ST-elevation myocardial infarction

Studies of outcome in relation to fibrinolysis and ischemia monitoring with on-line vectorcardiography.

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ABSTRACT

The treatment of acute myocardial infarction (AMI) has undergone a tremendous development during the last decades, and the most important factor is probably the introduction of reperfusion therapy aimed at preventing or limiting the myocardial injury.

It is of vital importance that patients with AMI are adequately monitored regarding the development of ECG changes during and after treatment to identify successful or failed reperfusion and to detect episodes of recurrent ischemia. Vectorcardiography (VCG) is one method for this purpose.

This series of studies was aimed at evaluating VCG as a method for detecting reperfusion and recurrent ischemia in patients with ST-elevation AMI who were treated with different reperfusion strategies. Specific changes in the VCG during the initial treatment phase, “reperfusion peaks,” were examined in detail. The influence of the fibrinolytic system and von Willebrand factor (vWF) on successful reperfusion and subsequent AMI and death after thrombolytic treatment with streptokinase (SK) was another main objective.

From the data in these studies it can be concluded that:

VCG is a relevant and easily used method for ischemia-monitoring in patients with AMI. A specific sign, the reperfusion peak, is associated with vectorcardiographic signs of reperfusion. This sign is observed both in patients treated with primary coronary angioplasty and in those who are treated with fibrinolytic agents. The reperfusion peak is associated with successful reperfusion and with larger infarcts, but by itself, the parameter has little prognostic significance. The recognition of the reperfusion peak is important since it can mimic severe ischemia. In an unfortunate situation the incorrect interpretation of the VCG could lead to premature treatment decisions that might even be harmful to the patient.

Streptokinase treatment of patients with AMI induced profound changes in the fibrinolytic system and vWF. A high tissue plasminogen activator (tPA) activity level (≥25 U/mL) early after the start of treatment, reflecting the fibrinolytic activity obtained by the given drug, was associated with successful reperfusion.

Pre-existing neutralising antibodies to SK were found to varying degrees in SK-treated patients. No association between SK-neutralising antibodies and the result of the treatment regarding successful reperfusion as judged by VCG was seen.

Pre-treatment levels of tPA activity, PAI-1 activity, PAI-1 mass-concentration and vWF had no correlation with the success of reperfusion therapy with SK or on the incidence of recurrent ischemia during the first 24 hours. Recurrent ischemia, however, was shown to be an independent risk factor for death within the first 1 year. Elevated levels of PAI-1 mass-concentration, and to some extent PAI-1 activity, after the start of SK treatment, were associated with a higher risk for death at one year, though not at five years.
ORIGINAL PAPERS

The thesis is based on the following papers, which will be referred to by their roman numerals. Permission is granted to reprint all the papers in the thesis.

Relationship between Fibrinolytic Activity following Streptokinase Treatment in Acute Myocardial Infarction and Vectorcardiographic signs of Reperfusion. 

The Effect of Streptokinase Neutralising Antibodies on Fibrinolytic Activity and Reperfusion Following Streptokinase Treatment in Acute Myocardial Infarction. 
*Journal of Internal Medicine* 2002;252:405-411.

Transient Increase in ST-segment Changes at Time of Reperfusion in Acute Myocardial Infarction Treated by Coronary Angioplasty. 

The electrocardiographic reperfusion peak in patients with ST-elevation myocardial infarction. 
*Submitted for publication*, 2006.

The influence of acute-phase levels of haemostatic factors on Reperfusion and Mortality in Patients with Acute Myocardial Infarction treated with Streptokinase. 
*Submitted for publication*, 2006.
CONTENTS

ABSTRACT .............................................................................................................. 4
ORIGINAL PAPERS ............................................................................................... 5
CONTENTS .............................................................................................................. 6
ABBREVIATIONS .................................................................................................. 8
INTRODUCTION .................................................................................................... 9
  Acute myocardial infarction ................................................................................. 9
  Atherosclerotic plaques .................................................................................... 9
  Haemostasis .................................................................................................... 10
  Von Willebrand factor (vWF) ....................................................................... 10
  Fibrinolysis ..................................................................................................... 11
  Tissue Plasminogen activator ....................................................................... 12
  Plasminogen activator inhibitor type 1 ........................................................... 13
  Platelets .......................................................................................................... 13
Summary ............................................................................................................. 13
Treatment ............................................................................................................ 14
Evaluation of treatment ........................................................................................ 14
AIMS ...................................................................................................................... 16
MATERIAL AND METHODS ............................................................................ 17
  Patients ............................................................................................................... 17
  Paper I................................................................................................................. 17
  Papers II and V. .................................................................................................. 17
  Paper III. ............................................................................................................. 19
  Paper IV.............................................................................................................. 19
  On-line vectorcardiography........................................................................... 20
  ST-VM and STC-VM ......................................................................................... 21
  QRS-VD ............................................................................................................. 22
  Recurrent ST episodes ....................................................................................... 22
  Reperfusion peak .............................................................................................. 22
  Laboratory Procedures....................................................................................... 23
    Fibrinolytic variables ...................................................................................... 23
    Streptokinase neutralising antibodies ........................................................... 23
  Statistics .............................................................................................................. 24
Contents

REVIEW OF THE RESULTS ........................................................................................................ 25
  Fibrinolytic variables, vWF, SK neutralising antibodies and outcome ......................... 25
    Papers I, II and V ........................................................................................................ 25
  Correlation with VCG-findings .................................................................................. 26
  Reperfusion, reperfusion peak, vectorcardiography and outcome ............................... 31
    Papers III and IV ...................................................................................................... 31

DISCUSSION ......................................................................................................................... 34
  Patients .......................................................................................................................... 34
  Streptokinase .............................................................................................................. 34
  Vectorcardiography .................................................................................................... 34
  Assessment of reperfusion ....................................................................................... 35
  Reperfusion peak ........................................................................................................ 36
  Recurrent ischemia ..................................................................................................... 38
  The Fibrinolytic system and von Willebrand factor .................................................... 38
  Cut-off levels for tPA activity, .................................................................................. 39
  Timing of the blood samples ..................................................................................... 40
  Relation to outcome .................................................................................................... 40
  Reperfusion and recurrent myocardial ischemia ....................................................... 40
  Mortality ....................................................................................................................... 41
  Streptokinase-neutralising antibodies ....................................................................... 42

CONCLUSIONS .................................................................................................................... 43

ACKNOWLEDGEMENTS ...................................................................................................... 44

REFERENCES .................................................................................................................... 46
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACE</td>
<td>angiotensin converting enzyme</td>
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<tr>
<td>CCU</td>
<td>coronary care unit</td>
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<tr>
<td>CK</td>
<td>creatine kinase</td>
</tr>
<tr>
<td>CK MB</td>
<td>creatine kinase iso-enzyme B</td>
</tr>
<tr>
<td>ELISA</td>
<td>enzyme-linked immunosorbent assay</td>
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<tr>
<td>MI</td>
<td>myocardial infarction</td>
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<tr>
<td>PAI-1</td>
<td>plaminogen activator inhibitor type 1.</td>
</tr>
<tr>
<td>PCI</td>
<td>percutaneous coronary intervention</td>
</tr>
<tr>
<td>QRS-VD</td>
<td>QRS vector difference</td>
</tr>
<tr>
<td>SD</td>
<td>standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>standard error of the mean</td>
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<tr>
<td>SK</td>
<td>streptokinase</td>
</tr>
<tr>
<td>SPECT</td>
<td>single photon myocardial perfusion emission computed tomography</td>
</tr>
<tr>
<td>STC-VM</td>
<td>ST Change Vector Magnitude</td>
</tr>
<tr>
<td>STEMI</td>
<td>ST-elevation myocardial infarction</td>
</tr>
<tr>
<td>ST-VM</td>
<td>ST Vector Magnitude</td>
</tr>
<tr>
<td>TIMI</td>
<td>thrombolysis in myocardial infarction</td>
</tr>
<tr>
<td>tPA</td>
<td>tissue plasminogen activator</td>
</tr>
<tr>
<td>UAP</td>
<td>unstable angina pectoris</td>
</tr>
<tr>
<td>uPA</td>
<td>urokinase-type plasminogen activator</td>
</tr>
<tr>
<td>VCG</td>
<td>on-line vectorcardiography</td>
</tr>
<tr>
<td>vWf</td>
<td>Von Willebrand factor</td>
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</table>
INTRODUCTION

Ischemic heart disease is one of the major causes of mortality and morbidity in the western world [1]. ST-elevation myocardial infarction (STEMI) is one of the manifestations of the acute coronary syndrome which also includes unstable angina. These two conditions are almost always due to atherosclerotic disease, e.g. the formation of the atherosclerotic plaque, disruption of an unstable plaque, various degrees of thrombus formation, and the resulting partial or total occlusion of the coronary vessel for various durations of time [2-8]. This dissertation will focus on STEMI, one of the most common causes of death in Sweden [9].

Acute myocardial infarction

Acute myocardial infarction (AMI) is defined as cardiac myocyte cell death due to prolonged ischemia [10] almost always caused by thrombosis formation on a coronary atherosclerotic plaque [11]. On rare occasions an AMI can be caused by spontaneous coronary artery dissection, coronary arteritis, coronary emboli, or coronary spasm [11]. The clinical presentation can vary from unstable angina pectoris (UAP), non-STEMI to STEMI depending on the presence of collaterals and the degree of obstruction. In STEMI there is usually a complete occlusion of the coronary artery resulting in ST-elevation on the ECG. Patients presenting with a new left bundle branch block (LBBB) on the ECG and clinical symptoms of AMI also belong to this classification. The diagnosis of myocardial infarction is based on a rise and fall of biochemical markers [12]. Almost all patients presenting with an STEMI develop a rise in biochemical markers diagnostic of myocardial infarction with a few exceptions called aborted myocardial infarctions, in which spontaneous reperfusion or very early reperfusion therapy may inhibit the development of myocyte cell death [13]. The spontaneous course in the early phase of an AMI is a highly dynamic process [14].

Atherosclerotic plaques

Atherosclerotic plaques are classified into different types according to the degree of severity [15]. Early lesions consisting of layers of macrophage foam cells and smooth muscle cells with extracellular lipid deposits are called “fatty streaks”. These early lesions can develop into complex, lipid-rich plaques which are susceptible to evolve into unstable plaques. The vulnerability of a plaque is a complex interaction between the size and consistency of the lipid-rich atheromatous core, the thickness of the fibrous cap covering the core and the ongoing inflammation and repair process within the fibrous cap [16]. At plaque rupture there is an exposure of substances such as collagen, lipids, smooth muscle cells and tissue factor which lead to activation and aggregation of platelets, thrombin generation and ultimately thrombus formation [6, 11]. This process is highly dynamic depending not only on the size and thrombogenicity of the ruptured plaque but also on the balance between the thrombogenic and the fibrinolytic systems [6].
**Introduction**

**Haemostasis**

The physiological function of the coagulation system is to secure haemostasis after vascular injury. A simplified outline of the haemostatic system is presented in Figure 1. The initial phase includes platelet adherence and aggregation at the site of the vascular injury and is called primary haemostasis. This is followed by activation of the coagulation system by tissue factor, contact activation and factors released from the activated platelets. Tissue factor is the key component necessary to trigger this system which ends up in the final common pathway leading to the production of thrombin. Thrombin induces cleavage of fibrinogen to soluble fibrin. Final cross-linking is provided by the activated factor XIII and the formation of a stable matrix where platelets and other formed elements are trapped – secondary haemostasis.

![Figure 1. A simplified outline of the haemostatic system.](image)

**Von Willebrand factor (vWF).**

Von Willebrand factor is a key-component in primary haemostasis. It mediates adhesion of platelets to the vascular endothelium and induces expression of the fibrinogen receptor on the platelets. The activated fibrinogen receptor (glycoprotein IIb/IIIa) binds fibrin to the platelets and initiates the platelet-platelet interaction necessary for their aggregation to a haemostatic plug. vWF is secreted from the endothelium and from megakaryocytes, a process stimulated by thrombin, fibrin and histamine [17]. Vascular injury or stress increases vWF synthesis, and it is also elevated in acute-phase inflammatory responses [18, 19]. The normal plasma concentration of vWF tends to increase with age and is highly variable in apparently healthy individuals with a range from 40 to 200 per cent of the mean value.
Fibrinolysis

After the formation of a haemostatic plug, clot lysis and vessel repair begin immediately. Circulating factors and mediators released from the endothelium regulate fibrinolysis. A schematic presentation of the fibrinolytic system is shown in Figure 2. Since fibrinolysis can be defined as the degradation of polymerised fibrin to prevent thrombus formation, the fibinolytic activity influences the growth and dissolution of a thrombus. Plasmin is the enzyme primarily involved in the breakdown of fibrin. It is synthesized in the liver as the inactive proenzyme plasminogen [20]. Plasminogen is activated to plasmin by tissue plasminogen activator (tPA). tPA is predominantly synthesised in endothelial cells and is the most important intravascular plasminogen activator. Activation is highly fibrin-specific and occurs both on the surface of a clot and on platelets [21-23]. This mechanism restricts the fibrinolytic response to the clot.

![Figure 2. A schematic presentation of the fibrinolytic system.](image)

The activation of the fibrinolytic system of tPA is balanced by circulating plasminogen activator inhibitor type 1 (PAI-1) which inhibits tPA and by α2-antiplasmin which inhibits plasmin, thereby preventing excessive fibrinolysis. In addition tPA is inactivated by the slow inhibitors C1-inhibitor, α1-antitrypsin and α2-macroglobulin. When tPA is acutely released from vascular endothelial cells it meets active PAI-1 in the circulation which results in rapid complex binding and inactivation of most of the tPA. Activation of plasmin by tPA takes place on the surface of the fibrin clot which means that fibrinolysis is locally restricted and does not become systemic [24]. tPA is present physiologically at low concentration in
Introduction

the plasma with approximately 1/3 being active. PAI-1 is in several-fold excess over tPA, and most of the tPA circulates as tPA-PAI-1 complex (Fig. 3). The distribution of fibrinolytic activators is influenced by the atherosclerotic process, and it has been shown that atherosclerotic vessels shows a marked predominance of fibrinolytic inhibition as compared with normal vessels [25]. Furthermore, an impaired capacity for tPA release has been observed in hypertensive patients [26]. The balance of tPA and PAI-1 in healthy individuals can be subtly changed, for example, by exercise or venous occlusion [27].

Figure 3. A schematic presentation of the relation between tPA antigen and activity and PAI-1 antigen and activity. The determination of total tPA includes free active tPA and tPA in complex with inhibitors. The determination of total PAI-1 includes free active PAI-1 and the tPA-PAI-1 complex.

Tissue Plasminogen activator

tPA is a serine protease synthesised in endothelial cells with a molecular weight between 65 and 75 kD. There is a basal tPA release that maintains a steady state plasma level and a regulated rapid release in which larger amounts of tPA can be secreted from intracellular storage to plasma in response to stimuli [28]. The local regulation of the release of tPA and the systemic levels of tPA may not always truly reflect the local fibrinolytic capacity at the organ level [29]. In the absence of fibrin, tPA has low affinity for plasminogen and accordingly a low activity. Normally 20 -30 % of tPA in the plasma is circulating in its biologically active form, and the rest is in its inactive, complex-bound form (Fig. 3) [29]. Elimination takes place in the liver, and liver blood flow is a determinant for plasma clearance of tPA [30]. Two receptors in the liver have been identified; one is the low-density lipoprotein receptor-related protein which binds both free tPA and the tPA-PAI-1 complex, the other is the mannose-dependent receptor. The half-life in plasma is short, only about 3-5 minutes [31].
Introduction

**Plasminogen activator inhibitor type 1**

PAI-1 is considered to be the most important inhibitor of fibrinolysis. It is a glycoprotein that belongs to the serine protease inhibitor family (serpins). The origin of the production of PAI-1 is not fully understood but endothelial cells, smooth muscle cells, adipocytes and hepatocytes have been proposed to be the main contributors [32-34]. PAI-1 is found in plasma, platelets, placenta and in extracellular matrix. Ninety per cent of PAI-1 is found in the platelets where it is stored in a latent form in the α-granulae and is released upon activation. The concentration of PAI-1 in plasma is about 10 ng/ml where most is bound to vitronectin which stabilizes PAI-1 in its active form and protects it from oxidation [35]. PAI-synthesis is stimulated by a wide variety of agents such as endotoxins, thrombin, insulin, various cytokines, oxidized LDL and other factors [34]. PAI-1 is present in a 3-8 fold excess over tPA, and when secreted it binds rapidly to tPA in a 1:1 ratio [27]. The affinity of the binding is strong and thought to be irreversible and the complex is cleared from the circulation by the liver with a half-life of approximately 20 minutes [24]. Since PAI-1 has the ability to bind to fibrin the PAI-1 concentration inside a clot is raised, even more so due to the release of PAI-1 from platelets.

**Platelets**

In the setting of an acute myocardial infarction platelets adhere to the subendothelial tissue and become activated after being exposed to the core of the ruptured plaque. The activated platelets express glycoprotein IIB/IIIA receptors on the surface. When these receptors are expressed the platelets are enabled to aggregate through cross-bridges of fibrinogen. During this process several vasoactive, inflammatory and pro-coagulative mediators are released.

**Summary**

An acute myocardial infarction should be regarded as a highly dynamic process. The initial plaque-rupture and thrombus formation is by no means a static situation. The fibrinolytic and thrombolytic factors on the one hand, and the pro-aggregational and thrombogenic factors on the other hand, create a process of both thrombus formation and dissolution which can be present during the time-course of the acute myocardial infarction. Spontaneous reperfusion of a myocardial infarction even before medical treatment is started is an established clinical finding and patients presenting a pattern with episodes of spontaneous reperfusion before the start of treatment have been reported to develop less myocardial damage [36]. Intermittent coronary occlusion has also been reported in patients both before and during treatment with intracoronary streptokinase [37].
**Introduction**

**Treatment**

The primary goal of reperfusion therapy in patients with AMI is to restore optimal myocardial blood flow. This is accomplished not only by restoring the blood flow in the epicardial vessels, but also by restoring perfusion on the cellular lever, tissue level reperfusion [38-40]. Other components of the treatment of AMI include, for example reducing oxygen demand and treating complications such as life-threatening arrhythmias and pulmonary oedema.

The care of patients with acute myocardial infarction has improved substantially over the last decades. The introduction of coronary care units in the 60s, resulting in a reduction in hospital mortality from 30-40% down to 18%, was the first step towards today’s modern care of these patients [41]. Other major improvements over the years are the use of Aspirin, lipid-lowering agents, ACE-inhibitors, beta-blockers, and in later years, the frequent use of coronary angiography and subsequent revascularisation.

The introduction of the use of fibrinolytic therapy to obtain reperfusion of the occluded coronary artery must be judged as one of the major advances for the treatment of patients with acute myocardial infarction. First, intravenous administration of streptokinase (SK) [42] and later recombinant t-PA (r-tPA) [43], were shown to markedly reduce mortality. The use of different reperfusion strategies to accomplish reperfusion of the infarct-related coronary artery in patients with acute myocardial infarction is today considered standard treatment [10]. Among the fibrinolytic drugs, SK is by far the cheapest. In comparative studies with r-tPA, there are slight disadvantages for SK regarding mortality and early (90 min) angiographic patency. However, bleeding complications are less common [44, 45]. Streptokinase activates the fibrinolytic system indirectly by forming a complex with plasminogen which converts the circulating and complex-bound plasminogen to a SK-plasminogen complex with the properties of activated plasmin. Neutralising antibodies against SK have been demonstrated after treatment with SK and after infections with streptococci [46-48]. Therefore it has been suggested that the presence of SK antibodies could reduce the thrombolytic effect of SK and it is recommended not to re-administer SK [10].

Recent pooled analyses of studies testing primary angioplasty against fibrinolytic therapy reveal a slight advantage for primary angioplasty in selected populations and when performed by highly qualified operators [49, 50], but fibrinolytic therapy is still the only acute treatment available for a vast majority of patients world-wide.

**Evaluation of treatment**

The goal of all types of reperfusion therapies is to accomplish a fast, complete and sustained reperfusion of the occluded coronary artery. The assessment of successful reperfusion has traditionally been dependent on coronary angiography
which has been (and still is by many) considered to be the gold-standard when evaluating the success of different types of pharmacologic reperfusion regimens. Measuring the TIMI-flow [51] (thrombolysis in myocardial infarction), where a TIMI 3 flow means a normal coronary flow early after treatment, has been a way to evaluate the effectiveness of different fibrinolytic agents. It has been shown that early achievement of a TIMI 3 flow is associated with a better prognosis [52]. There are, however, some drawbacks for coronary angiography: It is an invasive and costly procedure with a potential of causing complications for the patient. The angiogram showing patency or not, for example at 90 minutes, gives little information on the reperfusion at the microvascular level. It has been shown that the processes of thrombosis and vasomotor tone in the coronary arteries during a myocardial infarction are highly dynamic [14]. Serial angiograms of patients treated with tPA have revealed intermittent occlusions and reperfusion [53], a phenomenon also observed in patients treated with intracoronary SK [54].

In the era of primary coronary angioplasty the “no-reflow” phenomenon, supposed to be a result of microvascular injury in the infarct-zone [55], has been established as a clinical dilemma. Patients treated with primary coronary angioplasty who present this pattern have been reported to have a worse clinical outcome [56]. This is one example showing that patency of the epicardial vessel can not be interpreted as successful reperfusion on the cellular level. Some progress has been made concerning the use of coronary angiography for evaluating the state of the myocardium at a microvascular level. The use of “myocardial blush” as a marker of successful reperfusion is one method. The use of TIMI frame count, instead of the original TIMI flow classification is another. Others have used contrast echocardiography or single photon myocardial perfusion emission computed tomography (SPECT) for evaluation of reperfusion on the cellular level and for showing a disparity between epicardial patency and salvaged myocardium [57-59]. Several authors have discussed the best ways to identify successful reperfusion at both the microvascular and the epicardial level [38, 60, 61].

The use of different techniques for continuous monitoring of the ST-segment during the time-course of a myocardial infarction has been evaluated in a number of studies. This technique has several advantages since it is non-invasive, provides a continuous monitoring of the ischemic status of the patient, is quite easy to apply in almost all coronary care units, and provides both information on vessel patency and gives instant prognostic information. Several studies have addressed the identification of vessel patency after thrombolytic treatment [40, 62-68] as well as the possibility of collecting accurate and early prognostic information on patients treated with thrombolysis [40, 69-74] and with primary coronary angioplasty [39, 75, 76].
Aims

AIMS

With the use of on-line vectorcardiography (VCG) as a tool for detection of reperfusion and re-ischemia in patients treated for acute myocardial infarction, the aims have been:

- To further investigate the value of VCG in these patients.

- To evaluate the occurrence of and prognostic implications of the vectorcardiographic reperfusion peak in patients with AMI treated with fibrinolysis and/or coronary angioplasty.

- To investigate the levels and changes in the fibrinolytic system and von Willebrand factor before and during treatment of patients with AMI treated with SK.

- To relate these parameters (vectorcardiographic, fibrinolytic and vWF data) to the effect of reperfusion therapy and outcome (morbidity, mortality).

- To investigate the influence of SK-neutralising antibodies on the fibrinolytic response to treatment with SK and to successful reperfusion.
MATERIAL AND METHODS

Patients

All studies were approved by the Umeå University Research Ethics Committee (now called the Regional Ethical Review Board in Umeå).

Paper I

In the first study women and men admitted to the CCU in our hospital within 6 hours after the onset of nitroglycerine-resistant chest pain lasting for more than 20 minutes with a strong suspicion of myocardial infarction and no contraindication for streptokinase treatment were included. In addition, electrocardiographic signs of on-going transmural ischemia (ST segment elevation of $\geq 1$ mm in limb leads or $\geq 2$ mm in at least two precordial leads) were required. This group was treated with SK (Group A).

Forty consecutive patients were included. All the patients were treated with streptokinase $1.5 \times 10^6$ U iv for 60 minutes. A 500 mg dose of Aspirin was given orally on admission to the CCU, followed by 160 mg daily. Beta-blockers, iv nitroglycerine and anti-arrhythmic agents were given when needed according to clinical routine.

As a control group (Group B), we recruited 25 patients with AMI who had not been treated with thrombolytic agents. These patients had suffered chest pain lasting for more than 20 minutes but less than 6 hours, there was a strong suspicion of myocardial infarction, but there were either contraindications to SK (n=3) or electrocardiographic signs which did not fulfil the criteria for thrombolytic treatment (n=22). As a second control group (Group C), we recruited ten patients who had been referred to the CCU with chest pain but who did not develop a myocardial infarction.

Papers II and V.

These two studies were performed as a two-centre study with patients included at the CCU in our hospital and in a nearby county hospital (Skellefteå County Hospital). A total of 150 patients were included (100 patients in Umeå University Hospital and 50 patients in Skellefteå County Hospital). Eligible for the study were patients admitted to the CCU with symptoms of myocardial infarction, having had chest pain for more than 20 minutes but less than 12 hours. The inclusion criteria were electrocardiographic signs of ongoing transmural ischemia (ST-segment elevation of $\geq 0.1$ mV in limb leads or $\geq 0.2$ mV in two precordial leads), no contraindications for thrombolytic treatment, and the availability of VCG recording equipment. Patients with bundle branch block, AV block, pacemaker treatment or previous treatment with SK were excluded. All patients were treated with $1.5 \times 10^6$ U of SK i.v. for 60 minutes. Aspirin (500 mg orally) was given on admission or in
the ambulance, followed by 160 mg daily. Beta-blockers, nitroglycerine and anti-arrhythmic drugs were given according to clinical routine.

In Paper II, 104 of the patients were analysed regarding successful reperfusion assessed by VCG in relation to tPA activity as a measure of fibrinolytic response to treatment with SK and to the effect of pre-treatment SK antibodies.

In Paper V 139 patients were monitored with VCG and components of the fibrinolytic system and vWF were measured. The predictive value of the pre-treatment and acute-phase parameters were analysed with respect to reperfusion, reinfarction and mortality at one year and mortality at five years. Of all the 150 patients included, 11 were excluded due to repeated inclusion (n=1), withdrawn treatment (n=1), treatment with other thrombolytic drug than SK (n=1), bundle-branch block on arrival (n=2) or poor VCG recordings (n=6). Thus, 139 patients remained for the final analyses. Four of these patients developed bundle-branch block during the early phase of observation which precluded meaningful assessment of reperfusion or recurrent ischemia. The baseline clinical characteristics of the patients are presented in Table 1.

Table 1. Clinical and vectorcardiographic characteristics of the patients

<table>
<thead>
<tr>
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<th>Reperfusion according to VCG</th>
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<tr>
<td></td>
<td>NO</td>
<td>YES</td>
<td>p-value</td>
</tr>
<tr>
<td>Total n=135</td>
<td>n=63 (47)</td>
<td>n=72 (53)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>66.9 ± 10.0</td>
<td>72.0 ± 9.6</td>
<td>0.440</td>
</tr>
<tr>
<td>Male</td>
<td>43 (68)</td>
<td>39 (74)</td>
<td>0.493</td>
</tr>
<tr>
<td>Previous infarction</td>
<td>20 (32)</td>
<td>8 (11)</td>
<td>0.003</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>7 (11)</td>
<td>8 (11)</td>
<td>1.000</td>
</tr>
<tr>
<td>Hypertension</td>
<td>25 (40)</td>
<td>26 (36)</td>
<td>0.669</td>
</tr>
<tr>
<td>Previous CVS</td>
<td>4 (6)</td>
<td>3 (4)</td>
<td>0.581</td>
</tr>
<tr>
<td>Smokers</td>
<td>23 (37)</td>
<td>21 (29)</td>
<td>0.364</td>
</tr>
<tr>
<td>Medication at admission</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Beta-blockers</td>
<td>14 (23)</td>
<td>12 (16)</td>
<td>0.410</td>
</tr>
<tr>
<td>Aspirin</td>
<td>14 (23)</td>
<td>5 (7)</td>
<td>0.012</td>
</tr>
<tr>
<td>Diuretics</td>
<td>17 (27)</td>
<td>13 (118)</td>
<td>0.210</td>
</tr>
<tr>
<td>Anterior infarction</td>
<td>69 (59)</td>
<td>66 (45)</td>
<td>0.028</td>
</tr>
<tr>
<td>CK max</td>
<td>31.2 ± 25.6</td>
<td>37.0 ± 23.6</td>
<td>0.181</td>
</tr>
<tr>
<td>Delay (min)</td>
<td>333 ± 205</td>
<td>250 ± 212</td>
<td>0.002</td>
</tr>
<tr>
<td>Reischaemia</td>
<td>20 (33)</td>
<td>24 (33)</td>
<td>0.909</td>
</tr>
</tbody>
</table>

Data are presented as number of patients (%) or mean ± SD. (from paper 5)
**Paper III.**

The aim of Paper III was to analyse the incidence of the vectorcardiographic reperfusion peak and its possible value in patients with an acute myocardial infarction treated with angioplasty. Inclusion criteria were as follows: severe chest pain resistant to nitroglycerine, lasting for more than 20 minutes but less than 4 hours, ST-segment elevation > 1 mV in two contiguous standard leads or >2 mV in two contiguous chest leads, presenting at the emergency room at Umeå University hospital and no contraindications for thrombolytic treatment. Patients with bundle branch block or pacemaker-induced rhythm were excluded since reliable vectorcardiographic and electrocardiographic ST-segment analyses are precluded in these conditions. Thirty-two patients were included; twenty-four patients were treated with primary PCI (Primary Coronary Intervention) and eight with rescue PCI. Continuous ischemia monitoring with VCG was started immediately after admission to the coronary catheterisation laboratory.

**Paper IV**

One of the purposes of Paper IV was to study a daily-practice population of patients treated for AMI. The study included patients admitted to the CCU of Umeå University Hospital with a history of severe chest pain indicative of myocardial infarction. Inclusion criteria were as follows: chest pain less than 24 hours, ST elevation on scalar-ECG, no contraindications for thrombolytic treatment, availability of VCG recording equipment and significant initial VCG-changes [62, 71, 77]. Exclusion criteria were: bundle branch block, AV-block, pacemaker treatment, rescue angioplasty or previous inclusion in the study. Three-hundred fifty-five patients were included. Twenty of the patients entering the study were excluded for reasons as follows: rescue angioplasty (n=2), other causes of ST-elevation including pericarditis or old infarction (n=17) and repeated inclusion (n=1). All patients without significant deviation of ST-VM start (<100 μV) were excluded (n=66) [70, 71, 77]. Thus 269 patients were further analysed.

All patients were followed at least one year with respect to revascularisation and reinfarction except one patient who was lost for follow up at 1 year due to relocation abroad. Follow-up regarding death up to 5 years was performed for all except that one patient.

The baseline clinical characteristics are presented in Table 2.
Material and Methods

Vectorcardiography is based on the transformation of electrocardiographic signals into the vector–summation in the three orthogonal planes X, Y and Z using the Frank lead system described in 1956 [78] (Fig. 4). In the beginning, the use of vectorcardiography was limited to analyses of collected data since no real-time monitoring was possible. The development of a computer-based system for automated signal processing was performed by Martin Riiha and colleagues at the Chalmers Technical University, Gothenburg in the early 80s. Sederholm used this system to perform a number of studies on patients with acute myocardial infarction [79-83]. The system was further developed by Ortivus Medical AB, Täby, Sweden into the MIDA-system (Myocardial Infarction Dynamic Analysis). This system has been on the market for clinical use since 1986 and has undergone subsequent improvements to the ischemia-monitoring device we use today.

In all presented papers VCG were used for ischemia monitoring. Electrodes were placed according to Frank [78] (figure 4) and connected to a MIDA-1000 system or a CoroNet system, both developed by Ortivus Medical AB, Täby, Sweden. There are no relevant technical differences between these systems except for the signal storage rate that was 500 Hz in the MIDA system and 250 Hz in the CoroNet system. Beats were sampled and averaged for periods of 2 minutes (Papers I, II, IV, V) and for periods of 20 seconds (Paper III).

| Table 2. Clinical and vectorcardiographic characteristics of the patients |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                            | NO                          | YES                         |
| Total n = 269              | n =120 (45)                 | n =149 (55)                 | p-value                    |
| Age 66.6 ± 10.5            | 65.0 ± 10.8                 | 0.220                       |
| Female 33 (28)             | 39 (26)                     | 0.807                       |
| Previous infarction 35 (29)| 21 (14)                     | 0.002                       |
| Diabetes mellitus 23 (19)  | 15 (10)                     | 0.032                       |
| Hypertension 52 (44)       | 47 (32)                     | 0.041                       |
| Previous CVS 8 (7)         | 5 (3)                       | 0.207                       |
| Smokers 31 (27)            | 47 (32)                     | 0.363                       |
| Medication at admission    |                            |                            |
| Beta-blockers 40 (34)      | 32 (22)                     | 0.023                       |
| Aspirin 24 (20)            | 23 (15)                     | 0.296                       |
| Diuretics 27 (23)          | 21 (14)                     | 0.063                       |
| Anterior infarction 69 (59)| 66 (45)                     | 0.028                       |

Data are presented as number of patients (%) or mean ± SD. (from paper 4)

**On-line vectorcardiography**

Vectorcardiography is based on the transformation of electrocardiographic signals into the vector–summation in the three orthogonal planes X, Y and Z using the Frank lead system described in 1956 [78] (Fig. 4). In the beginning, the use of vectorcardiography was limited to analyses of collected data since no real-time monitoring was possible. The development of a computer-based system for automated signal processing was performed by Martin Riiha and colleagues at the Chalmers Technical University, Gothenburg in the early 80s. Sederholm used this system to perform a number of studies on patients with acute myocardial infarction [79-83]. The system was further developed by Ortivus Medical AB, Täby, Sweden into the MIDA-system (Myocardial Infarction Dynamic Analysis). This system has been on the market for clinical use since 1986 and has undergone subsequent improvements to the ischemia-monitoring device we use today.

In all presented papers VCG were used for ischemia monitoring. Electrodes were placed according to Frank [78] (figure 4) and connected to a MIDA-1000 system or a CoroNet system, both developed by Ortivus Medical AB, Täby, Sweden. There are no relevant technical differences between these systems except for the signal storage rate that was 500 Hz in the MIDA system and 250 Hz in the CoroNet system. Beats were sampled and averaged for periods of 2 minutes (Papers I, II, IV, V) and for periods of 20 seconds (Paper III).
Material and Methods

Figure 4. Frank leads. The lead placement and spatial axes are shown. Note that one lead is placed on the patient’s back. From these lead placements the 3 orthogonal components, X, Y and Z are derived.

The following vectorcardiographic parameters were studied:

**ST-VM and STC-VM**

ST-Vector Magnitude and ST-Change Vector Magnitude. ST-deviation was measured at 20 or 60 ms after the J-point. Conversion from J+20 to J+60 (or the opposite) was made possible by dedicated software from Ortivus Medical.

**ST-VM:** The absolute variable, ST-vector magnitude, was automatically calculated from the orthogonal leads according to the formula $\sqrt{X^2 + Y^2 + Z^2}$ in microvolts ($\mu$V) representing the total spatial ST-segment shift from the baseline.

**STC-VM:** The relative spatial difference between reference and current ST vector was calculated as follows: $\sqrt{(X_i - X_o)^2 + (Y_i - Y_o)^2 + (Z_i - Z_o)^2}$ in microvolts ($\mu$V) where ‘i’ represents the current measurement and ‘o’ represents the reference.

**ST-VM start:** The initial value of the spatial ST-vector magnitude (ST-VM).

**ST-VM max:** The maximal value recorded of ST-VM during the first 90 minutes after start of recording.

**ST-VM plateau:** The value when the trend curve, following a time of regression, has reached a stable value for at least 30 minutes.
The X, Y and Z coordinates of the ST-vector projected in the three perpendicular planes were used to analyse spatial rotational changes of the ST-vectors during transient increases of the ST-vector in Paper IV [84].

**QRS-VD**

QRS vector difference was defined as the area outlined between the reference complex and the current QRS complex in the three orthogonal leads: \(\sqrt{Ax^2 + Ay^2 + Az^2}\) (\(\mu\)Vs), where \(A\) represents the area outlined between the reference and the current QRS complex in the three orthogonal leads (X, Y and Z).

Changes in ST-VM, STC-VM and QRS-VD are calculated and presented in real-time as continuously updated trend curves. It is also possible to obtain a derived 12-lead ECG at each time point displayed on the screen.

**Recurrent ST episodes**

The cut-off level for regarding a recurrent episode of ST-VM or STC-VM elevation as significant was set to 50 \(\mu\)V according to previous studies [71, 74, 84-87].

**Reperfusion peak**

A reperfusion peak was defined as a transiently increased ST-VM of > 50 \(\mu\)V, measured from the start of the reperfusion peak, followed by a decrease of ST-VM within 20 minutes to a level lower than that at the start of the reperfusion peak. This definition of a reperfusion peak has been described in a previous study [88].

![Figure 5. A representative ST-VM trend curve of a patient with anterior ST-elevation infarction as displayed on-line. The arrows indicate where the start and plateau of ST-VM were measured and the reperfusion peak.](image-url)
Laboratory Procedures

Fibrinolytic variables

There are some precautions that have to be taken when samples for analysis of fibrinolytic variables are done. Due to the circadian rhythms of fibrinolysis, timing of samples must be defined and morning samples are recommended. This was, however, not applicable in our studies, since the timing of the samples were dependent upon the time for occurrence of the AMI. All venipunctures were done with a minimum of stasis and blood samples for fibrinolytic assays were drawn with a minimum of stasis into siliconized evacuated Stabilyte® tubes (Biopool, Umeå, Sweden) containing 1/10 volume of 0.5 mol/L acidified sodium citrate [89]. Immediate acidification of the sample is necessary to prevent PAI-1 from inactivating tPA. The samples were cold-centrifuged and the acidified plasma samples were frozen and stored at -70°C until analysis [90].

Blood samples for the analysis of vWF and the fibrinolytic variables were obtained immediately on arrival, before the start of the SK infusion, and after 4, 10, 16, 22 and 48 hours (Paper I), after 4 and 10 hours (Paper V) and after 4 hours (Paper II). For the vWF analyses, venous blood was drawn into siliconised venoject tubes containing 0.13 mol/L sodium citrate.

The fibrinolytic (tPA) activity was determined using a parabolic rate assay based on fibrin stimulation of the tPA-mediated conversion of plasminogen to plasmin [91]. The activity is reported in U/mL by referring to the activity of the International tPA Standard, coded 86/670 (National Institute for Biological Standards and Control, London).

The mass concentration of tPA in plasma was determined using an enzyme-linked immunosorbent assay (ELISA) [92].

PAI-1 activity was determined using the tPA assay [91], following the addition of an excess of tPA to the sample (40 IU/mL) and quantifying the remaining free enzyme as above. The activity is given in arbitrary U/mL, where 1 U of PAI-1 is defined as the amount of PAI-1 which inhibits 1 IU of the International tPA Standard (see above).

The reagent kits for assays of tPA and PAI-1 mass concentration (Imulyse) and tPA and PAI-1 activity (Spectrolyse/Fibrin) were purchased from Biopool AB, Umeå, Sweden.

Von Willebrand factor was measured with an ELISA [93], using reagents purchased from DAKO (Copenhagen, Denmark).

Streptokinase neutralising antibodies

The analysis of functionally neutralising antibodies to streptokinase (Paper II) was performed with a rapid bedside test, the thrombolysis assessment system (TAS™). The system is based on autologous clot lysis onset time as a quantitative measurement of the lytic response. The test cards contain thrombin allowing the
Material and Methods

rapid formation of a fibrin clot in the sample. Two test cards can be used; the one used in this study contained 100U/mL of SK, which will produce prolonged lysis times with samples containing high levels of antibodies to SK or inhibitors specific to SK or the SK-plasminogen complex. Thus, the method analysis the effect of neutralising antibodies to SK rather than the actual amount of SK antibodies. It is suggested that a lysis onset time of > 50 seconds indicates resistance to streptokinase treatment [94, 95].

Statistics

All quantitative observations were presented with descriptive statistical analyses, using mean, standard error of the mean (SEM), or standard deviation (SD). Student's t-test was used to compare the means unless otherwise specified. Chi-2, or when appropriate, Fisher's exact test were used to compare differences in outcome between groups

Paper I. Differences between sequential data were analysed by Friedman's two-way ANOVA and a p-value of ≤ 0.05 was considered significant. Selected comparisons were performed using Wilcoxon's rank-sum test, and due to repeated calculations, a p-value ≤ 0.01 was considered significant.

Paper II. Uni- and multivariate logistic regression analyses were performed with risk of no reperfusion as the dependent variable. Due to skewness of data and limited number of cases, predictors of outcome were tested as categorical variables and continuous variables were categorised when appropriate. Missing variables were treated as separate categories, which were omitted from the table.

Paper IV. Five-year mortality was calculated with the Cox regression analysis in comparison to traditional risk factors. The inter-observer accuracy was tested with a cross tabulation using the Cohen’s Kappa (κ). A kappa value greater than 0.75 represents excellent agreement beyond chance, while values below 0.40 represent poor agreement beyond chance. A kappa -value close to 1 indicates near perfect agreement [96].

Paper V. To test the relation between levels of risk determinants and the risk of recurrent ischemia, reinfarction and mortality Cox regression analysis was used to estimate hazard ratios (HR) and 95% confidence intervals (CIs) in univariate and multivariate models. In multivariate analysis we adjusted for traditional risk factors including age, sex, diabetes, hypertension, smoking and previous myocardial infarction. Since diabetes, hypertension, smoking and previous myocardial infarction had no impact on hazard ratios, only data adjusted for sex and ages are presented. Predictors of outcome were tested as continuous variables and were categorised when appropriate. Fibrinolytic variables and von Willebrand factor were categorised into tertiles in the Cox regression analysis. In the multivariate tests, missing values were replaced by the mean value for the referent group in the continuous variables and in the categorical variables categorized in a separate group. This allowed all subjects to be included in the multivariate Cox regression analyses.
REVIEW OF THE RESULTS

Fibrinolytic variables, vWF, SK neutralising antibodies and outcome

Papers I, II and V

These three papers studied patients with acute myocardial infarction treated with SK and monitored by VCG for the detection of reperfusion and/or episodes of recurrent ischemia.

Thrombolytic treatment with SK reduces mortality and preserves left ventricular function in patients with acute myocardial infarction [42, 44, 97]. In studies comparing the effect of tPA and SK, there is a slight disadvantage for SK regarding mortality and early (90-min) angiographic patency (approximately 50-60 % of occluded infarct-related arteries are successfully reopened by treatment with SK). On the other hand, bleeding complications are less common with SK [44, 45]. This suggests that at least one-third of patients treated with SK do not achieve successful thrombolysis. Different responses by the fibrinolytic system and vWF to SK may influence the outcome. Therefore, we characterized the activity of fibrinolytic and haemostatic variables during SK treatment to detect any possible relationship between the outcome of SK treatment and components of the fibrinolytic system and/or vWF.

In Paper I, which was planned as a pilot study for Paper II and V, the primary objective was to examine levels of tPA mass concentration and activity, PAI-1 mass concentration and activity and von Willebrand factor, immediately prior to and after SK treatment in patients with an evolving myocardial infarction as compared with patients with AMI who were not treated with SK. A secondary objective was to relate changes in the fibrinolytic and haemostatic systems to successful reperfusion.

The study included a SK-treated group (Group A), a control group of 25 patients with AMI who had not been treated with thrombolytic agents (Group B), and 10 patients (Group C) referred to the CCU with chest pain not developing an AMI.

Blood samples were obtained immediately on arrival at the CCU before the infusion of SK and after 4, 10, 16, 22 and 48 hours respectively. tPA activity and mass concentration, PAI-1 activity and mass concentration and vWF were analysed and are presented in Figure 6.

No significant differences were shown in plasma levels of PAI-1, tPA and vWF between the two AMI groups (A and B) at admission and at 22 and 48 hours. At 4 and/or 10 hours, significant differences were seen in all the studied variables, except for tPA mass concentration. Significant changes over time in all the fibrinolytic variables studied and vWF were observed in group A. In Group B, changes over time were less prominent and seemed to appear later; there were no significant changes in tPA mass concentration and tPA activity. In Group C, no significant changes over time were observed.
Correlation with VCG-findings

No patient in Group B showed VCG-signs of spontaneous reperfusion. In Group A, 20/36 (56%) patients were classified as being successfully reperfused.

No significant correlations between the studied haemostatic variables at admission and reperfusion as assessed by VCG were found.

Most of the SK-treated patients developed high and early peak levels of tPA activity. An arbitrary cut-off level for peak tPA activity, $\geq 30$ U/mL, indicating a substantial activation of the fibrinolytic system, was selected. In Group A, values above this cut-off level were observed within 10 hours in 16/20 (80%) in the group with VCG signs of successful reperfusion, whereas only 7/16 (44%) in the group without signs of reperfusion (p<0.05) showed high levels of tPA activity.

Based on these findings Papers II and V were performed.
In *Paper II*, 104 patients (34 female, 70 male, age $66.4 \pm 9.8$ years) with signs of acute myocardial infarction, were analysed. Fifty-five patients (53%) were classified as having been successfully reperfused. Blood samples for the analysis of neutralising antibodies to SK and for tPA activity were obtained immediately on arrival at the CCU before the infusion of SK. Another sample for tPA analysis was taken four hours later. There were no significant differences between the groups (reperfusion successful vs. unsuccessful) in terms of baseline characteristics including tPA activity on arrival ($0.96 \pm 2.1$ vs. $0.97 \pm 4.0$) except for time to treatment, which was longer in the group where reperfusion was unsuccessful ($4.0 \pm 2.4$ h vs. $6.2 \pm 4.3$ h, $p<0.05$).

The odds ratio for the risk of failed reperfusion was calculated in univariate and multivariate logistic regression models (Table 3). In short, no significant associations between levels of functionally neutralising SK antibodies and successful reperfusion were found. The presence of functionally neutralising SK antibodies was also tested with the suggested cut-off level of 50 seconds, but there was still no significant relationship to the success/failure of reperfusion (OR $2.02$, 95% CI: $0.85$-$4.01$).
### Table 3. Univariate and multivariate Logistic regression: risk of no reperfusion

<table>
<thead>
<tr>
<th></th>
<th>UNIVARIATE</th>
<th></th>
<th>MULTIVARIATE</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>SK neutralising antibodies</td>
<td>n</td>
<td>OR 95% CI</td>
<td>OR 95% CI</td>
<td></td>
</tr>
<tr>
<td>&lt; 51.3</td>
<td>34</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>51.3 - 62.7</td>
<td>34</td>
<td>2.65 0.98 - 7.10</td>
<td>2.66 0.76 - 9.33</td>
<td></td>
</tr>
<tr>
<td>&gt; 62.7</td>
<td>34</td>
<td>2.09 0.78 - 5.59</td>
<td>0.92 0.11 - 1.64</td>
<td></td>
</tr>
<tr>
<td>Time to treatment</td>
<td>Hours</td>
<td>1.17 1.03 - 1.32</td>
<td>1.17 1.02 - 1.35</td>
<td></td>
</tr>
<tr>
<td>tPA activity at 4 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 25</td>
<td>37</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>&gt; 25</td>
<td>63</td>
<td>0.18 0.08 - 0.44</td>
<td>0.17 0.06 - 0.51</td>
<td></td>
</tr>
<tr>
<td>Aspirin treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>86</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13</td>
<td>1.03 0.32 - 3.32</td>
<td>1.07 0.41 - 10.25</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>34</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>70</td>
<td>1.00 0.44 - 2.28</td>
<td>0.95 0.31 - 2.92</td>
<td></td>
</tr>
<tr>
<td>Smokers</td>
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<td></td>
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<td></td>
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<td>No</td>
<td>60</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>36</td>
<td>0.59 0.26 - 1.38</td>
<td>0.74 0.25 - 2.15</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>104</td>
<td>1.01 0.67 - 1.51</td>
<td>1.02 0.97 - 1.07</td>
<td></td>
</tr>
<tr>
<td>CVS</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>95</td>
<td>1.00</td>
<td>1.00</td>
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</tr>
<tr>
<td>Yes</td>
<td>5</td>
<td>0.28 0.03 - 2.58</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>91</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
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<td>7</td>
<td>0.87 0.18 - 4.13</td>
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<tr>
<td>Hypertension</td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>65</td>
<td>1.00</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>35</td>
<td>0.98 0.43 - 2.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>80</td>
<td>1.00</td>
<td>1.00</td>
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</tr>
<tr>
<td>Yes</td>
<td>20</td>
<td>0.50 0.46 - 3.26</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(from paper 2)

The development of a high tPA activity, > 25 U/mL, at four hours was associated with a significantly higher rate of reperfusion. Aspirin treatment, sex, smoking, age, a history of previous cerebrovascular disease, diabetes, hypertension or previous myocardial infarction did not show a significant association with the result of reperfusion therapy. In the multivariate analysis, high tPA activity at four
hours was associated with a higher rate of reperfusion, and a greater time to treatment was associated with a higher risk of failed reperfusion.

There was no significant correlation between functionally neutralising SK antibody levels and tPA activity at four hours \((r = -0.18, p = 0.08, \text{ Spearman's correlation})\).

In Paper V, all the 139 patients included were analysed. The aim of that study was to evaluate the influence of the pre-treatment levels, and the levels during the acute treatment phase, of components of the fibrinolytic system and vWF, on outcome defined as reperfusion by VCG and on reinfarction and mortality at one year and mortality at five years. Blood samples for the analysis of tPA activity, PAI-1 activity, PAI-1 mass concentration and vWF were obtained immediately on arrival and after 4 and 10 hours.

Successful reperfusion was seen in 53% (72 patients) and recurrent ischemia in 44/129 (34%) patients. The group with failed reperfusion had a longer delay to treatment, more often a previous AMI and more often an anterior location of the infarction than those with successful reperfusion. They also had smaller initial ST deviations on arrival indicating smaller myocardium at risk for infarction.

No significant association between pre-treatment levels of the measured fibrinolytic variables or vWF and reperfusion or recurrent ischemia was found. Only high ST deviation at arrival (OR 1.029, 95% CI 1.001-1.056) seemed to be associated with an increased risk for recurrent ischemia. No association between sex, age, delay to treatment, previous MI, smoking or previous medication and fibrinolytic response was found.

The previously reported (Paper II) association between the fibrinolytic response to SK and successful reperfusion was also found in this study where another 20 patients were included in the analysis (Table 4).

Table 4. Relation between fibrinolytic response to streptokinase and successful reperfusion in 134 patients with myocardial infarction.

<table>
<thead>
<tr>
<th>Reperfusion according to VCG</th>
<th>NO</th>
<th>YES</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n =62 (46)</td>
<td>n =72 (55)</td>
<td></td>
</tr>
<tr>
<td>tPA activity at 4 h &gt;25</td>
<td>31 (50)</td>
<td>53 (74)</td>
<td>0.003</td>
</tr>
<tr>
<td>tPA activity at 4 h &lt;25</td>
<td>31 (50)</td>
<td>19 (26)</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as n (%). (from paper 5)

In the analysis of risk for death at one and five years, diabetes, hypertension and smoking were omitted from further analyses, as they were not associated with increased risk in the univariate analysis. The presented data from the multivariate models were adjusted for age and sex. Further adjustment for previous myocardial infarction did not alter the results (data not shown).
The pre-treatment levels of the measured fibrinolytic variables were not related to mortality at one year. An association was seen with high PAI-1 mass concentration on admission and risk for death at five years, an association that was not present at one year. High levels of vWF on admission and after the SK treatment showed an association with increased risk for death during the first year and after five years in the univariate analysis. When adjusted for age and sex in the multivariate analysis this association disappeared. Elevated levels of PAI-1 mass concentration and PAI-1 activity after the start of SK treatment were associated with higher risk for death at one year both in the uni- and multivariate analyses, though not at five years. The results from the analysis of risk for death at five years are shown in Table 5.

Table 5. Univariate and multivariate Cox regression analysis, risk for death after five years. Except for sex, only variables significant or borderline significant (p<0.1) in the univariate analysis are shown.

<table>
<thead>
<tr>
<th></th>
<th>UNIVARIATE HR</th>
<th>95% CI</th>
<th>MULTIVARIATE HR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAI-mass at 0 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;16.7</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>&gt;38.9</td>
<td>2.48</td>
<td>1.07 – 5.50</td>
<td>2.92</td>
<td>1.24 – 6.90</td>
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<tr>
<td>vWF at 0 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;168</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>168 – 237</td>
<td>2.35</td>
<td>1.01 – 5.50</td>
<td>1.58</td>
<td>0.65 – 3.83</td>
</tr>
<tr>
<td>&gt;237</td>
<td>2.55</td>
<td>1.09 – 5.96</td>
<td>1.58</td>
<td>0.64 – 3.90</td>
</tr>
<tr>
<td>vWF at 4 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;216</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>216 – 280</td>
<td>1.56</td>
<td>0.69 – 3.52</td>
<td>1.28</td>
<td>0.56 – 2.89</td>
</tr>
<tr>
<td>&gt;280</td>
<td>2.04</td>
<td>0.93 – 4.96</td>
<td>1.44</td>
<td>0.64 – 3.23</td>
</tr>
<tr>
<td>vWF at 10 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;215</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>215 – 282</td>
<td>1.61</td>
<td>1.10 – 5.49</td>
<td>1.23</td>
<td>0.52 – 2.91</td>
</tr>
<tr>
<td>&gt;282</td>
<td>2.46</td>
<td>1.10 – 5.48</td>
<td>1.68</td>
<td>0.73 – 3.89</td>
</tr>
<tr>
<td>Age (years)</td>
<td>1.07</td>
<td>1.03 – 1.10</td>
<td>1.06</td>
<td>1.03 – 1.20</td>
</tr>
<tr>
<td>Male sex</td>
<td>1.36</td>
<td>0.75 – 2.46</td>
<td>0.75</td>
<td>0.40 – 1.30</td>
</tr>
<tr>
<td>Previous infarction</td>
<td>2.03</td>
<td>1.10 – 3.75</td>
<td>0.54</td>
<td>0.28 – 1.01</td>
</tr>
<tr>
<td>Medication at admission</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aspirin</td>
<td>2.34</td>
<td>1.21 – 4.52</td>
<td>1.52</td>
<td>0.19 – 4.72</td>
</tr>
<tr>
<td>Diuretics</td>
<td>2.95</td>
<td>1.65 – 4.52</td>
<td>2.07</td>
<td>1.11 – 3.85</td>
</tr>
<tr>
<td>CK max</td>
<td>1.01</td>
<td>0.99 – 1.02</td>
<td>1.02</td>
<td>1.01 – 1.03</td>
</tr>
<tr>
<td>Recurrent ischaemia</td>
<td>1.67</td>
<td>0.91 – 3.07</td>
<td>1.72</td>
<td>0.9 – 3.16</td>
</tr>
</tbody>
</table>

HR = hazard ratio, CI = confidence interval. (from paper 5)
Review of the Results

Recurrent ischemia during the first 24 h after the start of SK treatment was an independent risk factor for death at one year, but not at five years. Successful reperfusion according to the pre-specified, angiographically controlled criteria failed to show any significant association with outcome. However, when using the reduction of ST-VM by ≥ 50% within 60 min as criterion, a significant mortality reduction was seen at five years (HR 0.47, 95% CI 0.25 – 0.89), and a non-significant lower mortality was seen at one year (HR 0.44, 95% CI 0.17 – 1.12), and these results were not modified in the multivariate analysis.

Reperfusion, reperfusion peak, vectorcardiography and outcome

Papers III and IV

In Papers III and IV we analyzed the reperfusion peak in patients with AMI treated with primary- or rescue PCI (Paper III) and in patients treated with thrombolysis (Paper IV).

In Paper III a reperfusion peak was defined as: an increase in ST-VM of > 50 µV, starting within two min after the reopening of the infarct-related coronary artery, and that was followed by an immediate decrease to a level lower than that before the start of the reperfusion peak within 20 min, and that which fulfilled the predefined criteria for reperfusion.

Twenty-four patients were treated with primary PCI and eight patients with rescue PCI. A complete occlusion of the infarct-related coronary artery was found in all but one patient, in which a TIMI-1 flow was present at the time of angioplasty. The procedure was successful in all patients but a “no-reflow” phenomenon, as judged by the operator, was observed in three of the patients. Twenty patients (63%) developed a reperfusion peak. The mean duration of the reperfusion peak was 8.1 ± 6.5 min. The occurrence of a reperfusion peak and its possible relationships to other clinical variables are summarized in Table 6.

Table 6. Characteristics of patients with and without a reperfusion peak

<table>
<thead>
<tr>
<th></th>
<th>peak (n = 20)</th>
<th>no peak (n = 12)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ST-VM at start (µV)</td>
<td>404 (108-703)</td>
<td>188 (13-365)</td>
<td>0.002</td>
</tr>
<tr>
<td>CK-max (µkat/L)</td>
<td>48.8 (4.6-15.6)</td>
<td>25.9 (1.7-136)</td>
<td>0.162</td>
</tr>
<tr>
<td>CK MB-max (µkat/L)</td>
<td>4.8 (0.8-11.2)</td>
<td>2.8 (0.2-13.8)</td>
<td>0.207</td>
</tr>
<tr>
<td>LD-1 (µkat/L)</td>
<td>18.4 (25-260)</td>
<td>11.6 (2.1-46.9)</td>
<td>0.268</td>
</tr>
<tr>
<td>Time to treatment (min)</td>
<td>116 (25-260)</td>
<td>111 (10-245)</td>
<td>0.872</td>
</tr>
<tr>
<td>Beta blocker on admission</td>
<td>5 (25)</td>
<td>6 (50)</td>
<td>0.144</td>
</tr>
<tr>
<td>ASA on admission</td>
<td>3 (15)</td>
<td>2 (17)</td>
<td>0.377</td>
</tr>
<tr>
<td>Primary angioplasty</td>
<td>14 (70)</td>
<td>10 (83)</td>
<td>0.243</td>
</tr>
</tbody>
</table>

Figures indicate numbers of patients (%) or mean (range).
The patients who developed a peak presented with a significantly larger ST-VM, \( p=0.004 \) and developed significantly higher peak levels of creatine kinase (CK) \( p=0.014 \) and creatine kinase iso-enzyme B (CK MB) \( p=0.009 \).

The aim of *Paper IV* was to analyse the incidence of the reperfusion peak and to assess the relationship between a reperfusion peak and prognosis in a population of clinically routine patients with AMI treated with thrombolysis. The definition of a reperfusion peak in this population, with no angiographic control of reperfusion was: a transiently increased ST-VM of > 50 µV, measured from the start of the reperfusion peak, followed by a decrease within 20 min to a level lower than that before the start of the reperfusion peak. In this study the reduction of ST-VM > 50% within 60 min after the start of treatment was used as the definition of reperfusion. [72]

Inter-observer variation was tested with a Kappa-value of 0.82 for the assessment of reperfusion and 0.76 for reperfusion peaks.

A reperfusion peak was found in 111 /149 (75%) of the patients fulfilling the VCG criteria for successful reperfusion (41% of all patients) and in one patient not fulfilling those criteria. A trend graph from a representative patient is shown in Figure 5. In the group with successful reperfusion, the patients developing a peak had a non-significant trend towards larger initial ST changes indicating larger area of myocardium at risk, \( (\text{ST-VM} \mu \text{V})_{\text{start}} 278 \pm 135 \text{ vs. } 235\pm 115, p=0.079 \). No significant differences in five-year mortality between patients developing a reperfusion peak and those who did not in the group with successful reperfusion were found (HR 0.69, 95% CI 0.28-1.65, \( p=0.401 \)).

Successful reperfusion and a reperfusion peak were both powerful predictors of survival and freedom from reinfarction at one month, one year and at five years. Successful reperfusion proved to be the better predictor of the two and the hazard ratios (HR) corrected for age, sex, hypertension and diabetes are presented in Table 7.
Review of the Results

Table 7. Hazard Ratios corrected for sex, diabetes, hypertension and age at different points of measure. Only successful reperfusion and age are displayed

<table>
<thead>
<tr>
<th></th>
<th>HR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Death at 30 days</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful reperfusion</td>
<td>0.37</td>
<td>0.14 - 0.96</td>
<td>0.042</td>
</tr>
<tr>
<td>Age (increment by year)</td>
<td>1.048</td>
<td>0.99 - 1.10</td>
<td>0.065</td>
</tr>
<tr>
<td><strong>Death or new AMI at 30 days</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful reperfusion</td>
<td>0.39</td>
<td>0.19 - 0.77</td>
<td>0.007</td>
</tr>
<tr>
<td>Age</td>
<td>1.043</td>
<td>1.01 - 1.08</td>
<td>0.018</td>
</tr>
<tr>
<td><strong>Death at one year</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful reperfusion</td>
<td>0.52</td>
<td>0.26 - 1.01</td>
<td>0.054</td>
</tr>
<tr>
<td>Age</td>
<td>1.08</td>
<td>1.04 - 1.12</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Death or new AMI at one year</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful reperfusion</td>
<td>0.54</td>
<td>0.32 - 0.93</td>
<td>0.025</td>
</tr>
<tr>
<td>Age</td>
<td>1.06</td>
<td>1.03 - 1.09</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Death at five years</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Successful reperfusion</td>
<td>0.5</td>
<td>0.31 - 0.81</td>
<td>0.005</td>
</tr>
<tr>
<td>Age</td>
<td>1.06</td>
<td>1.03 - 1.09</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

(from paper 4)

The groups with failed vs. successful reperfusion showed significant differences in vectorcardiographic variables. The ST-VM (μV) start was significantly smaller in the group with failed reperfusion (215 ± 112 vs. 267 ± 131, p < 0.001). The development of VCG changes was slower in the group with failed reperfusion with a longer time to a plateau in ST-VM (211 ± 108 vs. 124 ± 98 min, p < 0.001). The difference between ST-VM start and ST-VM plateau, which has been proposed to indirectly reflect the amount of salvaged myocardium, was significantly greater in the patients with a presumed successful reperfusion (108 ± 94 vs. 182 ± 115 μV, p<0.001).
DISCUSSION

Patients

The patients included in these studies were recruited in order to study the “real-world” situation. However, there was one exception from the “real word.” In Paper III, primary PCI was not yet standard treatment at our clinic at the time of the study.

Our patients represent a typical hospital clinical practice with an infarct population of patients with a longer delay to treatment and higher age as compared with most randomised thrombolysis trials with selected populations.

For example, the mortality observed in Paper IV was greater than that in the GUSTO-trial (Global Utilisation of Streptokinase and Tissue Plasminogen Activator for occluded coronary arteries) at 30 days (7.4% vs 6.3-7.4%) and at 1 year (14.7% vs. 9.1-10.1%, respectively) [98].

Streptokinase

Why study streptokinase, is there anybody still using it? The answer to this question is – yes! Even-though some of these studies were started a long time ago, the information is still valid in the daily practice at present.

The two major advantages for SK, as compared to the newer fibrinolytic agents, are the lower cost and the lower risk for stroke with SK [45, 99]. In two randomised studies including 20,891 and 41,299 patients, respectively, no survival benefit for tPA, as compared with SK, was found [100, 101]. However, the accelerated tPA regimen introduced in GUSTO-1 was reported to result in 10 fever deaths per 1000 tPA-treated patients but also 3 additional strokes of whom one survived with a residual deficit. After that, one could assume that the incentive of funding from the pharmaceutical companies to support further comparative studies with SK was minimal. One could also speculate about why so little was done for tailoring SK therapy the same way that was done for tPA. There are actually reports from smaller groups of patients in which both an accelerated regimen for SK treatment and a double-bolus regimen for pre-hospital use, has been tested with promising results [102, 103]. These reports are from Eastern Europe and published in non-English journals. All in all, there must still be some doubt about the cost-benefit for some patient groups of the ten-fold more expensive modern fibrinolytic drugs compared with SK.

Vectorcardiography

One of the aims of this dissertation was to further evaluate the usefulness of ischemia monitoring with VCG for patients with ST-elevation AMI. When these studies were initiated, ST monitoring for the detection of ischemia or evaluation of reperfusion were not as well established as they are today. In order to properly
handle all the potential treatment options available today, we need an accurate, non-invasive method to obtain information about the dynamic changes in coronary flow in real-time.

Another important feature is the reproducibility of the results obtained with the method. This has been validated in two previous reports, both with encouraging results [104, 105]. In Paper IV we tested the inter-observer variation between two independent observers. They were blinded to patient outcome and analysed 125 VCG trend curves for the presence or absence of a reperfusion peak and successful reperfusion or not.

The testing of inter-observer validity resulted in a Kappa-value of 0.82 for the assessment of reperfusion and 0.76 for reperfusion peaks, both indicating good agreement between the two observers. This can be compared to the so called “gold-standard” coronary angiography, where reports of major differences between the clinical interpretation and the interpretation in core-labs have been presented [106, 107]. This mis-match has also been reported to result in inappropriate revascularisation procedures [108].

Thus, I suggest that this kind of information can be reliably obtained from VCG and in all five presented papers we used VCG for the detection of ischemia and evaluation of reperfusion.

**Assessment of reperfusion**

As pointed out earlier, the restoration of both normal epicardial flow and normal microvascular circulation is crucial for reducing myocardial cell damage. In the clinical situation, the possibility to rapidly and reliably judge whether or not the initiated reperfusion therapy has reached this goal is of fundamental value. The so called “golden standard” coronary angiography only provides a “snap-shot” picture of the situation in the coronary arteries, it is costly, invasive and for many patients, not easily available. It has also been shown not to adequately reflect the continuum of reperfusion [38]. The non-invasive detection of reperfusion in real-time with the help of ST monitoring, on the other hand, could easily be implemented for almost all patients.

**ST-VM resolution.**

A number of studies have addressed the issue of relation between ST resolution and angiographic patency with different ECG-methods [39, 62, 63, 65, 67, 68, 109-113]. The time for ST analysis and the time for angiography have been different, and the cut-off levels for ST-resolution have varied between 20% and 70%. Klootwijk et al. could not demonstrate any difference in the accuracy of three tested systems for continuous ST monitoring (continuous 12-lead ECG, VCG, three-lead Holter) in predicting reperfusion in the GUSTO-1 study [65]. This study used either ST-resolution at 90 or 180 min when relating changes in the VCG trend curves with angiographic patency [65]. Another, more complex definition, including ST-
VM decline 30 min prior to angiography of \( \geq 0.83 \, \mu V/min \) and/or QRS-VD increase \( \geq 0.10 \, \mu Vs/min \) and a visual evaluation of the trend curves until the first injection of contrast was used by Dellborg et al. [63]. It is more of a semantic dilemma whether ST-VM resolution at a specified time-point should be called “reperfusion” or be regarded as a non-invasive marker of prognosis. Conservative cardiologists, still believing in angiographic patency as the “golden standard”, might have trouble in accepting non-invasive markers of reperfusion. There are, however, data supporting the superiority of ST resolution over angiographic coronary flow in predicting prognosis [39]. There are also a number of studies in which ST resolution has been statistically correlated with prognosis [39, 74, 109, 114-117]. There has been a debate on what to measure, and at what time point. In 2000, de Lemos et al. suggested that ST resolution measured at 60 min, as compared to 90 min, provides better prognostic information in patients treated with lanoteplase or alteplase [118]. Three years later, Johanson et al. presented an analysis of patients treated with alteplase or TNK where the time to different grades of ST resolution (20, 30, 50, and 70%) was measured and related to 30-day mortality [72]. Their conclusion was that the optimal cut-off for ST-segment resolution is 50% measured at 60 min. This development over the years is the background to why we initially used the definition from Dellborg et al., and in Paper IV switched to the cut-off level proposed by Johanson et al.

Since SK has been shown to accomplish coronary artery patency later than tPA, and thereby can be suggested to have a slower onset of effect, it could be assumed that ST resolution at 60 min might be less appropriate in SK-treated patients. However, when we examined both of these two reperfusion criteria in Paper V, we found that successful reperfusion according to the angiographically controlled criteria [63] failed to show any significant association with outcome, but resolution of ST-VM by \( \geq 50\% \) within 60 min was associated with a significant mortality reduction at five years (HR 0.47, 95% CI 0.25 – 0.89), and a non-significantly lower mortality was seen at one year (HR 0.44, 95% CI 0.17 – 1.12).

In future studies and for the early clinical evaluation of patients treated with reperfusion therapy, there are reasons to use ST resolution by \( \geq 50\% \) within 60 min as a prognostic marker and as a sign of successful reperfusion.

**Reperfusion peak**

When the reperfusion peak first was observed and subsequently investigated in our experimental research lab in a closed-chest pig occlusion – reperfusion model, it was related to the size of the myocardium at risk and longer duration of occlusion i.e. larger infarctions [84]. When we observed patients who were treated with thrombolysis for AMI and were monitored by VCG in the clinic, we found that the reperfusion peak also appeared quite frequently among them. The results from the experimental studies and clinical observations made us wish to further examine the nature and possible importance of this, somewhat paradoxical, phenomenon. In the
first study (Paper III), we described the reperfusion peak in the setting of primary- or rescue PCI, a situation that has the advantage of exactly defining the time-point when the mechanical reperfusion of the coronary artery is achieved. ST-VM before coronary angiography was significantly larger (p=0.004) and peak enzyme levels were higher (p=0.014) in patients who developed a reperfusion peak. The larger infarctions, as measured by enzyme release, should not necessarily lead to the conclusion that the reperfusion peak indicates larger myocardial injury. A larger ST-VM at presentation indicates a larger myocardium at risk, and it is most likely that patients with a larger myocardium at risk develop larger infarcts when given the same treatment as patients with a small myocardium at risk. However, this observation might indicate that the reperfusion peak could be a sign of a reperfusion injury with accelerated cell death due to the abrupt onset of blood flow [119]. Shorter duration of occlusion of the coronary arteries (as compared with normal PCI), does not produce a reperfusion peak therefore suggesting that cell injury may be involved. This made us believe that the reperfusion peak was a sign of abrupt reperfusion of injured myocardium. Another finding supporting this assumption is that the analysis of the spatial amplitude and direction/rotation of the ST-vector (Paper IV) in a reperfusion peak, as compared with events of late recurrent ischemia, showed a striking resemblance.

The pathophysiological mechanism of the reperfusion peak is still unclear. It is likely that potassium homeostasis, as described earlier [120, 121], causes these rapid changes in repolarisation since the abrupt onset of reflow (wash out) and increased cellular membrane injury result in raised extracellular potassium levels. We have also observed in an occlusion-reperfusion pig model, an abrupt increase in potassium concentration measured by microdialysis from the infracted myocardium at the time of reperfusion and where a reperfusion peak was present (unpublished data).

In Paper V, where patients with AMI treated with thrombolysis were studied, we showed that a reperfusion peak was found in a total of 112/269 (42%) patients and in 111/149 (75%) of the patients fulfilling the VCG-criteria for successful reperfusion. This compares well with the findings in Paper III where we found a reperfusion peak in 20/32 (63%) patients treated with primary coronary angioplasty. The reperfusion peak appears to be as common among patients treated with thrombolysis having a successful reperfusion as it is for patients that are successfully reperfused by primary angioplasty.

The prognostic implications of the reperfusion peak were evaluated in Paper IV. One conclusion from those results was that the occurrence of a reperfusion peak did not add any vital information on prognosis when compared to successful reperfusion per se when reperfusion was defined as ST-VM resolution of \( \geq 50 \% \) at 60 minutes. These two entities are inter-related and there is good evidence for the positive prognostic information of ST-resolution. This does not mean that the
Discussion

reperfusion peak bears no importance on prognosis. Actually, it does, but when tested in a multi-variate model it could not match ST-VM resolution.

What then are the clinical implications of a reperfusion peak? We could not show any differences in mortality or risk for reinfarction during the first year after treatment among patients with assumed successful reperfusion with or without a reperfusion peak. ST-VM resolution of $\geq 50\%$ at 60 minutes was a better prognostic indicator than a reperfusion peak. Still, the reperfusion peak must be interpreted as a marker of reperfusion. Most important, there is a potential risk of misinterpreting the development of a reperfusion peak as aggravated ischemia. In clinical practice we have noticed that this can lead to inadequate treatment decisions such as unnecessary re-administration of a fibrinolytic agent or referring the patient for urgent rescue PCI which may not be needed, and may indeed confer additional risk for the patient.

Recurrent ischemia

The significance of ST-VM episodes of recurrent ischemia in the setting of an AMI [62, 71, 73, 74, 122, 123] and in patients with unstable angina pectoris have been evaluated in previous studies [87, 124-127]. Although not a major aim of these studies, the prognostic information yielded from the VCG registrations of our patients in Paper V confirmed that recurrent ST-VM episodes after thrombolysis are a significant indicator of worse prognosis. In this study (of only 139 patients) it proved to be an independent risk factor for death at one year (HR 2.40, CI 1.01 – 5.69), but not at five years.

The Fibrinolytic system and von Willebrand factor.

The knowledge about the influence of fibrinolytic and haemostatic systems on the success of SK treatment in AMI was limited when Papers I, II and V were initiated. As stated earlier, more than one-third of patients treated with SK do not achieve successful thrombolysis. Different responses by the fibrinolytic system to SK and to the AMI may influence the outcome. Thus, it would be of great value to characterize the activity of fibrinolytic and haemostatic variables before and during SK treatment in order to identify the subgroup of patients with poor response to SK treatment. This would give the opportunity to find an alternative or adjunctive treatment.

In Paper I, the primary objective was to examine the fibrinolytic system (levels of tPA mass concentration and activity, PAI-1 mass concentration and activity) and von Willebrand factor mass concentration, immediately prior to and after SK treatment in patients with an evolving myocardial infarction as compared with patients with AMI who were not treated with SK. A secondary objective was to compare changes in the fibrinolytic and haemostatic system and the clinical outcome defined as reperfusion assessed by VCG. In this study, increases in all the measured fibrinolytic variables during the initial phase of infarction were demon-
strated in the SK-treated group. Our results were confirmed in a later study published by Paganelli et al., [128]. Patients with AMI treated with r-tPA, SK or PCI were compared regarding levels of PAI-activity and PAI-1 mass concentration before and after the start of treatment. Patients treated with PCI showed no significant changes. Both patients treated with SK and r-tPA showed significant increases in both PAI-1 activity and PAI-1 mass concentration. The patients treated with SK had a more pronounced increase than those treated with r-tPA.

What is the potential mechanism behind this observed stimulation of the fibrinolytic system by SK? Levels of the fibrinolytic variables are influenced not only by an acute phase reaction but also by bacterial toxins and this may have played some role in the prominent increase in PAI-1 subsequently seen in the SK-treated group [129, 130]. Since r-tPA also induces an increase in PAI-1, some other mechanism is probably involved. One possible explanation could be that the treatment induces an increased release of tPA and PAI-1 from the endothelium.

The distinct increase in measured tPA activity early after the start of SK treatment is to be interpreted as a measure of the fibrinolytic response to the given drug. In this situation it does not reflect an abrupt increase in endogenous production or release. The method is based on measuring generated lytic activity against a plasmin chromogenic substrate, which therefore also responds to the activity of the SK-plasminogen complex. Two-thirds of the SK-treated patients in Paper I developed high and early peak levels of tPA activity at 4 h and 10 h, leaving one-third that did not display high tPA activity levels, thereby indicating that SK did not optimally activate the fibrinolytic system in those patients. This difference in response to SK was expressed as a significant difference in the likeliness to achieve successful reperfusion in the patients in Paper I. The patients who developed high and early peak-levels of tPA activity achieved successful reperfusion to a significantly higher extent than those who did not. When looking at the patients in Paper II, the same relation was found. The only remaining significant association with the result of SK treatment in the multivariate regression model was a lower risk of failed reperfusion in the group which developed high tPA activity (≥ 25 U/mL) at four hours (OR –0.17, 95% CI: 0.06-0.51). Paper V examined the same patients as in paper II adding another 20 patients analyzed regarding tPA levels and reperfusion. As expected, this did not change the results.

Cut-off levels for tPA activity.

In paper I we chose an arbitrary cut-off level for tPA activity at 30 U/mL measured at 4 or 10 hours after the start of treatment. This could be a subject for discussion, but the differences between the groups persisted when calculations were made with a cut-off level of 10 and 20 U/mL (p=0.024 and 0.019, respectively) but did not reach statistical significance when 40 U/mL was used (p=0.074). In Papers II and V, a cut-off level at 25 U/mL measured 4 hours after the start of treatment was
Discussion

used since it showed to be the best level to discriminate between failed and successful reperfusion in that larger group of patients.

**Timing of the blood samples**

Blood sampling started at admission and not at the onset of symptoms (which is an impossible task). Our main purpose was to study the time course of the fibrinolytic and haemostatic variables in AMI-treated with and without SK. In this situation, the time schedule did not allow analysis of the documented circadian variation in the fibrinolytic variables [131, 132].

Both coronary artery disease and acute inflammatory disease have been shown to blunt this normal circadian fluctuation [133, 134]. The time at which blood samples were taken is therefore unlikely to account for the pronounced differences in fibrinolytic and haemostatic variables observed between the patients treated with SK and those not treated with SK.

When using the best instrument invented so far, “the retrospectoscope”, the tPA activity should have been analyzed earlier after the start of treatment. Probably it would have been better to evaluate the effect of SK treatment already at 30 min or at 1 h, since the desired effect of the drug is to accomplish reperfusion as soon as possible after the administration.

**Relation to outcome**

The aim of Paper V was to evaluate if components of the fibrinolytic system and vWF are associated with the outcome of MI treatment with streptokinase. Outcome was defined as reperfusion and/or recurrent ischemia assessed by VCG, mortality and reinfarction at one year and mortality at five years.

**Reperfusion and recurrent myocardial ischemia**

No associations between pre-treatment levels of tPA activity, PAI-activity, PAI-mass concentration or von Willebrand factor and successful reperfusion were found. This is in contrast with the findings in previous studies [135, 136]. Neither could we demonstrate any association with episodes of recurrent ischemia.

The patients with failed reperfusion showed significantly higher levels of PAI-activity after 10 hours, and showed non-significantly higher levels already at 4 hours. This is in agreement with previously reported correlations between increased levels of PAI-1 and poor patency after thrombolysis with SK [128].

A local thrombus-stabilizing effect, which makes the clot resistant to lysis, has been proposed to be a possible mechanism by which PAI-1 blocks the fibrinolytic effect of SK [137]. Since most PAI-1 is released from endothelial cells and platelets at the site of the vessel injury and thrombus formation, circulating levels of PAI-1 could reflect the local effects of PAI-1. On the other hand, the dynamic
situation of an AMI may provoke rapid local changes that are not truly reflected in samples from venous blood. One must also consider the complexity of the fibrinolytic and haemostatic systems. A number of other factors like other inhibitors of tPA such as C1-inhibitor, α1-antitrypsin and α2-macroglobulin may be of importance, as well as other factors like thrombomodulin and platelet activation etc., but those were not measured.

One clinical implication of these results is that measuring the chosen fibrinolytic parameters in order to tailor thrombolytic therapy seems to be of negligible value.

Mortality

When presenting the results in Paper V, we chose not to show the results from the analysis of the combined end-point death or a new myocardial infarction during the first year, since it did not change the hazard ratios as compared with death only. Consequently, these data are not discussed. Patients expressing higher levels of PAI-1 activity after 10 hours and PAI-1 mass concentration after 4 and 10 hours suffered a higher risk for death at 1 year. This association was not seen at 5 years where only high pre-treatment levels of PAI-1 mass concentration were associated with higher risk for death. At five years, increasing age was the most important predictor of prognosis (as one could expect). Different mechanisms may be involved in the explanation of these observations. The more pronounced elevation of PAI-1 in the patients with a worse prognosis at 1 year could reflect the way the patients reacted to the acute manifestation of the thrombotic event. The association between elevated pre-treatment levels of PAI-1 mass concentration and death at 5 years could reflect an association with the habitual fibrinolytic state of the patients. One must also bear in mind the possibility of a type-1 error.

Von Willebrand factor stood out as a strong prognostic indicator for death at both 1 and 5 years in the univariate analyses. The risk increased for each tertile and this association was seen already at presentation, before the start of treatment. However, when adjusted for age, it could not be verified in the multivariate analysis. vWF levels are supposed to reflect endothelial dysfunction or damage to the endothelium. The association between vWF and the processes of thrombus formation or atherogenesis also suggests that high vWF levels may be a useful indirect indicator of atherosclerosis and/or thrombosis. However, there is limited information that increased vWF actually causes the progression of vascular disease [138]. In a recent study by Morange et al., vWF was identified as an independent risk factor for the development of coronary heart disease [139]. Our results indicate that vWF cannot be ruled-out as a weak predictor for future cardiac events. However, the failure to show any independent associations for events in the multivariate analysis suggest either that its role as a predictor might have been over interpreted in previous studies or that the present study lacked the power to verify
that association. A cynic could say that vWF in our study was a good, yet expensive, marker of increasing age.

**Streptokinase-neutralising antibodies**

The results in *Paper I* where 30% of the AMI patients treated with SK did not develop a high tPA activity at 4h and 10 h indicated that SK did not optimally activate the fibrinolytic system in those patients. A consequence of this could be that they were at a higher risk for unsuccessful thrombolysis. Since some individuals were resistant to SK to a certain degree, perhaps as a result of immunization, the next step was to investigate the presence of SK antibodies in relation to tPA activity and reperfusion in *Paper II*.

As described in *Papers I, II and V*, high peak levels and early onset of tPA activity was associated with successful reperfusion.

However, we were not able to demonstrate any obvious association between success or failure of reperfusion assessed by VCG, and the presence of functionally neutralising SK antibodies.

Neutralising antibodies against SK have been demonstrated after treatment with SK and after infections with streptococci [46-48, 140]. The presence of pre-treatment SK antibodies has been suggested to reduce the thrombolytic effect, although the results are not consistent [46, 47, 140, 141]. Allergic-like reactions to SK are reported in the large thrombolytic trials and vary from 3.5 to 5.7% (GUSTO-I, ISIS-2, ISIS-3, GISSI-1). The highest reported figure is from the GUSTO-I study in which 1,182 patients were treated with SK and 5.7% were reported to develop an SK allergic-type reaction. However, this had no impact on outcome measured as angiographic patency at 90 minutes, three or 24 hours, or left ventricular function [142].

The method for evaluating reperfusion (non-invasive or invasive) does not have any impact on the results. Thus, the divergent results of these studies may indicate that there is a methodological problem in the analysis of streptokinase antibodies. Different methods have been used, and there is no standardized method that has been shown to be “the gold-standard”. In the present paper we used the thrombolysis assessment system (TAS™), due to the potential clinical implication of a rapid bedside instrument for tailoring the thrombolytic therapy.

Since we were not able to demonstrate any significant correlation between the levels of functionally neutralising SK antibodies and the effect of SK on the fibrinolytic system expressed as tPA activity or on the clinical results, we conclude that SK antibodies in a patient population not previously given SK are of little or no importance. The levels of functionally neutralising SK antibodies in this population might have been too low to have any major impact on the clinical effect of SK therapy.
CONCLUSIONS

- On-line vectorcardiography was well suited for monitoring patients with AMI treated with different reperfusion strategies. The method was easy to use, and the interpretations of the data were reproducible.

- VCG recordings during the initial phase of an AMI provided important prognostic information. The resolution of ST-VM by ≥ 50% at 60 minutes was preferable as a prognostic indicator when evaluating the success of reperfusion therapy.

- The occurrence of a reperfusion peak during the minutes after the onset of reperfusion was a common finding in AMI patients treated at an early stage with PCI. The reperfusion peak was equally common among patients treated with thrombolysis having a successful reperfusion as it was when the patients were successfully reperfused using PCI. The occurrence of a reperfusion peak was less important than successful reperfusion regarding prognosis.

- The recognition of the reperfusion peak can be of clinical importance, since it can mimic aggravated ischemia which if misdiagnosed could lead to incorrect treatment decisions.

In patients with AMI treated with streptokinase;

- Streptokinase induced significant changes in the fibrinolytic system.

- No associations between pre-treatment levels of PAI-1 activity, PAI-1 mass concentration, tPA activity and von Willebrand factor with successful reperfusion or recurrent ischemia were found.

- There was a strong association between early, high levels of chromogenic substrate tPA activity (indicating a high fibrinolytic activity), and successful reperfusion. The high peak level and the early onset of tPA activity (reflecting streptokinase-plasminogen activity) were found in 70% of the patients.

- Pre-existing streptokinase antibodies had no significant influence on reperfusion and was not significantly correlated with the fibrinolytic activity obtained.
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