate evaluation of the relations to death and MI separately. The raised mortality associated with troponin elevation is evident in short- as well as long-term (73) and independent of inflammatory activity, ECG changes and impaired renal function

Table 3. Published studies on the predictive value of CRP elevation in unstable coronary artery disease. In order of sample-size.

Author	Included patients	Follow-up	CRP cut-off and method	End-point	Outcome
Liuzzo et al[17] 1994	UA n=31	In hospital	>3 vs.< 3 mg/l Elisa	Death, MI, Revasc. Death, MI	2/11 vs. 19/20 0/11 vs. 6/20
Thompson et al [13] 1995	Coronary angiog. SA, n=1026 UA, n=1346 Atypical CP, n=411	2 years	NA Nephelometric	Death, MI	CRP level for group with (death 40, MI 66) vs. without events (2700): 2.15±1.96 vs. 1.61± 1.38, p=0.01. Not independent when adjusting for fibrinogen.
Havercate et al[5] 1997	Coronary angiog. SA, n=743 UA, n=1030 Atyp. CP , n=326	2 years	>3.6 vs. ≤3.6 mg/l Elisa. D.L 0.05 mg/l	Coronary event 3.54% (n=75)	Adjusted OR 1.81 (23 vs. 51 events)
Oltrona et al[11] 1997	UA N=140	Hospital stay	> 10 vs ≤10 mg/l Nephelometric. D.L 1mg/l	Death, MI, Emerg. revasc. Death MI	13(n=5) vs. 28%(n=28), p=ns 0 vs. 0% 8 (n=3) vs. 4%(n=4), p=ns
Toss et al[6] 1997	UCAD N=965	5 months	>= 10 vs. 2-10 vs. < 2 mg/l Turbidimetric	Death, MI. 14.4% (n=138) Death. 4.4%(n=42)	16.1(n=52) vs. 15.1(n=47) vs. 11.8%(n=39), p=0.26 7.5 vs. 3.6 vs. 2.2%, p=0.003
Benamer et al[10] 1998	UCAD N=195	24h	>= 6 vs. < 6 mg/l Nephelometric	Death, MI, Emerg. revasc. Death, MI MI	11.1 vs. 12.8 % p= 0.71 5.1 vs. 7.7 %, p=0.47 4.3 vs. 7.7 %, p=0.31
Rebuzzi et al[21] 1998	UA N=102	3 months	> 3 vs. ≤3 mg/l Nephelometric	MI	24 (n=13) vs. 4% (n=2), p<0.01 Independent
Morrow et al[9] 1998	UCAD n=437	14 days	>15.5 vs. ≤15.5mg/l Nephelometric. D.L 0.1mg/l	Death	5.6% (n=6) vs. 0.3 % (n=1), p=0.001 Independent
Ferreiros et al[15] 1999	UA N= 105	90 days	>15 vs. < 15 mg/l Elisa. D.L 1 mg/l	Death, MI, Refract. angina Death, MI	73.3(n=22) vs. 34.7%(n=26), p< 0.001 Independent, OR 1.9 (1.2-8.3) 46.7(n=14) vs. 20.0%(n=15), p=0.008
Verheggen et al[16] 1999	UA N=211	Hospital stay mean 4.7 days	> 6.14 vs. < 1.20 mg/l Nephelometric. D.L 0.2mg/l	Refractory angina 36% (N=76)	Adjusted O.R 2.19 (0.94-5.11).
De Winter et al[34] 1999	UCAD‡ N=150	6 months	>5 vs. < 5 mg/l Nephelometric D.L 0.2 mg/l	Death, MI, re-UCAD 10% (N=15)	23% (n=14) vs. 1.1% (n=1) p<0.0001 Independent
Heeschen et al[8] 2000	High risk UCAD, (Capture-placebo) N=447	3 days 30 days 6 months 30 days 6 months	10 vs. ≤10 mg/l Nephelometric	Death, MI Death, MI Death, MI Death (n=13) MI (n=47) Death, MI Death, MI Death (n=23) MI (n=89)	8 vs. 10.3 %, p=0.41 7.6 vs. 14.1, p=0.03 9.5 vs. 18.9%, p=0.003 1.1 vs. 5.4%, p=0.005 8.4 vs. 13.5%, p=0.16 5.3 vs. 8.1%, p=0.04 6.2 vs. 14.9%, p<0.001 O.R 3.62, p<0.001 O.R 1.92, p=0.04
	High risk UCAD, (Capture-all) N=1081				
Lindahl et al[7] 2000	UCAD N=917	37 months	≥10 vs. 2-10 vs. <2 mg/l Turbidimetric	Cardiac death 13.5% (n=124)	16.5 (n=51)vs. 7.8(n=23) vs. 5.7% (n=18), p=0.001
James et al 2003	UCAD N=7108	30 days	≤1.84 vs.1.84-3.96 vs. 3.96-9.62 vs. > 9.62 mg/L Immunometric. D.L 0.1mg/L	Death MI	2% (n=36) vs. 3.3% (n=59) vs. 3.9% (n=69) vs. 6.3% (n=111); p< 0.001 5.5%(n=99) vs. 4.7%(n=84) vs.5.2%(n=93) vs. 5.9%(n=104) p=ns

SA denotes Stable angina pectoris; UA, Unstable angina pectoris; Atypical CP, Atypical chest pain; UCAD, Unstable coronary artery disease ie. unstable angina pectoris + non-ST elevation MI, Myocardial infarction; Emerg. revasc., Emergency revascularization; Refract. angina, Refractory angina. D.L, Detection Limit.

(85). Identification of patients with troponin elevation is also useful for targeting therapy with low-molecular weight heparin (86), glycoprotein IIb/IIIa inhibitors (87, 88) and early revascularisation (89) as these patients derive a particular benefit from these treatment modalities.

Markers of myocardial dysfunction

It is well recognised that elevation of BNP indicates a worse prognosis in patients with heart failure and after MI (48). Recently it has been shown that elevation of BNP as well as NT-proBNP levels obtained after the acute phase (median time 40 hours) in patients with a broad range of ACS, independently predicts mortality (90, 91). On multivariate analyses, BNP levels have not been associated with subsequent MI (91). However, the relationship between levels of BNP or NT-proBNP and other risk-markers in the assessment of risk in ACS patients has not been fully elucidated.

Renal dysfunction

Renal insufficiency is associated with a worse prognosis in a wide spectrum of patients with cardiovascular disease, including ACS (92–94). A part of the increased risk associated with reduced renal function is attributable to a large number of co-existing conditions such as age, diabetes and hypertension. Still however, renal failure itself has consistently been shown to be associated with a worse prognosis(95).

It has been proposed that renal dysfunction is a measure of the extent of vascular damage caused by a variety of insults on the endothelium. In many previous studies, patients with renal insufficiency have been excluded. Hence, the prognostic value of cardiac troponin and BNP in patients with and without renal dysfunction presenting with suspected acute coronary syndromes have not been defined.

Risk scores and combinations of risk markers

As several clinical, ECG and biochemical factors have been identified as independent markers of increased risk of subsequent cardiac events, the combinations of different markers have been evaluated. Several risk-scores have been developed from large clinical trials of ACS patients, by adding different indicators after weighing their relative importance (96). Thus, by combining BNP, CRP and troponin I, there was a near doubling of the mortality for each additional bio-marker that was elevated in a recent study of patients with ACS (97). The higher value of the risk-score, the more benefit from early interventional treatment is derived for ACS-patients according to the recently presented score from the FRISC II trial (98).

Before the present study was performed, neither the relations between levels of NT-proBNP and levels of markers of myocardial damage, inflammation and clinical risk factors nor the combination these markers for risk assessment had been elucidated.

(Therapies marked with bold text were evaluated in the study)
The aims of treatment in patients with unstable CAD are symptom relief, limitation of myocardial damage and prevention of future coronary events i.e. myocardial infarction and death. Anti-ischemic drugs reduce oxygen utilisation by decreasing heart rate, contractility and blood pressure and inducing vasodilatation. β-blocking agents exert their effects by inhibiting β-receptors in the myocardium and thereby decreasing oxygen consumption. Treatment of ACS patients with β-blockers is associated with a 13 % reduction in the progression to acute myocardial infarction (99). Nitrates and calcium channel blockers are vasodilating agents relieving pain but not convincingly shown to reduce coronary events (100).

Platelet inhibition

As platelet aggregation plays a central role in the pathogenesis of ACS, antiplatelet regiments are essential to reduce thrombus formation and peripheral embolization of thrombotic material in the coronary arteries.

Aspirin

Aspirin irreversibly inhibits cyclooxygenase, impairing prostacyclin metabolism and thromboxane A_2 synthesis predominantly in platelets. As a result, platelet activation and aggregation in response to collagen, ADP and thrombin is inhibited. However, at higher concentrations, thrombin and ADP still can activate platelets in the absence of thromboxane A_2.

A large number of trials have shown that aspirin reduces death and MI in patients in unstable angina, even in low doses (101). A loading dose of 300 mg is therefore recommended in all patients with ACS, followed by long term treatment (68).

ADP receptor inhibitors

Inhibition of the ADP receptor of the platelets results in decreased platelet aggregation. In a large clinical trial including patients with unstable angina confirmed by ECG changes or elevation of biochemical markers, Clopidogrel on top of aspirin was found to reduce the incidence of cardiac events significantly during a median follow-up of 9 months (102). Particularly, the incidence of MI was reduced. Furthermore, treatment with Clopidogrel in combination with aspirin is considered a routine treatment at least one month after implantation of a coronary stent due to its superior effect in reducing acute stent-thromboses (103).

Glycoprotein IIb/IIIa inhibitors

Activation of the GP IIb/IIIa receptor is considered the final common pathway in platelet aggregation. Blocking this receptor almost completely abolishes aggregation of platelets. **Abciximab** is the Fab fragment of a monoclonal antibody 7E3, binding with strong affinity to the GP IIb/IIIa receptor. In contrast to other GP

IIb/IIIa inhibitors, abciximab has a long half-life and binds to the vitronectin ($\alpha_v\beta_3$) and other receptors. By interaction with these receptors, abciximab has been suggested to influence also systems of coagulation and inflammation.

Glycoprotein IIb/IIIa receptor inhibitors have consistently been shown to reduce the rate of procedure related myocardial infarctions in patients undergoing percutaneous interventions in a large number of trials (104–108). Long term mortality is reduced in patients treated with abciximab and heparin in conjunction with coronary stenting as compared to patients on sole heparin treatment (109). Furthermore it has been shown that GP IIb/IIIa inhibitors, on top of aspirin and heparin, have reduced the rate of death and myocardial infarctions in special patient settings with unstable angina and non q-wave myocardial infarctions (110–113). The benefit has been shown to be most pronounced in high-risk patients with elevated troponin levels (87, 88) and in patients treated with early coronary interventions (52, 114). Before the performance of the GUSTO-IV trial, which constitutes the basis of the present study, no GP IIb/IIIa inhibitor had been evaluated in a pure non-interventional setting. However, in the CAPTURE trial the administration of abciximab to high-risk ACS patients for 18 to 24 hours before percutaneous intervention reduced the pre-intervention incidence of MI by 70% (relative risk reduction; 2.1% to 0.6 %, P=0.029) (105).

Several oral GP IIb/IIIa inhibitors have been tested in large trials without any evidence of benefit. In fact, the therapies have been associated with an increased incidence of bleedings and a modest increase in mortality (115), illustrating the potential risk with this type of treatment.

Coagulation inhibitors

Unfractionated heparin (UFH) is a heterogeneous mixture of sulphated polysaccarides of varying chain length increasing the effects of antithrombin, which most important way of action is inhibition of thrombin. UFH is effective in reducing the incidence of myocardial events in aspirin treated patients with unstable angina, although the number of clinical trials, and the number of patients included in those trials are relatively few (116). Nevertheless, in an international perspective, UFH treatment is regarded standard treatment in patients with unstable CAD.

Low-molecular weight heparins (LMWH) are fragments of unfractionated heparin that possess a greater anti-Xa activity in relation to anti-IIa (anti-thrombin) than UFH. In addition, LMWH, have several potential advantages over UFH (117, 118). The dose response is more predictable and reliable, the immunogenicity is reduced with less frequent thrombocytopenia, and finally there is less rebound effect after discontinuation of therapy. Other advantages from a practical point of view are; longer biological half-life enabling easier administration with subcutaneous injections and less need for monitoring the anticoagulant effect. There is convincing evidence that LMWH is more effective than placebo in reducing cardiac events (119). Furthermore, LMWH has been shown to be at least as effective as UFH in short-term. **Dalteparin** was shown to be similar to UFH (120), while

Enoxaparin was significantly more effective than UFH in the reduction of the cardiac events in large trials (121, 122).

The direct thrombin inhibitor Hirudin has been evaluated in two large scale trials and found to be slightly more effective than heparin in the short-term, without long-lasting effects. In spite of treatment with a combination of aspirin and short term LMWH, there is still a 15–25% risk for recurrent ischemic events the first month after the ACS (119) (121, 122). Thus, there is a compelling need for improvement of long-lasting treatment effects.

Complications due to antiplatelet and anticoagulation regimens

Thrombocytopenia may occur as a result of treatment with UFH as well as GP IIb/IIIa inhibitors. However, the incidence is fairly low and the condition is reversible upon discontinuation of the drug infusion. Mild thrombocytopenia (<100.000/ml) has been reported in 2.5–5.6 % and severe (<50.000/ml) in 0.9–1.6% among abciximab and heparin treated patients (123). With "small molecule" GpIIb/IIIa inhibitors, somewhat lower incidence numbers have been reported (124) (125).

Combining the anti-platelet properties of a GP IIb/IIIa inhibitor with the anticoagulant effect of a LMWH have theoretical advantages which may offer clinical improvements in the medical treatment of patients with ACS. The risk of bleedings may however outweigh the potential treatment benefits. Thus, close monitoring and reporting side effects of new pharmacological agents or combination of agents are therefore important. Acute coronary syndrome trials with GP IIb/IIIa inhibitors in addition to UFH have reported major bleeding levels around 1.4 to 10.8% and minor bleedings in up to 13.1% (126). The intervention rates and definitions for major and minor bleedings have however varied. The safety of abciximab and enoxaparin as well as tirofiban and enoxaparin has previously been evaluated in relatively small series and seemed to be well tolerated with low and similar bleeding rates (127, 128).

Coronary revascularisation

The difference between a treatment strategy including early coronary angiography and revascularisation if suitable, as compared to a conservative medical strategy and revascularisation only in case of recurrent ischemia or ischemia at a pre-discharge exercise test has been evaluated in a number of clinical trials. Since the planning of the GUSTO-IV trial, convincing evidence has emerged that an early invasive strategy is superior for patients with non-ST elevation ACS. In the FRISC II trial the composite primary endpoint of death or MI at 6 months was reduced significantly from 12.1% to 9.4% (2). At one-year follow-up there was a significant reduction in mortality in favour of the invasive strategy (129). The results were confirmed in the Tactics trial, in which the GP IIb/IIIa inhibitor tirofiban was used as adjunctive treatment in the invasive group (130). Also with enoxaparin as adjunc-

tive treatment, the primary composite endpoint was reduced with an interventional strategy in the RITA-III trial (131).

Depending on the extent and characteristics of the coronary lesions, as identified by coronary angiography, revascularisation may be carried out by either percutaneous coronary intervention (PCI) or coronary artery bypass grafting (CABG). In patients with left main disease or three-vessel disease, particularly in combination with left ventricular dysfunction, CABG is the treatment of choice by virtue of the well-documented reduction in mortality (132, 133). Due to a rapid progression in technology, including an increased utilisation of coronary stents and potent platelet inhibitors, PCI is however becoming increasingly used as the first line treatment alternative. In patients with one or two vessel disease the choice of PCI is indisputable but also in patients with three-vessel disease the results appear to be as good as with surgery (134). However, in the present study (GUSTO IV) a primary medical treatment was evaluated and early coronary angiography was discouraged.

AIMS

The aims were to investigate a large cohort of patients with non-ST elevation acute coronary syndrome regarding:

- Safety and efficacy of abciximab combined with dalteparin or unfractionated heparin.
- Activation of inflammation, coagulation and fibrinolysis and the influence of abciximab when added to dalteparin.
- The activation of inflammation and signs of myocardial damage for the separate prediction of death and myocardial infarction.
- Signs of myocardial dysfunction for the separate prediction of mortality and subsequent myocardial infarction.

MATERIALS AND METHODS

Patients

The Global Utilisation of Strategies To open occluded arteries-IV (GUSTO-IV) trial included 7800 patients with acute coronary syndromes between 1999 and 2000 (135) (figure 4) for evaluation of abciximab treatment without early revascularisation. The primary endpoint in the trial was the occurrence, of death (of any cause), or myocardial infarction (MI), within 30 days after randomisation.

In Scandinavia, Switzerland and selected sites in the US., 72 centres recruited all their patients (n=974) to the dalteparin substudy, referred to as the dalteparin cohort (figure 4). The non-dalteparin patients recruited in all other centres of the GUSTO IV trial (n=6826) are referred to as the UFH cohort. The primary objective of this substudy was to evaluate the rate of side-effects, during the initial seven days in

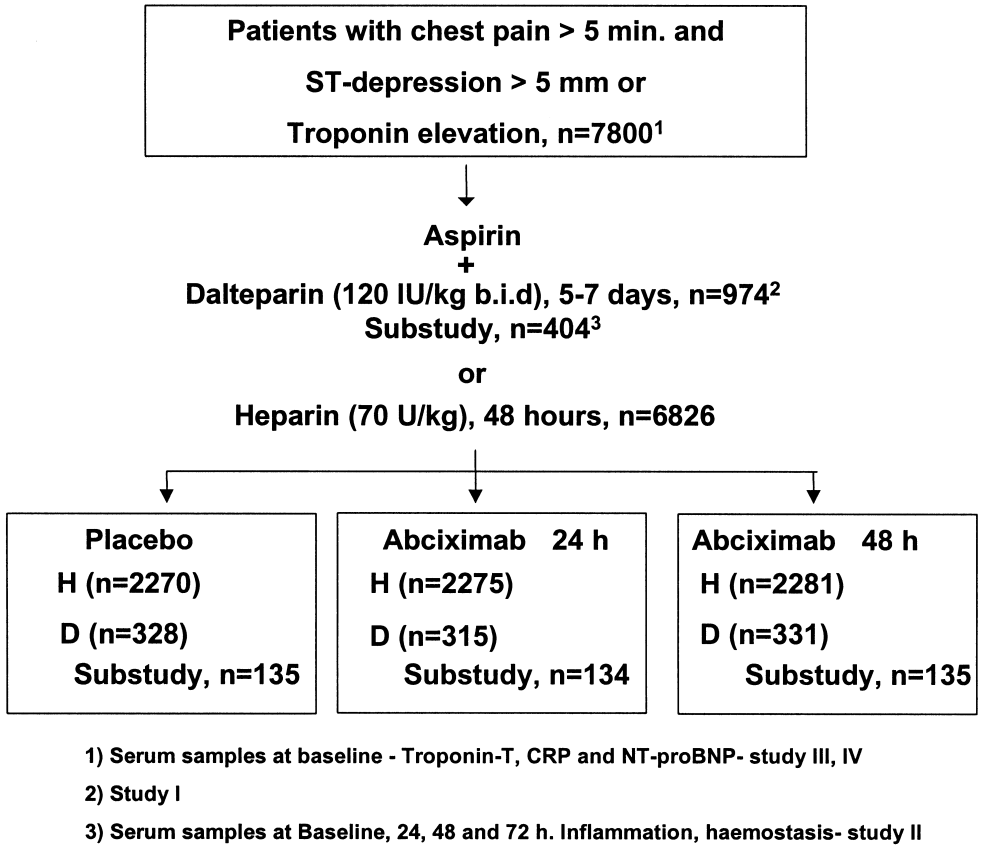


Fig. 4. Study design of the main GUSTO IV-ACS trial with the sub-studies included in the thesis indicated.

relation to the randomised abciximab or placebo treatment *within* the dalteparin cohort (randomised comparison) as well as *between* the dalteparin and UFH cohorts (non-randomised comparison).

A special blood-sampling program for repeated analyses of inflammation and coagulation factors performed at 28 selected Swedish sites, enrolling 404 patients, which constituted 74.3% of the patients recruited in Sweden and 42% of the patients in the dalteparin cohort.

Study Design

Inclusion

Patients with an acute coronary syndrome without persistent ST segment elevation were recruited. Eligible patients were B 21 years of age with one or more episodes of angina lasting B 5 min within the last 24 hours if they had either a positive cardiac troponin T or I test or B 0.5mm of transient or persistent ST-segment depres-

sion. Patients with a history of acute myocardial infarction distinct from the qualifying event within 10 days prior to enrolment were required to have new ST-segment depression and CK-MB levels below the upper limit of normal.

Exclusion

Exclusion criteria were myocardial ischemia precipitated by a condition other than atherosclerotic coronary artery disease, persistent ST-segment elevation myocardial infarction or new left bundle branch block, percutaneous coronary intervention (PCI) within previous 14 days, planned PCI or coronary bypass surgery within 30 days after enrolment, confirmed hypertension (systolic >180 mmHg or diastolic >100 mmHg), coexistent condition associated with limited life expectancy and a number of factors associated with an increased bleeding risk (135).

Randomised and concomitant treatment

The study was conducted in a double-blind fashion with patients randomly assigned to; abciximab therapy for 24 h (0.25 mg/kg bolus followed by a 0.125 ug/kg/min infusion up to a maximum of 10 ug/min for 24 h) followed by 24 h of placebo infusion, or abciximab therapy for 48 h (same bolus and infusion for total duration of 48 h), or matching placebo (bolus and 48 h infusion). All patients received 150–325 mg of aspirin as soon as possible after randomisation for long term treatment. The patients received a 70 U/kg unfractionated heparin (UFH) bolus (to a maximum of 5,000 U) followed by a continuous infusion of 10 U/kg/hour (to a maximum of 800 U/hour) titrated to maintain the aPTT between 50 and 70 seconds for 48 hours or dalteparin (120 IU/kg to a maximum of 10,000 IU) subcutaneously every 12 hours for 5–7 d or until a revascularisation procedure or discharge. Concomitant therapy with beta-blockers was strongly recommended. Use of all other cardiac medications was left to the discretion of the investigator.

Coronary angiography was discouraged during or within 12 hours after the completion of study agent infusion unless the patient had recurrent or continuing ischemia at rest associated with ischemic ST-T changes that was unresponsive to intensive medical therapy. If percutaneous coronary intervention was required during study drug administration, blinded crossover to active therapy with abciximab was provided.

Definitions of clinical endpoints

All patients had ECG examinations at baseline, 48 hours and at 30 days, as well as blood samples collected at baseline and 8, 16, 24, 36 and 48h after randomisation to be analysed in a central laboratory for CK-MB. Additional samples were to be collected at 0, 8, 16, and 24h after any ischemic episode lasting >20 minutes. MI was defined as either a new significant Q-wave (B 0.04 s or at least a quarter of the R-wave amplitude in two or more contiguous leads) or CK-MB B 3 times upper limit of normal. For patients with CK-MB elevation at entry, a new episode of chest-pain in combination with a new CK-MB elevation was required for MI diagnosis the initial 7 days as presented in detail in the GUSTO IV-ACS publication (135). Follow-

ing coronary by pass surgery a new significant Q-wave was the only criterion. A clinical endpoint committee blinded to treatment assignment adjudicated all suspected cases of myocardial infarction. During 30-days of follow-up, mortality and rate of adjudicated myocardial infarctions were recorded. At 12 months only all-cause mortality was collected.

Bleeding classification

Bleeding was classified as major, minor or insignificant by the TIMI criteria (136). Major bleeding during baseline hospitalisation was defined as either 1) intracranial haemorrhage or 2) bleeding associated with a haemoglobin drop greater than 50 g/L. Minor bleeding was defined as any of the following: 1) spontaneous gross hematuria or hematemesis; 2) observed blood loss with decrease of haemoglobin >3 but A50 g/L. Insignificant bleeding was defined as any bleeding not meeting the criteria for major or minor.

Statistical methods

According to the protocol the outcome analyses were made with the abciximab 24 hours and 48 hours treatment groups combined. The safety data were analysed for the abciximab groups separately but since there was no major difference between the abciximab groups these results were also presented with the groups combined. Comparisons were then made between the dalteparin and UFH cohorts. Comparisons between groups were made with Chi^2-test. Fischer's exact test was used for groups with small numbers. For continuous skewed variables a non-parametric, Mann-Whitney, test was performed. Differences between groups were presented as odds ratios O.R. with 95% confidence interval.

Differences in levels of biochemical markers were evaluated with non-parametric tests given that they were not normally distributed. Bivariate correlations were calculated with Spearman rank correlation coefficients. Significance tests of between-group comparisons were made with Kruskal-Wallis or Mann-Whitney tests and of within-group comparisons between different time-points with Wilcoxon signed rank test. Also, the change in median levels from the previous sample for the randomised treatment groups was evaluated with Kruskal-Wallis test.

The material was divided into quartiles on the basis of levels of biochemical markers. Means were expressed with one standard deviation for continuous variables and medians were shown with 25–75 percentiles for skewed variables. Differences in categorical base-line variables between quartiles were evaluated with Chi2 tests for trend. Differences between mean or median values for continuous variables were evaluated with one way ANOVA or Kruskal-Wallis tests as appropriate. Skewed variables were log transformed for calculation of independent associations between the variables in a multiple linear regression analysis. Kaplan-Meyer estimates were used for evaluation of the occurrence of the individual endpoints death and myocardial infarction after enrolment. Logistic regression analyses were per-

formed for evaluation of the significance of predictors of MI at 30 days and mortality at 30 days and one-year. Included variables are specified in detail in the respective paper.

Laboratory methods

Venous blood samples were drawn from direct punctures at randomisation, 24, 48 and 72 hours. The blood was collected into vacutainer tubes containing sodium citrate (0.13 M) or EDTA. Plasma was prepared within 30 minutes of collection by centrifugation at 2000 × *g* at room temperature for 20 minutes. After centrifugation serum was frozen at –20˚C in aliquots and sent for central laboratory analyses of CK-MB levels. One aliquot of the serum samples at baseline was stored at –70˚C and sent in batches of 500 to the Department of Clinical Chemistry, University Hospital, Uppsala, Sweden for analyses of troponin T, C-reactive protein (CRP) and NT-proBNP. One batch was unfortunately lost during transportation. At selected Swedish sites all patients were recruited to a special blood-sampling program for analyses of inflammation and coagulation factors from serum-samples obtained at baseline, 24 hours, 48 hours and 72 hours.

Troponin T levels were determined by a third generation assay on an Elecsys (Roche diagnostics) with the detection limit 0.01ug/L and a total coefficient of variance, CV of 8% at 0.05 ug/L and 4.1 to 6.0% between 0.1 to 11 ug/L.

CRP concentrations were measured with a high-sensitive chemiluminescent enzyme-labelled immunometric assay (Immulite CRP, Diagnostic Products Corporation) with a detection limit of 0.1 mg/L. and a total CV of 5.6% at 2 mg/L and 5% at 10 mg/L.

Plasma NT-proBNP was determined with a sandwich immunoassay on an Elecsys 2010. The analytical range extends from 20 to 35.000 ng/L. At the central lab, the total CV was 3.3% (n=21) at a level of 209 ng/L and 3.0% (n=21) at a level of 7431 ng/L. In a healthy population (n=407) matched to the FRISC II population (2, 3) for age (range 40–75 years) and gender (32% females) the 97.5 percentile was 290 ng/L.

Serum creatinine at baseline was analysed at the local laboratories for 7706 patients. The creatinine clearance rate was calculated with the Cockcroft and Gault equation with correction for gender: {(140-age) × (weight (kg)} / Serum creatinine (umol/L) (137).

Measurements of plasma levels of **Il-6, thrombin-antithrombin (TAT)** complexes and **tissue Plasminogen-activator (tPA)** antigen were performed using commercial enzyme-linked immunosorbent assay (Elisa) kits (Quantikine from R&D systems, Abingdon, UK, Enzygnost TAT, Behring Diagnostics, Marburg, Germany and Imulyse tPA, Biopool, Umeå, Sweden). **Soluble fibrin (SF)** was analysed using a quantitative spectrophotometric assay (Berichrom FM from Behring Diagnostics, Marburg, Germany). **Plasminogen-activator-inhibitor (PAI-1)** activity was determined by Chromolize PAI-1 (Biopool, Umeå, Sweden).

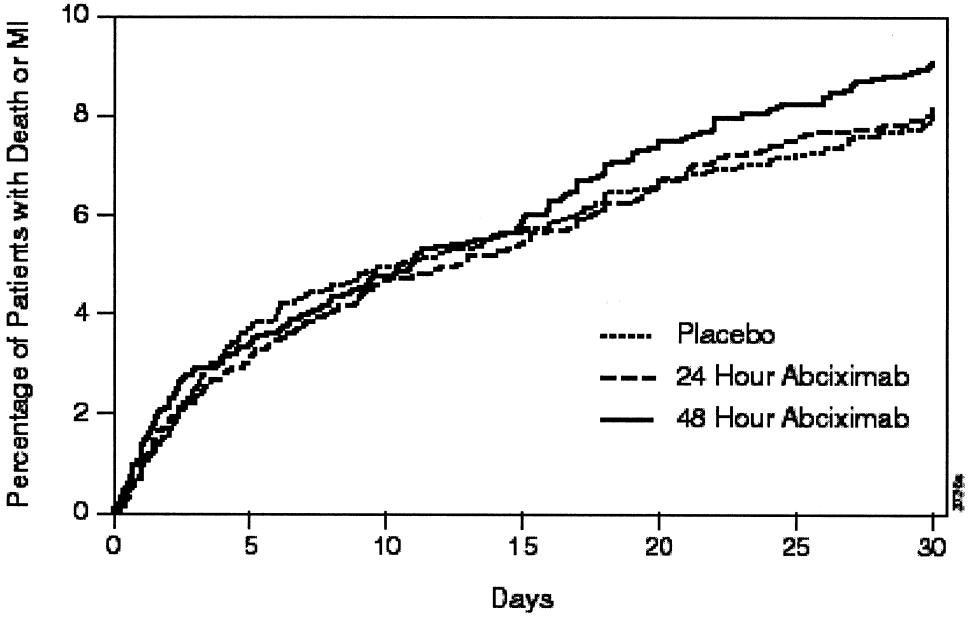


Fig. 5. Kaplan-Meyer analysis regarding the primary outcome (death or myocardial infarction) in the main GUSTO-IV trial during the initial 30-days.

RESULTS

The main GUSTO-IV trial

Baseline characteristics

As planned, GUSTO-IV-ACS enrolled 7800 patients between July 21, 1998 and April 21, 2000, in 458 hospitals in 24 countries. Western European sites enrolled 48% of the patients, Eastern Europe 31% and North America 14%. The mean age was 65 years (SD 11) and mean weight 77 kg (SD 14) and 62% were males. Most patients had a history of coronary artery disease, including myocardial infarction (31%), and previous revascularisation (16%). All baseline characteristics were balanced among the three treatment groups. At enrolment, 59% tested positive on a qualitative or quantitative assay for cardiac troponin T or I, 80% had ST-segment depression and 32% had both ST depression and an abnormal cardiac troponin.

Primary and secondary endpoints

The combined primary endpoint of death and MI at 30 days' follow-up was similar among the three treatment groups: 8.0% with placebo, 8.2% with abciximab 24 hours and 9.1% with abciximab 48 hours (figure 5). Moreover, no significant differences were apparent among the three treatment groups at any time point, 48 hours, 7 days and 30 days. The components of the combined endpoint were also evenly distributed among the three treatment groups. Within 30 days after enrolment coro-

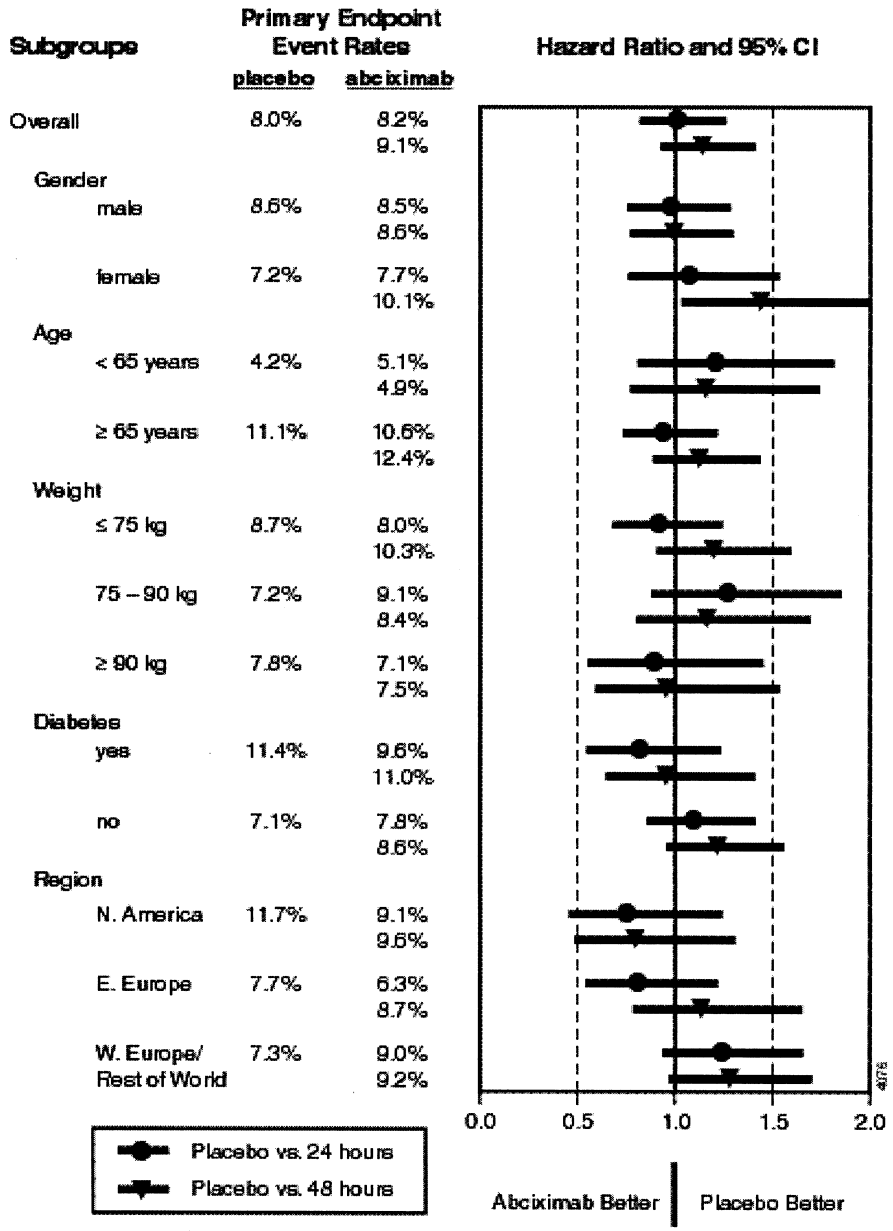


Fig. 6. Primary endpoint events (death or myocardial infarction at 30-days) in pre-specified subgroups of the main GUSTO-IV trial.

nary revascularisation was performed in 30% of patients: percutaneous coronary intervention (PCI) 19% and bypass surgery 11%. Yet, only few patients (2,5%) underwent revascularisation within 48 hours, while on study treatment (PCI 1.6%, bypass surgery 0.9%).

As might be expected, events (death and myocardial infarction) were more frequent among patients with high-risk characteristics, such as advanced age, ST-segment depression, elevated cardiac troponin or diabetes mellitus than among those without such characteristics. However, no significant treatment effect of abciximab was apparent in any of these subgroups (figure 6).

The dalteparin substudy of GUSTO-IV

Baseline characteristics

The randomised treatment groups were well matched regarding all baseline characteristics. However, as compared to the 6826 patients in the UFH treated cohort, the 974 patients in the dalteparin cohort constituted a higher risk population as indicated by higher age and weight, more prior by-pass surgery, more often an evolving myocardial infarction and a positive troponin test at baseline.

Safety

Major bleedings (not related to coronary artery by-pass surgery) were rare in all groups, although numerically doubled by abciximab treatment in both the UFH and

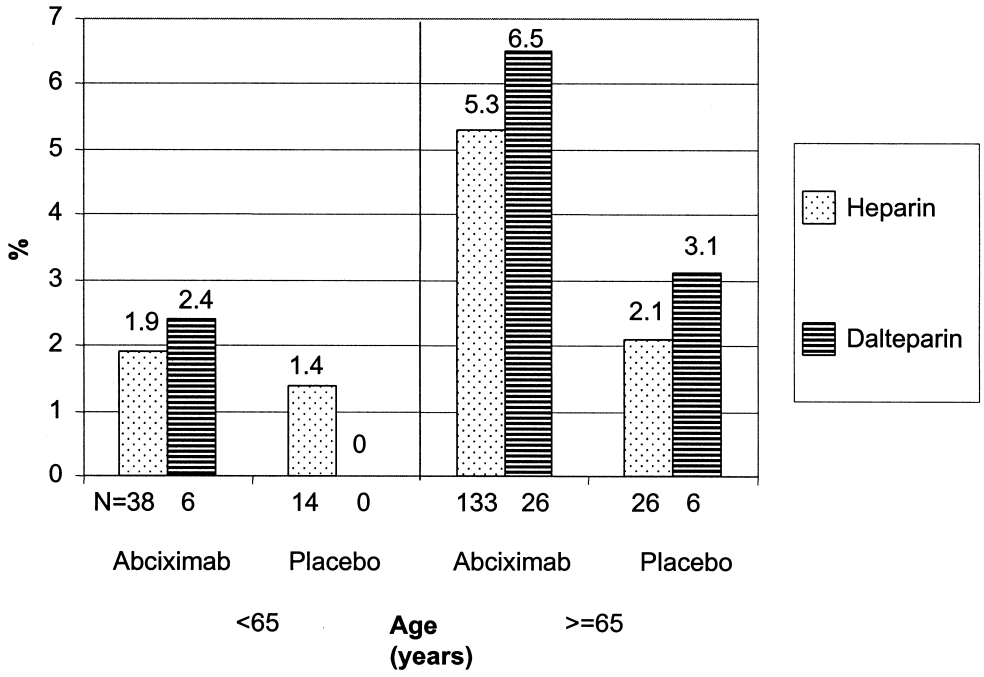


Fig. 8. Result of the primary outcome analysis of the dalteparin substudy of the GUSTO-IV for subgroups based on age. Major and minor bleedings presented for the abciximab and placebo groups in the unfractionated heparin and dalteparin cohorts.

Table 1. Safety at 7 days. Comparisons between placebo, abciximab (24 hours + 48 hours) and between dalteparin and heparin treated cohorts.

	Dalteparin		Heparin	
	Placebo N=328 %(N)	Abciximab N=646 %(N)	Placebo N=2270 %(N)	Abciximab N=4556 %(N)
Stroke	0 (0)	0.6 (4)	0.3 (7)	0.3 (14)
Intracr. haemorrhage‡	0 (0)	0.2 (1)	0 (0)	0.1 (4)
Major bleeding§	0.6 (2)	1.2 (8)	0.2 (5)	0.7 (34)**
Minor bleeding§	1.2 (4)	3.7 (24)*	1.5 (35)	3.0 (137)***
Insign. bleeding§	24.7 (81)†††	46.4 (300)***, †††	5.0 (113)	15.1 (690)***
Blood transfusion	4.9 (16)†††	4.3 (28)	1.9 (44)	3.0 (136)*
Platelet count				
<100.000/ml	1.5 (5)	3.1 (20)†††	0.9 (21)	6.3 (285)***
<50.000/ml	0.0 (0)	0.8 (5)	0 (1)	1.6 (73)***
Platelet transfusion	0.6 (2)	1.7 (11)	0.1 (3)	1.2 (53)***

*=p<0.05, **=p<0.01, ***=p<0.001 Abciximab versus placebo. † p<0.05, ††† p<0.001. Dalteparin versus heparin. ‡ Intracranial haemorrhage, § Bleedings according to TIMI-criteria, not related to coronary artery by-pass surgery.

dalteparin cohorts (table 1). Minor bleedings occurred at similar low rates in the dalteparin and the UFH groups but were also doubled by abciximab treatment in both cohorts (table 1). In the dalteparin cohort, major and minor bleedings occurred in 5.0% in the abciximab group as compared to 1.8% in the placebo group (O.R. 2.71; 1.14–6.41). In the UFH cohort the difference between the abciximab and placebo groups were similar (3.8% vs. 1.8%: O.R. 2.17; 1.52–2.99). Particularly elderly (> 65 years) patients had an increased risk of major and minor bleedings with abciximab (figure 8).

Stroke (any type) was an uncommon event in all groups in the trial (table 1). The highest stroke rate was found in the abciximab group (0.6%) of the dalteparin cohort, not statistically different from the placebo group (0%). The UFH treated patients experienced similar stroke rates (0.3% for both abciximab and placebo). Intracranial haemorrhage occurred only in one patient in the dalteparin cohort receiving abciximab 24 hours.

Thrombocytopenia was more common with abciximab than placebo in the whole patient population. Severe thrombocytopenia (<100.000/ml) occurred only with abciximab, both in the dalteparin- (0.8% vs. 0.0%, n.s.) and in the UFH cohort (1.6% vs. 0.0%, n.s.). Moderate thrombocytopenia (<50.000/ml) was also more frequent with abciximab than placebo both in the dalteparin (3.1% vs. 1.5%, ns) and in the UFH cohort (6.3% vs. 0.9%, p<0.001). Thus, during abciximab treatment the risk of thrombocytopenia was halved in the dalteparin as compared to the UFH cohort (p<0.01).

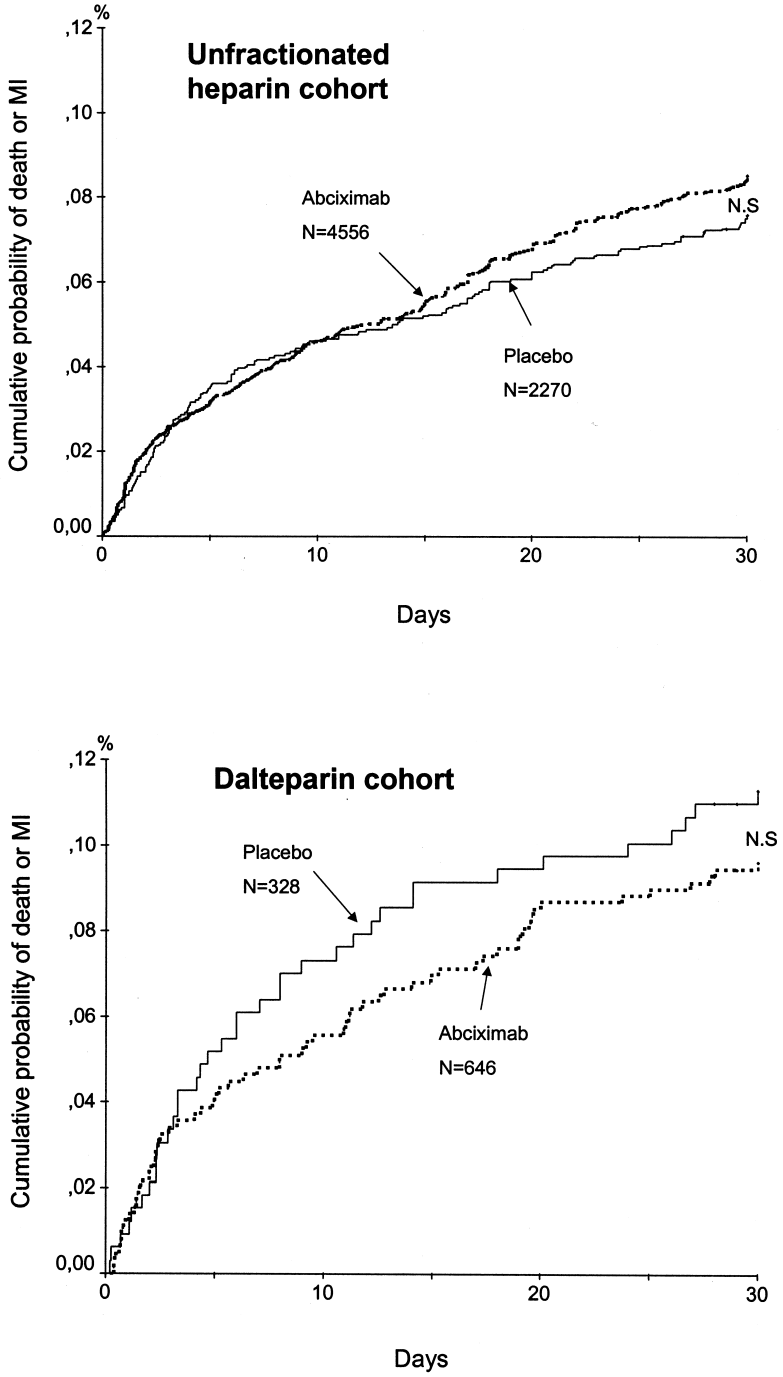


Fig. 7. Results of the secondary endpoint, the outcome analysis, in the dalteparin substudy of the GUSTO-IV depicted in Kaplan-Meyer curves for the unfractionated heparin and dalteparin cohorts respectively.

Efficacy

The secondary endpoint, outcome at 30 days, did not differ significantly between placebo and abciximab in the dalteparin substudy. In the dalteparin cohort the rate of death or MI in the abciximab groups vs. placebo was 9.6% vs. 11.3% (O.R. 0.85; 0.58–1.25) while in the UFH cohort the corresponding event rates were 8.5 % vs. 7.6 % (O.R. 1.12; 0.95–1.34) (figure 7). Thus, there was a trend for a higher event rate in the dalteparin cohort than in the UFH cohort (O.R. 1.27; 1.01–1.58). However, when correcting for other known predictors of an adverse outcome in a forward stepwise multiple regression analysis the dalteparin treatment did not remain an independent predictor of death or MI at 30 days.

Activation of coagulation, fibrinolysis and inflammation in relation to abciximab treatment

Coagulation

Levels of coagulation markers, TAT and SF, increased in the acute phase. Median TAT level increased significantly from baseline (3.1 ug/L) to 24 hours and stayed elevated at the same level at 48 hours (3.7 ug/L). At 72 hours the median level had decreased again and was not different from baseline. Median SF level also increased from baseline (20.0 ng/L) to 24 hours and remained elevated until 72 hours (23 nmol/L). At all time-points the median levels of the coagulation markers were similar in the three different randomised treatment groups. Neither the absolute nor the relative changes in median levels from previous sample differed between the groups at any time-point.

Fibrinolysis

The median tPA level showed a continuous rise from baseline (8.7 ug/L) until the last sample at 72 hours (17.5 ug/L). In contrast, the PAI-1 levels remained unchanged during the 72 hours sampling period. Neither the median levels at different time-points, nor the changes within the groups differed significantly between the randomised treatment groups.

Inflammation

The median level of Il-6 increased from baseline (5.4 ng/L) and reached its peak median level at 24 hours (7.8 ng/L). The median level decreased thereafter and was at 72 hours no longer statistically significant different from baseline. Median CRP level increased similarly from baseline (4.4 mg/L) to 24 hours (8.7 mg/L) but in contrast to Il-6, the median level remained significantly elevated until 72 hours. The median level of fibrinogen increased continuously from baseline (3.3 g/L) until the last sample at 72 hour (3.9 g/L). There were no differences in median levels or changes of levels of the inflammatory markers between the randomised treatment groups.

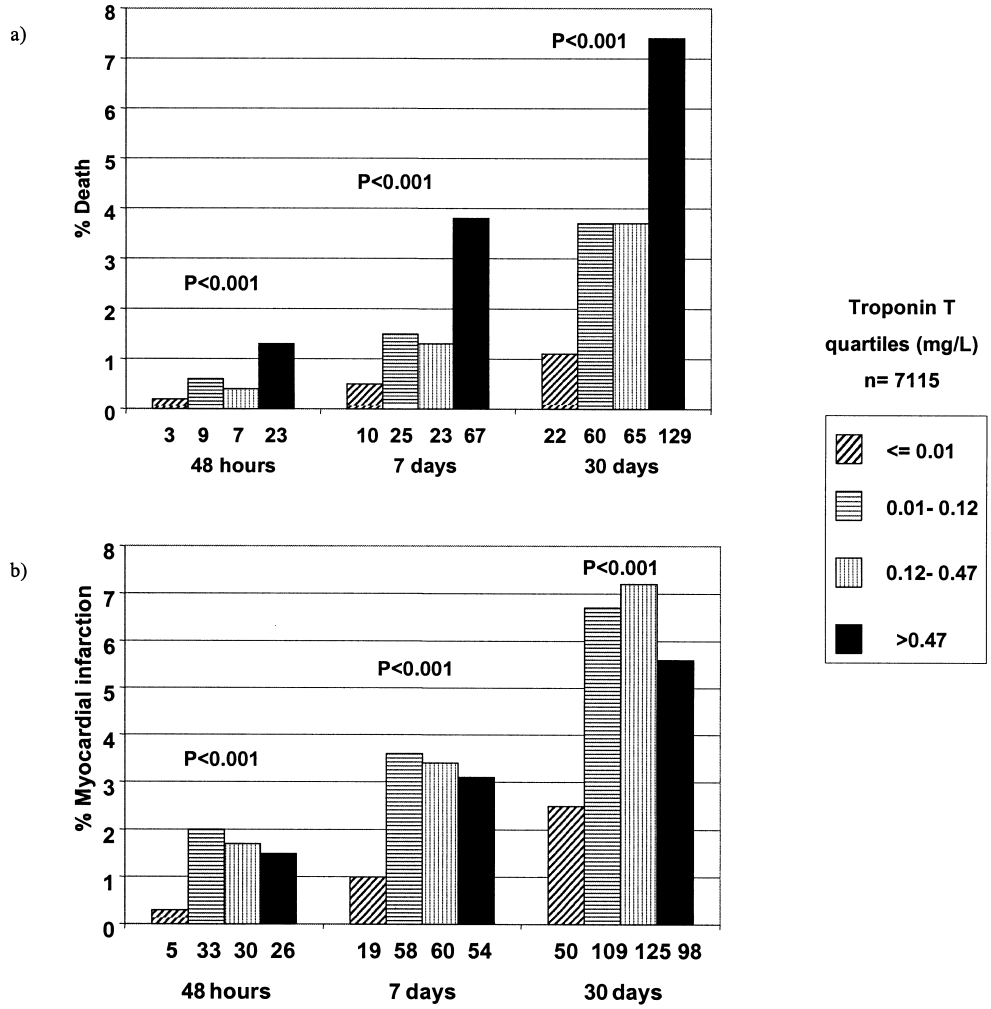


Fig. 9. Rate of a) death and b) MI at 48 h, 7 days and 30 days in relation to quartiles of Troponin T. The number of patients with events is noted under the bars.

Biochemical markers for prediction of coronary events

Markers of myocardial damage

The median time from the onset of the qualifying episode of ischemic chest pain to randomisation was 9.5 (5.0–16.6) hours. Troponin T analyses were available from 7115 (91.2%) of the patients. The troponin T-levels ranged from 0 to 17.3 ug/l and the quartile limits were 0.01, 0.12 and 0.47 ug/L. The rate of the primary combined endpoint of the GUSTO IV-ACS study, death or MI, was increasing with higher troponin T quartiles at all time-points of follow-up ($p<0.001$). Also mortality was markedly increasing with increased troponin T quartiles from 1.1% to 7.4% between the first to the fourth quartile at 30 days (figure 9 a). The rate of MI was

Table 4. Multiple logistic regression analyses on Troponin T and CRP as predictors of death, MI and death or MI at 30 days.

	Death		MI		Death or MI	
	O.R (95% C.I.)	P	O.R (95% C.I.)	P	O.R (95% C.I.)	P
Model 1						
TnT quartiles*	1.63 (1.43–1.87)	<0.001	1.23 (1.11–1.37)	<0.001	1.39 (1.27–1.52)	<0.001
CRP quartiles†	1.19 (1.05–1.35)	0.006	0.94 (0.85–1.04)	0.26	1.00 (0.92–1.09)	0.91
Model 2						
TnT (ug/l)						
>0.01 vs. A0.01‡	3.36 (2.10–5.34)	<0.001	2.48 (1.79–3.42)	<0.001	2.84 (2.14–3.76)	<0.001
CRP (mg/l)						
>1.84 vs. A1.84‡	1.72 (1.17–2.55)	0.009	0.76 (0.59–0.98)	0.033	0.93 (0.75–1.16)	0.53
Model 3						
TnT (ug/l)						
>0.47 vs. A0.47‡	2.47 (1.86–3.28)	<0.001	1.22 (0.79–1.32)	0.89	1.51 (1.23–1.86)	<0.001
CRP (mg/l)						
>9.62 vs. A9.62‡	1.31 (0.98–1.74)	0.07	0.98 (0.76–1.25)	0.85	1.07 (0.87–1.31)	0.55

O.R = Odds ratio, C.I. = confidence interval, MI = myocardial infarction. All three models included: age, male gender, body-weight, smoking, previous angina, stroke, heart failure, diabetes mellitus, hypertension, hypercholesterolemia, previous revascularization, previous myocardial infarction, current treatment with β-blockers and ACE-inhibitors, aspirin treatment prior to inclusion, ST-depression B0.5 mm and randomized treatment (abciximab 24 hours, 48 hours or placebo).

increasing from the first- to the second quartile and was at 30 days 2.5% vs. 6.7%. However, no difference was observed between the upper three quartiles (figure 9 b). There was even a trend to a lower rate of MI in the fourth quartile as compared to the third quartile (5.6% vs. 7.2%, p=0.055). In a multiple logistic regression analysis, increasing troponin T quartile was independently related to both death (O.R. 1.63; 1.43–1.87) and MI (O.R.1.23; 1.11–1.37) at 30 days (table 4). Any elevation of troponin T (> 0.01 ug/L vs. A 0.01) however, provided a larger differentiation of high and low risk regarding both death and MI (O.R. 3.36;2.10–5.34 and 2.48; 1.79–3.42 respectively)

Markers of inflammation

CRP analyses obtained at baseline were available from 7108 (91.1%) patients. The range of CRP-levels was 0 to 489 mg/L with quartile limits 1.84, 3.96 and 9.62 mg/L. The rate of the primary combined endpoint, death or MI was significantly increasing with higher CRP quartiles at 30 days: 7.1%, 7.3%, 8.1%, 10.5%, p=0.001. This difference was entirely driven by the difference in mortality, which was observed already at 48 hours (figure 10a). At 30 days, mortality increased from 2% in the first quartile to 6.3% in the fourth quartile with increased rates also from the first to the second and from the third to the fourth quartiles. However, at no time-point was there any relationship between the rate of MI and the quartiles of CRP (figure 10 b). In the multiple logistic regression analysis increasing CRP quar-

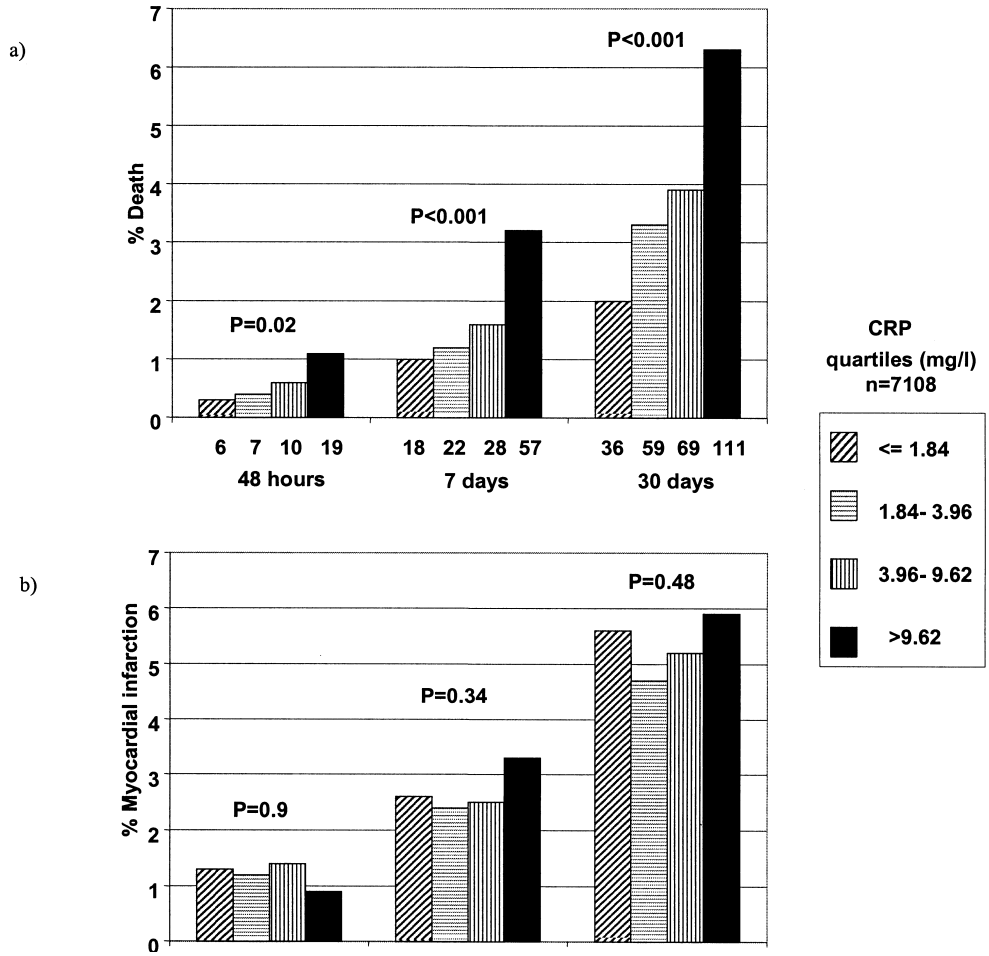


Fig. 10. Rate of a) death and b) MI respectively 48 h, 7 days and 30 days in relation to quartiles of CRP. The number of patients with events is noted under the bars.

tiles independently predicted 30-day mortality (O.R. 1.19; 1.05–1.35), while there was no relationship to subsequent MI (table 4).

Creatinine clearance

Patients with renal dysfunction are at high risk partly because of the high prevalence of multiple risk factors. In the GUSTO-IV population, a reduced creatinine clearance was significantly correlated with a large number of predictors of a worse outcome, such as diabetes, hypertension, age, heart failure, previous myocardial infarction (85) and elevation of CRP, troponin T and N-terminal proBrain Natriuretic Peptide (95). Still, a creatinine clearance below the 1st quartile (51 ml/min) was independently associated with mortality as well as subsequent myocardial infarction in multivariate analyses (95).

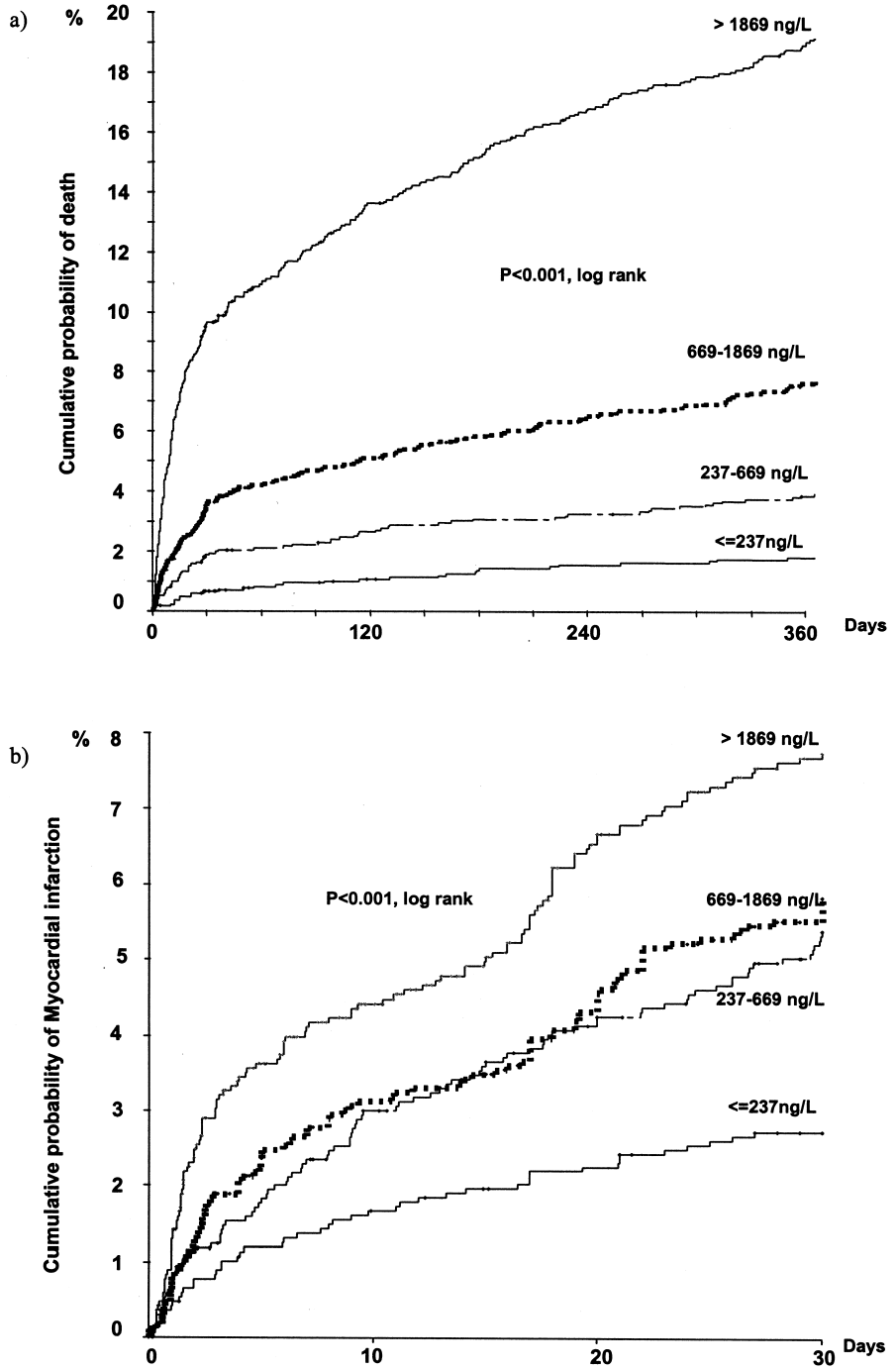


Fig. 11. Kaplan-Meier survival curves regarding probability of (a) death during one-year and (b) myocardial infarction during 30-days of follow-up for patient strata based on quartiles of NT-proBNP.

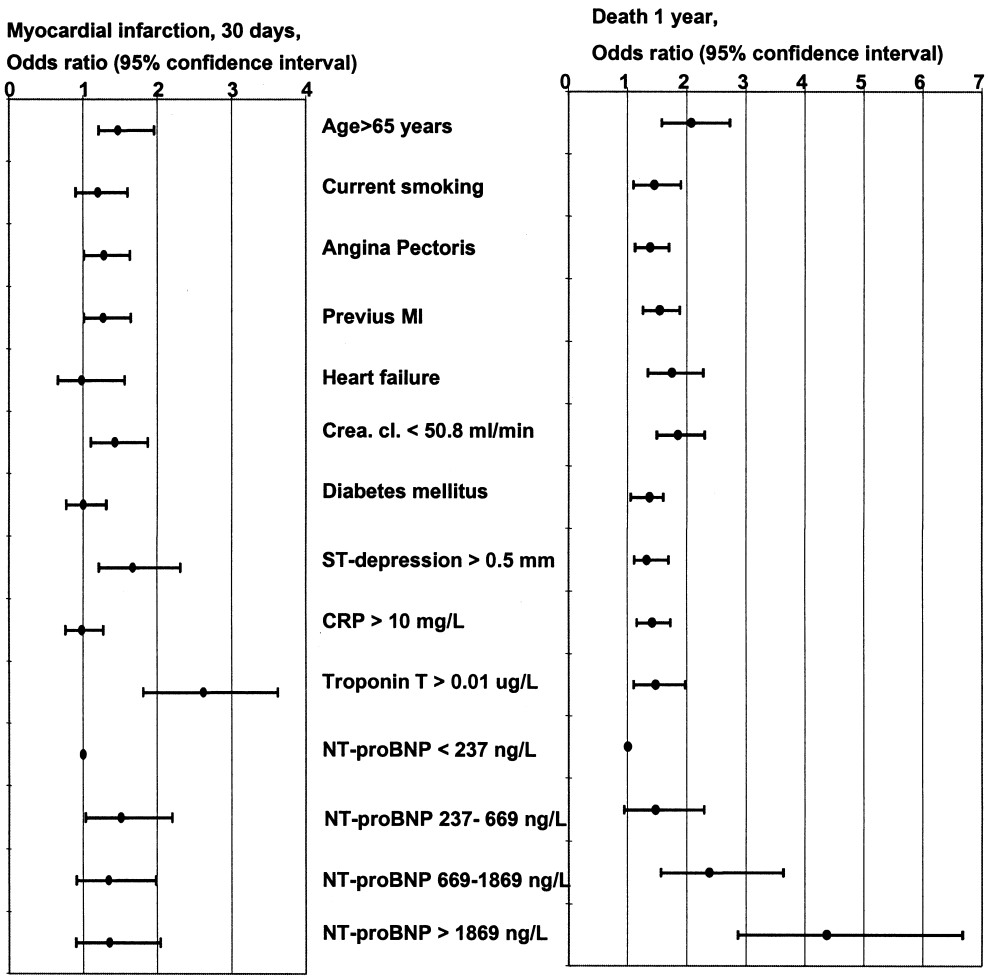


Fig. 12. Multiple logistic regression analyses for the prediction of myocardial infarction at 30 days and death at 1-year follow-up.

Hyperlipidemia (including elevated Lp(a) lipoprotein levels), the insulin resistance syndrome, and hyperhomocystinemia are other factors contributing to coronary artery disease in patients with renal dysfunction. The chronic anemia and volume overload associated with severe renal dysfunction, may be important contributors to an increased vascular stiffness, the development of heart failure and subsequent mortality. Other specific cardiovascular risk factors contributing to the vasculopathy induced by the renal disease include secondary hyperparathyroidism, increased sympathetic-nerve activity caused by afferent renal reflexes, elevated levels of oxidized low-density lipoprotein, endothelial dysfunction and diminished vascular nitric oxide production (138). Moreover, a reduced secretion of erytropoetin and insulin-like growth factor in patients with renal dysfunction may also specifi-

cally contribute to an increased risk of thrombotic cardiovascular events by an inhibition of vascular repair (139).

Markers of myocardial dysfunction

Determinations of NT-proBNP levels in serum samples obtained at randomisation were available from 6809 (87.3%) of the patients in the GUSTO-IV trial. The NT-proBNP levels ranged from 5.3 to 35000 ng/L with a median level of 669 (interquartile range 237–1869) ng/L.

Increasing levels of NT-proBNP were independently positively associated with age, female gender, current smoking, diabetes mellitus, hypertension, previous myocardial infarction and heart failure but negatively with body-weight, hypercholesterolemia and the occurrence of ST-depression at baseline. NT-proBNP levels were also associated with time from symptom onset and the magnitude of myocardial necrosis i.e. troponin elevation. In addition, NT-proBNP levels were associated with renal dysfunction and inflammatory activity as reflected by levels of creatinine and CRP.

There was an increased mortality among patients in increasing quartiles of NT-proBNP (figure 11). The Kaplan-Meier survival curves for the quartiles separated

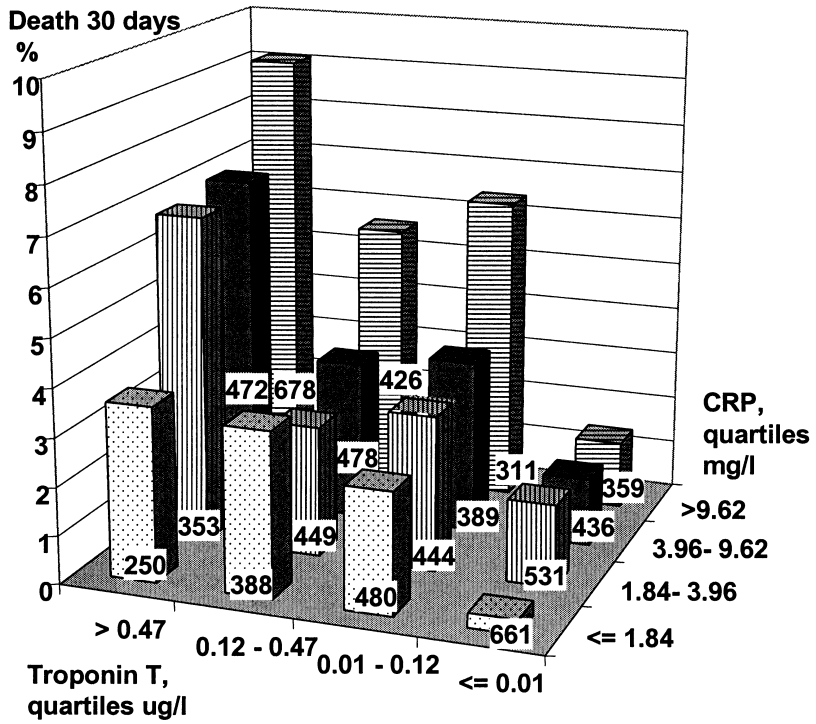
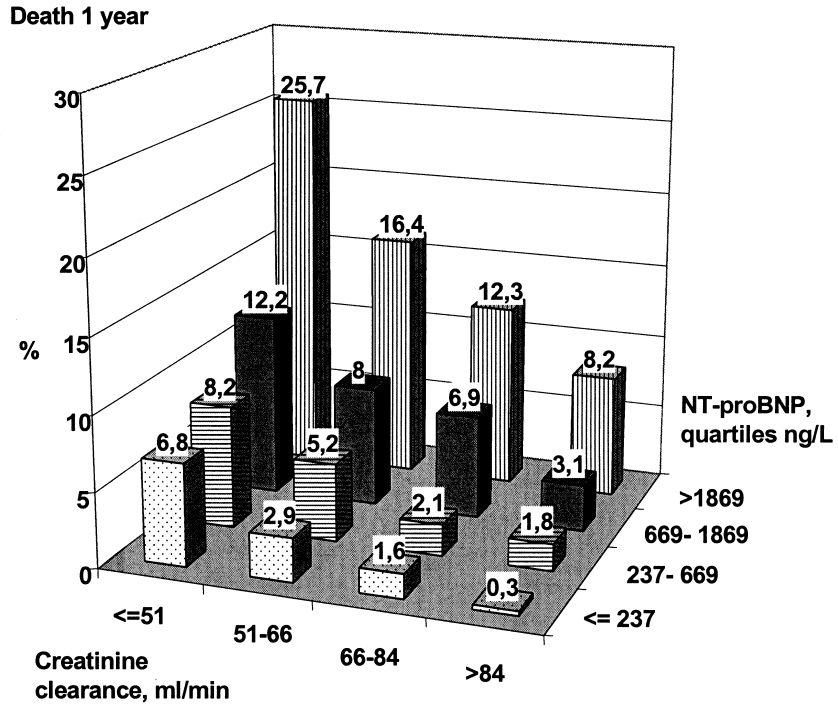
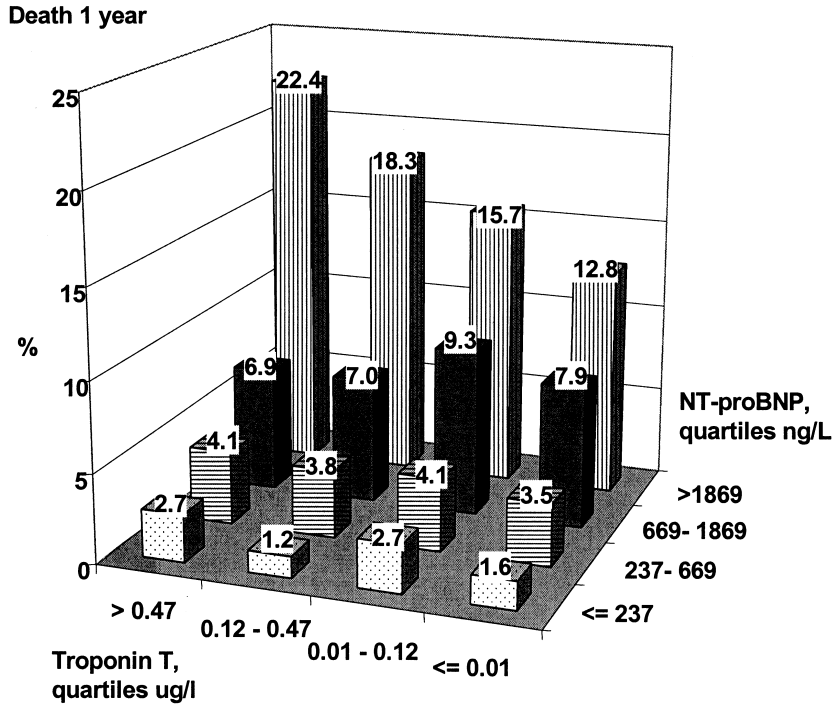


Fig. 13. Mortality at 30-days follow-up among strata of patients based on quartiles of Troponin-T and quartiles of C-reactive protein.


Death 1 year
30
25
20
15
10
5
0
%
25,7
16,4
12,3
8,2
12,2
8
6,9
8,2
6,8
5,2
3,1
2,9
2,1
1,8
1,6
0,3
<=51
51-66
66-84
>84
Creatinine clearance, ml/min
NT-proBNP, quartiles ng/L
>1869
669- 1869
237- 669
<= 237

a


Death 1 year
25
20
15
10
5
0
%
22.4
18.3
15.7
12.8
9.3
6.9
7.0
7.9
4.1
3.8
4.1
3.5
2.7
1.2
2.7
1.6
> 0.47
0.12 - 0.47
0.01 - 0.12
<= 0.01
Troponin T, quartiles ug/l
NT-proBNP, quartiles ng/L
>1869
669- 1869
237- 669
<= 237

b

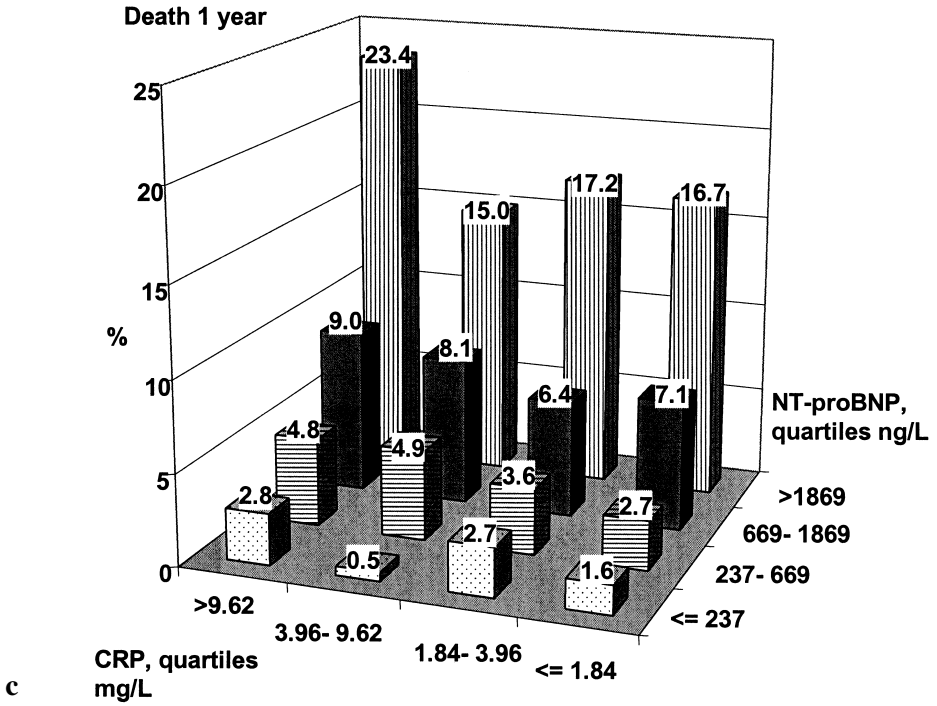


Fig. 14. Mortality at one-year follow-up among strata of patients based on quartiles of NT-proBNP and quartiles of creatinine clearance (a) Troponin-T (b) and C-reactive protein (c).

early. Already at 48 hours after randomisation the difference in mortality between the quartiles was statistically significant (p=0.001), with a mortality of 0.2% (n=3), 0.4% (n=6), 0.4% (n=7) and 1.4% (n=23) respectively. The separation of the curves continued throughout the first year after the index event (p<0.001, log rank). Thus, at one-year follow-up the mortality was 1.8 % in31, 3.9% in 66, 7.7% in 131 and 19.2% in 327 in the respective quartile. At one year there was an exponentially increasing mortality in the entire spectrum of NT-proBNP levels with a mortality of 0.4% in 3 in the lowest decile (<98 ng/L) and 27.1% in 185 in the highest decile (>4634 ng/L). In a multivariable logistic regression analysis, adjusting for a large number of predictors of long-term mortality, increasing quartiles of NT-proBNP still independently contributed to the prediction of 1-year mortality (figure 12).

The risk of subsequent MI after the index event was also increasing with increasing quartiles of NT-proBNP with an MI rate of 2.7% in 46, 5.4% in 91, 5.7% in 98 and 7.5% in 128 (p<0.001) for the respective quartile at 30-days follow-up (figure 11b). However, in a multivariable logistic regression analysis, age above 65 years, previous MI, creatinine clearance < 50.8 ml/min, angina pectoris, troponin T elevation (>0.01ug/L) and ST-depression at baseline, but not the level of NT-proBNP constituted independent predictors of myocardial infarction at 30 days (figure 12).

Combinations of biochemical markers

As both Troponin T and CRP were independent predictors of 30-day mortality (table 4), the prognostic value of combining these markers was also evaluated. The highest mortality at 30 days was found in the patients with both markers in the top quartiles, 9.1% and the lowest in the patients with both makers in the bottom quartiles, 0.3% (figure 13).

Finally, the long-term prognostic value of different combinations of NT-proBNP, troponin T, CRP and creatinine clearance were evaluated as all these markers were independent predictors of one-year mortality. A very low mortality was found in patients with NT-proBNP in the bottom quartile in combination with creatinine clearance in the top quartile (0.3%) or in combination with troponin T or CRP in the bottom quartile (1.6%) (figure 14a, b, c). The highest one-year mortality, 25.7%, was found in patients with levels of NT-proBNP in the top and creatinine clearance in the bottom quartile. A similar high mortality was found in patients with NT-proBNP in combination with troponin T or C-reactive protein levels in the top quartiles, 22.3 or 23.4 %.

DISCUSSION

Effect of abciximab and dalteparin treatment

There was no benefit with abciximab administered intravenously during the first 24 or 48 hours after enrolment of patients with acute coronary syndromes who did not undergo early coronary revascularisation. These findings are in sharp contrast to earlier investigations with abciximab in patients with refractory angina (105), in patients undergoing percutaneous coronary intervention (PCI) (104, 106, 140), and in studies with other parenteral glycoprotein IIb/IIIa receptor blockers (110, 111, 141). The reasons for these differences are unclear, and several possible explanations have been discussed.

The inclusion of patients was based on chest pain and either elevated cardiac troponin levels and/or ST segment depression >0.5 mm aiming at patients at a relatively high risk. Yet, the observed event rate in the placebo group, and in the trial overall was lower (8%) than expected (11%). It is possible that the minimal duration of chest pain required (5 minutes) and the low level of ST-segment depression required, allowed enrolment if relatively low risk patients. However, the event rate was in fact similar or even higher than in other ACS-trials when differences in endpoint definitions are taken into consideration (111, 119, 141, 142). In several subgroups of patients the event rate was clearly higher but there was still no effect of abciximab treatment in these groups (figure 2).

Based on the concept of a dominating role of platelet aggregation and coagulation activation in ACS, a combination of a GP IIb/IIIa inhibitor and a LMWH in the treatment of patients with non-ST elevation ACS, has several theoretical, in addition to the practical, advantages. Still, no treatment benefit of abciximab could be

observed in the dalteparin substudy that included a patient population at somewhat higher risk, resulting in significantly higher event rates than in the UFH cohort.

The GP IIb/IIIa inhibitor abciximab, in addition to its anti-thrombotic effect, also has been suggested to suppress the inflammatory response in unstable CAD. An anti-inflammatory effect might be related to abciximab cross-reacting with other integrin receptors (143). In the present study however, abciximab did not suppress the activation of inflammation in unstable CAD. This is in contrast to the findings in a substudy of EPIC (144) in patients undergoing percutaneous coronary intervention. In the latter the suppression of inflammatory activity may have been related to the prevention of myocardial damage at the time of the intervention by abciximab therapy. In GUSTO-IV no reduction in myocardial damage by abciximab was observed, and the rate of coronary interventions was very low, according to the trial design (135).

On the contrary, a possible pro-inflammatory effect of abciximab has emerged as one plausible explanation for unexpected lack of clinical benefit with abciximab in the GUSTO-IV ACS (145), as there even was a trend to a raised long-term mortality in the 48-hour abciximab group. In particular, an unfavourable trend with an excess in mortality was observed in patients with signs of inflammation and without evidence of ongoing intracoronary thrombosis, as reflected by elevated CRP levels and the absence of elevated cardiac troponin levels at enrolment (145). However, the results of the current study do not support such an explanation, as abciximab did not influence the development of indicators of inflammatory activity.

There is evidence that high dose dalteparin in placebo controlled ACS trials reduces thrombin generation and activity, as demonstrated by the reduction of the F1+2, TAT and SF levels (146). In the present study TAT and SF levels still increased during the initial 72 hours of dalteparin treatment, demonstrating an activation of the coagulation system. Although there was an activation of the coagulation and fibrinolytic system, there was no attenuation of the elevation of these by abciximab treatment. Previously a number of in vivo and in vitro studies have indicated that abciximab, by its blockade of the glycoprotein IIb/IIIa receptor, might have an indirect inhibiting effect also on the coagulation system (147, 148). However, the present study does not support any effect of abciximab on the coagulation system.

Abciximab, in contrast to clopidogrel, is ineffective in reducing platelet-leukocyte aggregates and p-selectin expression in vivo and even increases expression of p-selectin, presumably caused by “outside-in” signalling when abciximab binds to the GP IIb/IIIa receptor (149). Formation of platelet-leukocyte aggregates by platelet p-selectin binding to leukocytes at the site of vascular injury increases thrombin generation and might be an important mechanism that contributes to haemostasis and thrombosis (150). However, the present results also refute any procoagulative effect of abciximab, at least in patients treated with dalteparin and aspirin.

Thus, in a patient population with non-ST elevation ACS treated with aspirin and

Table 2. Independent predictors of bleedings in the GUSTO-IV ACS study.

	95% C.I for O.R		
Independent predictors for major and minor bleedings*	O.R	Lower	Upper
Age >65 years†	2.81	2.08	3.80
Abciximab treatment	1.62	1.37	1.91
PCI†† during initial 7 days	1.81	1.96	2.57

* Bleedings according to TIMI-criteria, not related to coronary artery by-pass surgery. † Prespecified cut-off for the outcome analyses and the mean age. †† Coronary intervention. Included variables: age, sex, weight, history of stroke or TIA, diabetes, hypertension, smoking, dalteparin cohort, randomised abciximab treatment, clopidogrel, aspirin, oral anticoagulant and other lmw-heparin usage.

low molecular weight heparin, the addition of abciximab infusion does not prevent an initial activation of the inflammation, coagulation or the fibrinolysis systems.

Safety of abciximab and dalteparin treatment

The safety of abciximab in addition to LMWH and aspirin in the treatment of ACS has not been investigated previously in a large-scale trial. In the current trial the numbers of major and minor bleedings were overall low and comparable to other trials of anti-thrombotic treatments in acute coronary syndromes (136). There were no significant differences in bleedings in the non-randomised comparison between full dose of dalteparin and a reduced dose of UFH (table 2).

However the addition of abciximab was associated with a doubling in major and minor bleedings, both within the UFH and dalteparin cohorts, in the present trial. Acute coronary syndrome trials with GP IIb/IIIa inhibitors in addition to UFH have reported major bleeding levels in up to 10.8% and minor bleedings in up to 13% (123, 126). The safety of abciximab and enoxaparin combined as conjunctive therapy to intervention was tested in the open label NICE 4 trial that included 818 patients which did not show an increased incidence of major bleedings or transfusions (0.2% major and 6.8 % minor bleedings, 1.2% transfusions) (151). Tirofiban and enoxaparin vs. UFH was evaluated in the ACUTE-II trial that included 525 patients presenting with unstable angina or suspected myocardial infarctions without ST-segment elevation and permitted coronary angiography after 24 hours. The combination treatment was well tolerated with low and similar bleeding rates in the two groups, i.e. major 1.0% vs.0.3% and minor 2.5 %vs. 4.3% (125). After the publication of the present study, a randomised comparison between enoxaparin and UFH in ACS-patients treated with eptifibatide has also been published. Major bleedings were less frequent (1.8% vs. 4.6%), while minor bleedings were more frequent for enoxaparin than UFH (30.3% vs. 20.8%) (152). Taking differences in definitions into account, these results are in accordance with the present findings of a similar rate of major and minor bleedings when UFH or dalteparin was combined with abciximab. The increased numbers of insignificant bleedings (<30 g/L fall in haemoglobin) in the dalteparin substudy was to a large extent attributable to the

subcutaneous heamatomas caused by dalteparin injections, which commonly create. Insignificant bleedings were particularly common for the patient groups with augmented risk of bleedings, such as elderly (Figure 8) and females.

As expected thrombocytopenia was more common with the addition of abciximab both to UFH and dalteparin. Severe thrombocytopenia was however rare. Therefore concerning the risk of thrombocytopenia at abciximab treatment LMWH seems preferable to abciximab.

Differential risk assessment with biochemical markers

Troponin and CRP

In concordance with other studies the current study showed that both troponin T and CRP were significant predictors of an adverse outcome in the early phase after an episode of ACS (31, 73, 75). In contrast to previous studies, both CRP and troponin T levels were available from a sufficiently large number of patients to allow prospective evaluation of their separate relationships to mortality and risk of MI. Furthermore, the independent associations to the different outcome events could also be demonstrated in multivariable analyses.

Increasing troponin T levels were associated with a continuous rise in mortality. In contrast, any detectable troponin T, i.e. above 0.01ug/L, was associated with a raised risk of MI without any further risk at higher troponin T levels. Increased CRP levels during the acute stage of unstable CAD were related to increased mortality in accordance with previous findings (31, 72, 73, 75). In this large patient cohort the relationship to increased mortality was evident early and further accentuated throughout the 30-day follow-up. There was however no association between the CRP levels and the risk of MI. No previous study on inflammatory markers in ACS has contained a sufficient number of patients enabling separation of the endpoints death and MI.

What might be the reason for the relationship between CRP and subsequent mortality but not MI in the acute phase of unstable CAD, in contrast to the well-established relationship between CRP elevation and subsequent coronary events in the chronic phase of atherosclerotic disease (69, 153–155)? In the acute phase of unstable CAD the elevation of CRP level is transient and to a large extent caused by an acute phase reaction (156). Some unstable CAD patients might have a hyperresponsiveness of the inflammatory system, which might exaggerate the acute phase reaction and increase the immune system reaction (157). Such a mechanism is supported by the observations of co-localisation of CRP and activated complement in infarcted myocardium (43). CRP may in itself contribute to inflammation by activation of complement that in turn may mediate myocardial damage, induce arrythmias and provoke contractile dysfunction (41). Such an interpretation is in accordance with the relationships between the CRP level and the occurrence of cardiac rupture, left ventricular aneurysm formation and mortality after acute MI (158). Thus, the CRP elevation in unstable CAD might indicate a different process than the low-grade CRP elevation that is associated to subsequent coronary events among healthy

individuals(69, 153, 159) and in the chronic phase of atherosclerosis after myocardial infarction (160). In unstable CAD as well as in chronic atherosclerotic disease there is a lasting elevation of the fibrinogen level (156) that might indicate an underlying chronic low-grade inflammatory condition that in both conditions is associated with a raised risk of later MI.

NT-proBNP in relation to other markers

In the present study, from a large cohort of patients of non-ST elevation ACS, we demonstrated that baseline levels of NT-proBNP are independently related not only to age and female gender (161) but also to low body-weight and renal dysfunction. Part of this relationship might be explained by an increased sensitivity to volume overload, as BNP levels have been shown to be secreted at a response to volume overload and to raised intra-cardiac pressure (162) irrespective of the cause. The present study also demonstrated that levels of NT-proBNP were independently related to clinical factors indicating any kind of cardiovascular damage or dysfunction supporting that elevation of BNP (or NT-proBNP) is a general indicator of reduced cardiac performance rather than a specific indicator of systolic dysfunction (163). Moreover, our study demonstrated that ongoing myocardial damage (i.e. minimal troponin elevation), time since start of myocardial ischemia and damage and the inflammatory response (i.e. CRP elevation) were related to the magnitude of elevation of NT-proBNP, further supporting the concept of BNP being a sensitive and rapid marker of reduced cardiac performance. This is in accordance with the recent report that BNP levels increase as a result of temporary occlusion of a coronary artery in conjunction with a coronary intervention (164) even when intracardiac filling pressures remained unchanged (165). In the present study NT-proBNP levels were negatively associated with the presence of ST-depression (> 0.5mm) at baseline which, however, might be attributable to the fact that ST-depression was part of the inclusion criteria and the low level of > 0.5 mm for the definition of ST-depression on admission.

Recently it has been shown that elevation of BNP as well as NT-proBNP levels obtained after the acute phase (median 40 to 72 hours after symptom onset) in patients with a broad range of ACS independently predict mortality (90, 91). In the present study we extended these results in a considerably larger population of non-ST elevation ACS, for NT-proBNP obtained already on admission, at a median 9.5 hours after symptom onset in accordance with a previous study from our group in an unselected chest pain population (166). Thereby, we could demonstrate that NT-proBNP predicted one-year mortality in patients with blood samples obtained within 5.0 hours (first quartile) as well as more than 16.6 hours (fourth quartile) after symptom onset. The present study also demonstrated that any elevation of NT-proBNP above the 97.5 percentile, 290 ng/L, in a healthy population matched for age and gender, seemed to be associated with an increased risk of death after the index event.

Despite the fact that the level of NT-proBNP was independently related to sev-

eral riskfactors, the NT-proBNP level still was the strongest independent indicator of mortality in the multivariate analysis. Also elevation of troponin T and CRP as well as reduced creatinine clearance rate (85) independently predicted an increased mortality. Accordingly, the combination of several of these markers allowed an even better stratification of future risk of fatal events. The combination of quartiles of increasing NT-proBNP levels and quartiles of decreasing creatinine clearance rates provided the best prediction of long-term mortality. Among patients in every quartile of creatinine clearance, mortality was increased with increasing quartiles of NT-proBNP. The combination of quartiles of NT-proBNP and either quartiles of CRP or troponin T provided a similar prediction of mortality. Interestingly however, elevated levels of troponin T seemed to contribute to an increased mortality only in patients with NT-proBNP levels in the top quartile. Thus, ACS-patients without myocardial dysfunction seem to tolerate even moderately large myocardial infarctions without a lethal outcome. On the other hand, ACS-patients with renal dysfunction, myocardial damage or increased inflammatory activity in addition to any reduction in cardiac performance, as indicated by a release of NT-proBNP, have a high risk of fatal complications to their heart disease.

In contrast to ST-depression and troponin elevation at baseline, the risk of subsequent MI at 30-days follow-up was not independently predicted by increasing levels of NT-proBNP in accordance with previous studies (167). The reason for this finding might be that BNP is a regulatory myocardial hormone which is not involved in the processes related to the rupture of a coronary plaques or formation of coronary thrombi. In contrast, elevation of BNP has been shown to predict sudden death in patients with heart failure (168). Thus, the release natriuretic peptides from ventricular myocytes, in response to increased wall-tension due to ischemia or volume overload, might indicate a propensity to develop ventricular arrythmias, ventricular rupture or terminal heart failure rather than myocardial infarction.

Clinical implications

What biomarkers should be recommended in the early evaluation of patients with unstable CAD (figure 15)? For prediction of MI troponin elevation is the strongest biomarker and also reduced creatinine clearance is independently associated with subsequent myocardial infarction. However, for prediction of mortality, several clinical as well as ECG and biochemical markers seem to be useful. On multivariable analysis, NT pro-BNP seems to be the strongest biochemical riskmarker. By virtue of being an unspecific marker of reduced cardiac performance NT proBNP seems to be very useful for selection of low-risk patients. A level below the 97.5 percentile (i.e. 290 ng/L) of a healthy population is associated with a very low mortality. In fact it also seems very unlikely that the patient has any significant heart disease at these low levels. Patients with elevation of NT pro-BNP on the other hand have increased risk of a fatal complication in relation to the level, which may

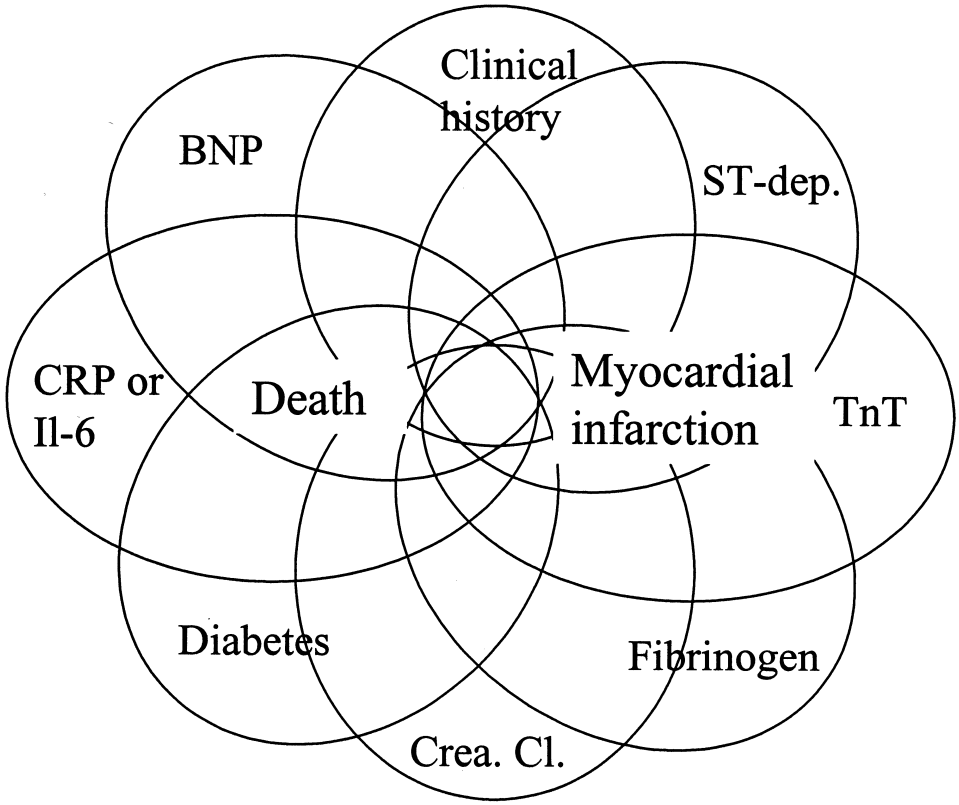


Fig. 15. Risk indicators in unstable coronary artery disease.

merit further investigation and treatment. It has been shown that Carvedilol treatment is particularly effective in patients with heart failure and elevated levels of NT-proBNP (169). However, it remains to investigate whether patients with high levels of NT-proBNP might derive a particular benefit from ACE-inhibition, early coronary interventions, implantable cardioverter-defibrillators (ICD) or other therapeutic modalities. Patients with any detectable troponin elevation have an increased risk of death and derive a particular benefit from early coronary intervention (89), GP IIb/IIIa inhibition (88) (87) and extended low molecular weigh heparin treatment (86) (170). Also a moderate reduction in creatinine clearance is independently associated with an increased mortality which should be increasingly recognised although not possible to treat. CRP elevation is particularly useful for prediction of long-term mortality. There is evidence that statin treatment reduce CRP levels among survivors of MI and that statin therapy is particularly beneficial for patients with CRP elevation, independent of lipid levels (171). Furthermore, ST-depression upon admission as well as several baseline factors are useful for prediction of mortality.

CONCLUSIONS

In patients with non ST-elevation ACS not scheduled for early revascularisation:

- Abciximab infusion on top of dalteparin and aspirin treatment was generally well tolerated although abciximab increased the number of bleedings as compared to placebo. In patients treated with abciximab, bleeding-rates were similar if combined with dalteparin than if combined with UFH.
- Addition of abciximab to standard treatment with dalteparin as the primary treatment of ACS, did not reduce the rate of cardiac events at 30-days follow-up.
- Despite dalteparin treatment there was a simultaneous activation of inflammation, coagulation and fibrinolytic systems not influenced by abciximab infusion.
- Baseline levels of troponin T and C-reactive protein were independently related to 30-day mortality. Any detectable elevation of troponin T, but not of C-reactive protein was also associated to a raised risk of subsequent MI. Concerning mortality, the combination of both markers provided a better risk stratification than either one alone.
- Increasing quartiles of NT-proBNP were independently related to short and long term mortality. The combination of NT-proBNP and creatinine clearance provided the best prediction of one-year mortality.
- A multimarker strategy with creatinine clearance, troponin, CRP and NT-proBNP together with ischemic ECG changes and clinical background characteristics has the potential to make risk assessment and clinical decision-making individualized and substantially improved.

SUMMARY

Patients with ACS constitute a heterogeneous population with different clinical history, extent and severity of the coronary artery disease. In the assessment of risk of cardiac events, several clinical as well as ECG and biochemical markers are useful. Different biochemical markers obtained on admission provide different and complementary prognostic information. By using a combination of biochemical markers an individualised and differentiated risk prediction can be made. Thus, with increasing levels of NT proBNP, CRP or troponin T there is a commensurate rise in short and long-term mortality that is independent of other risk indicators. Elevated levels of NT proBNP provide the strongest prediction of long-term mortality with a continuous increase in mortality in relation to the levels. The combination of NT-proBNP with creatinine clearance rate, or with levels of troponin T or CRP, provides a better risk stratification concerning the long-term risk of death in ACS patients than either one of the markers alone. At any detectable troponin level, in contrast to elevated levels of CRP or NT-proBNP, there is also a raised risk of a later myocardial infarction independent from other risk indicators.

Addition of abciximab to the standard treatment with UFH or LMWH and aspirin as primary treatment of ACS is not associated with any significant reduction in cardiac events but a doubled risk of bleedings. If abciximab is used the combination with dalteparin seems as safe as the combination with UFH, although nuisance bleedings are more common. On the other hand thrombocytopenia is more rare. Despite full dose low molecular weight heparin and aspirin treatment there is still a simultaneous activation of the inflammation, coagulation and fibrinolysis systems in non ST-elevation ACS. Prolonged treatment with abciximab has no influence on the activation of these systems. Therefore, more effective attenuation of the coagulation and inflammation systems as well as reduction of myocardial damage and dysfunction might be new objectives for pharmacological stabilisation of unstable CAD.

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