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Atherosclerosis, myocardial infarction and primary hemostasis: Impact of platelets, von Willebrand factor and soluble glycoprotein VI

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ABSTRACT

Introduction: Little is known about peril constellations in primary hemostasis contributing to an acute myocardial infarction (MI) in patients with already manifest atherosclerosis. The study aimed to establish a predicting model based on six biomarkers of primary hemostasis: platelet count, mean platelet volume, hematocrit, soluble glycoprotein VI, fibrinogen and von Willebrand factor ratio.

Materials and methods: The biomarkers were measured in 1.491 patients with manifest atherosclerosis of the Leipzig (LIFE) heart study. Three groups were divided: patients with coronary artery disease (900 patients) and patients with atherosclerosis and either ST-elevated MI (404 patients) or Non-ST-elevated MI (187 patients). Correlations were analyzed by non-linear analysis with Self Organizing Maps. Classification and discriminant analysis was performed using Learning Vector Quantization.

Results and conclusions.

The combination of hemostatic biomarkers is regarded as valuable tool for identifying patients with atherosclerosis at risk for MI. Nevertheless, our study contradicts this belief. The biomarkers did not allow to establish a predicting model usable in daily patient care. Good specificity and sensitivity for the detection of MI was only reached in models including acute phase parameters (specificity 0,9036, sensitivity 0,7937 in men; 0,8977 and 0,8133 in women). In detail, hematocrit and soluble glycoprotein VI were significantly different between the groups. Significant dissimilarities were also found for fibrinogen (in men) and von Willebrand factor ratio. In contrast, the most promising parameters mean platelet volume and platelet count showed no difference, which is an important contribution to the controversy concerning them as new risk and therapy targets for MI.

1. Introduction

Myocardial infarction (MI) is mainly caused by atherosclerosis [1]. While risk factors for atherosclerosis are extensively investigated, it remains controversial which factors lead to acute obstruction in patients with relevant atherosclerosis. Platelet adhesion and aggregation are central processes [1]. Beside platelets, subendothelial structures, coagulation proteins and rheologic factors are involved [2].

High platelet count (PLT) facilitates aggregation and increases secretion of thrombotic metabolites. Some authors confirmed a relationship between higher PLT and increased risk of adverse cardiovascular events [3–8], others did not [3,9–11]. The effect on thrombus composition was shown by Kovács et al. [12].

Platelets with higher mean platelet volume (MPV) may contain more granules and be more effective [13–17]. MPV has been described as risk factor for an ACS [14,17–19] and was correlated with the

Abbreviations: ACS, Acute coronary syndrome; CAD, Coronary artery disease; CRP, C-reaktive protein; FIB-C, Clauss' Fibrinogen; GPIb/IX/V, Glycoprotein Ib/IX/V; GPVI, Glycoprotein VI; Hct, Hematocrit; HPV, Hypothetic patient vector; MI, Myocardial infarction; MPV, Mean platelet volume; NSTEMI, Non-ST-elevation myocardial infarction; PCI, Percutaneous coronary intervention; PLT, Platelet count; sGPVI, Soluble glycoprotein VI; SOM, Self-organizing maps; STEMI, ST-elevation myocardial infarction; vWF, Von Willebrand factor; vWF-ratio, Von Willebrand factor ratio; vWF:RCo, Von Willebrand factor ristocetin cofactor activity; WBC, White blood cell count

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severity of coronary artery disease (CAD) or mortality in patients with ACS [5,9,16,20–22]. Others failed to confirm such associations [10,20,23].

In blood vessels, erythrocytes concentrate centrally, whereas platelets are pushed to the wall. If hematocrit decreases, platelets adhere less effectively. A correlation between anemia and mortality in patients with ACS was demonstrated [24], but pathophysiology is explained non-hemostaseological.

Fibrinogen (FIB-C) facilitates platelet adhesion and cross linking of thrombocytes [25,26]. Sponder et al. observed a predictive correlation between fibrinogen and severity of CAD, confirming other studies [27]. Further, an association of fibrinogen and MI in contrast to unstable angina pectoris was shown [28]. Xu et al. found a link between fibrinogen and adverse cardiac events in patients with CAD [29]. In contrast, the extensive analysis of Ndrepepa et al. failed to identify fibrinogen as a risk factor for all-cause mortality [30].

The adhesion between subendothelial collagen and GPIb/IX/V on platelet surface is mediated by von Willebrand factor (vWF) and activates thrombocytes [25,26,31–33]. Metaanalyses conclude that elevated vWF is associated with major cardiovascular risk factors [34–36]. Several studies dealt with the association between vWF and the risk of CAD, MI or death following CAD in healthy subjects. Results were heterogeneous [34–37] and little is known about the influence of the functionality of vWF expressed as ristocetin cofactor activity (vWF:RCo). We used vWF-ratio defined as vWF-antigen/ristocetin cofactor activity to overcome influence of acute phase reaction.

Glycoprotein VI (GPVI) is a collagen receptor on platelet surface mediating adhesion and activation [38], shed soluble GPVI (sGPVI) is a biomarker for platelet activation [39,40]. Higher expression of GPVI may lead to an easier activation of thrombocytes [41]. Elevated GPVI in patients with chest pain came along with higher risk for ACS and worse outcome. Further, GPVI was raised in patients with ambiguous ECG who finally suffered from ACS [42] and in patients with ACS in contrast to stable CAD. Patients with CAD presented with generally elevated levels of sGPVI [42]. Upregulated pathways for GPVI mediated platelet activation were found in patients with STEMI compared to CAD patients [41].

Recent works investigated antibodies against the GPVI-collagen-system as a target for antithrombotic therapy [43,44]. Diminishing the GPVI-function reduced platelet degranulation and platelet-endothelium interaction [45,46]. In a mouse model, the GPVI-antibody Revacept results in reduced infarct size [46] and in combination with low-dose thrombolysis in stroke, the therapy was effective but did not increase bleeding risk [47]. Further, it is under investigation in patients with stable CAD and symptomatic carotis stenosis, which highlights the importance of GPVI mediated adhesion of platelets in atherosclerosis [44].

In summary, the role of primary hemostasis in the development MI is still not known in detail. We aimed to identify risky constellations of primary hemostasis for MI in patients with CAD.

2. Materials and methods

Blood samples derive from 1491 patients from the Leipzig (LIFE) Heart Study [48]. All patients were suffering from coronary atherosclerosis with a lumen reduction of ≥50%. Group 0 includes only patients without MI and consists of patients either undergoing coronar-angiography for the first time due to clinical suspiscion of coronary artery disease or patients with already known coronary artery disease. In the following, these are called non-MI CAD patients. Group 1 patients presented in the Leipzig heart center with a first-time ST-elevation MI, Group 2 had first-time Non-ST-elevation MI. Coronary atherosclerosis was diagnosed by coronary angiography.

The Leipzig (LIFE) heart study complies with the ethical standards of the declaration of Helsinki and was approved by the medical ethics committee of the University of Leipzig, Germany (Reg. No 276 2005). Written informed consent has been obtained from all patients.

Venous blood samples were taken before invasive interventions in patients with first-time diagnosed coronary atherosclerosis, or independent of previous therapeutic interventions in patients with known CAD and 6 to 36 h after revascularization in patients with first-time MI. Parameters such as Hct, PLT and MPV were measured in fresh blood samples. Citrate plasma was frozen within two hours at −80 °C for later analysis.

2.1. Measurements

Hct, PLT and MPV were measured in EDTA blood using the analytical apparatus XE 2100 (Sysmex). Thawed citrate plasma was used for measurements of vWF antigen, vWF Ristocetin-cofactor-activity, Fibrinogen (Clauss) using an ACL TOP 700 (Instrumentation Laboratory®); sGPVI was measured using a sandwich-ELISA of Elabscience Biotechnology Co. Limited. CRP and WBC results were collected from basic data of the Leipzig (LIFE) Heart Study. We used vWF-ratio defined as vWF-antigen/ristocetin cofactor activity.

2.2. Statistics

WinSTAT® software and MATLAB® software were used. Normal distribution was tested by ShapiroWilkTest [49]. Parameters were compared by the Mann Whitney *U* test to be consistent also for non-Gaussian parameter distributions. The significance level α was chosen as $\alpha = 0{,}05$.

Correlations were analyzed by non-linear analysis with self-organizing maps (SOM) [50]. Patients' data is represented by 50 hypothetic patient vectors (HPVs). Hypothetical patient vectors represent the whole data cloud and visualize data distributions. Thus, a hypothetical patient shows the typical (averaged) behavior regarding the biomarkers of a certain subgroup of original patients. In this way, hypothetical patients act as local averages of the original patient data. By doing so, outliers are neglected and data noise is cancelled out [51].

SOM then realizes a non-linear mapping of the HPV data as a colored pixel arranged in an two-dimensional rectangular grid [52] visualizing correlations of the HPVs [53,54]. Conclusions such as “a typical patient with high levels of parameter A has on average high levels of parameter B and medial levels of parameter C etc.” are thus possible and help to identify correlating parameters [53]. The grid length ratio for the here analyzed data (optimized to represent similar patients by grid-neighbored hypothetical patients) was automatically determined by the method to be 2:1 (topology preserving mapping). Length ratio 10:5 and therefore 50 hypothetical patients were chosen, so each hypothetical patient takes a local average of approximately 30 (real) patients, which is minimum for meaningful averaging. A lower number of hypothetical patients would result in stronger averaging but weakens the spatial resolution of the SOM scales needed for correlation analysis.

Classification and discriminant analysis was performed using Learning Vector Quantization (LVQ) [55]. Based also on HPVs, LVQ generates a non-linear classification strategy, based on training of given samples, to distinguish the patients' data. Application of the trained LVQ to the available patients delivers a classification which can be compared to the true patient group. Evaluation of the corresponding contingency table yields sensitivity and specificity.

3. Results

Men and women were analyzed separately mainly because of the different reference range of hematocrit in men and women. Analyzing men and women together, a distinction between non-MI CAD and MI by means of hematocrit is not possible because the distribution of high hematocrit values for women overlap with the normal range for men.

Metric data was described by measures of central tendency. Differences in distribution were calculated. Table 1 presents data of

Table 1
Descriptive statistics of metric parameters (median, 25, 75 percentile), Mann Whitney's *U* test.

	Reference	Measures of central tendency			*p*-value	
	Range	Group 0[a]	Group 1[b]	Group 2[c]	Group 0[a] vs. 1[b]	Group 0[a] vs. 2[c]
Men						
age [years]	–	66 [57; 72]	56 [49; 68]	62 [51; 72]	1554E−15	0,003
BMI [kg/m²]	18,5– < 25	28,5 [26,0; 31,4]	27,8 [25,2; 30,8]	27,4 [25,4; 29,9]	0,009	0,006
CRP [mg/l]	< 5	2,3 [1,1; 5,1]	23,8 [10,1; 57,5]	13,3 [4,7; 36,2]	0,000*	0,000*
WBC [×10⁹/l]	3,5–9,8	7,1 [5,9; 8,5]	10,7 [8,7; 12,9]	9,2 [7,8; 10,6]	0,000*	0,000*
PLT [×10⁹/l]	140–360	223 [190; 257]	225 [191; 263]	217 [187; 255]	0,568	0,387
Hct [l/l]	0,400–0,530	0,424 [0,401; 0,445]	0,408 [0,382; 0,429]	0,415 [0,386; 0,441]	1703E−10	0,012
MPV [fl]	9,4–12,9	10,8 [10,2; 11,4]	10,8 [10,2; 11,4]	10,8 [10,3; 11,6]	0,518	0,869
FIB-C [g/l]	2,0–3,9	3,3 [2,8; 3,9]	4,0 [3,2; 5,1]	4,1 [3,3; 4,9]	0,000*	2087E−14
vWF-ratio [−]	–	1,26 [1,15; 1,50]	1,15 [1,07; 1,26]	1,21 [1,12; 1,34]	0,000*	0,001
sGPVI [ng/ml]	–	35,5 [31,4; 40,1]	40,0 [33,2; 46,6]	37,5 [32,9; 43,9]	2631E−12	0,005
Women						
age [years]	–	70 [60; 76]	67 [51; 76]	69 [58; 75]	0,116	0,521
BMI [kg/m²]	18,5– < 25	28,9 [26,1; 33,3]	28,2 [25,0; 31,3]	28,7 [26,3; 32,3]	0,045	0,853
CRP [mg/l]	< 5	2,9 [1,4; 6,3]	18,8 [6,7; 52,7]	20,2 [9,6; 43,1]	0,000*	1554E−14
WBC [×10⁹/l]	3,5–9,8	7,4 [6,3; 8,6]	10,5 [8,9; 12,8]	10,2 [8,7; 11,3]	0,000*	3264E−11
PLT [×10⁹/l]	140–360	257 [220; 299]	230 [196; 273]	251 [204; 282]	0,001	0,243
Hct [l/l]	0,360–0,480	0,402 [0,375; 0,420]	0,369 [0,341; 0,387]	0,382 [0,356; 0,403]	3597E−14	0,002
MPV [fl]	9,4–12,9	10,8 [10,1; 11,5]	10,9 [10,4; 11,4]	11,0 [10,4; 11,6]	0,712	0,423
FIB-C [g/l]	2,0–3,9	3,6 [3,1; 4,3]	3,6 [3,1; 4,4]	4,0 [3,3; 5,1]	0,480	0,021
vWF-ratio [−]	–	1,29 [1,16; 1,54]	1,13 [1,06; 1,26]	1,17 [1,09; 1,29]	4701E−12	1510E−04
sGPVI [ng/ml]	–	34,6 [31,3; 38,7]	39,0 [34,1; 43,9]	41,3 [33,0; 48,9]	1474E−05	0,001

* rounded.
[a] Patients with CAD without MI.
[b] Patients with CAD and STEMI.
[c] Patients with CAD and NSTEMI.

target parameters:

Hematocrit and sGPVI are significantly different between the groups in men and in women: median hematocrit is higher in group 0 (0,424 l/l in men; 0,402 l/l in women) versus group 1 (0,408 l/l in men; 0,369 l/l in women) respectively group 2 (0,415 l/l in men; 0,382 l/l in women), whereas median levels of sGPVI are lower in group 0 (35,5 ng/ml in men; 34,6 ng/ml in women) versus group 1 (40,0 ng/ml in men; 39,0 ng/ml in women) and group 2 (37,5 ng/ml in men; 41,3 ng/ml in women).

Significant dissimilarities were found for fibrinogen and vWF-ratio in men: median fibrinogen is lower in group 0 (3,3 g/l) versus group 1 (4,0 g/l) or group 2 (4,1 g/l), whereas median vWF-ratio is higher in group 0 (1,26) versus group 1 (1,15) respectively group 2 (1,21). PLT shows no difference in men: median is 223 Gpt/l in group 0, 225 Gpt/l in group 1 and 217 Gpt/l in group 2.

In women, median vWF-ratio is significantly higher in group 0 (1,29) versus group 1 (1,13) and group 2 (1,17) as well. Fibrinogen shows differences between group 0 and group 2 (median group 0 and 1: 3,6 g/l, group 2: 4,0 g/l). PLT shows differences in women between group 0 and 1: median is 257 Gpt/l in group 0, 230 Gpt/l in group 1 and 251 Gpt/l in group 2.

Median MPV shows no difference in both sexes (in men: 10,8 fl in all groups; in women: 10,8 fl in group 0, 10,9 fl in group 1, 11,0 fl in group 2).

Distribution of smoker status and diabetes is given in Table 2, age, BMI, CRP and WBC as further possible influencing factors are shown in Table 1.

Figs. 1 and 2 visualize the distribution of values in 50 hypothetical SOM-patients, derived from the measured data. One pixel depicts the same hypothetical patient in every 5 × 10 matrix. Visually similarly spread parameters were summarized in correlation clusters (Table 3).

In men, correlation cluster A consists of sGPVI, vWF-ratio, PLT, MPV, FIBC, CRP and WBC. Hct shows no SOM-correlation and is analyzed independently.

In women, sGPVI, vWF-ratio, FIBC, Hct, CRP and WBC form a correlation cluster. MPV and PLT are distributed inversely and collected in another correlation cluster.

Table 2
Descriptive statistics of smoking and diabetes.

	Group 0[a]	Group 1[b]	Group 2[c]
Men			
Total number	685	295	146
Smoker			
Current smoker	137 (20,0%)	160 (54,2%)	60 (41,1%)
Former smoker	362 (52,8%)	72 (24,4%)	53 (36,3%)
Non-smoker	186 (27,2%)	63 (21,4%)	33 (22,6%)
Diabetes			
Diabetes	249 (36,4%)	77 (26,1%)	46 (31,5%)
Non-Diabetes	436 (63,6%)	218 (73,9%)	100 (68,5%)
Women			
Total number	215	109	41
Smoker			
Current smoker	31 (14,4%)	46 (42,2%)	8 (19,5%)
Former smoker	41 (19,1%)	14 (12,8%)	6 (14,6%)
Non-smoker	143 (66,5%)	49 (45,0%)	27 (65,9%)
Diabetes			
Diabetes	85 (39,5%)	39 (35,8%)	17 (41,5%)
Non-Diabetes	130 (60,5%)	70 (64,2%)	24 (58,5%)

[a] Patients with CAD without MI.
[b] Patients with CAD and STEMI.
[c] Patients with CAD and NSTEMI.

Possible influence factors, which show no correlation to target parameters, are excluded from further calculation.

We investigated whether the LVQ model is able to sort the patients into the original group using the measured biomarkers. Sensitivity and specificity for different parameter constellations are given in Table 4.

Generally, in men and women, the distinction between non-MI CAD and STEMI is better or at least comparable to the distinction between non-MI CAD and overall MI for all biomarker sets, whereas the distinction between non-MI CAD and NSTEMI in both, women and men, offers especially lower sensitivities.

Focusing on the distinction between non-MI CAD and overall MI,

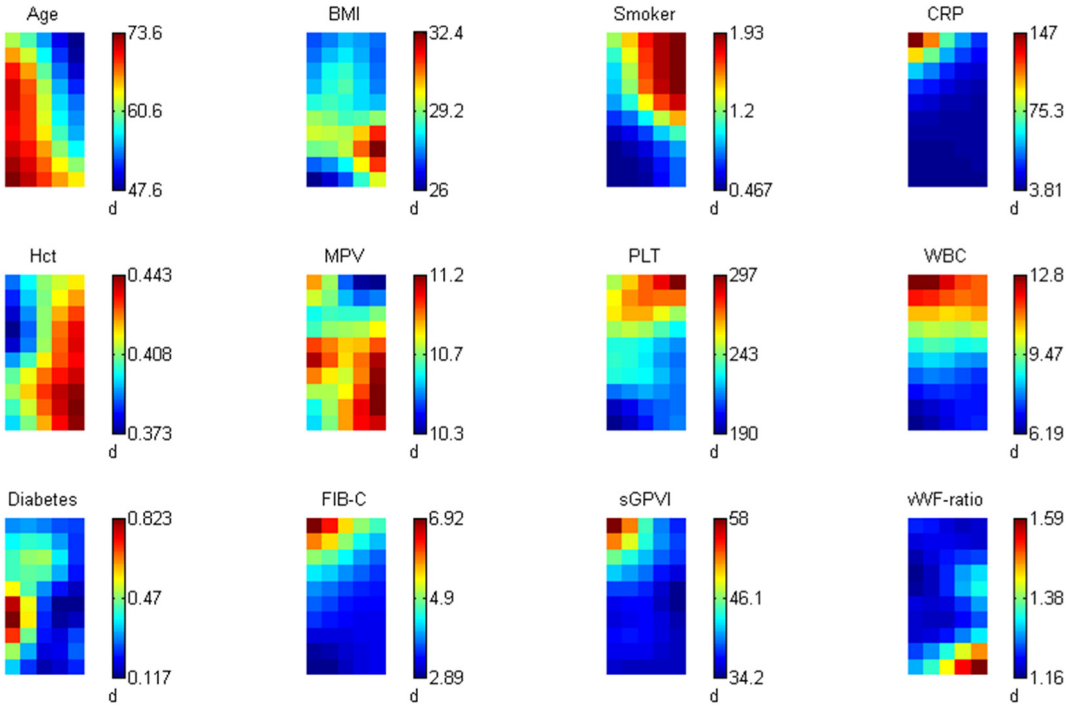

Fig. 1. SOM in men.
The colored scale visualizes the distribution of high and low values for parameters in 50 hypothetical SOM-patients. One pixel depicts the same hypothetical patient in every 5 × 10 matrix. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the set with all biomarkers and CRP and WBC offers the highest specificity and sensitivity in men (specificity 0,9036; sensitivity 0,7937). Correlation cluster A reveals lower values (specificity 0,9022; sensitivity 0,7891). No parameter set or single biomarker within correlation cluster A reaches comparable values. Correlation cluster B again offers lower values (specificity 0,8248; sensitivity 0,3492). In women, correlation cluster C presents highest specificity and sensitivity for the distinction between non-MI CAD and MI: the set with all biomarkers has a specificity of 0,8977 and sensitivity of 0,8133, in correlation cluster C specificity is 0,8977 and sensitivity is 0,8200. Single parameters or parameter sets within correlation cluster C or D do not offer comparable results.

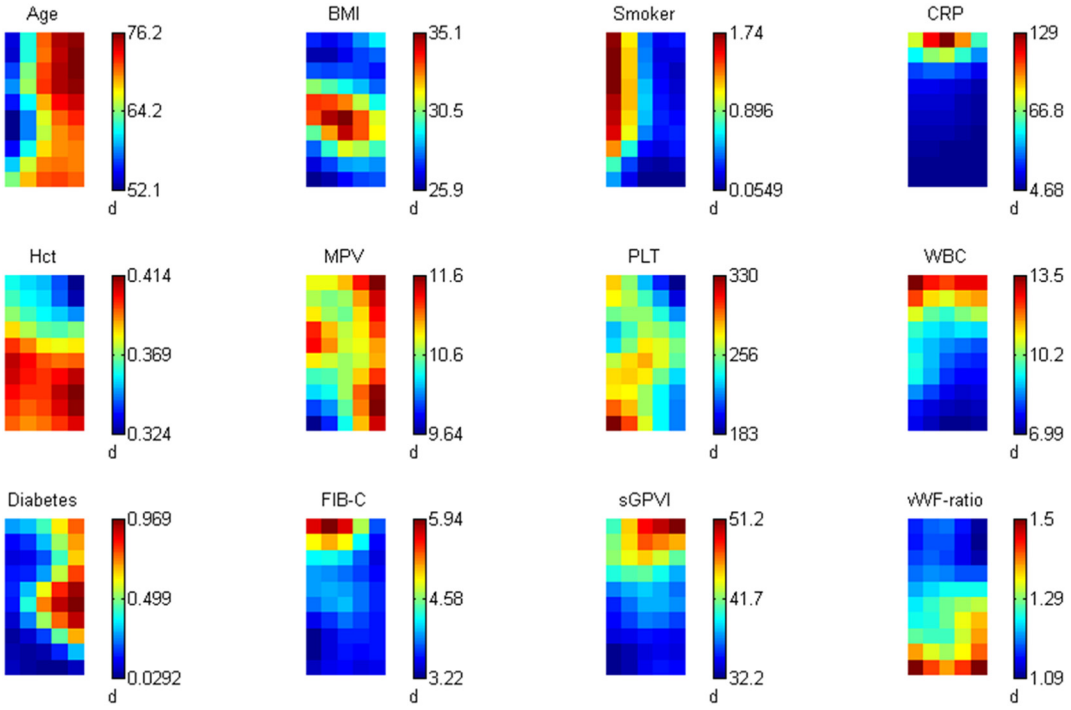

Fig. 2. SOM in women.
The colored scale visualizes the distribution of high and low values for parameters in 50 hypothetical SOM-patients. One pixel depicts the same hypothetical patient in every 5 × 10 matrix. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Correlation clusters.

Men	
Correlation cluster A	sGPVI, vWF-ratio, PLT, MPV, FIBC, CRP, WBC
Correlation cluster B	Hct
Unconfirmed influence parameters	Age, BMI, smoking status, diabetes
Women	
Correlation cluster C	sGPVI, vWF-ratio, FIBC, Hct, CRP, WBC
Correlation cluster D	PLT, MPV
Unconfirmed influence parameters	Age, BMI, smoking status, diabetes

4. Discussion

The risk to develop MI if atherosclerosis is manifest is still not predictable. The culprit lesion is often located proximal to the maximal stenosis [1]. Other factors besides degree of stenosis must influence time and severity of occlusion. We show the results of a large, well defined group of patients with manifest atherosclerosis with and without MI presenting data for primary hemostasis.

4.1. vWF-ratio

Previous studies show heterogeneous results for the association of vWF and different aspects of CAD [34–36]. Willeit et al. confirmed a positive relation between vWF and coronary heart disease [35].

Here, vWF and vWF:RCo were significantly different in patients with and without MI (data not shown). vWF is strongly influenced by acute-phase-reaction. This is not known for the protein's functionality, expressed as ratio of antigen and activity [56]. The vWF-ratio was significantly higher in the non-MI CAD group compared with the STEMI/NSTEMI groups. So, the portion of active vWF is higher in the MI groups. This finding confirms vWF functionality mediating the development of ACS and is in concordance with Rutten et al. [37].

An allocation of patients into their original groups was not successful using the vWF-ratio. We have to consider the vWF-ratio as irrelevant for a clinical risk score.

Anyhow, the measured differences point to a promising pathophysiological role of vWF and should be investigated in an experimental setting.

4.2. GPVI

sGPVI was significantly higher in our STEMI/NSTEMI groups compared with the non-MI CAD group, underlining previous publications [42] and suggesting an important role for platelet adherence in acute obstruction. As shown in animal models for stroke [47], our data supports the idea, that blocking the GPVI-mediated processes, i.e. by Revacept, would be effective in the very acute phase of infarction also in addition to anti-platelet therapies [57]. But sGPVI did not allow distinguishing between the three groups in a diagnosing model. We cannot postulate sGPVI as a promising parameter for everyday risk calculation.

Whether platelet bound GPVI plays a role in thrombus formation [41,45,46] becomes not clear, based on our analysis. Furthermore, the correlation of GPVI with acute-phase-markers hampers a meaningful interpretation.

4.3. MPV

We neither found differences in MPV, nor was MPV able to distinguish between the groups. Our results contrast with reports that describe a correlation between MPV and risk for ACS, reinfarction, severity of CAD, mortality after PCI or outcome [5,9,14,16–22]. The pathophysiologic concept is plausible, but differences in the named studies are low and only significant because measured in large groups. Furthermore, the evidence for a higher MPV leading to more active platelets is still lacking. Cesari showed an influence on the outcome for immature and larger platelets, but not for MPV [18]. In addition, others

Table 4
Results from the diagnosing LVQ model using different sets of biomarkers.

	Distinction non-MI CAD vs. MI		Distinction non-MI CAD vs. STEMI		Distinction non-MI CAD vs. NSTEMI	
	Specificity	Sensitivity	Specificity	Sensitivity	Specificity	Sensitivity
Men						
■ For all biomarkers and CRP + WBC	0,9036	0,7937	0,9401	0,8169	0,9708	0,3904
■ For correlation cluster A[a]	0,9022	0,7891	0,9328	0,8169	0,9620	0,4247
- For CRP + WBC only	0,8774	0,7642	0,9255	0,7424	0,9518	0,4247
- For CRP + WBC + sGPVI + vWF-ratio	0,8788	0.7823	0,9226	0,7831	0,9723	0,3562
- For PLT + MPV	0.9562	0.0998	0.9839	0,0203	10,000	0,0000
- For sGPVI only	0,8672	0,3243	0,8423	0,3932	0,9942	0.0274
- For vWF-ratio only	0,7577	0,4490	0,7577	0,4881	10,000	0,0000
- For PLT only	0,9562	0,0816	0,9591	0,0441	10,000	0,0068
- For MPV only	0,8365	0,2132	0,9606	0,0475	10,000	0,0000
- For FIB-C only	0,7869	0,5170	0,8686	0,4034	0,9883	0,0685
■ For correlation cluster B[b] [Hct only]	0,8248	0,3492	0,8613	0,2881	0,9912	0,0479
Women						
■ For all biomarkers and CRP + WBC	0,8977	0,8133	0,9116	0,8349	0,9860	0,4146
■ For correlation cluster C[c]	0,8977	0,8200	0,9070	0,8257	0.9860	0,4878
- For CRP + WBC only	0,8140	0,7933	0,8605	0,6972	0,9953	0.2927
- For CRP + WBC + sGPVI + vWF-ratio	0.9023	0.7533	0,9023	0,7706	0,9860	0,3659
- For sGPVI only	0,8233	0,4667	0,8047	0,4312	10,000	0,0000
- For vWF-ratio only	0,7488	0,5733	0,8744	0,3853	10,000	0,0000
- For FIB-C only	0,7349	0,4467	0,5953	0,4771	10,000	0,0000
- for Hct only	0,6791	0,7067	0,8419	0,4587	10,000	0,0000
■ For correlation cluster D[d]	0,7581	0,4267	0,8512	0,3303	10,000	0,0000
- For MPV only	0,8297	0,2933	0,8000	0,3394	0.5395	0,5122
- For PLT only	0,8419	0,3467	0,9860	0,0734	0,5023	0,5366

[a] sGPVI, vWF-ratio, PLT, MPV, FIB-C, CRP, WBC.
[b] Hct.
[c] sGPVI, vWF-ratio, FIBC, Hct, CRP, WBC.
[d] PLT, MPV.

used healthy controls [19]. Publications which could not find an association used study designs similar to our concept [10,20,23], concentrating on manifest CAD with or without MI. Indeed, Chu et.al show differences for MI vs. stable CAD or no coronary disease, but the definition of CAD was heterogeneous in the underlying publications.

Summarized, MPV seems not suitable for risk stratification in everyday patient care. This result is especially interesting against the background of MPV discussed as a most promising parameter [5,9,14,16–22].

4.4. Combined risk calculation

As far as we know, our study is the first that tried to develop a diagnosing model for risk stratification similar to the thrombophilia screening for venous thrombosis [58].

The combined diagnosing model includes six biomarkers and CRP and WBC to display the possible effect of an acute phase reaction following myocardial infarction.

The combined model reveals good specificity and moderate sensitivity for the distinction between non-MI CAD and overall MI and even better values for the distinction between non-MI CAD and STEMI, whereas the distinction between non-MI CAD and NSTEMI offers especially low sensitivity. As conclusion, a STEMI, provoked by plaque rupture, seems to be more influenced by primary hemostasis than a NSTEMI resulting from smaller thrombus formations.

Further, the dual model with CRP and WBC shows that the biggest part of the combined model's information is obtained by CRP and WBC and therewith by acute phase reaction. As the dual model is not as precise as the combined model, there must be further information in our target biomarkers. Unfortunately, it is not possible to specify the allocation of this further information to the target parameters. Neither of the models can respond to the question, whether a single parameter is able to distinguish between the groups prospectively by itself, independent of acute phase reaction. But following the results in Table 4, the latter seems improbable.

Nevertheless, that there is information uninfluenced by CRP and WBC within the target parameters reveals again a pathophysiological role of these parameters in the development of acute MI.

4.5. Limitations

First of all, time of blood sampling differed between patients with MI and non-MI CAD and results are particularly influenced by acute phase reaction following MI. In addition, results may be affected by antithrombotic therapy and the coronarangiographic interventions. Especially the anti-platelet therapy received in the context of acute MI may have changed platelet characteristics. In contrast, non-MI CAD patients also may have received anti-platelet therapy, e.g. because of already known coronary artery disease, former stroke or peripheral artery occlusive disease. Due to the general setting of the Leipzig (LIFE) Heart Study there was no opportunity to investigate platelet function. Since anti-platelet therapies would influence platelet functionality, measured by aggregometry methods, the need for such analysis in our setting is dispensable. Therefore, data about anti-platelet therapies was not reported here, even if medication is included in the central database of the study. So as groups were not matched based on anti-platelet therapy, results may be biased. Further, we used a retrospective investigation to establish prospective risk stratification, so final causality cannot be assumed and we have no information about the severity of MI or the course of biomarker levels.

5. Conclusion

Many parameters may contribute to a vascular occlusion based on present atherosclerosis, but none of the biomarkers measured here is yet identified to have sole responsibility. The combination in a diagnostic model is regarded to identify patients at high risk, but our LVQ-model did not reveal a clinical useful score. A prospective, longitudinal model in which patients with CAD are monitored and investigated until the endpoint MI would be favorable. However, especially our results concerning MPV, sGPVI and vWF-ratio are an important contribution to the controversial discussion regarding them as new risk- and therapy targets for MI.

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Contributors

JMV and TD collected the data, performed the analysis and wrote the manuscript, TV performed the calculation for LVQ and SOM. AT, RB and JT helped draft the manuscript and participated in study design and coordination. All authors read and approved the final manuscript.

Conflict of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

No prior presentation.

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