Expression of platelet glycoprotein VI is associated with transient ischemic attack and stroke

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Expression of Platelet Collagen Receptor Glycoprotein VI (GPVI) is Associated with Transient Ischemic Attack (TIA) and Stroke

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Abstract (246 words)

Background The platelet collagen receptor glycoprotein VI (GPVI) contributes significantly to platelet adhesion and thrombus formation. This study examined the platelet surface expression of GPVI in patients with transient ischemic attack (TIA) and stroke.

Methods and results We consecutively evaluated 205 patients, who admitted the stroke unit with symptoms for stroke. Surface expression of the platelet activation markers (GPVI, CD62P, GPIb) was determined by two-color whole blood flow cytometry. Patients with TIA as well as with stroke showed a significantly enhanced GPVI expression on admission compared to patients with non-ischemic (NI) events (TIA (mean fluorescence intensity (MFI) ± SD): 20.9±7.1 vs. NI: 16.2±3.9; p=0.002; stroke: 20.4±5.7 vs. NI; p=0.002), whereas CD62P surface expression showed a significant elevation in TIA and merely a trend in stroke (TIA vs. NI: 12.8±4 vs. 10.9±3.6; p=0.048; stroke: 12.6±7.9 vs. NI; p=0.145). Logistic regression analysis revealed that on admission GPVI was associated with stroke independent of conventional laboratory markers such as C-reactive protein, blood glucose, and creatine kinase. Using a receiver operating characteristic (ROC) curve on GPVI, we have determined the cut off value of 18.2 for stroke. The area under the curve was 0.683 (95%CI, 0.585 to 0.757). Thus, patients with enhanced GPVI expression levels (≥18.2) had a 2.4 fold relative risk for stroke. Patients with elevated platelet GPVI expression level had a poorer clinical outcome in cumulative event-free survival for stroke, myocardial infarction, and cerebro-/cardiovascular death at three-month follow-up (Log rank; p=0.045).

Conclusion Platelet GPVI surface expression is significantly enhanced in patients with TIA and stroke compared to patients with NI events. Determination of platelet-specific GPVI may be useful as an early biomarker for cerebral ischemia.
**Introduction**

Apart from acute coronary syndrome (ACS), acute ischemic stroke takes an enormous toll of life in the Western countries. Management of acute ischemic stroke is strongly based on clinical assessment. Early diagnosis of stroke is important for the determination of the type (ischemic or hemorrhagic) and the subtype classification (e.g. TOAST) in order to start proper treatment rapidly. Although the distinction between transient ischemic attack (TIA) and ischemic stroke has blurred over the last years, they share similar pathogenetic mechanisms.

Several new potential biomarkers have emerged to facilitate diagnosis of ischemic stroke. Beside markers for glial activation and neuronal and endothelial cell injury like S-100B, myelin basic protein, neuron-specific enolase (NSE), and soluble thrombomodulin, particularly markers for inflammation like C-reactive protein (CRP), matrix-metalloprotease-9 (MMP-9), interleukin-6 (IL-6), intercellular adhesion molecule-1 (ICAM-1) and platelet endothelial cell adhesion molecule-1 (PECAM-1) as well as von Willebrand factor (vWF) and P-selectin have been examined. However, despite of promising results with enhanced serum and/or plasma concentrations correlating to stroke and to clinical outcome, none of these biomarkers is established and considered to be useful in clinical routine yet. Likewise in patients with acute coronary syndrome (ACS), increases in biomarkers upstream from biomarkers of tissue damage or necrosis such as markers of inflammation and thrombosis may provide an earlier assessment of overall patient risk and help in identifying patients at risk of a cerebrovascular event. Platelets play a key role in arterial thrombosis, ACS and stroke. Exposure of collagen to the bloodstream in acute plaque rupture triggers sudden platelet activation and formation of a platelet plug. The following activation of the coagulant cascade leads to the development of a firm fibrin-containing thrombus and vessel occlusion.

Recently, we demonstrated that the surface expression of platelet collagen receptor
glycoprotein VI (GPVI) is elevated in patients with ACS and associated with acute coronary events.\textsuperscript{12,13}

The aim of the present study was to evaluate platelet GPVI levels in patients presenting with symptoms of acute cerebrovascular disease and to define GPVI as biomarker for acute stroke.

Methods

Study population and enrolment criteria

Our study population comprised 205 consecutive patients, who presented with symptoms of stroke. Among these patients, 133 (64.9\%) presented with stroke, 18 (8.8\%) with TIA, and 54 (26.3\%) with non-ischemic (NI) events, which served as controls. All of these patients have received cranial imaging (CT scan). 38 patients with stroke received a lysis therapy according to the CLOTBUST trial protocol.\textsuperscript{14}

The exclusion criteria were age below 18 years, inability and lack of informed consent. Clinical outcome was performed by a predefined structured telephone interview after a 3-month follow-up (92.2\%: 189 of 205 patients).

Definitions

\textit{TIA}: Transient ischemic attack was defined by the duration of symptoms of stroke less than 24 hours.\textsuperscript{3}

\textit{Stroke}: Stroke was considered with symptoms lasting more than 24 hours after the onset or with the evidence of an acute clinically relevant brain lesion in patients with rapidly vanishing symptoms using cranial imaging (CT scan).\textsuperscript{3}

\textit{TOAST}: The origin of cerebral ischemia was classified according to the Trial of Org10172 in Acute Stroke Treatment (TOAST) criteria according to Adams et al.: 1) large-artery
atherosclerosis, 2) cardioembolism, 3) small-vessel occlusion, 4) stroke of other determined etiology, 5) stroke of undetermined etiology.²

*Non-ischemic Events:* patients provided one of the following disorders: epileptic attacks, medication interaction, transient visual disorder, paroxysmal positioning vertigo, migraine with aura, psychogen, brain tumor, cerebral bleeding, alcohol abuse.

*NIHSS:* Stroke severity was assessed with the National Institutes of Health Stroke Scale (NIHSS).¹⁵

**Sample collection**

Blood was drawn from the anticubital vein on time of admission to hospital. The blood was filled into 5 mL citrate phosphate dextrose adenine vials, processed and analyzed immediately by flow cytometry as described.¹²,¹³,¹⁶

**FACS analysis**

The surface expression of the platelet receptors GPVI, CD62P, and GPIb was determined by two-color whole blood flow cytometry, as previously described.¹⁶,¹⁷ Mean fluorescence intensity (MFI) was used as the index of receptor expression. Fluorescein (FITC)-conjugated anti-CD62P (clone CLB-Thromb/6) and phycoerythrin (PE)-conjugated anti-CD42b (clone SZ2) monoclonal antibodies (mAb) were purchased from Immunotec, Beckman Coulter, Inc., USA. The fluorescein (FITC)-conjugated anti-GPVI mAb 4C9 was generated and characterized, as previously described.¹⁸,¹⁹

**Statistics**

A probability value of less than 0.05 was considered as statistical significant. Adjustment by possible confounders was performed by the multifactorial analysis of covariance for the decadic logarithm of GPVI. Association of the platelet GPVI surface expression was found to
be independent of medical treatment at the time of admission, cerebrovascular risk factors, age and gender. The association of GPVI after adjustment for conventional laboratory markers was assessed by the logistic regression analysis in patients under suspicion for stroke. Mean values between two categories were compared with a two-tailed unpaired $t$-test. In order to predict the individual risk for stroke by using the levels of GPVI, we calculated the receiver operating characteristic (ROC) curve.

A log-rank test (Mantel-Cox) was applied for the evaluation of associations between event and variables. All statistical analyses were performed using SPSS version 15.

Results

We consecutively investigated the platelet surface expression of collagen receptor GPVI in a total of 205 patients for suspected stroke. In all patients the diagnosis was determined by the duration of symptoms and by cranial imaging (CT scan). The demographic details are given in Table 1.

Patients with TIA (n=18) as well as with stroke (n=133) showed a significantly enhanced GPVI expression on admission compared to patients with non-ischemic (NI) events (n=54) (TIA (mean fluorescence intensity (MFI)$\pm$SD): 20.9$\pm$7.1 vs. NI: 16.2$\pm$3.9; p=0.02; stroke: 20.4$\pm$5.7 vs. NI; p=0.002), whereas CD62P surface expression showed a significant elevation in TIA and merely a trend in stroke (TIA vs. NI: 12.8$\pm$4 vs. 10.9$\pm$3.6; p=0.048; stroke: 12.6$\pm$7.9 vs. NI; p=0.145) (Figure 1A). GPIb expression tended to decrease with increased platelet activation (TIA vs. NI: 142.7$\pm$49.6 vs. 149.7$\pm$45; p=0.543; stroke: 138.1$\pm$42.7 vs. NI; p=0.447). There are no differences in the degree platelet activation between patients with TIA and those with ischemic stroke (GPVI: p=0.881; CD62P: p=0.293) (Figure 1A). Ischemic stroke was further itemized according to TOAST classification.\(^2\)
Patients with large-artery atherosclerosis (TOAST1) and cardioembolism (TOAST2) have shown significantly increased GPVI levels in contrast to patients with non-ischemic events (TOAST1: 20.1±6.1 vs. NI; p=0.002; TOAST2: 22.2±6.3 vs. NI; p=0.002), whereas CD62P surface expression showed no significant difference (TOAST1: 12±4.3 vs. NI; p=0.253; TOAST2: 14±12.1 vs. NI; p=0.328) ([Figure 1B](#)), nor GPIb expression presented with no significant difference (TOAST1: 138.3±41.8 vs. NI; p=0.448; TOAST2: 144.9±58.1 vs. NI; p=0.796). We found no correlation between platelet surface GPVI expression and the severity of stroke assessed by NIHSS (r=-0.19; p=0.789) ([Figure 1C](#)).¹⁵

To evaluate whether GPVI is associated with stroke independent of baseline conventional laboratory markers, we performed a logistic regression analysis that included C-reactive protein, blood glucose, and creatine kinase, determined at time on admission ([Table 2](#)). We found that GPVI platelet surface expression was independently associated with stroke ([Table 2](#)).

Comparison of the decadic logarithm of GPVI was adjusted by possible confounders such as age, gender, medical treatment and cerebrovascular risk factors.

To predict the individual risk for stroke by using the levels of GPVI, we calculated the receiver operating characteristic (ROC) curve ([Figure 2A](#)). The cut off value for stroke was 18.2. The area under the curve was 0.683 (95%CI, 0.585 to 0.757). Patients with GPVI levels of ≥18.2 had a 2.4-fold relative risk (95%CI, 1.4 to 4.2) for stroke ([Figure 2B](#)). Patients with an elevated platelet GPVI expression level (MFI≥18.2) had a poorer clinical outcome than patients with a lower GPVI expression level (MFI<18.2) ([Table 3](#)). Particularly, three-month follow-up reached a statistical significant level in cumulative event-free survival for stroke, myocardial infarction, and cerebro-/cardiovascular death (Log rank; p=0.045) ([Figure 3](#)). The clinical outcome in our patients was not influenced by the medication since patients had been administered comparable therapeutic regimes consisting of statins and antiaggregatory therapy ([Table 4](#)).
Discussion

The major findings of the present study are that (1) enhanced platelet collagen receptor GPVI expression is associated with TIA and stroke, (2) GPVI was associated with stroke independent of conventional laboratory markers such as C-reactive protein, blood glucose, and creatine kinase, and (3) patients with an elevated platelet GPVI expression level (MFI $\geq$18.2) had a poorer clinical outcome in cumulative event-free survival for stroke, myocardial infarction, and cerebro-/cardiovascular death at three-months follow-up.

Previous studies suggested that platelets play an important role in the development of transient brain ischemia or infarction.\textsuperscript{9,20,21} Platelet activation with enhanced P-selectin and CD63 expression has been reported with TIA and stroke.\textsuperscript{10,22} In particular, large-artery atherosclerosis (TOAST1) and cardioembolism (TOAST2) are the most frequent causes of brain ischemia.\textsuperscript{20} In our patients, TOAST1 and TOAST2 showed significantly increased levels of GPVI compared to patients with non-ischemic events indicating a similar thrombotic and platelet activation state in patients with large artery atherosclerosis and cardioembolism as cause for ischemic stroke. However, GPVI expression seems to be inappropriate to assess the severity of stroke, since we found no correlation between platelet surface GPVI expression and the NIHSS.

Early diagnosis of ischemic stroke with the help of adequate biomarkers may further improve early therapy and clinical outcome in patients.

There are several therapeutic regimes discussed on the appropriate management of high-risk patients, for it is challenging to dissociate the reduction of the risk of arterial thrombosis and increase of the risk of hemorrhagic and bleeding complications.\textsuperscript{21,24,25} Adding another second antiplatelet (clopidogrel) or anticoagulant (warfarin) medication to aspirin
may significantly reduce the risk of upcoming adverse events. However, others found that the use of a dual antiplatelet therapy was rather effective in symptomatic patients to reduce the rate of myocardial infarction, stroke, or death from cardiovascular causes. It is tempting to speculate that the collective of patients with increased platelet activation may profit from a dual antiplatelet therapy. Similarly to the recent findings of our prospective study in patients with acute coronary syndrome (ACS), symptomatic patients of TIA or stroke with an initially enhanced GPVI platelet expression (MFI≥18.2) had a poorer clinical outcome that may be improved by an intensified and timely antithrombotic therapy. Our previous findings suggested that high pretreatment platelet activation is associated with elevated platelet aggregability indicating a low response to standard antiplatelet therapy, when the conventional antiplatelet regimen is not highly effective in high-risk patients of ACS.

Specific therapeutic medical strategy may significantly influence prognosis in reducing the risk of TIA or stroke. Thus, patients with a recent TIA or stroke benefited from the administration of 80 mg atorvastatin per day in the reduction of overall incidence strokes and cardiovascular events. The rather detrimental clinical outcome in our patients with increased GPVI expression was not caused by the medication since patients had been administered comparable therapeutic regimes consisting of statins and antiaggregatory therapy.

Thus, future studies should unveil whether platelet-specific thrombotic GPVI might not only be a predictive biomarker but also a prognostic marker for further thromboischemic events. GPVI may help to define therapeutic strategies in patients with stroke.

Conclusion
We found a significantly enhanced expression of the platelet collagen receptor GPVI in patients with TIA and stroke compared to patients with non-ischemic events. Expression levels of GPVI determined on admission are strongly associated with stroke, independent of conventional laboratory markers such as C-reactive protein, blood glucose, and creatine kinase. Patients with an elevated platelet GPVI expression level had a poorer clinical outcome. Determination of the platelet-specific thrombotic marker GPVI may be useful as a biomarker for cerebral ischemia. Future studies should substantiate GPVI for its potential role for risk prediction and consider a possible prognostic value in order to improve risk stratification and guidance of treatment in patients, e.g. in dual antiplatelet therapy.

Acknowledgements

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Conflict of interest: non declared.

References


Legends to the Figures

**Figure 1: Platelet Collagen Receptor Glycoprotein VI Expression is Elevated in Patients With TIA and Stroke**

(A) Platelets of patients with non-ischemic events (NI), transient ischemic attack (TIA) and stroke were stained for the collagen receptor (GPVI) and P-selectin (CD62P) using monoclonal antibodies conjugated either to FITC or PE. Two-color whole blood flow cytometry was performed according to a standardized protocol and results were expressed as mean fluorescence intensity (MFI). (B) Expression of GPVI in patients according to the Trial of Org10172 in Acute Stroke Treatment (TOAST) classification. Patients with large-artery atherosclerosis (TOAST1) and cardioembolism (TOAST2) have shown significantly increased GPVI levels in contrast to patients with non-ischemic events (NI). Whiskers present 95% confidence intervals. (C) We found no correlation between platelet surface GPVI expression and the severity of stroke assessed by the National Institutes of Health Stroke Scale (NIHSS) \( r = -0.19; p = 0.789 \).\(^{15}\)

**Figure 2: Receiver Operating Characteristic (ROC) Curve and Relative Risk**

(A) Levels of platelet surface expression of GPVI mean fluorescence intensity (MFI) represented in a receiver operating characteristic (ROC) curve. GPVI cut off value for stroke is found at 18.2 MFI. Area under curve is 0.683 (95%CI, 0.585 to 0.757). (B) Patients with increased GPVI levels (MFI≥18.2) had a 2.4-fold relative risk (95%CI, 1.4 to 4.2) for stroke, patients with lower GPVI (MFI<18.2) presented a value of 0.5 (95%CI, 0.4 to 0.7).

**Figure 3: Cumulative Event-Free Survival**
Cumulative event-free survival considered stroke, myocardial infarction and cerebro-/cardiovascular death in three-month follow-up by telephone interview (92.2%: 189 of 205 patients). Patients with an elevated platelet GPVI expression level (MFI ≥ 18.2) had a poorer clinical outcome than patients with a decreased GPVI expression (Log rank; p=0.045).
Table 1. Baseline Patients’ Characteristics and Medical Treatment on Hospital Admission

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>All (n=205)</th>
<th>TIA (n=18)</th>
<th>Stroke (n=133)</th>
<th>Non-Ischemic (n=54)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age – yrs</td>
<td>71.6±12.9*</td>
<td>68.2±16.2*</td>
<td>73.1±11.6*</td>
<td>68.8±15.1*</td>
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<tr>
<td>Sex – no. (%)</td>
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<tr>
<td>Female</td>
<td>85 (41.5)</td>
<td>7 (38.9)</td>
<td>56 (42.1)</td>
<td>22 (40.7)</td>
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<td>Male</td>
<td>120 (58.5)</td>
<td>11 (61.1)</td>
<td>77 (57.9)</td>
<td>32 (59.3)</td>
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<tr>
<td>Cerebro-/Cardiovascular Risk Factors – no. (%)</td>
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<td>Arterial Hypertension</td>
<td>144 (70.2)</td>
<td>13 (72.2)</td>
<td>103 (77.4)</td>
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<td>Hyperlipidaemia</td>
<td>81 (39.5)</td>
<td>6 (33.3)</td>
<td>64 (48.1)</td>
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<td>Diabetes</td>
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<td>Family History of CVD</td>
<td>15 (7.3)</td>
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<td>11 (8.3)</td>
<td>4 (7.4)</td>
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<tr>
<td>Smoking</td>
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<td>2 (11.1)</td>
<td>31 (23.3)</td>
<td>9 (16.7)</td>
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<td>Atrial Fibrillation</td>
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<td>2 (11.1)</td>
<td>51 (38.3)</td>
<td>8 (14.8)</td>
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<td>Coronary Artery Disease</td>
<td>68 (33.2)</td>
<td>2 (11.1)</td>
<td>52 (39.1)</td>
<td>14 (25.9)</td>
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<tr>
<td>Medication – no. (%)</td>
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<td></td>
<td></td>
<td></td>
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<tr>
<td>ACE Inhibitors</td>
<td>49 (23.9)</td>
<td>2 (11.1)</td>
<td>32 (24.1)</td>
<td>15 (27.8)</td>
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<tr>
<td>Angiotensin Receptor Blockers</td>
<td>9 (4.4)</td>
<td>1 (5.6)</td>
<td>5 (3.8)</td>
<td>3 (5.6)</td>
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<tr>
<td>Beta Blockers</td>
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<td>7 (38.9)</td>
<td>39 (29.3)</td>
<td>15 (27.8)</td>
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<td>Statins</td>
<td>34 (16.6)</td>
<td>6 (33.3)</td>
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<td>8 (14.8)</td>
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<td>Aspirin</td>
<td>37 (18)</td>
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<td>Clopidogrel</td>
<td>3 (1.5)</td>
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<td>2 (1.5)</td>
<td>1 (1.9)</td>
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<tr>
<td>Vitamin K Antagonist</td>
<td>20 (9.8)</td>
<td>1 (5.6)</td>
<td>15 (11.3)</td>
<td>4 (7.4)</td>
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</table>

*mean ± standard deviation. CVD denotes cerebro-/cardiovascular disease, ACE angiotensin converting enzyme
Table 2. Predictive Value of GPVI for the Development of Stroke

<table>
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<tr>
<th>Parameters</th>
<th>P Value</th>
<th>Odds Ratio</th>
<th>95% CI for Odds Ratio</th>
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</thead>
<tbody>
<tr>
<td>GPVI (MFI)</td>
<td>0.003</td>
<td>1.199</td>
<td>1.065</td>
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<td>C-reactive protein (mg/dL)</td>
<td>0.800</td>
<td>1.019</td>
<td>0.882</td>
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<td>Blood Glucose (mg/dL)</td>
<td>0.117</td>
<td>0.990</td>
<td>0.978</td>
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<td>Creatine Kinase (U/L)</td>
<td>0.333</td>
<td>0.999</td>
<td>0.997</td>
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## Table 3. Results of Clinical Follow-up at 30 Days and 3 Months

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>All (n=189)</th>
<th>GPVI MFI&lt;18.2 (n=93)</th>
<th>GPVI MFI≥18.2 (n=96)</th>
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<tr>
<td><strong>At 30 Days</strong></td>
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<tr>
<td>Myocardial Infarction</td>
<td>3 (1.6%)</td>
<td>1 (1.1%)</td>
<td>2 (2.1%)</td>
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<tr>
<td>Stroke</td>
<td>4 (2.1%)</td>
<td>1 (1.1%)</td>
<td>3 (3.1%)</td>
</tr>
<tr>
<td>CV Death</td>
<td>7 (3.7%)</td>
<td>1 (1.1%)</td>
<td>6 (6.2%)</td>
</tr>
<tr>
<td>Composite MI/Stroke/CV Death</td>
<td>14 (7.4%)</td>
<td>3 (3.2%)</td>
<td>11 (11.5%)</td>
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<td><strong>At 3 Months</strong></td>
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<tr>
<td>Myocardial Infarction</td>
<td>5 (2.6%)</td>
<td>2 (2.2%)</td>
<td>3 (3.1%)</td>
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<tr>
<td>Stroke</td>
<td>6 (3.2%)</td>
<td>2 (2.2%)</td>
<td>4 (4.2%)</td>
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<tr>
<td>CV Death</td>
<td>11 (5.8%)</td>
<td>1 (1.1%)</td>
<td>10 (10.4%)</td>
</tr>
<tr>
<td>Composite MI/Stroke/CV Death</td>
<td>22 (11.6%)</td>
<td>5 (5.4%)</td>
<td>17 (17.7%)</td>
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</table>
Table 4. Medical Treatment of Follow-up Patients on Admission and after 3 Months

<table>
<thead>
<tr>
<th>Medication – no. (%)</th>
<th>All (n=189)</th>
<th>GPVI MFI&lt;18.2 (n=93)</th>
<th>GPVI MFI≥18.2 (n=96)</th>
<th>P Value</th>
</tr>
</thead>
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<tr>
<td><strong>On Admission</strong></td>
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<tr>
<td>ACE Inhibitors</td>
<td>47 (24.8)</td>
<td>21 (22.6)</td>
<td>26 (27.1)</td>
<td>0.055</td>
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<td>Angiotensin Receptor Blockers</td>
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<tr>
<td>Beta Blockers</td>
<td>59 (31.2)</td>
<td>27 (29)</td>
<td>32 (33.3)</td>
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<td>Statins</td>
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<td>2 (2.2)</td>
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<td>Vitamin K Antagonist</td>
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<td>11 (11.5)</td>
<td>0.193</td>
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<td><strong>At 3 Months</strong></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>ACE Inhibitors</td>
<td>69 (36.5)</td>
<td>37 (39.8)</td>
<td>32 (33.3)</td>
<td>0.074</td>
</tr>
<tr>
<td>Angiotensin Receptor Blockers</td>
<td>15 (7.9)</td>
<td>8 (8.6)</td>
<td>7 (7.3)</td>
<td>0.508</td>
</tr>
<tr>
<td>Beta Blockers</td>
<td>82 (43.4)</td>
<td>39 (41.9)</td>
<td>43 (44.8)</td>
<td>0.156</td>
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<tr>
<td>Statins</td>
<td>54 (28.6)</td>
<td>25 (26.9)</td>
<td>29 (30.2)</td>
<td>0.122</td>
</tr>
<tr>
<td>Aspirin</td>
<td>78 (41.3)</td>
<td>36 (38.7)</td>
<td>42 (43.8)</td>
<td>0.051</td>
</tr>
<tr>
<td>Clopidogrel</td>
<td>14 (7.4)</td>
<td>6 (6.5)</td>
<td>8 (8.3)</td>
<td>0.219</td>
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<tr>
<td>Vitamin K Antagonist</td>
<td>48 (25.4)</td>
<td>23 (24.7)</td>
<td>25 (26)</td>
<td>0.360</td>
</tr>
<tr>
<td>Aspirin+Dipyridamole</td>
<td>25 (13.2)</td>
<td>11 (11.8)</td>
<td>14 (14.6)</td>
<td>0.146</td>
</tr>
</tbody>
</table>
Bigalke et al., Figure 1

---

**A**

- P = 0.002
- P = 0.02
- P = 0.145
- P = 0.048

**B**

- P = 0.002
- P = 0.328
- P = 0.253

**Legend**

- **GPVI**
- **CD62P**

**Data**

- **No Ischemia**
  - GPVI: n = 54
  - CD62P: n = 18
- **TIA**
  - GPVI: n = 133
- **Stroke**
  - GPVI: n = 54
  - CD62P: n = 51

**MFI**

- Axis X: No Ischemia, TIA, Stroke, No Ischemia TOAST1, TOAST2
- Axis Y: MFI

---
Bigalke et al., Figure 1

C

\[ r = -0.19; \quad P = 0.789 \]
Bigalke et al., Figure 2

A

GPVI Cut Off for Stroke

Area: 0.683

B

Relative Risk

GPVI < 18.2

GPVI ≥ 18.2

0.3 0.5 0.8 1 1.5 2 2.5 3 4 5
Bigalke et al., Figure 3

Cumulative Event-Free Survival (MI, Ischemic Stroke, CV Death)

Log rank P=0.045

<table>
<thead>
<tr>
<th></th>
<th>No. at risk</th>
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</thead>
<tbody>
<tr>
<td>Cutoff GPVI (MFI) &lt;18.2</td>
<td>93</td>
<td>90</td>
<td>89</td>
<td>88</td>
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<tr>
<td>Cutoff GPVI (MFI) ≥18.2</td>
<td>96</td>
<td>85</td>
<td>81</td>
<td>79</td>
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<tr>
<td>Total</td>
<td>189</td>
<td>175</td>
<td>170</td>
<td>167</td>
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</table>