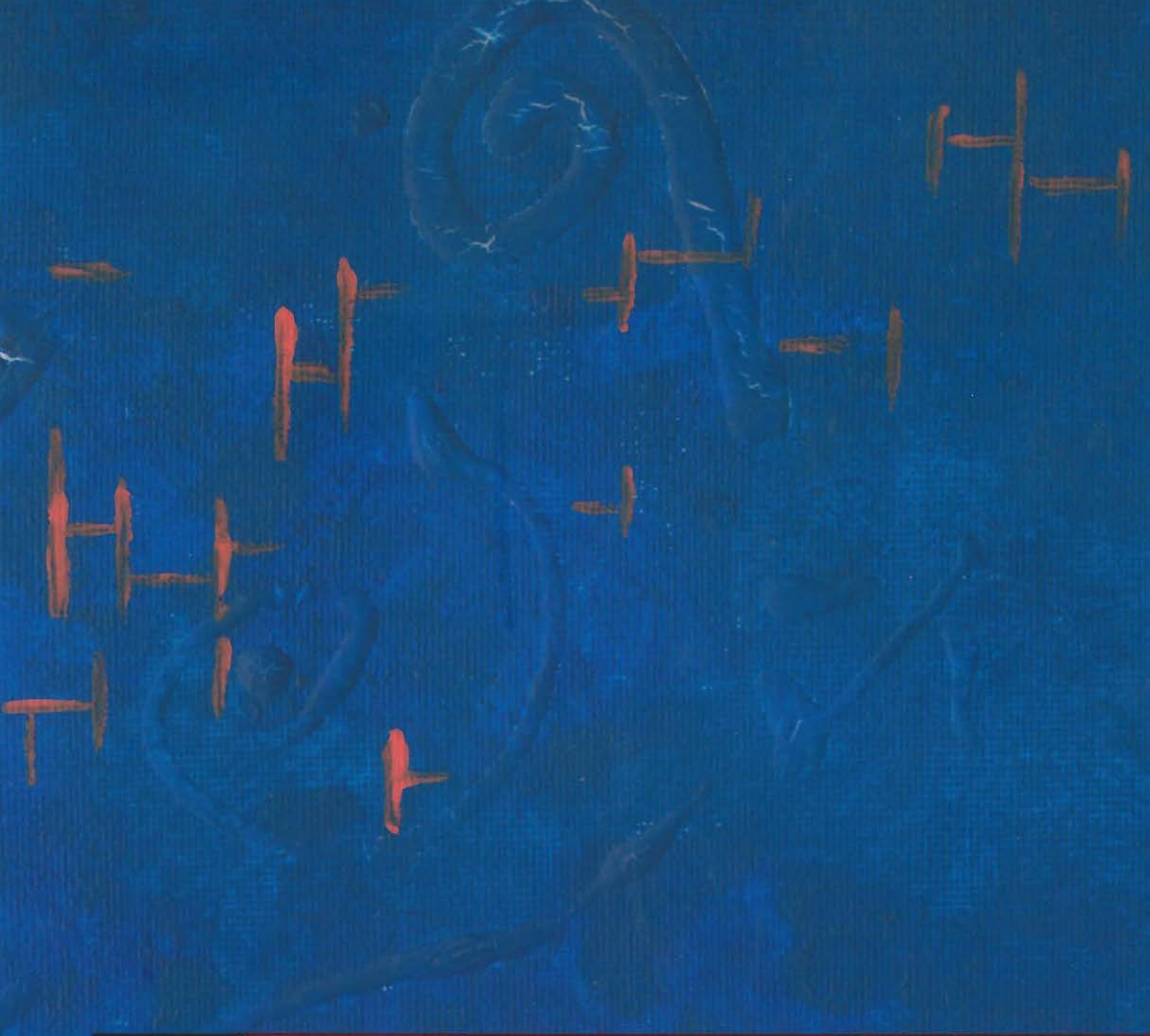

J.J.J. Smit

Platelets and White Blood Cells in Acute Coronary Syndromes

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Stellingen behorende bij het proefschrift

Platelets and White Blood Cells in Acute Coronary Syndromes

1. Trombotische complicaties komen na een Dotterbehandeling voor een hartinfarct vaker voor dan gerapporteerd wordt in gerandomiseerde onderzoeken (dit proefschrift).

2. Nieuwe testen van bloedplaatjesfunctie dienen plaats te vinden bij patiënten en niet bij gezonde vrijwilligers (dit proefschrift).

3. Bloedplaatjesfunctiemeting met behulp van ijzer is een reproduceerbare en gemakkelijk toepasbare manier om bloedplaatjesfunctie te meten bij patiënten die meerdere trombocytenaggregatieremmende middelen gebruiken ter behandeling van een hartinfarct (dit proefschrift).

4. Daling van het aantal witte bloedcellen is een krachtige voorspeller van geslaagde reperfusie en goede overleving bij patiënten met een hartinfarct (dit proefschrift).

5. De manier waarop witte bloedcellen en CRP prognose na een hartinfarct voorspellen, zijn verschillend van elkaar (dit proefschrift).

6. Witte bloedcellen zijn meer gerelateerd aan de neuro-humorale balans en zijn geen statische markers van inflammatie, in tegenstelling tot CRP (dit proefschrift).

7. Bij goed timemanagement kan een promotieonderzoek gedaan worden zonder dat gezin of klinisch werk er onder lijdt.

8. Als er maar genoeg geactiveerde bloedplaatjes in coronairarteriën zich ophopen, dan heb je een electrofysioloog nodig om de patiënt te helpen.

9. Het verkrijgen van het doctoraat moet je alleen nastreven als je wetenschapsbeoefening boeiend vindt.

10. Opleiding door Jan en Alleman, resulteert in goede en allround dokters.

11. In navolging van het postmodernisme, zal voldoende onderzoek naar bloedplaatjesfunctietesten vanzelf leiden tot implementatie van deze testen in de kliniek.

12. Kennis is weten wat men weet en weten wat men niet weet (Confucius).

13. Het mooiste wat we kunnen meemaken, is het raadselachtige: daar komt alle ware kunst, wetenschap en kennis uit voort (Albert Einstein).

Jaap Jan Smit, 23 april 2008

RIJKSUNIVERSITEIT GRONINGEN

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Voor Mei-Nga, Lisa en ?

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Chapter 1

General introduction

General introduction

Both platelets and leukocytes play an important role in the development of an acute coronary syndrome (ACS).[1-3] Platelet activation leading to a hyperactive state of the platelets and activated leukocytes by an enhanced inflammatory response are thought to form a crucial role in ACS.[4] Especially during primary percutaneous coronary intervention (PCI), stenting with stainless steel, a potent platelet activator, might further enhance thrombosis formation at the site of the already traumatized endothelium by plaque disrupture and PCI.5 Stent thrombosis is a major concern after primary PCI.[6-8] In contrast to restenosis, acute re-occlusion of the infarct related artery, especially after hospital discharge, causes acute reinfarction and is associated with substantial morbidity and mortality.[8-14] In patients with ACS, the relationship between the inflammatory state, level of platelet function and clinical outcome is, however, unclear. This thesis focuses on the prognostic implications of leukocytes and level of platelet aggregation in patients with ACS.

Platelet function and thrombus formation

Normal endothelium produces a number of platelet aggregation inhibitors.[15-17] After vessel wall damage by either a ruptured vulnerable atherosclerotic plaque or PCI, sub-endothelial components as collagen, adenosine diphosphate (ADP), thromboxane A2, serotonin, epinephrine and thrombin are exposed, causing platelet activation.[18-20] Adherence of platelets to the subendothelium and the subsequent activation cascade occurs by two different mechanisms: 1) shear stress induces von Willebrand factor (vWF) binding to the platelet GpIb-V-XI receptor with induction of intracellular signaling processes leading to integrin αIIbβ3 (GpIIb/IIIa) activation, 2) direct binding of platelets to subendothelial collagen by interaction with platelet receptors GpVI and integrin α2β1 (GpIa/IIa). This results in calcium mobilization and release of secondary agonists as ADP and thromboxane A2, with additional recruitment and activation of platelets.[21-23] Each secondary agonist binds to a specific platelet membrane receptor and signals via calcium mobilization the conformational

change of the GpIIb/IIIa receptor which becomes accessible for fibrinogen binding and consequent aggregation.

Platelet function tests

Several studies have correlated levels of platelet aggregation with clinical condition and outcome.[24-28] High platelet reactivity was found in patients who experienced stent thrombosis, whereas patients with clopidogrel resistance were at increased risk of recurrent atherothrombotic events.[24,25] After thrombolysis, higher platelet receptor occupancy was associated with better angiographic and electrocardiographic outcome.[26] Furthermore, in patients with ST elevation myocardial infarction (STEMI) undergoing primary PCI, higher levels of platelet aggregation inhibition by abciximab were associated with better myocardial reperfusion.[28] The introduction of novel anti-platelet strategies in atherosclerotic cardiovascular disease have also contributed to a heightened interest in monitoring the efficacy of anti-platelet therapy.[29-32] Since both inadequate platelet aggregation inhibition and bleeding complications are influenced by dose and type of anti-platelet therapy, platelet function measurement may possibly facilitate dosing and allow individual optimal aggregation inhibition.[33-36] Platelet function testing however, is not embedded into clinical practice, as no optimal, easy, reproducible and multipathway platelet aggregation test is available in clinical practice.

Light transmittance platelet aggregometry is generally considered to be the gold standard for determining platelet aggregation, but its relevance to in vivo platelet function is questionable, since aggregation is just one of the multiple aspects of platelet function.[37-39] Furthermore, the technical demands of the method make it difficult to use in daily practice.[37,38,40,41] Point-of-care assays may be useful for routine clinical use of platelet function testing and to identify patients who are at-risk of thrombotic events. Examples of such tests are adenosine diphosphate (ADP) induced platelet aggregation tests and the platelet function analyzer (PFA-100), an instrument providing a quantitative measurement of platelet adhesion and aggregation in whole blood flowing

through a small aperture under high shear stress conditions.[42-44] However, until now, an optimal method for platelet aggregation measurement has not yet been found to identify patients with increased levels of platelet aggregation at high risk for worse clinical outcome.

White blood cells

Inflammation plays an important role in atherosclerosis and the development of STEMI.[1-3] Elevated baseline white blood cell (WBC) counts in patients with STEMI have been associated with a poor prognosis. [1,45-47] In part, this can be explained by lower coronary patency rates in patients with elevated baseline WBC counts.[48] The relation between serial WBC count measurements and clinical outcome after acute myocardial infarction was recently published.[49] However, in that study, no angiographic parameters were investigated and the relationship with reperfusion success was not assessed. Furthermore, the population in this study was partly treated with primary PCI, and partly with thrombolysis. Therefore, the prognostic value of serial WBC count measurement in primary PCI patients remains to be investigated. Finally, it is unknown whether the WBC count in STEMI is related to other markers of inflammation.

The outline of this thesis

The main objective of this thesis is to investigate the prognostic value of platelet function and leukocytes in patients with an acute coronary syndrome.

Chapter 2 describes the incidence and predictors of subacute thrombosis after primary PCI for STEMI. In **Chapter 3** a study is presented investigating whether platelet aggregation inhibition by acetylsalicylic acid is comparable between patients with an acute coronary syndrome and healthy volunteers. Since the levels of platelet aggregation may correlate with underlying coronary disease, in **Chapter 4a** the prognostic value of the level of platelet aggregation

inhibition by GpIIb/IIIa blockers during STEMI is related to clinical outcome after primary PCI to assess whether less inhibition is associated with worse outcome. **Chapter 4b** is an overview of the role of the interindividual response to GpIIb/IIIa blockers and the role of platelet function testing in patients with an acute coronary syndrome. **Chapter 5** describes a novel method of platelet function measurement: Fe-Induced Platelet Aggregation (FIPA) measurement. In this chapter the feasibility of our new platelet function test and a comparison with existing platelet function tests is described. **Chapter 6** presents the relationship between signs of reperfusion after primary PCI for STEMI and serial WBC counts after PCI. **Chapter 7** addresses whether changes in leukocytes before and after primary PCI during STEMI is correlated to survival and reinfarction. Finally, **Chapter 8** is focused on the relationship between baseline WBC count and high-sensitive C-reactive protein and studies potential differences in prognostic value of both markers.

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Platelets

Chapter 2

Incidence and predictors of subacute thrombosis in patients undergoing primary angioplasty

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Abstract

Introduction

Subacute thrombosis (SAT) is a major concern in patients undergoing percutaneous coronary intervention (PCI). So far, only few data are available on characteristics and outcome of patients with SAT after primary PCI for ST elevation myocardial infarction (STEMI).

Methods

From 1997-2001, 1548 unselected consecutive patients underwent primary PCI for STEMI as part of a randomized controlled trial stenting vs. balloon angioplasty. All patients received acetylsalicylic acid (500 mg i.v.) and heparin (5.000 IU) before the procedure. After stenting, all patients received ticlopidine 250 mg daily (before July 1999) or clopidogrel 75 mg daily (after July 1999) for one month. Five percent of patients received glycoprotein IIb/IIIa blockers. We prospectively recorded incidence and characteristics of patients with SAT during one year follow-up.

Results

SAT occurred in 4.1% (63/1548) and reinfarction in 6.0% of patients. The incidence of SAT did not change over time (1997:8/175 (4.6%),1998:8/325 (2.5%),1999:13/358 (3.6%), 2000:22/426 (5.2%), 2001:12/264 (4.5%)). SAT occurred in 39/63 (62%) patients during hospital stay. The incidence did not differ between patients after ticlopidine 23/681 (3.4%) or clopidogrel 40/867 (4.6%, p=0.222). Univariate predictors of SAT were: patients with an LAD stenosis (5.4% vs. 2.9%, p=0.016), with Killip class>1 at presentation (8.6% vs. 3.7%, p=0.007) and in patients who received a stent (5.1% vs. 2.7%, p=0.022). After multivariate analysis, Killip class>1 on admission was the only independent predictor of SAT (OR 2.26, 95%CI 1.14-4.47, p=0.019). SAT was associated with a higher mortality at long-term follow-up (15% vs. 7%, p=0.026).

Conclusions

In a prospectively recorded, unselected consecutive series of patients undergoing PCI for STEMI, SAT occurred in 4.1% of patients at one-year follow-up. Signs of heart failure on admission, anterior myocardial infarction and stenting were predictors of SAT.

Key words: subacute thrombosis, catheterization, STEMI, prognosis, PCI

Introduction

In the treatment of patients with an acute myocardial infarction (AMI), primary percutaneous coronary intervention (PCI), anti-thrombotic agents and pre-hospital initiation of reperfusion therapy to reduce coronary occlusion time resulted in major improvement in patency of the infarct related vessel (IRV), reduction in infarct size, improvement in ejection fraction and decrease in morbidity and mortality.[1,2] One of the major remaining concerns in the treatment of AMI patients is the occurence of subacute thrombosis (SAT).[3-5] In contrast to restenosis, acute re-occlusion of the infarct artery, especially after hospital discharge, causes acute reinfarction and is associated with substantial morbidity and mortality.[5-11] Some predicting factors have been reported for elective PCI procedures as diabetes, age, long and complex lesions, post-intervention lumen dimensions, and procedure related abnormal lesion morphology as dissections and thrombus formation.[12-14] Until now, only a few studies, with substantial patient exclusion, have been published on the characteristics and clinical outcome of patients with SAT after primary PCI for AMI.[5] In these studies, it was not clear whether the reinfarction was caused by SAT from target vessel reocclusion. Better knowledge of the outcome and predictors of SAT after AMI might lead to case-specific adjustments in the anti-thrombotic regime. Therefore, we prospectively recorded complications, characteristics and incidence of angiographic proven SAT of an unselected series of patients presenting with an AMI who underwent primary PCI as part of a randomized controlled trial of stenting vs. balloon angioplasty at our institution.[15]

Methods

Patients

As part of the stenting vs. balloon randomized trial, from 1997 until 2001, all patients presenting with a ST elevation myocardial infarction (STEMI) within the first 6 hours, or between 6 and 24 hours if they had persistent symptoms and evidence of ongoing ischemia were enrolled for the study. Except inability

to give informed consent, no other exclusion criteria were applied. The study was approved by the Institutional Review Board. STEMI was defined as > 30 minutes chest pain together with more than 0.2 mV (anterior myocardial infarction) or 0.1 mV (non-anterior myocardial infarction) ST elevation in two contiguous ECG leads. Detailed method descriptions and outcome of randomization have previously been reported.[15] In brief, all patients presenting with a STEMI underwent immediate coronary angiography and primary PCI when possible. Coronary intervention was performed according to standard procedure.[16] All patients received 500 mg acetylsalicylic acid intravenously, 5000 IU of unfractionated heparin intravenously before PCI and continued with 100 mg oral acetylsalicylic acid afterwards. After stenting, all patients received ticlopidine 250 mg daily (before July 1999) or clopidogrel 75 mg daily (after July 1999) for one month. Glycoprotein (Gp) IIb/IIIa blockers were used at the discretion of the physician.

Angiographic core laboratory

All angiographic data were analyzed by an independent core-lab (Diagram Zwolle, the Netherlands) and scored by two observers who were unaware of randomization or outcome data. At coronary angiography, the initial injection was used to assess TIMI flow of the infarct related vessel (IRV). The IRV was selected based on electrocardiographic localization of ischemia and angiographic appearance of the lesion. Judgment of IRV flow was made on initial contrast injection according to the TIMI classification.[17] Successful angioplasty was defined as a less than 50% diameter stenosis and TIMI 3 flow of the IRV. Myocardial blush grade and distal embolization were defined as previously described.[18,19]

Study outcome

Recurrent myocardial infarction was recorded at one year follow-up as previously described.[20] SAT was defined as documented vessel occlusion at the site of the PCI during recurrent infarction at one year follow-up. Total death from all causes was also recorded.

Statistics

Statistical analysis was performed with the SPSS 12.0 statistical package. Continuous data were expressed as mean ± standard deviation, and categorical data as percentage, unless otherwise denoted. The analysis of variance and the chi-square test were appropriately used for continuous and categorical variables respectively. The difference in event rates between stenting and balloon angioplasty during the follow-up period were assessed by the Kaplan-Meier method using the log-rank test. A multiple logistic regression analysis on all clinical and angiographic parameters mentioned in the tables except from reinfarction, re-PCI and mortality was performed to identify independent predictors of SAT according to intention-to-treat analysis. Cross-overs (either from balloon to stent or from stent to balloon were added as an additional variable. The stepwise selection of variables and estimation of significant probabilities were computed by means of maximal likelihood ratio tests. The chi-square value was calculated from the log of the ratio of maximal partial likelihood functions. The additional value of each category of variables added sequentially was evaluated on the basis of the increases in the overall likelihood statistic ratio. A p-value of < 0.05 was considered statistically significant.

Results

Patients

During the 5-year study period, 1702 consecutive patients with STEMI were admitted to our hospital. Nineteen patients were excluded from the study because of death before randomization or inability to obtain informed consent. The remaining 1683 patients were randomized for the stenting/balloon angioplasty study. After coronary angiography, in 114 patients there was no indication for angioplasty and in 21 patients PCI failed. Therefore, a total of 1548 (785 in stent group and 763 in balloon group) actually underwent primary angioplasty and formed the study population of our study. In 357 patients cross-over to the other treatment arm was performed. Cross-over was observed in 109 (13.9%) patients randomized to stent and in 214 (28%) randomized to

balloon angioplasty. In 93 (6.0%) patients, a reinfarction was detected at one-year follow-up, in 87 patients repeat coronary angiography was performed and in 84 patients, a re-PCI was performed. The reasons for withholding repeat coronary angiography were: death before angiography (1), urgent CABG (1), post-CABG infarction (1) and non ST elevation myocardial infarction (3). Reasons for not performing re-PCI were: no significant angiographic lesion (2) and diffuse coronary sclerosis (1). The average duration of hospitalization was 4.57 ± 5.05 days. The average follow-up was 565 ± 409 days. There were no patients lost to follow-up. In 5% of patients GpIIb/IIIa blockers were used.

Study outcome

The incidence of SAT at 1 year follow-up was 4.1% (63 of 1548 patients). Patient characteristic and complications were presented in table 1. The angiographic characteristics of patients with SAT are shown in table 2. The incidence of SAT decreased rapidly after hospital discharge (figure 1). The incidence of SAT did not change over time (1997: 8/175 (4.6%), 1998: 8/325 (2.5%), 1999:13/358 (3.6%), 2000: 22/426 (5.2%), 2001: 12/264 (4.5%)). SAT occurred in 23/681 (3.4%) patients treated with ticlopidine therapy and in 40/867 (4.6%) patients treated with clopidogrel (p=0.222).

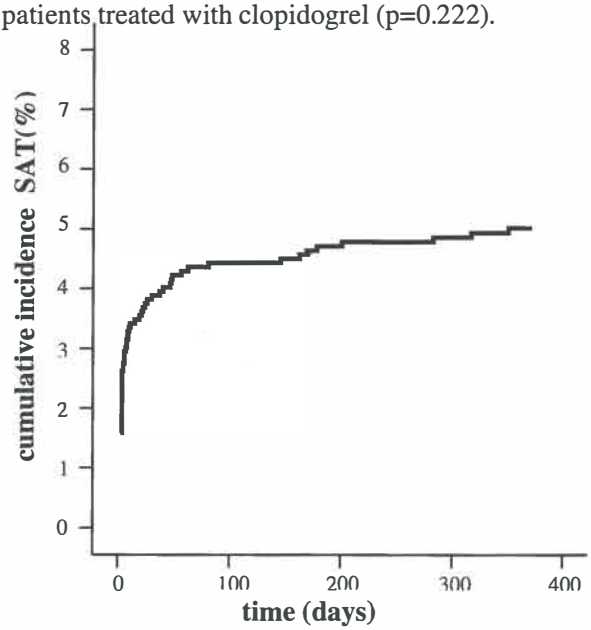

Figure 1. Cumulative incidence of SAT (%) during one year follow-up.

Univariate predictors of SAT were: patients with an LAD stenosis (5.4% vs. 2.9%, OR 1.88, 95% CI 1.12-3.15, p=0.016), an anterior myocardial infarction (5.2% vs. 3.0%, OR 1.75, 95% CI 1.04-2.94, p=0.032), with Killip class>1 at presentation (8.6% vs. 3.7%, OR 2.47, 95% CI 1.26-4.87, p=0.007) and in patients who received a stent (5.1% vs. 2.7%, OR 1.89, 95% CI 1.09-3.30, p=0.022). After multivariate analysis, including Killip class > 1, anterior myocardial infarction, randomization to stenting and cross-overs in the final model, Killip class>1 on admission was the only independent predictor of SAT (OR 2.26, 95%CI 1.14-4.47, p=0.019). In the multivariate analysis anterior myocardial infarction (OR 1.69, 95% CI 1.01-2.85, p=0.050) was a borderline significant predictor, and cross-overs (OR 1.51, 95% CI 0.84-2.72, p=0.168) and stenting (OR 1.58, 95% CI 0.93-2.68, p=0.089) were not independent predictors of SAT. SAT was associated with a higher mortality at one-year follow-up (15% vs. 7%, OR 2.35, 95% CI 1.16-4.73, p=0.026). The Kaplan Meier Curve in figure 2A shows enhanced SAT free survival in patients who were actually treated with stenting or balloon angioplasty. Figure 2B and 2C show the intention-to-treat analysis and subgroup analysis of the cross-over patients. As expected, cross-over was associated with an increased incidence of SAT.

Table 1. Patient characteristics

	SAT n=63 (4.1%)	no SAT n=1485 (95.9%)	p-value
age (mean ± SD)	61.1 ± 11.7	60.3 ± 11.6	0.61
women (n)	17/63 (27.0%)	336/1485 (22.6%)	0.42
diabetes mellitus (n)	3/63 (4.8%)	157/1485 (10.6%)	0.14
smoker (n)	31/63 (49.2%)	742/1485 (49.2%)	0.91
hypertension (n)	18/63 (28.6%)	407/1485 (27.4%)	0.84
hypercholesterolemia (n)	12/63 (19.0%)	311/1485 (20.9%)	0.72
prior myocardial infarction (n)	4/63 (6.3%)	159/1485 (10.7%)	0.27
prior angioplasty (n)	3/63 (4.8%)	66/1485 (4.4%)	0.76
prior CABG (n)	0/63 (0%)	31/1485 (2.1%)	0.64
prior stroke or TIA	2/63 (3.2%)	48/1485 (3.2%)	1.00
cumulative ST elevation > 15mm (n)	16/49 (32.7%)	242/1078 (22.4%)	0.10
LVEF < 30% (n)	7/54 (13.0%)	160/1225 (13.1%)	0.98
anterior infarction (n)	39/63 (61.9%)	715/1485 (48.1%)	0.032
Killip class > 1 on admission (n)	11/63 (17.5%)	117/1485 (7.9%)	0.010
medication after discharge for STEMI			
beta-blockers (n)	52/62 (83.9%)	1265/1462 (86.5%)	0.55
acetylsalicylic acid (n)	54/62 (87.1%)	1352/1461 (92.5%)	0.14
clopidogrel (n)	40/63 (63.5%)	827/1485 (55.7%)	0.22
ticlopidine (n)	23/63 (36.5%)	658/1485 (44.3%)	0.22
ACE-inhibitor (n)	40/62 (64.5%)	756/1462 (51.7%)	0.05
statin (n)	34/62 (54.8%)	855/1458 (58.6%)	0.55
spironolacton (n)	0/62 (0%)	14/1462 (1.0%)	1.00
coumarin (n)	9/62 (14.5%)	124/1462 (8.5%)	0.10
diuretic (n)	7/62 (11.3%)	104/1462 (7.1%)	0.21
calciumantagonists (n)	0/62 (0%)	55/1462 (7.1%)	0.21
nitrates (n)	16/62 (25.8%)	213/1462 (14.6%)	0.02
GpIIb/IIIa blockers (n)	4/63 (6.3%)	76/1485 (5.1%)	0.77
reinfarction (n)	63/63 (100%)	30/1485 (2.0%)	<0.001
re-PCI (n)	63/63 (100%)	21/1485 (1.4%)	<0.001
all-cause mortality at one year (n)	9/63 (14.8%)	104/1485 (7.0%)	0.026

CABG = coronary artery bypass grafting, LVEF = left ventricle nuclear ejection fraction

Table 2. Angiographic characteristics

	SAT n=63 (4.1%)	no SAT n=1485 (95.9.3%)	p value
balloon (n)	18/63 (28.5%)	640/1485 (43.1%)	0.022
stent (n)	45/63 (71.4%)	845/1485 (56.9%)	0.003
culprit vessel			
LAD (n)	39/63 (61.9%)	689/1485 (46.4%)	0.016
LCx (n)	7/63 (11.1%)	206/1485 (13.9%)	0.53
RCA (n)	16/63 (25.4%)	561/1485 (37.8%)	0.047
LM (n)	1/63 (1.6%)	8/1485 (1.6%)	0.31
graft (n)	0/63 (0%)	21/1485 (1.4%)	1.00
diseased vessels			
1	30/63 (47.6%)	698/1485 (47.0%)	0.92
2	23/63 (36.5%)	466/1485 (31.4%)	0.39
3	10/63 (15.9%)	315/1485 (23.0%)	0.31
preprocedure			
TIMI 3 flow	8/63 (12.7%)	217/1482 (14.6%)	0.67
minimal luminal diameter (mm, mean ± SD)	0.29 ± 0.44	0.30 ± 0.47	0.86
reference diameter (mm, mean ± SD)	2.76 ± 0.51	2.94 ± 0.59	0.17
stenosis (%,mean ± SD)	89.22 ± 15.9	89.50 ± 15.90	0.89
length of stenosis (mm, mean ± SD)	9.59 ± 3.33	10.02 ± 4.63	0.68
postprocedure			
TIMI 3 flow	58/63 (92.1%)	1298/1475 (88.0%)	0.33
TIMI < 3 flow	5/63 (7.9%)	177/1475 (12.0%)	0.33
MBG 3	26/62 (41.9%)	596/1471 (40.5%)	0.82
minimal luminal diameter (mm, mean ± SD)	2.24 ± 0.40	2.28 ± 0.55	0.57
reference diameter (mm, mean ± SD)	2.85 ± 0.46	2.98 ± 0.56	0.07
stenosis (%,mean ± SD)	20.95 ± 9.48	23.22 ± 13.26	0.18
length of stenosis (mm, mean ± SD)	11.68 ± 6.85	11.56 ± 6.68	0.88
successful PCI	58/63 (95.1%)	1287/1484 (86.7%)	0.22

LAD = left anterior descending coronary artery, LCx = left circumflex coronary artery, RCA = right coronary artery, LM = left main coronary artery, MBG = myocardial blush grade

Figure 2a

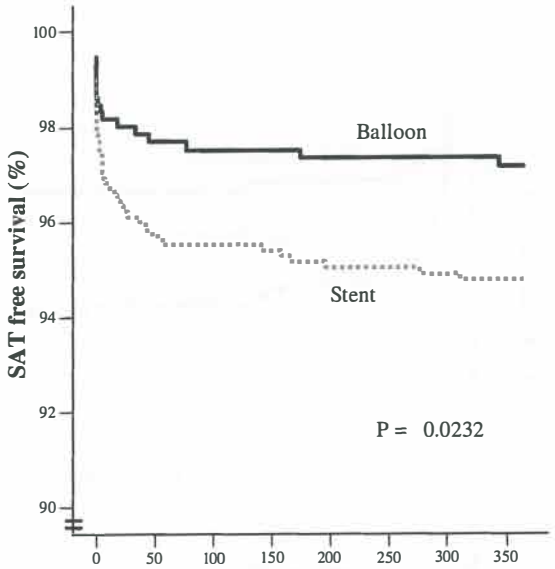

Figure 2b

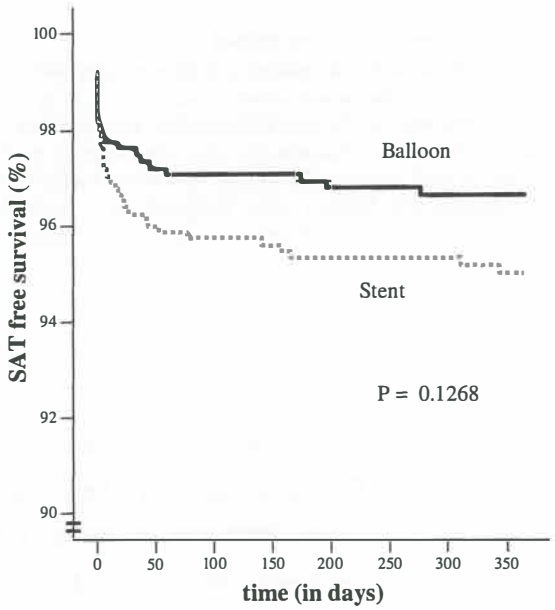

Figure 2c

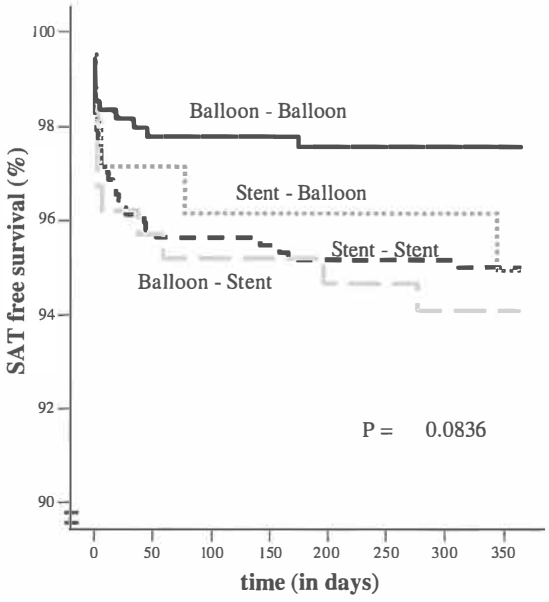

Figure 2. Kaplan Meier curve of SAT free survival.
Patients who are treated with balloon angioplasty compared to patients treated with stenting according to the actual-treatment analysis (A, p= 0.0232), according to the intention-to-treat analysis (B, p=0.1268), and divided into four subgroups, according to initial randomization allocation and final treatment (C, p=0.0836). Stent-stent = randomized to stent and actually treated with stent. Balloon-stent = randomized to stent but treated with balloon only. Stent-balloon = randomized to balloon but treated with stent. Balloon-balloon = randomized to balloon and actually treated with balloon.

Discussion

This is the first prospective study to assess the incidence and risk factors of angiographic proven subacute IRV re-occlusion in a large cohort of unselected STEMI patients undergoing primary PCI. The incidence of SAT was 4.1%. Sixty-two percent of SAT occurred during initial hospital stay. Late symptomatic infarct artery re-occlusion, after discharge until one-year follow-up occurred less often. Signs of heart failure on admission was an independent predictor of SAT. No differences over the course of the 5-year study period in the incidence of SAT were detected.

Only few studies, with substantial patient exclusion, have currently been published on the characteristics and outcome of patients with SAT or reinfarction after primary PCI for AMI. Kernis reported in a meta-analysis of AMI patients, excluding patients with cardiogenic shock and high bleeding risk, the following significant predictors of one-month reinfarction: Killip class > 1, LVEF < 50%, final coronary stenosis > 30%, post-PCI coronary dissection and post-PCI intracoronary thrombus.[5] Furthermore, it was not clear whether reinfarction was caused by symptomatic documented target vessel reocclusion.

In the PAMI studies, 2.1% of patients suffered reinfarction within one month after primary PCI for AMI.[5] In a meta-analysis, Keeley et al. reported 3% of patients after primary PCI for AMI experienced reinfarction at 6-18 months follow-up.[21] In several other randomized AMI thrombolytic trials, an incidence of in-hospital reinfarction of 2,5% to 4,7% was reported.[8,10,22,23] Therefore, our study seems to have a comparable incidence of angiographic proven SAT as compared with the incidence of reinfarction reported in these previous studies. However, our study shows that in 24 of 87 (28%) patients reinfarction was not caused by reocclusion of the IRV. Therefore, by just counting the incidence of reinfarction, the incidence of SAT might be overestimated.

In concordance with literature, SAT was associated with a higher mortality.[6,7,14] In contrast to restenosis, SAT causes sudden onset of symptoms and signs of recurrent AMI, which is associated with substantial morbidity and mortality, especially in the out-of-hospital setting.[5-11] An important independent predictor of subacute occlusion after PCI for STEMI was Killip class > 1 on admission. In contrast to the study of Kernis and Cheneau, in which postprocedure lumen dimensions and lesion morphology were found to predict reinfarction, no angiographic predictors of SAT, except from LAD stenosis could be determined in our study, although there was a trend for increased incidence of SAT in smaller IRV size.[5,14] Our study complements and expands on previous studies, which also reported signs of heart failure (Killip class > 1) and LVEF<30%, as predictors of reinfarction.[5] Killip class > 1 and LVEF<30% are common after large myocardial infarctions.[24] The question remains whether

incomplete platelet aggregation inhibition in these patients might have contributed to early IRV re-thrombosis. Glycoprotein IIb/IIIa blockers were used in only 5% of patients.

Furthermore, stenting of the IRV was also associated with an increased incidence of SAT in contrast to previous studies.[25] However, most stent studies were performed in highly selected patient groups.[26-30] This study randomized patients before angiography and therefore only few patients were excluded. No informed consent was the only exclusion criterium in this study. As SAT is relatively rare, large numbers of patients are required to find a significant difference between stented and non-stented patients. All previous studies were too small to individually detect such a difference. Since stenting is often used as bail-out for dissections caused by balloon angioplasty, increased platelet activation and thrombus formation at the site of the vessel injury seems to be a reasonable explanation for the increased re-occlusion after stenting. Additionally, in the setting of hyperactive platelets in an acute myocardial infarction, stenting with stainless steel, a potent platelet activator, might further enhance thrombosis formation at the site of the already traumatized endothelium by plaque disrupture and PCI procedure.[31] The relation between SAT and stents has also been extensively described.[3,26-30] Only few clinical studies have been performed to define the optimal level of platelet aggregation to prevent SAT and reinfarction in patients suffering an acute myocardial infarction.[32] Prevention of SAT by selecting high risk patients groups for more extensive platelet aggregation inhibition might be subject to further studies in the future. Furthermore, although SAT after hospital discharge was infrequent, prolonged clopidogrel therapy up to or beyond one year might be suggested for the prevention of late SAT.

Study limitations

GpIIb/IIIa blockers were used in only 5% of patients, mostly as bail-out treatment. However, in daily practice the number of patients using GpIIb/IIIa

blockers is also limited, as is shown in a large registry study in another unselected patient cohort.[33] However, our study clearly suggests that GpIIb/IIIa blockers, which have shown to reduce the incidence of SAT, are highly indicated especially in patients with an anterior myocardial infarction or who present with heart failure. Our post-stenting antiplatelet regimes have been modified during the study period, when it became clear that clopidogrel has a similar effect as ticlopidine.[34-36] Furthermore, this study was performed before drug-eluting stents were widely introduced, which might change the number of SAT, especially after discharge.[37-39] However, since not all patients are treated with drug-eluting stents, our study highlights the importance of treatment with optimal platelet inhibitors in STEMI patients at high risk for developing SAT. Finally, SAT may occur silent in large myocardial infarctions. Therefore, clinical assessment of subacute thrombosis may underestimate the true incidence of SAT.

Conclusion

In this prospectively recorded, unselected consecutive series of patients undergoing PCI for STEMI, SAT occurred in 4.1% of patients at one-year follow-up. In 38% of patients, SAT occurred after hospital discharge. Signs of heart failure on admission was an independent predictor of SAT. Case-specific adjustments in the peri-PCI anti-platelet regime in STEMI patients on the basis of risk profile for SAT might further decrease the incidence of SAT after PCI.

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Chapter 3

Impaired platelet inhibitory effect of a single dose acetylsalicylic acid in patients with unstable coronary artery syndrome in comparison with healthy volunteers

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Abstract

Introduction

Controversial reports have been published about the efficiency of potent platelet inhibitors in patients with coronary artery syndrome (CAS). Therefore, we questioned whether a functional change of platelets affects the patients response to acetylsalicylic acid (ASA).

Methods

Nineteen consecutive patients presenting with unstable coronary syndrome and fifteen healthy volunteers were included. No platelet inhibitory drugs or coumarine were used in both groups before the study. Platelet aggregation tests were performed on baseline samples and after a single dose of ASA. Afterwards, all patients underwent coronary angiography to exclude non-CAS.

Results

In the patient group (n=15 after exclusion) no significant increase in bleeding constant, using a platelet function analyzer, after ASA was found in contrast to the control group. The maximal velocity and the maximal percentage optical platelet aggregation using ADP was significantly more reduced in the control group. ASA did not significantly reduce the thromboxane-B_2 production in the patient group.

Conclusions

ASA has less platelet inhibitory effects in patients with unstable CAS in comparison with healthy volunteers. Platelets, in the hyperactive state of unstable CAS, prove to be less subject to inhibition. This might add to the explanation of the lack of efficiency of platelet inhibitory drugs to prevent thrombotic

complications after percutaneous coronary intervention and platelet aggregation onto stent surfaces in patients with acute CAS.

Key words: unstable coronary artery disease, ASA, healthy volunteers, acetylsalicylic acid, platelet inhibition, aggregation

Introduction

Acetylsalicylic acid (ASA) is one of the corner stones in modern strategy to stabilize coronary artery syndrome. Platelet activation and aggregation plays an important role in the mechanism of (un)stable coronary artery syndrome.[1] Inhibition of platelet aggregation and thrombus formation is thought to be the most important mechanism of action of ASA. It exerts its primary antithrombotic effects through the irreversibly inhibition of type one cyclooxygenase (COX), thereby reducing thromboxane A_2 production and amplifying the platelet aggregatory response to a variety of stimuli including collagen, thrombin, adenosine-5'-diphosphate (ADP) and epinephrine.[2-5] However, ASA has proved to be a weak platelet inhibitor, affecting only the cyclooxygenase pathway, and is known to have a specific group of non-responders.[6-11] Furthermore, conflicting clinical outcome have been published about the use of in vitro potent platelet inhibitors in patients with coronary artery syndrome.[9,12-14] These controversies contributed to the increasing use of combination of inhibitors against the ADP-mediated pathway of aggregation and GpIIb/IIIa receptor antagonists, particularly after stenting. Therefore, we questioned whether a functional change of platelets in patients, related to their disease, affects their response to ASA. In this study, we compared the effect of a single low-dose ASA on platelet function of patients with unstable coronary artery syndrome and healthy volunteers.

Methods

Patients

After informed consent, 19 consecutive patients presenting with unstable coronary syndrome were included into this study. Unstable coronary artery disease was described as at least 30 minutes angina pectoris at rest and new ST depression on the electrocardiogram (> 0.1mm) and/or positive Troponine-T at three or six hours after start of the discomfort. Furthermore 15 healthy volunteers, without a history of chest pain or suspicion of coronary artery syndrome were included. Exclusion criteria were use of ASA or other platelet

aggregation inhibitors in the former two months, coumarine derivates, known clotting deficiencies, renal function disorders, ST elevation as well as pre-menopausal women. In our hospital subsequent percutaneous coronary intervention, following coronary angiography, is performed in most patients presenting with signs and symptoms of unstable coronary artery syndrome. Patients not receiving coronary angiography or patients without significant (defined as decrease in luminal diameter over 70%) decrease in vessel luminal diameter were as yet excluded from the study. Above a starting dose of 100 mg non-enteric coated oral ASA, the patients did not receive any other platelet inhibitors or coumarine derivates during the study. After the study all patients received standard treatment with, among others, a loading dose of intravenous ASA. Furthermore Creatine Kinase (CK, U/L) and MB levels (U/L) were measured to evaluate myocardial infarction (CK-MB >24 U/L or >6% of CK levels, if CK >200 U/L).

Blood sampling

Before and 75-105 minutes after oral intake of 100 mg ASA, a venous sample of 25 mL blood was withdrawn after short congestion using a 20 Gauche needle and a syringe for minimal platelet activation. Immediately thereafter the blood was poured partly into 0.32% sodium citrate and partly into indomethacin-citrate medium (0.05 mg/mL – 0.32%). Indomethacin-citrate blood was centrifuged (10 minutes, 1100 x g) to obtain platelet poor plasma (PPP).

Platelet function tested by PFA-100

The platelet function was measured using a platelet function analyzer (PFA-100, Dade Behring, Marburg GmbH,Germany), a newly developed instrument that provides a quantitative measurement of platelet adhesion and aggregation in whole blood flowing through a small aperture under high shear conditions. The aperture (147 μm) was coated with 2 μg type I collagen and 50 μg epinephrine bitartrate. The closure time of the aperture, referred in the text as bleeding constant, is an indicator of platelet function. As a control, a cartridge

coated with 2 µg type I collagen and 50 µg ADP to measure platelet dysfunction not caused by ASA, was used in each sample.

Optical platelet aggregation using ADP

To investigate the platelet aggregation we used final concentrations of 2.0 µmol/L and 5.0 µmol/L ADP (Sigma, St. Louis, Mo, USA) as an aggregation stimulus. Therefore whole blood was used to prepare platelet rich plasma (PRP) by centrifugation at room temperature at 95 g for 10 minutes and platelet poor plasma (PPP) by centrifugation at room temperature at 1100 g for 10 minutes. The platelet count in the PRP was kept between 300 and 400 x 10^9 platelets per liter by dilution with PPP. The recording of the aggregability was derived from light transmission measurements (Chronolog, Havertown, MI, USA). For each sample the maximal aggregation of 90% was determined as the amount of light transmitted through the sample of background plasma without platelets and base aggregation of 10% was determined as the amount of light transmitted through the platelet suspension. The maximum second wave aggregation percentage of PRP and the maximum slope per minute were used as parameters for this study.

Thromboxane B_2

PPP obtained from the indomethacin collected blood was used for biochemical assay of thromboxane B_2 (EIA, Cayman Chemical Company, Ann Arbor, Michigan, USA).

Statistics

Before data analysis all non-categorical data were tested and found normal distributed according to the Kolmogorov-Smirnov goodness-of-fit test. An unpaired one-tailed student's t-test was used to compare the difference in bleeding constant, ADP aggregation parameters and thromboxane concentrations between the patient and control group. An unpaired two-tailed student's t-test was used to test the difference between the non-categorical patient characteristics. Chi-square tests were used to investigate differences between categorical data. A p-value of < 0.05 was considered statistically

significant. All hematological, and biochemical data are expressed as mean and standard error of the mean, unless otherwise indicated.

Results

Four of the included patients were excluded from the study. In one patient no significant coronary artery syndrome was established during coronary angiography and in another patient no coronary angiography was performed. One excluded patient was given Aspegic in high dose before the second blood sample was taken. The fourth patient was excluded due to failure of laboratory testing within one hour after blood sampling. The patient characteristics did not differ significantly in both groups (Table 1). In four patients non ST elevation myocardial infarction was discovered (maximal CK-MB 404, 415, 32, 47 U/L after 8, 12, 12, 7 hours).

Table 1. Characteristics of patients and controls (mean ± standard deviation). No significant differences were observed between the two groups

variable	patient (n=15)	control (n=15)
age (yrs)	53 ± 9	61 ± 15
women	0.46	0.13
diabetes mellitus	0	0
cigarette use	0.26	0.40
history of hypertension	0.26	0.20
hypercholesterolaemia	0.13	0.13
familial hypercholesterolaemia	0	0
positive family history of CAD*	0.53	0.20
platelet count (K x 10^3 / μL)	387 ± 123	450 ± 116
number of diseased coronary arteries†	2.2 ± 1.1	not evaluated

Myocardial infarction or unstable angina within the age of 60 in first degree family members; CAD = coronary artery disease, † Number of coronary arteries with decrease in luminal diameter of ≥ 70%

Platelet function tested by PFA-100

The bleeding constant after administration of ASA using the collagen-epinephrine cartridges was not significantly enhanced in the patient group as compared to the control group (Figure 1). The increase in bleeding constant

was significantly higher in the control group in comparison with the patient group (Figure 2). On ADP cartridges, the bleeding constant decreased significantly in all samples after ASA intake in contrast to the samples before ASA (Figure 3).

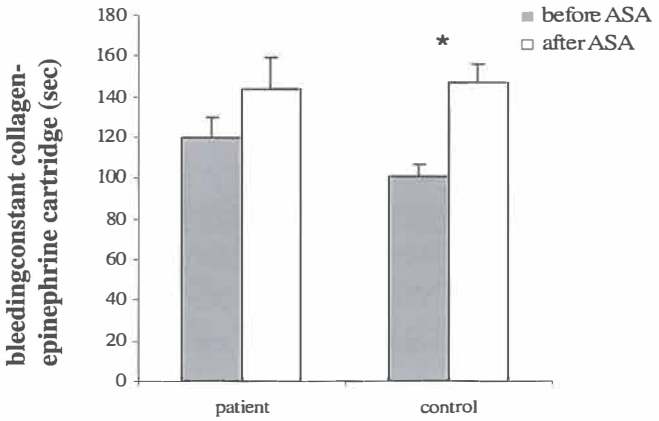

Figure 1. Bleeding constant after ASA administration was not significantly enhanced in patients as compared to controls (p-value: 0.101 vs. 0.001, indicated by asterix).

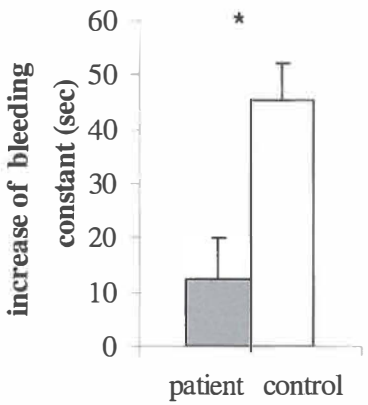

Figure 2. Increase of bleeding constant after ASA administration was significantly enhanced in the control group as compared to the patient group (p-value: 0.001, indicated by asterix).

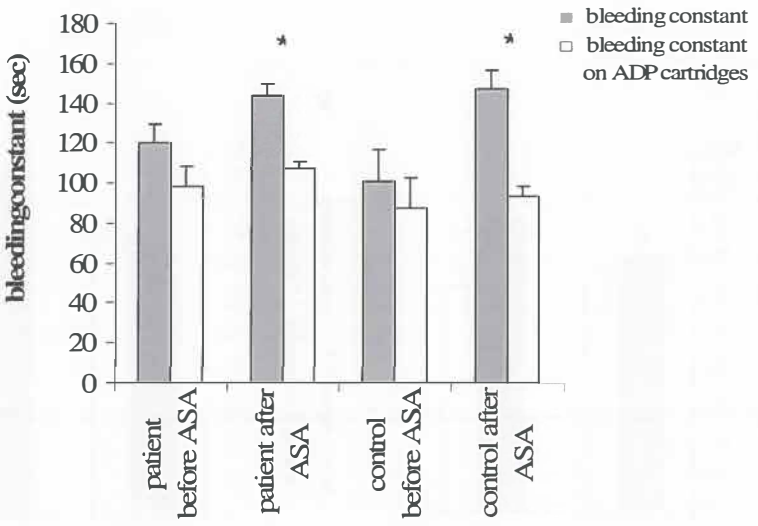

Figure 3. Bleeding constant on ADP cartridges decreased significantly (indicated by asterix) in all samples after ASA administration (p-value patients 0.02, controls 0.00002) in contrast to the samples before ASA administration (p-value patients 0.06, controls 0.05).

Optical platelet aggregation using ADP

The decrease of the maximal aggregation and maximal slope of aggregation using 2.0 μmol/L and 5.0 μmol/L ADP in control group was clearly noted (p-value for all groups significant, Figure 4). The patient group, however, did not show a significant decrease in the aggregation. The differences of decrease in aggregation between patients and controls were significant for all parameters. Furthermore, the ADP aggregation in the pre-ASA sample in both concentrations was significantly less pronounced in the patient group than in the control group. The maximal ADP aggregation using 5.0 μmol/L ADP resulted in maximal outcome in both groups before and after ASA, and was therefore not a sensitive marker.

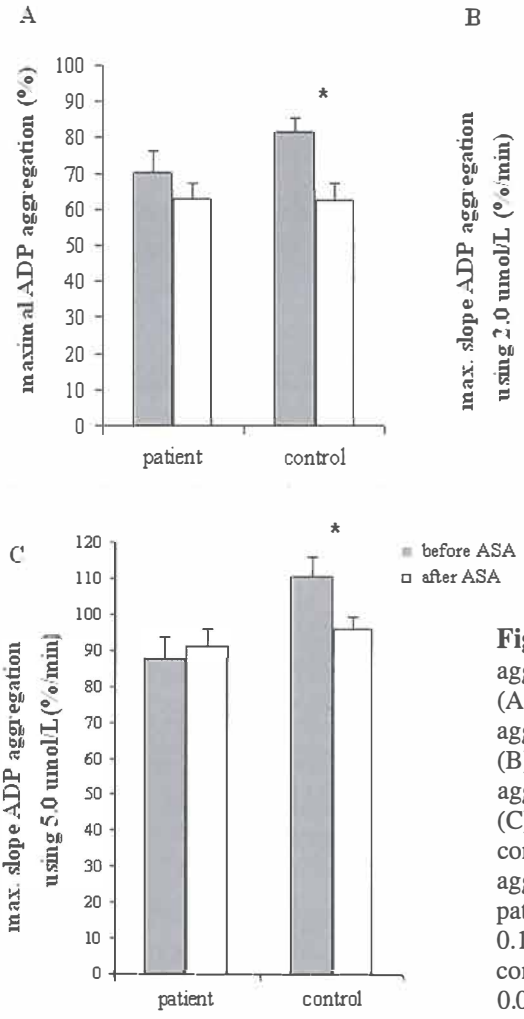

Figure 4. The maximal platelet aggregation using 2.0 µmol/L ADP (A), the maximal slope platelet aggregation using 2.0 µmol/L ADP (B) and the maximal slope platelet aggregation using 5.0 µmol/L ADP (C) were shown in patients and in controls. ASA did not reduce platelet aggregation in test A,B and C in the patient group (p-value respectively: 0.17,0.39,0.34) in contrast to the control group (p-value respectively: 0.0019, 0.001, 0.017, indicated by asterix). The differences in decrease of platelet aggregation after ASA between patients and controls were all significant (p-value respectively: 0.016,0.006,0.001). The ADP aggregation in the pre-ASA sample was significantly less pronounced in the patient group as compared with the control group (p-value A,B,C respectively 0.07,0.008,0.005).

Thromboxane B_2

The thromboxane levels in patients with unstable coronary artery syndrome were reduced after ASA, but not statistically significant (Figure 5). In healthy volunteers, the only moderately increased thromboxane levels before ASA resulted in a significant decrease until minimal levels after ASA (p-value 0.04). The thromboxane levels after ASA in the patient group were comparable to the thromboxane levels before ASA in the control group.

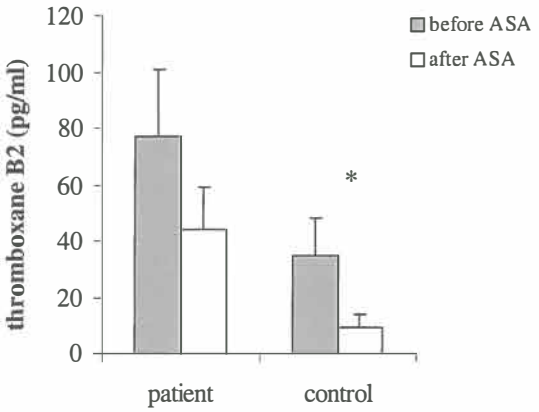

Figure 5. Thromboxane B_2 levels were not significantly reduced in the patient group as compared to the control group (p-value 0.12 vs. 0.04, indicated by asterix).

Discussion

ASA is one of the most common used platelet aggregation inhibitors in modern cardiology.[15] However, a comparison between patients with unstable coronary artery syndrome and healthy volunteers regarding the anti-platelet effect of ASA has never been made. In patients with stable angina, thromboxane B_2 production after atrial pacing-induced ischaemia was completely inhibited by a low dose ASA.[16] Our study showed that in patients with unstable coronary artery syndrome ASA has less platelet inhibitory effects as compared with healthy volunteers. Although only a small study population was investigated, most parameters used exhibited a significant difference in the anti-platelet effect between patients and healthy volunteers. The results indicate that platelets in the hyperactive state of unstable coronary artery disease are

significantly less aggregable by ADP (the baseline sample). ASA did not affect the platelet aggregation response to ADP at all, in contrast to the group of healthy volunteers. The calculation of the difference between before and after a dose ASA, therefore, appeared to be a sensitive model for measurement of the platelet inhibitory effect between the two groups.

In our study, we chose a low-dose aspirin to investigate the difference between patients and healthy volunteers. We tested platelet inhibition markers at baseline and after 75-105 minutes. Since ASA is rapidly absorbed in the upper gastrointestinal tract, maximal inhibition of platelet function has been reported within 60 minutes.[17] For detection of platelet inhibition by ASA we used three different platelet function tests. We measured the bleeding constant using the PFA-100 to determine the platelet aggregation by collagen and epinephrine stimulation under high shear stress conditions. Although recently developed, the PFA has proved to be a sensitive marker of platelet function, especially the ASA-induced inhibition of platelet aggregation.[11,19-20] Using the ADP cartridge we showed the absence of ASA before the oral administration and we proved the measurability of ASA. Secondly the classical optical platelet aggregation using low dose of ADP was used to test the ADP dependent mechanism of platelet aggregation, which is only partly inhibited by ASA.[21] The only parameter which did not show a significant reduced anti-platelet effect of ASA was the measurement of the maximal ADP aggregation using 5.0 µmol/L ADP. This was probably due to excess of stimulation, resulting in a near-maximal aggregation of platelets in all groups. Thirdly, thromboxane B_2, the active metabolite of thromboxane A_2 was measured, indicating the most direct ASA-induced platelet inhibition. In previous studies a correlation between thromboxane production and hyperactive platelets in unstable coronary artery disease was found, but appeared to be too weak to function as a diagnostic biochemical marker of unstable coronary artery syndrome.[1,6,22-24] In our study the thromboxane production in patients was, according to the literature, high in the pre-ASA sample. After ASA intake the thromboxane production was not significantly reduced, and still above the pre-ASA sample in the control group,

reflecting the hyperactive state of platelets during unstable coronary artery disease.

Coronary artery thrombosis superimposed on a disrupted atherosclerotic plaque has emerged as the pivotal pathophysiologic event in unstable coronary syndromes. A cascade of platelet activation leading to a hyperactive state of the platelets, in which many activation receptors are expressed on their cell membranes, is thought to form a crucial role in this process.[1] Recently, a series of bioactive prostaglandin (PG)F_2-like compounds have been discovered that are produced from arachidonic acid by lipid peroxidation, catalyzed by oxygen free radicals.[25] One of these products (8-iso-$PGF_2\alpha$) is known to contribute to platelet activation.[26,27] Increased oxidant stress in unstable coronary syndrome is thought to increase the 8-iso-$PGF_2\alpha$ and might therefore enhance and continue platelet aggregation.[27,28] Other stimuli such as catecholamines (providing a link between emotional stress and coronary artery syndrome), shear-stress, synergistically working with epinephrine, and thrombin also have been proposed as thrombotic agents able to overcome the inhibitory effects of ASA.[29-33] Combination of elevated cathecholamines and a rough stenosed lumen causing turbulent flow with increased shear stress have been suggested to result in aspirin insensitive platelets.[34] In addition, the ruptured atherosclerotic plaque leading to unstable angina also exposes materials that may provoke thrombosis by mechanisms that ASA does not block, such as tissue factor.[35] Furthermore, the mentioned prostaglandines (especially 8-iso-$PGF_2\alpha$) were thought to stimulate an extraplatelet pathway of thromboxane production and therefore provide a biochemical link between the increased oxidant stress in unstable coronary artery syndrome and an aspirin insensitive thromboxane biosynthesis found in unstable coronary artery patients.[28,36] Due to the resulting prolonged activation platelets might already have formed too many activation receptors on their cell membranes for ASA to conduct sufficient platelet inhibition. This has been reported in small series of patients, in which despite antiplatelet therapy, significant platelet activation, detected as PAC-1 expression, has been reported in patients with unstable coronary artery disease.[37,38] Finally, COX-2, instead of COX-1 was proposed to contribute to

the aspirin resistance in patients with coronary artery disease.[39,40] COX-2, found in vascular endothelium, smooth muscle cells, and in varying amounts in platelets, provides an alternate pathway for prostaglandin H_2 and finally thromboxane A_2 production in platelets.[39] Since ASA is a 170 times less potent COX-2 than COX-1 inhibitor, the variation in COX-2 expression might explain variations in platelet thromboxane production and aggregation response after ASA. All these factors might add to an explanation for the relative insensitivity of platelets in patients with unstable coronary artery syndrome as compared to healthy volunteers found in our study.

Although the patients in the investigated patient group were thought to have truly unstable form of coronary artery syndrome, a certain patient selection could not be excluded. All included patients were presenting for the first time with chest discomfort in a hospital, thereby not representing the entire group of unstable coronary artery syndrome of a cardiologist's practice. This should be marked as a limitation of our study.

Conclusions

Since platelets importantly contribute to the pathogenesis of arteriosclerosis and to the restenosis after vascular transplant surgery, studies to platelet response may help to understand the proper treatment of arteriosclerosis.[41] In this study we found a remarkable difference in the extent of platelet inhibition of a common used platelet inhibitor between patients with unstable coronary artery syndrome and healthy volunteers. We therefore conclude, that the diminished platelet inhibitory response to ASA might be related to a functional change of platelets in patients with an unstable coronary artery syndrome. Further questions about differences in platelet inhibition between patients with stable angina or myocardial infarction will be subject of further research. An important additional implication of our study is that data regarding testing of anti-platelet drugs on healthy volunteers should be interpreted with great caution.

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Platelet micro-aggregation inhibition in patients with an acute myocardial infarction pretreated with tirofiban and relationship with angiographic and clinical outcome

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Abstract

Introduction

The relationship between the level of platelet aggregation inhibition in patients with an acute myocardial infarction (AMI) and their clinical outcome is unknown.

Methods

In patients with an AMI included in the On-TIME trial and transferred to the primary percutaneous coronary intervention center (PCI) of Zwolle, who were pre-treated with Tirofiban on top of acetylsalicylic acid and heparin, platelet micro-aggregation inhibition was assessed on admission and immediately after PCI, using the Sysmex K4500 platelet micro-aggregation measurement. The level of platelet micro-aggregation inhibition was compared to angiographic and clinical outcome. Patients were randomized to early, pre-hospital initiation of Tirofiban, or to initiation in the catheterization laboratory. Therefore, the effect of Tirofiban on platelet micro-aggregation inhibition could additionally be determined by measuring baseline platelet micro-aggregation also at entrance into the hospital.

Results

In 412 of 463 (89%) patients platelet micro-aggregation inhibition was measured after receiving Tirofiban. There was no difference between the four quartiles of the level of platelet micro-aggregation inhibition with regard to distal embolization, TIMI-3 flow and blush grade 3 post-PCI, mean corrected TIMI frame count, ejection fraction, enzymatic infarct size and percentage ST-segment resolution (p-value: 0.91, 0.97, 0.46, 0.94, 0.73, 0.33, 0.72 resp.). The baseline platelet micro-aggregation inhibition in patients treated with Tirofiban was 38% ± 25% (mean ± SD), and in the patients treated with placebo 14% ± 22% ($p < 0.001$).

Conclusions

We found no correlation between the level of platelet micro-aggregation inhibition after Tirofiban and outcome. Whereas, only modest increase in platelet micro-aggregation inhibition was observed after a commonly used dose of Tirofiban.

Introduction

The relationship between the level of platelet aggregation inhibition and clinical outcome in patients undergoing primary percutaneous coronary intervention (PCI) for an acute myocardial infarction (AMI) is still unclear.[1] In initial dose-finding trials in humans, a regimen necessary to obtain > 80% receptor blockade or < 20% of baseline ADP-induced platelet aggregation was strived for.[2-4] The extent of platelet aggregation inhibition varies between the different types and dosages of anti-platelet agents.[5-8] In particular, patients with an acute coronary artery syndrome may, related to their disease, not reach the same level of platelet aggregation inhibition in response to platelet inhibitors as healthy volunteers.[9] In patients with an AMI, the relation between level of platelet aggregation inhibition and clinical outcome after glycoprotein (Gp) IIb/IIIa blockers has never been investigated. Understanding this relationship may lead to a more tailored treatment of patients with an AMI. Patients with more activated platelets and reduced response to GpIIb/IIIa blockers may have worse clinical outcome. Therefore, in this substudy of the On-TIME trial, we compared the level of platelet aggregation inhibition after a commonly used dose of Tirofiban with angiographic and electrocardiographic outcome, infarct size and left ventricular (LV) function in patients with an AMI. In the On-TIME trial, patients were randomized into early pre-hospital initiation of Tirofiban or to initiation in the catheterization laboratory.[10] Therefore, the effect of Tirofiban on platelet aggregation inhibition could additionally be determined by measuring baseline platelet aggregation at entrance into the hospital.

Methods

Patients

For precise methods description of our study we refer to the main finding of the On-TIME trial.[10] In brief, in this prospective, randomized, double-blinded study, patients were included if there was more than 30 minutes of chest pain with more than 0.2 mV (anterior myocardial infarction, MI) or 0.1 mV (non-

anterior MI) of ST elevation in 2 contiguous ECG leads and the ability to perform primary angioplasty within 6 hours after the start of symptoms. Patients over 80 years of age, women less than 50 years of age, patients who were treated with thrombolytic therapy in the previous 24 hours, patients on warfarin or acenocoumarol within the last 7 days and patients with a contraindication to GpIIb/IIIa blockade were excluded. Patients with severe heart failure or cardiogenic shock (Killip class III or IV) and patients on hemodialysis were also excluded. The protocol was approved by our institution's Review Board and Ethical Committee. Before transportation, oral informed consent was obtained in all patients by either a physician or a specialized ambulance nurse. The day after the angioplasty procedure, written informed consent was obtained. After receiving a bolus of 5.000 IU of unfractionated heparin and 250mg of acetylsalicylic acid intravenously, patients were randomized to an intravenous bolus of Tirofiban (Merck & Co., Inc., Whitehouse Station, New Jersey, USA, 10μg/kg) followed by a maintenance infusion (0.15 μg/kg/min) or infusion with placebo. Emergency transportation was performed after pre-information of arrival of the patient at the catheterization laboratory. At coronary angiography, the initial injection was used to assess TIMI flow of the infarct related vessel (IRV). After coronary angiography, but before angioplasty, all patients received a second bolus of study drug intravenously (Tirofiban in case of initial treatment with placebo and placebo in case of initial treatment with Tirofiban). After the second study drug, all patients were treated with open label Tirofiban (maintenance infusion, 0.15 μg/kg/min) for 24 hours. Post PCI, all patients were treated with Clopidogrel (300 mg loading dose followed by 75 mg daily for a month), acetylsalicylic acid, beta blockade, statin therapy and ACE inhibition.

Platelet micro-aggregation inhibition

For measurement of the platelet micro-aggregation inhibition, we used the recently described Sysmex K 4500 method.[11] Blood samples for assessment of platelet micro-aggregation inhibition were taken at three different time points: (1) at hospital entrance, after having received the first dose of study medication

in the ambulance, (2) after PCI (after receiving all anti-platelet drugs), and (3) 3 hours after PCI. For platelet micro-aggregation inhibition measurement, blood samples were collected in plastic tubes containing EDTA and tubes containing PPACK with 20 μM/L adenosine diphosphate (ADP, Plateworks, Helena Laboratories, Beaumont, Texas). A routine platelet count was performed on each sample using a routine blood cell counter (Sysmex K4500, Sysmex Corp., Kobe Japan). The platelet count in the EDTA tube was used as a reference. In the presence of the agonist ADP, platelets aggregate and associate. As the aggregated platelets exceed the threshold limitations for platelet size, they are no longer counted as individual platelets. The ratio between the non-aggregated platelets in the agonist sample and the platelet count in the reference tube was calculated as the platelet micro-aggregation inhibition. In our laboratory, we showed a correlation coefficient of 0.90 between the Sysmex K4500 and the ICHOR point-of-care platelet analyzer (Helena Laboratories, Beaumont, TX) to validate the Sysmex K4500 platelet micro-aggregation measurement.[12,13]

Angiographic and electrocardiographic outcome, infarct size and LV function

All angiographic data were analyzed by an independent core-lab (Diagram Zwolle, the Netherlands) and scored by an observer who was unaware of randomization or outcome data. Judgment of IRV flow was made on initial contrast injection according the TIMI classification.[14] Successful angioplasty was defined as a less than 50% diameter stenosis and TIMI 3 flow of the IRV. Corrected TIMI frame count and myocardial blush grade were defined as previously described.[15,16] Distal embolization, enzymatic infarct size (lactate dehydrogenase, LDHQ48), nuclear ejection fraction, percentage ST segment resolution after PCI, and 30-day and one-year death from all causes were also assessed. Recurrent myocardial infarction was defined as a new increase in creatin kinase (CK)-MB fraction of more than 3 times the upper limit of normal whether or not accompanied by chest pain/and or ECG changes and present in two separate blood samples.

Statistics

Statistical analysis was performed with the SPSS 12.0 statistical package. Continuous data were expressed as mean ± standard deviation, and categorical data as percentage, unless otherwise denoted. The analysis of variance and the chi-square test were appropriately used for continuous and categorical variables respectively.

Results

Patients

From June 2001 to November 2002, 507 patients were randomized to either pre-hospital initiation of Tirofiban (early group, n=251) or to cath-lab initiation of Tirofiban (late group, n=256). The platelet micro-aggregation inhibition substudy was performed only in patients admitted to our hospital. Platelet micro-aggregation inhibition measurements were performed in 412 of 463 (89%) patients recruited in Zwolle: 205 patients in the early group and 207 patients in the late group. Baseline characteristics of these patients are described in table 1. Early treatment with Tirofiban was started a median of 59 minutes earlier than late treatment, and 112 minutes earlier than second blood sampling.

Platelet micro-aggregation inhibition

The level of platelet micro-aggregation inhibition pre-PCI and post-PCI is presented in figure 1. The baseline platelet micro-aggregation inhibition in patients treated with Tirofiban was 38% ± 25%, and in the patients treated with placebo 14% ± 22% ($p < 0.001$). After PCI the platelet micro-aggregation inhibition in the early group was similar to the aggregation inhibition in the late group: 44% ± 27% vs. 40% ± 26% ($p = 0.09$). The correlation between platelet micro-aggregation inhibition, measured in all patients, after Tirofiban (sample 2) and angiographic and clinical outcome is shown in table 2.

Table 1. Baseline characteristics of patients in which platelet micro-aggregation inhibition was determined.

	Early group (n=205)	Late group (n=207)	p-value
age (mean yrs ± SD)	63 ± 9	61 ± 11	0.05
male gender (n, %)	178 (81.0)	184 (80.7)	0.96
diabetes mellitus (n,%)	19 (8.6)	24 (10.6)	0.48
Previous infarction (n,%)	16 (6.8)	24 (10.6)	0.16
Previous PCI* (n,%)	11 (5.0)	13 (5.8)	0.73
Previous CABG† (n,%)	5 (2.3)	5 (2.6)	1.00
Smoking‡ (n,%)	131 (60.1)	151 (67.7)	0.10
hypercholesterolemia (n,%)	46 (20.9)	58 (25.7)	0.24
hypertension (n,%)	56 (25.5)	69 (30.5)	0.23
family history of CAS§ (n,%)	92 (41.8)	88 (39.3)	0.59
anterior infarction (n,%)	92 (43.6)	98 (45.2)	0.75
one-vessel disease	91 (42.7)	104 (47.1)	0.36

*PCI = primary coronary intervention, †CABG = coronary artery bypass grafting, ‡current or previous smoking, §CAS = coronary artery syndrome

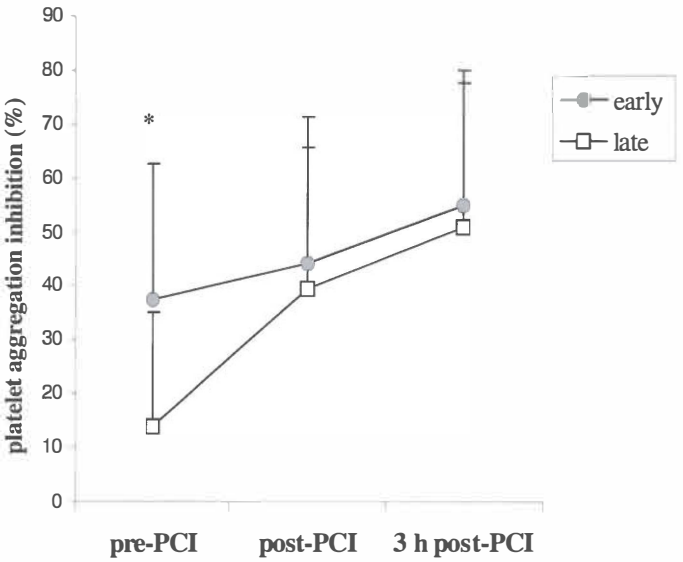

Figure 1. Platelet micro-aggregation inhibition before, immediately after and 3 hours after PCI in patients receiving Tirofiban before the first pre-PCI sample (early group) and in patients receiving Tirofiban after the first pre-PCI sample (late group). The difference between the level of platelet micro-aggregation inhibition in patients receiving Tirofiban (early group) or placebo (late group) was significant (asterix, $p < 0.001$).

Table 2. Clinical and angiographic outcome in patients with an acute myocardial infarction after Tirofiban divided into 4 levels of platelet micro-aggregation inhibition.

Platelet micro-aggregation inhibition (%, mean)	1st quarter 9.6 (n = 104)	2nd quarter 28.7 (n = 102)	3rd quarter 50.7 (n = 103)	4th quarter 78.7 (n=103)	p-value
distal embolization post-PCI (%)	9.3	8.9	11.8	11.1	0.91
TIMI-3 flow post-PCI (%)	91.7	89.8	92.7	91.3	0.97
blush grade 3 post-PCI (%)	60.0	48.9	57.3	53.3	0.46
mean corrected TIMI frame count (±SD)	26.0 ± 12.6	27.1 ± 18.3	25.8 ± 14.3	25.6 ± 14.4	0.94
ejection fraction (%,± SD)	45.5 ± 10.5	45.4 ± 12.2	46.4 ± 8.7	44.5 ± 10.9	0.73
LDHQ48 (U/L, ± SD)	1996 ± 256	1753 ± 223	1090 ± 128	1595 ± 203	0.33
30-day mortality (%)	0	1.0	1.0	1.0	0.79
30-day mortality + reinfarction (%)	1.0	1.0	4.0	1.0	0.27
one-year mortality(%)	1.0	3.1	2.0	2.9	0.73
one-year mortality + reinfarction (%)	3.0	8.3	6.9	4.9	0.39
% STT resolution (median, 25-75%)	81.8 (62.5-100)	83.4 (69.1-94.8)	83.6 (70.3-100)	82.4 (69.7-100)	0.72

In this table, patients were divided into four groups according to quartiles of the level of platelet micro-aggregation inhibition. No difference between the four quartiles of the level of platelet micro-aggregation inhibition was found in terms of distal embolization, TIMI-3 flow, blush grade 3, mean corrected TIMI frame count, ejection fraction, LDHQ48 and percentage STT resolution (p-value: 0.91, 0.97, 0.46, 0.94, 0.73, 0.33, 0.72 resp.). No difference was found in angiographic and clinical outcome in patients treated with early initiation in comparison with late initiation of Tirofiban (table 3).

Table 3. Clinical and angiographic outcome in patients with an acute myocardial infarction treated with early versus late initiation of Tirofiban.

	early group (n=205)	late group (n=207)	p-value
distal embolization post-PCI (%)	10.2	10.3	0.99
TIMI-3 flow post-PCI (%)	90.8	92.0	0.67
blush grade 3 post-PCI (%)	53.6	56.5	0.58
mean corrected TIMI frame count (±SD)	27.0 ± 16.1	25.3 ± 13.6	0.33
ejection fraction (%,± SD)	44.6 ± 10.5	46.2 ± 10.6	0.19
LDHQ48 (U/L, ± SD)	1665 ± 1613	1622 ± 1641	0.83
30-day mortality (%)	1.0	0.5	0.62
30-day mortality + reinfarction (%)	2.5	1.0	0.28
one-year mortality(%)	2.0	2.5	1.00
one-year mortality + reinfarction (%)	5.0	6.5	0.52
% STT resolution (median, 25-75%)	80 (62.5-100)	84.3 (71.2-100)	0.24

Discussion

This is the first study in which the relationship between platelet micro-aggregation inhibition, after a commonly used GpIIb/IIIa blocker, and angiographic and clinical outcome was prospectively investigated in a large cohort of patients with an AMI. Platelet activation aggregation and coronary artery thrombosis superimposed on a disrupted atherosclerotic plaque has emerged as the pivotal pathophysiologic event in coronary artery syndromes. GpIIb/IIIa receptor blockers, which block the final common pathway leading to platelet aggregation, might improve the patency of the infarct related vessel in patients with an AMI.[10] It has been questioned whether the commonly used dose of Tirofiban is sufficient to achieve optimal platelet aggregation inhibition.[11] However, the minimal level of platelet aggregation inhibition to improve outcome has never been specified. In this study, we did not find a relationship between the level of platelet micro-aggregation inhibition and angiographic and clinical outcome after PCI and Tirofiban for AMI. Even in

the quartile of patients who had a mean platelet micro-aggregation inhibition of <10% on Tirofiban, no increase in distal embolization, LDHQ48 level, or deterioration in TIMI-3 flow, blush grade 3, mean corrected TIMI frame count, ejection fraction, or percentage STT resolution was noticed. This seems to be in contrast to the GOLD study, in which the level of platelet aggregation was related to major adverse cardiac events in a population non-urgent PCI patients.[1] The occurrence of major adverse cardiac events (MACE) was doubled in the group of patients with a level of platelet aggregation inhibition < 95%, 10 minutes after PCI. However, after 1 and 24 hours and in the group of patients with an aggregation inhibition < 80% 10 minutes after PCI, no correlation was found between platelet aggregation inhibition and occurrence of MACE. Direct comparison of the GOLD study data and our study must be interpreted with caution because of differences in the method of platelet aggregation testing used and differences in study population.

Variations in the method of platelet aggregation inhibition measurement might also contribute to the lack of relationship between the level of platelet aggregation inhibition and angiographic and clinical outcome. Subtle differences in blood sampling and in sample handling might have substantial effect on the outcome of the sensitive platelet aggregation tests. In the former study using the Sysmex K 4500 method however, clear differences were measured between the different types of antiplatelet agents used in AMI patients before PCI.[11] Whether the Sysmex K 4500 method, which resembles the Plateletworks method (Helena Laboratories), is the optimal method of platelet micro-aggregation measurement remains subject of discussion. In the recent study of Lau, a relationship was found between the ability of clopidogrel to inhibit platelet aggregation, measured with a cell counter on a Plateletworks platform, and use of atorvastatin.[17] However, these results were questioned because of small patient size and non-randomized retrospective methods used, as well as lack of clinical evidence of adverse effects of atorvastatin on clopidogrel in a post hoc analysis of the CREDO trial.[18,19] Lack of clinical significance of the effects of atorvastatin on platelet inhibitory effect of clopidogrel might add to the discussion whether the correct method op platelet

aggregation was used. Furthermore, it is not clear whether sole measurement of platelet aggregation, which is only part of thrombus formation is of clinical importance. Since thrombus formation is a consequence of platelet activation, adhesion and platelet aggregation, additional measurement of the other two pathways might be indicated to predict clinical outcome, for example subacute stent thrombosis, after an AMI.

Although we did not find a correlation between platelet micro-aggregation inhibition and clinical outcome, a small number of studies have demonstrated that platelet function tests can predict clinical outcome in patients with an acute coronary artery syndrome.[20] In a recent publication of Frossard et al. enhanced platelet function using the PFA-100 was found in patients with a ST elevation myocardial infarction (STEMI) and higher Creatine Kinase (CK) MB levels in contrast to non-STEMI and lower CK-MB levels.[21] Platelet function using the PFA-100 might even predict CK-MB fraction in the STEMI subgroup in this study. Furthermore, circulating monocyte-aggregates were found to be a possible early marker of an AMI.[22] High concentrations of CD40L in unstable angina patients may be able to predict greater clinical benefit of abciximab.[23] Neither of these markers, however, have been adequately studied to become part of daily care in treatment of AMI patients.

An additional finding of our study was the only modest increase in platelet micro-aggregation inhibition after Tirofiban (14 ± 1.6% vs. 38 ± 1.7% in placebo vs. Tirofiban). This observation is in concordance with the recently published study, in which maximal 84% of the platelets were inhibited in the group of myocardial infarction patients receiving the highest dose of Tirofiban, and maximal 55% after a common dose of Reopro.[11] This study used the same method of platelet micro-aggregation measurement. It supports the hypothesis that in high-risk patients undergoing PCI for AMI, in the setting of a large thrombogenic and inflammatory stimulus, higher doses of platelet antagonists may be needed to achieve sufficient high levels of inhibition to prevent major cardiac adverse events.

Study limitations

Although we studied the largest population so far, it was relatively too small to study clinical adverse events with a limited incidence as in our study. The lack of events in the population studied, might also be further decreased by the lack of platelet function assessment in patient who died during PCI. Therefore, a correlation between platelet micro-aggregation inhibition and clinical outcome in AMI patients should be further investigated in a larger population. Furthermore, the optimal method of platelet aggregation testing remains subject to discussion. The lack of predictive value of our platelet micro-aggregation test might also be explained by the fact that the ADP mediated platelet aggregation was the only pathway of platelet activation measured in our platelet micro-aggregation test. Finally, conclusions regarding the relationship between patients with high levels of platelet inhibition and clinical outcome seem not to be justified based on this study, because in only very few patients a high level of platelet micro-aggregation inhibition was measured. It is however unknown, whether ADP mediated platelet aggregation is the major pathophysiologic pathway of platelet aggregation leading to thrombotic complications in an acute coronary syndrome. This could be marked as another limitation of our study. A platelet function test measuring multiple pathways of platelet function might be of higher predictive value in predicting outcome in AMI patients.

Conclusions

In this largest study so far, we found no correlation between the level of platelet micro-aggregation inhibition after Tirofiban and angiographic and clinical outcome in patients with an AMI, treated with primary PCI. Furthermore, only modest increase in platelet micro-aggregation inhibition after a commonly used dose of Tirofiban, was noted. Further studies on the relationship between the level of platelet aggregation inhibition and clinical outcome seem relevant for understanding the mechanism of acute coronary artery syndromes and the response to therapy. New insight in this matter might

lead to a more tailored regime of platelet aggregation inhibitors in patients with an acute coronary syndrome.

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Does Glycoprotein IIb/IIIa resistance exist?

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editorial

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Does Glycoprotein IIb/IIIa resistance exist?

Platelet function testing is not embedded into routine clinical practice, because no optimal, easy, reproducible and multipathway platelet aggregation test can be accomplished in vitro. Only recently, the relationship between the level of platelet aggregation inhibition by platelet inhibitors and clinical outcome in acute myocardial infarction became more clear.[1-5] High platelet reactivity was found in patients who experienced stent thrombosis, and patients with clopidogrel resistance were at increased risk of recurrent atherothrombotic events.[1,2] Furthermore, in ST elevation myocardial infarction (STEMI) increased levels of platelet aggregation were found as compared to unstable angina or control patients.[4] In a thrombolysis study, higher platelet receptor occupancy was coupled with better angiographic and electrocardiographic outcome.[3] Finally, in STEMI patients undergoing primary percutaneous intervention (PCI), higher levels of platelet aggregation inhibition by abciximab were recently found to be associated with better myocardial reperfusion.[5] Therefore, measuring platelet function in patients with an acute coronary artery syndrome is gaining interest to select patients at high risk of an unfavorable thrombotic event.

In the study of Van Werkum et al. in this edition of the Netherlands Heart Journal, platelet function is measured in patients with an acute coronary syndrome randomized to abciximab, high-dose tirofiban or placebo. Only 40-50% of platelets were inhibited in the abciximab treated patients, whereas platelet inhibition reached 80% in the patients treated with high-dose tirofiban. The authors refer to studies using TARGET trial dosing, in which low-dose tirofiban achieved only 60-66% platelet inhibition and resulted in more procedure-related ischemic events than abciximab which resulted in 90-95% platelet inhibition (using optical light aggregometry).[6,7] Therefore, Van Werkum et al. used the high dose tirofiban instead, for comparison with abciximab and placebo. Nevertheless, even in the high-dose tirofiban, there was still considerable platelet aggregation in vitro (20% of platelets). In early dose-finding studies a level of platelet aggregation inhibition of >80% was strived for.[8,9] The study results of Van Werkum et al. show striking similarities

with a previous study which used the same platelet function test.[10] Again only 46% platelet aggregation inhibition was found in the abciximab group and 86% platelet aggregation inhibition in de high-dose tirofiban group. The explanation for this 'drug resistance', might be related to the dose of the drug or the underlying disease. This lack of optimal platelet inhibition could be described as Glycoprotein (Gp) IIb/IIIa 'resistance'. However, does 'resistance' against an antiplatelet therapy exist?

Platelet function tests and 'resistance'

In the past years the terms aspirin 'resistance' and clopidogrel 'resistance' have emerged to describe different phenomena: 1) inability to prevent thrombotic complications, 2) a platelet aggregation inhibition, measured by in vitro platelet function tests, below certain cut-off values, 3) insufficient inhibition of plasma or urinary biochemical markers for platelet aggregation. 'Resistance' as description of interindividual variability in outcome of a platelet function test is incorrect, because the definition of resistance is dependent on a cut-off value. The cut-off values for aspirin or clopidogrel resistance are highly variable in the literature. Above all, most of the cut-off values are defined by measuring healthy volunteers. The response of patients with unstable coronary artery disease or myocardial infarction is, however, different from outcome of a platelet function test in healthy volunteers.[11] In the setting of an acute coronary syndrome, hyperaggregable platelets circulate which require more extensive antiplatelet therapy. For example in one of the most recent articles of Gurbel et al., the cut-off value for the bleeding time measured by PFA-100 collagen cartridge was 193 seconds.[12] This value however, is derived from the manufacturers, which is based on a normal value in healthy volunteers. Since these values can hardly be extrapolated to patients with coronary artery disease, defining aspirin or clopidogrel resistance based on cut-off values derived from healthy volunteers is inadequate. Another argument against describing the interindividual platelet aggregation response to an antiplatelet drug as 'resistant' is the normal pharmacodynamic and pharmacokinetic variability.[13] It seems more likely that the interindividual response to aspirin and clopidogrel is

a normally divided bell-shaped reaction, depending on both pharmacodynamic and pharmacokinetic variability and the underlying disease and type of used platelet function test, rather than a separate group of patients unable to respond to the drug.[14] Therefore, also GpIIb/IIIa 'resistance' is probably better described as a normal interindividual response to GpIIb/IIIa blockers.

Conclusion

Just as aspirin and clopidogrel 'resistance', GpIIb/IIIa blocker 'resistance' is very much dependent on the type of assay or type of platelet function test used. An internationally accepted definition of resistance to antiplatelet drugs is lacking. Therefore, it remains to be determined whether results of platelet function testing can be used as surrogate for the clinical effectiveness of these drugs. Therefore, it is still unknown whether the high-bolus dosage of Tirofiban is safe and effective in patients undergoing primary PCI. The On-TIME 2 trial currently recruiting patients in the Netherlands and Germany will try to answer this question by measuring platelet function in patients with STEMI undergoing primary PCI randomized to high-bolus tirofiban or placebo.[15]

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Fe-Induced Platelet Aggregation measurement: a novel method to measure platelet function in stenting for ST elevation myocardial infarction

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submitted

Abstract

Introduction

Iron and (stainless) steel are potent platelet aggregation activators, and may be involved in stent thrombosis, a potential life threatening complication after intracoronary stenting. Current platelet function tests are suboptimal, possibly because of inappropriate agonists and/or lack of reproducibility. We tested the feasibility and reproducibility of a novel platelet function test using stainless steel as an agonist and compared it with other platelet function tests.

Patients and Methods

In 111 patients with ST elevation myocardial infarction, duplo measurement of Fe-Induced Platelet Aggregation (FIPA) were performed after treatment with clopidrogel, acetylsalicylic acid and as part of a randomized controlled trial of tirofiban or placebo. Within one hour, citrated blood samples drawn from the femoral sheath before primary percutaneous coronary intervention (PCI), were added to 100 mg of low carbon steel and after mixing with vortex for 5 seconds the samples were incubated for 15 minutes at room temperature. The ratio between the non-aggregated platelets in the agonist sample and a platelet count in a reference sample x 100% was calculated as the platelet aggregation inhibition. FIPA was compared to platelet aggregation measurement using ADP as a stimulus and the platelet function analyzer PFA-100®.

Results

FIPA measurement was highly reproducible (correlation coefficient $R = 0.942$, $p < 0.001$ between the duplo samples). FIPA correlated well with ADP induced platelet aggregation ($R = 0.83$) but weakly with PFA-100 bleeding time ($R = 0.56$). FIPA could be measured in patients in which platelet aggregation could not be measured by PFA-100 or after ADP.

Conclusion

This study shows the reproducibility of a novel, easy applicable platelet function test using stainless steel as an agonist. The test was highly reproducible and correlated well with validated platelet function tests using ADP. Furthermore, FIPA could be measured in patients using potent platelet inhibitors as tirofiban plus clopidogrel and acetylsalicylic acid in contrast to other platelet function tests. Therefore, it revealed to be a suitable test for measurement of platelet aggregation inhibition in patients undergoing stenting for ST elevation myocardial infarction, using multiple antiplatelet regimes. Whether it is related with clinical outcome remains to be examined.

Introduction

Iron and (stainless) steel are potent platelet aggregation activators, and this has been considered as a limitation of using these materials in endovascular stents.[1-7] This is particularly of importance since (subacute) stent thrombosis is a potential life threatening complication after intracoronary stenting.[8] Current platelet function tests are not optimal, possibly because of inappropriate agonists and lack of reproducibility.[9] We developed a platelet function test using stainless steel as an agonist. In this study, we tested the feasibility of Fe-Induced Platelet Aggregation (FIPA) measurement and compared our FIPA test with other platelet function tests in ST elevation myocardial infarction (STEMI) patients before percutaneous coronary intervention (PCI).

Methods

It concerns a substudy of 111 patients of the On-TIME-2 pilot study. The On-TIME-2 study is a randomized, open label, investigator closed, multicenter trial to evaluate the value of pre-hospital administration of additional high dose (bolus 25 μg/kg, followed by a maintenance infusion of 0.15μg/kg/min) tirofiban vs. placebo (standard treatment) in STEMI patients on improving the extent of myocardial reperfusion (primary endpoint).[10] The protocol was approved by our institution's Review Board and Ethical Committee and written informed consent was obtained from all patients. PCI was performed immediately after coronary angiography in all patients. All patients were treated with clopidogrel (600 mg loading dose followed by 75 mg daily for one year), acetylsalicylic acid, beta blockade, statin therapy and angiotensin converting enzyme inhibition. In our substudy, additional blood samples were collected in citrate (0.109 M) in plastic tubes before PCI, but after having received study medication (tirofiban or placebo) in addition to 600 mg oral clopidogrel, 5000 IU heparin intravenously and 500 mg acetylsalicylic acid intravenously. The samples were drawn from the femoral sheath at the start of catheterization, before additional heparin infusion, using the Vacutainer® system.

Fe-induced platelet aggregation

Platelet aggregation was measured, using AISI 434 low carbon steel as a stimulus. Citrate anticoagulated whole blood was used, collected within one hour before platelet function testing. Duplicate samples of 2cc citrated blood were added to tubes containing 100 mg steel wool (Haemoscan, Groningen, Netherlands) and after 5 seconds mixture on a vortex (type MIX TM01, Retsch, Germany) incubated for 15 minutes at room temperature. Subsequently, platelet count was performed on each sample using a routine blood cell counter (Sysmex K4500, Sysmex Corp., Kobe, Japan) and on a reference tube. In the presence of the agonist steel, platelets aggregate and adhere to the steel surface. As the aggregated platelets exceed the threshold limitations for platelet size, they are no longer counted as individual platelets. The ratio between the non-aggregated platelets in the agonist sample and the platelet count in the reference tube without steel multiplied by 100% was used as the platelet aggregation inhibition.

ADP induced platelet aggregation

For measurement of the ADP platelet aggregation inhibition, we used the Sysmex K4500 method.[11] Blood samples were collected in plastic tubes containing EDTA and tubes containing PPACK with 20 µM/L adenosine diphosphate (ADP, Plateletworks®, Helena Laboratories, Beaumont, Texas). A routine platelet count was performed on each sample. The platelet count in an EDTA tube was used as a reference. In the presence of the agonist ADP, platelets aggregate and associate. As the aggregated platelets exceed the threshold limitations for platelet size, they are no longer counted as individual platelets. The ratio between the non-aggregated platelets in the agonist sample and the platelet count in the reference tube x 100% was calculated as the platelet aggregation inhibition. In our laboratory, we reported a correlation coefficient of 0.90 between the Sysmex K4500 and the ICHOR point-of-care platelet analyzer (Helena Laboratories, Beaumont, TX) to validate the Sysmex K4500 platelet aggregation measurement.[12,13]

PFA-100®

Platelet function was measured using a platelet function analyzer, (PFA-100, Dade Behring, Marburg GmbH,Germany), an instrument that provides a quantitative measurement of platelet adhesion and aggregation in whole blood flowing through a small aperture under high shear conditions.[14-17] The aperture (147 μm) is coated with 2 μg type I collagen and 50 μg epinephrine bitartrate. The closure time of the aperture, referred to as bleeding constant, is an indicator of platelet function.

Statistics

Statistical analysis was performed with the SPSS 12.0 statistical package. Continuous data were expressed as mean ± standard deviation, and categorical data as percentage, unless otherwise denoted. The analysis of variance and the chi-square test were appropriately used for continuous and categorical variables respectively. For comparison of the reproducibility of the duplo FIPA measurements and comparison with other platelet function tests, a Spearman Rho correlation coefficient was determined using the Pearson product moment correlation. Furthermore, a Bland-Altman graph was drawn to compare the two different outcome values of the duplo FIPA sample with the mean of these values. The limits of agreement, the borderline of 2 standard deviations from the mean were determined to investigate clinical usefulness. A p-value of < 0.05 was considered statistically significant.

Results

From April 2005 to December 2005, platelet aggregation was measured in 111 consecutive patients, who were randomized to either pre-hospital high-dose tirofiban (n=53) or placebo (n=58). Baseline characteristics of these patients are described in table 1.

Table 1. Baseline characteristics of patients according to randomization

	group 1 (n=53)	group 2 (n=58)	p-value
male gender	77%	71%	0.424
hypertension	40%	36%	0.711
diabetes	8%	10%	0.745
smoking	54%	42%	0.212
hypercholesterolemia	23%	17%	0.476
family history	43%	42%	0.891
previous angina	43%	36%	0.439
previous myocardial infarction	13%	12%	0.857
previous PCI*	8%	5%	0.707
previous CABG$	4%	5%	1.000
previous CVA#	0%	0%	
age (years, ± SD)	61.9 ± 11.7	63.3± 10.8	0.540
blood pressure systolic (mm Hg, ± SD)	142 ± 25	130 ± 26	0.024
blood pressure diastolic (mm Hg, ± SD)	86 ± 15	80 ± 19	0.071
heart rate (bpm)	75 ± 21	75 ± 20	0.872
length (cm, ± SD)	176.0 ± 8.7	174.8 ± 9.0	0.589
weight (kg, ± SD)	82 ± 12	83 ± 16	0.583

*PCI = percutaneous coronary intervention, $CABG = coronary artery bypass grafting, #CVA = cerebral vascular accident

FIPA

The duplo measurement of the Fe induced platelet aggregation test showed good reproducible results (R = 0.942, p < 0.001, figure 1). In 72% of the patients who received high dose tirofiban, platelet aggregation inhibition was below 80%. The Bland Altman graph shows the mean of both duplo measurements and limits of agreement of plus or minus 20% (figure 2). There was no association between an increase in FIPA inhibition and increased difference between the two measurements.

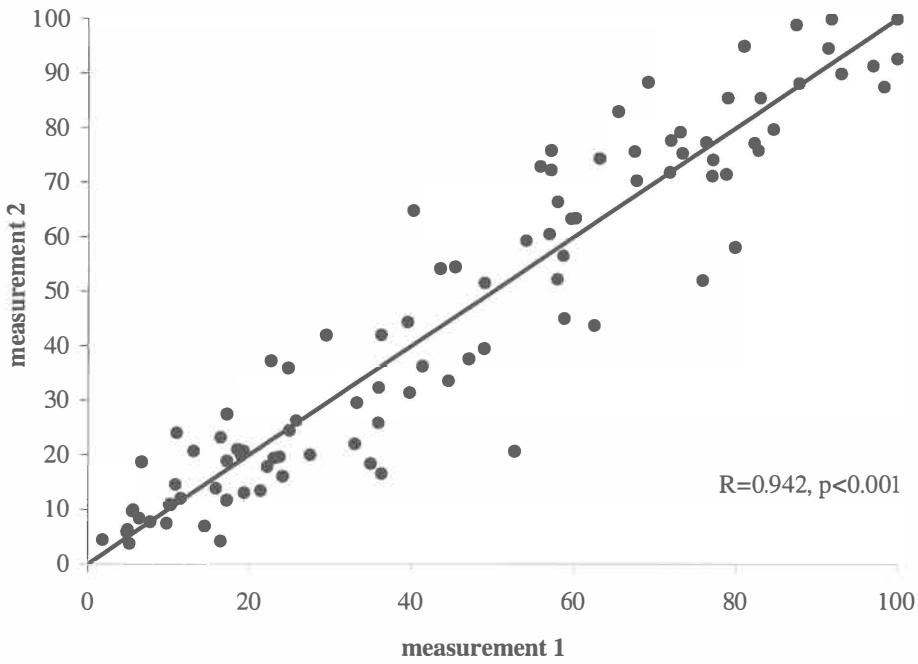

Figure 1. Duplo measurement and correlation of the FIPA inhibition (R = 0.942, p < 0.001)

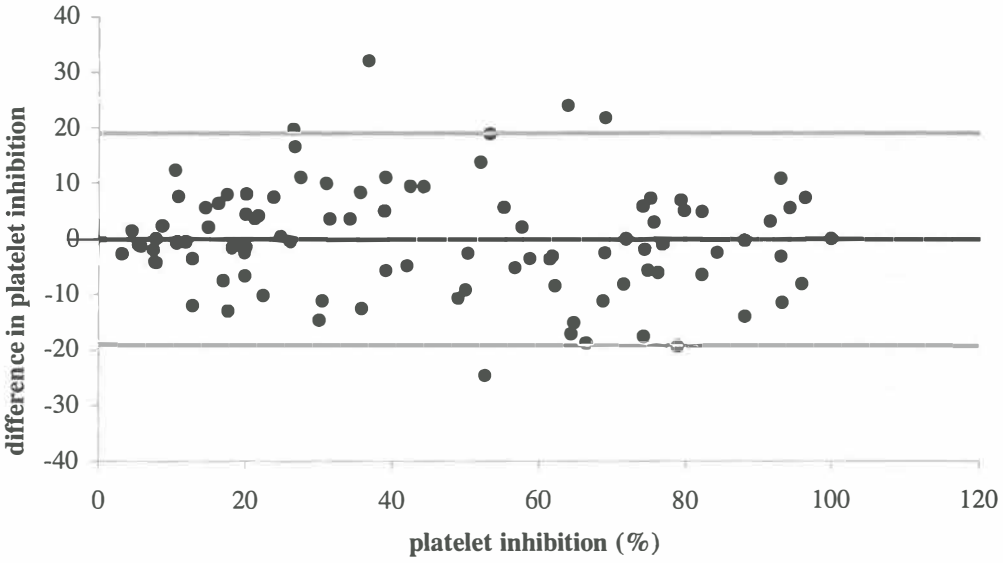

Figure 2. Bland Altman graph of the mean of both duplo measurements of FIPA inhibition.

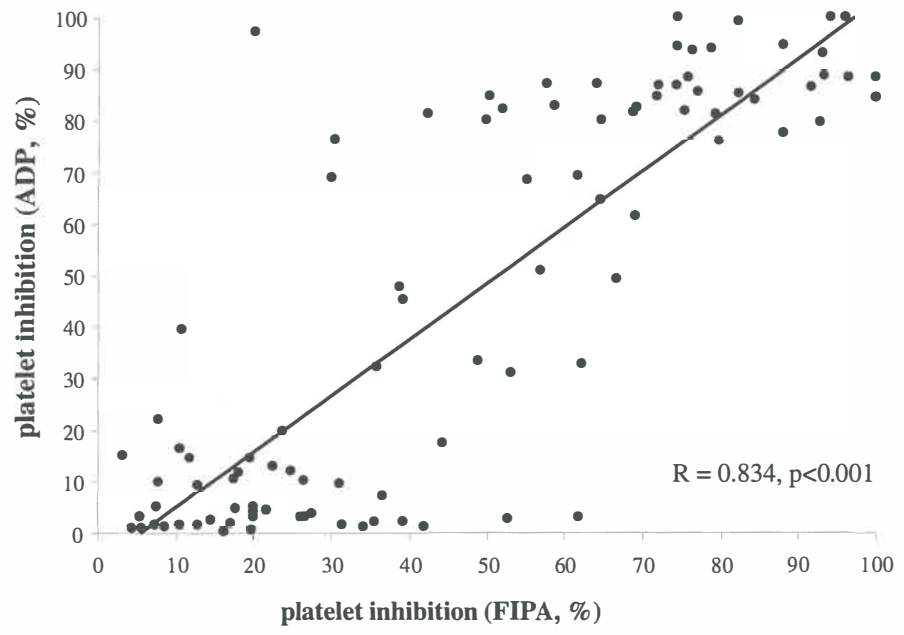

Figure 3. Comparison of FIPA inhibition and ADP induced platelet aggregation inhibition.

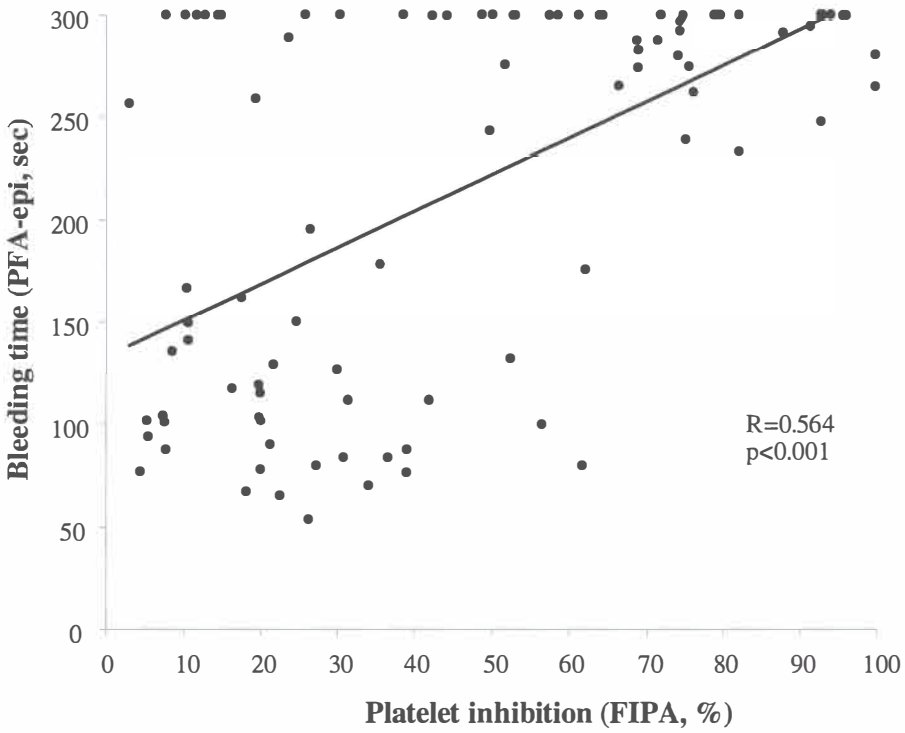

Figure 4. Comparison of FIPA inhibition and PFA-100 bleeding time.

Comparison with other tests

FIPA correlated well with ADP induced platelet aggregation (R = 0.834, p-value < 0.001, figure 3), but there was only a weak association between FIPA and the quantitative platelet adhesion measurement by PFA-100 (R = 0.564, p-value < 0.001, figure 4). FIPA could be measured also in patients in which platelet aggregation could not be measured by PFA-100 or after ADP (figure 3,4). As compared to PFA-100 only few patients had maximal platelet inhibition using the FIPA test, indicating that FIPA measurement can be performed in patients with more extensive platelet inhibition (figure 4). As compared to the ADP induced platelet aggregation inhibition, in few patients minimal platelet aggregation inhibition was found, indicating that platelet function could be measured in patients with maximal platelet activation (figure 3).

Discussion

In the present study, we tested the feasibility and reproducibility of a novel platelet function test using stainless steel as an agonist for platelet aggregation. Our test had good reproducibility and platelet aggregation could even be measured in patients with enhanced levels of platelet aggregation inhibition or activation in contrast to other platelet function tests.

Antiplatelet therapy has been increasingly important in patients with coronary artery disease, particularly in those with acute coronary syndrome (ACS) and in those undergoing PCI.[18] The intended platelet aggregation inhibition should be a balance between prevention of thrombotic complications on one side and bleeding risks on the other side.[11,19] Platelet function measurement may facilitate individual dosing and type of antiplatelet therapy. Platelet function testing is not embedded into routine clinical practice, because no optimal, easy, reproducible and multipathway platelet aggregation test can be accomplished in vitro. Furthermore, only few studies have associated platelet aggregation inhibition with clinical outcome in myocardial infarction. Conflicting results have been present in particular in patients with subacute thrombosis after stenting.[20-25] High platelet reactivity was found in patients who experienced stent thrombosis, and patients with clopidogrel resistance were at increased risk of recurrent atherothrombotic events.[20,21] Furthermore, in STEMI increased levels of platelet aggregation (related to infarct size) were found as compared to unstable angina or control patients.[22] A thrombolysis study reported platelet receptor occupancy to be associated with better angiographic and electrocardiographic outcome.[23] Finally, in STEMI patients undergoing primary PCI, increased inhibition of platelet aggregation by abciximab was recently found to be associated with better myocardial reperfusion.[24] However, in our recent On-TIME-1 study, no relationship was found between the levels of platelet aggregation inhibition and clinical outcome in STEMI patients undergoing primary PCI.[25] The lack of correlation between platelet aggregation inhibition and clinical outcome might be explained by the small number of major adverse cardiac events by exclusion of moderate heart failure patients, or it could be due to an insufficient method of platelet aggregation measurement

at that time of the study. In the On-TIME-1 study we used ADP as an agonist for platelet aggregation inhibition measurement. It is questionable whether this single pathway platelet aggregation agonist is sufficient to simulate the multipathway platelet aggregation in the acute coronary syndrome. We invented a simple, platelet aggregation test for practical clinical use, using a multipathway agonist for platelet aggregation, resembling stents in coronary arteries. This platelet function test might identify patients with increased levels of platelet aggregation, who might be at high risk for worse clinical outcome and be in need of tailored antiplatelet regimes. Our FIPA test will be used in the ON-TIME-2 study to identify patients at high risk for thrombotic complications after primary PCI for STEMI.

Stainless steel activates both platelet adhesion, aggregation and thromboxane release.[1-7] The potent platelet activation characteristics of stainless steel might be due to coverage of stainless steel with other proteins than albumin such as fibrinogen, von Willebrand factor, IgG, fibronectin.[26] As a consequence, the protective cover of albumin, which does not interact with platelets, is less present in stainless steel surfaces resulting in increased platelet activation by steel surfaces.[26]

In the present study, we found an association between FIPA and ADP induced platelet aggregation. Therefore, our FIPA test might be importantly ADP regulated. However, since FIPA is a multipathway stimulus for platelet aggregation, it might better resemble the clinical platelet aggregation by metal stents in the setting of an acute coronary syndrome. However, no golden standard platelet function test is available. Therefore, we compared our FIPA test to two other platelet function tests instead of one.

Bleeding time measurement, in patients with an acute myocardial infarction, using PFA-100, before anti-platelet therapy administration was correlated inversely with the extend of myocardial cell necrosis.[22] In a study of mixed stable and acute coronary syndrome patients, shortened bleeding time with PFA-100 (<190s) was associated with a higher risk for the reoccurrence of cardiovascular events.[27] A disadvantage of PFA-100 is that bleeding time is highly dependent on the levels of von Willebrand factor.[28,29] As a result, high

von Willebrand factor levels may mask the inhibitory effects of anti-platelet therapy because the high-shear rates will promote direct binding of vWF to GpIIb/IIIa. It has been suggested that clopidogrel is not able to inhibit the platelet plug formation under the high concentrations of collagen and ADP in the cartridges of the PFA-100.[30,31] Since patients were randomized to either high dose Tirofiban or placebo on top of clopidogrel and acetylsalicylic acid in the On-TIME-2 study, might explain the diversity in the results of the PFA-100 bleeding time found in our study.

Limitations

Since thrombosis of coronary arteries is known to be the result of a multifactorial interplay of substance release from endothelial wall, activation of platelets, shear stress due to roughness of the internal vessel wall and mechanical complications of PCI, inflammation markers released by the immune system and thrombogenicity of stent material as well as form and geometry of stents, it is wrong to suggest an in-vitro platelet aggregation test using stainless medical steel to represent the complete platelet aggregation response in patients with an acute STEMI undergoing primary PCI.[32] Another limitation is the lack of comparison with the Verify-now® test.

Conclusion

In this pilot study, Fe Induced Platelet Aggregation measurement showed to be a novel, feasible and easy method for platelet function measurement, resembling the agonist for platelet aggregation in stents in coronary arteries. Furthermore, FIPA could be measured in acute STEMI patients using potent platelet inhibitors as GpIIb/IIIa blockers plus clopidogrel and acetylsalicylic acid in contrast to other methods of platelet function.

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White blood cells

Chapter 6

Successful reperfusion for ST elevation myocardial infarction is associated with a decrease in white blood cell count

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Abstract

Introduction

Elevated white blood cell (WBC) count on admission in patients with ST segment elevation myocardial infarction (STEMI) has been associated with an adverse prognosis. Whether successful reperfusion by primary percutaneous coronary intervention (PCI) is associated with a decrease in WBC count is unknown.

Methods

In this subanalysis of the On-TIME trial, WBC count was measured on admission and 6 and 24 hours after primary PCI for STEMI (n = 364). Angiographic measurements of reperfusion, including TIMI-flow and myocardial blush grade were compared to changes in WBC count.

Results

Restoration of TIMI 3 flow by primary PCI was associated with a significant decrease in median WBC count (11.5(9.7-14.2), 10.7(9.0-12.5) 9.9(8.5-11.5) at baseline, 6 and 24 hours), whereas after unsuccessful PCI (TIMI<3 flow) WBC count remained elevated (12.5(9.5-14.6), 12.1(9.9-14.4), 11.4(9.2-15.2)). Improved myocardial blush was also related to a decrease in WBC count. After multivariate analysis, improved myocardial perfusion (TIMI 3 flow and myocardial blush grade 3) was an independent predictor of decrease of WBC count after PCI.

Conclusion

Impaired myocardial reperfusion after primary PCI for STEMI is associated with persistent WBC elevation.

Keywords: change of WBC count, leukocytes, myocardial reperfusion

Introduction

Inflammation plays an important role in atherosclerosis and the development of ST elevation myocardial infarction (STEMI).[1-3] White blood cell (WBC) count may be a marker of inflammation and elevated baseline WBC counts in patients with STEMI have been associated with increased infarct size and higher in-hospital and long-term mortality.[4-9] Successful and sustained reperfusion is a strong predictor of prognosis in patients with STEMI.[10] Whether reperfusion is associated with a decrease in inflammatory markers is unknown. Therefore, we measured serial WBC counts, before and after primary percutaneous coronary intervention (PCI) in patients with a STEMI included in the On-TIME trial and assessed whether WBC count changes were associated with angiographic measurements of reperfusion.

Methods

Patients

It concerns a sub-analysis of data from the On-TIME trial, a prospective, double-blinded, randomized controlled trial investigating early versus late initiation of Tirofiban in STEMI.[11] In brief, patients were included if there was more than 30 minutes of chest pain with more than 0.2 mV (anterior myocardial infarction, MI) or 0.1 mV (non-anterior MI) of ST elevation in 2 contiguous ECG leads and the ability to perform primary angioplasty within 6 hours after the start of symptoms. Patients over 80 years of age, women less than 50 years of age, patients who were treated with thrombolytic therapy in the previous 24 hours, patients on warfarin or acenocoumarol within the last 7 days and patients with a contraindication to glycoprotein (Gp) IIb/IIIa blockade were excluded. Patients with severe heart failure or cardiogenic shock (Killip class III or IV) and patients on haemodialysis were also excluded. The protocol was approved by our institution's Review Board and Ethical Committee and informed consent was obtained in all patients. At coronary angiography, the initial injection was used to assess TIMI flow of the infarct related vessel

(IRV). PCI was performed immediately after coronary angiography. Post PCI, all patients were intended to be treated with Clopidogrel (300 mg loading dose followed by 75 mg daily for a month), acetylsalicylic acid, beta blockade, statin therapy and angiotensin converting enzyme inhibition. In 70% of patients stents were used.

WBC count

Blood samples were taken for routine WBC measurement on admission (before PCI) and 6 and 24 hours after PCI (Sysmex K4500, Sysmex Corp., Kobe Japan). Change (Δ) of WBC count was defined as the WBC count after 6 or 24 hours minus the WBC count at admission. The primary objective of our sub-analysis was to associate success of reperfusion with a change in WBC count.

Angiographic outcome

All angiographic data were analyzed by an independent core-lab (Diagram Zwolle, the Netherlands) and scored by an observer who was unaware of randomization, WBC counts or outcome. Judgment of IRV flow was made on initial contrast injection according the TIMI classification.[12] Successful angioplasty was defined as a less than 50% diameter stenosis and TIMI 3 flow of the IRV. Myocardial blush grade were defined post-PCI as previously described.[10] Enzymatic infarct size (lactate dehydrogenase, LDHQ48) was also assessed. Intra-coronary thrombus was noted as previously described.[11]

Statistics

Statistical analysis was performed with the SPSS 12.0 statistical package. Continuous data were expressed as median (first-third quartile), and categorical data as percentage, unless otherwise denoted. Significance of differences in WBC counts over time was tested by the paired t-test. The analysis of variance and the chi-square test were appropriately used for continuous and categorical variables respectively. A multiple logistic regression analysis was performed to identify independent correlates of WBC count decrease. Candidate variables were: early use of Tirofiban, gender, age, previous myocardial infarction, previous coronary artery bypass grafting (CABG), previous PCI,

hypercholesterolemia, hypertension, diabetes mellitus, family history of coronary artery syndrome, smoking, Killip class>1, TIMI flow, myocardial blush grade, anterior myocardial infarction, enzymatic infarct size, time onset of complaints to PCI < 90 minutes and baseline WBC count. Change in WBC count was dichotomized and a decrease of WBC count was defined as a decline more than the median change of WBC count. The stepwise selection of variables and estimation of significant probabilities were computed by means of maximal likelihood ratio tests. The chi-square value was calculated from the log of the ratio of maximal partial likelihood functions. The additional value of each category of variables added sequentially was evaluated on the basis of the increases in the overall likelihood statistic ratio. A p-value of < 0.05 was considered statistically significant.

Results

From June 2001 to November 2002, 507 patients were included in the On-TIME trial. Three patients died before WBC count could be measured after PCI and in 140 patients one of the serial WBC counts was missing. WBC measurements at different time points were performed in 364 of 507 (72%) recruited patients. Higher baseline WBC counts were measured in older patients, smokers and those who had TIMI-0 flow before PCI. The baseline characteristics of patients in which all WBC counts were measured and those in which not all WBC counts were measured were shown in table 1. Median WBC count decreased from 11.4 (9.7-14.2) 10^9/L before PCI to 10.6 (9.0-12.7) and 9.9 (8.5-11.6) 10^9/L, 6 and 24 hours after PCI respectively (p<0.001). The median decrease of WBC count was 0.8 10^9/L after 6 hours and 1.4 10^9/L after 24 hours. WBC count decrease after 6 hours was significantly higher in smokers and those with lower systolic blood pressure on admission (table 2). After 24 hours, WBC count was particularly decreased in smokers, in patients without diabetes mellitus, those without previous CABG and with a lower heart rate (table 3).

Successful PCI was associated with a decrease in WBC count (11.5 (9.7-14.2), 10.7 (9.0-12.5) and 9.9 (8.5-11.5) at baseline, 6 and 24 hours), whereas after unsuccessful PCI, WBC count remained elevated (resp. 12.5 (9.5-14.6), 12.1 (9.9-14.4) and 11.4 (9.2-15.2), for difference between two groups: $p = 0.80$, $p = 0.025$, $p = 0.002$, figure 1). Enhanced myocardial blush grade was also significantly related to a decrease in WBC count (WBC count at baseline, 6 and 24 hours for MBG 3: 11.6 (9.7-14.3), 10.6 (9.0-12.1), 9.7 (8.3-11.3), for MBG<3: 11.5 (9.7-14.4), 11.5 (9.6-13.3), 10.4 (8.8-12.8), $p = 0.713$, $p = 0.007$, $p < 0.001$, figure 2).

To assess the independent association between reperfusion and a decrease in WBC count, multivariate analyses were performed. Variables included in the final model were TIMI<3 flow post-PCI, myocardial blush grade <3, previous CABG, baseline WBC count, smoking and anterior infarction. After multivariate analysis, decreased coronary flow after PCI (TIMI<3 flow after 6 hours: OR 2.61 (95% confidence interval: 1.04-6.59) p=0.042, TIMI<3 flow after 24 hours: OR 7.68 (2.58-22.91) p<0.001) was an independent predictor of WBC count change smaller than the median WBC count change. Myocardial blush grade was not an independent predictor of change in WBC count (MBG<3 after 6 hours: OR 0.69 (0.40-1.20) $p = 0.188$, MBG<3 after 24 hours: OR 1.47 (0.83-2.63), $p = 0.187$)

Table 1. Baseline characteristics and clinical outcome of patients with and without WBC count measurement.

	WBC count (n=364)	without WBC count (n=143)	p-value
age (mean yrs ± SD)	61.7 ± 10.7	61.4 ± 11.0	0.793
male gender	80.5%	77.6%	0.469
diabetes mellitus	9.9%	12.1%	0.460
previous infarction	8.5%	7.9%	0.827
previous PCI*	5.5%	4.3%	0.583
previous CABG†	2.5%	1.4%	0.735
smoking‡	65.0%	63.4%	0.734
hypercholesterolemia	21.4%	27.3%	0.159
hypertension	25.5%	36.4%	0.015
family history of CAS§	37.7%	44.9%	0.148
anterior infarction	44.6%	47.2%	0.611
one-vessel disease	45.5%	40.6%	0.382
Killip class > 1	15.2%	18.5%	0.392
blood pressure systolic (mm Hg ± SD)	134.7 ± 25.8	132.5 ± 27.4	0.410
blood pressure diastolic (mm Hg ± SD)	81.4 ± 16.7	78.6 ± 19.6	0.124
heart rate (BPM ± SD)	72.6 ± 19.4	75.1 ± 20.8	0.204
MBG 3¥	51.5%	52.4%	0.874
MBG 2¥	36.8%	32.4%	0.407
MBG 0,1¥	11.7%	15.2%	0.336
distal embolization pre-PCI*	16.5%	18.2%	0.822
distal embolization post-PCI*	10.3%	12.3%	0.644
TIMI 3 flow pre-PCI*	16.4%	18.1%	0.656
TIMI 0,1 flow pre-PCI*	65.6%	51.2%	0.004
TIMI 3 flow post-PCI*	89.9%	93.5%	0.273
TIMI 0,1 flow post-PCI*	1.2%	3.7%	0.099
reinfarction	0.6%	0%	1.000
all-cause mortality <30 days	1.1%	5.4%	0.009
all-cause mortality <one year	3.6%	5.5%	0.359
intra-coronary thrombus	28.3%	29.1%	0.909
LDHQ48 (U/L, median, first-third quartile)	1311.5 (558.0-2536.8)	701.0 (290.0-1338.0)	< 0.001
baseline WBC count ≤ 11.4 10^9/L	50.3%	54.1%	0.480
acetylsalicylic acid at discharge	98.1%	89.9%	<0.001
beta-blocker at discharge	90.4%	81.4%	0.007
ace inhibitor at discharge	54.9%	39.5%	0.003
statin at discharge	87.9%	72.9%	<0.001
clopidogrel at discharge	69.8%	69.8%	0.008

*PCI = primary coronary intervention, †CABG = coronary artery bypass grafting, ‡current or previous smoking, §CAS = coronary artery syndrome, ¥MBG = myocardial blush grade

Table 2. Baseline characteristics and angiographic outcome compared to ΔWBC count 6 hours after PCI.

	ΔWBC count: median (first-third quartile)		
	with characteristic	without characteristic	p-value
age ≥ 65 years	-0.70 (-2.1–0.80)	-0.90 (-2.9-0.4)	0.063
male gender	-0.80 (-2.55-0.60)	-0.7 (-2.2-0.8)	0.430
diabetes mellitus	0.15 (-2.35-1.48)	-0.90 (-2.48-0.60)	0.075
previous infarction	-1.0 (-2.3-0.50)	-0.80 (-2.45-0.65)	0.988
previous PCI*	-1.05 (-2.65-0.88)	-0.80 (-2.4-0.60)	0.922
previous CABG†	-0.1 (-0.90-1.25)	-0.80 (-2.5-0.60)	0.155
smoking‡	-1.00 (-2.90-0.60)	-0.6 (-2.00-0.70)	0.052
hypercholesterolemia	-1.05 (-2.33-0.55)	-0.75 (-2.5-0.60)	0.662
hypertension	-0.70 (-1.85-0.90)	-0.80 (-2.6-0.60)	0.201
family history of CAS§	-0.90 (-2.5-0.60)	-0.75 (-2.4-0.70)	0.640
anterior infarction	-0.70 (-2.28-0.88)	-0.90 (-2.7-0.50)	0.140
one-vessel disease	-0.90 (-2.90-0.70)	-0.75 (-2.28-0.60)	0.507
Killip class > 1	-0.45 (-3.03-1.10)	-0.80 (-2.40-0.50)	0.495
blood pressure systolic > 134 mm Hg	-0.70 (-1.93-0.70)	-1.0 (-3.28-0.50)	0.030
blood pressure diastolic > 81 mm Hg	-0.70 (-2.08-0.60)	-1.0 (-3.0-0.50)	0.091
heart rate > 73 BPM	-0.65 (-2.40-0.93)	-1.0 (-2.6-0.4)	0.104
early Tirofiban initiation	-1.10 (-3.00-0.70)	-0.80 (-2.10-0.50)	0.441
MBG 3¥	-1.0 (-2.88-0.58)	-0.60 (-2.10-0.88)	0.009
MBG 2¥	-0.80 (-2.2-0.60)	-0.80 (-2.60-0.70)	0.660
MBG 0,1¥	0.30 (-1.10-2.20)	-0.90 (-2.60-0.60)	0.001
distal embolization pre-PCI*	-0.30 (-2.20-0.55)	-0.45 (-2.40-1.10)	0.881
distal embolization post-PCI*	-1.50 (-3.0-0.50)	-0.70 (-2.40-0.70)	0.315
TIMI 3 flow pre-PCI*	-0.60 (-2.80-0.70)	-0.80 (-2.40-0.60)	0.671
TIMI 0,1 flow pre-PCI*	-0.80 (-2.40-0.60)	-0.70 (-2.38-0.78)	0.476
TIMI 3 flow post-PCI*	-0.90 (-2.58-0.60)	0.25 (-2.0-2.25)	0.013
TIMI 0,1 flow post-PCI*	0.45 (-1.78-2.23)	-0.80 (-2.40-0.60)	0.296
intra-coronary thrombus	-1.0 (-2.63-0.43)	-0.75 (-2.30-0.80)	0.168
LDHQ48 (U/L) < median	-0.60 (-2.30-0.60)	-0.60 (-1.90-0.85)	0.361
baseline WBC count ≤ 11.4 10^9/L	0.10 (-1.10 – 1.20)	-2.10 (-3.90 - -0.40)	<0.001

*PCI = primary coronary intervention, †CABG = coronary artery bypass grafting, ‡current or previous smoking, §CAS = coronary artery syndrome, ¥MBG = myocardial blush grade

Table 3. Baseline characteristics and angiographic outcome compared to ΔWBC count 24 hours after PCI.

	ΔWBC count: median (first-third quartile)		
	with characteristic	without characteristic	p-value
age ≥ 65 years (n=)	-1.2 (-2.8-0.78)	-1.6 (-4.05-0.4)	0.047
male gender	-1.4 (-3.35-0.6)	-1.1 (-2.8-0.30)	0.602
diabetes mellitus	0.05 (-2.9-1.38)	-1.5 (-3.3-0.30)	0.031
previous infarction	-1.6 (-2.8-1.0)	-1.4 (-3.35-0.50)	0.781
previous PCI*	-1.05 (-2.65-0.88)	-1.4 (-3.28-0.50)	0.706
previous CABG†	1.4 (-1.15-3.95)	-1.5 (-3.4-0.50)	0.014
smoking‡	-1.65 (-3.98-0.28)	-0.90 (-2.60-1.10)	0.003
hypercholesterolemia	-1.8 (-3.0-0.73)	-1.35 (-3.5-0.50)	0.773
hypertension	-1.30 (-3.05-0.55)	-1.5 (-3.5-0.50)	0.456
family history of CAS§	-1.30 (-3.75-0.40)	-1.45 (-3.10-0.63)	0.978
anterior infarction	-1.15 (-2.28-0.88)	-1.60 (-3.9-0.50)	0.034
one-vessel disease	-1.50 (-3.90-0.50)	-1.40 (-2.88-0.63)	0.295
Killip class > 1	-0.90 (-3.60-1.10)	-1.50 (-3.40-0.20)	0.287
blood pressure systolic > 134 mm Hg	-1.35 (-2.7-0.33)	-1.75 (-4.08-0.50)	0.132
blood pressure diastolic > 81 mm Hg	-1.35 (-2.68-0.73)	-1.70 (-0.40-0.20)	0.060
heart rate > 73 BPM	-1.30 (-2.7-0.80)	-1.6 (-3.9-0.10)	0.034
early Tirofiban initiation	-1.40 (-3.75-0.70)	-1.40 (-3.10-0.40)	0.673
MBG 3¥	-1.80 (-4.00—0.10)	-0.90 (-2.43-0.90)	<0.001
MBG 2¥	-1.30 (-2.7-0.50)	-1.60 (-3.80-0.40)	0.193
MBG 0,1¥	0.30 (-1.70*-2.10)	-1.50 (-3.50-0.20)	< 0.001
distal embolization pre-PCI*	-1.30 (-2.30-0.60)	-0.90 (-2.93-1.03)	0.917
distal embolization post-PCI*	-1.35 (-3.10-0.83)	-1.50 (-3.38-0.38)	0.460
TIMI 3 flow pre-PCI*	-1.10 (-3.0-1.40)	-1.50 (-3.30-0.35)	0.235
TIMI 0,1 flow pre-PCI*	-1.55 (-3.50-0.30)	-1.10 (-2.80-0.93)	0.152
TIMI 3 flow post-PCI*	-1.60 (-3.48-0.20)	0.25 (-1.20-1.40)	0.001
TIMI 0,1 flow post-PCI*	1.00 (0.075-1.93)	-1.40 (-3.30-0.40)	0.032
intra-coronary thrombus	-1.60 (-3.55- -0.08	-1.30 (-3.10-0.73)	0.187
LDHQ48 (U/L) < median	-1.40 (-3.05-0.70)	-1.30 (-2.90-0.50)	0.757
baseline WBC count ≤ 11.4 10^9/L	-0.10 (-1.50 – 1.40)	-3.0 (-4.85- -1.30)	<0.001

*PCI = primary coronary intervention, †CABG = coronary artery bypass grafting, ‡current or previous smoking, §CAS = coronary artery syndrome, ¥MBG = myocardial blush grade

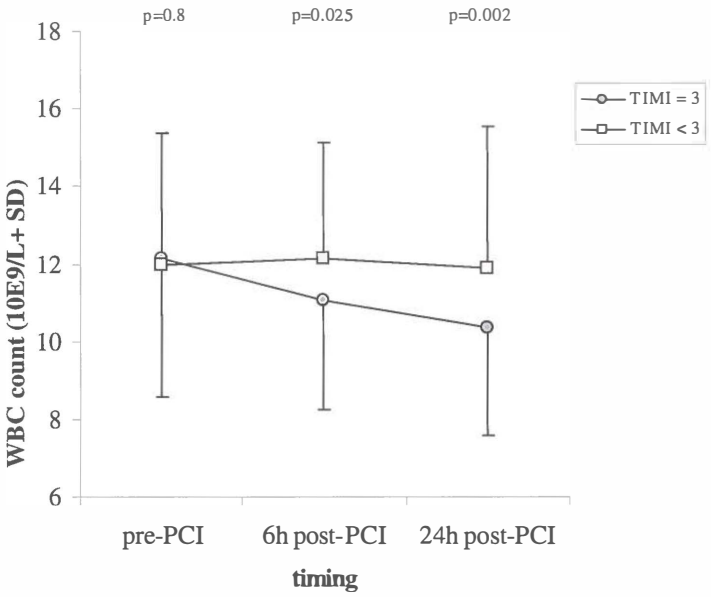

Figure 1. Serial WBC counts in patients with or without succesful PCI (TIMI <3) for STEMI.

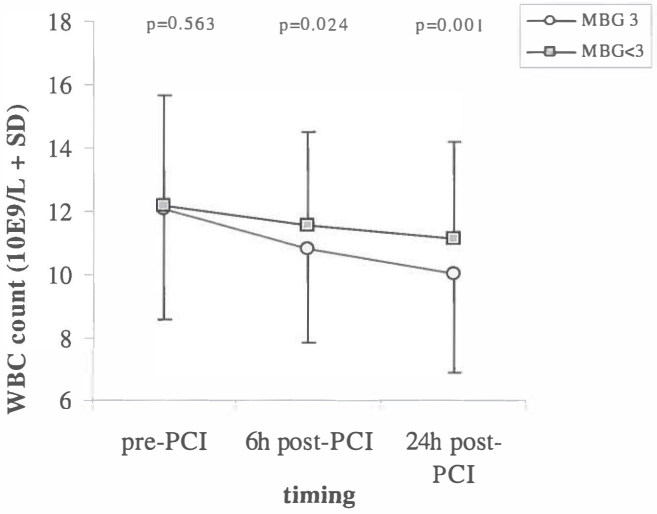

Figure 2. Serial WBC counts in patients with or without optimal myocardial blush
(MBG 3).

Discussion

This is the first study showing that WBC count after an acute STEMI decreases within hours and is related to the success of reperfusion therapy as reflected by TIMI flow and myocardial blush grade. Baseline WBC count has been associated with poor clinical outcome in patients with stable angina, unstable angina as well as non-STEMI as STEMI.[4-9,13-15] In part, this can be explained by lower coronary patency rates in patients with elevated baseline WBC counts.[14] The relation between serial WBC count measurements and clinical outcome after acute myocardial infarction was recently published in patients treated with thrombolysis or primary PCI.[16] However, in that study, no angiographic parameters were investigated and no relationship with success of reperfusion was assessed. In our study, change in WBC count (as soon as 6 hours after PCI) reflected success of PCI. Therefore, change in WBC count may be an early marker of reperfusion.

Leukocytosis is a marker of inflammation and is associated with the inflammatory response at the site of plaque in patients with an acute myocardial infarction.[1,17,18] As part of this inflammatory reaction, cytokines like IL-6, IL-8 and CD40 ligand trigger upregulation of monocyte tissue factor expression, which may facilitate the extrinsic pathway of the coagulation cascade.[19,20] Activated leukocyte receptors can further activate thrombosis.[20] Leukocyte aggregated vessel plugging and thrombus formations are thought to lead to ischemic damage, further enhanced by oxygen-free radical formation and complement activation by the activated leukocytes.[21-25] A high state of inflammation is suggested to reflect clinically more significant and unstable coronary artery disease.[17] However, our study shows that the WBC count is a dynamic and early marker, dependent on success of reperfusion. Therefore, WBC count may be a marker of an acute stress reaction, comparable to hyperglycemia or brain natriuretic peptide, rather than a marker of increased general inflammation.[26] If so, change in WBC count in patients with STEMI reflects neuro-humoral imbalance instead of the state of inflammation of the coronary arteries.

Limitations

As a substudy of the On-TIME trial, this study was not designed to investigate the relation between WBC count and angiographic outcome. Therefore, the relationship between change in WBC count and myocardial perfusion must be interpreted with caution and investigated in larger trials. Furthermore, in 28% of patients either one or more WBC measurements were missing. Since some patients died before all WBC counts could be measured, the characteristics from the original study differ from our study population. However, since myocardial perfusion is worse in patients who do not survive their STEMI, the relationship between reperfusion and the change in WBC count might be even more pronounced in these patients. Because the relation between change in WBC was not expected to be correlated with angiographic outcome shortly after PCI we did not measure change in WBC counts within 6 hours after PCI. These data would have given additional insight into the change of WBC very shortly after PCI.

Conclusions

WBC count decreased over time after successful reperfusion after primary PCI for STEMI, but remained elevated in patients with unsuccessful reperfusion. WBC count might therefore emerge as an early marker for adverse events shortly after PCI.

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Chapter 7

Change of white blood cell count more prognostic important than baseline values after primary percutaneous coronary intervention for ST elevation myocardial infarction

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Abstract

Introduction

Elevated white blood cell (WBC) count in patients with ST-segment elevation myocardial infarction (STEMI) on admission has been associated with an adverse prognosis. Recently, we found that impaired myocardial reperfusion after primary percutaneous coronary intervention (PCI) for STEMI is associated with persistent WBC elevation. It is unknown whether persistent WBC count elevation is also associated with impaired clinical outcome and whether this is even more important than a single baseline WBC count.

Methods

In this subanalysis of the On-TIME trial, WBC count was measured on admission, as well as 6 and 24 hours after admission in 364 patients undergoing primary PCI for STEMI. Baseline and change in WBC (ΔWBC) count were compared with ST resolution, LDHQ48, ejection fraction, reinfarction and one year all-cause mortality.

Results

Mean WBC count decreased from 12.0 ± 3.4 before PCI to 11.0 ± 3.0 and 10.5 ± 3.1, 6 and 24 hours after admission respectively (mean ± standard deviation). In one-year survivors WBC count decreased after 6 and 24 hours: ΔWBC -0.9 ± 0.8 and -1.5 ± 0.9 resp., in contrast to patients who died: ΔWBC 0.8 ± 2.9 and 0.9 ± 4.4 (p-value for difference after 6 and 24 hours: 0.010 and 0.002). After multivariate analysis, persistent elevation of WBC counts after PCI was an independent predictor of one-year mortality, whereas WBC count on admission was not.

Conclusion

The change of WBC count after primary PCI for STEMI has more prognostic importance than a single baseline WBC count. In assessing the predictive value of WBC count, the timing of blood sampling should be taken into account.

Keywords: white blood cell count, leukocyte, prognosis, inflammation, STEMI

Introduction

Elevated baseline white blood cell (WBC) counts in patients with ST segment elevation myocardial infarction (STEMI) have been associated with increased infarct size and higher in-hospital and long-term mortality.[1-6] Persistent WBC count elevation may be an even more important predictor of a poor prognosis than a single baseline WBC count. However, the relationship between serial WBC measurements and clinical outcome in patients with STEMI is unknown. Therefore, we measured serial WBC counts, before and after primary percutaneous coronary intervention (PCI) in patients with a STEMI and assessed whether WBC count changes were associated with clinical outcome.

Methods

Patients

It concerns a sub-analysis of data from the On-TIME trial, a prospective, double-blinded, randomized controlled trial investigating early versus late initiation of Tirofiban in STEMI.[7] In brief, patients were included if there was more than 30 minutes of chest pain with more than 0.2 mV (anterior myocardial infarction, MI) or 0.1 mV (non-anterior MI) of ST elevation in 2 contiguous ECG leads and the ability to perform primary angioplasty within 6 hours after the start of symptoms. Patients over 80 years of age, women less than 50 years of age, patients who were treated with thrombolytic therapy in the previous 24 hours, patients on warfarin or acenocoumarol within the last 7 days and patients with a contraindication to glycoprotein (Gp) IIb/IIIa blockade were excluded. Patients with severe heart failure or cardiogenic shock (Killip class III or IV) and patients on hemodialysis were also excluded. The protocol was approved by our institution's Review Board and Ethical Committee and informed consent was obtained in all patients. All patients underwent primary PCI immediately after hospital admission. Post-PCI, all patients were treated with Clopidogrel (300 mg loading dose followed by 75 mg daily for a month), acetylsalicylic acid, beta blockade, statin therapy and ACE inhibition.

WBC count

Blood samples were taken for routine WBC measurement on admission, before PCI and 6 and 24 hours after admission, after PCI (Sysmex K4500, Sysmex Corp., Kobe Japan). ΔWBC was defined as the WBC count after 6 or 24 hours minus the WBC count at admission. The primary objective of our subanalysis was to compare WBC count and change in WBC count (ΔWBC) with clinical outcome.

Clinical outcome

Enzymatic infarct size (lactate dehydrogenase, LDHQ48), nuclear ejection fraction (EF), ST-segment resolution after PCI and one-year death from all causes were recorded.[8] High enzyme release was defined as a LDHQ48 release more than the median. More pronouncement of impairment of ejection fraction was defined as ejection fraction below the median. Complete ST resolution was defined as described before[8]. Recurrent myocardial infarction was defined as a new increase in creatin kinase (CK)-MB fraction of more than 3 times the upper limit of normal whether or not accompanied by chest pain / and or ECG changes and present in two separate blood samples or not.[7]

Statistics

Statistical analysis was performed with the SPSS 12.0 statistical package. Continuous data were expressed as mean ± standard deviation of mean, and categorical data as percentage, unless otherwise denoted. Significance of differences in WBC counts over time was tested by the paired t-test. The analysis of variance and the chi-square test were appropriately used for continuous and categorical variables respectively. For univariate and multivariate analysis, WBC count was dichotomized and a decrease of WBC count was defined as a decline more than the median change of WBC count. Heart rate and blood pressure were also dichotomized at the median. The Cox hazard method was used to identify independent predictors of one-year mortality.

Results

From June 2001 to November 2002, 507 patients were included in the On-TIME trial. Three patients died before WBC count could be measured after PCI and in 140 patients (28%) one of the serial WBC counts was missing. WBC measurements at all different time points were performed in 364 of 507 (72%) recruited patients. Median LDHQ48 was 1311.5 U/L and median ejection fraction was 45%. The mean time between hospital admission and first balloon inflation was 46 ± 23 minutes. A higher baseline WBC count was associated with age ≤ 65 years, smoking, no previous coronary artery bypass grafting (CABG) or high enzyme release (table 1).

Mean WBC count decreased from 12.0 ± 3.4 before PCI to11.0 ± 3.0 and 10.5 ± 3.1, 6 and 24 hours after admission respectively (mean difference: -1.0 ± 3.0, $p < 0.001$, and -1.5 ± 3.2, $p < 0.001$ resp.). In table 2, the relationship between ΔWBC count and baseline characteristics and clinical outcome is shown after 6 and 24 hours.

WBC count decrease after 6 hours was significantly higher in patients with lower systolic blood pressure on admission. After 24 hours, WBC count was particularly decreased in smokers, those without previous coronary artery bypass grafting (CABG) and patients with complete ST segment resolution.

Table 1. Baseline characteristics and clinical outcome and relationship with baseline WBC count

	WBC count (mean ± SD), n		
	with characteristic	without characteristic	p-value
age ≥ 65 years	(11.25 ± 3.01), 164	(12.53 ± 3.66), 200	< 0.001
male gender	(12.06 ± 3.47), 293	(11.55 ± 3.28), 71	0.266
diabetes mellitus	(11.21 ± 2.83), 36	(12.04 ± 3.49), 328	0.167
previous infarction	(11.25 ± 2.85), 31	(12.02 ± 3.48), 333	0.233
previous PCI	(11.34 ± 2.96), 20	(11.99 ± 3.46), 344	0.406
previous CABG	(9.00 ± 2.42), 9	(12.03 ± 3.43), 355	0.009
smoking*	(12.64 ± 3.43), 236	(10.67 ± 3.08), 127	<0.001
hypercholesterolemia	(11.60 ± 2.96), 78	(12.05 ± 3.55), 286	0.304
hypertension	(11.63 ± 3.60), 93	(12.07 ± 3.38), 271	0.284
family history of ACS	(12.12 ± 3.50), 137	(11.84 ± 3.39), 226	0.449
anterior infarction	(12.07 ± 3.25), 160	(11.83 ± 3.49), 199	0.506
one-vessel disease	(12.08 ± 3.38), 157	(11.73 ± 3.37), 188	0.342
Killip class > 1	(11.71 ± 3.37), 50	(12.08 ± 3.49), 279	0.481
RR systolic > 134 mm Hg	(11.72 ± 3.09), 166	(12.36 ± 3.80), 164	0.092
RR diastolic > 81 mm Hg	(12.02 ± 3.49), 152	(12.10 ± 3.48), 175	0.844
heart rate > 73 BPM	(11.94 ± 3.36), 158	(12.04 ± 3.53), 195	0.800
early Tirofiban initiation	(11.96 ± 3.46), 185	(11.95 ± 3.42), 179	0.983
complete ST segment resolution	(11.95 ± 3.40), 235	(11.76 ± 3.29), 90	0.641
high enzyme release	(12.70 ± 3.54), 125	(10.83 ± 3.03), 125	< 0.001
ejection fraction > 45% (n)	(11.69 ± 3.38), 140	(12.15 ± 3.32), 146	0.245
reinfarction 30 days (n)	(10.13 ± 1.97), 4	(11.97 ± 3.45), 359	0.286
one-year mortality	(12.28 ± 3.60), 13	(11.94 ± 3.42), 348	0.723

CABG = coronary artery bypass grafting, ACS = acute coronary syndrome, PCI = primary coronary intervention, *current or previous smoking

Table 2. Baseline characteristics and clinical outcome compared to ΔWBC count 6 and 24 hours after PCI

	ΔWBC count (mean ± SD) 6 hours			ΔWBC count (mean ± SD) 24 hours		
	with characteristic	without characteristic	p-value	with characteristic	without characteristic	p-value
age ≥ 65 years	-0.68 ± 2.36	-1.15 ± 2.97	0.105	-1.10 ± 2.72	-1.75 ± 3.52	0.053
male gender	-0.98 ± 2.79	-0.76 ± 2.43	0.527	-1.48 ± 3.34	-1.36 ± 2.56	0.787
diabetes mellitus	-0.38 ± 2.80	-1.00 ± 2.71	0.194	-0.63 ± 2.99	-1.54 ± 3.21	0.105
previous infarction	-0.95 ± 2.09	-0.94 ± 2.78	0.980	-1.16 ± 3.03	-1.48 ± 3.22	0.591
previous PCI	-1.10 ± 2.34	-0.93 ± 2.74	0.794	-1.75 ± 2.70	-1.44 ± 3.23	0.676
previous CABG	0.13 ± 1.41	-0.96 ± 2.74	0.231	1.27 ± 3.23	-1.52 ± 3.17	0.010
smoking*	-1.09 ± 2.94	-0.63 ± 2.22	0.120	-1.78 ± 3.42	-0.83 ± 2.65	0.006
hypercholesterolemia	-1.02 ± 2.30	-0.92 ± 2.83	0.777	-1.39 ± 2.84	-1.47 ± 3.29	0.847
hypertension	-0.73 ± 2.33	-1.01 ± 2.84	0.387	-1.41 ± 2.77	-1.47 ± 3.34	0.880
family history of ACS	-1.01 ± 2.54	-0.89 ± 2.83	0.671	-1.47 ± 2.94	-1.43 ± 3.35	0.908
anterior infarction	-0.67 ± 2.48	-1.13 ± 2.87	0.108	-1.08 ± 2.75	-1.73 ± 3.45	0.056
one-vessel disease	-1.13 ± 2.80	-0.86 ± 2.27	0.322	-1.73 ± 3.10	-1.33 ± 2.72	0.195
Killip class > 1	-0.44 ± 4.01	-1.07 ± 2.48	0.143	-0.76 ± 4.58	-1.65 ± 2.90	0.073
RR systolic > 134 mm Hg	-0.65 ± 2.28	-1.28 ± 3.14	0.035	-1.33 ± 2.64	-1.70 ± 3.71	0.293
RR diastolic > 81 mm Hg	-0.72 ± 2.38	-1.22 ± 3.04	0.099	-1.24 ± 2.82	-1.79 ± 3.52	0.122
heart rate > 73 BPM	-0.77 ± 2.73	-1.14 ± 2.74	0.198	-1.18 ± 2.94	-1.79 ± 3.38	0.077
early Tirofiban initiation	-1.01 ± 3.01	-0.86 ± 2.39	0.602	-1.49 ± 3.56	-1.42 ± 2.79	0.850
complete ST segment resolution	-1.06 ± 2.85	-0.51 ± 2.29	0.098	-1.65 ± 3.28	-0.82 ± 2.75	0.033
high enzyme release	-0.56 ± 3.08	-0.89 ± 2.31	0.340	-1.12 ± 3.70	-1.32 ± 2.90	0.637
ejection fraction > 45% (n)	-1.24 ± 2.52	-0.79 ± 2.57	0.132	-1.79 ± 2.88	-1.45 ± 2.81	0.313
reinfarction 30 days (n)	0.7 ± 3.68	-0.95 ± 2.71	0.228	-0.38 ± 4.56	-1.46 ± 3.19	0.500
one-year mortality	0.82 ± 5.48	-1.00 ± 2.56	0.018	0.76 ± 7.10	-1.53 ± 2.95	0.011

CABG = coronary artery bypass grafting, ACS = acute coronary syndrome, PCI = primary coronary intervention, *current or previous smoking

Only 13 patients died within one year (3.6%). Univariate predictors of mortality were age (OR 1.08, 95% CI 1.01-1.16, p = 0.017), Killip class > 1 at admission (OR 5.13, 95%CI 1.50-17.54, p = 0.009), enzymatic infarct size (OR

8.55, 95% CI 1.05-69.43, p = 0.045) and change of leukocytes after 6 hours (OR 1.21, 95% CI 1.02-1.42, p = 0.025) and 24 hours (OR 1.17, 95% CI 1.02-1.33, p = 0.021). Increased baseline WBC count was not an univariate predictor of mortality (OR 1.03, 95% CI 0.88-1.20, p = 0.722 and table 1). However, decreasing WBC count after PCI was associated with survival at one year (figure 1).

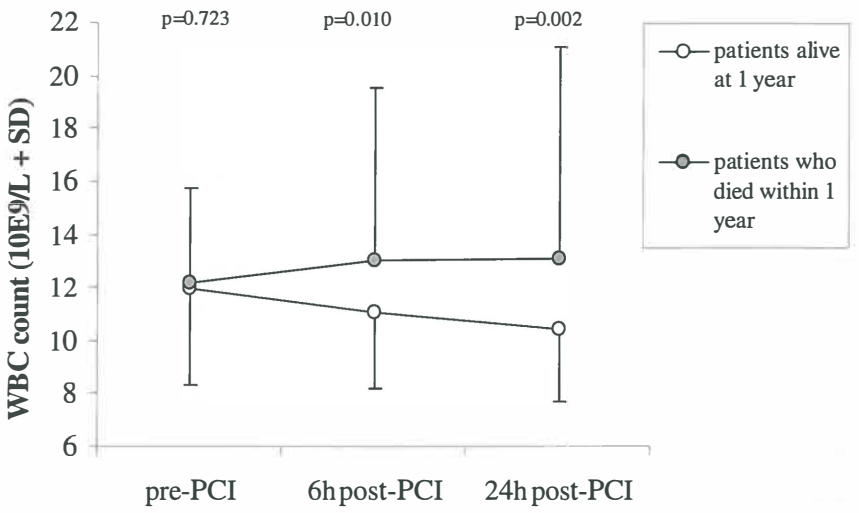

Figure 1. Survival after STEMI is associated with decrease in WBC count after PCI.

For multivariate analysis we included: baseline WBC count, age, gender, smoking and WBC count change after 6 or 24 hours into the final model. Increase of WBC count after PCI was an independent predictor of one-year all-cause mortality (ΔWBC count after 6 hours: OR 1.28, 95% CI 1.07-1.53, p = 0.007; ΔWBC count after 24 hours: OR 1.22, 95% CI 1.06-1.42, p = 0.007, whereas baseline WBC count was not significantly associated with mortality (WBC count after 6 hours: OR 1.22, 95% CI 0.99-1.50, p = 0.061; WBC count after 24 hours: OR 1.20, 95% CI 0.98-1.45, p = 0.075).

Discussion

This is the first study suggesting that the change of WBC count after PCI may be more prognostic important in patients with STEMI treated with primary PCI

than a single baseline WBC measurement. In our study, persistent WBC elevation after PCI for STEMI was associated with increased mortality, whereas elevated baseline WBC count was no univariate or multivariate predictor of mortality.

Thirty years ago the association between leukocyte count and a myocardial infarction was made for the first time.[1] Since then, baseline WBC count has been associated with clinical outcome in patients with stable angina, unstable angina as well as non ST elevation myocardial infarction and ST elevation myocardial infarction.[2-6,9-11] The relation between serial WBC count measurements and clinical outcome after acute myocardial infarction was recently published for the first time in a combined study population of patients treated with either thrombolysis or primary PCI.[12] At the same time, we showed that impaired myocardial reperfusion resulted in lack of decrease of WBC count after PCI for STEMI.[13] The impaired prognosis of patients with persistent elevated serial WBC counts after STEMI found in the present study, might be explained by the worse myocardial perfusion after primary PCI in patients with persistent elevated WBC counts.[13] Since outcome after STEMI is largely determined by the success of PCI (sustained reperfusion), change in WBC counts after PCI might be a more important predictor of outcome than single baseline WBC count measurement.[14] In the current study, the relationship between change in WBC count after PCI and mortality was clearly found, whereas the relationship between baseline WBC count and outcome was only weak. The lack of a significant association between baseline WBC count and clinical outcome might be the result of a low-risk study population treated with primary PCI within only 46 minutes on average, selected after 24 hours survival with a limited sample size in comparison with previous trials.[9-11]

In concordance with former studies, younger patients, smokers and patients with lower ejection fraction had higher baseline WBC counts.[13] In addition, we also found younger patients to show less decrease in WBC counts. In contrast to our study, Patel found that a decrease in WBC count was associated with a lower enzymatic infarct size.[12] Successful reperfusion resulting in more pronounced impairment of enzymatic infarct size might explain reduced mortality in patients with reduction in WBC count after PCI.

Leukocytosis is a marker of inflammation and is associated with the inflammatory response at the site of plaque and in the myocardium in patients with an acute myocardial infarction.[15-17] As part of this inflammatory reaction, cytokines like IL-6, IL-8 and CD40 ligand trigger upregulation of monocyte tissue factor expression, which may facilitate the extrinsic pathway of the coagulation cascade.[18,19] Activated leukocyte receptors can further activate thrombosis.[19] Leukocyte aggregated vessel plugging and thrombus formations are thought to lead to ischemic damage, further enhanced by oxygen-free radical formation and complement activation by the activated leukocytes.[20-24] A high state of inflammation may reflect clinical more significant and unstable coronary artery disease.[16] However, our study shows that the WBC count is a dynamic and early marker of outcome after reperfusion. Therefore, WBC count may be a marker of an acute stress reaction, comparable to hyperglycemia or BNP, rather than a marker of increased general inflammation.[25] If so, change in WBC count in patients with STEMI reflects neuro-humoral imbalance in stead of the state of inflammation of the coronary arteries. Despite the novelty of our findings, the difference in absolute change of WBC count between survivors and non-survivors seems too small to be a clinical marker of poor prognosis, but it can stimulate further research.

Limitations

As a substudy of a randomized trial, this study was not designed to investigate the relation between WBC count and clinical outcome, and in 28% of patients, some of the serial WBC counts were missing. Therefore, the relationship between change in WBC count and clinical outcome must be interpreted with caution and confirmed in larger trials. Since patients were excluded in which no serial WBC counts were measured due to early fatality, the baseline characteristics differ from the original study.

Conclusion and implications

The change of WBC count after primary PCI for STEMI is a stronger predictor of mortality than a single baseline WBC count. Therefore, dynamic WBC changes emerge as an early marker for adverse events after PCI. Furthermore, WBC count in patients with STEMI might be better characterized as a marker of neuro-humoral imbalance rather than a static marker of inflammation.

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Chapter 8

Comparison of usefulness of C-reactive protein versus white blood cell count to predict outcome after primary percutaneous coronary intervention for ST elevation myocardial infarction

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Abstract

Introduction

White blood cell (WBC) count and high-sensitive C-reactive protein (hs-CRP) are both used as markers of inflammation and prognosis after a ST elevation myocardial infarction (STEMI), but it is unknown whether they have independent prognostic value. We investigated the association and independent prognostic importance of WBC and hs-CRP after STEMI.

Methods

In this subanalysis of the On-TIME trial, in 490 of 507 (97%) patients either WBC count or CRP, and in 362 (71%) patients both WBC count and CRP were measured on admission before primary percutaneous coronary intervention.

Results

There was no significant correlation between WBC count and CRP ($R = 0.08$). Higher levels of CRP were associated with a reinfarction or death within 1 year (mean hs-CRP 14.2 ± 20.4, vs. 6.1 ± 14.2, $p = 0.006$), but CRP was not associated with enzymatic infarct size (lactate dehydrogenase, LDHQ48) or left ventricular ejection fraction. A higher baseline WBC count was associated with larger LDHQ48 and lower left ventricular ejection fraction, but not with one year reinfarction or death.

Conclusions

Although both WBC count and CRP are markers of inflammation and predictors of outcome after STEMI, we did not find a correlation between baseline WBC count and CRP levels in patients treated with primary percutaneous coronary intervention for STEMI. The mechanisms by which WBC counts predict outcome were myocardial infarct size related whereas CRP were not.

Keywords: hs-CRP, WBC count, STEMI, inflammation, prognosis, CRP

Introduction

Inflammation plays an important role in the development and prognosis of acute coronary syndromes.[1-3] C-reactive protein (CRP) is a sensitive marker of inflammation and has been associated with outcome after an acute myocardial infarction in multiple studies.[4-11] Elevated baseline white blood cell (WBC) counts in patients with ST segment elevation myocardial infarction (STEMI) have also been associated with a poor prognosis.[5,12-16] However, no comparison of high-sensitive (hs) CRP and WBC count has ever been made in STEMI patients undergoing primary percutaneous coronary intervention. There are few data available trying to find a mechanism by which hs-CRP or WBC count predict clinical outcome. Therefore, we sought to investigate the association between, and independent prognostic value of hs-CRP and WBC count and its association with angiographic outcome and left ventricular ejection fraction in patients with STEMI undergoing primary percutaneous coronary intervention.

Methods

Patients

It concerns a sub-analysis of data from the On-TIME trial, a prospective, double-blind, randomized controlled trial investigating early versus late initiation of Tirofiban in STEMI.[17] In brief, 507 patients were included if there was > 30 minutes of chest pain with > 0.2 mV (anterior myocardial infarction) or 0.1 mV (non-anterior myocardial infarction) of ST elevation in 2 contiguous electrocardiographic leads and the ability to perform primary angioplasty within 6 hours after the start of symptoms. Patients > 80 years of age, women < 50 years of age, patients who were treated with thrombolytic therapy in the previous 24 hours, patients on warfarin or acenocoumarol within the last 7 days and patients with a contraindication to glycoprotein IIb/IIIa blockade were excluded. Patients with severe heart failure or cardiogenic shock (Killip class III or IV) and patients on haemodialysis were also excluded. The protocol was approved by our institution's Review Board and Ethical Committee and

informed consent was obtained from all patients. At coronary angiography, the initial injection was used to assess TIMI flow of the infarct related vessel. Percutaneous coronary intervention was performed immediately after coronary angiography. In 70% of patients stents were used. Post percutaneous coronary intervention, patients were to be treated with clopidogrel (300 mg loading dose followed by 75 mg daily for a month), acetylsalicylic acid, beta blockade, statin therapy and angiotensin converting enzyme inhibition.

hs-CRP

The plasma concentration of hs-CRP were measured in venous blood drawn on admission to the hospital using an immunoturbidimetric assay (Modular; Roche Diagnostics GmbH, Mannheim, Germany) covering a range of 0.1 to 20 mg/L. For values below the limit of detection, the lower limit was used for statistical analysis.

WBC count

Blood samples were taken for routine WBC measurement on admission, before percutaneous coronary intervention (Sysmex K4500, Sysmex Corporation., Kobe Japan). The primary objective of our sub-analysis was to associate baseline WBC count and CRP with angiographic and clinical outcome and to investigate the correlation between CRP and WBC count.

Angiographic and clinical outcome

All angiographic data were analyzed by an independent core-lab (Diagram Zwolle, the Netherlands) and scored by an observer who was unaware of randomization, WBC counts or outcome. Judgment of infarct related vessel flow was made on initial contrast injection according the TIMI classification.[18] Successful angioplasty was defined as a less than 50% diameter stenosis and TIMI 3 flow of the infarct related vessel. Myocardial blush grade were defined post percutaneous coronary intervention as previously described.[19] Enzymatic infarct size (LDHQ48), ejection fraction (< 30%), reinfarction rate and one year all-cause mortality were also assessed, as previously described.[20]

Statistics

Statistical analysis was performed with the SPSS 13.0 statistical package. Continuous data were expressed as mean ± standard deviation (SD), and categorical data as percentage, unless otherwise denoted. Significance of differences in WBC counts over time was tested by the paired t-test. The analysis of variance and the chi-square test were appropriately used for continuous and categorical variables respectively. A multiple logistic regression analysis was performed to identify independent correlates of WBC count and CRP in the population in which both CRP as WBC counts were measured using all parameters from tables 2 and 3. The stepwise selection of variables and estimation of significant probabilities were computed by means of maximal likelihood ratio tests. For analysis of the multivariate predictors of infarct size, LDHQ48 was dichotomized at the median. Blood pressure and heart rate were also dichotomized at the median. The chi-square value was calculated from the log of the ratio of maximal partial likelihood functions. The additional value of each category of variables added sequentially was evaluated on the basis of the increases in the overall likelihood statistic ratio. A p-value of < 0.05 was considered statistically significant.

Results

In 490 patients (97%) included in the On-TIME trial either WBC count or CRP was measured. A WBC count was available in 473 of 507 (93%), and in 379 (75%) patients CRP was measured. Both markers were available in 362 (71%) patients. The baseline characteristics are shown in table 1. There were no important differences between total included patients in the On-TIME trial and those in which either or both WBC count and CRP were determined. The baseline characteristics according to elevated CRP or WBC count are shown in table 2. There was no significant correlation between WBC count and CRP (figure 1, R=0.08). Angiographic and clinical outcome data are shown in table 3.

Table 1. Baseline characteristics of patients according to measurement of white blood cell count or C-reactive protein or both

	WBC count measured (n=473)	CRP measured (n=379)	both measured (n=362)
age ≥ 65 years	43%	43%	42%
male gender	81%	81%	82%
diabetes mellitus	11%	10%	11%
previous infarction	8%	8%	8%
previous PCI	5%	5%	6%
previous coronary artery bypass grafting	2%	2%	3%
smoking[a]	64%	65%	64%
hypercholesterolemia	23%	23%	23%
hypertension	28%	29%	29%
family history of acute coronary syndrome	40%	40%	40%
anterior infarction	45%	46%	47%
one-vessel disease	45%	48%	48%
Killip class > 1	16%	16%	16%
blood pressure systolic > 134 mm Hg	50%	50%	50%
blood pressure diastolic > 81 mm Hg	45%	45%	45%
heart rate > 73 BPM	46%	46%	47%
early Tirofiban initiation	50%	52%	49%
acetylsalicylic acid at discharge	97%	98%	99%
beta-blocker at discharge	89%	90%	91%
ace inhibitor at discharge	52%	53%	54%
statin at discharge	85%	89%	90%
clopidogrel at discharge	79%	80%	81%

[a]current or previous smoking

Table 2. Baseline characteristics of patients according to C-reactive protein and white blood cell count (n=490)

	C-reactive protein (mg/L, mean ± SD)			white blood cell count (10^9/L, mean± SD)		
	with characteristic	without characteristic	p-value	with characteristic	without characteristic	p-value
age ≥ 65 years	6.64 ± 11.87 (n = 162)	6.64 ± 13.75 (n = 217)	0.999	11.10 ± 3.14 (n=201)	12.57 ± 3.79 (n = 272)	< 0.001
male gender	6.12 ± 11.71 (n = 306)	8.85 ± 17.17 (n = 73)	0.106	12.07 ± 3.63 (n = 381)	11.42 ± 3.42 (n = 92)	0.117
diabetes mellitus	7.76 ± 16.76 (n = 39)	6.54 ± 12.51 (n = 338)	0.579	11.25 ± 3.22 (n = 50)	12.03 ± 3.64 (n = 423)	0.151
previous infarction	9.56 ± 19.30 (n = 29)	6.42 ± 12.34 (n = 348)	0.213	10.97 ± 2.73 (n = 39)	12.04± 3.66 (n = 433)	0.076
previous PCI	8.74 ± 12.44 (n = 201)	6.55 ± 13.03 (n = 357)	0.463	11.02 ± 2.80 (n = 25)	12.00 ± 3.63 (n = 448)	0.186
previous CABG	11.66 ± 13.09 (n = 9)	6.54 ± 12.99 (n = 368)	0.243	8.88 ± 2.18 (n = 11)	12.02 ± 3.60 (n = 462)	0.004
smoking (current or previous)	6.60 ± 12.43 (n = 241)	6.83 ± 14.18 (n = 131)	0.871	12.73 ± 3.55 (n = 298)	10.61 ± 3.32 (n = 167)	< 0.001
hypercholesterol-emia	6.08 ± 10.15 (n = 88)	6.84 ± 13.76 (n = 289)	0.630	11.79 ± 3.31 (n = 108)	12.00 ± 3.68 (n = 364)	0.593
hypertension	7.51 ± 12.84 (n = 109)	6.32 ± 13.07 (n= 68)	0.421	11.73 ± 3.76 (n = 132)	12.03 ± 3.54 (n = 341)	0.407
family history of ACS	5.90 ± 12.37 (n = 150)	7.14 ± 13.43 (n=225)	0.366	12.19 ± 3.89 (n = 186)	11.79 ± 3.39 (n = 283)	0.232
anterior infarction	5.70 ± 11.20 (n = 170)	7.36 ± 14.30 (n = 197)	0.222	12.02 ± 3.37 (n = 207)	11.96 ± 3.74 (n = 250)	0.850
one-vessel disease	6.07 ± 12.54 (n = 178)	7.18 ± 13.51 (n = 193)	0.414	12.20 ± 3.73 (n = 192)	11.70 ± 3.45 (n = 235)	0.149
Killip class > 1	8.65 ± 17.70 (n = 56)	6.53 ± 12.56 (n = 287)	0.285	11.47 ± 3.46 (n = 67)	12.11 ± 3.67 (n = 360)	0.189
RR systolic > 134 mm Hg	7.11 ± 13.94 (n = 171)	6.63 ± 13.09 (n = 173)	0.743	11.77 ± 3.43 (n = 214)	12.21 ± 3.79 (n = 216)	0.209
RR diastolic > 81 mm Hg	5.96 ± 10.99 (n = 154)	7.26 ± 14.95 (n = 187)	0.368	12.07 ± 3.59 (n = 191)	11.96 ± 3.65 (n = 236)	0.754
HR > 73 BPM	8.73 ± 16.73 (n = 170)	5.11 ± 8.69 (n = 196)	0.008	11.90 ± 3.63 (n = 209)	12.07 ± 3.62 (n = 249)	0.613
early Tirofiban initiation	7.16 ± 13.57 (n = 182)	6.16 ± 12.39 (n = 197)	0.452	12.00 ± 3.54 (n = 235)	11.89 ± 3.67 (n = 238)	0.751
ASA at discharge	6.42 ± 12.79 (n = 364)	10.95 ± 10.32 (n = 7)	0.352	11.97 ± 3.62 (n = 454)	10.52 ± 3.08 (n = 15)	0.125
beta-blocker at discharge	6.39 ± 12.35 (n = 334)	7.51 ± 16.06 (n = 37)	0.614	11.98 ± 3.61 (n = 418)	11.52 ± 3.63 (n = 51)	0.399
ACE inhibitor at discharge	6.84 ± 14.12 (n = 198)	6.11 ± 11.01 (n = 173)	0.585	12.05 ± 3.49 (n = 242)	11.80 ± 3.73 (n = 227)	0.443
statin at discharge	6.23 ± 12.53 (n = 331)	8.71 ± 14.45 (n = 40)	0.247	12.00 ± 3.48 (n = 399)	11.51 ± 4.26 (n = 70)	0.299
clopidogrel at discharge	6.03 ± 11.76 (n = 298)	8.42 ± 16.12 (n = 73)	0.152	11.82 ± 3.51 (n = 371)	12.33 ± 3.96 (n = 98)	0.220

Table 3. Angiographic and clinical outcome of patients according to C-reactive protein and white blood cell count.

	C-reactive protein (mg/L, mean ± SD)			white blood cell count (10^9/L, mean± SD)		
	with characteristic	without characteristic	p-value	with characteristic	without characteristic	p-value
TIMI 0,1 flow pre-PCI	7.61 ± 15.44 (n = 231)	4.68 ± 6.63 (n = 140)	0.034	12.48 ± 3.41 (n = 287)	11.17 ± 3.68 (n = 172)	< 0.001
TIMI 3 flow post-PCI	6.69 ± 13.48 (n =313)	6.23 ± 9.81 (n = 33)	0.848	12.13 ± 3.54 (n = 381)	11.98 ± 3.41 (n = 38)	0.800
LDHQ48 > median	7.64 ± 13.23 (n = 103)	6.46 ± 12.43 (n = 121)	0.493	12.78 ± 3.52 (n = 134)	10.82 ± 3.12 (n = 154)	< 0.001
ejection fraction < 30% (n)	6.19 ± 9.83 (n = 28)	5.69 ± 10.58 (n = 253)	0.810	13.09 ± 3.73 (n = 34)	11.85 ± 3.38 (n = 253)	0.047
all-cause mortality < one year	13.76 ± 13.37 (n = 10)	6.36 ± 12.77 (n = 356)	0.072	12.16 ± 3.58 (n = 16)	11.93 ± 3.61 (n = 449)	0.809
all-causes one-year mortality + reinfarction	14.23 ± 20.38 (n = 20)	6.11 ± 14.23 (n = 346)	0.006	12.69 ± 3.82 (n = 30)	11.89 ± 3.59 (n = 435)	0.243

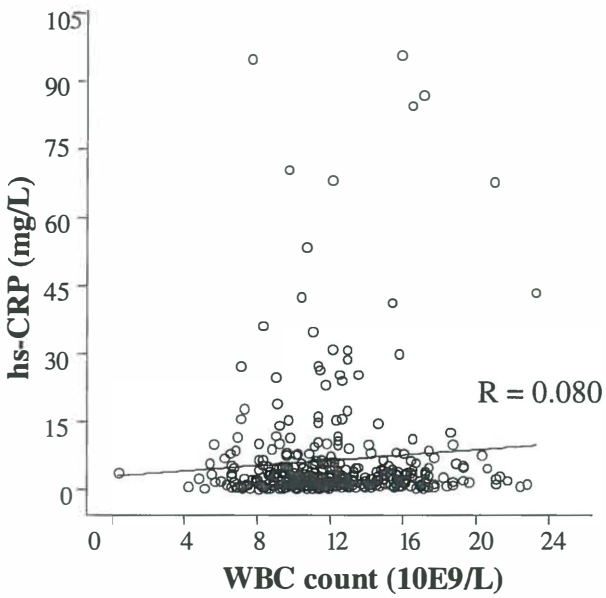

Figure 1. Correlation between WBC count and CRP (n=362).

Higher level of CRP (OR 1.03, 95% CI 1.01-1.05, p=0.012) was a significant predictor of the combined endpoint of reinfarction and one-year mortality,

whereas WBC count was not statistically associated with worse outcome (OR 1.09, 95% CI 0.96-1.23, p = 0.202). To assess independent predictors of the combined endpoint, multivariate analyses were performed. Variables included in the final model were age, gender, baseline WBC count and CRP. Higher levels of CRP (OR 1.03, 95% CI 1.01-1.06, p = 0.008) and older age (OR 1.08, 95% CI 1.02-1.14, p = 0.009) were associated with an increased risk of reinfarction or death at long-term follow-up. Although there was a trend, WBC count was again not statistically significantly associated with an increased incidence of death and/or reinfarction (OR 1.13, 95% CI 0.98-1.31, p = 0.09).

The median LDHQ48 was 1154 (95% CI 440-2507). Univariate predictors of increased enzymatic infarct size (LDHQ48 > median) were: hypotension (systolic blood pressure ≤ median: 134 mmHg: OR 1.93, 95% CI 1.10-3.37, p = 0.021) and baseline WBC count (OR 1.18, 95% CI 1.08-1.28, p < 0.001). After multivariate analysis, including age, gender, CRP, baseline WBC count and hypotension in the final model, WBC count (OR 1.19, 95%CI 1.09-1.31, p < 0.001) and hypotension (OR 1.82, 95%CI 1.01-3.27, p = 0.046) were independent predictors of increased infarct size.

Univariate predictors of ejection fraction <30% were an anterior wall myocardial infarction (OR 8.51, 95% CI 2.85-25.36, p < 0.001) and increased WBC count (table 3). After multivariate analysis, including anterior infarction, age, gender, WBC count and CRP into the final model, anterior infarction OR 9.61 (95% CI 3.16-29.19, p < 0.001) was the only independent predictor, whereas CRP had no association (OR 0.99, 95% CI 0.94-1.04, p = 0.72). WBC count was borderline significantly associated with a lower left ventricular ejection fraction (OR 1.14, 95% CI 1.00-1.31, p = 0.05).

Discussion

This is the first study comparing the prognostic importance of CRP and WBC counts on outcome after primary percutaneous coronary intervention for STEMI. No relationship between CRP and WBC count was found, and the mechanisms by which WBC counts predict outcome are infarct size related, in contrast to CRP.

Higher levels of CRP have been associated with worse outcome after acute myocardial infarction in multiple studies.[4-11] Even in stable condition, 25 days after a myocardial infarction, CRP significantly predicted mortality (HR 4.94, 95%CI 1.13-21.6).[21] Our findings are consistent with a study by Brunetti, in which CRP levels were predictors of 6-month major adverse cardiac events.[6] However, they found also no correlation between ejection fraction, angiographic findings and CRP levels.

Elevated baseline WBC counts in patients with STEMI have been associated with increased infarct size and higher in-hospital and long-term mortality.[5,12-16] That no relation between WBC count and reinfarction plus mortality was shown in our study is probably due to the small sample size and low event rate in the On-TIME-1 study. It should be expected that WBC counts predict long-term clinical outcome as it was associated with lower left ventricular ejection fraction and larger enzymatic infarct size.

Although both markers are used as markers of inflammation, the difference in mechanism of prognosis found in our study and former studies, suggests different underlying pathophysiological mechanisms.[22] Especially, since we recently found leukocytes to vary rapidly, depending on the success of reperfusion, WBC count might be a marker of neuro-humoral balance rather than a static marker of inflammation, in contrast to CRP.[23] On the other hand, fluctuation in CRP levels on the basis of the state of the coronary syndrome

(STEMI, non-STEMI or unstable angina) are known and CRP levels normally increase during the development of a acute coronary syndrome.[6,24,25] In these studies, however, only top level of CRP was correlated with clinical outcome and the importance of a change in CRP was not studied. Further investigation into the prognostic value of change in both CRP and leukocytes during the infarction is needed to support our theory. CRP might be a static marker of inflammated coronary arteries, and might therefore be used to identify high-risk patients, who could benefit from aggressive anti-inflammatory therapy.[26]A randomized clinical trial of statin therapy suggests that attributable risk reductions achieved by statins are more enhanced in the presence of elevated CRP.[27] The finding that higher baseline WBC counts were measured in younger patients and smokers was reported before.[15,16,28,29] Furthermore, smoking status was not significantly associated with CRP levels in a study of Abdelmouttaleb, as in our study.[30]

Limitations

As a substudy of the On-TIME trial, this study was not fully designed to investigate the relation between WBC count, CRP and angiographic outcome. Therefore, the relationship between WBC count, CRP and angiographic and clinical outcome must be interpreted with caution and investigated in larger trials. Since some patients died before blood samples could be measured, the characteristics from the original study are not entirely identical to our study population.

Conclusions

Although both WBC count and hs-CRP are markers of inflammation and predictors of outcome after a myocardial infarction, we did not find a correlation between baseline WBC count and hs-CRP levels in patients treated with primary percutaneous coronary intervention for STEMI. The mechanisms

by which WBC counts predict outcome were infarct size related whereas hs-CRP were not.

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Chapter 9

Summary and conclusions

Summary and conclusions

In this thesis, we have studied the role of leukocytes and platelets as well as methods to measure platelet aggregation, in the clinical management of patients presenting with acute coronary syndromes. We have tried to describe the incidence and to identify predictors of adverse cardiac events with platelet function tests or inflammatory markers. This will aid in the future identification of patient-specific therapeutic targets in patients with acute coronary syndromes.

In **Chapter 2** we have studied prospectively the incidence and predictors of subacute thrombosis in an unselected consecutive series of patients after primary percutaneous coronary intervention (PCI) for ST elevation myocardial infarction (STEMI). Subacute thrombosis occurred in 4.1% of patients during one-year follow-up. Signs of heart failure on admission, anterior myocardial infarction and stenting were predictors of subacute thrombosis. Previous studies have been performed in selected patient groups and have shown a lower incidence of subacute thrombosis or did not provide systematic angiographic evidence of target vessel occlusion. Therefore, this is one of the first studies in which a high incidence is reported of an important complication of primary PCI for STEMI in a large unselected patient population.

In **Chapter 3** we have tried to identify potential explanations for the lack of effectiveness of platelet aggregation inhibitory drugs to prevent thrombotic complications after primary PCI for an acute coronary syndrome. We investigated whether changes in functional characteristics of platelets as part of the underlying disease altered the platelet inhibitory response to a single oral dose of acetylsalicylic acid in patients with an acute coronary syndrome as compared with healthy volunteers. Acetylsalicylic acid had less platelet inhibitory effects in patients with unstable coronary artery disease in comparison with healthy volunteers.

In **Chapter 4a** the prognostic value of the level of platelet aggregation inhibition by GpIIb/IIIa blockers during primary PCI for STEMI was

investigated. No correlation was found between the level of platelet aggregation inhibition with tirofiban and clinical outcome. Furthermore, only a modest increase in platelet aggregation inhibition was observed after the usual dose of tirofiban, indicating a hyperactive state of platelets and incomplete inhibition of platelet aggregation during STEMI.

In **Chapter 4b** the role of the interindividual response to GpIIb/IIIa blockers and potential importance of platelet function testing in patients with an acute coronary syndrome is discussed. Since there is no international accepted definition of 'resistance' to an antiplatelet drug, it remains difficult to use results of platelet function testing as surrogate for the clinical effectiveness of these drugs. As current platelet function tests perform suboptimal, due to inappropriate agonists or lack of reproducibility, we developed a novel method of platelet function measurement: Fe-Induced Platelet Aggregation measurement (FIPA).

In **Chapter 5** the feasibility of this new platelet function test and a comparison with existing platelet function tests was described. FIPA measurement was a feasible and easy applicable method for platelet function measurement, resembling the trigger for platelet aggregation of stent placement in coronary arteries. Furthermore, FIPA could be measured in STEMI patients using potent platelet inhibitors as GpIIb/IIIa blockers in addition to clopidogrel and acetylsalicylic acid in contrast to other methods to assess platelet function.

In **Chapter 6** the relationship between signs of reperfusion after primary PCI for STEMI and serial white blood cell (WBC) counts after PCI is presented. Impaired myocardial reperfusion (TIMI<3 flow and myocardial blush grade<3) after primary PCI for STEMI was associated with persistent WBC elevation within hours after PCI, whereas WBC counts decreased over time after successful reperfusion. Improved myocardial perfusion was an independent predictor of a decrease in WBC count after PCI.

In **Chapter 7** we addressed whether changes in leukocyte count after primary PCI for STEMI were correlated to survival and reinfarction. Persistent WBC elevation after primary PCI was associated with increased mortality, whereas elevated baseline WBC count was no univariate or multivariate predictor of

mortality. We concluded that the change of WBC count after primary PCI for STEMI has more prognostic importance than a single baseline WBC count. Furthermore, the timing of blood sampling should be taken into account when the predictive value of WBC count is assessed.

Finally, **Chapter 8** is focused on the relationship between baseline WBC count and high sensitive C-reactive protein (hs-CRP). We studied differences in prognostic value of both markers. Although both WBC count and hs-CRP are markers of inflammation and predictors of poor outcome, no correlation was found between baseline WBC count and hs-CRP after primary PCI for STEMI. An increased WBC count was associated with a larger infarction size as reflected by enzymatic infarct size (LDHQ48) or left ventricular ejection fraction, while hs-CRP levels were related to the rate of reinfarction or death after 1 year. Both markers are used as markers of inflammation, but there is a fundamental difference in mechanism by which they impact on prognosis. This suggests different underlying pathophysiological mechanisms. Especially, since we found WBC counts to respond rapidly, in relation to the success of reperfusion therapy, WBC count may be a marker of the actual neuro-humoral balance more than just a static marker of inflammation, in contrast to CRP.

Final comments

Although it is known that platelet and leukocytes play an important role in development of an acute coronary syndrome, the role of measurement of platelet function and inflammatory markers in clinical practice to predict the outcome of an acute coronary syndrome is largely unknown.

Platelets

The incidence of thrombotic events after primary PCI for STEMI is higher in clinical practice than usually reported in randomized clinical trials. Antiplatelet regimes are not as effective in patients with acute coronary syndromes compared to healthy volunteers. Furthermore, bleeding risks are known to

increase when full-dose antiplatelet regimes are administered to all unselected patients. Therefore, expanding our knowledge about platelet function will provide a basis for patient-specific tailored antithrombotic therapy. However, as shown in this thesis, the assessment of platelet function remains a major challenge. We have developed a new, applicable and promising platelet function test using stainless steel as an agonist. However, this should be confirmed in a larger patient population, using different anti-platelet drugs. Furthermore, we are awaiting the results of the predictive value of our FIPA test in the ongoing On-TIME-2 study, in which the test will be correlated with clinical outcome in a large STEMI population.

White blood cells

Our perspective on the role of leukocytes during an acute myocardial infarction has changed fundamentally during our research. Since leukocyte counts varied quickly, and are in particular responsive to success of reperfusion, variation in leukocytes has emerged as a powerful predictor of mortality and has more prognostic implications than baseline levels. Furthermore, the mechanisms by which leukocytes predict mortality and reinfarction are related to infarct size. This is in contrast to hs-CRP, a well known marker of inflammation and predictor of outcome in patients with acute coronary syndromes. These findings imply different underlying pathophysiological mechanisms by which leukocytes and hs-CRP are related to clinical outcome. Therefore, leukocytes are markers of changes in neuro-humoral balance rather than static markers of inflammation, in contrast to CRP. Further research is needed to elaborate on this hypothesis.

Future research will be focused on the elucidation of the underlying pathophysiologic mechanisms and will further expand the role of measurements of leukocytes, platelets and platelet aggregation in the rapidly evolving field of percutaneous coronary interventions in patients presenting with an acute coronary syndrome.

Chapter 10

Samenvatting en conclusies

Samenvatting en conclusies

In dit proefschrift, onderzochten we de rol van leukocyten en bloedplaatjes als ook methoden om bloedplaatjesaggregatie te meten bij patiënten die zich presenteren met een acuut coronair syndroom. We hebben getracht de incidentie en voorspellers van een slechte cardiale uitkomst te beschrijven door bij deze patiënten bloedplaatjesaggregatie en markers van ontsteking te meten. In de toekomst zouden de uitkomsten van dit onderzoek nuttig kunnen zijn om patient-specifieke therapeutische maatregelen te treffen bij patiënten die zich presenteren met een acuut coronair syndroom.

In **hoofdstuk 2** hebben we prospectief de incidentie en voorspellers van subacute trombose bestudeerd in een ongeselecteerde opeenvolgende serie van patiënten die primaire percutane coronaire interventie (PCI) ondergingen voor een ST-elevatie-myocard-infarct (STEMI). Subacute trombose trad op bij 4.1% van de patiënten gedurende de follow-up van een jaar. Tekenen van hartfalen bij binnenkomst in het ziekenhuis, voorwandmyocardinfarct en stent-plaatsing bleken voorspellers te zijn van subacute trombose. Eerdere studies, die verricht werden in geselecteerde patiëntengroepen, lieten een lagere incidentie van subacute trombose zien en leverden geen angiografisch bewijs van target-vesselafsluiting. Dientengevolge is dit een van de eerste studies waarin een hoge incidentie wordt gerapporteerd van een belangrijke complicatie van primaire PCI voor een acuut STEMI in een grote ongeselecteerde populatie.
In **hoofdstuk 3** hebben we getracht om mogelijke verklaringen te vinden voor het gebrek aan effectiviteit van antiplaatjesmedicijnen ter preventie van deze trombotische complicatie na primaire PCI voor acute coronaire syndromen. Wij hebben onderzocht of functionele veranderingen in de bloedplaatjes, veroorzaakt door de onderliggende ziekte, de bloedplaatjesaggregatieremming door een enkele dosis acetylsalicylzuur hebben veranderd bij patiënten met een acuut coronair syndroom in vergelijking met gezonde vrijwilligers. Acetylsalicylzuur veroorzaakte minder bloedplaatjesinhibitie bij patiënten met een instabiel coronair syndroom dan bij gezonde vrijwilligers.

In **hoofdstuk 4a** wordt de prognostische waarde van de mate van bloedplaatjesaggregatie-inhibitie door Gp IIb/IIIa blokkers voor primaire PCI voor STEMI geëvalueerd. Er is geen relatie tussen het niveau van bloedplaatjesaggregatie-inhibitie door Tirofiban en klinische uitkomsten gevonden. Voorts was er alleen een bescheiden stijging in de mate van bloedplaatjesaggregatie-inhibitie door een gebruikelijke dosering van Tirofiban te zien. Dit wijst op een hyperactieve toestand van de bloedplaatjes en op incomplete bloedplaatjesaggregatie-inhibitie tijdens een STEMI.
In **hoofdstuk 4b** wordt de rol van de interindividuele reactie op Gp IIb/IIIa en het potentiële belang van bloedplaatjesfunctietesten bij patienten met een acuut coronair syndroom bediscussieerd. Aangezien er geen internationale gestandaardiseerde definitie is van resistentie voor een antiplaatjesmedicijn, kunnen uitslagen van bloedplaatjesfunctietesten niet worden gebruikt als surrogaat voor de klinische effectiviteit van deze medicijnen. Vanwege de gebrekkige resultaten van huidige bloedplaatjesaggregatietesten door verkeerde of onvolledige agonisten of gebrek aan reproduceerbaarheid, hebben we een nieuwe methode ontwikkeld om bloedplaatjesfunctie te meten, namelijk de. Fe-Induced Platelet Aggregation (FIPA) test.
In **hoofdstuk 5** evalueren we de toepasbaarheid en reproduceerbaarheid van deze nieuwe plaatjesfunctietest en vergelijken we de test met bestaande bloedplaatjesfunctietesten. De FIPA test is toepasbaar, gemakkelijk te verrichten en maakt gebruik van een vergelijkbare trigger voor bloedplaatjesaggregatie als stents in coronairarterieën. Verder kon, in tegenstelling tot andere methoden van plaatjesfunctiemeting, FIPA worden gemeten bij patiënten die uitgebreide bloedplaatjesremming door middel van acetylsalicylzuur, clopidogrel en Gp IIb/IIIa blokkers gebruikten.

In **hoofdstuk 6** wordt de relatie tussen tekenen van reperfusie na primaire PCI voor een STEMI en seriële witte–bloedceltelling (WBC-telling) onderzocht. Een verminderde myocardiale perfusie (TIMI< 3 flow of myocardial blush grade < 3) na primaire PCI voor STEMI, is geassocieerd met persisterende WBC-verhoging in de uren na PCI. De WBC-telling daalde enkele uren na een

succesvolle PCI. Verbeterde myocardiale doorbloeding bleek een onafhankelijke voorspeller van de daling in WBC-telling na primaire PCI.

In **hoofdstuk 7** wordt behandeld of verandering in WBC-telling ook geassocieerd is met overleving en reïnfarct. Persisterend verhoogde WBC na primaire PCI waren geassocieerd met een verhoogd sterftegetal. Een verhoogde basaalwaarde van de WBC-telling was geen univariate of multivariate voorspeller van mortaliteit. Wij concluderen dat verandering van het aantal witte bloedcellen na primaire PCI voor STEMI meer prognostische waarde heeft dan de basaalwaarde van de WBC-meting. Daarom lijkt het moment van bloedafname van belang om de voorspellende waarde van de WBC-telling in te schatten.

Ten slotte hebben wij ons in **hoofdstuk 8** gericht op de relatie tussen de basaalwaarde van de WBC-telling en hoog-sensitieve C-reactieve proteëne (hs-CRP) meting. Verder werd de voorspellende waarde van afzonderlijke metingen vergeleken. Hoewel zowel WBC als hs-CRP bekende markers zijn van ontsteking en bekende voorspellers van klinische uitkomsten, werd er geen correlatie tussen de beide markers gevonden na primaire PCI voor STEMI. Een verhoogde WBC-telling was geassocieerd met een grotere infarctafmeting (gemeten door LDHQ48 en linkerventrikelejectiefractie), hs-CRP was gerelateerd aan reïnfarcten en sterfte na één jaar. Hoewel beide markers worden gebruikt als markers van ontsteking, was er een fundamenteel verschil in het mechanisme waarop ze de prognose voorspellen. Dit suggereert een verschillend onderliggend pathofysiologisch mechanisme waarop de twee markers prognose voorspellen. In het bijzonder omdat we hebben gevonden dat witte bloedcellen snel reageren al naar gelang van het succes van reperfusietherapie, zouden witte bloedcellen eerder een marker van neuro-humorale balans kunnen zijn in plaats van een statische marker van inflammatie zoals CRP.

Slotopmerkingen

Alhoewel bloedplaatjes en witte bloedcellen een belangrijke rol spelen bij het ontstaan en de ontwikkeling van het acute coronaire syndroom, blijft de rol van bloedplaatjesaggregatiemeting en van markers van inflammatie in de klinische praktijk nog onduidelijk.

Bloedplaatjes

De incidentie van trombotische events na primaire PCI voor STEMI is groter in de klinische praktijk dan vaak gerapporteerd wordt in gerandomiseerde klinische trials. Antiplaatjesregimes zijn niet zo effectief bij patiënten met een acuut coronair syndroom in vergelijking tot gezonde vrijwilligers. Verder blijken de bloedingsrisico's toe te nemen als in een ongeselecteerde patiëntenpopulatie een maximale dosis en maximaal aantal antiplaatjesmedicatie worden toegediend tijdens het acute coronaire syndroom. Daarom is toenemende kennis over individuele bloedplaatjesfunctie door bloedplaatjesfunctietesten van eminent belang voor patiënt-specifieke dosisaanpassingen. De meting van bloedplaatjesaggregatieremming blijft echter een grote uitdaging. We hebben een nieuwe, gemakkelijk toepasbare plaatjesfunctietest ontwikkeld die gebruik maakt van roestvrij staal als agonist. Of deze FIPA-test klinisch voorspellende waarde heeft, zal moeten blijken uit de lopende On-TIME 2 studie, waarin de test zal worden gecorreleerd aan klinische uitkomsten in een grote STEMI-populatie.

Witte bloedcellen

Ons perspectief van de rol van witte bloedcellen tijdens het acute myocardinfarct is veranderd tijdens het onderzoek. Aangezien witte bloedcellen snel wisselen in aantal, in het bijzonder als reactie op reperfusie, blijkt variatie in witte bloedcellen een krachtigere voorspeller van mortaliteit dan de basaalwaarde van de WBC-telling. Verder lijken de mechanismen waarop witte bloedcellen mortaliteit en reïnfarct voorspellen, gerelateerd te

zijn aan infarctgrootte. Dit is tegengesteld aan hs-CRP, die een bekende marker van inflammatie en een krachtige voorspeller van klinische langeretermijnuitkomsten bij patiënten met een acuut coronair syndroom is. Deze bevindingen suggereren een verschillend onderliggend pathofysiologisch mechanisme waarop witte bloedcellen en CRP klinische uitkomsten voorspellen. Witte bloedcellen zijn wellicht meer markers van de neuro-humorale balans in plaats van statische markers van inflammatie, in tegenstelling tot CRP. Om deze stelling te onderbouwen is verder onderzoek nodig.

Toekomstig onderzoek zal zich moeten richten op verduidelijking van onderliggende pathofysiologische mechanismen en de rol van witte bloedcellen, bloedplaatjes en bloedplaatjesaggregatiemeting in het snel ontwikkelende terrein van de primaire PCI voor patiënten die zich presenteren met een acuut coronair syndroom.

Curriculum vitae

Jaap Jan Johannes Smit was born on May 7 th in Harlingen, the Netherlands. After attending secondary school at the 'Christelijk Gymnasium' in Leeuwarden (1987-1989) and the 'Menso Alting College' in Hoogeveen (1989-1993) he studied medicine at the 'Rijksuniversiteit Groningen' in Groningen (1993-2000). During this period he studied philosophy during evening lectures at the same university. He also studied two years classical percussion at the Conservatory of Groningen during this period (1996-1998). He performed his scientific research stage at the Thorax-center/biomaterials department of university of Groningen investigating the effect of leukocyte filtration during cardiopulmonary bypass surgery (dr. W. van Oeveren). After graduation in 2000 (cum laude), he worked half a year as a resident in cardiology at the Isala klinieken, location Weezenlanden in Zwolle before starting his cardiology training at the same hospital. He was trained at the department of internal medicine (dr. J.G. van der Star and dr. M.A. Alleman) in the first two years and afterwards at the department of cardiology at location Sophia (dr. H. Oude Luttikhuis) and location de Weezenlanden (dr. J.C.A. Hoorntje). During his training for cardiology, from 2000 until 2007, he started scientific research resulting in this thesis. Since the end of his training for cardiologist in January the 1st of 2001 he works as a fellow electrophysiology at the department of cardiology of the Isala klinieken Zwolle. He will end his fellowship on January the 1st of 2009.

He is married to Mei-Nga Smit-Wu and is proud father of Lisa Maria Smit (2006) and is hoping to become father for the second time in May 2008.

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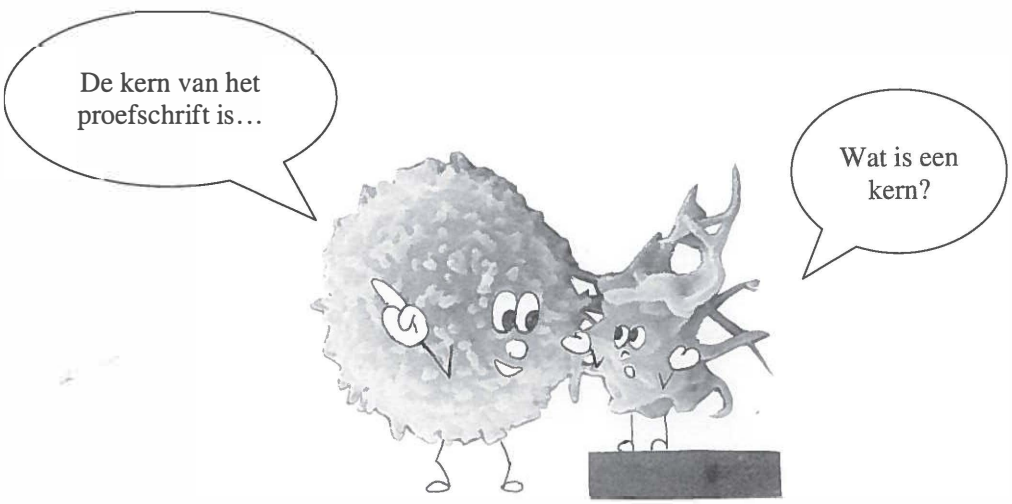
Einstein wist al dat er een grotere wereld bestaat dan de op axioma's gebaseerde wetenschappelijke wereld. Naar **Hem** die dat alles gemaakt heeft, gaat de allergrootste dank uit.


De kern van het proefschrift is…
Wat is een kern?