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Low Levels of High-Density Lipoprotein Cholesterol Are Linked to Impaired Clopidogrel-Mediated Platelet Inhibition

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Abstract

Low high-density lipoprotein cholesterol (HDL-C) levels are an independent predictor of ischemic events in patients with atherosclerotic cardiovascular disease. This may in part be due to decreased clopidogrel-mediated platelet inhibition in patients with low HDL-C. We investigated the association of HDL-C with on-treatment platelet reactivity to adenosine diphosphate (ADP) in 314 patients on dual antiplatelet therapy with clopidogrel and aspirin undergoing angioplasty and stenting. Platelet P-selectin expression was assessed by flow cytometry, and platelet aggregation was determined by the VerifyNow $P2Y_{12}$ assay and the Impact-R. High-density lipoprotein cholesterol levels were inversely associated with P-selectin expression and the VerifyNow $P2Y_{12}$ assay (both $P \leq .01$). Moreover, we found a positive correlation of HDL-C with surface coverage by the Impact-R ($P = .003$). Patients with low HDL-C ($\leq$35 mg/dL) exhibited a significantly higher P-selectin expression in response to ADP and higher platelet aggregation by the VerifyNow $P2Y_{12}$ assay and the Impact-R than patients with normal HDL-C (>35 mg/dL; all $P < .05$). High on-treatment residual platelet reactivity by the VerifyNow $P2Y_{12}$ assay occurred significantly more frequently in patients with low HDL-C levels than in those with normal HDL-C (47.4% vs 30.1%, $P = .01$). In conclusion, low HDL-C is linked to impaired clopidogrel-mediated platelet inhibition after angioplasty and stenting.

Keywords

high-density lipoprotein cholesterol, high on-treatment residual platelet reactivity, clopidogrel, dual antiplatelet therapy, angioplasty, stenting

Introduction

Dual antiplatelet therapy with aspirin and an adenosine diphosphate (ADP) $P2Y_{12}$ receptor antagonist is prescribed after angioplasty and stenting to prevent detrimental platelet activation.[1-3] Despite the emergence of new ADP receptor antagonists, clopidogrel remains a frequently administered $P2Y_{12}$ inhibitor. This is mainly due to the fact that newer agents, such as prasugrel and ticagrelor, are only approved for patients with acute coronary syndromes (ACS). Moreover, both increased the risk of bleeding compared to clopidogrel, and prasugrel yielded no net clinical benefit in patients with prior ischemic stroke and in those with low body weight and aged 75 years or older.[4,5] However, the antiplatelet efficacy of clopidogrel is influenced by several factors ranging from age,[6] diabetes,[7] renal function,[8] and comedication[9] to cytochrome P450 2C19 and 2C9 carrier status.[10-12] High on-treatment residual platelet reactivity to ADP (HRPR) occurs in 30% to 40% of clopidogrel-treated patients and is associated with adverse cardiovascular events.[13]

High-density lipoprotein cholesterol (HDL-C) represents an important atheroprotective factor, and it is well established that low HDL-C levels are associated with adverse cardiovascular events in patients with coronary artery disease.[14-16] Previous studies suggest that HDL-C inhibits platelet activation in vivo and ex vivo.[17] Briefly, HDL-C may lower platelet membrane cholesterol and modulate the membrane raft–associated receptor clusters glycoprotein (GP) Ib and FcγRII.[18,19] Hereby, thiol acylation of GPIbβ and GPIX by palmitate is reduced resulting in less platelet aggregation and adhesion to von Willebrand factor.[20] Further, under inflammatory conditions, platelet function can be attenuated by secretory phospholipase A2-HDL.[21] Moreover, it has been shown that the infusion of reconstituted HDL is highly effective at inhibiting platelet hyperactivity in

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diabetic patients,[22] and platelet reactivity was enhanced in patients with ACS and low HDL-C.[23]

Due to these previous studies, we hypothesized that low HDL-C may be linked to the response to clopidogrel therapy in patients with atherosclerotic cardiovascular disease. We therefore investigated the association of HDL-C with on-treatment residual platelet reactivity to ADP in patients undergoing angioplasty and stenting.

Methods

Study Population

The study population consisted of 314 patients on daily aspirin (100 mg/d) and clopidogrel (75 mg/d) therapy after percutaneous intervention with stent implantation; of the 314 included patients, 170 underwent peripheral artery stenting, 37 carotid artery stenting, and 107 patients underwent coronary artery stenting. All patients with peripheral and carotid artery stenting received bare-metal stents and those with coronary artery stenting received drug-eluting stents. All interventions were elective, and all patients were in a stable clinical condition at the time of the angioplasty procedure.

Exclusion criteria were a known aspirin or clopidogrel intolerance (allergic reactions and gastrointestinal bleeding); therapy with vitamin K antagonists (warfarin, phenprocoumon, and acenocoumarol) or direct oral anticoagulants (rivaroxaban, apixaban, dabigatran, and edoxaban); treatment with ticlopidine, dipyridamole, or nonsteroidal anti-inflammatory drugs; a family or personal history of bleeding disorders, malignant paraproteinemia, myeloproliferative disorders, or heparin-induced thrombocytopenia; severe hepatic failure; known qualitative defects in thrombocyte function; a major surgical procedure within 1 week before enrollment; a platelet count <100 000 or >450 000/μL; and a hematocrit <30%.

The study protocol was approved by the Ethics Committee of the Medical University of Vienna in accordance with the Declaration of Helsinki, and written informed consent was obtained from all study participants.

Blood Sampling

Blood was drawn by aseptic venipuncture from an antecubital vein using a 21-gauge butterfly needle (0.8 × 19 mm; Greiner Bio-One, Kremsmünster, Austria) 24 hours after the percutaneous intervention. To avoid procedural deviations, all blood samples were taken by the same physician applying a light tourniquet which was immediately released, and the samples were mixed adequately by gently inverting the tubes. After the initial 3 mL of blood had been discarded to reduce procedurally induced platelet activation, blood was drawn into a 3.8% sodium citrate Vacuette tube (9 parts of whole blood and 1 part of sodium citrate 0.129 mol/L; Greiner Bio-One, Kremsmünster, Austria) for evaluations by flow cytometry into a 3.2% sodium citrate Vacuette tube (9 parts of whole blood and 1 part of sodium citrate 0.109 mol/L; Greiner Bio-One, Kremsmünster, Austria) for the VerifyNow $P2Y_{12}$ assay and into a Vacuette tube containing lithium heparin (18 IU/ mL) for the determinations by the Impact-R.

P-Selectin Expression

The expression of P-selectin was determined in citrate-anticoagulated blood as previously described.[24] In brief, whole blood was diluted in phosphate-buffered saline (PBS) to obtain 20×10^3/mL platelets in 20 mL and incubated for 10 minutes without agonists and after in vitro exposure to suboptimal concentrations of ADP (final concentration: 1 μmol/L; Dynabyte, Munich, Germany). The platelet population was identified by staining with anti-CD42b (5 mL of clone HIP1, allophycocyanin labeled, final dilution 1:9; Becton Dickinson, San Jose, California), and the expression of P-selectin was determined by the binding of the monoclonal antibody anti-CD62 p-phycoerythrin (PE; 5 mL of clone CLB-Thromb6, final dilution 1:9; Immunotech, Beckman Coulter, Fullerton, California). Isotype-matched control antibodies were used in separate vials for the determination of nonspecific binding. After 15 minutes of incubation in the dark, the reaction was stopped by adding 500 mL of PBS, and samples were acquired immediately on an FACS Calibur flow cytometer (Becton Dickinson) with excitation by an argon laser at 488 nm and a red diode laser at 635 nm at a rate of 200 to 600 events per second. Fluorescein isothiocyanate and PE-labeled beads were used to compensate manually for the fluorescein isothiocyanate signal into the PE channel and vice versa. Platelets were gated in a side scatter versus FL4 dot plot. A total of 10 000 events were acquired within this gate. Positive analysis regions for P-selectin were set with appropriate nonspecific controls. The gated events were further analyzed in histograms for FL-1 and FL-2 for P-selectin, using the Cell-Quest Pro software (Becton Dickinson). Standard Becton Dickinson Calibrite beads were used for daily calibration of the cytometer.

VerifyNow P2Y12 Assay

The VerifyNow system (Accumetrics, San Diego, California) was performed as published.[25] It measures platelet $P2Y_{12}$ receptor blockade and is based upon the ability of activated platelets to bind fibrinogen. Citrate anticoagulated whole blood is automatically dispensed from the blood collection tube into the assay device by the instrument. Adenosine diphosphate is incorporated into the assay channel to induce platelet activation, and light transmittance increases as activated platelets bind and aggregate fibrinogen-coated beads. The instrument measures this change in optical signal and reports results in $P2Y_{12}$ reaction units (PRU). With this assay, higher PRU reflects greater ADP-inducible platelet aggregation.

Impact-R

The Impact-R (DiaMed, Cressier, Switzerland) was performed as described previously.[25] It is a commercially available development of the cone and plate analyzer.[26,27] In brief, 130 μL of lithium heparin-anticoagulated whole blood were placed on the

polystyrene plate. Shear stress was immediately applied (2050 s-1) using a acrylnitril-butadien-styrene cone. Plates were then washed with tap water and stained with May-Grünwald solution following the manufacturer's instruction. Samples were analyzed with an inverted light microscope connected to an image analyzer (Galai, Migdal Haemek, Israel). As in previous reports,[28,25] only platelet adhesion determined by examination of the percentage of total area covered with platelets (surface coverage, %SC) was used for the statistical analyses. Seven images were collected from each run, and medians of these were calculated by the analyzing system. In the ADP-response test, the whole blood sample was preincubated with 1.36 μmol/L ADP (DiaMed, Cressier, Switzerland) for 1 minute under gentle mixing (10 rpm) prior to the Impact-R test. This led to platelet activation and microaggregates formation in the tube resulting in reduced platelet adhesion to the well as reflected by reduced SC. The reduction was attenuated in samples from patients responding well to clopidogrel, while full reduction was observed in patients with residual ADP-inducible platelet aggregation, despite clopidogrel treatment.

High-Density Lipoprotein Cholesterol Measurement

Serum analyses of HDL-C were performed as described previously without prior separation with polyethylene glycol-modified enzymes and sulfated α-cyclodextrin.[29]

Statistical Analysis

A sample size calculation was based on the observed mean ± standard deviation of residual ADP-inducible platelet aggregation by the VerifyNow $P2Y_{12}$ assay (204 [89] PRU) in 80 patients on dual antiplatelet therapy with aspirin and clopidogrel after angioplasty with stent implantation.[25] We calculated that we needed to include 300 patients to detect a 20% relative difference in ADP-inducible platelet aggregation by the VerifyNow $P2Y_{12}$ assay between patients without and with low HDL-C with a power of 84% (using a 2-sided α level of .05). To compensate for potential technical problems, we included 14 additional patients.

Statistical analysis was performed using the SPSS (IBM SPSS version 24, Armonk, New York). Median and interquartile range of continuous variables are shown. Categorical variables are given as number (%). Spearman rank correlation was used to assess the correlations of HDL-C with platelet activation and aggregation parameters. We performed Mann-Whitney *U* tests to detect differences in continuous variables. The χ^2 test was used to assess differences in categorical variables. Multivariate linear regression analyses were used to adjust for patient characteristics that were significantly different between patients without and with low HDL-C. Covariates for adjustment were selected based on univariate analyses (*P* value <.1), including age, sex, hypertension, hyperlipidemia, diabetes, active smoking, number of implanted stents, hemoglobin, white blood cell count, platelet count, serum creatinine, high-sensitivity C-reactive protein, low-density lipoprotein cholesterol, triglycerides, use of statins, angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, calcium channel blockers, and proton pump inhibitors (PPIs). Two-sided *P* values <.05 were considered significant.

Table 1. Clinical, Laboratory, and Procedural Characteristics of the Patient Population.[a]

Characteristics	HDL ≤ 35mg/dL, n = 58	HDL > 35mg/dL, n = 256	*P*
Demographics			
Age, years	62 (56.8-69)	66 (58-76)	.02
Male sex, n (%)	48 (83)	156 (61)	<.01
Medical history			
Hypertension, n (%)	51 (88)	232 (91)	.54
Hyperlipidemia, n (%)	53 (91)	239 (93)	.59
Diabetes mellitus, n (%)	27 (47)	74 (29)	.01
Smoking, n (%)	26 (45)	105 (41)	.6
Stent implantation, n (%)	58 (100)	256 (100)	1
No. of stents/patient	1 (1-2)	1 (1-2)	.46
Laboratory data			
LDL-C, mg/dL	79 (57-105)	98 (73-126)	<.001
Triglycerides, mg/dL	166 (125-207)	140 (107-191)	.04
Serum creatinine, mg/dL	1.05 (0.9-1.25)	1.02 (0.89-1.18)	.31
High-sensitivity C-reactive protein, mg/dL	0.79 (0.32-2.19)	0.79 (0.33-1.81)	.9
Hemoglobin, g/dL	12.8 (11.6-13.6)	13.2 (12-14.3)	.05
Platelet count, 109/L	200 (173-250)	210 (176-253)	.38
WBC, 10^9/L	8.3 (7.2-10.6)	8.4 (6.8-10.3)	.69
Medication			
Statins, n (%)	56 (97)	243 (95)	.6
ACE inhibitors or ARB, n (%)	52 (90)	219 (86)	.41
Calcium channel blockers, n (%)	18 (31)	78 (31)	.93
Proton pump inhibitors, n (%)	41 (71)	124 (48)	<.01

Abbreviations: ACE, angiotensin-converting enzyme; ARB, angiotensin receptor blockers; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; WBC, white blood cell count.
[a]N = 314.

Results

Low HDL-C was defined as an HDL-C concentration ≤35 mg/dL (0.9 mmol/L); low and normal HDL-C were seen in 58 (18.5%) and 256 (81.5%) patients of the study population, respectively. Clinical, laboratory, and procedural characteristics of the overall study population and of patients without and with low HDL-C levels are given in Table 1. Patients with normal HDL-C were significantly older than patients with HDL-C below 35 mg/dL ($P < .02$). In patients with low HDL-C, male sex ($P < .1$) and diabetes ($P = .1$) were more common. Further, PPIs were prescribed more often in patients with low HDL-C than in those with normal HDL-C ($P < .01$). Patients with low HDL-C levels also had lower LDL-C ($P < .001$) but higher triglyceride levels ($P < .05$; Table 1).

Genotyping of cytochrome 2C9 (CYP2C9) and cytochrome 2C19 (CYP2C19) was available in 286 (91.1%) patients of the study population. The occurrence of loss-of-function (LOF)

Table 2. Correlations of Laboratory Parameters With Platelet Surface P-selectin Expression in Response to ADP and Platelet Aggregation by the VerifyNow $P2Y_{12}$ Assay and the Impact-R.

Characteristics	P-Selectin	VerifyNow $P2Y_{12}$	Impact-R
Triglycerides	$r = .144$, $P = .012$	$r = .027$, $P =$ ns	$r = -.069$, $P =$ ns
LDL-C	$r = -.066$, $P =$ ns	$r = -.170$, $P = .003$	$r = .113$, $P = .048$
Creatinine	$r = .031$, $P =$ ns	$r = .183$, $P = .001$	$r = -.117$, $P = .041$
CRP	$r = .051$, $P =$ ns	$r = -.028$, $P =$ ns	$r = -.103$, $P =$ ns
Hemoglobin	$r = -.072$, $P =$ ns	$r = -.338$, $P < .001$	$r = -.246$, $P < .001$
Platelet count	$r = -.1$, $P =$ ns	$r = -.155$, $P = .006$	$r = .195$, $P = .001$
White blood cell count	$r = -.035$, $P =$ ns	$r = -.153$, $P = .007$	$r = .212$, $P < .001$

Abbreviations: ADP, adenosine diphosphate; CRP, C-reactive protein; LDL-C, low density lipoprotein cholesterol; ns, not significant.

polymorphisms of CYP2C9 and CYP2C19 was not significantly different between patients with low and normal HDL-C levels (CYP2C9 LOF: 5 [9.4%] of 53 patients vs 37 [15.9%] of 233 patients, $P = .2$; CYP2C19 LOF: 15 [28.3%] of 53 patients vs 72 [30.9%] of 233 patients, $P = .7$).

In a first step, we assessed the correlations of HDL-C with residual ADP-inducible platelet activation and aggregation. We found significant inverse correlations of HDL-C levels with ADP-inducible platelet surface P-selectin expression ($r = -.16$, $P = .007$) and platelet aggregation by the VerifyNow $P2Y_{12}$ assay ($r = -.14$, $P = .01$), reflecting increasing platelet activation and aggregation with decreasing HDL-C. Moreover, we found a positive correlation of HDL-C with SC by the Impact-R ($r = .17$, $P = .003$). Since the latter is inversely associated with residual ADP-inducible platelet reactivity, this positive correlation also suggests increasing platelet aggregation with decreasing HDL-C.

Besides HDL-C, there was a significant correlation of platelet activation as measured by P-selectin expression in response to ADP with triglycerides and of platelet aggregation by the VerifyNow $P2Y_{12}$ assay with LDL-C, creatinine, hemoglobin, platelet count, and white blood cell count. Further, platelet aggregation by the Impact-R correlated with LDL-C, creatinine, hemoglobin, platelet count, and leukocytes (Table 2). Moreover, we compared all platelet markers between patients without and with hypertension, diabetes, hyperlipidemia, and active smoking. Thereby, we found no significant differences in P-selectin expression in response to ADP. However, as previously published, residual ADP-inducible platelet aggregation by the VerifyNow $P2Y_{12}$ assay and the Impact-R was significantly lower in smokers than in nonsmokers (Table 3).[12,30]

In a second step, we compared residual ADP-inducible platelet activation and aggregation between patients without and with low HDL-C levels. Patients with low HDL-C exhibited a significantly higher platelet surface expression of P-selectin in response to ADP than patients with normal HDL-C concentrations (15.9 mean fluorescence intensity [MFI]: [8.9-25.5 MFI] vs 11.5 MFI: [7-19 MFI], $P = .02$; Figure 1 A). Moreover, patients with low HDL-C had higher residual ADP-inducible platelet aggregation by the VerifyNow $P2Y_{12}$ assay and the Impact-R than patients with normal HDL-C (VerifyNow $P2Y_{12}$ assay: 222 PRU [149-301 PRU] vs 190 PRU [127-250 PRU], $P = .02$; Impact-R: 2.9% SC [1.7%-4.4% SC] vs 4% SC [2%-6% SC], $P = .006$; Figure 1 B and C).

Table 3. Differences in Platelet Surface P-Selectin Expression in Response to ADP and Platelet Aggregation by the VerifyNow $P2Y_{12}$ Assay and the Impact-R Between Patients Without and With Hypertension, Diabetes, Hyperlipidemia, and Active Smoking.

Characteristics	P-Selectin (MFI)	VerifyNow $P2Y_{12}$ (PRU)	Impact-R (SC)
Hypertension			
Yes	12.5 (7.8-19.4)	199 (133-269)	3.7 (1.9-5.6)
No	9.3 (6.2- 21.2)	185 (91-236)	4.3 (2.4-6.9)
P	.157	0226	.115
Hyperlipidemia			
Yes	12 (7.6-19.7)	194 (128-264)	3.7 (1.9-5.7)
No	12 (5-17.5)	213 (145-282)	4.2 (1.9-5.8)
P	.412	.470	.941
Diabetes mellitus			
Yes	13.5 (8.3-20.7)	210 (134-281)	4.2 (1.9-6.1)
No	11.4 (7.3-19.1)	192 (129-250)	3.7 (2-5.7)
P	.189	.138	.825
Smoking			
Yes	11.8 (7.2-19.2)	180 (126-216)	4.1 (2.3-6)
No	12.3 (7.6-20)	220 (137-296)	3.1 (1.8-5.4)
P	.942	<.001	.011

Abbreviations: ADP, adenosine diphosphate; MFI, mean fluorescence intensity; PRU, P2Y12 reaction units; SC, surface coverage.

The associations of low HDL-C with residual ADP-inducible platelet reactivity by the VerifyNow $P2Y_{12}$ assay and the Impact-R remained statistically significant after adjustment for differences in clinical characteristics between patients with low and normal HDL-C by multivariate linear regression analyses (Table 4; both $P \leq .01$). Moreover, there was a strong trend toward higher platelet surface P-selectin expression in patients with low HDL-C after multivariate regression analysis (Table 4; $P = .07$). Of note, triglyceride levels remained significantly associated with both platelet activation and aggregation by the applied test methods after multivariate regression analyses (Table 4; all $P < .05$).

Finally, we compared the incidence of HRPR by the VerifyNow $P2Y_{12}$ assay between patients with low and normal HDL-C levels, respectively. The HRPR by the VerifyNow $P2Y_{12}$ assay was defined according to a recent consensus

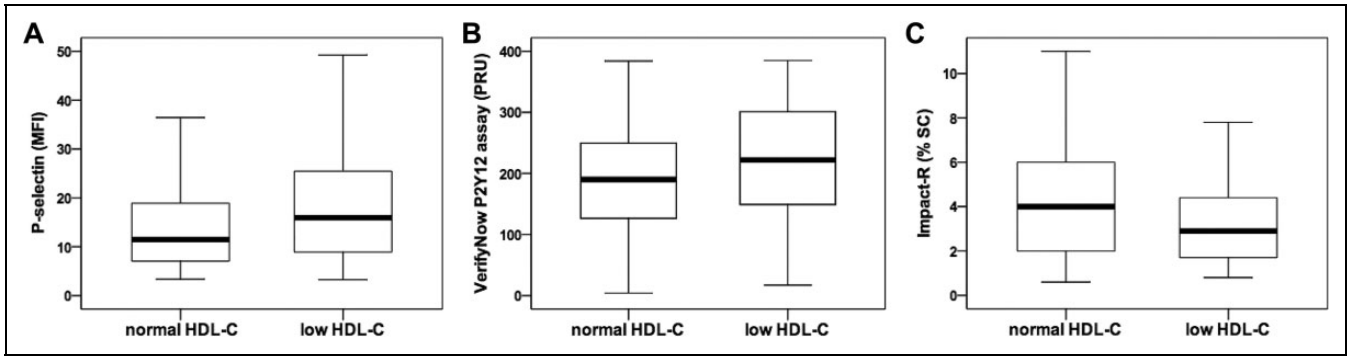

Figure 1. A, Platelet surface expression of P-selectin in response to ADP in patients with normal and low HDL-C concentrations (n = 314). B, Residual ADP-inducible platelet aggregation by the VerifyNow $P2Y_{12}$ assay in patients with normal and low HDL-C concentrations (n = 314). C, Residual ADP-inducible platelet aggregation by the Impact-R in patients with normal and low HDL-C concentrations (n = 314). The boundaries of the box show the lower and upper quartile of data and the line inside the box represents the median. Whiskers are drawn from the edge of the box to the highest and lowest values that are outside the box but within 1.5 times the box length. ADP indicates adenosine diphosphate; HDL-C, high-density lipoprotein cholesterol.

Table 4. Multivariate Linear Regression Analyses of Age, Sex, Diabetes, HDL-C, Hemoglobin, Triglycerides, LDL-C, and the Use of Proton Pump Inhibitors for Platelet Surface P-Selectin Expression and Platelet Aggregation by the VerifyNow $P2Y_{12}$ Assay and the Impact-R.

	P-Selectin			VerifyNow $P2Y_{12}$			Impact-R		
Characteristics	B	CI	*P*	B	CI	*P*	B	CI	*P*
Age	0.21	0.06 to 0.35	.01	2.95	2.11 to 3.78	<.001	−0.02	−0.05 to 0.01	.11
Sex	−2.15	−5.68 to 1.37	.23	−26.66	−47.07 to −6.25	.01	0.56	−0.07 to 1.19	.08
Diabetes	0.74	−2.67 to 4.14	.67	−1.27	−21.03 to 18.5	.9	0.74	0.13 to 1.35	.02
Low HDL-C	3.94	−0.25 to 8.13	.07	36.88	12.2 to 61.56	.004	−0.83	−1.58 to −0.08	.03
Hemoglobin	−0.62	−1.7 to 0.45	.26	−15.38	−21.68 to −9.09	<.001	0.42	0.22 to 0.61	<.001
Proton pump inhibitors	2.36	−0.84 to 5.56	.15	−0.32	−18.85 to 18.2	.97	−0.25	−0.82 to 0.32	.39
Triglycerides	0.02	0.00 to 0.04	.04	0.16	0.05 to 0.28	.01	−0.004	−0.01 to −0.001	.02
LDL-C	0.02	−0.03 to 0.06	.46	−0.03	−0.27 to 0.21	.83	0.01	−0.00 to 0.01	.16

Abbreviations: B, regression coefficient; CI, confidence interval; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

document on the definition of HRPR.[31] Thereby, a threshold of >235 PRU was applied to identify patients with HRPR. With use of this cutoff value, HRPR by the VerifyNow $P2Y_{12}$ assay was found in 104 (33.1%) patients of the study population. The HRPR was significantly more frequent in patients with low HDL-C than in those with normal HDL-C levels (47.4% vs 30.1%, $P = .01$).

We did not assess long-term clinical outcomes of the study population. However, all patients stayed at the hospital for at least 24 hours after the percutaneous intervention. During this time, we did not observe any adverse ischemic or bleeding events.

Discussion

Our study investigated the associations of HDL-C with on-treatment residual platelet activation and aggregation in response to ADP in patients undergoing angioplasty with stent implantation. We found a significant association of continuous HDL-C levels with residual ADP-inducible platelet activation and aggregation. In detail, there was an inverse correlation between HDL-C and ADP-inducible platelet surface P-selectin expression as well as between HDL-C and platelet aggregation by the VerifyNow $P2Y_{12}$ assay signifying enhanced platelet activation with decreasing HDL-C levels. In addition, we observed a positive correlation between HDL-C and SC by the Impact-R, supporting the abovementioned results. Moreover, we observed higher platelet activation as assessed by ADP-inducible platelet surface P-selectin expression and enhanced platelet aggregation by the VerifyNow $P2Y_{12}$ assay and the Impact-R in patients with low HDL-C.

Previous studies used different definitions of low HDL-C for male and female patients.[32] This was based on the observation of different physiologic HDL-C levels between men and women and the related risk of mortality due to cardiovascular ischemic disease.[33] However, studies applying different HDL-C cutoff values for male and female patients often also failed to show significant associations of HDL-C with ischemic cardiovascular events.[34-36]

Since an HDL-C $\leq$35 mg/dL was associated with adverse ischemic outcomes in male and female patients in several large studies,[37-39] we decided to apply this threshold for men and women in our study.

After adjusting for differences in clinical characteristics between patients with low and normal HDL-C levels, low HDL-C remained independently linked to increased platelet aggregation in response to ADP as measured by the VerifyNow $P2Y_{12}$ assay and the Impact-R. In addition, there was a strong trend toward higher platelet surface P-selectin expression in patients with low HDL-C after multivariate adjustment. Finally, HRPR by the VerifyNow $P2Y_{12}$ assay occurred more frequently in patients with low HDL-C than in those with normal HDL-C levels. As observed in previous studies,[6] age emerged as an independent influencing factor for on-treatment platelet activation and aggregation as measured by P-selectin expression and the VerifyNow $P2Y_{12}$ assay, respectively.[6,40] Hemoglobin was independently associated with platelet aggregation as measured by the VerifyNow $P2Y_{12}$ assay and the Impact-R, which is also in line with prior results.[41] Triglyceride levels remained independently associated with both platelet activation and aggregation by the applied test methods after multivariate regression analyses. This is in line with previous reports of increased platelet reactivity in hypertriglyceridemia.[42] Since postprandial hyperglycemia is associated with platelet activation,[43,44] blood sampling in our patient collective was performed after overnight fasting.

As previously published, influencing factors for clopidogrel-mediated platelet inhibition are to a certain extent assay dependent, and not all influencing factors are captured by the same test system.[45] This may be due to the different methodology of the various test systems.[45]

Low HDL-C levels are an established risk factor for adverse cardiovascular events.[46] In a post hoc analysis of the Treating to New Targets study, HDL-C was predictive of major cardiovascular events in patients with coronary heart disease and statin treatment.[15] Furthermore, the Targeted Platelet Inhibition to Clarify the Optimal Strategy to Medically Manage Acute Coronary Syndromes (TRILOGY ACS) substudy on HDL-C and platelet function reported a higher risk of long-term cardiovascular and all-cause death in 9064 patients with unstable angina or non-ST-segment elevation myocardial infarction and HDL-C levels below 30 mg/dL.[46] In this study, the patients underwent medical management with clopidogrel or prasugrel plus aspirin without coronary revascularization and were followed through 30 months.[46] Increasing evidence suggests that the association of low HDL-C with adverse ischemic events may be explained by the interaction of HDL-C with platelets resulting in a modulation of platelet function.[21,47,48] Previous studies showed that oxidized HDL-C suppresses agonist-induced platelet aggregation.[49,50] The modification of HDL-C by oxidation is very similar to that mediated by secretory phospholipase A2, and in both, the content of lysophosphatidylcholines in HDL-C is elevated.[21] Recently, Curcic et al showed that secretory phospholipase A2-HDL inhibits platelet activation and aggregation induced by several agonists as measured by P-selectin expression, GP IIb/IIIa activation, and superoxide production in washed platelets or platelet-rich plasma after incubation with HDL and human recombinant type V secretory phospholipase A_2 in samples of healthy volunteers.[21] In addition, pathways of platelet activation were inhibited as demonstrated by decreased phosphorylation of the kinases Akt and ERK 1/2 as measured by Western blot analyses in washed platelets of healthy volunteers.[21]

In 2011, Tselepis et al reported an inverse association of HDL-C with platelet activation parameters (as measured by P-selectin expression) in patients with ACS and dual antiplatelet treatment with aspirin and clopidogrel.[23]

We extend the current knowledge by providing data on the association of HDL-C with platelet activation and aggregation in patients on dual antiplatelet therapy as assessed by different platelet function tests. The surface expression of P-selectin is regarded as a very sensitive marker of platelet activation, and the interaction of platelet surface P-selectin with P-selectin glycoprotein ligand 1 on leukocytes is crucial for tethering of these cells to activated platelets.[51] The VerifyNow $P2Y_{12}$ assay is a rapid and fully standardized point-of-care device. Various studies linked HRPR as assessed by the VerifyNow $P2Y_{12}$ assay with the occurrence of atherothrombotic events after angioplasty and stenting.[12,13,31,52] The Impact-R is based on prestimulation of platelets by ADP followed by the detection of nonactivated platelets via application of high shear stress.[27,53] Due to the effort required for the assay, it has not become a routine method for platelet function analysis, and only few data are available on this test, so far. We observed a lower SC in the Impact R test after stimulation with ADP, that is, higher on-treatment residual platelet reactivity, in patients with low HDL-C compared to those with normal HDL-C levels. To date, therapeutic concepts of HDL-C elevation by niacin have failed to enter clinical practice due to an increase in adverse events.[54,55] The side effects ranged from an interference with diabetes control, an increased incidence of diabetes, infections, and bleeding to serious adverse events related to the gastrointestinal and musculoskeletal system. However, the recent publication of the results from the Randomized Evaluation of the Effects of Anacetrapib through Lipid Modification (REVEAL) trial demonstrated a feasible and clinically safe option for HDL-C elevating therapy, with anacetrapib significantly lowering major coronary events in patients with atherosclerotic vascular disease.[56] In detail, 30 449 patients were treated with atorvastatin and either 100 mg of anacetrapib once daily or placebo resulting in 104% higher HDL-C levels and 18% lower non-HDL-C levels in the anacetrapib group.[56] Based on the results of our study, one may speculate that the positive effect of HDL-C elevation on cardiovascular outcomes could at least in part be due to a better response to antiplatelet therapy. However, the clinical benefit might also be due to a reduction in LDL-C levels.[56] Unfortunately, there will be no further progress with anacetrapib because the manufacturer decided not to apply for its clinical use in patients.

Fibrate therapy may be accompanied by a significant increase in HDL-C.[57,58] However, all patients with lipid-lowering drugs in our study cohort were treated exclusively with statins.

A limitation of our study is the lack of long-term clinical outcome data. Another potential bias might be the difference in patient number after stratification into the 2 subgroups based on HDL-C levels. However, as outlined in the statistical analysis section, our study was adequately powered to reveal a 20% relative difference in ADP-inducible platelet aggregation by the VerifyNow $P2Y_{12}$ assay between patients without and with low HDL-C. The percentage of patients on PPIs was high in our patient cohort because in Austria, PPIs are frequently administered to prevent upper gastrointestinal bleeding in patients on dual antiplatelet therapy. Prescription was based on the individual decision of the supervising physician. The administration of PPIs may have an impact on clopidogrel-mediated platelet inhibition. Lower levels of the active metabolite of clopidogrel and higher residual platelet reactivity to ADP were described in clopidogrel-treated patients who concomitantly received PPIs.[59-61] This may be due to a drug interaction at the level of hepatic biotransformation of clopidogrel to its active metabolite by the cytochrome P450 enzyme system and was seen particularly in patients receiving omeprazole.[59,61] However, in the prospective and randomized Clopidogrel and the Optimization of Gastrointestinal Events Trial (COGENT), the authors observed no difference in the occurrence of adverse cardiovascular outcomes between clopidogrel-treated patients on omeprazole therapy and those receiving placebo.[62] Accordingly, the clinical relevance of the potential interaction between clopidogrel and omeprazole is questionable. In our study population, only 11 (3.5%) patients received omeprazole. Furthermore, we adjusted for the use of PPIs by multivariate regression analyses. Therefore, it seems unlikely that the differences in the use of PPIs between patients with low and normal HDL-C affected our results.

In conclusion, low HDL-C is linked to impaired clopidogrel-mediated platelet inhibition after angioplasty and stenting. Studies are warranted to further investigate the underlying mechanisms and to determine whether HDL-C elevating strategies directly affect residual platelet reactivity during antiplatelet therapy.

Authors' Note

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