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Einfluss von klinischen Risikofaktoren auf postinterventionelle Komplikationen bei Patienten mit akutem Koronarsyndrom, behandelt mit perkutaner Koronarintervention.

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Kaiser, Bastian

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Dekan: Professor Dr. I. B. Autenrieth

1. Berichterstatter: Professor Dr. T. Geisler
2. Berichterstatter: Professor Dr. T. Bakchoul

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Table of contents

List of figures and tables

List of abbreviations

ACS:	Acute coronary syndrome
ACE-inhibitor:	Angiotensin-converting-enzyme inhibitor
ADP:	Adenosine diphosphate
AUC:	Area under the curve
ASA:	Acetylsalicylic acid
BARC:	The bleeding academic research consortium
BMI:	Body mass index
BMS:	Bare metal stent
CAD:	Coronary artery disease
CAG:	Coronary angiography
CABG:	Coronary artery bypass graft
DEB:	Drug eluting balloon
DES:	Drug eluting stent
ECG:	Electrocardiogramme
ESC:	European society of cardiology
GRACE:	Global registry of acute coronary events
GUSTO:	The global use of strategies to open occluded arteries
GPI:	Glycoprotein IIb/IIIa-inhibitor
HMG-CoA-reductase:	3-Hydroxy-3-Methyl-Glutaryl-CoEnzyme A reductase
LDL:	Low density lipoprotein
MACE:	Major adverse cardiac events
MEA:	Multiple electrode aggregometry
MI:	Myocardial infarction
NOAC:	Novel (or non-vitamin K) oral anticoagulants
NSTE-ACS:	Non-ST-elevation acute coronary syndrome
NSTEMI:	Non-ST-elevation myocardial infarction
PCI:	Percutaneous coronary intervention
ROC:	Receiver operating characteristics curves
SAP:	Stable angina pectoris

STEMI:	ST-elevation myocardial infarction
TIA:	Transient ischemic attack
TIMI:	Thrombolysis in myocardial infarction
TRAP:	Thrombin receptor activating peptide
TuePIC:	Tübingen platelet investigative consortium
UAP:	Unstable angina pectoris

1. Introduction

1.1. Epidemiology of non-ST-Elevation acute coronary syndrome (NST-ACS)

Over the years of 2005-2009 the proportion of patients suffering an acute coronary syndrome (ACS) of all hospital admissions stayed constant at about 1.3% throughout Germany. Whereas the number of patients admitted with ST-elevation myocardial infarction (STEMI) decreased, non-ST-elevation myocardial infarction (NSTEMI) patients increased continuously, especially throughout the age group of 65-89 years old. In 2009 61.5% of all ACS patients were considered NSTEMI, versus 50.5% in 2005 and showed to be consistently more frequent than STE-ACS since.

Out of all ACS patients 76% were 60 years and beyond, with an average age of 71.9 years in NSTEMI patients in 2009. There was a 1.7-higher rate of ACS in men than in women. In-hospital mortality is about 2-fold higher in STEMI patients, but at four years after admission there is a two-fold higher death rate in NSTE-ACS patients, likely caused by a higher rate of co-morbidities, like diabetes and renal failure in NSTE-ACS patients. [23, 11, 38, 63] There are similar numbers in the United States of America, where approximately 70% of the more than 780.000 ACS patients each year suffered an NSTE-ACS. [2]

1.2. Pathophysiology of NSTE-ACS

An ACS is caused by an imbalance between supply and demand of myocardial oxygen, leading to ischemia of muscle tissue, mostly as a result of a limited flow of oxygenated blood caused by an obstruction in the affected coronary artery. Most common (type I myocardial infarction) cause of obstruction in ACS is the deposition of cholesterol in the arteries leading to inflammatory processes and formation of an atherosclerotic plaque over time. Crucial for the development of plaques is an endothelial dysfunction, mostly caused by cardiovascular risk factors, like arterial hypertension, hyperlipidemia or diabetes mellitus. A higher permeability for lipoproteins results in low density lipoprotein (LDL) molecule accumulation within the sub-endothelium of the coronary artery and initiation of an inflammatory response. Oxidation of the accumulated LDL

leads to an amplification of the inflammation process and expression of adhesion molecules. Monocytes develop into macrophages and absorb the oxidized LDL particles via their „scavenger receptor“. Those cells are now called „foam cells“ and make up the early arteriosclerotic lesions known as „fatty streaks“. Fatty streaks usually don't cause symptoms. The inflammatory process induces a migration and proliferation of smooth muscle cells into the Intima and secretion of collagen, forming a cap around the core of the atheroma. A lower percentage of collagen in relation to the liquid, lipid rich core and destabilization of the cap by several molecules, like proteases produced by activated macrophages and T-cells results in a more vulnerable plaque and higher risk of rupture of the fibrous cap, responsible for approximately 60-70% of coronary thrombosis [28, 35, 65]. The sudden disruption of a fibrous cap in the culprit lesion, acting as a stimulus for thrombogenesis is again triggering the formation of a thrombus resulting in an interruption of blood flow. Platelets play an important role in formation of early atherogenic lesions and thrombus formation. Platelets interact with sub-endothelial tissue to get activated and initiate tethering, rolling and adhesion. Following adhesion, platelets secrete pro-inflammatory and pro-coagulatory factors, like adenosine diphosphate (ADP). This process results in recruitment of monocytes, auto-activation of platelets and progress of atherogenesis. [25] 30% of ACS result from plaque erosion, meaning endothelial apoptosis leading to luminal thrombosis without communication with the necrotic core and is especially seen in young patients [35]. Most plaque ruptures (66%-78%) occur from vulnerable lesions with stenosis smaller than 50%, and only a small percentage of below 5% from lesions with above 70% stenosis of the coronary artery.

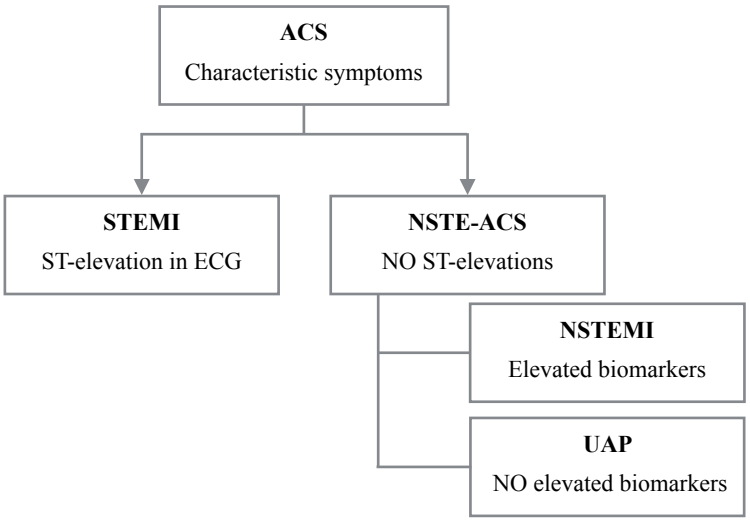
Typ II myocardial infarctions are caused by other possible, but less common reasons for myocardial necrosis and include: coronary vasospasm, progressive luminal narrowing, inflammatory mechanisms like vasculitis, coronary dissection or extrinsic factors like anemia or severe hypotension. [53]

Several cardiovascular risk factors have been described to significantly increase the risk of an ACS, including: high blood cholesterol, high blood pressure, positive family history, current smoking habit and diabetes mellitus. Typical symptoms of an ACS

include: prolonged anginal pain at rest being the most common clinical presentation with about 80% , de novo angina, destabilization of stable angina or post ACS angina. Atypical symptoms are often seen in female patients, elderly and patients suffering from diabetes or renal failure and include epigastric pain or isolated dyspnoe as an angina equivalent. Additional symptoms such as dyspnoe, sweating, nausea or syncope may also be present. [2, 56]

A non ST-elevation-acute coronary syndrome is defined as a typical onset of symptoms with no persistent ST-elevation in the electrocardiogram (ECG). There may be ST-depression, transient ST-elevation or T-wave inversions present, but not required for diagnosis. The umbrella term of NSTE-ACS is further subdivided, depending on additional laboratory work up. It includes:

1. Non-ST-elevation myocardial infarction (NSTEMI), where cardiac biomarkers of necrosis, like troponin and CK/CK-MB are elevated and display a more severe injury of myocardial tissue.
2. Unstable angina pectoris (UAP), where cardiac biomarkers present negative.



Flowchart 1: ACS definition by diagnostic means.

1.2. Treatment of a NSTE-ACS

The in-hospital mortality of NSTEMI patients has declined between 2005 and 2009 to about 9.9% of all patients, showing a lower mortality rate in comparison to STEMI patients in which mortality increased to 12.2%. There was an increased use of coronary angiography throughout 2005 to 2009 in all ACS patients, so that in 2009, 55% of all NSTEMI patients underwent coronary angiography (CAG). Following CAG, 66.5% of NSTEMI patients underwent percutaneous coronary intervention (PCI) in 2009, out of which 95% were administered a intracoronary stent (55.8% being drug eluting stents (DES)). The data showed an increase in mortality in patients receiving PCI to 3.8% in NSTEMI patients, with a significantly lower rate in mortality, when using drug eluting stents vs. bare metal stents (BMS) (3.8% vs. 6.3% in 2009). In-hospital mortality of patients treated with anti-platelet agents was much lower at around 5% in NSTEMI patients. [23]

1.2.1. Conservative therapy options

Additional or singular administration of drugs in patients with NSTE-ACS have a prognostic and symptomatic value. They have shown to reduce the risk of further ischemic events, like stent stenosis, new myocardial infarction (MI) or stroke and have a positive influence with a significant reduction in mortality. Typically administered medication in patients being admitted with NSTE-ACS include a platelet aggregation inhibitor in form of acetylsalicylic acid (ASA) 150-300mg, often in combination with an ADP-receptor antagonist. A ß-receptor inhibitor is recommended in patients with ischemic symptoms and no contraindications. An anticoagulant like heparin, bivalirudine or a GPIIb/IIIa-inhibitor may be used additionally in patients with high risk profile. For the symptomatic release of angina complaints nitrolingual may be administered. Post-interventional, the patient receives a combination of a dual platelet inhibition, including ASA lifelong and an ADP-receptor antagonist for up to 12 months. Additionally, for secondary prevention a HMG-CoA reductase inhibitor (statins) to lower blood lipid levels, a ß-blocker and a angiotensin-converting-enzyme (ACE)-inhibitor are prescribed. An anticoagulant like cumarine or one of the NOACs may

become required as part of a triple anticoagulant therapy in certain cases, like atrial fibrillation. [53]

1.2.2. Invasive strategy options

There have been several studies comparing outcomes of an early invasive treatment strategy versus a conservative strategy or more selective invasive strategy. Definition of the time span for early invasive strategy differed between studies making them harder to compare. Overall, there have been inconclusive findings on benefits and risks for NSTE-ACS patients depending on the strategy of treatment.

The 2007 ICTUS-trial four year follow-up showed a higher risk for primary endpoints for the early invasive strategy and no significant difference in all-cause mortality between the two study groups. [29] Other studies, like 1999 FRIST II, 2001 Cannon et al., 2002 RITA-3-trial, 2006 Lindqvist et al., 2009 Mehta et al. and 2013 Tekin et al. all evaluated data, that suggested a significant reduction of their defined primary endpoint in an early invasive strategy in NSTE-ACS patients.

The evaluation of a benefit on overall mortality between study groups differed among these studies. Only Mehta et al. and Tekin et al. showed a significant reduction in all-cause mortality in patients receiving an early invasive strategy. Lindqvist et al. showed a benefit in mortality for patients receiving early invasive treatment, only when the patients were younger than 70 years old. [43, 62] FIRST-II, Cannon et al., the RITA-3-trial and the ICUTS-trial four year follow-up all showed no significant difference in mortality between both strategies. [11, 30, 36, 29, 22]

Most of the studies included an evaluation of the risk of major bleeding incidences to analyze adverse effects of an early therapy option. Cannon at al. showed no significant increase in thrombolysis in myocardial infarction (TIMI) major bleeding, but the RITA-3-trial and the ICTUS-trial-4 year follow-up showed a significant higher rate of major bleeding events in patients of the early invasive group. [11, 30, 29]

2016 Fanning et al. analyzed eight prospective randomized controlled trials and compared clinical outcomes of a routine invasive versus a selective invasive strategy in NSTE-ACS patients. The data showed no significant benefit to all-cause mortality for

the routine invasive strategy, but it did show a risk reduction for MI, refractory angina and re-hospitalization. Patients receiving a routine invasive strategy showed a higher rate in post interventional bleeding events and an increased risk for procedural-related MI. Therefore Fanning et al. came to the overall conclusion to support a more selective approach for the use of invasive strategy in NSTE-ACS patients. [19]

The European Society of Cardiology (ESC), just like Fanning at al., concludes in their 2015 NSTEMI guideline that there is no significant benefit to an overall recommendation for early invasive strategies for all incoming patients suffering a NSTE-ACS alike. They rather recommend an early risk stratification at point of admission of each individual and initiate an invasive strategy, if eligible, depending on that risk stratification as explained in the segment below. The treatment and the timing of treatment can be individualized to minimize the risk of unnecessary adverse bleeding events and decrease the occurrence of primary endpoints and overall mortality. [53]

1.2.3. Risk stratification

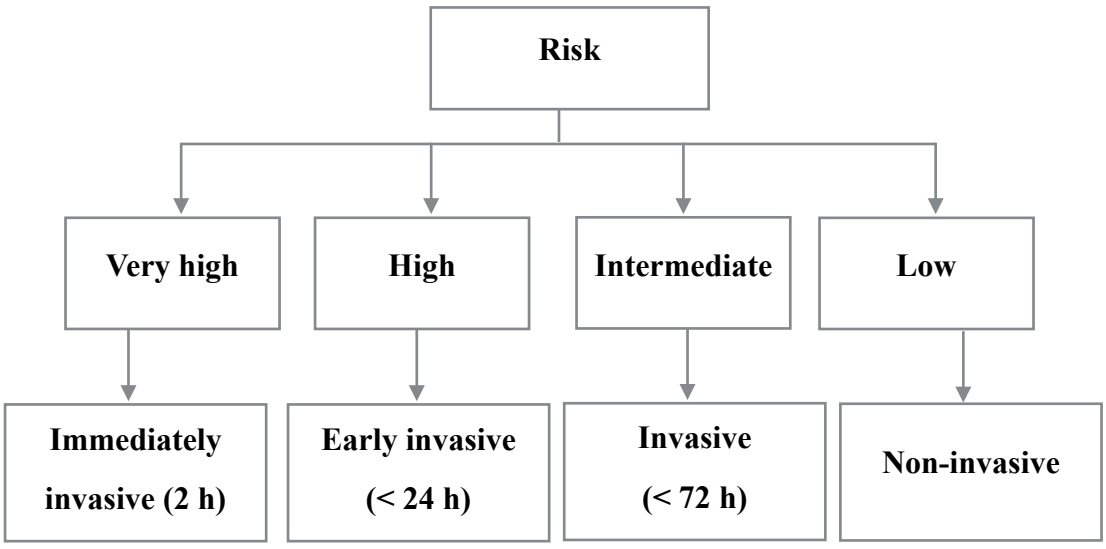
Treatment possibilities and timing recommendations of invasive strategies for incoming patients suffering from NSTE-ACS differ depending on their initial risk stratification. Therefore a careful assessment of risks and benefits of an invasive strategy for each individual should be performed and evaluated at time of presentation. The risk stratification classifies four subgroups of risk criteria with decreasing severity from very high risk to high risk to intermediate risk and low risk (Table 1). Presentation of these different risk criteria in patients determine the time span and necessity of an invasive treatment strategy. [2]

Very-High Risk	High Risk	Intermediate Risk	Low Risk
Hemodynamic instability or cardiogenic shock	Rise or fall in cardiac troponin compatible with MI	Diabetes mellitus	Any characteristics not mentioned
Recurrent or ongoing chest pain refractory to medical treatment	Dynamic ST- or T-wave changes (symptomatic or silent)	Renal insufficiency (GFR < 60mL/Min)	
Life-threatening arrhythmias or cardiac arrest	GRACE score >140	LVEF <40% or congestive heart failure	
Mechanical complications		Early post-infarction angina	
Acute heart failure		Prior PCI	
Recurrent dynamic ST-T-wave changes, particularly with intermittent ST-elevation		Prior CABG	
		GRACE risk score > 109 and < 140	

Table 1: NSTE-ACS risk stratification criteria.

Invasive coronary angiography (CAG) remains the main management strategy for NSTE-ACS patients in order to confirm and identify culprit lesions and therefore establish the indication for PCI treatment. CAG is a minimal invasive procedure that uses a radiocontrast agent, which is injected into the contrary arteries via a catheter, that is inserted into the artery via a mostly radial or femoral access site, and guided to the opening of the coronary arteries under x-ray control. The contrast material is injected into the coronary arteries. Using X-ray imaging the blood flow can be visualized on a monitor. Coronary stenosis therefore can be identified as a narrowing or complete

obstruction of the vessel. Alternative, less invasive approaches to CAG, like CT-angiography may be used for diagnosis in patients with a high risk profile or critical contraindications for CAG. The time window for CAG in NSTEMI patients is defined by the initial risk stratification. The 2015 European Society of Cardiology-Guidelines classify four different categories with different time windows on when to perform CAG. Patients who meet at least one of the very-high risk criteria as defined above should receive CAG as soon as possible, but at least within two hours. When meeting at least one of the high risk criteria, CAG should be performed within 24 hours. The time window is expanded to 72 hours, when the patient meets at least one of the intermediate risk criteria category. For patients, who do not meet any of these criteria an invasive strategy is not recommended. [53]



Flowchart 2: Risk stratification system for treatment decision.

Percutaneous coronary intervention, when indicated, usually directly follows the CAG to treat stenosis and restore blood flow to the myocardial muscle tissue. Treatment by PCI can involve different procedure options to minimize coronary stenosis. Balloon angioplasty, a procedure that uses the inflation of a balloon inside the blockage of the artery to compress the atherosclerotic plaque and expand the artery, is the most commonly used procedure. An advanced development is the drug eluting balloon (DEB), that is coated with an anti proliferative substance (for example Paclitaxel) which

is instantly released at the site of stenosis and is supposed to lower the risk of re-stenosis. Additional to widening the stenosis via ballon, the implantation of a stent into the obstructed area of the coronary artery is often indicated. A stent is implanted to keep the coronary artery open after ballooning. There are different types of stent material regularly used during PCI. Bare metal stents provide a mechanical framework and are not specifically coated. Drug eluting stents are coated with an anti-proliferative substance such as Paclitaxel, Everolismus or Sirolismus among others to slow down endothelialization of the stent and reduce the risk of in-stent restenosis. This process increases the risk of stent thrombosis and makes a prolonged usage of a ADP-P2Y12-inhibitor necessary. [4]

1.3. P2Y12-inhibitors

Prior to percutaneous coronary intervention, incoming patients suffering a NSTE-ACS receive a loading dose of an ADP-P2Y12-inhibitor, as for example clopidogrel 300mg/ 600mg or ticagrelor 180 mg as part of a dual platelet inhibition in combination with Aspirin to reduce the risk of thrombotic events and life threatening stent thrombosis.

1.3.1. Characteristics of P2Y12-inhibitors

Adenosine diphosphate (ADP) is soluble activator of platelet activation and aggregation. It binds a G-protein coupled receptor (P2Y) and results in a shape change of the platelet and secretion of vasoactive and procoagulant substances. Those substances further enforce platelet activation and coagulation. P2Y12-inhibitors prevent ADP from blinding the receptor site and starting the intracellular signaling process that results in further coagulation.

There are two groups of P2Y12-inhibitors most commonly used:

1. Thienopyridines, like clopidogrel and prasugrel, are irreversible inhibitors of the ADP-P2Y12-receptor.
2. Cyclopentyl-triazolo-pyrimidine, like ticagrelor, is a direct and reversible ADP-receptor antagonist.

Both types of P2Y12-inhibitors interfere with the ADP-induced platelet aggregation, therefore causing a reduction in ischemic events, but also an increased risk of adverse bleeding events.

clopidogrel is a prodrug that needs to be converted into an active metabolite by cytochrom P450 (CYP)-enzymes and therefore has a delayed onset with a maximum inhibition effect after five to six days. Administering a loading dose of 300-600 mg of clopidogrel reduces the delayed onset to three to five hours, which is important in the treatment of NSTE-ACS.

Prasugrel and ticagrelor are faster acting drugs with an onset of effect (50% inhibition of thrombotic aggregation) after about 30 minutes. Because of CYP-polymorphisms, clopidogrel also shows a substantial inter-individual variability. Platelet reactivity can be tested via ADP-Multiplate Analyzer to identify non-responders to clopidogrel. [12]

1.3.2. Ticagrelor vs. clopidogrel

Platelet aggregation seems to be more rapid and effective with less inter-individual variability with ticagrelor compared to clopidogrel. The DISPERSE-2 study showed that ticagrelor achieved greater levels of inhibition than clopidogrel in NSTE-ACS and no significant difference in major bleedings between the clopidogrel and the ticagrelor-group [10]. The same results concluded by Wallentin et al. (PLATO trial) showing that ticagrelor significantly decreases the risk of primary endpoints in comparison to clopidogrel in patients with ACS. They also stated no significant difference in major bleedings seen between these two groups. Ticagrelor, as a reversible antagonist of ADP receptors, additionally has the advantage of a much more rapid termination of effect on platelet aggregation and therefore faster management in treatment of an adverse bleeding complication. [66]

1.3.3. Prasugrel vs. clopidogrel

A 2007 study comparing outcomes and adverse effects in patients with ACS and scheduled PCI showed a significant reduction for ischemic events, when loaded and use of post interventional treatment with prasugrel versus clopidogrel. This association

was shown for stent thrombosis, myocardial infarction and urgent revascularization. The study also showed a significant higher rate of major and fatal bleeding complications throughout the prasugrel group, therefore suggesting a careful assessment of risks and benefits for each individual [68]. The 2014 ACCOAST-PCI study further supports these findings on the increase in adverse bleeding incidences following treatment with prasugrel, but could show no significant decrease in ischemic events. The cohort of the ACCOST-PCI study was limited to NSTEMI patients and suggest deferring the treatment with prasugrel until a decision about revascularization is made. [47]

1.3.4. Need of P2Y12-inhibitor as pre-treatment strategy

In regard to patients with ST-Elevation Myocardial Infarction (STEMI) the 2001 PCI-CLARITY-Study was able to show a significant decrease in cardiovascular death, MI and stroke in patients pretreated with clopidogrel. Also, there was no increase in TIMI major or minor bleeding recorded, supporting a strategy of early and routine use of clopidogrel ad hoc treatment in all STEMI patients [54]. Therefore the 2013 AHA/ ACCF guidelines for the management of STEMI state a class-I-recommendation and a B-level of evidence for the use of a loading with clopidogrel, prasugrel or ticagrelor as well as long-term treatment with either one of these [46]. Looking at the treatment of NSTE-ACS patients, it seems that studies and guidelines are not as conclusively as they are for the treatment of STEMI, especially in regard to the timing of P2Y12-inhibitors.

1.3.5. Guidelines on P2Y12-inhibitors

The American Heart Association and the American College of Cardiology don't recommend the „upfront-use“ of prasugrel in patients with NSTE-ACS in the 2014 AHA/ACC Guidelines following the findings of the ACCOAST-study. They do give a class I-recommendation grade and an A-level of evidence for an initial loading dose of 300-600 mg clopidogrel in NSTE-ACS patients. Also they have a class-I-recommendation and an A-level of evidence for administering a loading dose of a P2Y12-receptor inhibitor before PCI, naming clopidogrel or ticagrelor as possible options [2]. The 2015 European society of cardiology (ESC) guidelines for the

treatment of NST-ACS make no recommendation for or against the ad hoc usage of a pretreatment with clopidogrel or ticagrelor, hence no studies for the optimal timing of administration are available. But they also state a class-I recommendation with an A-level of evidence for the addition of a P2Y12-inhibitor to Aspirin as a loading dose as well as long-term administration. Ticagrelor or prasugrel are recommended to all patients with moderate or high-risk for ischemic events, prasugrel to patients proceeding to PCI and clopidogrel only for patients who cannot receive ticagrelor, nor prasugrel. But just like the 2015 AHA/ACC-guidelines they are giving only a B-level of evidence and a class-III-recommendation for the usage of prasugrel as upfront pretreatment in NSTE-ACS patients without known coronary artery anatomy based on the findings of the ACCOAST-trial. [53]

Pro

Most guidelines at the time suggesting a beneficial use of pre-treatment and a continuous administration of clopidogrel following PCI, take into account that the 2001 double blind, randomized PCI-CURE study showed, that in patients with NSTE-ACS undergoing PCI, the pre-treatment plus long-term administration of clopidogrel in combination to Aspirin was associated with a lower rate of cardiovascular death, myocardial infarction, or any revascularization by about a third. Additionally, it showed no significant increase in major, nor minor bleedings between the pre-treatment and the no-pre-treatment group. Therefore suggesting an overall benefit of a clopidogrel pre-treatment-strategy in patients with NSTE-ACS undergoing PCI. [45]

Following studies, including the 2001 Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators, investigating the benefit and adverse effects of an upfront use of clopidogrel in patients concluded an association with a lower risk of major coronary events. They showed a reduced risk for death of cardiovascular cause, but not call cause mortality. In regard to bleeding events, studies differed from only showing an increase in minor bleedings in a 2012 systematic review to an increased risk for major bleedings in the CURRENT-OASIS-7 study. [7, 70, 44]

However by having a faster access to PCI following CAG nowadays and the more frequent usage and development of newer, more potent P2Y12-inhibitors, there is a need for updated studies to evaluate the overall benefit of ad-hoc therapy in NSTE-ACS patients. There are more current studies suggesting an at least more limited benefit of pre-treatment and a higher risk of bleeding by administering platelet aggregation inhibitors early on in NSTE-ACS patients.

Contra

The ACCOAST-PCI study has shown that patients receiving a prasugrel 30 mg loading dose as pre-treatment have a three-fold higher incidence of TIMI major bleeding and six-fold higher incidence of life threatening bleeding in comparison to patients receiving a placebo. The study also suggested that there is no significant benefit to a pre-treatment with prasugrel, when looking at the reduction of primary endpoints. [47] Bellemain-Appaix et al. systematic review and meta-analysis of 2014 investigated the benefit of thienopyridine pretreatment in NSTEMI patients. It showed no significant reduction in any-cause death, cardiovascular death, stroke, myocardial infarction or urgent revascularization. However, it did state a significant association between the pretreatment with P2Y12-receptor antagonists and the occurrence of minor and major bleeding. This was shown for all NSTE-ACS patients and specifically for those patients receiving PCI, overall suggesting to reconsider the immediate pretreatment with P2Y12-Inhibitors. [6]

1.3.6. Timing of administration of P2Y12-inhibitors

When evaluating the correct treatment strategy of NSTE-ACS patients to further limit the risk of bleeding without increasing the risk of ischemic events, it is crucial to consider the impact of timing of a possible anti platelet therapy in regard to diagnosis by CAG and final treatment by PCI.

Manoukian et al. published in 2007 that the timing of aspirin, thienopyridine, and antithrombin administration related to angiography did not influence major bleeding. [39] The multicentre trial PRAGUE-8-Study investigated primary endpoints and

bleeding complications between a group of patients with stable angina pectoris receiving 600 mg clopidogrel >6 h before CAG and a group of patients with stable angina pectoris (SAP) receiving 600 mg clopidogrel after CAG and only in case of PCI. The study showed that the pre-CAG-clopidogrel group had an increased risk of minor bleeding complications, especially in those receiving PCI. [2] The 2002 published Steinhubl et al. showed no significant reduction of incidence in death, myocardial infarction or urgent revascularization at 28 days in patients being administered a pretreatment loading dose of clopidogrel three to less than six hours before undergoing PCI. But patients being administered 300 mg clopidogrel at least six hours before PCI showed a relative risk reduction of about 38.6% in combined end points suggesting a benefit of early administration of clopidogrel. [58]

The knowledge of the coronary anatomy and stenosis by CAG showed to be important, when identifying the need and benefit of an immediate PCI-treatment, even becoming part of the 2015 ESC guidelines, when looking at the recommendation for usage of prasugrel depending on wether the coronary anatomy is known. Nowadays PCI is usually being performed immediate after diagnosis of stenosis by coronary angiography. Considering the findings above, which suggest administration of anti-platelet medication as early as possible when undergoing PCI, an anti-platelet therapy needs to be administered in advance of CAG in order to reach its full effect at time of PCI. But this sequence of events further limits the chance to identify patients, who will not benefit from an ad-hoc P2Y12-receptor inhibitor treatment, but might even have an increased risk of bleeding and therefore calls for a new way to identify and evaluate patients with a high-risk for bleeding events, when administered pre-treatment anti-platelet drugs.

1.4. Bleeding complications

When discussing the necessity versus the adverse effects of an early treatment of NSTE-ACS patients with a P2Y12-inhibitor, it is important to know the likelihood of bleeding after PCI as well as the impact of the bleed on the overall outcome. Even though most patients (81.9%) with ACS undergoing PCI don't develop a major bleeding [33],

periprocedural bleeding is still one of the most frequent complications of PCI with reported incidences of major bleeding as high as 5.4%, close to the reported incidences of refractory ischemia, MI, or death. [39, 49, 33] Minor bleedings were recorded at 12.7% of the patients during the study of Kinnaird et al., who also showed no significant association to antithrombotic treatment. [33]

1.4.1. Known risk factors

P2Y12-inhibitors

Anti-thrombotic medication increases the risk in bleeding events, because of their desired effect on platelet aggregation. The ACCOST-PCI study, for example, showed that prasugrel has a significantly higher incidence in minor and major bleeding complications. [47, 68]

Clinical factors

Other Studies have shown age, sex, elevated creatinine, white blood cell count and anaemia as independent risk factors for bleeding in ACS patients. [40] Other predictors proved to be lower weight, heart rate, low systolic blood pressure and lower baseline hematocrit in NSTEMI patients. [61]

1.4.2. Preventive measures

Drug regime

When expecting a higher risk for bleeding events in patients it is possible to change the regime of the medication used pre- and periinterventional. For example the usage of clopidogrel instead of the more potent prasugrel or ticagrelor, when expecting a risk in bleeding, because of its lower inhibition rate of the ADP receptor. Another possibility is the use of Bivalirudine, rather than Heparin and abandon the use of GPIIb/IIIa-receptor antagonists to further reduce the risk in bleeding. [60, 59, 32]

PCI-access site

The use of a less susceptible access site to introduce the catheter is a common way to further prevent bleeding. The access site via radial artery should be preferred over the femoral artery, if possible. It significantly reduces access site complications, is easier to compress in case of bleeding and provides a more limited space for spreading, reducing the risk for major bleeding events. It also allows the mobility of the patient without increasing the risk of bleeding. The radial access site shows a significant reduction in mortality in patients with ACS after two years compared to femoral arterial access. [53, 31, 1, 24]

Postprocedual sealing systems

Following coronary intervention a sealing system may be used in patients to reduce the risk of bleeding from the exit site of the catheter. Some studies have indicated a significantly shorter time to hemostasis and a higher reduction in local complications in comparison to manuell compression. These systems also show a risk of lower limb ischemia, arterial stenosis and device entrapment. [55, 34, 17]

1.4.3. Impact of bleedings on outcome/mortality

Mortality

Non-coronary artery bypass graft (CABG)-related major bleeding is an independent risk factor for one-year mortality showing a significant increase in risk of death within and after 30 days. The 30-day mortality after major bleeding was six-fold higher than in patients without major bleeding events, which makes bleeding events a stronger independent risk factor than MI. [39] This was supported by further studies, all showing minor and even stronger major bleeding events to be significant independent risk factors for 30-day and one year mortality [41, 49, 33, 20] Further, the prognostic impact and risk of mortality was directly correlated to the severity of the bleeding. Only isolated hematoma showed no significant effect on mortality. [40, 20, 41, 16] For example, the bleeding academic research consortium (BARC) was able to observe an association between a BARC type three-bleeding, comparable to TIMI major and minor and the

global use of strategies to open occluded arteries (GUSTO) moderate and severe bleedings, and the two-year mortality. Within the BARC-type three patients, there was a correlation between severity of the subgroups and the mortality risk (3a<3b<3c). The shown association was regardless of the time passed after PCI, nor the regime of anti-platelet-therapy used. [64]

Ischemic events

A significant increase in risk of ischemic and hemorrhagic stroke within 30-days following a major bleed could be shown. After 30-days there was a reduction in risk showing no significant increase for risk of stroke any longer. [16] Patients suffering major bleeding events had a three-fold higher rate of ischemic events in the first 30-day, significantly higher rates of MI, unplanned revascularization, a nearly six-fold higher risk for stent thrombosis. [39] Patients suffering a major bleeding had a significantly prolonged hospital stay. [39, 33]

No impact

Studies showed that CABG-related major bleeding did not significantly increase the risk of mortality and cardiac death, which may be interesting in the context of withholding antithrombotic therapy before knowing the necessity of CABG in ACS patients. [40, 41, 64]

1.5. Aims

The 2014 ACCOAST-PCI study showed for NSTEMI patients undergoing PCI, that a pretreatment strategy with prasugrel was not associated with a significant benefit on ischemic events. Furthermore, they did show a significant increase in major and life-threatening bleeding complications, when pretreated with prasugrel. This has led them to the conclusion to defer the pretreatment with prasugrel, which has been incorporated into the newer guidelines for NSTE-ACS therapy. Besides the ACCOAST-PCI study, there are several other studies analyzing the benefits and adverse effects of a pretreatment strategy in NSTE-ACS patients, coming to different conclusions. Up to date guidelines still support a general pretreatment strategy.

The aim of this retrospective study of NSTE-ACS patients undergoing PCI after being pretreated with an ADP-antagonist, is to analyze the risk factors of post-interventional bleeding rates. We aimed to analyze usual clinical risk factors, timing and type of the loading with ADP-antagonist and platelet reactivity accessed by multiple electrode aggregometry (MEA). Furthermore, major adverse events following PCI were recorded. The objective of the evaluated risk profile was to categorize incoming patients by bleeding risk more accurately and directly at time of admission. Being able to categorize patients by their individual risk profile will then again help to decide whether an early preventive strategy may be indicated to avert adverse events and improve overall outcome. A preventive, more cautious approach may then be taken, as described above, in form of different medication being used, different arterial access site for catheter installation or the use of sealing systems.

There is only little research on the timing of the loading with a P2Y12-inhibitor and its consequences on the probability of adverse events, which is why we also categorized patients into four different subgroups depending on their loading time. By doing so, we were hoping to evaluate differences in probability of bleeding incidences and primary endpoints depending on the time category of their pretreatment.

By adding a three months follow-up with all patients recording primary endpoints, we were also including the outcome into the overall analysis. This allowed us to compare outcomes between both cohorts, bleeding versus non-bleeding, but also between the kinds of P2Y12-inhibitors administered and their different loading times. Having an assessment of the outcome, as well as the probability of adverse bleeding events, allows us to compare benefits and disadvantages of certain strategies to one another, leading to a more valid recommendation for treatment options.

2. Methods

2.1. Study population

The study was conducted as a retrospective analysis. The study population consisted of patients recruited in Tuebingen Platelet Investigative Consortium (TuePIC) trial in the department of cardiology at the university hospital of Tuebingen (UKT). From 2547 patients admitted between 2011 to 2014, 439 patients were included into this study according to following criteria.

Inclusion criteria

Patients diagnosed with NSTE-ACS including NSTEMI and unstable angina pectoris (UAP), who received a P2Y12-inhibitor prior to PCI were included.

Exclusion criteria

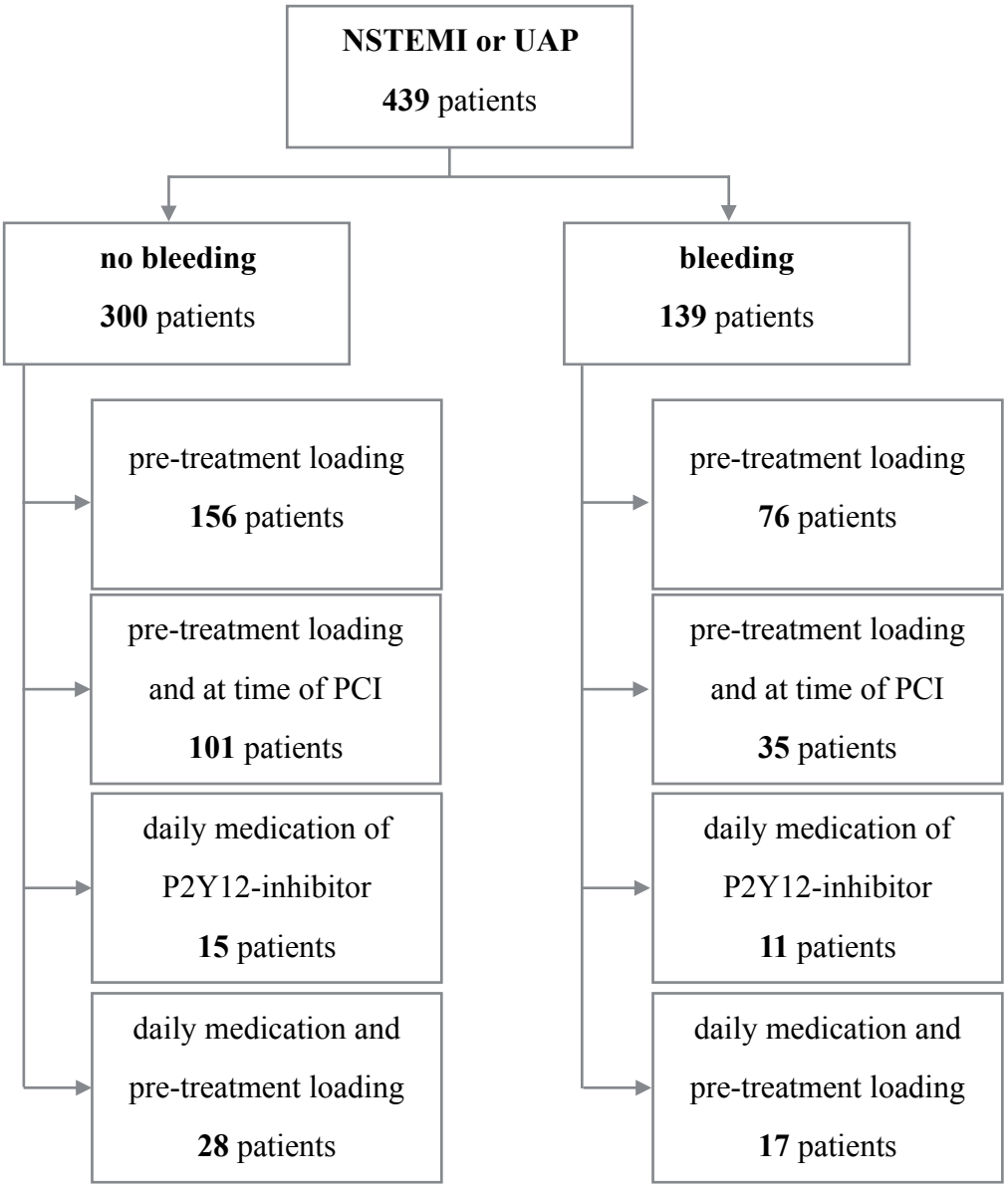
Patients with ST-elevation myocardial infarction on admission or patients not receiving coronary angiography after diagnosis were not included into the study group.

Patients suffering from NSTE-ACS, but not being administered an ADP-P2Y12-inhibitor at all or only at the time of percutaneous coronary intervention were also excluded from the study.

Patients data including clinical baseline characteristics, used P2Y12-receptor antagonist, follow-up (major adverse cardiac events (MACE) and bleeding events) and platelet function analysis were obtained from the TuePIC database and patients' files. Data were anonymized for the analysis. The cohort was divided into two subgroups depending on documented bleeding occurrences following PCI. These events were retrospectively researched by browsing the TuePIC database, physician letters and other documentation by the UKT for each patient. The control group consists of 300 patients with NSTE-ACS, who did not suffer any documented post-PCI bleeding event. 139 patients suffered a documented bleeding event within three months after PCI, meeting the TIMI-Bleeding Score, BARC-Classification and Gusto Score.

For further evaluation of the effect of timing of the administration of the P2Y12-inhibitor on the occurrence of post interventional bleeding, patients were stratified into four groups depending on the time of receiving a loading dose: The first subgroup

consisted of a total of 232 patients receiving a pre-treatment loading dose only. The second group included patients receiving a pre-treatment, as well as a loading at the time of PCI, consisting of 136 patients. The third group included all 26 patients, where the P2Y12-inhibitor was already part of their daily medication. The last group is made up of patients with a P2Y12-inhibitor as part of their daily medication, who additionally received a pre-treatment loading dose and includes 45 patients altogether.



Flowchart 3: Categorization of cohorts and subgroups.

2.2. Bleeding classification

For better comparison of patients, we used three bleeding scores: TIMI, GUSTO and BARC. By the use of these scores, we were able to classify the severity of the bleed in all patients suffering a bleeding event following intervention (event group). Most bleeding events were not initially classified by the physicians in charge at the time of hospitalization. Therefore, the classification of severity was mostly done retrospectively, after research of clinical description, sonographic measurements and laboratory findings.

2.2.1. TIMI bleeding classification

The TIMI Bleeding Classification was developed to classify hemorrhage in STEMI patients and differs between non-CABG- and CABG-related bleeding. It consists of three categories with increasing severity, including minimal, minor and major. For non-CABG related bleedings, as applicable for this cohort, it uses a combination of clinical observations, like overt signs and location of the bleed and fatal outcome. It also puts a big emphasis on laboratory data, especially drops in hemoglobin or/and hematocrit to evaluate the bleeding severity. [42, 9] The CABG-related classification uses localization, need for intervention, chest tube output and fatality of the bleed to classify the severity, but has not been used throughout this study cohort.

The TIMI bleeding classification was developed on STEMI patients, which differs from our NSTEMI study group, but still gives us a valid comparison of hemorrhage severity between patients.

2.2.2. GUSTO score

The global use of strategies to open occluded arteries classification (GUSTO) is a clinically driven score that differentiates bleedings into three subgroups with increasing severity.

Severe or life-threatening bleed consisting of intracerebral hemorrhage or resulting in substantial hemodynamic compromise requiring treatment. Moderate bleeding requiring blood transfusion, but not resulting in hemodynamic compromise and mild bleeding,

which does not meet the criteria above. It differs from other definitions, in that it doesn't take changes of hemoglobin into account. Because of this only clinically driven criterion, the severity tracks well with the risk of MI and death but shows a lack of objective standardization. [42, 51]

The GUSTO-Score was developed in the fibrinolytic era and was done on STEMI patients, which again differs from the drug treatment and patient cohort within this study group.

2.2.3. BARC-classification

The score by the bleeding academic research consortium (BARC) classification was developed to standardize the assessment of bleeding events and use a numeric categorization with increasing severity of the bleed, instead of descriptive terms like major or minor bleeding, that are being used in other classification systems. However, in order to compare patient cohorts by the severity of the bleeding event, we chose to define each bleeding, categorized BARC three or higher as a „major bleeding“. The BARC score uses five categories to classify a bleed, which derive from a combination of clinically and laboratory based criteria that were intergraded from already exciting scores, like GUSTO and TIMI. [42, 64] Eikelboom et al. was able to show that the BARC classification presents the strongest correlation of the real clinical condition, when compared to TIMI and GUSTO bleeding scores, therefore suggesting the preference of the use of BARC over GUSTO and TIMI. [16] For the evaluation of outcomes, like MI or death following adverse bleeding complications, a study by Rao et al. showed that it seems to be more crucial to look at clinical criteria and the need for blood transfusion instead of laboratory findings. The increased risk of short or intermediated death or MI with an increasing severity in GUSTO bleeding complication in patients persisted after adjusting for transfusion. This was not true, when using the more laboratory driven TIMI bleeding score. Therefore, suggesting a higher correlation of outcome to clinical criteria versus laboratory findings. [51]

2.3. Risk scores

Determination of the pre-treatment bleeding risk for each patient was done by using the ACUITY score and the CRUSADE score. Additionally we determined the mortality risk of each patient by applying the TIMI-risk score and the global registry of acute coronary events (GRACE) mortality score.

2.3.1. Bleeding events

ACUITY-HORIZON

It is used to predict the risk for non-CABG related major bleedings in patients with ACS within 30 days. It uses seven independent predictors: female sex, advanced age, elevated serum creatinine and white blood cell count, anemia, presentation of STEMI/ NSTEMI or UAP and usage of antithrombotic medications in form of heparin + glycoprotein IIb/IIIa-inhibitor (GPI) or Bivalirudin. Depending on the score, the risk for bleeding is categorized into 4 different groups: low, moderate, high and very high with an increasing risk for major bleeding. It also showed a higher rate of bleeding complications in patients with STEMI than NSTEMI and again higher than in patients with UAP. [40]

The ACUITY-HORIZON trials included STEMI patients additionally to NSTEMI and UAP, which is a different setting compared to our study cohort. But, more crucially it is derived from patients receiving either Bivalirudin or a GPI, which was rarely administered throughout our cohort and is not the standard form of treatment for NSTEMI. This may lead to a restricted use for this particular risk score in NSTEMI patients nowadays.

CRUSADE

A second risk score used for this study to predict the occurrence of bleeding is the 2009 CRUSADE risk score. It was derived from a study on NSTEMI patients and is being used to predict the risk of in-hospital major bleeding. The score uses the patient characteristics age, heart rate, systolic blood pressure, hematocrit and creatinine clearance, as well as signs of CHF on admission, diabetes mellitus and prior vascular

disease, that showed to be significantly associated with a higher risk in bleeding events. [61] The added sum of weighted scores out of these eight predictors, ranges from one to 100 and is divided into quintiles with increasing bleeding risk. Starting with a) very low with a score of below 21, b) low, with a score between 21 and 30, c) moderate, with a score of 31 to 40, d) high risk, with a score of 41 to 50 and d) very high risk, with a score of above 50.

The CRUSADE risk score is derived from a cohort that is better comparable to our study group. Both cohorts consist of NSTEMI patients, even though we also included UAP patients. Differing from our own criteria, patients taking warfarin at home were excluded from the CRUSADE study. Anticoagulants are a common medication among patients and are known to result in an increased bleeding risk, making them an important factor to be analyzed, when trying to get a complete bleeding risk profile for the patient. Also the CRUSADE study did not look at cohorts depending on their pretreatment with a P2Y12-inhibitor and was only developed in regard to major bleedings.

Accuracy of bleeding risk scores

A 2015 meta-analysis of nine studies looking at the accuracy of bleeding scores in patients presenting with myocardial infarction showed that, when looking at all ACS patients, CRUSADE and ACUITY-Score performed similarly, when compared to GRACE. CRUSADE was the only one externally validated for NSTEMI.[51]

2.3.2. Mortality

TIMI risk score

The 2001-developed score by Antman EM et al. UAP or NSTEMI patients aims at predicting the probability of ischemic events, like MI, urgent revascularization and all-cause mortality. The study was able to identify seven significant predictors, including age, risk factors for coronary artery disease (CAD), known CAD, severe angina, use of Aspirin, ST-deviation $\geq$ 0.5 mm and elevated cardiac enzymes. [3] The results of the TIMI risk score are calculated by adding one point for each assessed risk factor. When

patients show a risk score of zero or one point, they should be further risk stratified. When showing higher risk scores, more aggressive intervention may be necessary.
The TIMI risk score was derived from a similar cohort, including UAP and NSTEMI patients. But they did randomly assign either heparin/Fondaparinux therapy versus placebo and did not include different P2Y12-inhibitor regimes into account. Also, because of low incidence at trial, the TIMI risk score does not include heart failure, which is a significant risk factor for death, as shown by Granger et al. [26, 27]

GRACE mortality score

Developed in 2002 by Granger CB et al., it uses eight factors to evaluate the probability of all-cause death during hospitalization for all ACS patients. The highest increase in death was shown by the Killip-classification (two-fold), followed by age (1.7-fold), making them the most relevant predictors. Blood pressure, cardiac markers, cardiac arrest on admission, ST-segment deviation, heart rate and creatinine levels also showed to be significant predictors. The GRACE score seems to be a beneficial tool to predict all cause death in STEMI, NSTEMI and UAP patients, having shown no impact of the presence of ST-elevation for the determination of risk of death. [27] The GRACE risk score is divided into three different risk categories representing the probability of in-hospital death. A score from 1 to 108 (low risk) correlates with an in-hospital death of below one percent, a score of 109 to 140 (intermediate risk) with one to three percent and a score of 141 to 372 (high risk) with above three precent.
Differing from our study cohort, the GRACE cohort included all forms of ACS, including 39% of the patients being admitted with STEMI, and only a small fraction of the cohort was treated with clopidogrel.
In 2004 the GRACE-6-months post discharge prediction model identified nine variables, again for all ACS patients, to predict all-cause mortality within six months of discharge. Equivalent to the 2002 GRACE mortality score, the calculated risk score is divided into risk categories correlating with the percentage of the 6-months post discharge mortality. The low risk category including a risk score of one to 88 correlates with a six-months mortality of below three percent, the intermediate risk (score of 89 to

118) with three to eight percent and the high risk category with a mortality of above eight percent for non-STE-ACS. [15]

Accuracy of mortality risk scores

In NSTE-ACS, quantitative assessment of ischemic risk by means of scores is superior to the clinical assessment alone. The GRACE risk score provides the most accurate stratification of risk, both on admission and at discharge. [53] The comparison of the risk scores TIMI, PURSUIT and GRACE in 2005 by Gonçalves et al. analyzed that out of these scores the GRACE risk score showed to have the best discriminatory accuracy for major adverse cardiac events, whereas the TIMI-risk score showed the lowest at 30-days and one-year. For the long term prognosis all three scores showed higher accuracies. [26] Accordingly the 2015 ESC guideline for NSTEMI uses the GRACE risk score categorization, as one of the possible risk stratification criteria to evaluate the need and the necessary time window of an invasive strategy in NSTEMI patients, having a class-I-recommendation and a A-level of evidence. [53]

2.4. Statistical analysis

Continuous data are presented as mean ± SD, categorical variables are expressed as number (%). Equality of distribution of categorical variables between subgroups was analyzed by chi-squared test. For analysis of predictors for bleeding events univariate logistic regression analysis was used. In the analysis

- Clinical observations: age, sex, body mass index (BMI)
- Laboratory findings: renal insufficiency, troponin, CK/CK-MB (NSTEMI vs. UAP)
- Echocardiographic data: heart insufficiency (LVEF)
- Prior medical history: prior ACS or PCI/ACB-OP, diabetes mellitus, hypertension, hyperlipidemia
- Prior drug intake: anticoagulants, P2Y12-inhibitors
- Platelet function analysis: ADP-Multiplate data

- Pre- and peri-interventional therapy: treatment option, P2Y12-inhibitor loading therapy, change of ADP-antagonist, use of sealing system
- Follow-up data: MACE at three months

Factors with a significance level of $p < 0.1$ in univariate analysis were included into multivariate model. Multivariate analysis was then used to identify independent predictors of bleeding. For comparison of categorical and continuous data a two-sided p-value of < 0.05 was considered statistically significant. All statistical tests were performed with IBM SPSS Statistics software, version 21.0.

2.4.1. Platelet function analysis

Platelet function analysis was performed via ADP-Multiplate analyzer in order to integrate the platelet function into the evaluation of the individual bleeding risk of patients. Other platelet activators, that were also measured via Multiplate were not of high interest for the context of this study and therefore not further analyzed and evaluated. A total of 421 out of 439 patients (95,9%) received measurements of their platelet function.

The P2Y12-receptor, on the surface of blood platelets, is one of the mechanisms used to regulate platelet aggregation. It is a G-protein coupled protein for ADP. After binding an ADP-molecule, it leads to platelet activation and supports the clotting process. Therefore, it can be used as a target for P2Y12-antagonists, like clopidogrel, prasugrel and ticagrelor, to prevent thromboembolism. Measurement of the platelet function via ADP-Multiplate analyzer shows the degree of inhibition reached by the administered P2Y12-inhibitor. It also allows classification of each patient, receiving a P2Y12-inhibitor, in regard to their individual responsiveness to the administered medication. This is usually done, when administering clopidogrel. The patient is categorized as a) normal-responder, b) low-responder or c) non-responder correlating with an increasing risk for ischemic events in low- or non-responders and can be used as indication to change treatment protocol. On the other hand normal responders and patients with high

responsiveness of a certain platelet activator antagonist may show a higher risk for bleeding events.

Functionality of the multiplate analyzer:

The multiplate analyzer uses impedance aggregometry. It is a method, that continuously records the electrical impedance between two metal sensor electrodes inside the test cuvette. Adhesion and aggregation of platelets on the surface of these electrodes will result in change of impedance , which is continually recorded by the analyzer.

The patient sample consists of whole blood and is primarily anticoagulated with the thrombin antagonist Hirudin. For this reason, after admission of the patient and consent to the study procedure, a blood panel was drawn for the laboratory work up as well as the multiplate analyzer. The sample is diluted with saline and incubated at 37 °C for about three minutes before adding the chosen platelet agonist.

There are different inductors of platelet aggregation, which can each be evaluated individually via multiplate:

- ASP-I-Test is conducted via the activator arachidonic acid and is typically influenced by the administration of ASA.
- ADP-Test, where ADP is used as an activator and is affected by P2Y12-inhibitors and is therefore the platelet function test of choice, evaluated in this study.
- Thrombin receptor activating peptide (TRAP-6), which stimulates the thrombin receptor independently is used as negative and quality control of platelet aggregation. This is also why Hirudin must be used. The TRAP-6 activator also includes the platelet inhibition by GPIIb/IIIa-antagonists and is therefore a necessary control, when evaluating platelet function.
- COL-test using collagen as inductor of platelet aggregation.
- RISTO-test using Ristocetin as platelet agonist.

The patients blood was usually analyzed for all of the aggregation agonists mentioned above, except for the Ristocetin. For this particular study cohort however, only the

ADP-test was of specific interest, because of its correlation to P2Y12-inhibitor administration. The chosen activator is added to the whole blood of the patient and allows the quantitative in vitro ascertainment of platelet aggregation by detecting changes in impedance over time. The results are usually depicted in form of a graph showing the change in impedance over time from which the area under the curve in [U] is calculated. This parameter is the most useful in assessing platelet function and was used within this study group. Further parameters are the maximum aggregation in [AU] showing as the maximum height of the curve and the velocity of aggregation in [U/min].

Different factors may affect the result of the multiplate, including thrombocytopathy, thrombocytopenia and all medication influencing the platelet aggregation. The reference value for the ADP-test in non anti-coagulated whole blood sample is 57-113 U, which depicts the 5th to 95th percentile. When using anticoagulation in form of P2Y12-inhibitors the value is expected to be lower.
A 2009 cross validation of the multiple electrode aggregometry to different systems testing platelet function in patients came to the conclusion that the greatest signal magnitude for clopidogrel and Aspirin was found in multiplate analysis. [57]

2.5. Follow-up

To evaluate possible short-term consequences on outcome we included a three months follow-up with each patient. This was done by phone call with the patient or close relatives, additionally to retrospectively checking newer physician letters and re-hospitalizations at the hospital of Tuebingen (UKT).

The following primary endpoints were included in the follow-up:

- Stroke
- Transient ischemic attack (TIA)
- ACS
- Stent thrombosis
- Pulmonary embolism
- Revascularization
- Death

All 439 patients received a three months follow-up. None was completely lost within this time span.

3. Results

3.1. Baseline characteristics

439 patients met the necessary criteria and were included into this study. Out of which 174 patients (39.6%) were hospitalized because of UAP and 265 patients (60.4%) were diagnosed and treated for a NSTEMI.

Baseline patients' characteristics are shown in table 2. 69.7% of the patients are male and 30.3% female with an overall mean age of 68 years (CI 67 bis 69). 31.9% suffered from diabetes, 49.8% had hyperlipidemia, 35.1% were current smoker and 81.3% were previously diagnosed with hypertension. The mean body mass index (BMI) was 27.8 kg/m2 (CI 27.4 - 28.3). Adipositas defined as BMI > 30 kg/m^2 was seen in 27% of all patients.

The mean left ventricular function (LVEF, %) was at 50.4% and the mean creatinine at 1.1 mg/dL with a calculated mean GFR (Cockcroft-Gault) of 84.6 ml/min/1.73m^2, representing a KDOQI stadium G2. Baseline medication showed that in 17.4% a P2Y12-inhibitor was already included and 9.3% of the patients used an anticoagulant. Prior MI was recorded in 29.1% and prior need of PCI in 36%.

ADP-antagonists loading characteristics are shown in table 3. 80.9% of patients were administered or already taking clopidogrel, 14.4% were treated with ticagrelor and 4.8% with prasugrel. The combined group of newer ADP-antagonists made up 19.1%.

Timing of the loading was done in 52.8% prior to PCI, in 31% prior and at time of PCI, 10.3% already used a P2Y12-inhibitor and were loaded additionally at time of PCI and 5.9% were only having the P2Y12-inhibitor already as premedication.

Baseline characteristics	**Study cohort n = 439**	**Cohorts depending on bleeding occurrence**		**p-value**
		Control group n = 300	**Event group n = 139**	
Gender m/f (%)	69.7/30.3	71.7/28.3	65.5/34.5	0.214
Age (years)	68 (67-69)	67	70.6	3
BMI (mean)	27.8 (27.4-28.3)	27.4	27.7	0.507
Adipositas BMI > 30 (%)	26.9	25.5	28.3	0.508
Diabetes mellitus (%)	31.9	28.6	38.7	0.078
Hyperlipidemia (%)	49.2	49	51.5	0.708
Hypertension (%)	80.4	80.6	82.4	0.714
Current smoker (%)	34.9	37.5	30	0.127
GFR (mean)	84.6	89	80.2	**0.060**
GFR < 60%	35.7	28.5	34.1	**0.031**
Left ventricular function (mean, %)	50.4	51.9	48.8	
LVEF < 55%	48.7	48.2	50.0	0.371
UAP vs NSTEMI	38.7 vs 61.3	41.9 vs 58.1	35.5 vs 64.5	0.251
Baseline medication				
Prior P2Y12-inhibitor (%)	17.4	14.4	20.4	**0.097**
Prior anticoagulant (%)	26.9	24	33.3	0.170
Prior MI (%)	29.1	26.8	31.4	0.247
Prior PCI (%)	36,0	34.9	37	0.652
Peri-interventional				

Baseline characteristics	Study cohort n = 439	Cohorts depending on bleeding occurrence		p-value
		Control group n = 300	Event group n = 139	
Sealing system (%)	43.7	42.7	46	0.517
Stent (%)	80	76.7	87.1	**0.011**
BMS (%)	12.5	11.7	14.4	0.426
DES (%)	64.9	62.7	69.8	0.147

Table 2: Baseline characteristics

Baseline loading characteristics	Patients n = 439	Cohorts depending on bleeding occurrence		p-value
		Control group n = 300	Event group n = 139	
Clopidogrel (%)	80.9	84	74.1	0.014
Ticagrelor (%)	14.4	11.3	20.9	0.008
Prasugrel (%)	4.8	4.7	5	0.018
New ADP-antagonist	16.9	13.7	24.2	0.017

Table 3: Loading characteristics

3.1.1. Peri-interventional treatment

Stent

An intracoronary stent was placed in 351 cases (80%) and only 87 patients (19.8%) were not treated via stent. 2.5% received a bare metal stent (BMS) as well as a drug eluting stent (DES) and one patient (0.2%) had no documentation of treatment option.

Access site

With 431 patients (98.2%), the majority was accessed via femoral artery for the procedure, whereas only three patients (0.7%) were done by radial artery access. In the case of four patients (0.9%) the access way was not known and one person (0.2%) was done by radial and by femoral artery.

Sealing system

A protective sealing system used at puncture site to prevent bleeding events was implanted post-interventional in 192 patients (43.7%). 245 patients (55.8%) were not treated with a sealing system and for two patients (0.5%) the status was unknown. Out of all sealing systems being used, 37.1% of all patients received AngioSeal, 4.8% were treated with ExoSeal, 1.4% with ProGlide. 0.5% received a TR-band following intervention via radial artery.

Control group versus event group

Stent

76.7% received a stent implantation of any kind within the control group and 87.1% within the event group. A significantly higher percentage of patients out of the event group compared to the control group received treatment via stent, which showed to be true for both stent types. 14.4% versus 11.7% of patients were treated with BMS and in 69.8% versus 62.7% of the patients a DES was placed. Only 12.2% of patients in the event group were not receiving a stent during PCI, but 23.3% of the control group were not treated by stent implantation. The data shows that there was a significantly higher rate of patients treated via stent of the coronary artery within the event group ($p = 0.011$), suggesting a higher risk of bleeding, when using a stent. This was true for BMS- and DES-implantation. On all the patients being treated via stent, the event group makes up about 34.5%, which is not significantly larger than the proportion made up by this cohort on all the patients (31.6%). The same can be shown for DES and BMS implantation separately, suggesting no significant increase of risk in bleeding for patients being treated with stent implantation.

Sealing system

Comparison shows that a sealing system has been used more frequently, but not significantly so, throughout the event group with a total of 64 patients (46%) compared to 128 patients (42.7%) in the control group (p = 0.517). This distribution was true for all sealing system, except for the use of ProGlide with very small patient numbers respectively. 35.7% of the patients in the control group were treated via AngioSeal and 4.7% with ExoSeal versus 40.3% and 5% in the event group. Out of the control group, 56.7% compared to only 53.9% of the event group were not treated with a protective system following intervention.

Paradoxically, the amount of usage of an angio sealing system, used to protect bleeding after PCI, was more frequent among patients suffering a bleeding. This may be explained by the fact, that those patients were already considered at greater risk of a bleed and therefore were more frequently treated with protective sealing systems. The proportions made up by both cohorts on all sealing systems used, does not seem to vary significantly from their proportion on the study cohort (33.3% versus 31.6%).

3.2. Bleeding complications

3.2.1. Overall bleeding events

Out of all 439 patients included in the study 139 (31.6%) suffered a documented bleeding event after PCI, making up the event group. 300 patients (68.3%) did not show any signs of a post interventional bleeding complication (control group). (Fig. 1)

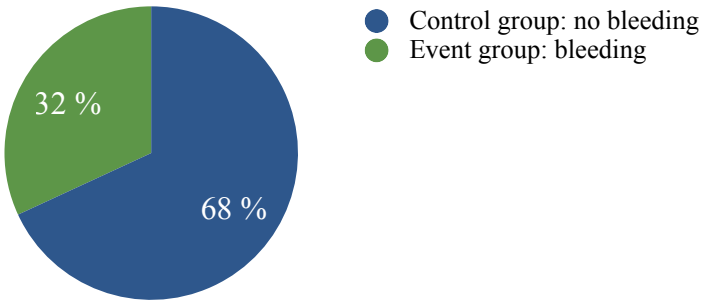


Fig. 1: Proportion of occurred bleedings of entire study cohort.

Bleeding severity

The event group consisted of 84 patients that were classified as TIMI minimal, 47 as TIMI minor and 7 as TIMI major bleedings. One patient was not classified via TIMI bleeding score. (Fig. 2)

Classified by the GUSTO-Score, 123 patients suffered a mild bleeding, seven were classified as moderate- and again seven as severe bleeding complication. (Fig. 3) Using the BARC Classification, 78 patients suffered a type one bleeding, 43 a type two bleeding, eight patients suffered a type 3a bleeding, four patients a typ 3b bleeding, three suffered a type 3c bleeding and one patient a type four bleeding. There was no occurrence of type five bleeding throughout the entire study cohort. Altogether, 16 patients had a major bleeding event, defined by us as being classified BARC three or above. (Fig. 4)

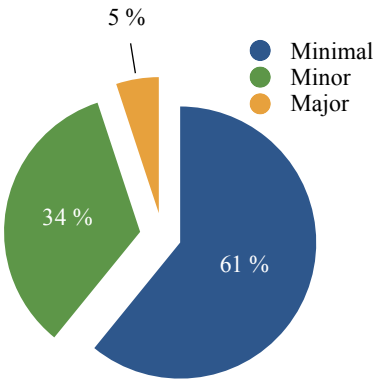


Fig. 2: TIMI

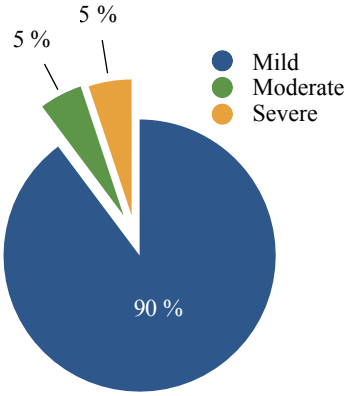


Fig. 3: GUSTO

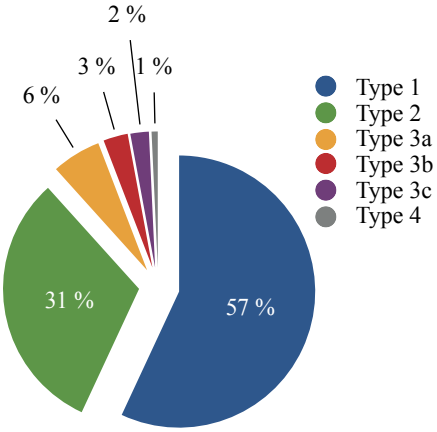


Fig. 4: BARC

Fig. 2,3 and 4: Proportion of bleeding severity (%) on all bleeding events by classification score used.

Out of all patients of the event group, major bleedings made up 5.1%, when classified via TIMI or GUSTO and 11.5%, when classified via BARC. There was a significant (two to three fold) higher initially calculated mean major-bleeding risk for the entire study population, when compared to the observed occurrence of major bleeding events after intervention (4.7% and 6% risk with ACUITY and CRUSADE respectively vs.1.6% major bleeding events when classified TIMI/GUSTO, p = 0.011). There was no significant difference when compared to BARC-classification of major bleeding (3.6%,

p = 0.114). When comparing initial bleeding risk stratification with occurred bleeding events for the event group only, there is no significant difference for major bleeding, when classified by TIMI/GUSTO, but a significant difference, when evaluated via BARC (p = 0.607 and p = 0.050 for BARC). (Fig. 5)

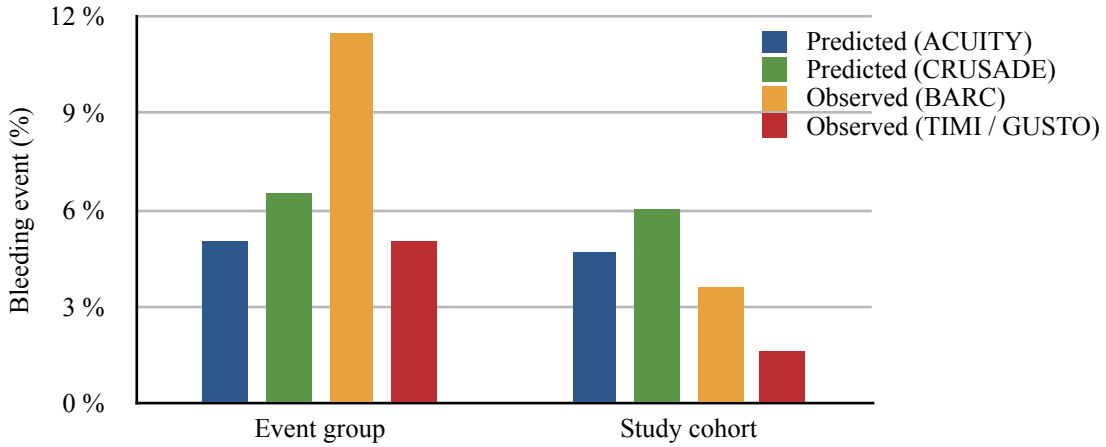


Fig. 5: Calc. risk vs. occurrence of major bleed (p=0.607 and p=0.050).

Clopidogrel versus new ADP-antagonists

355 patients (80.9%) received clopidogrel and 84 patients (19.1%) received one of the newer P2Y12-inhibitors, including either ticagrelor or prasugrel.

When looking at differences between the amounts of occurred bleedings after PCI, the newer P2Y12-inhibitors were associated with higher risk of overall bleeding events in comparison with clopidogrel. (44.3% vs. 28.2% for the new inhibitors vs. clopidogrel respectively, p = 0.017, Fig. 6). This difference was driven by minor bleedings (TIMI minimal or minor 40.5% vs. 27.9% for new inhibitors vs. clopidogrel respectively, p = 0.05, TIMI major 1.4% vs. 2.4%, p=ns)

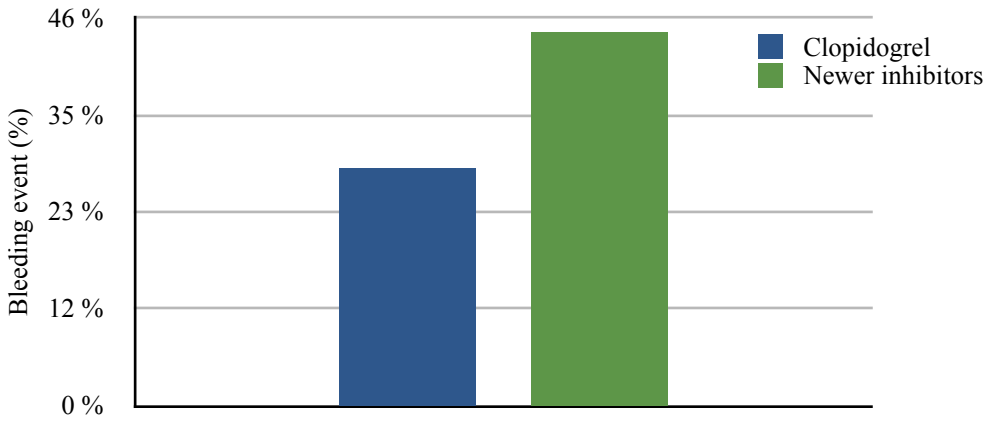


Fig. 6: Total bleedings clopidogrel vs. new P2Y12-inhibitors (p=0.017).

Timing of P2Y12-inhibitor administration

The cohort, which received a pre-PCI loading only, included a total number of 232 patients (52.8%). 26 patients (5.9%) only had a P2Y12-Inhibitor as part of their pre-medication, 136 patients (31%) received a loading dose prior to PCI as well as at the time of PCI and 45 patients (10.3%) used a P2Y12-inhibitor as pre-medication as well as receiving a loading dose during PCI. We did not find significant differences concerning overall bleeding complications according to different loading strategy. There were 32.8%, 42.3%, 25.7% and 37.8% bleeding complication in patients with pre-PCI loading, pre medication only, pre-PCI with second loading and pre medication with second loading respectively. (p = 0.211, Fig. 7)

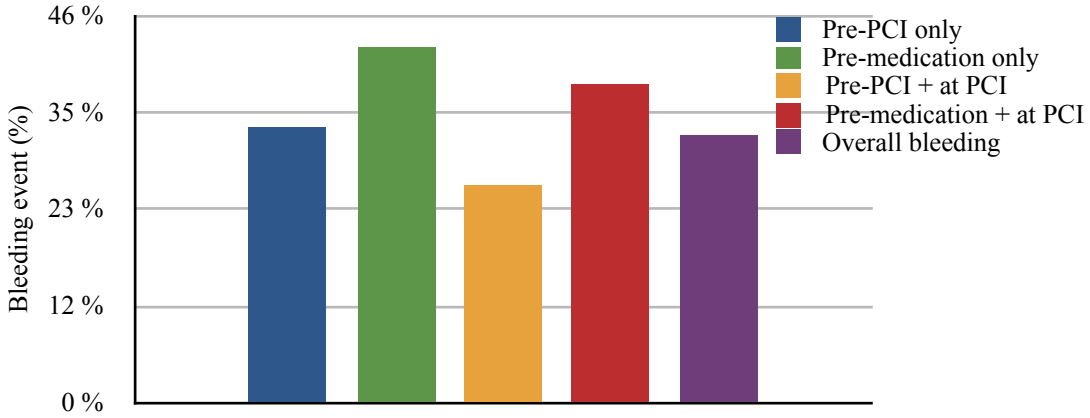


Fig. 7: Rate of bleeding events depending on timing of loading (p = 0.211).

Evaluating the data regarding TIMI major bleedings alone did not show a significant difference. There were 6.6%, 0.0%, 5.9%, 0.0%, TIMI major bleeding complication in patients with pre-PCI loading, pre medication only, pre-PCI with second loading and pre medication with second loading respectively. (p = 0.166; Fig. 8).

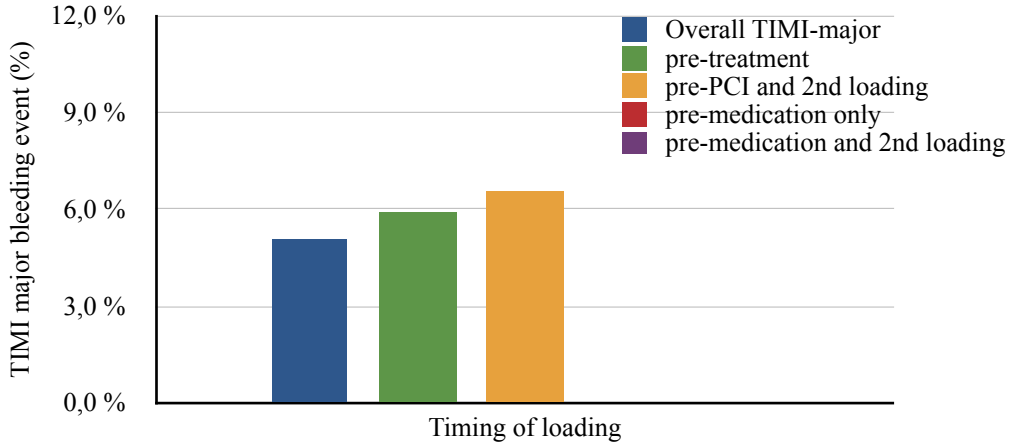


Fig. 8: Proportion of patients of loading categories suffering a TIMI major bleeding (p = 0.166).

3.3. Risk factors

3.3.1. Bleeding risk scores

CRUSADE

The mean CRUSADE risk score for the entire study population was calculated at 26.5 (CI 25.0 - 28.0), being considered a low-risk category with a risk of in-hospital major bleeding of approximately six percent. Evaluation of the CRUSADE risk score for each cohort individually showed, that the control group had a calculated mean CRUSADE score of 24.6, whereas the event group averaged a higher mean CRUSADE score of 29.4. Accordingly, both cohorts would also individually be classified as low risk, but ranging from approximately 5.7% risk of in-hospital major bleeding for the control group to about 6.5% risk for the event group. (Fig. 9)

ACUITY

The mean ACUITY risk score was calculated at 14.8 points (CI 14.0 - 14.6), which is considered a moderate risk category with an expected non-CABG major bleed within 30

days of approximately 4.7%. The mean ACUITY risk score for the control group was 13.9 points resulting in a classification of moderate risk with a risk of major bleeding of about 4.7%. The event group showed to be a high risk classification, with a mean ACUITY risk score of 16.5 points, translating to a risk of major bleed of approximately 5 to 6% within 30 days. (Fig. 9)

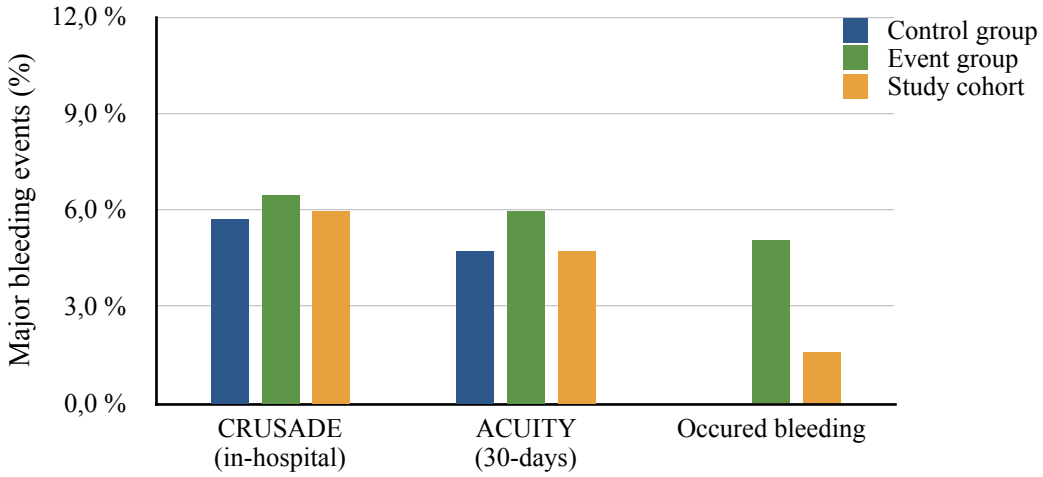


Fig. 9: Bleeding risk by CRUSADE and ACUITY and occurred major bleeding (%) for both cohorts.

3.3.2. ADP-Multiplate

Out of all 439 patients included into the study, 95.9% received an ADP-multiplate analyzing to further determine the platelet function in regard to ADP-receptor interaction, whereas 18 patients (4.1%) did not receive a measurement of their platelet function via multiplate. The overall mean ADP test for all 421 patients was calculated at 27.2 U. Patients with bleeding events showed significant higher platelet inhibition (14.6 U vs. 20.2 U for patients with and without bleedings respectively, $p = 0.012$) at time of steady-state (Fig. 10). The data shows a significant lower mean ADP-multiplate test in patients suffering a bleeding event compared to patients without any bleeding events, suggesting a low ADP-test to be a significant predictor for the increase in risk of adverse bleeding events.

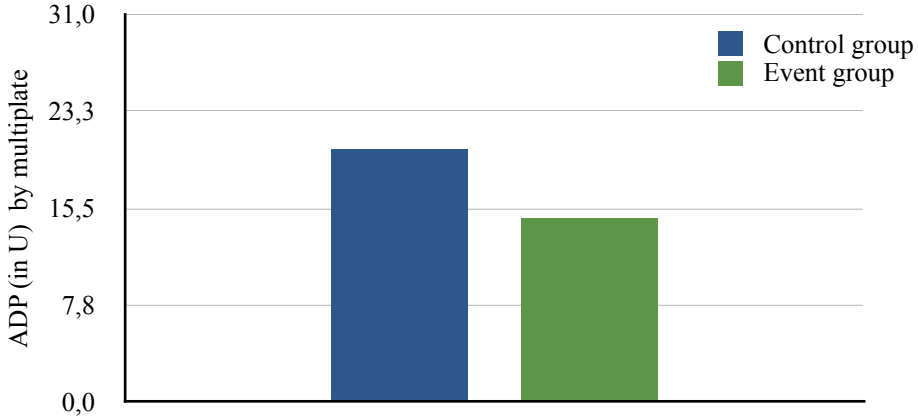


Fig. 10: Mean ADP in control vs. event group in steady state (p = 0.012).

P2Y12-inhibitor depending mean ADP

Patients receiving a new ADP-Antagonists showed a significant lower ADP-test compared to patients receiving clopidogrel. (23.0 U and 30.6 U respectively, p= 0.017; Fig. 11).

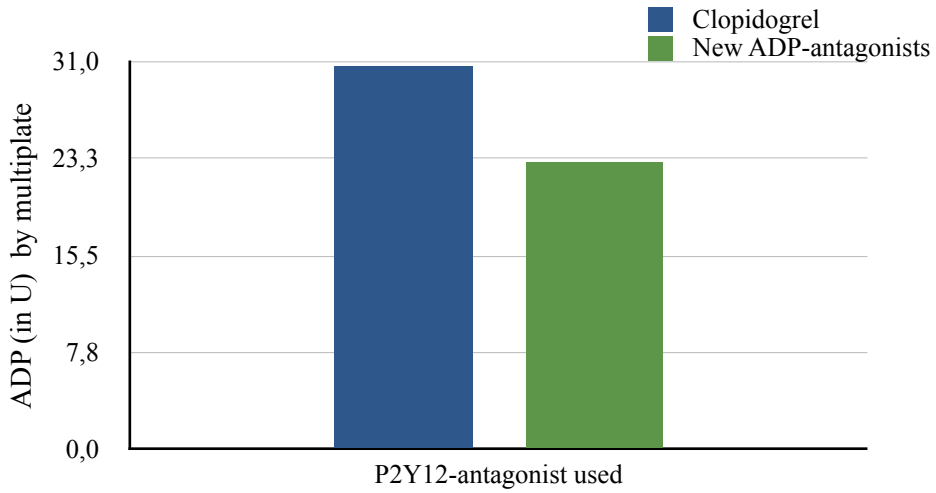


Fig. 11: ADP-Multiplate for clopidogrel vs. new ADP-antagonists (p = 0.017).

3.3.3. Risk factor variables

The following five variables showed to be significant predictors of an increase in adverse bleeding events in NSTE-ACS patients following PCI and P2Y12-inhibitor pre-treatment ($p < 0.1$; table 4) and were included in a multivariate analysis: Age at time of intervention, usage of newer P2Y12-inhibitor, diabetes mellitus, reduced renal function (GFR < 60%) and the ADP-multiplate data.

In the multivariate analysis only the ADP-multiplate and diabetes mellitus remained a significant predictor of bleeding events (table 5). Age, usage of newer P2Y12-inhibitor and decreased renal function were no longer significant.

	B	Odds ratio	95% C.I.for EXP(B)		P
			Lower	Upper	
Age at intervention	0.028	1.028	1.010	1.047	**0.003**
Gender	0.273	1.314	0.854	2.019	0.214
BMI (kg/m2)	0.013	1.013	0.975	1.052	0.507
New ADP-inhibitor	0.593	1.810	1.110	2.951	**0.017**
IAP versus NSTEMI	0.243	1.275	0.842	1.932	0.251
Hypertension	0.099	1.104	0.652	1.869	0.714
D.M.	0.383	1.466	0.958	2.244	**0.078**
HLP	0.077	1.081	0.721	1.620	0.708
GFR (CockroftGault)	0.005	0.995	0.990	1.000	**0.060**
Renal insufficiency (GFR < 60%)	0.484	1.623	1.045	2.521	**0.031**
Anticoagulants	0.460	1.584	0.821	3.054	0.170
ADP-Multiplate	0.033	0.968	0.994	0.993	**0.012**
MACE	0.183	1.201	0.629	2.293	0.578
Heart insufficiency (LVEF < 55%)	0.240	1.271	0.752	2.147	0.371
Sealing system used	0.134	1.143	0.763	1.712	0.517
prior MI	0.261	1.298	0.835	2.016	0.247
Secondary loading	0.195	1.215	0.809	1.825	0.348

Table 4: Statistical significance of risk factor variables.

	B	Odds ratio	95% C.I.for EXP(B)		P
			Lower	Upper	
Age at intervention	0.004	1.004	0.974	1.034	0.804
New ADP-antagonist	0.318	0.727	0.315	1.682	0.457
Diabetes mellitus	0.827	2.287	1.216	4.300	**0.010**
Renal insufficiency	0.473	1.604	0.705	3.654	0.260
ADP-Multiplate	0.028	0.972	0.947	0.998	**0.034**

Table 5: Multivariate analysis

Other risk factors tracked did not show to be significant predictors for post interventional bleed in NSTE-ACS patients after ADP-antagonists pretreatment ($p > 0.1$), including: gender, BMI, UAP vs. NSTEMI, hypertension, HLP, use of anticoagulants beforehand, MACE, heart insufficiency defined as LVEF below 55%, the usage of a sealing system after intervention, prior MI and administration of a secondary loading dose in patients (table 4).

3.4. Follow-up

3.4.1. Mortality risk scores

The mean GRACE mortality score was 96.7 (CI 94.2 - 99.1), which is considered a low risk category with an in-hospital mortality of below one percent and an intermediate risk category for a six months post-discharge mortality of three to eight percent. When calculating the risk score for each subgroup individually, it showed a score of 100.1 for the event group and a risk score of 94.5 for the control group. Both cohorts would still be classified as low risk category for in-hospital mortality and intermediate risk category for six-months post discharge mortality for NSTE-ACS patients.

The mean TIMI risk score was at 2.9 (CI 2.8 - 3.0) giving an approximately 13% risk of all-cause mortality, new or recurrent MI or severe recurrent ischemia at 14-days.

Calculating the TIMI risk score for each cohort individually shows a score of 3.1 for the control group and a score of 2.8 for the event group.

Comparing the calculated mean risk of mortality at time of hospitalization with the overall mortality at the three months follow-up showed a lower percentage of death than expected with 1.6% versus 3-8% in the GRACE score and 13%, including the risk of other primary end points, in the TIMI risk score (Fig. 12).

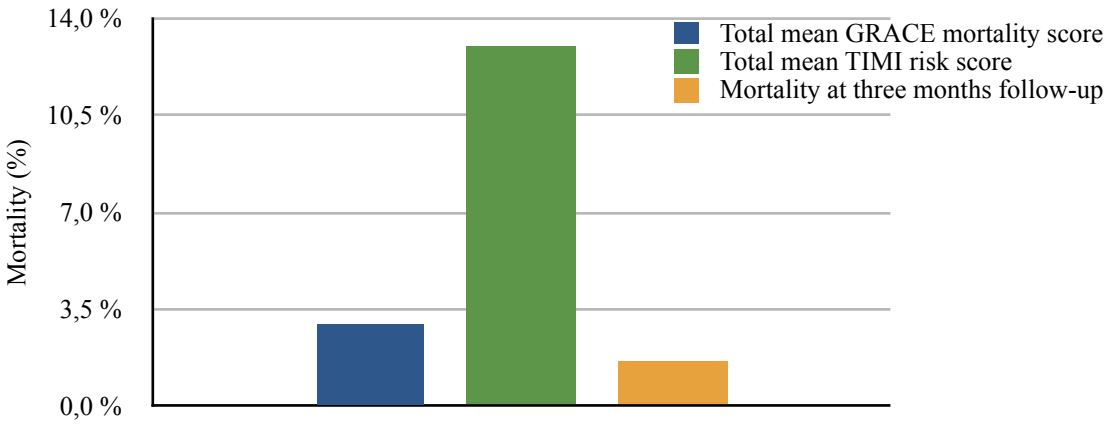


Fig. 12: Calculated mortality risk versus observed mortality at three months.

3.4.2. Overall outcome

The three months follow-up concluded that out of 439 patients, 82% of the patients suffered no further event. 79 patients (18%) reported one of the primary endpoints as defined before. 1.4% suffered a stroke and 0.2% a TIA, 6.6% were re-hospitalized, because of an ACS, 0.5% were treated for stent thrombosis, 7.7% had to receive a re-PCI and 1.6% had died within the given time span. No patients were completely lost to follow-up (Fig. 13).

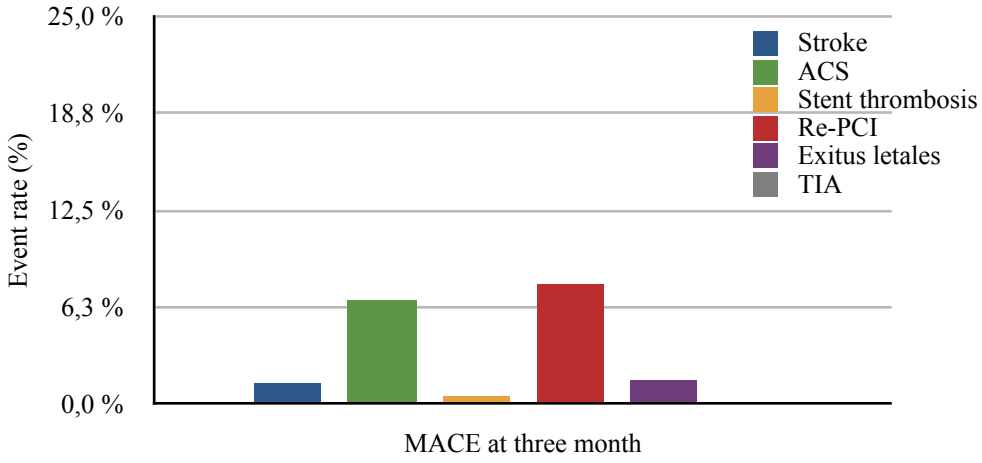


Fig. 13: Event rates at three months follow-up of the entire study cohort.

3.4.3. Timing of P2Y12-inhibitor

25% of patients in the control group and 23.5% in the event group receiving a loading dose of an ADP-antagonist at time of PCI additionally to their prior medication had to be re-hospitalized because of a recurrent ACS. This proportion is significantly higher than the proportion of recurrent ACS on all patients, being 6.6%. It is also higher than the calculated mean TIMI risk score for primary endpoints of about 13%, which includes the risk of MI (Fig. 14 and 15). There were two patients in the loading at PCI + prior medication group and none in the other loading groups that suffered an ACS within the first 5 days after initial PCI, which showed to be significant (p = 0.001).

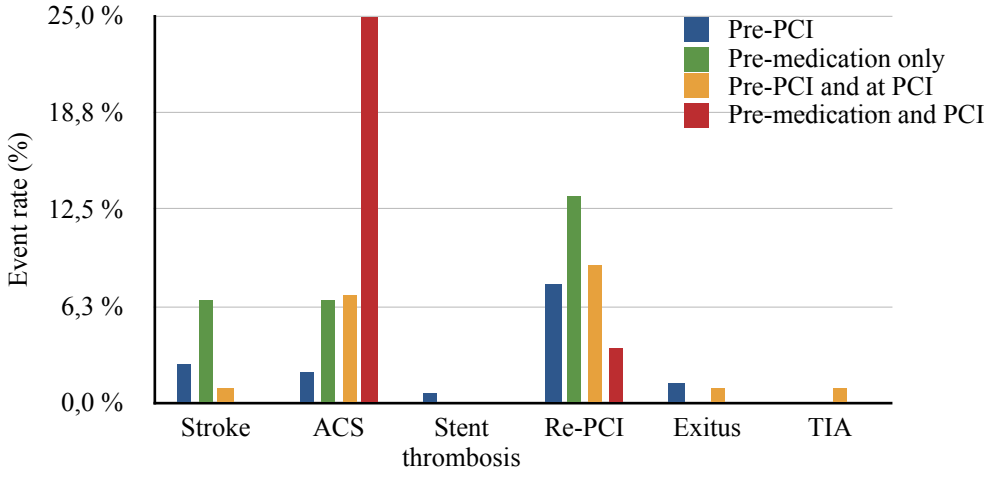


Fig. 14: Follow-up depending on loading time in the control group.

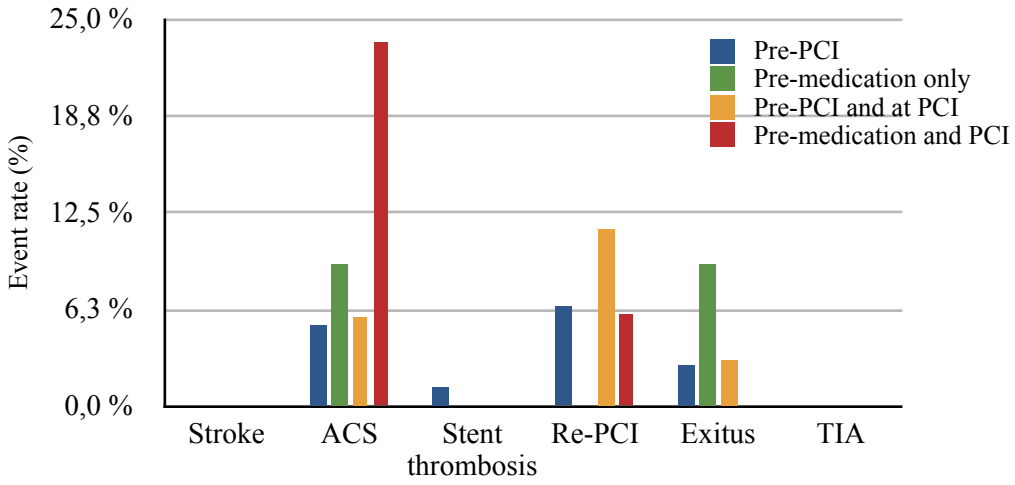


Fig. 15: Follow-up depending on loading time in the event group.

3.4.4. Control group versus event group

In the control group six patients suffered a stroke within three months making up 2% and one patient suffered a TIA (0.9%), whereas in the event group none of the patients stated to have had a stroke, nor a TIA. In the control group three patients died, making up one percent, whereas four patients (2.9%) out of the event group have died during the same time interval ($p = 0.144$). The higher percentage of death within this cohort correlates with the higher pretreatment mortality risk calculated via GRACE and TIMI risk score (Fig 16). However, in both cohorts and in all patients the percentage of death is lower than the mortality risk that was initially determined. The data also states no significant difference in the likelihood of occurrence of primary endpoints at the three months follow-up between both groups ($p = 0.792$).

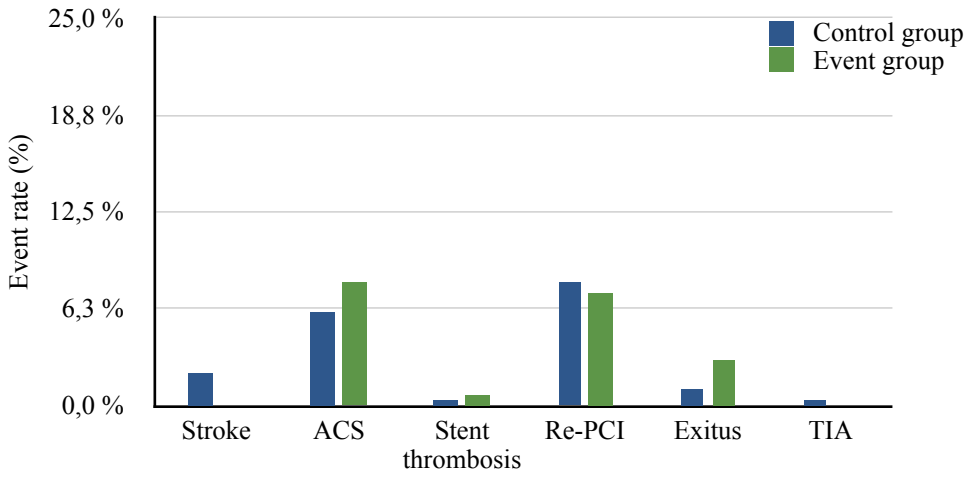


Fig. 16: Rate of primary endpoints at three months follow-up. (p = 0.792)

3.4.5. Clopidogrel versus new ADP-antagonists

Of the 355 patients loaded with clopidogrel, 1.4% suffered a stroke or TIA, 5.4% were re-hospitalized because of ACS, 0.5% suffered a stent thrombosis and 7% needed a revascularization. In the group of 84 patients loaded with one of the new P2Y12-inhibitors the numbers were 2.4%, 11.9%, 0% and 10.7% respectively. It shows no significantly higher risk for the combined ischemic endpoints (p = 0.640), but for ACS occurrence alone a significant higher risk in the new P2Y-12-inhibitor group (p = 0.028; Fig. 17).

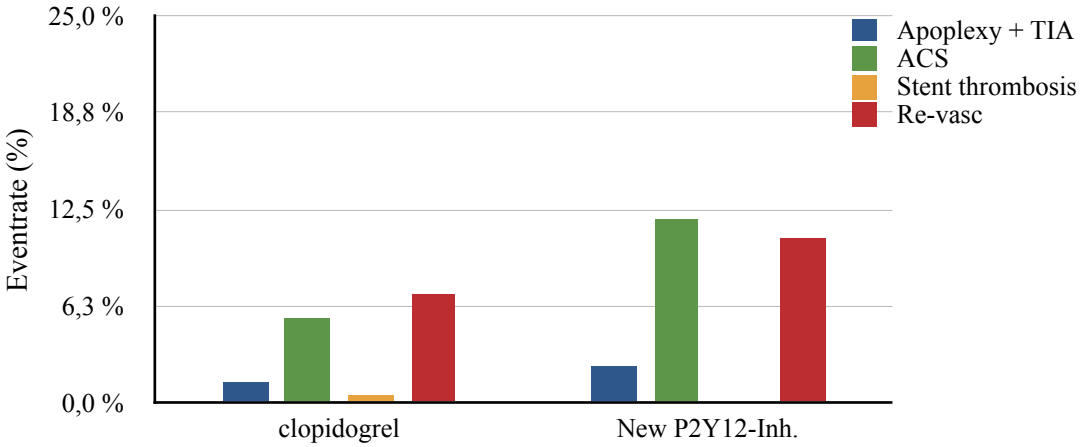


Fig. 17: Rate of primary endpoints at three months follow-up in comparison between clopidogrel and new P2Y12-inhibitor. (p= 0.640 (combined), p = 0.028 (ACS alone))

4. Discussion

4.1. General bleeding outcomes

The analyzed data showed that post interventional bleeding, with an occurrence rate of 32% still is a significant adverse complication in NSTE-ACS patients undergoing PCI. Most bleeding events were located at puncture site and classified as being minor. Only 5 to 11.5%, depending on the classification score used, of the documented bleeding events were classified as being major. Those numbers are comparable to the data presented by earlier studies [26, 56, 29] and lower than the initially calculated risk of major bleeding throughout the study population. The overall bleeding rate of 32% seemed to be a little higher than described in most previous studies [29]. This may be the case, because of a more detailed documentation and retrospective research in regard to minor bleeding events, since most described minor bleedings were small hematomas at puncture site.

The results show a similar distribution of bleeding severity among TIMI and GUSTO classification, but a more than two times higher rate of „major“ bleeding, when classified via BARC (1.6% for TIMI and GUSTO versus 3.6% for BARC). This may be the result of the more detailed classification that is made by BARC, making up more categories and initially avoiding a description as major or minor, but instead to number the categories with increasing severity. We chose to define a BARC three score or higher as being major, which may have led to a higher percentage of major bleeding, when classified via BARC, as compared to GUSTO and TIMI.

The study shows that bleeding events after percutaneous intervention is a common and potentially serious complication, that needs to be taken into consideration before performing PCI. The possible negative influence of bleeding events on overall mortality has been stated by other studies and described in the introduction. These findings on a worse outcome after occurred bleeding, however, could not be supported by the analysis of the three months follow-up in this study as shown below.

Overall the data supports the need for better evaluation tools for the risk of bleeding complications before undergoing PCI or receiving a P2Y12-inhibitor and preferably at point of admittance in order to further decrease these adverse complications in the future.

4.2. Risk factor analysis

Primary aim

After evaluation of sixteen different patient characteristics, we were able to identify five variables to be significant predictors of bleeding events following PCI in NSTE-ACS patients. These variables included: age at intervention, diabetes mellitus, ADP-Multiplate analysis data, renal insufficiency with a GFR < 60% and the usage of a newer P2Y12-inhibitor in form of ticagrelor or prasugrel. After multivariate analysis only the variables diabetes mellitus and the measured ADP-multiplate data stayed significant. The other three predictors mentioned did not stay significant.

The measured platelet function via ADP-multiplate analyzer was able to show that patients, who suffered a post interventional bleeding event had significantly higher platelet inhibition. This result sustains that a higher platelet inhibition correlates well to a higher risk of adverse bleeding events after PCI and could be used to evaluate patient risk. An ADP-multiplate testing is a rather time consuming process, which does not seem to be useful to wait on for emergency patients presenting with NSTE-ACS. It could however be analyzed at point of admission, before treatment with P2Y12-inhibitors and then be used after further evaluation for possible preventive measures during or after PCI.

Unfortunately, the study was not able to state enough significant risk factors of post interventional bleeding occurrences in NSTE-ACS patients to create receiver operating characteristics (ROC) curves to compare to the existing ACUITY and CRUSADE score. Furthermore we were not able to create the bleeding risk stratification score as anticipated by this study, because of the missing significance of risk factors and their influence on bleeding events. We can only conclude five risk factors that showed to be significant, of which only two remained significant after multivariate analysis being diabetes mellitus and low ADP-multiplate scores.

The study only included a total of 439 patients, which may be too small of a study population to create a significant risk profile for patients. Risk factors described by other studies, like the baseline use of anticoagulants, were hardly represented within this study group. Only 41 patients were using anticoagulants as baseline medication, which

showed to have a significance level of 0.170 in our statistical analysis. We believe that those variables, among others, might show a bigger impact on bleeding risk than represented by this study population, and could be used as starting point for further studies and hypotheses. Risk factors of bleeding events previously described by CRUSADE and ACUITY [40, 61], like advanced age, female sex, signs of CHF and prior MI did not hold up to be significant throughout our data analysis of NSTE-ACS patients.We believe that since CRUSADE and ACUITY were designed in times of different therapy strategies we do need further investigation to create better risk stratification for todays patients and treatment options.

Secondary aim

The comparison of different pre-treatment P2Y12-inhibitor loading times in regard to post interventional bleeding occurrences in NSTE-ACS patients showed that none of the collected data was able to state a significant correlation. Therefore the findings of the study suggest, that the timing has no significant impact on the likelihood of a bleeding event. This showed to be the case regardless of the severity of the evaluated bleed. Therefore, according to our data, a pretreatment loading strategy has no significant impact on the adverse events after PCI of the NSTEMI patient. This showed to be the case in our collective of patients, which was mainly treated with clopidogrel, less often with ticagrelor. In difference to the ACCOAST-PCI trial, we saw hardly any use of prasugrel in our collective, so that we can make no further statement to underline or contradict the findings of the ACCOAST trial, that showed no benefits, but an increase in adverse bleeding events with prasugrel-pretreatment in NSTEMI patients. Given that the correlation was only analyzed for different timings all in regard to pre-treatment loading options and not in comparison to a loading strategy at the time of PCI only, we believe that further studies may be necessary to compare an overall pretreatment strategy to a loading strategy at time of PCI. So far the best possible timing for a P2Y12-inhibitor treatment is still not proven and remains uncertain for incoming NSTE-ACS patients.

In regard to the kind of P2Y12-inhibitors being used we were able to show, that the patients treated by newer ADP-antagonists, ticagrelor and prasugrel, showed to have significantly higher platelet inhibition, when compared to clopidogrel. Also there was a significant higher rate in bleeding, when using one of the newer ADP-antagonists with 42.9% versus 29.3%, when receiving clopidogrel. The use of different P2Y12-inhibitor could not be proven significant in a multivariate analysis. The data from the multiplate analysis (platelet inhibition) however still showed to be significant throughout the multivariate analysis and may be a starting point for further investigation of the risk in the new P2Y12-inhibitors. After adjusting for severity categorization via GUSTO and TIMI, where 5.6% of the patients receiving a newer ADP-antagonist and suffering a bleeding event were classified as being a TIMI major bleed. In the clopidogrel group, the proportion of TIMI-major bleeds made up 4.9% out of all bleeding events respectively. Since we expected and stated a higher occurrence rate in bleeding, when using one of the newer ADP inhibitors, after showing a significant lower ADP-measurement, we would explain the missing significance in the multivariate analysis of the data by the small patient numbers treated with one of the newer P2Y12-inhibitors throughout the study. Throughout the time span of intervention taken place, most of the patients were still loaded with clopidogrel, instead of one of the newer ADP-antagonists. After the pivotal studies TRITON-TIMI-38 in 2007 [68] for the use of prasugrel versus clopidogrel and the PLATO-trial in 2009 [66] for the use of ticagrelor versus clopidogrel the distribution between these P2Y12-inhibitors used has shifted. Both trials showed a reduced ischemic event rate in patients with ACS for the use of ticagrelor / prasugrel versus clopidogrel. The increasing use of newer P2Y12-inhibitors and has not been taken into account in any of the bleeding risk scores available so far and most studies have been performed on populations mostly consisting of cohorts using clopidogrel. This, unfortunately, has also been the case for this study. Therefore we believe this topic to be of interest for further studies with a bigger study population tested for better comparison.

4.3. Follow-up

The overall occurrence of tracked primary endpoints after three months was with a total of 18% lower than the calculated risk scores anticipated.

The follow-up data presented no significant difference in the occurrence of primary endpoints between both study groups. Therefore, in contrast to earlier studies, we cannot conclude that occurred bleeding events in NSTE-ACS patients have a negative impact on the risk of primary endpoints at three months. There was only a slightly higher rate of stroke and TIA (2.9% in the control group versus 0% in the event group) in patients not suffering a postinterventional bleeding event. This data would be explainable by a lower rate of platelet inhibition throughout the control group, which has also been shown by the ADP-Multiplate measurements. A lower inhibition of platelet aggregation leads to a higher risk of ischemic events. Other cofounders, like post interventional P2Y12-inhibitor treatment and patient compliance were not tested and would also affect the risk of further ischemic events. The higher, but not significant, mortality rate in the event group of 2.9% versus 1% in the control group tracks well with the higher initially calculated risk of mortality. It could be due to a higher risk profile and comorbidities by these patients.

It did show a significant higher occurrence of ACS within the subgroup of patients receiving an additional loading dose to their preexisting treatment with an ADP-antagonist. This result seems paradox, considering that the loading with an ADP-antagonist is supposed to reduce the risk of primary ischemic endpoints, but may be a confounder. It is possible that especially patients with a higher initial risk of ischemic events were given a second loading dose. These findings may also be explained by the overall small number of only 45 patients within this subgroup and the overall small patient numbers of the study.

Patients treated with a newer ADP-antagonist showed to make up a higher proportion on recurrent PCI, ACS and stroke after three months compared to the clopidogrel-group, of which only the occurrence rate in ACS stayed significant ($p = 0.280$). These findings also seem paradox, since the newer P2Y12-inhibitors have a higher effect on platelet inhibition, which could also be shown in the multiplate data. These results may again be

explained by the effect as a confounder, where the patients receiving the newer ADP-antagonist might have shown the higher initial risk for ischemic events. And again this subgroup consisted of small numbers of patients being treated with one of the newer inhibitors throughout the study group. Also the longterm treatment with P2Y12-inhibitors, preventing ischemic events, as well as patients compliance were not further taken into account.

4.4. Outlook

This study did show that bleeding complications are still a common problem following PCI and that ADP-multiplate analysis is a valid tool to evaluate platelet function and the risk of bleeding. Unfortunately we were not able to create a scoring system to classify NSTE-ACS patients at point of admission depending on their individual risk of bleeding. Other than the ADP-multiplate data and diabetes mellitus, the taken risk variables did not stay significant after multivariate analysis. Further studies with a bigger study population may be necessary to evaluate more risk factors and especially the role of newer P2Y12-inhibitors on occurring bleeding complications. Also the best timing of administration of the P2Y12-inhibitor is still unknown and may be subject for further studies.

We believe that the two bleeding risk scores being used, CRUSADE and ACUITY, both have their individual weaknesses, when applied to nowadays NSTE-ACS patient population. They both derived from a time with a bigger emphasis on bivalirudine or GPI-usage and a limited evaluation of different P2Y12-inhibitors and their timing of administration in regard to PCI treatment. Therefore, we believe a more accurate risk score, adapted to the treatment options of our time is vital in the evaluation of the risk of bleeding in NSTE-ACS patients at time of admission. This will help us in deciding on the right treatment option and timing and reduce the occurrence of adverse complications doing so. To create such a score, further, more adapted studies with bigger study populations will be necessary.

5. Limitations

Over all the study conclude is a retrospective analysis with a small number of patients, which limits the extent of the possibility to transfer these results to the general practice, but gives an idea of possible starting point for further studies and investigations.

1) Recall bias

The case control study design holds the risk of a recall-bias. In this case, especially that patients don't accurately recall certain risk factors to the right extent or follow-up data gets lost or is not remembered to the right extent.

2) Selection bias

Selection bias may occur in form that patients are not equally loaded with the same kind of P2Y12-inhibitor or different dosages within the subgroups. Also an exact selection of a matched control group is difficult to achieve and may lead to biased results. There was no cohort with loading only at time of PCI as control to the timing of P2Y12-inhibitor. Within the different risk factors being responsible for a higher risk in bleeding we cannot exclude all the confounders that may intervene with these results. There also may have been other factors before or after P2Y12-inhibitor treatment and PCI effecting the risks of bleeding in patients that are not known to us and have therefore not been included.

3) Different interpretation

The severity of the bleeding of the patients may have been interpreted differently by different doctors, especially when clinical diagnosis seemed sufficient and there was no laboratory panel or imaging. Also there may have been a different level of accurate documentation of bleedings, which we used to identify and classify the patients with. This could lead to missing bleeding events that were not accurately documented.

4) Follow-up

In the three months follow-up there may be circumstances for patients to drop out of the personal follow-up, for example being unable to get ahold of anymore or simply not wanting to be actively participating any longer. Also there may be a recall bias on whether there were certain end points taken place, for example not exactly knowing the

cause of death or further primary end points in a patient by their relatives being questioned.

5) P2Y12-inhibitors

There was no difference made between the three P2Y12-inhibitors used, so that there might be different outcomes when looking at each one of them individually. A possibility of confounding would also be the dosage of ADP-antagonists that was given and may vary between patients. We mainly saw the use of clopidogrel 600 mg as a loading dose prior to PCI, which may not reflect today routine with an increase in the usage of especially ticagrelor and prasugrel, as well.

6. Conclusion

Bleeding events following PCI are still a common complication among NSTE-ACS patients. This study was able to show a significant increase in the risk of bleeding for increasing inhibition measured via ADP-multiplate and for one of the cardiovascular risk factors, being diabetes mellitus after multivariate analysis. Therefore patients with a higher platelet inhibition are more likely to suffer a bleeding event after PCI. The initially shown increase of bleeding events, when using one of the newer ADP-antagonists did not stay significant in the multivariate analysis. There was no statistically significant increase in risk of bleeding in any of the other risk factors tested, nor depending on the timing of loading the patient. Unfortunately, we could not identify enough significant risk factors with a correlation to an increased risk in bleeding events to create a ROC curve to compare it to the existing ACUITY and CRUSADE risk scores. Also the data could not be used to create our own significant risk score to evaluate the risk of bleeding in incoming NSTE-ACS patients before treating via PCI, as anticipated as primary aim of this study. Following these results the only significant way to get a better idea of the bleeding risk of the individual patient would be by ADP-multiplate analysis, which seems not practical in terms of daily use before treating via PCI.

However, this study only included a small number of patients. Especially, when comparing P2Y12-inhibitors used, the group receiving one of the newer inhibitors was still very small. The study did show a significant higher platelet inhibition by newer P2Y12-inhibitors via ADP-multiplate data, but no significant increase in risk of bleeding throughout this subgroup. With nowadays increasing numbers of patients treated with one of the newer ADP-antagonists, this unknown role may be subject of evaluation in further studies.

The timing of pretreatment loading seems to have no impact on the occurrence of bleeding throughout our study group. Further studies however may compare the risks to a loading strategy at time of PCI and take the increased usage of ticagrelor and prasugrel into account.

Looking at adverse events the study showed a signifiant increase in the occurrence of ACS at three months after PCI, when using a newer ADP-antagonist. This paradox finding may be a confounder, since patients with higher ischemic risks may be more likely to receive one of the newer ADP-antagonists in an overall small subgroup receiving ticagrelor or prasugrel. The total end point of ischemic events was a little higher in patients receiving one of the new ADP-antagonists, but not significantly so. These findings contradict other studies (PLATO and TRITON-TIMI) and may be due to the small numbers of patients within these groups. We believe that further studies with better subgroups representing todays distribution of P2Y12-inhibitors used are needed to evaluate this complex topic better. There was no significant difference found between the two study groups or between different loading intervals in regard to ischemic end points at the three months follow-up.

We believe that further studies are necessary to investigate the bleeding risk, different loading times and dosages. These studies should consist of patient cohorts representing today's treatment options better, the more frequent usage of new ADP-antagonists, as well as the radial access site as primary access site used. With these data we hope a better and up to date risk score can be created to evaluate bleeding risk before treatment and enable a more personalized strategy.

7. Abstract

Introduction

This retrospective study was conducted at the university hospital of Tuebingen and included NSTE-ACS patients receiving a PCI throughout the years of 2011 to 2014. A total of 439 patients was included into study. We divided the cohort into two groups. The control group, making up 300 patients without any adverse bleeding event and the event group with 139 patients, all suffering a documented post interventional bleeding incidence. The primary aim of the study was to evaluate the overall risk for occurrence of adverse bleeding events in NSTE-ACS patients following PCI. Secondly we were looking to identify a risk profile for NSTE-ACS patients in order to create a score to better identify patients at risk for bleed. That again would allow the clinician to categorize the patient at time of admission and depending on classification change treatment protocols towards more preventive measures to reduce the risk of post interventional bleeding events.

Methods

We retrospectively analyzed 16 different factors to identify risk variables significantly associated with a higher risk in adverse bleeding events in NSTE-ACS patients undergoing PCI. Additionally we evaluated different times of loading and compared clopidogrel to one of the newer P2Y12-inhibitors in regard to bleeding events and occurrence of MACE after three months of hospitalization. Each patient got measurements of their ADP-inhibition via ADP-multiplate analysis. A three months follow-up was done with all patients.

Results

Out of all the variables tested five risk factors proved to show significant, including: diabetes mellitus, ADP-multiplate scores, renal insufficiency (GFR < 60%), age at intervention and the usage of a new P2Y12-inhibitor. In the multivariate analysis the following two variables persisted to be significant: the ADP-multiplate data and diabetes mellitus. The other three variables unfortunately did not stay significant.

There was no significant difference between clopidogrel and new ADP-antagonists in regard to bleeding events. Also there was no significant difference between both study cohorts in the risk for ischemic events at the three months follow-up. It did show a significant higher risk for ACS after five days and three months for patients with an ADP-antagonist in their prior medication, who received an additional loading at time of PCI.

Discussion

We were able to show that postinterventionel bleeding still states a risk after PCI, even though it did not show a significant difference for the midterm outcome at the three months follow-up. The ADP-multiplate seems to bee a valid tool to measure the amount of platelet inhibition and the risk of bleeding, but is not clinically useful for the ad hoc therapy decision. Therefore we believe further studies are necessary to create a risk scheme for a more individualized treatment strategy for todays NSTE-ACS patients.

8. Zusammenfassung

Einleitung

Die retrospektive Studie wurde im Rahmen der TuePIC-Studie der Abteilung Kardiologie des Universitätsklinikums Tübingen durchgeführt und involvierte insgesamt 439 NSTE-ACS Patienten, welche mittels PCI behandelt worden. Wir bildeten zwei Kohorten, die Kontrollgruppe, bestehend aus 300 Patienten, welche alle kein Blutungsereignis hatten und die Eventgruppe mit 139 Patienten mit dokumentiertem postinterventionellem Blutungsereignis.

Das primäre Ziel der Studie war es zunächst das Gesamtrisiko für das Eintreten von Blutungsereignissen nach PCI in NSTE-ACS Patienten besser abzuschätzen. Vor allem aber dann verschiedene Patientenvariablen auf deren Zusammenhang mit einem erhöhten Blutungsrisiko zu analysieren um ein Risikoprofil erstellen zu können. Mit diesem Risikovariablen erhofften wir uns ein Score zu erstellen, mit dessen Hilfe es möglich ist Patienten bei Aufnahme, in Bezug auf ihr individuelles Blutungsereignis, kategorisieren zu können und dann nach Bedarf das Therapieprotokoll anpassen zu können, um postinterventionelle Blutungen zu verhindern.

Methoden

Wir haben 16 verschiedene Variablen getestet um einen signifikanten Zusammenhang mit einem erhöhten Blutungsrisiko in NSTE-ACS Patienten nach PCI zu untersuchen. Außerdem haben wir verschiedene Loadingzeitpunkte, sowie den Vergleich zwischen der Gabe von Clopidogrel gegenüber einem den neuen P2Y12-Inhibitoren in Bezug auf ein erhöhtes Blutungsrisiko und dem auftreten von MACE im follow-up nach drei Monaten evaluiert. Alle Patienten erhielten eine Messung der ADP-Inhibierung via ADP-Multiplate. Diese Daten wurden ebenfalls in Bezug auf das Blutungsrisiko evaluiert. Es erfolgte eine dreimonatiges follow-up mit allen Patienten.

Ergebnisse

Aus allen getestet Variablen konnten wir fünf Faktoren finden, die sich als signifikant herausstellten. Dazu gehörten: Alter bei Intervention, Diabetes Mellitus, Nieren-insuffizienz (GFR < 60%), die ADP-Multiplate Daten und die neuen P2Y12-Inhibitoren. In der Multivariante Analyse persistierten nur die ADP-Multiplate Daten und Diabetes mellitus signifikant. Die weiteren Variablen zeigten sich nicht signifikant. Im follow-up zeigte sich ein signifikant erhöhtes Auftreten von ACS innerhalb von 5 Tagen und 3 Monaten bei Patienten die eine Vormedikation mit einem P2Y12-inhibitor erhielten und zudem während der PCI erneut geloaded wurden. Ansonsten gab es keine signifikanten Unterschiede während der follow-ups.

Diskussion

Postinterventionelle Blutungsereignisse sind weiterhin eine häufige Komplikation in NSTE-ACS Patienten. Diese Studie konnte einen signifikanten Zusammenhang zwischen den ADP-Multiplate Daten und dem Vorliegen eines Diabetes mellitus mit einem erhöhten Risiko für postinterventionelle Blutungen aufzeigen. Leider war es uns auf Grund der fehlenden Signifikanz weiterer Risikofaktoren nicht möglich den angestrebten Risikoscore zu entwickeln, welcher die bessere Einschätzung des Blutungsrisikos des Patienten bei Aufnahme ermöglicht.

Bezüglich unserer zweitrangigen Hypothesen konnten wir keinen signifikanten Unterschied zwischen den verschiedenen Loadingzeitpunkten und dem post-interventionellem Blutungsrisiko aufweisen. Da wir nur Loadingzeitpunkte vor PCI untersuchten, ist ein Vergleich zum Loading während PCI eine mögliche Thematik in späteren Studien.

Der Vergleich zwischen Clopidogrel und den neuen P2Y12-Inhibitoren zeigte eine signifikant höhere Einschränkung der Plättchenfunktion im ADP-Multiplate. Es konnte aber in der Studie kein signifikant erhöhtes Blutungsrisiko der Gruppe mit neuen P2Y12-Inhibitoren gezeigt werden. Da diese Gruppe in der Studie sehr klein war, wird dies ein Thema für folgende Studien sein, um den Einfluss, der immer häufiger verwendeten, neueren P2Y12-Inhibitoren auf das Blutungsrisiko zu untersuchen.

Das vermehrte Auftreten von ACS in der Patientengruppe mit P2Y12-inhibitor Vormedikation und Loading während der PCI sehen wir am ehesten als cofounder und bedingt durch die geringe Studienpopulation und noch geringere Subgruppengröße.
Es werden weitere Studien nötig sein, um das Risiko für Blutungen, vor allem in Anbetracht der neuen Therapiestrategien, wie z.B. neue ADP-Antagonisten und radialer Zugang, der NSTE-ACS Patienten zu beurteilen und eine aktuellere Möglichkeit der Risikoerfassung darzustellen um eine personalisierte Therapie möglich zu machen.

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Die Arbeit wurde in dem Universitätsklinikum Tübingen unter Betreuung von Prof. Dr. Tobias Geisler durchgeführt.
Die Konzeption der Studie erfolgte in Zusammenarbeit mit Prof. Dr. Geisler, stellvertretender ärztlicher Leiter und Dr. Michal Droppa, Facharzt für Kardiologie.

Sämtliche Recherche und Auswahl, sowie Zusammenstellung der patientenbezogenen Daten wurden von mir eigenständig durchgeführt.
Die Multiplate Messungen wurden durch die Mitarbeiter der Arbeitsgruppe Kardiologie, Prof. Dr. Geisler mit Unterstützung durch Fr. Latev im Labor Gawaz durchgeführt.

Die statistische Auswertung erfolgte eigenständig nach Beratung und mit Hilfe durch Dr. Michal Droppa. Alle Grafiken, Flussdiagramme und Tabellen wurden eigenständig durch mich erstellt.

Ich versichere das Manuskript selbständig verfasst zu haben und keine weiteren, als die von mir angegebenen Quellen verwendet zu haben.

Stuttgart, den 16.07.2019

Bastian Kaiser