Early preloading with P2Y12 inhibitors in patients presenting with acute coronary syndromes without persistent ST-segment elevation

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DIPLOMA THESIS

Academic year:

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LIST OF ABBREVIATIONS

ACC - American College of Cardiology

ACS – Acute coronary syndrome

AHA - American Heart Association

AMI – Acute myocardial infarction

ASA – Acetylsalicylic acid

BARC – Bleeding Academic Research Consortium

CABG – Coronary artery bypass grafting

CAD – Coronary artery disease

DAPT – Dual antiplatelet treatment

DM – Diabetes mellitus

ESC – European Society of Cardiology

GPIIb/IIIa – Glycoprotein GPIIb/IIIa

LVEF – Left ventricular ejection fraction

MACCE – Major adverse cerebrovascular and cardiovascular events

NCDR – National Cardiovascular Disease Registry

NSTE-ACS – Non-ST-elevation acute coronary syndromes

NSTEMI - Non-ST-elevation myocardial infarction

PCI – Percutaneous coronary intervention

RCT – Randomized controlled trial

STEMI – ST-elevation myocardial infarction

TIMI – Thrombolysis In Myocardial Infarction

UA – Unstable angina

1. INTRODUCTION

1.1 Introductory remarks on the acute coronary syndrome

Acute coronary syndrome (ACS) refers to a spectrum of clinical signs and symptoms that occur due to decreased blood flow in coronary arteries thus resulting in myocardial ischemia and in some cases, myocardial injury and necrosis. It can be complicated with mechanical valvular problems and cardiac arrest while ongoing ischemia can cause electrical or hemodynamic instability leading to cardiogenic shock. ACS is clinically classified as ST-segment elevation myocardial infarction (STEMI), and non-ST-segment elevation myocardial infarction (NSTEMI), and unstable angina (UA) based on symptoms, dynamic changes captured on the 12-lead ECG, and laboratory findings, particularly biomarkers reflecting myocardial injury (troponins). NSTEMI and UA are collectively called NSTE-ACS (1,2).

1.1.1 Epidemiological data on ACS (societal burden, incidence)

ACS is the leading cause of death and loss of disability-adjusted life years (DALY) worldwide, and approximately 7 million deaths and 128 DALY's are lost every year (3). Cardiovascular illness has a large impact on the economy, accounting for one-third of the predicted 47$ trillion lost in non-communicable diseases (NCD) over the next 20 years. Low- and middle-income (LMIC) nations account for approximately two-thirds of all ACS DALYs and more than half of all deaths. Several of these low-income countries have seen exponential economic growth together with lifestyle changes, which have increased the prevalence of ACS risk factors and death rates. The epidemiological transition provides a valuable insight into understanding the emergence of ACS in low- and middle-income countries. Populations, in general, begin with low life expectancies where mortality is driven by common infections, malnutrition, and disease and injuries connected to childbirth and early infancy. With improvement in agriculture and sanitation, the cause of deaths tends to shift towards NCDs, in particular, ACS and malignancies as the leading cause of death. As malignancies and ACS become more manageable and easier to prevent, we see the burden of these diseases shift over to the elderly population. ACS is now among the top five causes of death in every region of the world except for Sub-Saharan Africa (3).

Higher rates of STEMI are seen in men compared to women, and for NSTE-ACS women are more likely to have unstable angina compared to men (4). In Croatia, similar trends have been observed in the cohort involving 1550 ACS patients. Women had a higher prevalence of NSTEMI and UA than men while the index ACS event tended to occur, on average, with 7

years of delay in women compared to men. Similarly, women were significantly less treated with PCI and underwent coronary angiography to a lesser degree than men (5). The median age of ACS presentation in the United States is 68 years, with a male-to-female ratio of roughly 3:2. Some patients have a history of stable angina (chronic coronary syndrome - CCS), while others are experiencing their first symptoms of coronary artery disease (CAD) because of ACS. ACS is predicted to affect more than 780 000 people in the United States per year, and NSTE-ACS will be found in approximately 70% of the patients. Cardiac and non-cardiac comorbidities are more likely to be seen in patients with NSTE-ACS than those with STEMI (5,6). NSTEMI and UA are major causes of mortality and morbidity in Western countries and are responsible for approximately 2.5 million hospital admission annually (7). Re-infarction and hospital deaths affect 5-10% of patients with NSTE-ACS and bear significantly worse prognosis than in patients with CCS (7). Even with optimal treatment, 5-10% of the patients will suffer recurrent myocardial infarction (MI) or death. Compared to patients suffering STEMI, NSTE-ACS patients have a higher long-term risk for recurrent MI and death (7). Finally, among patients with NSTE-ACS, sudden cardiac death represents the largest proportion of cardiovascular deaths at 30 days among patients enrolled in cardiovascular trials focused on NSTE-ACS (8).

1.1.2 Pathophysiology of acute coronary ischemia (ACI)

Acute coronary ischemia (ACI) occurs when there is a mismatch between oxygen demand and oxygen supply for the myocardium. Like most other muscle tissues, the myocardium exhibits a correlation between pre-contraction tension and contraction velocity. Increased velocity equals increased energy demand which necessitates an increase in oxygen consumption. Even a small change in the pre-contraction ventricular volume is associated with a large change in wall tension (2, 9). Rupture or erosion of an unstable atheromatous plaque is the most frequent culprit in ACI, while coronary spasm, arteritis, and spontaneous coronary dissection are less common causes of coronary ischemia (7, 10). Plaque erosion is more commonly associated with NSTE-ACS while plaque rupture predominates in STEMI (11). Occlusion of a coronary artery by ruptured atherosclerotic plaque is the most common cause of ACI. Foam cells, smooth muscles cells, and lipids are being exposed due to the ruptured plaque resulting in local thrombin production and deposition of fibrin. The thrombin and fibrin facilitate platelet aggregation and adhesion which leads to the creation of an intracoronary thrombus. Initiation of cardiac ischemia happens when the thrombotic atherosclerotic plaque partially or completely occludes an epicardial coronary artery. The size of the infarction is

determined by the extent of the ischemic area, the duration and intermittency of the coronary blockage, the amount of residual collateral blood flow, and the level of coronary microvascular dysfunction (12). The infarct progresses in a wavefront pattern that begins in the subendocardial layers and is at risk of extending into the subepicardial layers with persistent coronary occlusion. Myocardial infarctions across different species vary due to the differential innate collateral circulation and resistance to myocardial ischemia. By analysis of biomarkers and use of magnetic resonance imaging (MRI) researchers have found that 30-50% of the cardiac tissue at risk of ischemia is viable for 4-6 hours after onset of anginal symptoms. At 12 hours after coronary occlusion there is still viable cardiac tissue, and the infarct size can be reduced by doing emergent coronary reperfusion (2, 7, 13).

1.1.3 Clinical presentation

There are variations across race, age, sex, and the history of patients in the terms of ACS presentation. We generally divide the clinical signs and symptoms into two main groups "*typical*" and "*atypical",* which tend to overlap each other based on the sources and studies used. There are different sources for descriptions used for typical symptoms in ACS. Widely accepted and a cited description is Heberden's description. A more precise and integrating description are the and guidelines of the National Heart Attack Alert Program (NHAAP) (14-16). Heberden's description of typical symptoms is as following: "*left sided substernal/angina pain as a strangling sensation worsened by exertion and relieved by rest, that radiated to the left arm*" (17). NHAAP describes the typical presentation as: "*pain, if present, is described as pressure, tightness, or heaviness. It may radiate to the neck, jaw, shoulders, back, or one or both arms. The pain may also be described as indigestion or heartburn with associated nausea and/or vomiting. Additional symptoms in the absence of pain may include shortness of breath, weakness, dizziness, lightheadedness, or loss of consciousness* ", (18). Three studies define the best prognostic atypical symptoms like nausea, diaphoresis dyspnea, syncope, or pain primarily localized to the arm, neck, jaw, or abdomen. Also, epigastric pain, or back pain, or pain that was described as burning, stabbing, characteristic of indigestion, or other is considered atypical. Diaphoresis in presence of other ACS symptoms has shown to be a good predictor for the probability of STEMI, rather than NSTEMI. Moreover, S3 or S4 gallop and pulmonary crackles might be auscultated and present on physical examination. If the patient has severe hypotension and/or cardiogenic shock, it's more likely to be STEMI rather than NSTE-ACS (14-16). Finally, it is also important to highlight that some cardiovascular researchers call for an action to

abandon the *"typical" vs. "atypical"* dichotomy in ACS presentation as it might be misleading (15). A recent study found that typical symptoms are more common and provide greater predictive value in women than in men with acute MI (19).

1.1.4 Diagnosis

The diagnosis of ACS is generally based on five diagnostic tools: 12-lead ECG, clinical history and examination, cardiac biomarkers, imaging, and stress testing. ECG allows for an affordable, quick, and non-invasive way of identifying transient or clear ST-segment elevation or depression, T wave inversion, bundle branch blocks, and changes after nitrates are given. Medical history is important to set the appropriate diagnosis and to give a correct treatment. A physical examination can eliminate differential diagnoses such as pneumothorax, pericarditis, or pleuritis and give clues to possible cardiac problems such as hemodynamic instability and ventricular failure. Myocardial damage can be detected by checking the cardiac-specific troponin (cTn) I or T, and creatine kinase MB (CKMB) levels. These markers do not differentiate between ischemic and non-ischemic causes of myocardial injury, and one should consider differentials as end-stage renal disease, myocarditis, and pulmonary embolism as possible causes for the increase. Troponin I and T are sensitive markers for detecting myocardial necrosis. Levels starts to increase 2-3 hours (h) after injury, peaking at 24 h and persist for 1-2 weeks. CKMB is less reliable and specific than troponin I and T for myocardial injury. Circulating levels of CKMB start to increase after 4 h, peaking at 24 h and go back to baseline after 48-72 h. The short duration of CKMB makes it suitable to monitor any possible new myocardial infarctions (reinfarctions). Furthermore, transthoracic echocardiography can be of great help in evaluating the systolic and diastolic function of the ventricles, regional wall motion abnormalities (RWMA), pericardial effusion, and gross valvular abnormalities in ACS. Cardiac magnetic resonance (CMR) imaging is increasingly used and allows for more precise evaluation of myocardial damage, myocardial dysfunction, infarct distribution and size, and myocardial hemorrhage both acutely and in follow-up examinations (7, 20, 21-24).

The European Society of Cardiology (ESC) recently proposed a diagnostic algorithm to *"rule in"* or *"rule out"* the possibility of ACS by integrating findings obtained from physical examination and vital signs, 12-lead ECG, values of high-sensitivity cardiac troponin (hs-cTn) assays at baseline (hour 0), and dynamics of troponin change (if they occur) within 1, 2 or 3 hours. This diagnostic algorithm is shown in **Figure 1**.

In short, diagnosis of ST-elevation myocardial infarction (STEMI) is primarily made based on the 12-lead ECG recording and additional troponin measurement is a redundant step in such cases. On the other hand, ECG changes in NSTEMI and UA are much more intricate and can widely range from being non-specific and discrete to being dramatic and obvious. Such changes might involve T-wave inversion or flattening, biphasic T-waves, poor R-wave progression and/or ST-segment depression and other minor electrocardiogram abnormalities. However, it should be kept in mind that many ECG patterns that do not meet formal STEMI criteria can still represent significant coronary occlusion, especially in the setting of persistent ischemic chest pain, hemodynamic instability, and dynamic ECG changes. Such ECG patterns are also termed *"STEMI-equivalents"* and they must be timely recognized because such patients are not infrequent in clinical practice and they need to be emergently reperfused (25). Finally, NSTEMI and UA are in the borderline cases differentiated by the levels of cardioselective enzymes such as troponins reflecting myocardial injury - in UA these biomarkers will not be elevated in circulation, while in NSTEMI they will.

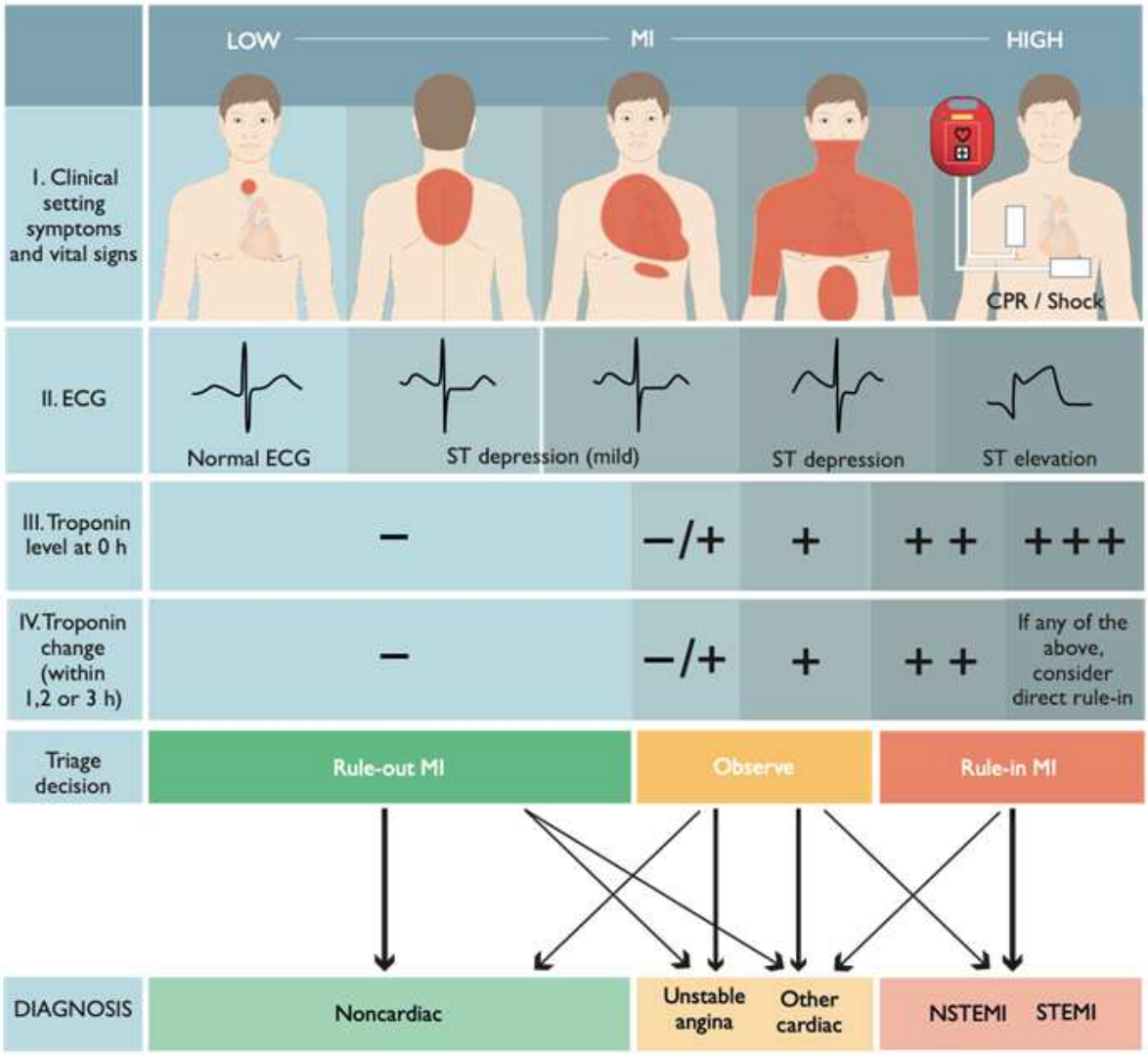


Figure 1. Diagnostic algorithm and triage in the acute coronary syndrome according to European Society of Cardiology (ESC) NSTE-ACS guidelines (taken from Collet JP, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J*. 2021;42:1289–367)

Several rule-in and rule-out algorithms based on contemporary high-sensitivity troponin assays are used nowadays to rule out or rule in NSTE-ACS diagnosis in hemodynamically stable patients. By using such algorithms, clinicians in the Emergency Department can either rule out NSTE-ACS diagnosis and discharge patients home or to the ward, they can further observe patients or they might admit (rule-in) patients in the ward or to the coronary care unit (CCU). One of such algorithms that can be applied among non-differentiated patients with suspected NSTE-ACS is the 0-1 hour algorithm that is also endorsed by the ESC (as shown in **Figure 2**).

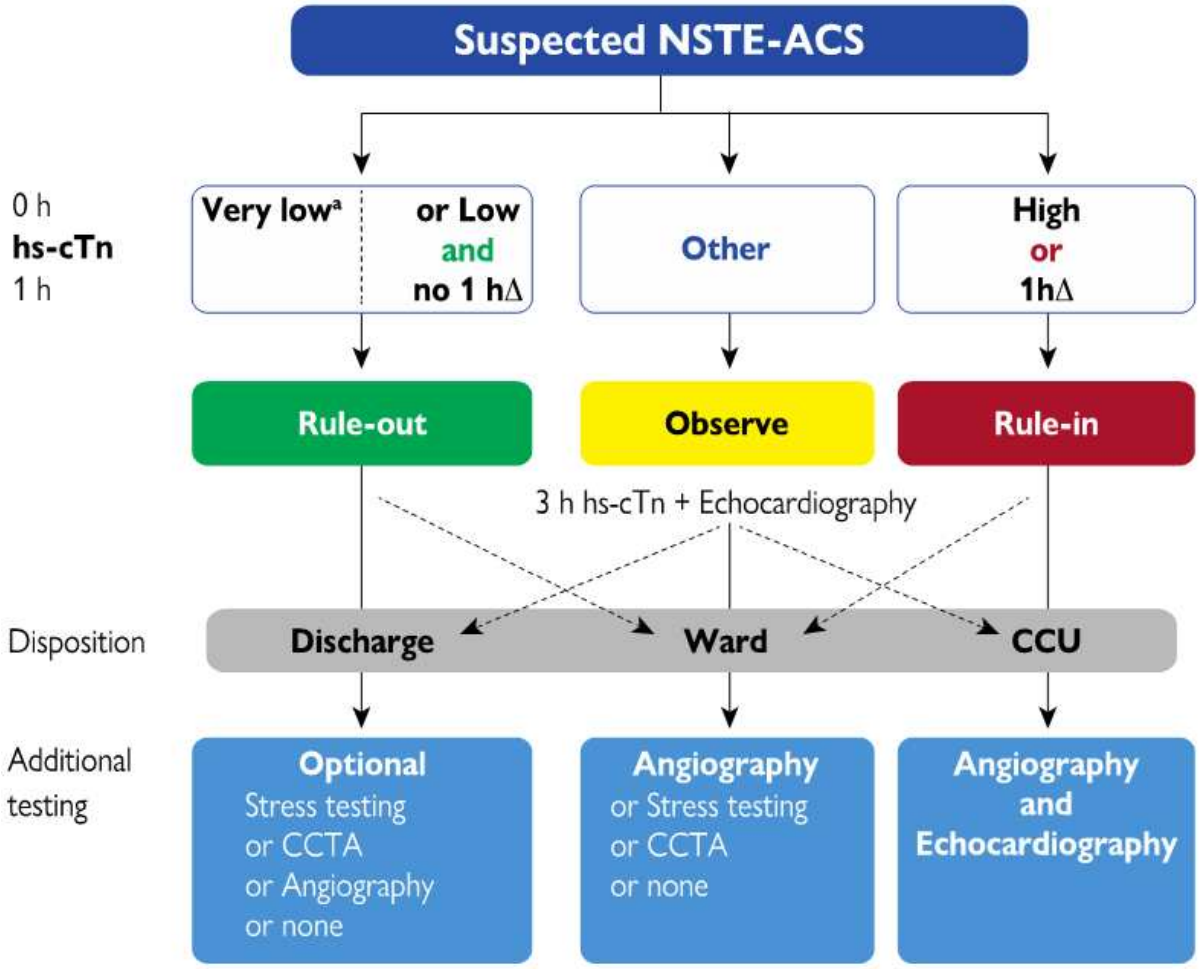


Figure 2. A 0-to-1 hour algorithm by the ESC for the rule-in or rule-out of NSTE-ACS diagnosis (taken from Collet JP, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J.* 2021;42:1289–367).

1.1.5 Pharmacological treatment

Majority of the drugs used in the treatment of ACS work by increasing oxygen supply or decreasing the oxygen demand of the heart by changing the hemodynamics of arterial blood pressure, contractility, heart rate, and thrombus formation. Classes of the most relevant drugs used to treat ACS and its complications are discussed further.

Nitroglycerin reduces the myocardial oxygen demand through endothelium-independent vasodilation of coronary arteries and ischemic areas by decreasing preload and afterload. Nitrates may cause reflex tachycardia which increases oxygen demand unless a beta-

blocker is given concomitantly. Nitroglycerin should be given intravenously in acute settings with heart failure but may be used topically or orally if the patient is pain-free and there is no refractory or recurrent ischemia (1, 2, 20).

Morphine is a potent anxiolytic and analgesic through its interaction with the opioid receptors and should be administered in patients who present with refractory ischemic-related symptoms after 3 doses of nitroglycerin (2, 26).

Beta-blockers (BB) (*e.g.* carvedilol, bisoprolol, metoprolol, esmolol) reduce the myocardial oxygen demand by decreasing cardiac contractility and heart rate through antagonism of the $ß_1$-adrenergic receptors in the myocardium. Beta-blockers are used to decrease cardiac ischemia, reinfarction and are preferred anti-anginal agents of choice in those without contraindications. BB should be given within 24 hours after onset of ACS, and the dose should be adjusted to reach the desired heart rate of 50-60 beats/min. Early administration doesn't reduce short-term mortality. However, it increases long-term survival (1, 2, 16, 27).

Calcium channel blockers (CCB) reduce the oxygen demand of the heart by improving the coronary blood flow and limit the myocardial contraction by preventing calcium from moving through the L-type calcium channels present in cardiac muscles and smooth muscle cells of blood vessels. The dihydropyridine CCB's (*e.g.* amlodipine and nifedipine) primarily act on the calcium channels in the smooth muscle cells of blood vessels to cause vasodilation, preventing vasospasm and reducing the coronary artery vascular resistance and afterload. The non-dihydropyridine CCB's (*e.g.* verapamil and diltiazem) reduce the heart rate by acting on the conduction system of the heart. CCB's are recommended for patients who have refractory or recurrent symptoms after treatment with the full dose of nitrates and BB, or for patients with contraindications for BB (1, 2, 16, 20).

Inhibitors of the renin-angiotensin-aldosterone system (RAAS) lower blood pressure by reducing systemic vascular resistance (SVR) through vasodilatation and other mechanisms. Angiotensin-Converting-Enzyme (ACE) inhibitors (*e.g.* captopril and enalapril) prevents the conversion of angiotensin I to angiotensin II by inhibiting ACE (1, 2). ACE inhibitors have been shown to decrease mortality early as 24 hours after administration. If ACE inhibitors are badly tolerated or the patient is refractory to treatment it can be substituted with angiotensin-receptor-blockers (ARB) (*e.g.* losartan). ARB's block the action of angiotensin-II and show similar benefits on mortality for ACS as in ACE inhibitors (28). Selective aldosterone receptor blockers (*e.g.* eplerenone) have been shown to reduce morbidity and mortality in patients with

MI that is complicated with left ventricular dysfunction, chronic heart failure (CHF), or diabetes mellitus (DM). Nonselective aldosterone inhibitors (*e.g.* spironolactone) have shown benefits in non-MI cases of heart failure with ischemic etiology (1, 2, 29).

Anticoagulants are agents that increase clotting time to reduce the risk of blood coagulation and thrombus formation. They should be introduced immediately after the onset of ACS (2). Unfractionated heparin (UFH) together with acetylsalicylic acid (ASA) has commonly been used for the treatment of unstable angina and minor myocardial injury before the era of dual antiplatelet therapy (DAPT), revascularization, and early invasive management. The supporting evidence for using UFH as monotherapy is limited, however, the anticoagulant effect shows lower death rates and MIs compared to monotherapy with ASA. Low-Molecular-Weight Heparin (LMWH) (*e.g.* enoxaparin, nadroparin, dalteparin) blocks the activity and formation of thrombin by acting on factor Xa and factor IIa. LMWH is superior to placebo in ASA-treated patients. LMWH is also more practical, can be administered subcutaneously, and is at least as effective as UFH for anticoagulation, and is less likely to induce heparin-induced thrombocytopenia (HIT). LMWH does not need monitoring and is similar to UFH in terms of bleeding risk (2, 16, 20).

Direct thrombin inhibitors (e.g. bivalirudin, argatroban) distinguish themselves from other anticoagulants by inhibiting clot-bound thrombin directly without the need for antithrombin. Thrombocytopenia is not common, and they do not interact with plasma proteins. Studies have shown that bivalirudin as monotherapy is non-inferior to the standard therapy of UFH/LMWH combined with GP IIb/IIa receptor inhibitors, and bivalirudin caused a significantly lower amount of major bleeding in comparison. Fondaparinux is a factor Xa inhibitor and the only selective inhibitor for activated factor X available for use in clinical settings. For it to be active it requires an antithrombin factor. Activated partial thromboplastin time (APTT) and activated clotting time (ACT) is not affected by fondaparinux, and monitoring of anti-Xa activity is not required. Studies show that fondaparinux is non-inferior to enoxaparin in reducing the incidence of primary outcomes of death, and bleeding was 50% lower in the patients using fondaparinux (2, 20).

Antiplatelet agents work by inhibiting platelet aggregation and serve as prophylaxis for arterial ischemic events such as coronary artery disease, cerebrovascular incidents, and peripheral artery disease. The DAPT consisting of ASA and clopidogrel has been considered essential in the treatment of ACS. With the development of newer and more effective adenosine diphosphate antagonists (ADP) (*e.g.* P_2Y_{12} inhibitors such as ticagrelor and prasugrel) and the

glycoprotein IIb/IIIa inhibitors (GPI) (*e.g.* abciximab, eptifibatide, tirofiban) we may see a future shift in the treatment strategies for ACS. Cangrelor is the rapid onset and potent P_2Y_{12} inhibitor and is the only one approved for intravenous use (30). A regimen known as *"triple therapy"* including ASA, clopidogrel, and an anticoagulant can in some select cases be given to patients with a high risk of systemic thrombotic events such as in the initial stages of stenting and in patients with atrial fibrillation (31).

ASA prevents platelet aggregation by irreversibly blocking cyclooxygenase-1 (COX-1), which inhibits the synthesis of thromboxane A_2 and prevents platelet activation. Since platelet activation can happen through alternative pathways it's often necessary to add additional agents like adenosine diphosphate antagonists (ADP). ADP antagonists block the P_2Y_{12} receptor on platelets, preventing the activation and aggregation process. Their effectiveness in DAPT by prevention of unfavorable cardiovascular events in thrombotic patients is well established. In patients who cannot tolerate ASA, a potent P_2Y_{12} inhibitor monotherapy may be administered. The P_2Y_{12} inhibitors among themselves have different pharmacological properties such as binding capacity, the onset of action, elimination time, and metabolism. These pharmacological characteristics will be mentioned later (2, 20).

GPIs interfere with cross-linking of the platelets in the common pathway of fibrinogen which inhibits the aggregation of platelets (31). Use of GPI's is generally restricted to patients with unstable angina who undergo percutaneous coronary intervention (PCI) and cannot receive pretreatment with P_2Y_{12} inhibitors. Due to the fast onset of action, GPIs are under investigation for being used as a *"bridging"* strategy in patients who are on DAPT and need to undergo surgery (32).

A graphical depiction summarizing antithrombotic treatment in NSTE-ACS including antiplatelet and anticoagulation drugs is shown in **Figure 3**.

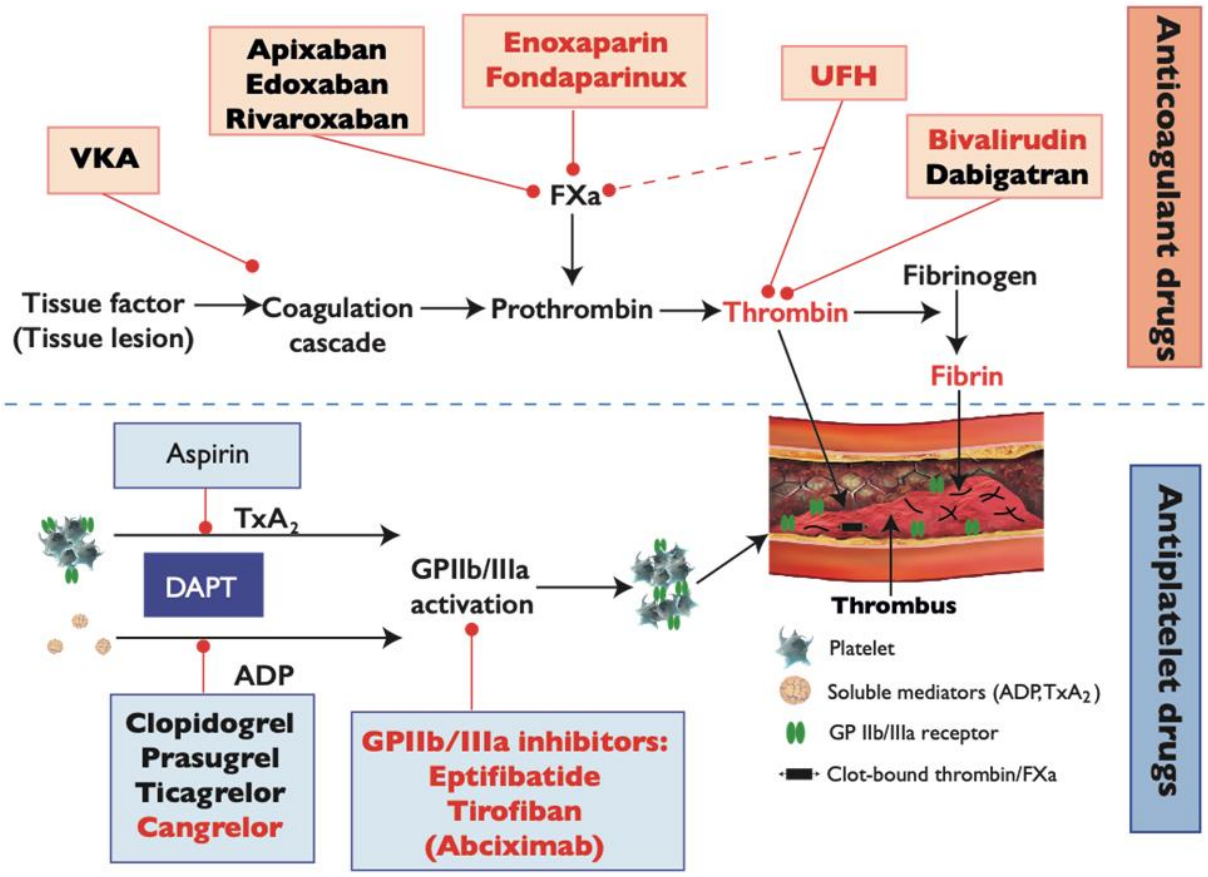


Figure 3. Antithrombotic treatment and pharmacological targets in non-ST-segment elevation acute coronary syndrome (taken from Collet JP, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J.* 2021;42:1289–367)

1.1.6 Percutaneous and surgical coronary revascularization

Coronary artery bypass grafting (CABG) and percutaneous coronary intervention (PCI) are established revascularization interventions that have been available for more than 40 years. The goal of revascularization should be to improve the quality of life and prognosis of patients with ischemic heart disease. In most cases, PCI is the best and most suitable option to treat a fully occluded coronary vessel and provide immediate reperfusion.

A number of considerations and limitations need to be evaluated before deciding which type of revascularization should be performed. These include the short- and long-term risks, patient preferences and estimation if procedure will improve the patient's quality of life and extend the life expectancy (2, 33). Revascularization should be administered within 12 hours to

all eligible patients presenting with STEMI symptoms, and PCI is the procedure of choice if done within 90 minutes from first medical contact to device time (21). However, CABG is a better choice in patients with complex multivessel disease, especially if it is complicated with diabetes mellitus (2). In patients with stable ischemic heart disease the benefit is less clear, and only CABG can prolong the life expectancy (34). Survival benefits for early invasive coronary angiography (<24 hours) in NSTE-ACS patients are inconsistent but are in general recommended for NSTE-ACS patients who have electrical or hemodynamic instability and refractory angina. Early invasive coronary angiography is also recommended for NSTE-ACS patients who have been previously stabilized and do not have severe comorbidities (*e.g.* pulmonary and liver failure or cancer) or other strong contraindications (2, 21).

1.2 Antiplatelet drugs used in NSTE-ACS

Platelet activation and aggregation are central themes of the pathogenesis in ACS, and DAPT is considered a gold standard in providing antithrombotic cascade and preventing recurrent myocardial infarctions and post-MI-related deaths (31). Targets of antiplatelet agents are shown in the **Figure 4**.

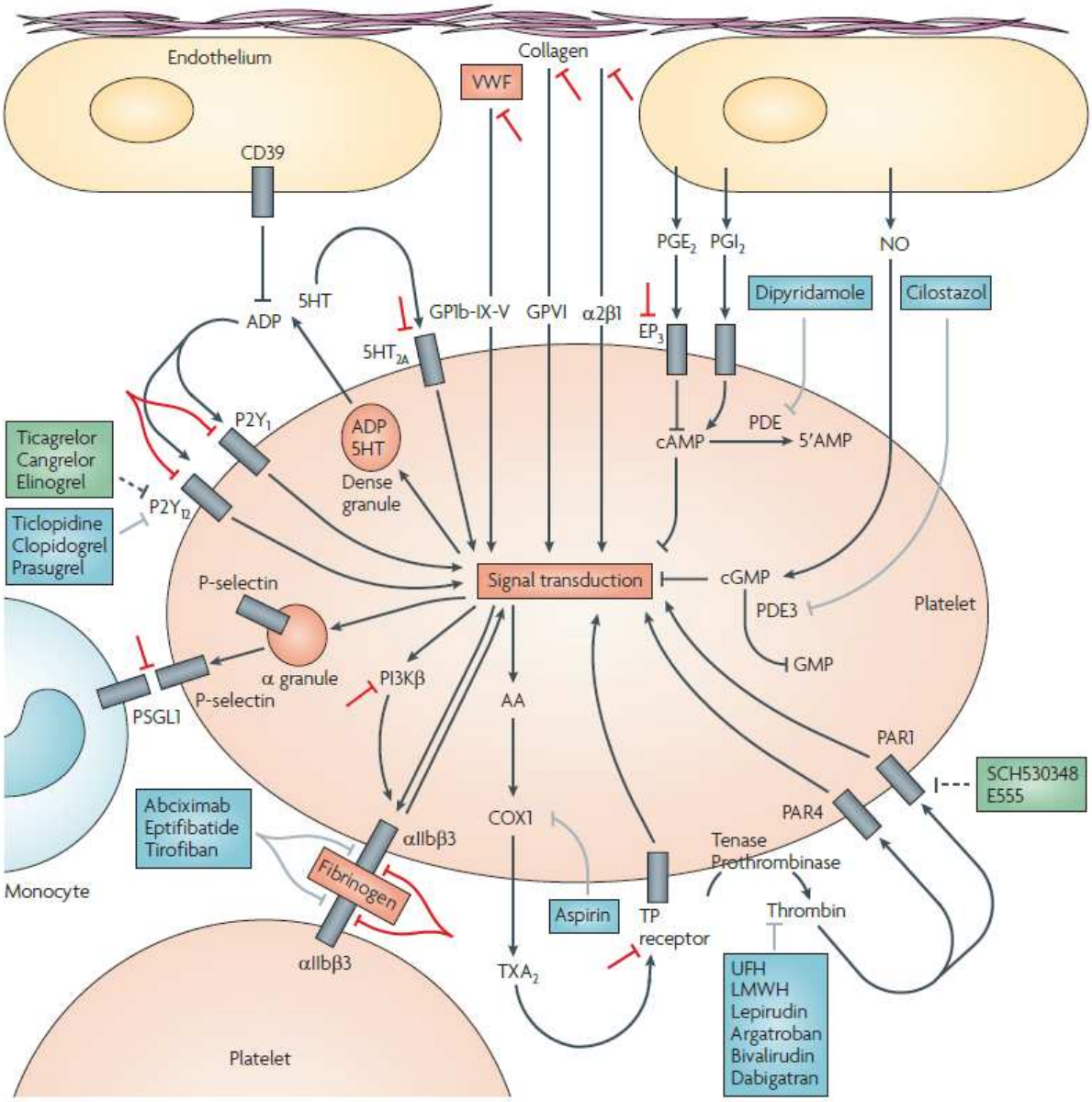


Figure 4. Cellular targets of antiplatelets (taken from Michelson AD. Antiplatelet therapies for the treatment of cardiovascular disease. *Nat Rev Drug Discov*. 2010;9:154-69

1.2.1 Acetylsalicylic acid (ASA)

ASA is an irreversible inhibitor of COX1, preventing platelet activation and aggregation. ASA constitutes a foundational component DAPT regimen and is a standard first-line therapy in patients with NSTE-ACS. The use of ASA is proven to significantly improve clinical outcomes by lowering the risk of recurrent MI and death (31).

1.2.2 Inhibitors of P_2Y_{12} receptors

P_2Y_{12} receptor inhibitors are ADP antagonists that interfere with platelet aggregation by reversible or irreversible binding of the purinergic receptor P_2Y_{12} that is localized on the surface of platelets (31). Ticlopidine was the first oral P_2Y_{12} inhibitor (first generation) to be approved but had unfavorable hematological effects such as neutropenia and thrombocytopenic purpura which made its clinical use limited (35).

Clopidogrel is a second generation of P_2Y_{12} inhibitors and has been widely used but has shown poor compliance and, therefore, lead to the development of newer agents like prasugrel and ticagrelor. Furthermore, about 4% to 30% of patients treated with conventional doses of clopidogrel do not achieve adquate antiplatelet response and this is also known as *„clopidogrel resistance"* (36). Clopidogrel binds irreversibly to the receptor requiring hepatic metabolization like prasugrel, but prasugrel is more efficient causing the faster onset of action and offset of effects while demonstrating a more potent antiplatelet effect. Ticagrelor in comparison has a faster onset of action and offset of effect, and is more efficient because it irreversibly binds to the platelet receptor and doesn't require hepatic metabolization. However, ticagrelor has been linked to importan side-effects such as disorders of cardiac rhythm (conduction disturbances such as atrioventricular block) and dyspnea. Until recently all P_2Y_{12} inhibitors were only available as oral drugs which requires the patient to be conscious and not intubated (31). Novel agents like cangrelor and selatogrel allow for intravenous (cangrelor) and subcutaneous (selatogrel) administration thus mitigating all potential shortcomings of oral agents. Cangrelor and selatogrel reversibly bind to the receptor and do not require hepatic activation giving them an onset of action and offset of effects in a matter of minutes and hours instead of days. These pharmacological properties allow for little to no surgical delay compared to the oral agents who usually require 3 to 7 days for completing „wash-out". Selatogrel as of 2020 has yet to complete Phase 3 trials that are currently ongoing (31, 37). Pharmacological properties of P2Y12 inhibitors are shown in **Table 1**.

Table 1. Pharmacological properties of P_2Y_{12} receptor inhibitors

	Oral administration				*Intravenous*	*Subcutaneous*
	Ticlopidine	**Clopidogrel**	**Prasugrel**	**Ticagrelor**	**Cangrelor**	**Selatogrel**
P_2Y_{12} binding	Irreversible	Irreversible	Irreversible	Reversible	Reversible	Reversible
Prodrug	Yes	Yes	Yes	No	No	No
Onset of action		Delayed: 2-6 h	Rapid: 0.5-4 h	Rapid: 0.5-2 h	Immediate: 2 min.	
Offset of effect		3-10 days	5-10 days	3-4 days	30-60 min.	
Delay to surgery		5 days	7 days	5 days	No delay	

Table adjusted from Collet JP, Thiele H, Barbato E, Barthélémy O, Bauersachs J, Bhatt DL, et al. 2020 ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation. *Eur Heart J*. 2021;42:1289–367

1.3 Concept of P_2Y_{12} preloading in NSTE-ACS: pros and cons

Early preloading with P_2Y_{12} inhibitors before any anticipated invasive management or surgical procedure has been used as a treatment strategy to reduce the risk of adverse cardiovascular events in patients presenting with ACS. Preloading is defined as giving oral P_2Y_{12} inhibitors in a high (loading) dose in addition to ASA before undergoing coronary angiography and at the time when the coronary anatomy is still unknown (31). The timing, dose, and agent of choice have been the subject of a recent debate, and the benefits need to be evaluated against the potential risks and complications. In other words, risks of ischemia and bleeding should be carefully considered in each particular patient and decision of treatment needs to be personalized. Preloading is used with the main intention of inhibiting platelet activation to reduce the risk of stent thrombosis post-PCI and new thrombus formation elsewhere (38). However, the antithrombotic effects of P_2Y_{12} inhibitors may increase the risk of bleeding complications and they may delay the coronary intervention for patients planned to undergo the CABG procedure. The onset of action for the most widely used P_2Y_{12} inhibitor clopidogrel is several hours, and administration is typically done before the coronary angiography. Since about 65% of patients that are diagnosed with ACS will eventually require PCI it causes unnecessary bleeding risk for the remaining 35% of patients (37, 39). Finally, the *net* clinical benefit of preloading in patients with ACS undergoing PCI has been examined in

several studies (37, 40). The main theoretical rationale for using P_2Y_{12} preloading in NSTE-ACS is to allow maximal platelet inhibition at the time of PCI procedure. On the other hand, the no-preloading strategy assumes the initiation of P_2Y_{12} inhibitor during the PCI procedure or shortly after it, therefore, maximum platelet inhibition is achieved at a later time. The difference between that time and the time needed for maximum platelet inhibition in the preloading strategy constitutes a potential window of benefit with respect to stent thrombosis risk as shown in **Figure 5**. As it can be appreciated from that image, the potential of benefit with early P_2Y_{12} preloading lies within the area with slanted grey lines, between the red (no preloading) and green (preloading) curves. Moreover, the risk of stent thrombosis (black curve) is highest in the early period following PCI and generally greatly diminishes after the first 30 days (so-called "vulnerable period").

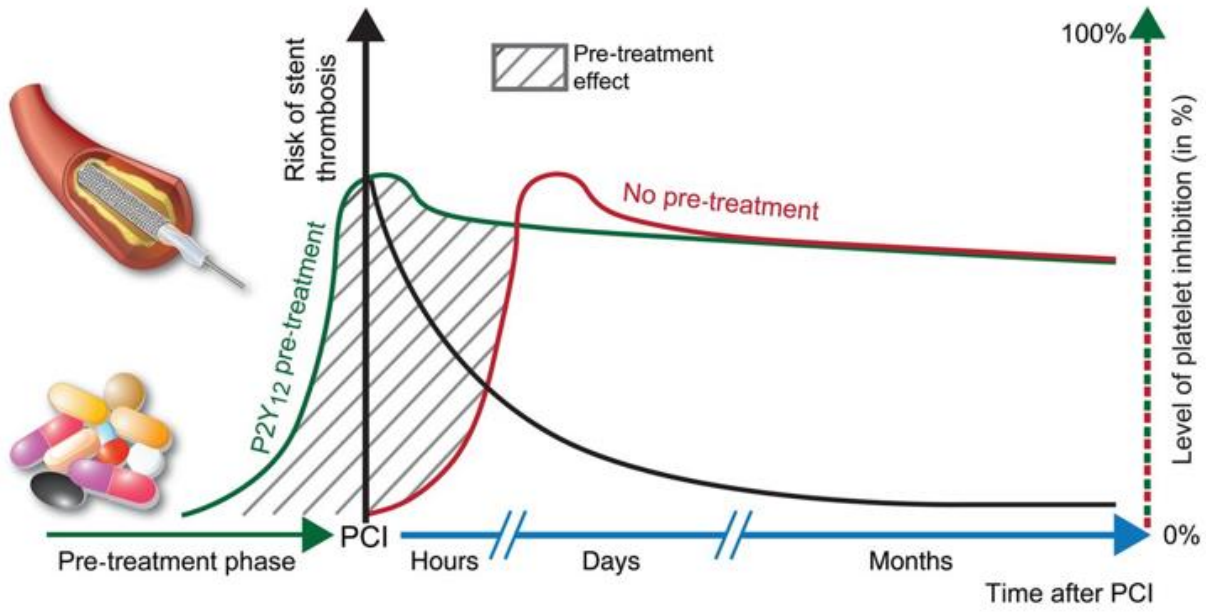


Figure 5. Level of platelet inhibition over time with respect to P_2Y_{12} strategy used (preloading *vs*. no preloading) and with respect to PCI and the risk of stent thrombosis (Taken from Sibbing D, Kastrati A, Berger PB. Pre-treatment with P2Y12 inhibitors in ACS patients: who, when, why and which agent? *Eur Heart J*. 2016;37:1284-95

1.4 Role of P_2Y_{12} preloading in NSTE-ACS as per current international guidelines

The European Society of Cardiology (ESC) had a long record of recommending preloading with P_2Y_{12} inhibitors in NSTE-ACS patients until the most recent guidelines were published in 2020. The 2020 ESC guidelines state that: "*It is not recommended to administer routine pre-treatment with a P_2Y_{12} receptor inhibitor in patients in whom coronary anatomy is not known and an early invasive management is planned*". This statement has been backed up with class III, level A recommendation. On the other hand, guidelines allow the pretreatment with a P_2Y_{12} inhibitor in patients with NSTE-ACS who are not planned to undergo an early invasive strategy and that do not have high bleeding risk (IIB class recommendation, level C). Such important change in recommendations is based on relevant trial and registry data that demonstrated a lack of any ischemic benefit, with a significant increase in the risk of bleeding with early P_2Y_{12} preloading strategy in NSTE-ACS patients. In comparison, the 2014 American College of Cardiology and American Heart Association (AHA/ACC) guidelines do not mention the use of P_2Y_{12} inhibitor preloading in NSTE-ACS, but only vaguely state that they are indicated in the treatment of myocardial ischemia in general (2).

The aim of the present thesis was to provide a perspective on the totality of the evidence regarding the use of P_2Y_{12} preloading in NSTE-ACS in the form of systematic review and meta-analysis aggregating data from randomized controlled trials in this setting.

2. OBJECTIVES

2.1 Aims

The aims of the present study were to investigate the effects of early P_2Y_{12} inhibitor preloading (pretreatment) *vs.* no preloading in randomized controlled trials that enrolled patients presenting with acute coronary syndromes without persistent ST-segment elevation (NSTE-ACS) with respect to the following short-term outcomes:

a) **Composite endpoint of ischemia at 30 days** – including the events of death, non-fatal myocardial infarction, stroke or transient ischemic attack and/or urgent target vessel revascularization and/or stent thrombosis
b) **Composite endpoint of bleeding at 30 days** – including the events of major bleeding, minor bleeding requiring intervention or hospitalization, and/or other clinically relevant bleeding events, as adjudicated by study investigators and international bleeding classifications such as BARC (Bleeding Academic Research Consortium), NCDR (National Cardiovascular Disease Registry) Cath PCI (Percutaneous Coronary Intervention) and TIMI (Thrombolysis in Myocardial Infarction)
c) **Net adverse clinical events (NACE) at 30 days** – an aggregated net harm outcome consisting of both composites of ischemia and bleeding

2.2 Hypotheses

With respect to the prespecified aims of the study, the following hypotheses were proposed:

a) Early P_2Y_{12} preloading will be similar to no preloading strategy in patients with NSTE-ACS concerning the risk of ischemic events at 30 days.
b) Early P_2Y_{12} preloading will be associated with a higher risk of bleeding at 30 days, compared to no P2Y12 preloading strategy among patients with NSTE-ACS.
c) Early P_2Y_{12} preloading will be inferior to no P_2Y_{12} preloading strategy with respect to reduced risk of NACE at 30 days.

3. PATIENTS AND METHODS

3.1 Study design

This diploma thesis was envisioned as a systematic review of the literature and meta-analysis of randomized controlled trials (RCTs) investigating the impact of early P_2Y_{12} inhibitor preloading in patients with NSTE-ACS on the short-term (30 days) composite outcomes of ischemia, bleeding and net adverse clinical events (NACE). No prespecified protocol was registered before performing this analysis and no Ethics Committee approval from the University of Split School of Medicine was required for the study of this design. This study was carried out under the Department of Pathophysiology, University of Split School of Medicine.

3.2 Search strategy

The search strategy was developed by the student mentor (JAB) while the search of electronic databases was independently carried out by the student (BM) and student mentor (JAB). Electronic databases included in the search were the National Library of Medicine – PubMed, Ovid MEDLINE, Cochrane Central Register of Controlled Trials, Ovid Journals (full text), and SCOPUS. These databases were manually searched to obtain full records of original articles (RCTs) that investigated the early preloading strategy with P_2Y_{12} inhibitors in patients with NSTE-ACS. The search was limited to records published in relevant peer-reviewed journals in the English language from 2000 until 2021. Similarly, only clinical studies involving adult human subjects were considered. The date of the last search was performed on August 3rd, 2021. No grey literature search was performed and no external authors were contacted to provide additional data or to obtain additional studies. Both the student and mentor independently performed the literature search, deleted duplicate records, screened available titles and abstracts for relevance, and classified obtained studies as *„excluded“* or requiring further assessment or additional clarification. Such studies were labeled as *„potential for inclusion“*. Finally, prespecified eligibility and exclusion criteria were applied consistently among potentially inclusive studies. If there was a discrepancy between the two investigators concerning the search strategy, this was resolved by the joint discussion involving the opinion of the external expert and healthcare professional from the Department of Pathophysiology, University of Split School of Medicine.

3.3 PICOS principle and inclusion/exclusion criteria

To be eligible for potential inclusion in the analysis, obtained studies had to satisfy a number of inclusion criteria according to PICOS principles (**P**atient, problem, or population/**I**ntervention/**C**omparison/**O**utcomes/**S**tudy design) questions, as follows:

1. **Patient population:** Patients with NSTE-ACS including its clinical subtypes – unstable angina and NSTEMI, regardless of the intent concerning invasive management
2. **Intervention:** patients with NSTE-ACS had to receive a high (preloading) dose of P_2Y_{12} inhibitor of any type (clopidogrel, prasugrel, ticagrelor), before any type of invasive management (if planned), on top of the guideline-directed standard of care treatment that is routinely administered in NSTE-ACS
3. **Comparison:** patients in the control group would need to not receive early P_2Y_{12} preloading (pretreatment) or would need to be given a placebo pill added to standard of care treatment
4. **Outcome:** the primary outcomes of interest were composite endpoint of ischemia, a composite endpoint of bleeding, and the composite endpoint of net adverse clinical events (NACE) at 30 days. Composite ischemia endpoint consisted of events at 30 days including cardiovascular death or all-cause death, non-fatal myocardial infarction, urgent target vessel revascularization, stent thrombosis, stroke or transient ischemic attack (TIA), and need for the glycoprotein GP IIb/IIIa bailout. Composite of bleeding included bleeding events that were adjudicated and defined differently across included trials. For this analysis, we included all life-threatening, major bleeding, and minor bleeding events that required intervention or hospitalization.
5. **Study design:** studies had to be designed and executed as RCTs to be considered for the potential inclusion in the analysis.

Studies were considered for potential inclusion only if the length of follow-up was designed to capture at least 30 days following randomization to experimental or control treatment.

We excluded studies in the following circumstances:

1. If the study had a non-RCT design (*i.e.* observational and/or non-randomized study)
2. If the study did not report on any of the principal outcomes of interest or it did not report a number of events regarding primary outcomes in both experimental and control groups; if the study did not provide basic data on study length, description of the main

baseline characteristics relevant for the studied population such as age, sex, PCI receipt, periprocedural characteristics, P_2Y_{12} inhibitor type, timing and dose

3. If the study enrolled patients with stable coronary artery disease, *i.e.* patients with stable angina or those with chronic coronary syndromes (CCS)
4. If the study was not designed to investigate early preloading of P2Y12 inhibitor in NSTE-ACS
5. If the study investigated preloading with some other antithrombotic compound other than P_2Y_{12} inhibitors licensed for this use (clopidogrel, ticagrelor, prasugrel)
6. If the study was a duplicate report without additional or updated outcome data

3.4 Data items and extraction

Both the student (BM) and student mentor (JAB) independently extracted data from the included studies by using pre-designed, piloted extraction forms containing baseline study information such as author's first and last name, study design, the total number of patients, and a number of patients stratified by experimental/control group, P_2Y_{12} inhibitor dosing, timing, and administration route, sex distribution in the experimental and control group, the mean age of experimental and control group, description of the control treatment (placebo pill or no P_2Y_{12} preloading added to standard treatment), percentage of diagnostic coronary angiography and PCI procedures performed in the whole study sample as well as the use of stents and type of stents (if available). Previously elaborated prespecified primary outcomes of interest were captured in the same form. For each study, we also extracted the prevalence of comorbidities including arterial hypertension, diabetes mellitus, hypercholesterolemia, smoking as well as a history of myocardial infarction. Furthermore, the prevalence of multivessel coronary artery disease (defined as significant stenosis in at least two epicardial coronary vessels as determined by diagnostic angiography) and or left main (LM) disease were captured.

3.5 Risk of Bias (RoB) assessment

Cochrane's Risk of Bias 2 (RoB 2) tool, as recommended by the Cochrane Collaboration has been used to assess the individual risk of bias of each included study (41, 42). RoB 2 assessment is designed to evaluate a fixed set of domains of bias, such as trial design, conduct, and reporting. Within each domain, a series of signalling questions are asked aiming to elicit

information about trial characteristics that might modify risk of bias. RoB 2 assessment was independently performed by the student (BM) and student mentor (JAB) while potential discrepancies were resolved by consultation with the third investigator and healthcare professional from the Department of Pathophysiology, University of Split School of Medicine.

3.6 Statistical analysis (quantitative synthesis)

Data analysis was performed by adhering to Cochrane Collaboration recommendations and PRISMA statement (43).

Risk ratio (RR) with 95% confidence intervals (95% CI) was used as the main summary measure for effect estimates on prespecified dichotomous outcomes (composite endpoint – yes/no). Random-effects model with Mantel-Haenszel statistical method was applied for the meta-analysis. Meta-analysis was performed by using Review Manager software (RevMan, version 5.4, The Cochrane Collaboration, 2020). Chi-square test of heterogeneity and Higgins I^2 statistic of inconsistency were used to assess heterogeneity across studies. Studies with an I^2 statistic of 25% to <50% were considered to have low heterogeneity; 50% to 75% - moderate heterogeneity, and those with I^2 statistic >75% were considered to have a high heterogeneity. *P*-values were two-tailed and results were considered statistically significant if $P<0.05$.

4. RESULTS

4.1 Study inclusion and risk of bias assessment

A total of 603 records were screened after duplicate records were removed. Out of these records, 420 were excluded because they did not pertain to acute coronary syndromes but other forms of cardiovascular disease. Finally, full texts were obtained for 58 records and were analyzed for potential inclusion in qualitative synthesis and meta-analysis. This resulted in five (5) randomized controlled trials being included in the data analysis as shown in **Figure 1**.

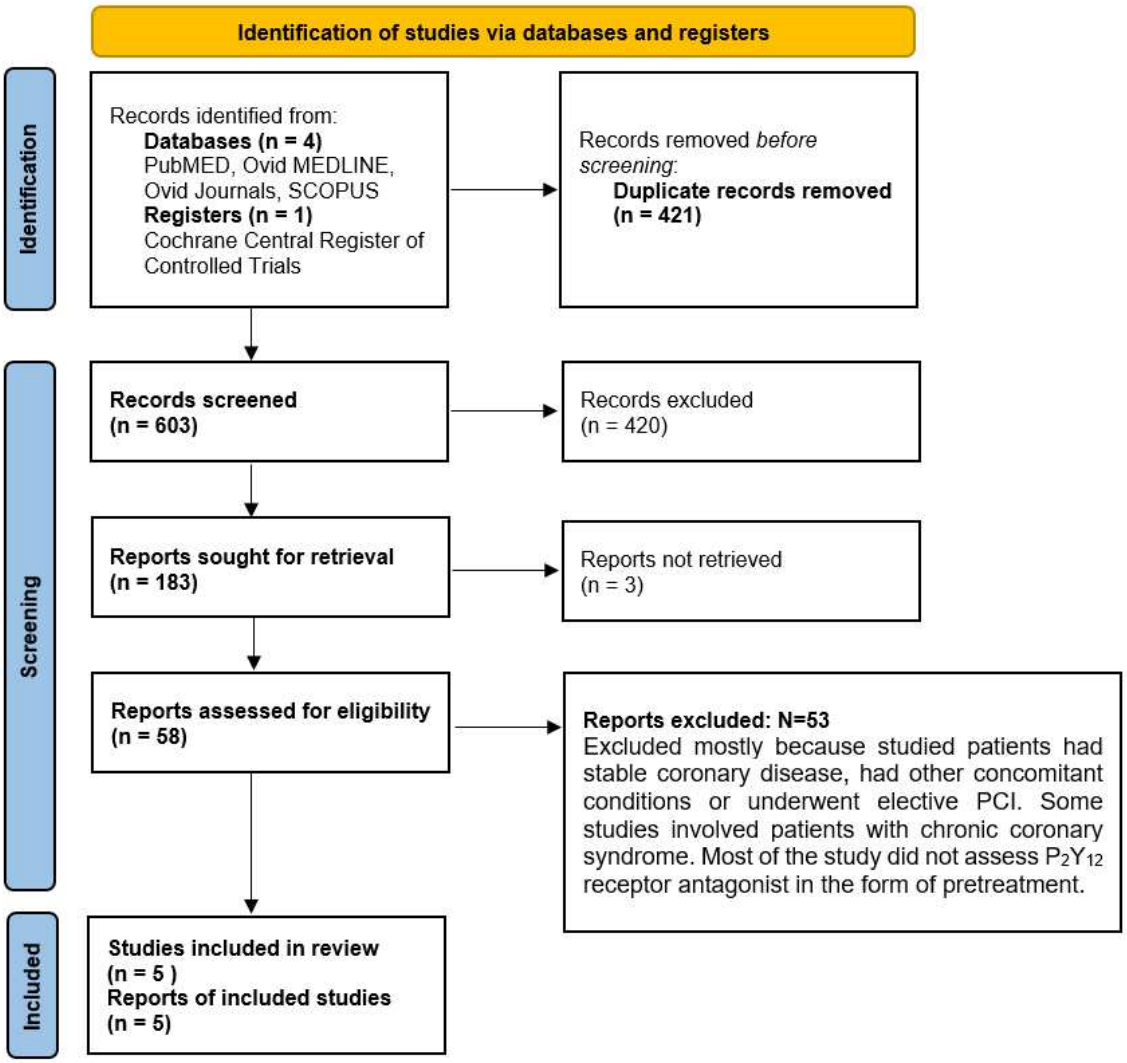


Figure 1. PRISMA flow diagram of study inclusion

Risk of bias assessment 2 (RoB) was independently performed for each of included five RCTs by the principal thesis author (BM) and thesis mentor (JAB). Summary of the RoB 2 for

each included trial is presented in **Figure 2** while the percentage of low risk or some concerns regarding the risk of bias judgments of included trials is shown in **Figure 3**. This analysis showed that only one trial (CREDO 2002) was qualified as having some concerns regarding the bias due to deviations from intended intervention and due to this trial was conservatively graded as having *„some concerns"* for the overall risk of bias domain. Included trials generally had a low risk of bias with respect to the randomization process, deviations from intended interventions, missing outcome data, measurement of the outcomes, and selection of the reported results.

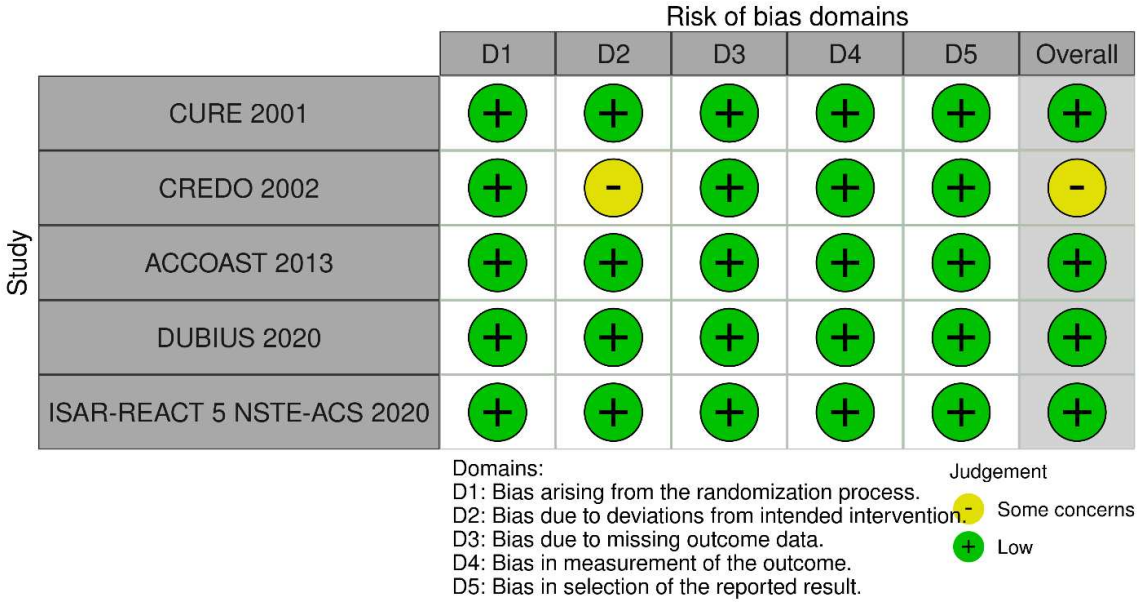


Figure 2. Risk of bias 2 (RoB 2) summary including authors' judgements about each risk of bias domain for each included study

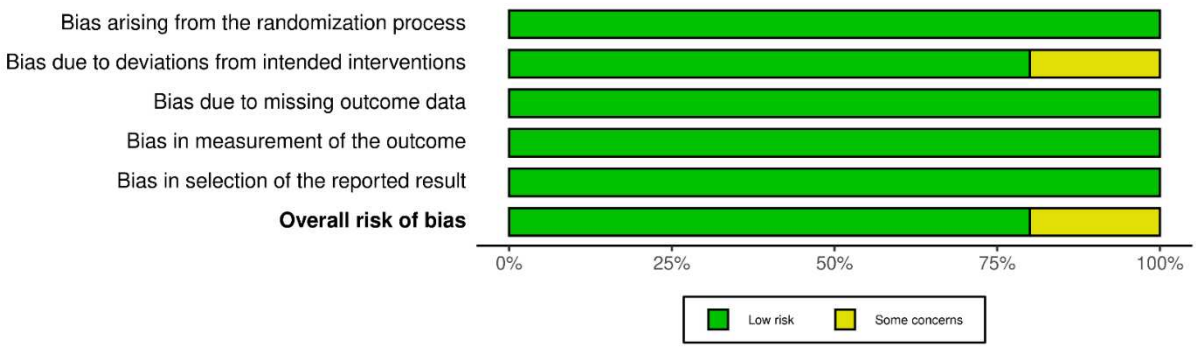


Figure 3. Risk of bias 2 (RoB 2) graph of review authors' judgements about each risk of bias domain presented as percentages across all included studies

4.2 Baseline characteristics of included randomized controlled trials and basic description of enrolled patient population

As demonstrated in **Table 1**, all included trials were designed as randomized controlled trials that examined the early preloading of P_2Y_{12} receptor inhibitor (as added to acetylsalicylic acid as a part of dual antiplatelet treatment - DAPT) *vs.* no P_2Y_{12} preloading strategy. In CURE and CREDO trials, preloading doses of 300 mg clopidogrel were administered *per os*. Third generation P_2Y_{12} inhibitors were used in ACCOAST trial (preloading dose of 30 mg prasugrel) and the DUBIUS trial (ticagrelor preloading before angiography in 95% of cases in the upstream group, and ticagrelor and prasugrel in the downstream group – 50% and 47% of cases, respectively). Similarly, ISAR-REACT 5 trial NSTE-ACS substudy examined the use of a preloading dose of 180 mg ticagrelor *vs.* 60 mg prasugrel administered after coronary angiography. The control group in all trials either received a placebo pill or no P_2Y_{12} inhibitor administered early as a part of the preloading patient management. All interventions were examined on top of the standard of care management that is stipulated per international guidelines for the diagnosis and management of NSTE-ACS. All trials reported ischemic outcomes as the primary outcome (such as death from cardiovascular or cerebrovascular causes, recurrent nonfatal myocardial infarction, stroke, and urgent revascularization), and all trials reported bleeding events (either as being the part of the primary outcome or as one of the secondary outcomes). Regarding trial sponsorships, three trials received full or partial funding from the pharmaceutical industry (CURE, CREDO, and ACCOAST) while two remaining trials were investigator-led and free of commercial bias (DUBIUS and ISAR-REACT 5). Three trials were conducted in Europe (ACCOAST in France, DUBIUS in Italy, and ISAR-REACT 5 in Germany), one in the United States (CREDO), and one in Canada (CURE).

Table 1. Baseline characteristics of included randomized controlled trials

Study	Patient population	Preloading drug	Intervention *vs.* comparator	Primary outcome(s)	Study type	Funding
Yusuf et al. 2001 CURE trial N=12562	**UA** 9414 patients **MI** 3148 patients	Clopidogrel 100%	Clopidogrel 300 mg loading at presentation followed by 75 mg once daily + Aspirin *vs.* Aspirin + Placebo Both P.O.	Death from cardiovascular causes, nonfatal myocardial infarction, or stroke	Multicenter RCT	Supported by Sanofi-Synthelabo and Bristol-Myers Squibb
Steinhubl et al. 2002 CREDO trial N=2116	**UA** 1117 patients **RECENT MI** 290 patients	Clopidogrel 100%	Clopidogrel 300 mg loading those *vs.* Placebo Along with standard-of-care treatment 3 to 24 hours prior to PCI Both P.O.	Composite of death, MI, and stroke in the intent-to-treat population	Multicenter RCT	This study was supported by a grantfrom the Bristol-Myers Squibb/Sanofi -Synthelabo partnership
Montalescot et al. 2013 ACCOAST trial N=4033	**NSTE-ACS** 4033/4033 (100%)	Prasugrel 100%	Prasugrel 30 mg loading dose prior to angiography *vs.* Placebo prior to angiography Notes: in patients that had PCI additional 30 mg was given in early loading and 60 mg in the control group Both P.O.	The primary composite end point was the first occurrence of death from cardiovascular causes, myocardial infarction, stroke, urgent revascularization, or the need for rescue therapy with glycoprotein IIb/IIIa inhibitors through day 7 after randomization	Multicenter RCT	Funded by Daiichi Sankyo and Eli Lilly; ACCOAST ClinicalTrials. gov number, NCT0101528 7
Tarantini et al. 2020 DUBIUS trial N=1449	**NSTEMI** 1073 patients **UA** 286 patients	Upstream (preloading): Ticagrelor 95%, Prasugrel 2 %, Clopidogrel 3%. Downstream (no preloading): Ticagrelor 50% Prasugrel 47% Clopidogrel 2%	Ticagrelor pre-treatment before angiography *vs.* No pre-treatment with ticagrelor before angiography. Both P.O.	Composite of death from vascular causes non-fatal myocardial infarction (MI), or non-fatal stroke and major or fatal bleeding	Multicenter RCT	Funded by the Italian Societ y of Interventional Cardiology (SICI-GISE)

Valina et al. 2020 ISAR-REACT 5 trial NSTEMI substudy N=2365	**NSTEMI** 1855 patients **UA** 510 patients	Ticagrelor 1179 patients Prasugrel 1186 patients	Loading group: Ticagrelor 180 mg loading prior to coronary angiography *vs.* No loading group: Prasugrel 60 mg after coronary angiography Both P.O.	Composite of death, myocardial infarction, or stroke at 1 year and the safety endpoint was Bleeding Academic Research Consortium (BARC) class 3, 4, or 5bleeding at 1 year.	Multicenter RCT	Funded by the German Center for Cardiovascular Research and Deutsches Herzzentrum München; ISAR-REACT 5 ClinicalTrials.gov number, NCT01944800

Abbreviations: MI-myocardial infarction; NSTE-ACS-Non-ST-elevation acute coronary syndromes; NSTEMI-Non-ST-elevation myocardial infarction; P.O.-per os; UA-unstable angina;

As shown in **Table 2**, the age of enrolled patients ranged from 61.5 to 66.0 years while more than two-thirds were men. In all trials, with the exception of the CURE trial, nearly all patients with NSTE-ACS underwent diagnostic coronary angiography while in the CURE trial less than half underwent such procedure. As expected, rates of percutaneous revascularization were substantially lower and ranged from only 21.2% in the CURE trial to 86% in the CREDO trial. More contemporary trials that used newer and more potent P_2Y_{12} agents were also associated with high or exclusive use of DES platforms in the setting of PCI. For example, proportional rates of DES use for PCI were 59%, 100%, and 88.9% in ACCOAST, DUBIUS, and ISAR-REACT 5 trials, respectively, while CURE and CREDO trials used BMS platforms.

Table 2. Baseline characteristics of patients and procedures in included trials

Study	Age, years M ± SD or Median (IQR)	Men N/N (%)	Underwent CA N/N (%)	Underwent PCI N/N (%)	Stent use % and DES use %
Yusuf et al. 2001 CURE trial N=12562	Experimental 64.2±11.3 Control 64.2±11.3	7726/12562 (61.5%)	5491/12562 (43.7%)	2658/12562 (21.2%)	N/A Stent type - BMS
Steinhubl et al. 2002 CREDO trial N=2116 patients	Experimental 61.5±11.2 Control 61.8±11.0	1510/2116 (71.4%)	2116/2116 (100%)	1815/2116 (86%)	Stents used in 1615/2116 (76%) of patients that had PCI Likely BMS stents were used
Montalescot et al. 2013 ACCOAST trial N=4033 patients	Experimental 63.8 Control 63.6	2923/4033 (72.5%)	3994/4033 (99%)	2770/4033 (68.7%)	DES used in 1635/2770 (59%) of patients that had PCI
Tarantini et al. 2020 DUBIUS trial N=1449 patients	Downstream 65 (56-73) Upstream 64 (72-57)	1097/1449 (75.7%)	1408/1449 (99.2%)	970/1449 (72%)	All patients that underwent PCI received DES
Valina et al. 2020 ISAR-REACT 5 trial NSTEMI substudy N=2365	Ticagrelor 66.0±11.7 Prasugrel 65.6±12.0	1753/2365 (74.1%)	2352/2365 (99.5%)	1809/2365 (76.5%)	DES used in 1608/1809 (88.9%) of patients that had PCI

Abbreviations: BMS-bare metal stent; CA-coronary angiography; DES-drug-eluting stent; PCI-percutaneous coronary intervention;

4.3 Comorbidities of patients enrolled in included trials

As it can be appreciated from **Table 3**, overall about one-quarter (22.8%) of patients with NSTE-ACS had diabetes mellitus, one-half were smokers (49.1%), nearly two-thirds had arterial hypertension (62.6%) while the prevalence of hypercholesterolemia was 56.1%.

Moreover, 26.8% of patients from the total trial population had a positive history of myocardial infarction while nearly half of patients (49.3%) had multivessel and/or left main coronary artery disease determined by diagnostic angiography (this percentage was based on data available from ACCOAST and ISAR-REACT 5 trials, other studies did not disclose information on these angiographic variables).

Table 3. Prevalence of comorbidities such as diabetes mellitus (DM), prior myocardial infarction (MI), smoking, hypercholesterolemia (HCX), arterial hypertension (AH), and multivessel disease (MVD) and/or left main (LM) disease

Study	**DM** N/N (%)	**Prior MI** N/N (%)	**Smoking** N/N (%)	**HCX** N/N (%)	**AH** N/N (%)	**MVD and/or LM disease** N/N (%)*
Yusuf et al. 2001 CURE trial N=12562	2840/12562 (22.6%)	4044/12562 (32.2%)	7631/12562 (60.7%)	N/A	7392/12562 (58.8%)	N/A
Steinhubl et al. 2002 CREDO trial N=2116 patients	560/2116 (26.5%)	719/2116 (33.9%)	652/2116 (30.8%)	1580/2116 (74.7%)	1450/2116 (68.5%)	N/A
Montalescot et al. 2013 ACCOAST trial N=4033 patients	820/4033 (20.3%)	578/4033 (14.3%)	1340/4033 (33.2%)	1814/4033 (45.0%)	2504/4033 (62.1%)	1661/4033 (41.2%)
Tarantini et al. 2020 DUBIUS trial N=1449 patients	333/1449 (23%)	245/1449 (17.0%)	769/1449 (53.1%)	675/1449 (46.6%)	942/1449 (58.1%)	N/A
Valina et al. 2020 ISAR-REACT 5 trial NSTEMI substudy N=2365	580/2365 (24.5%)	444/2365 (18.8%)	671/2365 (28.4%)	1523/2365 (64.4%)	1803/2365 (76.2%)	1492/2365 (63%)

*multivessel disease defined as the coronary artery disease involving ≥2 epicardial vessels and/or left main (LM) disease (combined endpoint)

Abbreviations: AH-arterial hypertension; HCX-hypercholesterolemia; MI-myocardial infarction; MVD-multivessel disease;

4.4 Rates and definitions of adverse events

The number and rates of ischemic events that occurred in each trial with detailed descriptions of ischemic endpoints are shown in **Table 4** while number and rates of bleeding events with detailed descriptions of these endpoints are provided in **Table 5.**

Table 4. Ischemic composite at 30 days with definitions and number of events

Study	**Type of the study from which data is extracted**	**Preloading with P_2Y_{12} inhibitor** **N of events**	**No preloading with P_2Y_{12} inhibitor** **N of events**	**Ischemic endpoint definition and timeframe of measurement** *(as included in the statistical analysis)*
Yusuf et al. 2001 CURE trial	RCT, double-blinded	**275/6259** (4.4%)	**346/6303** (5.5%)	Composite endpoint of death from cardiovascular causes, nonfatal MI and stroke during the first 30 days after randomization
Steinhubl et al. 2002 CREDO trial	RCT, double-blinded	**61/900** (6.8%)	**76/915** (8.3%)	Composite endpoint of 28-day incidence of death, nonfatal MI and urgent target vessel revascularization in the intention-to-treat population
Montalescot et al. 2013 ACCOAST trial	RCT, double-blinded	**203/2037** (10.0%)	**195/1996** (9.8%)	Composite endpoint of death from cardiovascular causes, nonfatal MI, stroke, urgent revascularization, or glycoprotein GP IIb/IIIa bailout at 30 days
Tarantini et al. 2020 DUBIUS trial	RCT, open label, adaptive	**21/711** (3.0%)	**18/721** (2.5%)	Composite endpoint of ischemic events including all-cause death, nonfatal MI, stent thrombosis, target vessel revascularization, stroke or TIA at 30 days of follow-up
Valina et al. 2020 ISAR-REACT 5 trial NSTE-ACS substudy	NSTE-ACS substudy**	**At 30-days 40/1179** (3.4%)	**At 30-days 33/1186** (2.8%)	Primary composite endpoint of death, MI, or stroke during the 1-year follow-up and at 30 days*

*data at 30 days provided directly from the study authors as per reasonable request

**Post-randomization subgroup defined as randomized comparison

Table 5. Bleeding composite at 30 days with definitions and number of events

Study	Type of the study from which data is extracted	Preloading with P_2Y_{12} inhibitor N of events	No preloading with P_2Y_{12} inhibitor N of events	Bleeding endpoint definition and timeframe of measurement *(as adjudicated per study investigators' definition)*
Yusuf et al. 2001 CURE trial	RCT, double-blinded	**533/6259** (8.5%)	**317/6303** (5.0%)	Composite endpoint of life-threatening, major or minor bleeding events at 30 days. Major bleeding episodes were defined as substantially disabling bleeding, intraocular bleeding leading to the loss of vision, or bleeding necessitating the transfusion of at least 2 units of blood. Major bleeding was classified as life-threatening if the bleeding episode was fatal or led to a reduction in the hemoglobin level of at least 5 g per deciliter or to substantial hypotension requiring the use of intravenous inotropic agents, if it necessitated a surgical intervention, if it was a symptomatic intracranial hemorrhage, or if it necessitated the transfusion of 4 or more units of blood. Minor bleeding episodes included other hemorrhages that led to the interruption of the study medication.
Steinhubl et al. 2002 CREDO trial	RCT, double-blinded	**83/1053** (7.9%)	**62/1063** (5.8%)	Composite endpoint of any major and minor bleeding event at 28 days, as defined by TIMI bleeding criteria***[3]
Montalescot et al. 2013 ACCOAST trial	RCT, double-blinded	**58/2037** (2.8%)	**29/1996** (1.5%)	Composite safety endpoint of all CABG-related or non-CABG-related TIMI*** major bleeding events at 30 days[3]
Tarantini et al. 2020 DUBIUS trial	RCT, open-label, adaptive	**14/711** (1.9%)	**12/721** (1.6%)	Composite endpoint consisting of bleeding events adjudicated as BARC[6] type 3,4, or 5 bleeding* during at 30 days of follow-up
Valina et al. 2020 ISAR-REACT 5 trial NSTE-ACS subanalysis	NSTE-ACS substudy#	**At 30 days** **40/1159** (3.5%)	**At 30 days** **27/963** (2.8%)	Composite safety endpoint consisting of bleeding events adjudicated as BARC[6] type 3, 4, or 5 bleeding* during at 1-year of follow-up and at 30-days¶

Abbreviations: BARC-Bleeding Academic Research Consortium; CABG-coronary artery bypass grafting; MACCE-major adverse cardiovascular and cerebrovascular events; NCDR-National Cardiovascular Disease Registry; TIMI-Thrombolysis in Myocardial Infarction

***BARC bleeding scale:** type 0 – no bleeding; type 1 - bleeding that is not actionable and does not cause the patient to seek unscheduled performance of studies, hospitalization, or treatment by a health-care professional; may include episodes leading to self-discontinuation of medical therapy by the patient without consulting a health-care professional; type 2 - any overt, actionable sign of hemorrhage (e.g., more bleeding than would be expected for a clinical circumstance, including bleeding found by imaging alone) that does not fit the criteria for type 3, 4, or 5 but does meet at least one of the following criteria: requiring nonsurgical, medical intervention by a health-care professional, leading to hospitalization or increased level of care, or prompting evaluation; type 3a – evident bleeding with a decrease in the hemoglobin level of 3 to 5 g per deciliter or any transfusion; type 3b – evident bleeding with a decrease in the hemoglobin level of 5 g or more per deciliter or an evident bleeding leading to cardiac tamponade, surgical intervention, or the use of intravenous vasoactive agents; type 3c – vision comprising intraocular bleeding or intracranial hemorrhage; type 4 – CABG-related bleeding; type 5a – probable fatal bleeding; type 5b – certain fatal bleeding

****NCDR CathPCI** Bleeding events including any in-hospital major bleeding event associated with any of the following: 1) hemoglobin drop of ≥3 g/dL; 2) transfusion of whole blood or packed red blood cells; or 3) procedural intervention/surgery at the bleeding site to reverse/stop or correct the bleeding. https://www.ncdr.com/WebNCDR/docs/default-source/public-data-collection-documents/cathpci_v4_codersdictionary_4-4.pdf?sfvrsn=2;accessed

*****TIMI bleeding criteria:** TIMI major bleeding - defined as intracranial haemorrhage or bleeding with a haemoglobin decrease of >5 g/dL or haematocrit decrease of >15%; TIMI minor bleeding – adjudication dependent on whether or not there is an identifiable source of blood loss. If a bleeding site is found, then TIMI minor bleeding is defined as a haemoglobin decrease of >3 g/dL or a haematocrit decrease of >10%; if no site is found, then it is defined as a haemoglobin decrease of >4 g/dL or haematocrit decrease of >12%; TIMI minimal bleeding - defined as any clinically overt sign of haemorrhage that is associated with a haemoglobin decrease of <3 g/dl or a haematocrit decrease of <9%

¶Data at 30 days were provided directly by the study authors as per reasonable request. Bleeding events were assesed in the modified intention to treat population and after accouting for the competing risks of death, as defined in the Supplementary Appendix file of the original ISAR-REACT 5 Trial[8]

#Post-randomization subgroup defined as randomized comparison

Abbreviations: BARC-Bleeding Academic Research Consortium; CABG-coronary artery bypass grafting; MACCE-major adverse cardiovascular and cerebrovascular events; NCDR-National Cardiovascular Disease Registry; TIMI-Thrombolysis in Myocardial Infarction

4.5 Effects of interventions

4.5.1 Composite of ischemic events at 30 days

All trials contributed to effect estimates with an overall of 22207 patients with NSTE-ACS. Early P_2Y_{12} preloading *vs.* no preloading in patients with NSTE-ACS was associated with a 7% reduction in the relative risk of ischemic event at 30 days (RR 0.93, 95% CI 0.79–1.09), however, this effect was not significant (Z=0.94, *P*=0.350) (**Figure 4**). A low degree of heterogeneity was detected for this endpoint across trials (Tau^2=0.01, I^2=41%, *P*=0.150).

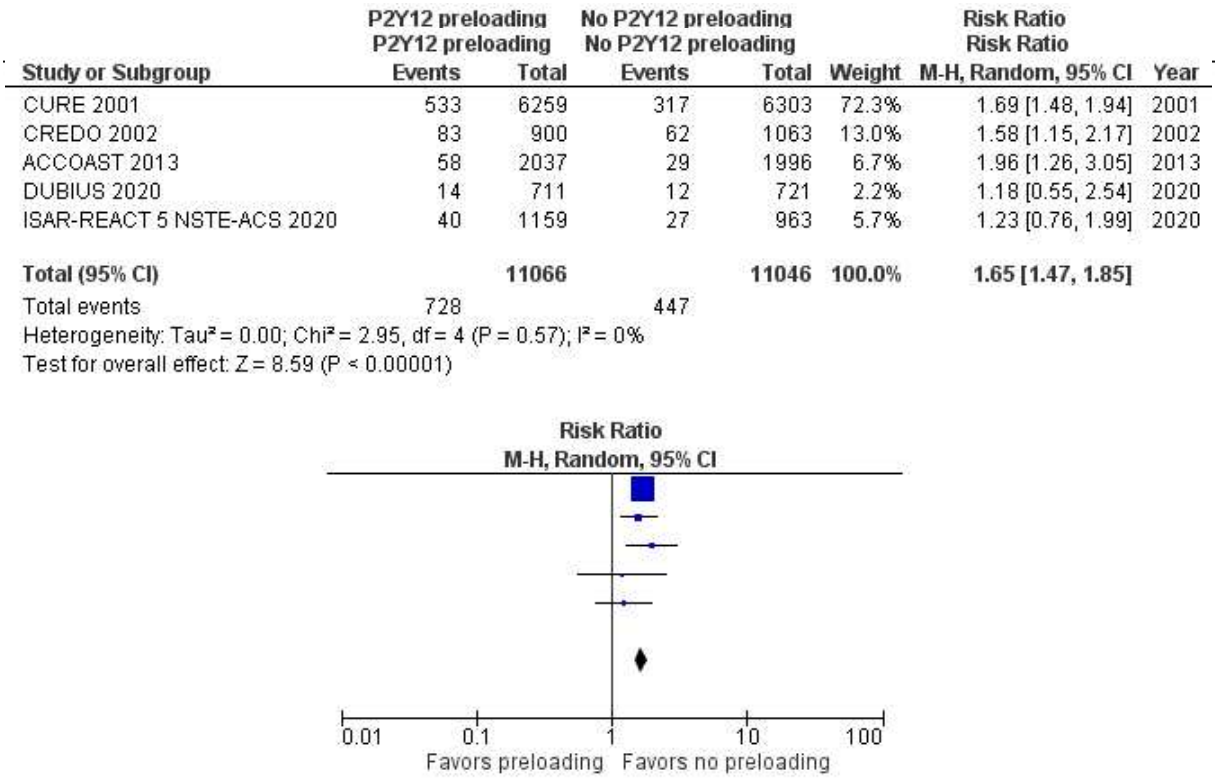
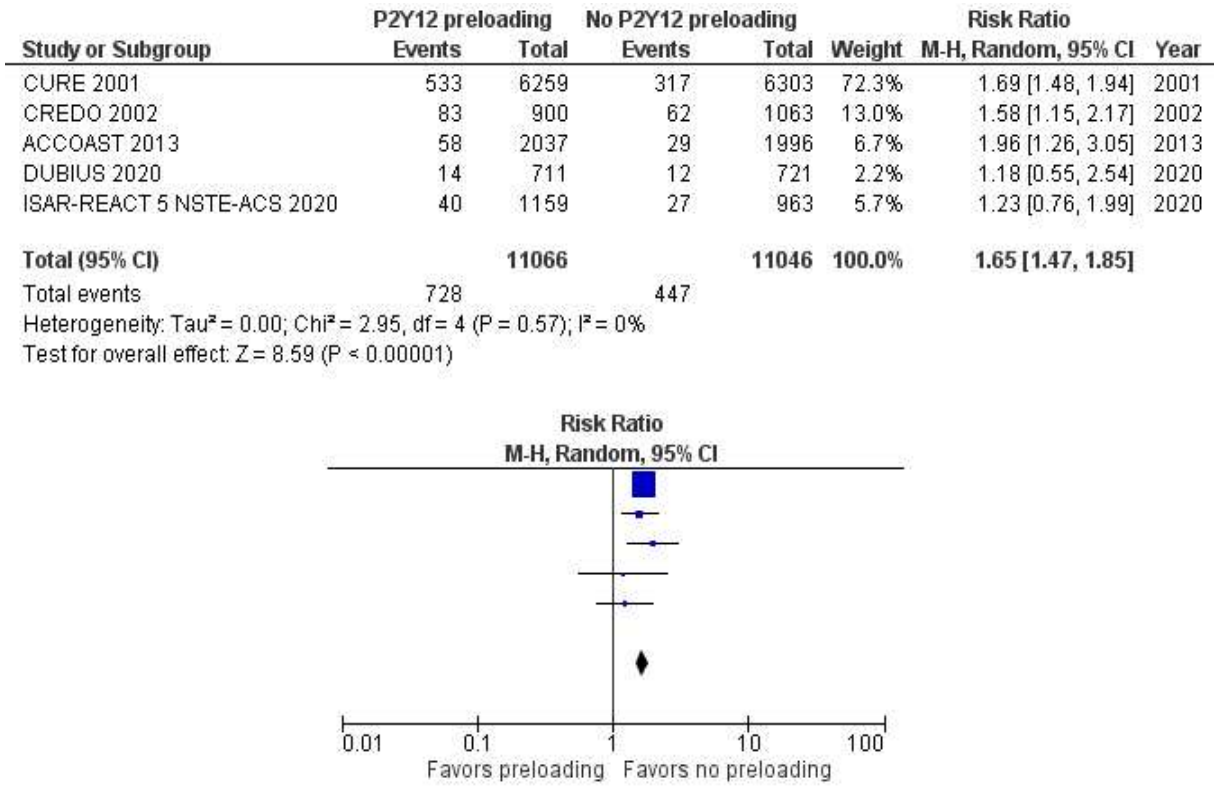


Figure 4. Relative risk (RR) of experiencing ischemic event at 30 days in NSTE-ACS if early P_2Y_{12} preloading strategy was used *vs.* if it was not (control)

4.5.2 Composite of bleeding events at 30 days

All trials contributed to effect estimates with an overall of 22112 patients with NSTE-ACS. Early P_2Y_{12} preloading *vs.* no preloading in patients with NSTE-ACS was associated with a 65% increase in the relative risk of bleeding event at 30 days (RR 1.65, 95% CI 1.47–1.85), and this result was highly significant (Z=8.59, P<0.001) (**Figure 5**). No heterogeneity was detected for this endpoint across trials (Tau^2=0.00, I^2=0%, P=0.570).



Study or Subgroup	P2Y12 preloading Events	Total	No P2Y12 preloading Events	Total	Weight	Risk Ratio M-H, Random, 95% CI	Year
CURE 2001	533	6259	317	6303	72.3%	1.69 [1.48, 1.94]	2001
CREDO 2002	83	900	62	1063	13.0%	1.58 [1.15, 2.17]	2002
ACCOAST 2013	58	2037	29	1996	6.7%	1.96 [1.26, 3.05]	2013
DUBIUS 2020	14	711	12	721	2.2%	1.18 [0.55, 2.54]	2020
ISAR-REACT 5 NSTE-ACS 2020	40	1159	27	963	5.7%	1.23 [0.76, 1.99]	2020
Total (95% CI)		**11066**		**11046**	**100.0%**	**1.65 [1.47, 1.85]**	
Total events	728		447				

Heterogeneity: Tau² = 0.00; Chi² = 2.95, df = 4 (P = 0.57); I² = 0%
Test for overall effect: Z = 8.59 (P < 0.00001)

Figure 5. Relative risk (RR) of experiencing bleeding event at 30 days in NSTE-ACS if early P_2Y_{12} preloading strategy was used *vs.* if it was not (control)

4.5.3 Net adverse clinical events (NACE) at 30 days

All trials contributed to effect estimates with an overall of 22207 patients with NSTE-ACS. Early P_2Y_{12} preloading *vs.* no preloading in patients with NSTE-ACS was associated with a 19% increase in the relative risk of net adverse clinical events at 30 days (RR 1.19, 95% CI 1.11–1.29), and this result was highly significant (Z=4.62, *P*<0.001) (**Figure 6**). No heterogeneity was detected for this endpoint across trials (Tau^2=0.00, I^2=0%, *P*=0.690).

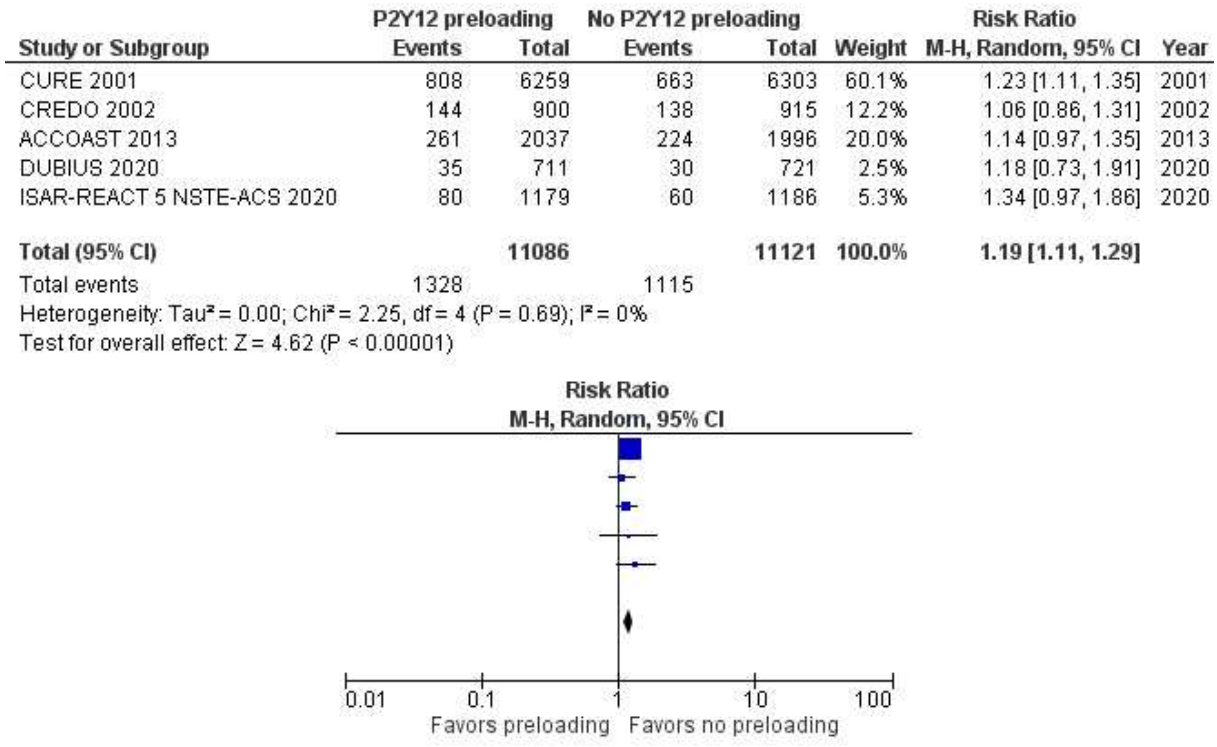


Figure 6. Relative risk (RR) of experiencing net adverse clinical event (NACE) at 30 days in NSTE-ACS if early P_2Y_{12} preloading strategy was used *vs.* if it was not (control)

5. DISCUSSION

The concept of using preloading (pretreatment) with P_2Y_{12} inhibitors in patients with NSTE-ACS has been challenged recently and in the latest ESC guidelines, this treatment modality is contraindicated (class III, level of evidence A recommendation). According to current guidelines, these agents should not be initiated in patients with NSTE-ACS until coronary anatomy is known (as determined by diagnostic coronary angiography) and in whom an early invasive management is planned. Such rationale has been dominantly based on three major publications providing relevant evidence. The first publication provided randomized data from ACCOAST trial (Comparison of Prasugrel at the Time of Percutaneous Coronary Intervention or as Pretreatment at the Time of Diagnosis in Patients with Non-ST Elevation Myocardial Infarction) that showed how preloading strategy with prasugrel in NSTE-ACS did not provide any ischemic benefit but significantly increased risk of bleeding (40). In support of this, observational data from the Swedish Coronary Angiography and Angioplasty Registry (SCAAR) encompassing nearly 65 thousand patients with NSTE-ACS showed that preloading with P_2Y_{12} receptor antagonists was not associated with improved clinical outcomes but instead increased the likelihood of bleeding by nearly 50% (44). In the most recent randomized ISAR-REACT 5 trial, it has been demonstrated that deferred P_2Y_{12} administration (prasugrel) after or at the time of coronary angiography was superior to ticagrelor-based preloading (45).

The most recent pre-specified study of ISAR-REACT 5 trial that enrolled patients with NSTE-ACS and that was published after the ESC guidelines were presented, showed how deferred prasugrel administration was associated with a 41% lower risk of the composite endpoint of death, MI or stroke during the 1-year follow-up, compared to ticagrelor preloading while preloading strategy was also associated with the higher risk of bleeding (46). Taken together, available data suggest that early preloading strategy with P_2Y_{12} inhibitors might be harmful in NSTE-ACS patients as it has marginal effect on mitigating ischemia but significantly increases risk of bleeding and subsequent complications.

For these reasons, the goal of the present thesis was to provide a unique perspective on the totality of evidence coming from randomized trial data regarding the role of P_2Y_{12} inhibitor preloading in NSTE-ACS. In this analysis, seminal randomized studies designed to examine early P_2Y_{12} preloading strategies in NSTE-ACS patients were included and data were updated with the latest randomized trial data coming from the ISAR-REACT 5 (Prospective, Randomized Trial of Ticagrelor Versus Prasugrel in Patients With Acute Coronary Syndrome; NCT01944800) NSTE-ACS substudy and DUBIUS trial (Downstream Versus Upstream Strategy for the Administration of P_2Y_{12} Receptor Blockers In Non-ST Elevated Acute

Coronary Syndromes With Initial Invasive Indication [DUBIUS]; NCT02618837). Moreover, in addition to undertaking the analysis with updated trial data, we performed the net adverse clinical events (NACE) analysis by combining composite ischemic and bleeding events that are both important for the outcomes of these patients and bear important downstream implications. By executing such analysis we aimed to most precisely show the net clinical benefit or harm associated with the P_2Y_{12} preloading strategy in this population.

The main results of this thesis show that the P_2Y_{12} preloading in NSTE-ACS did not provide ischemic benefits since it was associated with a non-significant 7% relative risk reduction in composite ischemia endpoint. On the other hand, the preloading strategy was associated with a significant increase in relative risk of bleeding by 65%, compared to the strategy that did not utilize preloading. Finally, an integrative NACE analysis demonstrated the overall harm of P_2Y_{12} preloading in NSTE-ACS since this intervention was associated with a 19% relative risk increase in the cumulative endpoint aggregating both ischemic and bleeding events.

Such updated and expanded results on this topic are in favor of the recommendation raised in the latest ESC guidelines for the diagnosis and management of NSTE-ACS. There are several important points that should be discussed based on the presented findings.

Firstly, preloading with P_2Y_{12} inhibitors before coronary anatomy is known might be deleterious in a sizeable proportion of patients that might present with acute conditions mimicking NSTE-ACS such as aortic dissection or major bleeding complications that might be life-threatening like intracranial bleeding. For example, acute Stanford type A aortic dissection is often misdiagnosed as an ACS while subsequent administration of antiplatelet treatment was shown to be a strong determinant for the timing and clinical outcomes of the dissection (47). Similarly, many other conditions with ACS-like presentation but not related to coronary artery disease would have no use of P_2Y_{12} preloading such as myocarditis, stress cardiomyopathy (Takotsubo), suspicion of NSTE-ACS in the setting of various tachyarrhythmias, and similar (48).

Secondly, a high number of patients with NSTE-ACS will likely have a severe coronary disease (three-vessel and/or left main disease) at presentation that might not be appropriate for percutaneous revascularization and would instead be referred to bypass surgery. In such patients, preloading with P_2Y_{12} inhibitors might unnecessarily delay the CABG procedure as 3 to 7 days are required for the wash-out of antiplatelet drug effects, and the risk of periprocedural

bleeding would likely be enhanced in these patients if such procedure would need to be done urgently. Similarly, these patients would be subjected to prolonged hospital stay and this would increase healthcare costs.

To illustrate the importance of these ramifications we could use recent data obtained from a Croatian cohort of patients with NSTE–ACS in whom coronary angiography was performed (49). In this sample, more than one-third of patients had the three-vessel disease while 56.2% of patients had the multivessel disease (defined as significant stenosis in at least 2 epicardial coronary vessels) while about 1 in 10 patients presented with significant left main stenosis. Furthermore, even as much as 37.3% of all NSTE-ACS patients were referred to CABG surgery. Equally important, patients with NSTE-ACS tend to have a high comorbidity burden and this fact, along with severe coronary disease might likely predispose them to revascularization *via* CABG surgery instead of PCI. For example, in the aforementioned Croatian cohort of NSTE-ACS patients, the prevalence of diabetes was about 30%, nearly two-thirds had arterial hypertension while hypercholesterolemia was documented in half of the patients. Similarly, in the NSTE-ACS cohort from the landmark ISAR-REACT 5 trial, the prevalence of multivessel disease was 63.4%, while 38.3% of patients had three-vessel or left main disease (46). Similarly, one-third of patients had diabetes mellitus. Based on such observations, it becomes clear that a large proportion of NSTE-ACS patients will be diabetics with complex CAD thus at a substantially increased likelihood of being referred to CABG. Therefore, for the number of reasons elaborated earlier, routine P_2Y_{12} preloading in these patients might be harmful, and withholding these agents would allow for more personalized decision-making in the NSTE-ACS setting based on angiographic and procedural variables.

Equally important, non-CABG-related bleeding is a relevant complication and involves entities such as gastrointestinal bleeding (GIB) and catheterization access bleeding. A recent study showed that the relative risks of GIB and non-CABG major bleeding were 22% and 18% higher, respectively, for third-generation P_2Y_{12} inhibitors such as ticagrelor and prasugrel, compared to 2nd generation P_2Y_{12} inhibitor clopidogrel (50). Employment and routine use of radial access for PCI can significantly mitigate access-related bleeding complications.

Finally and equally important, periprocedural use of newer and more potent P_2Y_{12} inhibitors such as ticagrelor and prasugrel allows for the administration of these agents after diagnostic angiography is performed and before PCI. Of note, the onset of effect of both agents when loading dose is administered is rapid, and efficacious platelet inhibition can be achieved as early as within 30 minutes to up to 2 hours with ticagrelor and up to 4 hours with prasugrel.

This makes the use of these agents practical in modern interventional cardiology practice and allows for the deferral of these drugs in NSTE-ACS patients that would be better candidates for CABG than PCI. Such notions seem to be concordant with the results reported in this thesis. For example, more contemporary trials in our analysis (ACCOAST, DUBIUS, and ISAR-REACT 5 NSTE-ACS) seem to show a trend towards better outcomes with no P_2Y_{12} preloading strategy in NSTE-ACS compared to older and historic trials (CURE and CREDO). There are some relevant differences between these trials that should be discussed. For example, older trials exclusively used clopidogrel as a P_2Y_{12} loading agent, substantially fewer patients underwent percutaneous revascularization procedures and if they did old stent designs such as bare-metal stents were deployed with high risks of early and mid-term restenosis. Furthermore, patients in these trials experienced substantially longer mean times to angiography. The beneficial signal obtained from more contemporary trials concerning no preloading strategy might possibly be explained by the use of more potent P_2Y_{12} inhibitors such as prasugrel and ticagrelor periprocedurally and substantially higher use of the early invasive approach in NSTE-ACS patients, as well as deployment of new generation modern DES platforms that elicit less endothelial damage and are generally more biocompatible with the coronary vessel tissue.

It is also important to highlight that there are potential pitfalls associated with the withholding of P_2Y_{12} preloading in NSTE-ACS patients and this must be considered in select clinical scenarios. For example, some patients with NSTE-ACS might wait too long to receive coronary angiography and these delays in care might be harmful if patients did not receive potent P_2Y_{12} inhibition early. This might be a significant problem in areas without developed tertiary-care infrastructure with respect to PCI networks or in less accessible areas where long transfers of NSTE-ACS patients to PCI-capable centers are common and unpredictable. Such patients might benefit from early ischemic protection while awaiting coronary angiography thus deriving a benefit from earlier use of potent P_2Y_{12} inhibitors. However, in centers with established NSTE-ACS protocols in which most patients undergo early invasive management and are administered potent P_2Y_{12} inhibitors at the table in the cath lab, early P_2Y_{12} preloading is likely unnecessary and might only cause harm and downstream bleeding complications.

Possible limitations of this meta-analysis are that no search of grey literature was performed and only randomized data were included for the analysis that might not entirely reflect real-world clinical practice. Furthermore, some trials such as DUBIUS had very low event rates thus data from this trial might provide limited information regarding the outcomes of interest. Another limitation is that there was a notable difference in the proportion of invasive

management across these trials as older trials tended to treat more patients conservatively by using optimal medical treatment, used less potent P_2Y_{12} agents (clopidogrel only), had significantly longer mean times to angiography, and performed percutaneous reperfusion with bare-metal stents. Therefore, the results presented herein should be interpreted with having these trial characteristics in mind. On the other hand, low heterogeneity or no heterogeneity was detected for all three main outcome analyses and a more conservative random-effects model was used for all effect estimates.

In conclusion, presented results show that early preloading with P_2Y_{12} inhibitors in patients with NSTE-ACS provides no relevant ischemic benefits and significantly increases the risk of bleeding at 30 days. Similarly, *net* harm analysis confirmed that early preloading in these patients is overall significantly associated with worse short-term clinical outcomes in this population. Finally, these updated results support the most recent guideline-directed recommendation that routine early P_2Y_{12} preloading in NSTE-ACS patients should be avoided in most clinical scenarios, especially if coronary anatomy is unknown and in patients in whom early invasive management is planned.

6. CONCLUSIONS

Based on the quantitative and meta-analytic synthesis of obtained data from randomized controlled trials investigating the use of early P_2Y_{12} preloading in patients with NSTE-ACS, we can conclude the following:

1. Early P_2Y_{12} preloading strategy in patients with NSTE-ACS was no different than no P_2Y_{12} preloading strategy with respect to risk of ischemic events at 30 days.

2. Early P_2Y_{12} preloading was associated with a non-significant 7% relative risk reduction of composite ischemic endpoint at 30 days, compared to no P_2Y_{12} preloading strategy.

3. Early P_2Y_{12} preloading strategy was associated with a significant 65% relative risk increase in the composite endpoint of bleeding at 30 days, compared to no P_2Y_{12} preloading strategy.

4. Early P_2Y_{12} preloading strategy was associated with a significant overall 19% increase in the relative risk of net adverse clinical events (NACE), a net-harm endpoint aggregating both ischemic and bleeding events at 30 days.

5. Taken together, presented data suggest that routine early preloading of NSTE-ACS patients with P_2Y_{12} inhibitors should be avoided since this strategy provided no significant ischemic benefits but significantly increased risk of bleeding.

6. Caution when interpreting these results should be exercised since a strategy with no P_2Y_{12} preloading while being a justified approach in the majority of clinical scenarios, might potentially be harmful in patients with long delays to coronary angiography and if early invasive management is not feasible as these patients might be unprotected in terms of ischemic risks if P_2Y_{12} inhibition is not initiated early.

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8. SUMMARY

Objectives: The aims of this study were to investigate the impact of early P_2Y_{12} inhibitor preloading (pretreatment) *vs.* no preloading on short-term clinical outcomes in patients with acute coronary syndromes without persistent ST-segment elevation (NSTE-ACS).

Patients and methods: Meta-analysis and quantitative synthesis were performed by including five randomized controlled trials (RCTs) that examined P_2Y_{12} preloading in NSTE-ACS patients. Primary outcomes of interest were composite endpoints of ischemia, bleeding, and net adverse clinical events (NACE) at 30 days since randomization. Risk ratio (RR) with 95% confidence intervals (95% CI) was used as the main summary measure while a random-effects model with the Mantel-Haenszel method was used to populate results of meta-analysis.

Results: A total of 5 RCTs enrolling 22207 patients with NSTE-ACS contributed to observed effect estimates. Four trials were at low risk of bias (RoB) while one trial had some concerns regarding RoB. Trials dominantly enrolled men and acetylsalicylic acid was used concomitantly as a second antiplatelet agent. Early P_2Y_{12} preloading was similar to no preloading strategy among NSTE-ACS patients concerning the risk of ischemic events at 30 days (RR 0.93, 95% CI 0.79-1.09, P=0.350; low heterogeneity detected – I^2=41%). On the other hand, the P_2Y_{12} preloading strategy was associated with a significant 65% increase in the relative risk of a bleeding event at 30 days, compared to no P_2Y_{12} preloading strategy (RR 1.65, 95% CI 1.47-1.85, P<0.001; no heterogeneity detected – I^2=0%). Finally, P_2Y_{12} preloading was associated with a significant 19% increase in the relative risk of NACE at 30 days (RR 1.19, 95% CI 1.11-1.29, P<0.001; no heterogeneity detected – I^2=0%).

Conclusion: Early P_2Y_{12} inhibitor preloading in NSTE-ACS did not improve ischemic outcomes and significantly increased the risk of bleeding at 30 days, compared to a treatment strategy that did not use preloading. Similarly, early P_2Y_{12} preloading was significantly associated with a higher risk of NACE at 30 days. Taken together, obtained data suggest that an early preloading strategy with P_2Y_{12} inhibitors should be avoided in NSTE-ACS.

9. CROATIAN SUMMARY

Naslov rada: Rani pretretman korištenjem inhibitora P_2Y_{12} receptora u bolesnika koji se prezentiraju s akutnim koronarnim sindromom bez perzistentne elevacije ST segmenta

Ciljevi: Glavni ciljevi ove studije su bili istražiti utjecaj ranog pretretmana korištenjem inhibitora P_2Y_{12} receptora u usporedbi s načinom liječenja bez pretretmana s inhibitorima P_2Y_{12} receptora na kratkoročne kliničke ishode u bolesnika s akutnim koronarnim sindromom bez perzistentne elevacije ST segmenta (NSTE-ACS).

Pacijenti i metode: Kvantitativna analiza i meta-analiza su izvršene uključivanjem pet randomiziranih kliničkih studija koje su istraživale rani pretretman s inhibitorima P_2Y_{12} receptora u bolesnika s NSTE-ACS. Glavni ishodi od interesa su bili mjereni unutar 30 dana od randomizacije, a uključivali su ishemijske događaje, značajna krvarenja i sveukupne neželjene kliničke događaje (kompozitni ishod). Omjer rizika (RR) sa 95%-tnim intervalima pouzdanosti (95% CI) je korišten kao glavna mjera ishoda, a model s nasumičnim učincima i Mantel-Haenszel algoritmom je korišten za meta-analizu.

Rezultati: Analizirano je pet randomiziranih kliničkih istraživanja koja su uključila ukupno 22,207 bolesnika sa NSTE-ACS. Četiri istraživanja su imala nizak rizik od pristranosti, a jedno istraživanje imalo je moguće elemente pristranosti. Navedena istraživanja su uglavnom uključila muške ispitanike, a acetilsalicilna kiselina korištena je u svim studijama kao drugi antiagregacijski lijek. Rizik od ishemijskih događaja unutar 30 dana bio je sličan bez obzira na strategiju ranog liječenja sa pretretmanom ili bez (RR 0,93, 95% CI 0,79-1,09, P=0,350; niska razina heterogenosti – I^2=41%). S druge strane, strategija ranog pretretmana s inhibitorima P_2Y_{12} receptora bila je značajno povezana s 65% višim relativnim rizikom za krvarenje unutar 30 dana u usporedbi sa strategijom liječenja bez pretretmana (RR 1,65 95% CI 1,47-1,85, P<0,001; bez prisutne heterogenosti – I^2=0%). Konačno, korištenje ranog pretretmana s inhibitorima P_2Y_{12} receptora bilo je značajno povezano s 19% višim relativnim rizikom sveukupnih neželjenih kliničkih događaja unutar 30 dana u usporedbi sa strategijom liječenja bez pretretmana (RR 1,19, 95% CI 1,11-1,29, P<0,001; bez prisutne heterogenosti – I^2=0%).

Zaključci: Strategija ranog pretretmana korištenjem inhibitora P_2Y_{12} receptora nije poboljšala ishemijske ishode i značajno je povećala rizik za krvarenje unutar 30 dana u usporedbi sa strategijom liječenja bez pretretmana. Jednako tako, strategija ranog pretretmana s inhibitorima P_2Y_{12} receptora bila je povezana sa značajno višim rizikom od svekupnih neželjenih događaja unutar 30 dana. Konačno, prikazani rezultati ne podržavaju strategiju ranog pretretmana s inhibitorima P_2Y_{12} receptora u bolesnika sa NSTE-ACS.

10. CURRICULUM VITAE

Personal data:

Name and surname:	Bjørnar Marcelius
Date of birth:	October 25th 1989 in Levanger, Norway
Languages:	Norwegian (native), English (fluent)
Current residence:	Selbu, Norway
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Education:

2015 – 2021	University of Split, School of Medicine, Croatia
	Doctor of Medicine (M.D.)
2011 – 2014	Atlantis Medical University College, Norway
	Bachelor of Science in Nutrition (B.Sc.)

Work experience:

2021 – (*present*)	Lærdal Hospital, Department of Internal Medicine, Norway
	Medical student with licence
2014 – 2019	St. Olavs Hospital, Department of Psychiatry, Norway
	Nursing assistant

Projects:

2014 – 2014	Volunteer for the Visayans, Nutrition Project, Philippines
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