




The Effect of Protease-Activated Receptor-1 (PAR-1) Inhibition on Endothelial-Related Biomarkers in Patients with Coronary Artery Disease

Dennis W. T. Nilsen^{1,2}  Michelle Røysland¹ Thor Ueland^{3,4} Pål Aukrust^{4,5,6} Annika E. Michelsen^{4,5} Harry Staines⁷ Ståle Barvik¹ Frederic Kontny^{2,8} Jan Erik Nordrehaug^{1,2} Vernon V. S. Bonarjee¹

¹ Department of Cardiology, Stavanger University Hospital, Stavanger, Norway

² Department of Clinical Science, University of Bergen, Bergen, Norway

³ Department of Clinical Medicine, Thrombosis Research Center, UiT – The Arctic University of Norway, Tromsø, Norway

⁴ Faculty of Medicine, University of Oslo, Oslo, Norway

⁵ Research Institute of Internal Medicine, Oslo University Hospital, Rikshospitalet, Oslo, Norway

⁶ Section of Clinical Immunology and Infectious Diseases, Oslo University Hospital, Rikshospitalet, Oslo, Norway

⁷ Sigma Statistical Services, Balmullo, United Kingdom of Great Britain and Northern Ireland

⁸ Drammen Heart Center, Drammen, Norway

Address for correspondence Dennis W. T. Nilsen, MD, PhD, Department of Cardiology, Stavanger University Hospital, Helse Stavanger HF, PB 8100, 4068 Stavanger, Norway (e-mail: dnilsen1@gmail.no).

Thromb Haemost 2023;123:510–521.

Abstract

Background Vorapaxar has been shown to reduce cardiovascular mortality in post-myocardial infarction (MI) patients. Pharmacodynamic biomarker research related to protease-activated receptor-1 (PAR-1) inhibition with vorapaxar in humans has short follow-up (FU) duration and is mainly focused on platelets rather than endothelial cells. **Aim** This article assesses systemic changes in endothelial-related biomarkers during vorapaxar treatment compared with placebo at 30 days' FU and beyond, in patients with coronary heart disease.

Methods Local substudy patients in Norway were included consecutively from two randomized controlled trials; post-MI subjects from TRA2P-TIMI 50 and non-ST-segment elevation MI (NSTEMI) patients from TRACER. Aliquots of citrated blood were stored at -80° C. Angiotensin-2, angiotensin-like 4, vascular endothelial growth factor, intercellular adhesion molecule-1, vascular cell adhesion molecule-1, E-selectin, von Willebrand factor, thrombomodulin, and plasminogen activator inhibitor-1 and -2 were measured at 1-month FU and at study completion (median 2.3 years for pooled patients).

Results A total of 265 consecutive patients (age median 62.0, males 83%) were included. Biomarkers were available at both FUs in 221 subjects. In the total population, angiotensin-2 increased in patients on vorapaxar as compared with placebo at 1-month FU ($p=0.034$). Angiotensin-like 4 increased ($p=0.028$) and plasminogen activator inhibitor-2 decreased ($p=0.025$) in favor of vorapaxar at final FU. In post-

Keywords

- ▶ PAR-1 inhibition
- ▶ vorapaxar
- ▶ myocardial infarction (MI)
- ▶ post-MI
- ▶ NSTEMI
- ▶ biomarkers
- ▶ endothelium

received

July 4, 2022

accepted after revision

October 20, 2022

article published online

December 31, 2022

DOI <https://doi.org/>

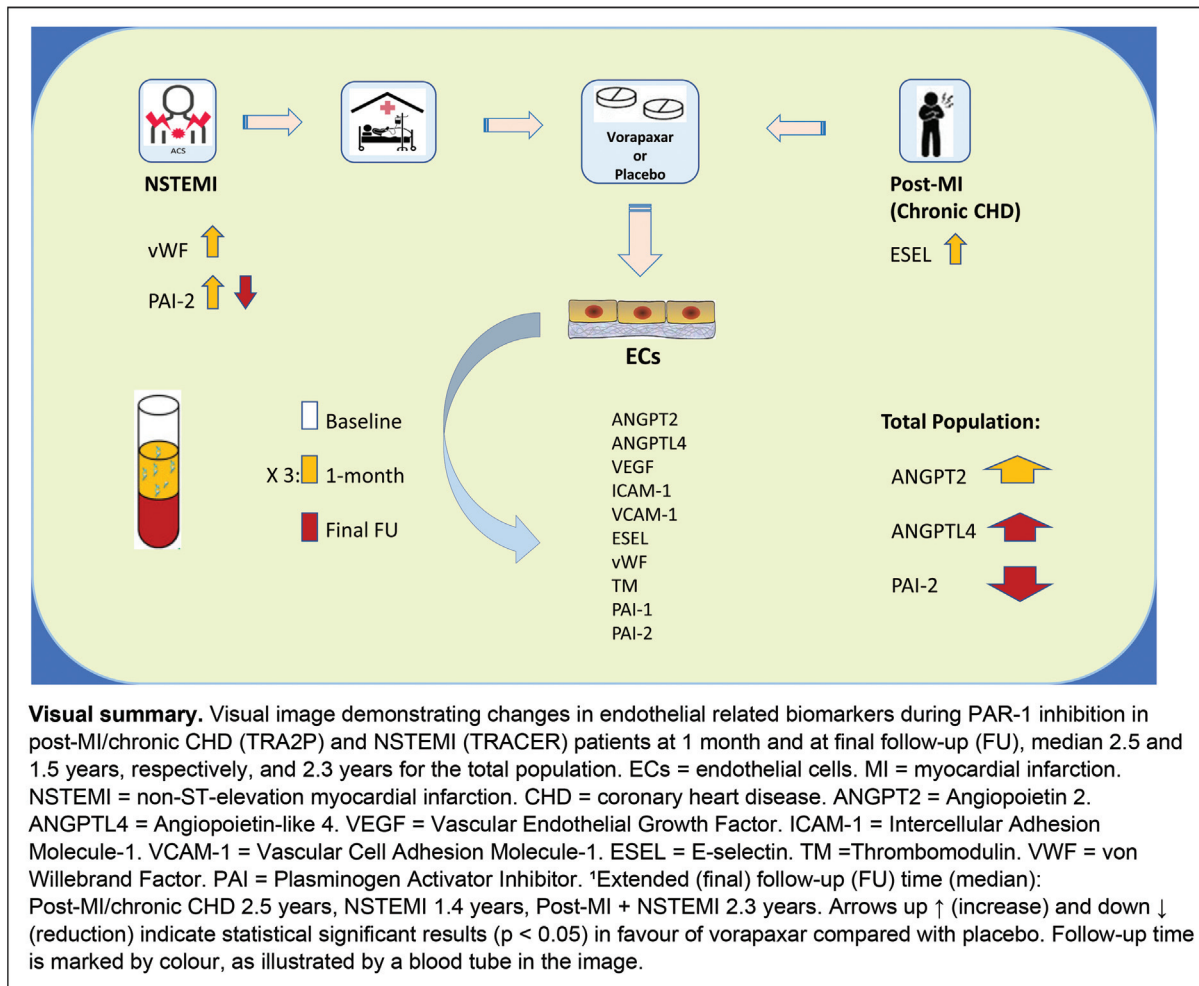
10.1055/s-0042-1760256.

ISSN 0340-6245.

© 2022. The Author(s).

This is an open access article published by Thieme under the terms of the Creative Commons Attribution-NonDerivative-NonCommercial-License, permitting copying and reproduction so long as the original work is given appropriate credit. Contents may not be used for commercial purposes, or adapted, remixed, transformed or built upon. (<https://creativecommons.org/licenses/by-nc-nd/4.0/>)

Georg Thieme Verlag KG, Rüdigerstraße 14, 70469 Stuttgart, Germany



MI subjects, a short-term increase in E-selectin favoring vorapaxar was observed, $p = 0.029$. Also, a short-term increase in von Willebrand factor ($p = 0.032$) favoring vorapaxar was noted in NSTEMI patients.

Conclusion Significant endothelial biomarker changes during PAR-1 inhibition were observed in post-MI and NSTEMI patients.

Introduction

Protease-activated receptor-1 (PAR-1) is expressed in a large number of different cell types and is abundantly expressed by both platelets and endothelial cells.^{1,2} The G protein-coupled PAR-1 is irreversibly activated by thrombin¹ and by activated factor X (FXa).^{3,4} Activation of PAR-1 stimulates complex intracellular signaling networks. Receptor crosstalk mechanisms differ and contribute to a diversity of signal transduction and receptor-trafficking processes, resulting in multiple physiological effects.⁵ However, whereas low concentrations of thrombin and FXa may provide endothelial protective actions by PAR-1-mediated routes under physiological conditions,^{4,6} high and persistently elevated levels could promote thrombus formation and enhanced endothelial cell activation.

Clinical trials on vorapaxar, a potent inhibitor of PAR-1,⁷ such as TRA2P-TIMI 50⁸ and TRACER,⁹ have investigated vorapaxar's efficacy to reduce future cardiovascular events in stable post-myocardial infarction (MI) patients and in patients suffering an acute coronary syndrome (ACS), respectively. Biomarker research related to PAR-1 inhibition with vorapaxar in humans has essentially focused on platelets and to some degree on endothelial cells.¹⁰ However, follow-up (FU) time has been short.

To further elucidate the effects of vorapaxar, we predefined a subproject at three study sites in Norway, examining both short and late effects of vorapaxar on a wide range of markers of endothelial cell activation, also reflecting inflammation and platelet activation, in subjects from the TRA2P-TIMI 50 study, in which the included patients had undergone a MI 2 weeks to 12 months earlier, and from the TRACER

Table 1 Endothelial biomarkers

	Description and functional aspects
Angiopoietin-2 (ANGPT2)	Involved in angiogenesis, inflammation and progression of atherosclerosis. ^{4,21,22} Potential prognostic biomarker in cardiovascular disease (CVD). ^{11,23,24} Released from Weibel-Palade bodies of endothelial cells (ECs). ²¹ Upregulated by inflammatory stimuli, counteracting the vascular protective effects of angiopoietin-1 (ANGPT1) ^{11,21,22}
Angiopoietin-like 4 (ANGPTL4)	Involved in angiogenesis, inflammation and progression of atherosclerosis. ^{4,21} Potential prognostic biomarker in CVD. ^{23,25} Highly expressed on ECs in response to ischemia ^{25,26} and is involved in angiogenesis ^{15,16,27} and metabolism regulation. ^{16,25,28} Full-length ANGPTL4 is cleaved into an N-terminal and a C-terminal fragment, which display different biological functions. ²⁵ Full-length ²⁸ and soluble N-terminal fragment are mainly involved in lipid metabolism. C-terminal fragment is mainly produced by endothelial cells and is involved in regulation of vascular integrity and angiogenesis ²⁸
Vascular endothelial growth factor (VEGF)	Potent angiogenic factor, promoted by ANGPT2 ²⁹
E-selectin (ESEL)	One of the three members of the selectin family, expressed on ECs in response to inflammation. Plays a role in cell adhesion to the vascular endothelium, and regulates leukocyte recruitment during inflammation ^{30,31}
Intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion protein-1 (VCAM-1)	Plays a role in cell adhesion to the vascular endothelium together with ESEL. A firm union and transmigration of leucocytes depend on the binding to ICAM-1 and VCAM-1. ^{30,31} Adhesion molecules separated from the cell surface may reflect their expression on the endothelial surface ³¹
Thrombomodulin (TM)	High-affinity thrombin receptor expressed on the ECs surface. ^{32–34} Serves as a cofactor of thrombin-catalyzed activation of protein C, counteracting the procoagulant effects of thrombin ^{32,33}
von Willebrand factor (VWF)	Synthesized within ECs and is either directly released or stored in the Weibel-Palade bodies. ^{19,35} Upon release, VWF rapidly unfolds into ultralong strings, docking on the endothelium and acting as a bridging molecule, promoting platelet adherence and aggregation ³⁵
Plasminogen activator inhibitor-1 (PAI-1)	Main inhibitor of tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA). Elevated PAI-1 is a risk factor for thrombosis and atherosclerosis ³⁶
Plasminogen activator inhibitor-2 (PAI-2)	Weak inhibitor of fibrinolysis. ²⁰ Present in high quantities in the placenta and only in low concentrations in quiescent ECs. ^{12,20} Rapidly stimulated by a variety of inflammatory mediators. Only small quantities are exported from ECs due to lack of N-terminal signal peptide. Its physiological importance is still being investigated ^{37,38}

study (one study site) consisting of non-ST-segment elevation MI (NSTEMI) patients.

The selected biomarkers in this study included mediators with a major role in leukocyte and endothelial interactions (i.e., E-selectin [ESEL], intercellular adhesion molecule-1 [ICAM-1], and vascular cell adhesion molecule-1 [VCAM-1]), angiogenic factors with effects on inflammation (angiopoietin-2 [ANGPT2], angiopoietin-like 4 [ANGPTL4], and vascular endothelial growth factor [VEGF]), mediators with antifibrinolytic (plasminogen activator inhibitor-1 [PAI-1] and [PAI-2]) and antithrombin (thrombomodulin [TM]) effects, and a mediator promoting endothelial-platelet interaction (von Willebrand factor [VWF]). For more details, see ► **Table 1**.

Methods

Study Population

Patients were recruited consecutively from two large international, multicenter, randomized, double-blind, clinical trials. In the “Trial to Assess the Effects of Vorapaxar in Preventing Heart Attack and Stroke in Patients with Atherosclerosis” (TRA2P-TIMI 50), ClinicalTrials.gov Identifier: NCT00526474,⁸ three sites in Norway, with the main site located in Stavanger, participated in a biomarker substudy, in

which patients who had suffered an MI 2 weeks to 12 months earlier, were included. At the Stavanger site, patients were also recruited from the “Thrombin Receptor Antagonist for Clinical Event Reduction in ACS (TRACER)” trial, ClinicalTrials.gov Identifier: NCT00527943,⁹ in which NSTEMI patients were included, applying a similar study protocol as for the TRA2P-TIMI 50 substudy. Recruitment of patients in the main trials was ongoing when the subproject was launched. Patients in both TRA2P-TIMI 50 and TRACER were treated with a daily maintenance dose of 2.5 mg vorapaxar, with a loading dose of 40 mg in TRACER, or matching placebo. Background medication was recorded for all patients. Median FU length in the main studies was 30 months for TRA2P-TIMI 50 and 502 days for TRACER, respectively, and locally the median FU time was 29.9 months for the former and 540 days for the latter, as provided by the main trial organizers.

Laboratory Measurements

Treatment effects on 10 biomarkers related to endothelial function were studied in citrated plasma processed from venous blood harvested at baseline, 1-month FU, and prolonged FU. Samples were stored at -80°C in aliquots containing 0.25 mL citrated plasma. Plasma levels of ANGPT2,

ANGPTL4, ICAM-1, VCAM-1, ESEL, TM, PAI-1, PAI-2 (R&D Systems, Minneapolis, Minnesota, United States) and VEGF-A (PeproTech, Cranbury, New Jersey, United States) were measured in duplicate by enzyme immunoassays (EIAs), applying commercially available antibodies in a 384 format, and using a combination of a SELMA (Jena, Germany) pipetting robot and a BioTek (Winooski, Vermont, United States) dispenser/washer. Absorption was read at 450 nm with wavelength correction set to 540 nm using an enzyme-linked immunosorbent assay plate reader (Bio-Rad, Hercules, California, United States). Plasma VWF levels were measured by EIAs, as described above with antibodies obtained from Dako (Glostrup, Denmark), using a polyclonal antibody for coat (A0082) and a horseradish peroxidase-conjugated polyclonal antibody for detection (P02256). Parallel-diluted pooled human plasma from 10 random samples was used as standard. Intra- and interassay coefficients of variation were < 10% for all EIAs. Units were - ng/mL, except for VEGF (pg/mL) and VWF (AU).

Statistics

Absolute values were reported as median (Q1–Q3). Differences in baseline characteristics were assessed by the Kruskal–Wallis test for continuous data and the chi-squared test for categorical data. The Mann–Whitney *U* test was used to test for the equality of the median of two samples, comparing treatment differences and intragroup changes for (1) both patient groups collectively (pooled), (2) post-MI patients (chronic coronary heart disease [CHD]) at the three sites, (3) NSTEMI patients recruited at the site in Stavanger, and (4) males and females separately. Statistical significance was set at $p < 0.05$, whereas statistical trends were defined as p -values between 0.05 and 0.1, also used in view of the hypothesis-generating nature of this study. As this was an exploratory study, no adjustment was made for multiplicity and no power calculations were undertaken.

Results

In all, 328 patients (age median 61.0 years [Q1–Q3: 55.0–68.0 years], males 82%) were included. Pending and directly following approvals by the Ethics Committee of Northern Norway, 265 (age median 62.0 [Q1–Q3: 55.0–68.0 years], males 83%) consecutive subjects with at least one change from baseline value were included in the pooled biomarker study. At the three TRA2P-TIMI 50 sites, there were 186 patients; of these, 158 patients were included at the largest center located in Stavanger. From TRACER, 79 patients were all included at the site in Stavanger. Biomarkers were available in 221 patients at both 30 days' and median 2.3 years' FU. There were no statistical differences in important baseline variables between the vorapaxar and placebo groups, counting all patients (► **Supplementary Table S1**, available in the online version), or those with available biomarkers (► **Table 2**).

Absolute biomarker values for the pooled study and by clinical subgroups at baseline and at early and final (late) FU, respectively, are shown in ► **Tables 3–5**.

Biomarker Changes between Treatment Arms

Within-group changes from baseline to 1-month- and late FU, respectively, and treatment-induced differences in the pooled study and by clinical subgroups are shown in ► **Table 6**. ► **Supplementary Figs. S1–S5** (A and B, available in the online version) illustrate the changes for each biomarker in the pooled study. Intergroup p -values for each biomarker are summarized in ► **Supplementary Tables S2–S4** (available in the online version), for the pooled study and by clinical subgroups and sex, respectively.

A significant overall increase in ANGPT2 and ANGPTL4 in favor of vorapaxar as compared with placebo patients was observed for pooled patients. However, whereas the increase for ANGPT2 ($p = 0.034$) was noted at 1-month FU, mainly contributed by males ($p = 0.018$), the increase in ANGPTL4 was observed at late FU ($p = 0.028$), and was also statistically present in males ($p = 0.045$), as shown in ► **Table 6** and ► **Supplementary Table S3** (available in the online version), respectively.

Between-group trends in ANGPT2 were noted for both post-MI ($p = 0.062$) and NSTEMI patients ($p = 0.081$) at 1-month FU, whereas clinical group-related treatment differences were not quite that obvious during late FU, with p -values between 0.1 and 0.2 (► **Table 6**). The only statistically significant between-treatment difference was noted for ANGPTL4 at late FU in NSTEMI patients ($p = 0.045$).

No significant intergroup difference in biomarker changes were noted for VEGF during treatment with vorapaxar as compared with placebo, ignoring an increase during short-term intervention in females belonging to the TRACER group, due to a small sample size.

ICAM-1 and VCAM-1 in the total study population and in the clinical subgroups of TRA2P-TIMI 50 and TRACER, respectively, were unaffected by treatment, whereas VCAM-1 increased significantly ($p = 0.038$) by PAR-1 inhibition in the TRA2P female population during short-term treatment with vorapaxar as compared with placebo, as shown in ► **Supplementary Table S4** (available in the online version).

ESEL increased ($p = 0.029$) in the post-MI (chronic CHD) group of patients, but not in the total study population, during short-term (1 month) treatment with vorapaxar (► **Table 6**). These changes were limited to males ($p = 0.018$), as shown in ► **Supplementary Table S3** (available in the online version), and were not present at late FU.

TM was unaffected by vorapaxar treatment as compared with placebo, regardless of patient group and sex, whereas a trend toward an increase in VWF in the pooled population was noted in favor of vorapaxar at 1-month FU ($p = 0.075$), mainly related to changes in NSTEMI patients ($p = 0.032$) and in males of that group ($p = 0.030$), as shown in ► **Table 6** and ► **Supplementary Table S3** (available in the online version), respectively.

No intergroup difference in PAI-1 change was noted in the pooled population or in the subgroups during intervention with vorapaxar as compared with placebo. PAI-2 decreased significantly in favor of vorapaxar as compared with placebo

Table 2 Baseline profile of pooled post-MI and NSTEMI patients with measured biomarkers ($n=265$) at Norwegian sites participating in the biomarker subproject, applying chi-squared [1] and Kruskal–Wallis [2] tests

Baseline variables	Total	Placebo	Vorapaxar	p -Value
	($N=265$)	($N=123$)	($N=142$)	
Age (y) median (Q1–Q3)	62.0 (55.0–68.0)	63.0 (55.0–69.0)	61.0 (55.0–68.0)	0.668 [2]
Males, n (%)	220 (83.02)	104 (84.55)	116 (81.69)	0.536 [1]
Baseline BMI < median	144 (54.34)	63 (51.22)	81 (57.04)	0.343 [1]
Hist. of MI/AP, n (%)	228 (86.04)	103 (83.74)	125 (88.03)	0.315 [1]
Index diagnosis				
STEMI, n (%)	91 (34.34)	40 (32.52)	51 (35.92)	0.254 [1]
NSTEMI, n (%)	168 (63.40)	82 (66.67)	86 (60.56)	
AMI other, n (%)	6 (2.26)	1 (0.81)	5 (3.52)	
Hist. of PCI, n (%)	179 (67.55)	79 (64.23)	100 (70.42)	0.283 [1]
Hist. of HF, n (%)	15 (5.66)	8 (6.50)	7 (4.93)	0.580 [1]
Hist. of HT, n (%)	111 (41.89)	50 (40.65)	61 (42.96)	0.704 [1]
Hist. of DM, n (%)	31 (11.70)	15 (12.20)	16 (11.27)	0.815 [1]
Hist. of smoking, n (%)	197 (74.34)	90 (73.17)	107 (75.35)	0.685 [1]
Current smoker, n (%)	55 (20.75)	21 (17.07)	34 (23.94)	0.169 [1]
CrCl (mL/min) median (Q1–Q3)	107.0 (82.9–136.7)	107.1 (83.1–136.6)	106.6 (82.9–136.9)	0.759 [2]
Aspirin, n (%)	261 (98.49)	120 (97.56)	141 (99.30)	0.248 [1]
Thienopyridines, n (%)	188 (70.94)	87 (70.73)	101 (71.13)	0.944 [1]
Statins, n (%)	251 (94.72)	117 (95.12)	134 (94.37)	0.784 [1]

Abbreviations: AMI, acute myocardial infarction; BMI, body mass index; CrCl, creatinine clearance; DM, diabetes mellitus; HF, heart failure; Hist., history; HT, hypertension; MI/AP, myocardial infarction or angina pectoris; NSTEMI, non-ST-segment elevation myocardial infarction; PCI, percutaneous coronary intervention; Q, quartile; STEMI, ST-segment elevation myocardial infarction.

in the total study population ($p=0.025$) at late FU, essentially driven by changes in the post-MI (chronic CHD) patients ($p=0.054$), whereas vorapaxar induced a borderline significant increase ($p=0.05$) in NSTEMI (TRACER) patients during short-term therapy (►Table 6). Males were the main contributors to these changes ($p=0.045$), as shown in ►Supplementary Table S3 (available in the online version).

All statistically significant biomarker changes in patients on vorapaxar as compared with those on placebo in the pooled study and by subgroups are summarized in ►Fig. 1.

Discussion

Short- and late effects of PAR-1 inhibition with vorapaxar on 10 biomarkers related to endothelial function were assessed in a combined analysis of two similar substudies based on TRA2P-TIMI 50⁸ recruiting post-MI subjects and TRACER⁹ recruiting NSTEMI patients, respectively. Comparing changes in biomarkers between treatment groups, we found that PAR-1 inhibition (1) increased ANGPT2 and ANGPTL4 during short- and late (final) FU, respectively, and (2) decreased PAI-2 at final FU, mainly driven by changes in post-MI patients, although a borderline significant transient increase in PAI-2 during short-term treatment was noted in NSTEMI patients. Whether the main findings reflect poten-

tial harmful effects of this treatment modality remains to be determined.

The vorapaxar-induced increase in ANGPT2 and ANGPTL4 in the pooled population would suggest that their expression is upregulated by PAR-1 inhibition. The increase in ANGPT2 was more pronounced in NSTEMI (TRACER) patients than in post-MI (TRA2P-TIMI 50) patients (in the present context classified as chronic CHD), possibly due to a more prominent expression of ANGPT2 in acute- as compared with chronic CHD, as previously reported.¹¹ ANGPT2 possesses inflammatory properties and acts as a chemoattractant.¹² An increase in this mediator may inflict damage during healing through an endothelial destabilizing effect, alleviated in the presence of angiopoietin-1 and VEGF.^{13,14} With regard to ANGPTL4, levels in placebo-treated NSTEMI patients dropped significantly from baseline, possibly indicating that ANGPTL4 expression is increased during the acute event. Furthermore, the overall vorapaxar-induced increase in ANGPTL4 at late FU would indicate that increased expression persists during PAR-1 inhibition. This could in the long run, at least theoretically, exert a negative impact on angiogenesis, lipid metabolism, and atherosclerotic development.^{15,16} Conversely, experimental studies suggest that overexpression of ANGPTL4 in endothelial cells may be protective and reduce lesion area and macrophage content in the endothelium wall.^{17,18} Furthermore, in subjects with metabolic syndrome

Table 3 Absolute biomarker values (median [Q1–Q3]) according to treatment groups (vorapaxar vs. placebo), assessed in pooled post-MI (chronic CHD) and NSTEMI patients at 1-month and extended follow-up (median 2.3 years), respectively

Biomarker	Baseline		1-month follow-up		Extended follow-up	
	Placebo (N = 123)	Vorapaxar (N = 142)	Placebo (N = 122)	Vorapaxar (N = 140)	Placebo (N = 108)	Vorapaxar (N = 116)
ANGPT2	3.72 (2.97, 4.47)	3.63 (2.82, 4.47)	3.74 (3.03, 4.62)	3.77 (2.97, 4.79)	3.69 (3.06, 4.64)	3.51 (2.79, 4.29)
ANGPL4	9.68 (7.61, 12.40)	9.52 (7.76, 11.92)	8.80 (7.12, 11.76)	9.32 (7.32, 12.00)	9.51 (8.45, 12.16)	10.08 (8.09, 12.58)
VEGF	41.56 (28.21, 69.02)	49.41 (31.70, 82.74)	43.23 (29.28, 65.17)	50.46 (32.17, 97.59)	39.91 (28.88, 63.73)	46.11 (26.45, 79.08)
ICAM-1	173.20 (142.67, 197.60)	176.80 (154.40, 205.60)	172.30 (144.84, 195.40)	178.59 (157.61, 201.76)	168.86 (148.90, 196.04)	174.77 (142.50, 203.32)
VCAM-1	568.13 (440.00, 719.50)	575.46 (454.27, 706.60)	554.00 (439.00, 745.00)	606.91 (467.75, 744.08)	556.63 (454.63, 797.56)	567.56 (450.16, 789.63)
ESEL	19.78 (13.41, 26.10)	19.75 (13.80, 26.83)	19.15 (12.90, 25.40)	19.85 (14.20, 28.90)	20.90 (15.29, 27.70)	19.85 (13.80, 25.96)
TM	4.49 (3.77, 5.49)	4.53 (3.75, 5.75)	4.58 (3.76, 5.48)	4.67 (3.90, 5.73)	4.65 (3.96, 5.36)	4.67 (3.72, 5.65)
VWF	125.04 (110.50, 158.50)	133.00 (109.50, 161.34)	125.10 (105.45, 147.00)	133.02 (108.00, 158.00)	131.86 (106.75, 150.00)	132.05 (102.82, 156.49)
PAI-1	4.12 (2.47, 6.04)	3.60 (2.44, 5.91)	4.23 (2.69, 7.33)	4.17 (2.62, 6.61)	3.14 (1.93, 5.45)	3.16 (1.96, 4.97)
PAI-2	56.03 (37.95, 68.41)	53.01 (40.56, 68.83)	54.43 (39.80, 67.42)	53.43 (41.45, 68.31)	53.34 (40.14, 73.13)	51.57 (38.11, 64.75)

Abbreviations: ANGPT2, angiotensinogen-converting enzyme 2; ANGPTL4, angiotensin-like 4; CHD, coronary heart disease; ESEL, E-selectin; ICAM, intercellular adhesion molecule; MI, myocardial infarction; NSTEMI, non-ST-elevation myocardial infarction; PAI, plasminogen activator inhibitor; Q, quartile; TM, thrombomodulin; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor.

Table 4 Absolute biomarker values (median [Q1–Q3]) according to treatment groups (vorapaxar vs. placebo), assessed in post-MI (chronic CHD) at 1-month and extended follow-up (median 2.5 years), respectively

Biomarker	Baseline		1-month follow-up		Extended follow-up	
	Placebo (N = 84)	Vorapaxar (N = 102)	Placebo (N = 84)	Vorapaxar (N = 100)	Placebo (N = 74)	Vorapaxar (N = 87)
ANGPT2	3.80 (3.03, 4.59)	3.81 (2.85, 4.65)	3.69 (3.03, 4.56)	3.72 (2.93, 4.77)	3.93 (3.36, 4.71)	3.36 (2.70, 4.23)
ANGPL4	9.48 (7.12, 11.64)	9.24 (7.60, 11.84)	8.96 (7.48, 11.80)	9.32 (7.16, 11.92)	9.56 (8.56, 12.16)	10.32 (8.00, 13.04)
VEGF	37.13 (24.10, 69.55)	42.13 (26.35, 63.08)	38.99 (25.65, 64.59)	44.63 (27.60, 74.88)	41.60 (24.52, 69.72)	38.99 (24.24, 74.65)
ICAM-1	178.13 (158.97, 204.72)	180.83 (156.60, 210.20)	178.93 (153.42, 195.00)	183.24 (158.20, 205.90)	171.70 (156.00, 202.00)	170.86 (143.20, 203.60)
VCAM-1	587.02 (449.25, 736.55)	581.07 (455.00, 690.64)	550.25 (427.71, 722.56)	585.78 (444.80, 686.75)	542.52 (440.00, 776.00)	553.46 (429.22, 748.44)
ESEL	20.65 (15.88, 25.85)	19.95 (15.00, 25.90)	19.45 (15.10, 25.20)	20.05 (15.40, 26.65)	20.90 (16.40, 26.70)	19.80 (14.20, 24.40)
TM	4.45 (3.83, 5.38)	4.56 (3.95, 5.52)	4.43 (3.85, 5.35)	4.64 (3.97, 5.64)	4.48 (3.99, 5.09)	4.55 (3.71, 5.28)
VWF	121.54 (110.28, 150.20)	136.24 (110.50, 161.34)	123.31 (104.93, 146.13)	132.11 (109.50, 149.62)	132.79 (108.50, 151.11)	137.00 (109.50, 159.48)
PAI-1	4.51 (2.86, 6.94)	3.64 (2.57, 6.48)	4.59 (3.10, 8.16)	4.06 (2.27, 6.61)	3.41 (2.09, 7.04)	3.03 (1.99, 4.67)
PAI-2	57.53 (41.82, 68.49)	56.25 (43.80, 71.20)	56.65 (43.45, 77.91)	55.62 (42.48, 70.29)	53.34 (41.66, 76.10)	51.32 (37.01, 64.98)

Abbreviations: ANGPT2, angiotensinogen-converting enzyme 2; ANGPTL4, angiotensin-like 4; CHD, coronary heart disease; ESEL, E-selectin; ICAM, intercellular adhesion molecule; MI, myocardial infarction; PAI, plasminogen activator inhibitor; Q, quartile; TM, thrombomodulin; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor.

Table 5 Absolute biomarker values (median [Q1–Q3]) according to treatment groups (vorapaxar vs. placebo), assessed in NSTEMI patients at 1-month and extended follow-up (median 1.5 years), respectively

Biomarker	Baseline		1-month follow-up		Extended follow-up	
	Placebo	Vorapaxar	Placebo	Vorapaxar	Placebo	Vorapaxar
	(N = 39)	(N = 40)	(N = 38)	(N = 40)	(N = 34)	(N = 29)
ANGPT2	3.39 (2.58, 4.37)	3.08 (2.55, 3.80)	3.74 (2.90, 4.62)	4.01 (3.20, 5.21)	3.38 (2.56, 4.47)	3.84 (3.15, 4.66)
ANGPL4	10.90 (8.10, 15.30)	10.13 (8.00, 12.89)	8.42 (6.71, 11.74)	9.36 (7.82, 12.51)	9.40 (8.38, 12.71)	8.94 (8.17, 12.09)
VEGF	44.79 (39.41, 63.28)	59.18 (44.11, 105.00)	48.84 (39.41, 72.67)	76.00 (50.74, 103.52)	39.55 (33.80, 51.82)	62.19 (45.87, 86.63)
ICAM-1	151.15 (113.31, 178.44)	166.50 (140.70, 189.86)	159.93 (117.03, 195.60)	171.51 (146.60, 196.33)	162.26 (127.58, 188.36)	179.89 (134.40, 203.05)
VCAM-1	550.52 (429.62, 667.90)	572.53 (444.29, 745.08)	652.35 (471.87, 826.95)	672.01 (518.53, 938.76)	571.06 (482.44, 824.02)	669.07 (530.56, 839.86)
ESEL	17.32 (7.98, 26.23)	18.38 (7.39, 29.59)	14.39 (7.81, 26.06)	18.08 (8.36, 37.65)	20.93 (13.07, 34.98)	22.41 (12.23, 33.87)
TM	4.57 (2.70, 5.71)	4.50 (2.63, 5.95)	4.95 (3.35, 6.03)	4.89 (3.37, 6.80)	5.27 (3.87, 7.09)	4.75 (3.81, 6.32)
VWF	139.00 (111.00, 161.50)	131.25 (96.00, 160.25)	127.75 (106.00, 147.50)	140.00 (102.75, 170.75)	123.75 (106.00, 141.00)	114.00 (98.00, 152.00)
PAI-1	3.43 (1.28, 4.92)	3.42 (1.61, 5.41)	3.75 (1.32, 5.51)	4.25 (2.71, 6.44)	2.56 (1.76, 4.20)	3.60 (1.50, 6.61)
PAI-2	46.81 (30.39, 68.41)	48.64 (35.41, 62.03)	43.46 (32.14, 63.13)	49.47 (40.56, 64.98)	51.98 (36.81, 59.97)	51.74 (40.17, 63.59)

Abbreviations: ANGPT2, angiotensinogen 2; ANGPTL4, angiotensin-like 4; ESEL, E-selectin; ICAM, intercellular adhesion molecule; NSTEMI, non-ST-segment elevation myocardial infarction; PAI, plasminogen activator inhibitor; Q, quartile; TM, thrombomodulin; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor.

Table 6 Between-group differences in biomarker levels assessed by the Mann–Whitney test in pooled and by study post-MI (chronic CHD) and NSTEMI patients, respectively, at 1-month (1-M) and extended (EXT) follow-up^a in vorapaxar- versus placebo-treated patients

Time and marker	Treatment arm	N	Pooled post-MI and NSTEMI median change	p-Value between treatments	N	Post-MI median change	p-Value between treatments	N	NSTEMI median change	p-Value between treatments
1-M: ANGPT2	PL	122	-0.044	0.034	84	-0.210 ^c	0.062	38	0,390 ^c	0.081
	V	140	0.150 ^c		100	0.060		40	0,720	
EXT: ANGPT2	PL	108	-0.015	0.559	74	-0.045	0.148	34	0.210	0.185
	V	116	-0.090		87	-0.180		29	0.510	
1-M: ANGPTL4	PL	122	-0.560	0.304	84	-0.200	0.699	38	-1.607	0.162
	V	140	-0.400		100	-0.160		40	-1.292	
EXT: ANGPTL4	PL	108	-0.120	0.028	74	0.440	0.196	34	-1.467 ^c	0.045
	V	116	0.560 ^d		87	0.720		29	0.000	
1-M: VEGF	PL	122	2.384	0.794	84	0.424	0.611	38	4.046	0.704
	V	140	0.000		100	-0.848		40	4.690	
EXT: VEGF	PL	108	-2.804	0.266	74	-0.002	0.156	34	-5.233	0.591
	V	116	-3.071		87	-2.863		29	-7.051	
1-M: ICAM-1	PL	122	-0.724	0.554	84	-6.298	0.487	38	6.927	0.908
	V	140	-1.782		100	-6.081		40	11.010	
EXT: ICAM-1	PL	108	1.803	0.675	74	-7.700	0.700	34	5.789	0.689
	V	116	-0.200		87	-7.200		29	14.784	
1-M: VCAM-1	PL	122	-10.000	0.120	84	-28.500	0.201	38	67.494	0.165
	V	140	21.000		100	-16.750		40	136.163	
EXT: VCAM-1	PL	108	26.836	0.727	74	-5.824	0.658	34	96.546	0.549
	V	116	27.000		87	2.000		29	107.991	
1-M: ESEL	PL	122	0.035	0.135	84	-0.700 ^b	0.029	38	1.358	0.849
	V	140	0.505		100	0.500		40	0.509	
EXT: ESEL	PL	108	0.640	0.816	74	-0.100	0.959	34	2,250	0.684
	V	116	0.050		87	-0.600		29	2,632	
1-M: TM	PL	122	0.038	0.478	84	-0.090	0.481	38	0.309	0.830
	V	140	0.118		100	0.020		40	0.476	
EXT: TM	PL	108	0.065	0.959	74	-0.057	0.606	34	0.571	0.772
	V	116	0.105		87	-0.050		29	0.549	
1-M: VWF	PL	122	-6.419 ^d	0.075	84	-4.034	0.522	38	-8.750 ^c	0.032
	V	140	-0.500		100	-0.576		40	2.000	
EXT: VWF	PL	108	-1.864	0.867	74	0.661	0.477	34	-10.000	0.146
	V	116	-3.457		87	-4.034		29	1.500	
1-M: PAI-1	PL	122	0.164	0.634	84	0.240	0.796	38	-0.103	0.202
	V	140	0.214		100	0.202		40	0.711	
EXT: PAI-1	PL	108	-0.697	0.884	74	-0.767	0.815	34	-0.603	0.577
	V	116	-0.599		87	-0.810		29	-0.218	
1-M: PAI-2	PL	122	-0.699	0.142	84	-0.161	0.601	38	-2.383	0.050
	V	140	1.347		100	1.407		40	1.186	

Table 6 (Continued)

Time and marker	Treatment arm	N	Pooled post-MI and NSTEMI median change	p-Value between treatments	N	Post-MI median change	p-Value between treatments	N	NSTEMI median change	p-Value between treatments
EXT: PAI-2	PL	108	2.069	0.025	74	0.595	0.054	34	5.009	0.328
	V	116	-0.613		87	-1.559		29	0.832	

Abbreviations: ANGPT2, angiotensin 2; ANGPTL4, angiotensin-like 4; CHD, chronic heart disease; ESEL, E-selectin; ICAM, intercellular adhesion molecule; MI, myocardial infarction; NSTEMI, non-ST-segment elevation MI; PAI, plasminogen activator inhibitor; PL, placebo; TM, thrombomodulin; V, vorapaxar; VCAM, vascular cell adhesion molecule; VEGF, vascular endothelial growth factor; VWF, von Willebrand factor.

Note: Significance levels for within-group changes were indicated in the presence of statistically significant between-group differences.
^aExtended follow-up time (median): post-MI (chronic CHD) patients 2.5 years, NSTEMI patients 1.5 years, pooled MI and NSTEMI patients 2.3 years. Statistical significance was set at $p < 0.05$, whereas statistical trends were defined as p -values between 0.05 and 0.1 due to the hypothesis-generating design of this study.

^b $p = 0.05-0.1$.

^c $p < 0.05$.

^d $p < 0.01$.

and low-grade inflammation, ANGPTL4 was found to be independently and negatively associated with carotid atherosclerosis measured by 3-T magnetic resonance imaging.¹⁸ However, on the whole, an increase in ANGPT2 and ANGPTL4 could potentially reflect hitherto unrecognized harmful effects of PAR-1 inhibition.

We found a reduction in PAI-2 levels by vorapaxar as compared with placebo at late FU in the pooled cohort. Within-treatment variations of PAI-2 were rather inconspicuous, which may be due to endothelial cells containing and exporting only small amounts of PAI-2.¹² Although the synthesis of PAI-2 can be rapidly stimulated by inflammatory

Statistically significant biomarker changes during PAR-1 inhibition

Follow-up period	One Month				Extended		
	All	Post-MI	NSTEMI	NSTEMI	All	NSTEMI	All
Patient poulation							
Biomarker	ANGPT2	ESEL	VWF	PAI-2	ANGPTL4	ANGPTL4	PAI-2
Units	ng/mL	ng/mL	AU	ng/mL	ng/mL	ng/mL	ng/mL
p-value	0.034	0.029	0.032	0.050	0.028	0.045	0.025

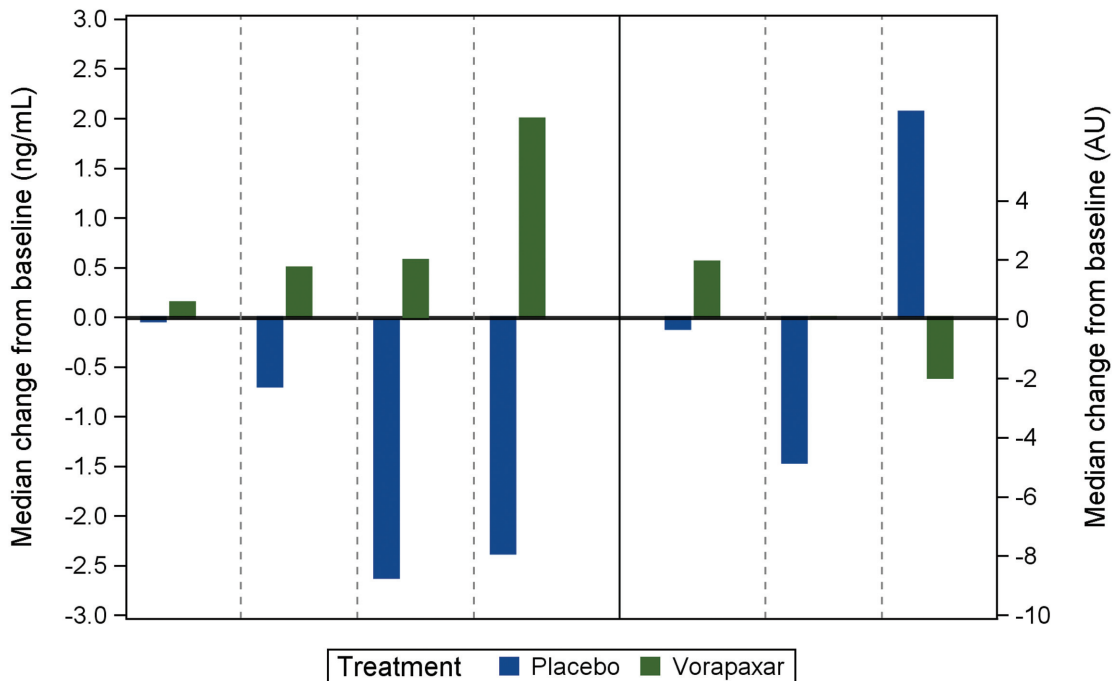


Fig. 1 Statistically significant biomarker changes on vorapaxar as compared with placebo in pooled patients and by post-myocardial infarction (MI) (chronic coronary heart disease [CHD]) and ST-segment elevation MI (NSTEMI) subgroups.

signaling,¹⁹ it is a weak inhibitor of fibrinolysis as compared with PAI-1.²⁰ Thus, the clinical significance of the overall modest effects of PAI-2 inhibition requires further investigation.

As compared with placebo, vorapaxar induced a significant increase in E-selectin, a specific marker for endothelial cell activation, in stable post-MI (TRA2P) subjects at 1-month FU. These subjects will have recovered after their MI and will be in a steady-state condition. A potential activating effect on the endothelium was also seen in NSTEMI patients (TRACER), in whom VWF levels, a biomarker with endothelial cells as its main contributor, were maintained by PAR-1 inhibition, in contrast to a significant drop in the placebo-treated patients at both 1-month and late FU. Although these findings may suggest endothelial activating effects of PAR-1 inhibition by vorapaxar, their clinical significance is at present unclear.

Study Limitations and Strengths

Recruitment was consecutive from two randomized, double-blind, clinical trials. Despite a slightly uneven number of patients in the active treatment- as compared with the placebo group, baseline characteristics were well balanced. Furthermore, storage of plasma was optimal at -80°C and biomarker analysis was run blindly and simultaneously for all samples.

As there are several biomarkers, each would need a different number of patients for power calculations, and, therefore, this was not performed. The testing procedure was run as a modification of the original assay kits, which may affect the absolute biomarker values obtained, whereas changes will not be affected by the modified approach. Furthermore, all subjects were recruited from a Norwegian population, and, therefore, our results may not necessarily be generalizable.

In conclusion, several endothelial-related biomarkers were significantly affected by PAR-1 inhibition as compared with placebo during short and late FU in patients recruited from two large-scale randomized trials assessing clinical outcome in post-MI and NSTEMI subjects, respectively. Our data may suggest some harmful effects of PAR-1 inhibition on markers of endothelial cell activation. If this could have contributed to the lack of effects on mortality in both the TRACER and TRA2P-TIMI 50 study is at present unclear, but should be taken into account in future studies with PAR-1 inhibitors.

What is known about this topic?

- Antiplatelet effects of vorapaxar have been thoroughly investigated.
- A short-term pharmacodynamic study with vorapaxar as compared with placebo has been performed in NSTEMI patients.
- Large RCTs with vorapaxar versus placebo have demonstrated reduced combined cardiovascular events in patients on vorapaxar, with no effect on total mortality.

What does this paper add?

- In this study we have focused on endothelial-related biomarkers.
- Patients with both acute and chronic coronary heart disease were included.
- Variations in biomarker levels during short- and long-term PAR-1 inhibition with vorapaxar versus placebo were studied.
- We provide novel information on long-term effects of PAR-1 inhibition in the total population, as well as in the two subpopulations.
- Our results suggest that negative effects on some prognostic biomarkers may arise from PAR-1 inhibition.

Ethical Statement

This study was approved by the Ethics Committee of Northern Norway and the biobank was licensed by the Ethics Committee of South-Eastern Norway. Biobank no. 10104, ref. 2009/330.

Conflict of Interest

None declared.

Acknowledgment

The authors wish to express their gratitude to the TIMI Group, Duke investigators, and coordinators and local officials at MSD, for facilitation, assistance, and general support. MSD also provided funding for this study.

References

- 1 Vu T-KH, Hung DT, Wheaton VI, Coughlin SR. Molecular cloning of a functional thrombin receptor reveals a novel proteolytic mechanism of receptor activation. *Cell* 1991;64(06):1057-1068
- 2 O'Brien PJ, Molino M, Kahn M, Brass LF. Protease activated receptors: theme and variations. *Oncogene* 2001;20(13):1570-1581
- 3 Coughlin SR. Protease-activated receptors in hemostasis, thrombosis and vascular biology. *J Thromb Haemost* 2005;3(08):1800-1814
- 4 Feistritz C, Riewald M. Endothelial barrier protection by activated protein C through PAR1-dependent sphingosine 1-phosphate receptor-1 crossactivation. *Blood* 2005;105(08):3178-3184
- 5 Gieseler F, Ungefroren H, Settmacher U, Hollenberg MD, Kaufmann R. Proteinase-activated receptors (PARs) - focus on receptor-receptor-interactions and their physiological and pathophysiological impact. *Cell Commun Signal* 2013;11:86
- 6 Ten Cate H, Guzik TJ, Eikelboom J, Spronk HMH. Pleiotropic actions of factor Xa inhibition in cardiovascular prevention: mechanistic insights and implications for anti-thrombotic treatment. *Cardiovasc Res* 2021;117(09):2030-2044
- 7 Chackalamanni S, Wang Y, Greenlee WJ, et al. Discovery of a novel, orally active himbacine-based thrombin receptor antagonist (SCH 530348) with potent antiplatelet activity. *J Med Chem* 2008;51(11):3061-3064
- 8 Morrow DA, Braunwald E, Bonaca MP, et al; TRA 2P-TIMI 50 Steering Committee and Investigators. Vorapaxar in

- the secondary prevention of atherothrombotic events. *N Engl J Med* 2012;366(15):1404–1413
- 9 Tricoci P, Huang Z, Held C, et al; TRACER Investigators. Thrombin-receptor antagonist vorapaxar in acute coronary syndromes. *N Engl J Med* 2012;366(01):20–33
 - 10 Storey RF, Kotha J, Smyth SS, et al. Effects of vorapaxar on platelet reactivity and biomarker expression in non-ST elevation acute coronary syndromes. *Thromb Haemost* 2014;11:883–891
 - 11 Lee KW, Lip GYH, Blann AD. Plasma angiopoietin-1, angiopoietin-2, angiopoietin receptor tie-2, and vascular endothelial growth factor levels in acute coronary syndromes. *Circulation* 2004;110(16):2355–2360
 - 12 Boncela J, Przygodzka P, Papiewska-Pajak I, Wyroba E, Cierniewski CS. Association of plasminogen activator inhibitor type 2 (PAI-2) with proteasome within endothelial cells activated with inflammatory stimuli. *J Biol Chem* 2011;286(50):43164–43171
 - 13 Saharinen P, Eklund L, Alitalo K. Therapeutic targeting of the angiopoietin-TIE pathway. *Nat Rev Drug Discov* 2017;16(09):635–661
 - 14 Akwii RG, Sajib MS, Zahra FT, Mikelis CM. Role of angiopoietin-2 in vascular physiology and pathophysiology. *Cells* 2019;8(05):471
 - 15 Guo L, Li S-Y, Ji F-Y, et al. Role of Angptl4 in vascular permeability and inflammation. *Inflamm Res* 2014;63(01):13–22
 - 16 Mattijssen F, Kersten S. Regulation of triglyceride metabolism by Angiopoietin-like proteins. *Biochim Biophys Acta* 2012;1821(05):782–789
 - 17 Georgiadi A, Wang Y, Stienstra R, et al. Overexpression of angiopoietin-like protein 4 protects against atherosclerosis development. *Arterioscler Thromb Vasc Biol* 2013;33(07):1529–1537
 - 18 Xu L, Guo ZN, Yang Y, et al. Angiopoietin-like 4: a double-edged sword in atherosclerosis and ischemic stroke? *Exp Neurol* 2015;272(272):61–66
 - 19 Rondaj MG, Bierings R, Kragt A, van Mourik JA, Voorberg J. Dynamics and plasticity of Weibel-Palade bodies in endothelial cells. *Arterioscler Thromb Vasc Biol* 2006;26(05):1002–1007
 - 20 Medcalf RL. Plasminogen activator inhibitor type 2: still an enigmatic serpin but a model for gene regulation. *Methods Enzymol* 2011;499:105–134
 - 21 Thomas M, Augustin HG. The role of the angiopoietins in vascular morphogenesis. *Angiogenesis* 2009;12(02):125–137
 - 22 Eklund L, Kangas J, Saharinen P. Angiopoietin-Tie signalling in the cardiovascular and lymphatic systems. *Clin Sci (Lond)* 2017;131(01):87–103
 - 23 Aarsetøy R, Ueland T, Aukrust P, et al. Angiopoietin-2 and angiopoietin-like 4 protein provide prognostic information in patients with suspected acute coronary syndrome. *J Intern Med* 2021;290(04):894–909
 - 24 Iglesias MJ, Kruse LD, Sanchez-Rivera L, et al. Identification of endothelial proteins in plasma associated with cardiovascular risk factors. *Arterioscler Thromb Vasc Biol* 2021;41(12):2990–3004
 - 25 Olshan DS, Rader DJ. Angiopoietin-like protein 4: a therapeutic target for triglycerides and coronary disease? *J Clin Lipidol* 2018;12(03):583–587
 - 26 Le Jan S, Amy C, Cazes A, et al. Angiopoietin-like 4 is a proangiogenic factor produced during ischemia and in conventional renal cell carcinoma. *Am J Pathol* 2003;162(05):1521–1528
 - 27 Cazes A, Galaup A, Chomel C, et al. Extracellular matrix-bound angiopoietin-like 4 inhibits endothelial cell adhesion, migration, and sprouting and alters actin cytoskeleton. *Circ Res* 2006;99(11):1207–1215
 - 28 Dijk W, Kersten S. Regulation of lipoprotein lipase by Angptl4. *Trends Endocrinol Metab* 2014;25(03):146–155
 - 29 Lobov IB, Brooks PC, Lang RA. Angiopoietin-2 displays VEGF-dependent modulation of capillary structure and endothelial cell survival in vivo. *Proc Natl Acad Sci U S A* 2002;99(17):11205–11210
 - 30 Bevilacqua MP, Nelson RM. Selectins. *J Clin Invest* 1993;91(02):379–387
 - 31 Macías C, Villaescusa R, del Valle L, et al. Endothelial adhesion molecules ICAM-1, VCAM-1 and E-selectin in patients with acute coronary syndrome [in Spanish]. *Rev Esp Cardiol* 2003;56(02):137–144
 - 32 Esmon CT, Owen WG. Identification of an endothelial cell cofactor for thrombin-catalyzed activation of protein C. *Proc Natl Acad Sci U S A* 1981;78(04):2249–2252
 - 33 Boffa MC, Karmochkine M. Thrombomodulin: an overview and potential implications in vascular disorders. *Lupus* 1998;7(Suppl 2):S120–S125
 - 34 Ben Shimon M, Lenz M, Ikenberg B, et al. Thrombin regulation of synaptic transmission and plasticity: implications for health and disease. *Front Cell Neurosci* 2015;9:151
 - 35 Peyvandi F, Garagiola I, Baronciani L. Role of von Willebrand factor in the haemostasis. *Blood Transfus* 2011;9(Suppl 2):s3–s8
 - 36 Vaughan DE. PAI-1 and atherothrombosis. *J Thromb Haemost* 2005;3(08):1879–1883
 - 37 Ritchie H, Jamieson A, Booth NA. Regulation, location and activity of plasminogen activator inhibitor 2 (PAI-2) in peripheral blood monocytes, macrophages and foam cells. *Thromb Haemost* 1997;77(06):1168–1173
 - 38 Colognato R, Slupsky JR, Jendrach M, Burysek L, Syrovets T, Simmet T. Differential expression and regulation of protease-activated receptors in human peripheral monocytes and monocyte-derived antigen-presenting cells. *Blood* 2003;102(07):2645–2652