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HDL cholesterol levels and endothelial glycocalyx integrity in treated hypertensive patients

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Endothelial dysfunction indicates target organ damage in hypertensive patients. The integrity of endothelial glycocalyx (EG) plays a vital role in vascular permeability, inflammation and elasticity, and finally to cardiovascular disease. The authors aimed to investigate the role of increased HDL cholesterol (HDL-C) levels, which usually are considered protective against cardiovascular disease, in EG integrity in older hypertensive patients. The authors studied 120 treated hypertensive patients older than 50 years were divided regarding HDL-C tertiles in group HDL_H (HDL-C ≥ 71 mg/dL, upper HDL-C tertile) and group HDL_L (HDL-C < 71 mg/dL, two lower HDL-C tertiles). Increased perfusion boundary region (PBR) of the sublingual arterial microvessels (ranging from 5 to 9 μm) using Sideview Darkfield imaging (Microscan, Glycocheck) was measured as a non-invasive accurate index of reduced EG thickness. PBR 5-9 was significantly decreased in group HDL_H ($P = 0.04$). In the whole population, HDL-C was inversely but moderately related to PBR 5-9 ($r = -0.22$, $P = 0.01$). In a multiple linear regression analysis model, using age, BMI, smoking habit, HDL-C, LDL-C, and office SBP, as independent variables, the authors found that BMI ($\beta = 0.25$, $P = 0.006$) independently predicted PBR 5-9 in the whole population. In older hypertensive patients, HDL-C ranging between 71 and 101 mg/dL might moderately protect EG and subsequently endothelial function. Future studies in several groups of low- or high-risk hypertensives are needed in order to evaluate the beneficial role of extremely elevated HDL-C regarding cardiovascular risk evaluation as well as endothelial glycocalyx as a novel index of target organ damage in essential hypertension.

1 | INTRODUCTION

Arterial hypertension is recognized as a major risk factor for cardiovascular disease (CVD). At the beginning, it leads to subclinical or target organ damage (TOD), an intermediate stage in the cardiovascular (CV) disease continuum as well as an important determinant of CV risk.[1] Signs of TOD (left ventricular hypertrophy, reduced coronary flow reserve, increased carotid intima-media thickness, microalbuminuria, aortic stiffness, endothelial dysfunction) should be investigated thoroughly in hypertensive patients.

Cardiovascular risk assessment tools assume that elevated high-density lipoprotein cholesterol (HDL-C) is a favorable CV risk factor, since plasma HDL-C levels are inversely associated with CV risk.[2-4] Indeed, elevated HDL-C levels are inversely related to coronary

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events, ischemic stroke, and carotid atherosclerosis even after adjustment for lipid and non-lipid risk factors in populations without known cardiovascular disease (CVD).[5] Moreover, the appearance of coronary artery disease is being delayed by 3-5 years in those patients with elevated HDL-C compared to patients with low or normal HDL-C levels.[6]

The endothelial glycocalyx (EG) represents a barrier of glycoproteins and proteoglycans between blood cells and endothelial vascular surface. EG damage, caused by inflammatory or atherogenic factors, leads to enhanced vascular sensitivity to prothrombotic, vasoactive, and atherogenic stimuli and is observed in newly diagnosed untreated hypertensives.[7] Subsequently, the integrity of EG has become evident regarding vascular homeostasis.[8,9] Novel imaging techniques assess EG function through measurements of the perfused boundary region (PBR) of the luminal wall of the sublingual microvessels ranging from 5 to 25 μm. PBR, a cell-poor layer, includes the most luminal part of EG that does allow red cell penetration. An increased PBR indicates a loss of EG barrier properties and is a marker of reduced EG thickness and endothelial dysfunction.[10]

We hypothesized that elevated HDL-C levels, as a favorite cardiovascular risk factor, should play a positive role in EG integrity leading to endothelial function protection. In the present study, we aimed to investigate the role of extremely increased HDL-C in EG integrity in older treated patients with essential hypertension.

2 | MATERIAL AND METHODS

2.1 | Study population

From a cohort of 250 consecutive patients with essential hypertension visiting our hospital outpatient hypertension clinic as new referrals or scheduled follow-ups between November 2015 and September 2017, we studied a group of 120 treated hypertensives aged ≥50 years. All patients in the study group were subjected to the following examinations: (a) office blood pressure (BP) measurements in the hypertension outpatient clinic; (b) blood sampling for routine blood chemistry examination; (c) fasting lipid profile measurement in a core laboratory (total cholesterol, triglycerides, HDL-C, and LDL); (d) standard 12-lead electrocardiogram; (e) carotid-femoral pulse wave velocity measurement (PWV) in order to evaluate arterial stiffness; and (f) perfused boundary region (PBR) of the sublingual arterial microvessels (diameter ranging from 5 to 9 μm) measurement in order to evaluate EG integrity.

Patients were advised to continue their antihypertensive and hypolipidemic treatment on the day of the examination since any short-term interruption of the treatment should not have an effect on our results.

Patients with untreated hypertension, secondary hypertension, congestive heart failure, previous myocardial infarction, stroke, cardiac valve diseases, history of coronary artery intervention and/or by-pass grafting, atrial fibrillation, renal insufficiency (estimated glomerular filtration rate, eGFR ≥60 mL/min/1.73 m^2 using the formula CKD-EPI), overt proteinuria (urine albumin levels ≥300 mg/24 hour), non-cardiovascular diseases, pregnancy, and hormone replacement treatment (estrogens and/or progesterone preparations) were excluded from the study in order to study a homogenous hypertensive group.

Informed consent had been obtained during the initial visit of the study which was approved by the ethical committee of the hospital.

2.2 | Diagnostic workup

2.2.1 | Office BP measurement

Morning office BP was measured in the hospital outpatient clinic, approximately in the same morning hour of the day, by the same cardiologist with a mercury sphygmomanometer (first and fifth phases of Korotkoff sounds taken as systolic [SBP] and diastolic blood pressure [DBP], respectively) after the patients had rested for a period of 5-10 minute in the sitting position. Two measurements were taken at 1-minute intervals, and the average was used to define office SBP and DBP. Office pulse pressure (PP) was calculated as SBP minus DBP, while mean BP was defined as DBP plus one-third of the PP. Patients were advised to avoid smoking or coffee at least for 2 hours before examination. Since all patients were already diagnosed and treated hypertensives with scheduled follow-ups in our hospital outpatient hypertension clinic, we did not proceed in additional BP estimation with out of hospital measurements (24 hour ABPM or HBPM). We characterized as controlled hypertensives those patients with office SBP ≤140 mmHg.

2.2.2 | Carotid-femoral pulse wave velocity (PWV)

Arterial stiffness was estimated by an automatic carotid-femoral PWV measurement using a Complior SP (Artech Medical, France), a computerized device that permits automatic calculation of PWV. Time delay between the recorded carotid and femoral arterial waves was recorded, while distance separating the transducers was superficially measured resulting in a PWV calculation as the average of at least 10 cardiac cycles. The same examiner, who was blinded to the patient's history, performed all measurements. Patients were advised to avoid smoking or coffee at least for 2 hours before examination. PWV ≤10 m/s was consider as normal.[1,11] Simultaneously to PWV estimation, central systolic blood pressure was also automatically calculated.

2.2.3 | Endothelial glycocalyx

We measured the PBR of the sublingual arterial microvessels (with diameter ranging from 5 to 9 μm) using Sidestream Darkfield imaging (Microscan, Glycocheck, Microvascular Health Solutions Inc., Salt Lake City, UT, USA).[12-14] Sidestream Darkfield imaging provides a direct, non-invasive, operator independent, easy to perform, and fast method (duration of ~3 minute) for the assessment of the EG. It has standardized methodology, it provides multiple measurements of sample sites (>3000 vascular segments of sublingual microvasculature) and estimation of the EG integrity of the microvessels ranging

from 5 to 25 μm in diameter, and it has a very good reproducibility[14] and thus is proposed as a valid method to assess endothelial integrity by the European Society of Cardiology Working Group on Peripheral Circulation.[10] The inter- and intra-observer variabilities of the PBR measurements were 5.2% and 4.3%, respectively.

2.2.4 | Lipid profile

All patients were subjected in fasting lipid profile measurement in a core laboratory (total cholesterol, triglycerides, HDL-C, and LDL). Blood samples were drawn by a clean venipuncture (20-gauge needle) from an antecubital vein under control venous stasis in reclined position. HDL-C levels were measured in a core laboratory. Both lipid profile estimation and PBR measurement were performed the same day of each patient's evaluation.

Subsequently, hypertensive patients were divided regarding HDL-C levels (median HDL-C = 58 mg/dL, mean HDL-C = 63 mg/dL) in tertiles:

1. Lower HDL-C tertile ranging between 23 and 46 mg/dL, median (25-75 IQR) value = 35 (31-38) mg/dL.
2. Middle HDL-C tertile ranging between 47 and 70 mg/dL, median (25-75 IQR) value = 63 (56-67) mg/dL.
3. Upper HDL-C tertile ranging between 71 and 101 mg/dL, median (25-75 IQR) value = 78 (74-87) mg/dL.

Hypertensives in the two lower HDL-C tertiles were included in the low HDL-C group (HDL_L, n = 79, HDL-C < 70 mg/dL) while those patients in the upper HDL-C tertile in the high HDL-C group (HDL_H, n = 41, HDL-C > 71 mg/dL).

2.3 | Statistical analysis

Variables were tested by the Kolmogorov-Smirnov test to assess the normality of distribution. Since almost all variables (triglycerides and central PP excluded) were normally distributed, they are expressed as mean ± standard deviation. Triglycerides and central PP are expressed as median value plus 25%-75% interquartile range (IQR). Paired Student's *t* test was used in order to compare numeric differences within groups, while Mann-Whitney test was used for categorical variables. ANOVA was used in order to compare PBR differences between the HDL-C tertiles. Pearson's correlation was used to identify the relations between PBR 5-9 and HDL-C levels as well as the other variables in total population.

Multiple linear backward regression analysis was performed in order to explore independent relations of PBR 5-9 measurements in

TABLE 1 Demographic and clinical characteristics of the two HDL-C subgroups (HDL_H and HDL_L)

	Group HDL_L (HDL-C < 71 mg/dL)	Group HDL_H HDL-C ≥ 71 mg/dL	*P*[a]
Characteristics			
N	79	41	
Age (years)	66 ± 9	67 ± 10	0.57
Males (%)	45 (57%)	6 (15%)	*<0.001*
Weight (kg)	84 ± 14	73 ± 11	*<0.001*
BMI (kg/m^2)	31 ± 4	29 ± 4	*0.01*
Current smokers (%)	25 (32%)	7 (17%)	0.08
Diabetes mellitus	22 (28%)	4 (10%)	*0.02*
Total cholesterol (mg/dL)	189 ± 41	210 ± 34	*<0.01*
Triglycerides (mg/dL)	128 (93-187)	76 (62-112)	*<0.001*
LDL-C (mg/dL)	119 ± 37	121 ± 31	0.70
HDL-C (mg/dL)	48 ± 15	80 ± 9	*<0.001*
Treatment with statins	48 (61%)	19 (48%)	0.17
Office SBP (mmHg)	145 ± 17	148 + 19	0.51
Office DBP (mmHg)	87 ± 10	87 ± 12	0.80
Office PP (mmHg)	58 ± 14	61 ± 17	0.24
Central SBP (mmHg)	136 ± 23	141 ± 20	0.25
Central PP (mmHg)	44 (37-62)	42 (54-62)	0.13
PWV (m/s)	13 ± 2.5	12 ± 2	0.10
PBR 5-9 (μm)	1.22 ± 0.1	1.17 ± 0.1	*0.01*

Significant differences are shown in italics.
BMI, body mass index; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; PBR, perfusion boundary region; PP, pulse pressure; PVW, pulse wave velocity; SBP, systolic blood pressure.
[a]Statistical difference between hypertensive patients with higher and lower HDL-C levels.

total population. Age, weight, smoking habit, HDL-C, LDL-C, and office SBP were forced into the regression analysis model as independent variables since they play a significant role in endothelial function.

We also provided subgroup analyses in whole population as followed: (a) smokers vs non-smokers, (b) diabetics vs non-diabetics, (c) statin-treated vs non-treated patients, and (d) controlled vs uncontrolled hypertensives in order to investigate any differences in HDL-C and PBR_{5-9} levels within the above-mentioned groups as well as the relationship between HDL-C and PBR_{5-9} in each group separately.

The level of statistical significance was determined as a *P* value <0.05. Statistical analysis was performed on a SPSS 21 version (SPSS Inc., Chicago, IL, USA).

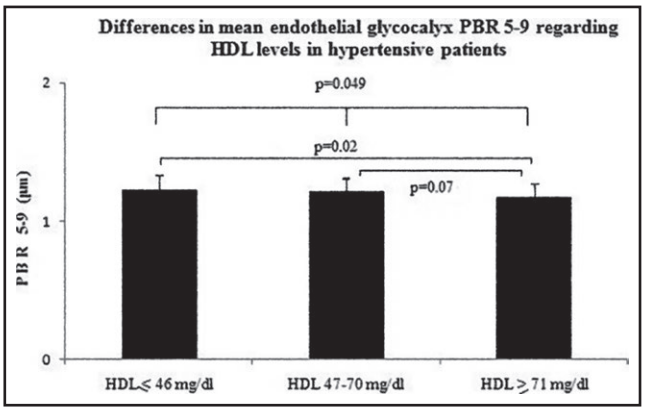

FIGURE 1 Differences in mean PBR_{5-9} levels between HDL-C tertiles

3 | RESULTS

Demographic and clinical characteristics of both HDL_H and HDL_L groups are listed in Table 1. Patients in both groups had similar age, central and brachial systolic and diastolic BP as well as PP, smoking habit, LDL-C levels, and PWV. On the other hand, patients in HDL_H group were mostly females (*P* < 0.001) and less obese (*P* < 0.01), fewer suffered from diabetes mellitus (*P* = 0.02), they had a better profile regarding total cholesterol (*P* < 0.01) and triglycerides (*P* < 0.001), and finally PBR 5-9 (Figure S1), representing endothelial dysfunction, was significantly decreased (*P* = 0.01).

3.1 | EG and HDL-C in the whole population

We found that mean ± SD PBR 5-9 was 1.23 ± 0.1 μm (lower HDL-C tertile), 1.22 ± 0.1 μm (middle HDL-C tertile), and 1.17 ± 0.1 μm (upper HDL-C tertile). Using ANOVA, we found that PBR 5-9 was significantly decreased across the HDL-C tertiles (*P* = 0.049) with its lower value appearing in the upper HDL-C tertile (HDL-C ≥ 71 mg/dL). Additionally, PBR 5-9 was decreased in hypertensive patients in the upper HDL-C tertile compared with either those patients in the middle (*P* = 0.07) or in the lower HDL-C tertile (*P* = 0.02) (Figure 1).

Using Pearson's univariate analysis in the whole population, we found that (a) HDL-C was inversely but moderately related to PBR 5-9 (*r* = −0.22, *P* = 0.01) (Figure 2) as well as the smoking habit (*r* = −0.24, *P* = 0.008) and (b) both HDL-C and PBR 5-9 were moderately related to the BMI (*r* = −0.22, *P* = 0.01 and *r* = 0.25, *P* = 0.006, respectively).

In a multivariate regression analysis, where the following parameters sex, age, BMI, smoking habit, diabetes mellitus, HDL-C, LDL-C, and office SBP were inserted as independent variables, we found that only BMI independently but moderately predicted PBR 5-9 levels in the whole population (β = 0.25, *P* = 0.006).

3.2 | EG and HDL-C in the hypertensives with smoking habit

Both groups, smokers (n = 32, mean age 62 ± 7, 18 males) and non-smokers (n = 88, mean age 68 ± 10, 33 males), had similar BMI, LDL-C levels, and PBR 5-9. However, smokers were younger (*P* = 0.06) with lower central (*P* = 0.004) and brachial systolic BP (*P* = 0.007), PWV (*P* = 0.01), and HDL-C levels (*P* = 0.008). We found that there was a trend toward an inverse moderate relationship between HDL-C and PBR 5-9 (*r* = −0.34, *P* = 0.06) only in the smoker group.

3.3 | EG and HDL-C in the hypertensives with diabetes mellitus

Both groups, diabetics (n = 26, mean age 71 ± 8, 13 males) and non-diabetics (n = 94, mean age 65 ± 9, 38 males), had similar central and brachial systolic BP and PBR 5-9. However, diabetics were older (*P* = 0.002) and more obese (*P* = 0.04) with lower LDL-C (*P* < 0.001) and HDL-C levels (*P* = 0.002) and increased PWV (*P* = 0.004). An inverse moderate relationship between HDL-C and PBR 5-9 (*r* = −0.20, *P* = 0.04) was revealed in non-diabetics group.

3.4 | EG and HDL-C in hypertensives treated with statins

Both groups, treated with statin (n = 68, mean age 68 ± 9, 28 males) and non-treated with statin patients (n = 52, mean age 64 ± 9, 23 males), had similar central and brachial systolic BP, PWV, HDL-C levels, and PBR 5-9. Additionally, treated with statins patients were older (*P* = 0.02) and had lower LDL-C levels (*P* < 0.001) compared to non-treated with statins patients. In the non-treated with statins patients, an inverse moderate relationship between HDL-C and PBR 5-9 (*r* = -0.28, *P* = 0.04) was revealed.

3.5 | EG and HDL-C in controlled hypertensives

Our hypertensive population was divided in controlled group and uncontrolled group of patients using the cut-off level of office SBP=140 mmHg. Both groups, controlled (n = 47, mean age 63 ± 8, 29 males) and uncontrolled hypertensive patients (n = 73, mean age 68 + 9, 24 males), had similar BMI, LDL-C, HDL-C levels, and PBR 5-9. However, the controlled hypertensives were younger (*P* = 0.003),

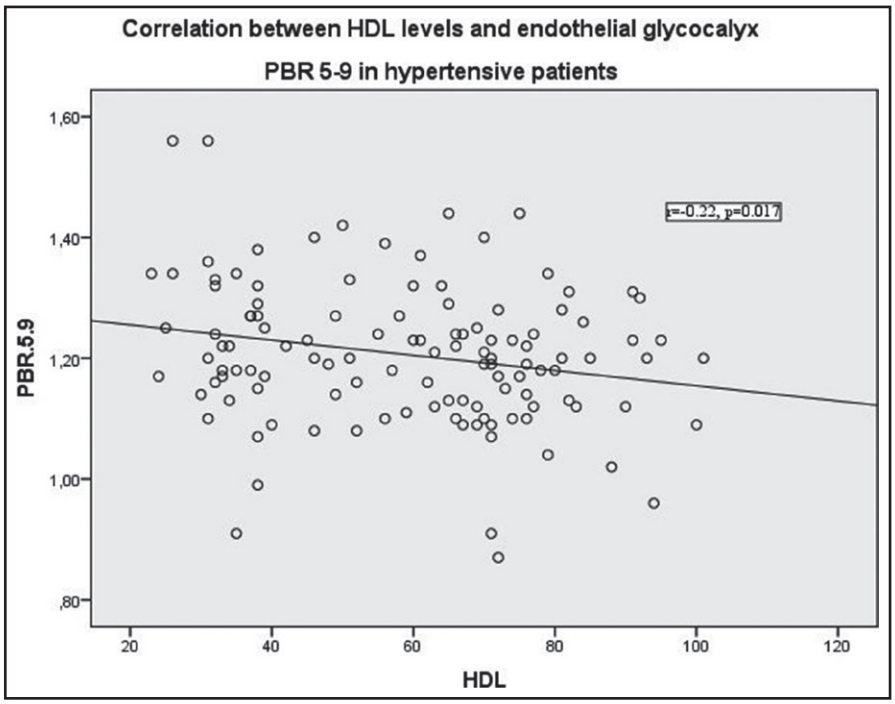

FIGURE 2 Correlation between PBR_{5-9} (μm) and HDL-C levels (mg/dL) in the whole study population

mostly men ($P = 0.008$) with a higher incidence of smoking habit (40% vs 18%, $P = 0.01$) and with lower central and brachial systolic BP and PP ($P < 0.001$) as well as PWV ($P < 0.001$). An inverse relationship between HDL-C and PBR 5-9 ($r = -0.40$, $P = 0.006$) was revealed only in the controlled hypertensives.

4 | DISCUSSION

In the present cross-sectional study, we tried for the first time to explore the role of extremely increased HDL-C levels regarding endothelial protection in older hypertensive patients using a new technique which estimates EG integrity. The primary end point of the study is that PBR 5-9, an index of EG dysfunction, is significantly decreased in older treated hypertensives with HDL-C ≥ 71 mg/dL compared with those patients having HDL-C < 71 mg/dL. The inverse non-independent and moderate relationship between HDL-C and PBR 5-9 in the whole studied hypertensive population should be mentioned as a secondary end point.

Current guidelines recommend measuring HDL-C for risk estimation and before initiation of lipid-lowering therapy.[15] The prognostic importance of HDL-C as a modifiable risk factor for cardiovascular disease has been identified since the first reports of the Framingham study, and 40 years later, HDL-C still stands for cardiovascular protection.[2,3] The atheroprotective function of HDL-C is based on the reverse cholesterol transport, the maintenance of endothelial cell homeostasis, and its potent antioxidant properties.[16,17] However, HDL-C can become pro-inflammatory in the presence of atherosclerosis and probably a synergistic role of low HDL-C and inflammation exists on the atherosclerotic disease progression from subclinical lesions to clinical events.[18] Raising HDL-C pharmacologically has not proven beneficial in randomized clinical trials[19] and has even paradoxically been associated with increased mortality in one study.[20] Additionally, extremely increased HDL-C levels as an independent and modifiable risk factor have been also challenged recently.[21,22] It is noteworthy that the association between HDL-C and both cardiovascular disease and mortality across a wide range of concentrations does not appear to be causal. Instead, it seems that the association between HDL-C and all-cause mortality might have a U-shape, with the lowest risk for all-cause mortality described in HDL-C levels equal to 73 and 93 mg/dL (in men and women, respectively).[21] An attractive hypothesis has been proposed to explain this phenomenon. Impaired functional HDL-C characteristics due to certain gene mutations are implicated in HDL-C metabolism (CETP, ABCA1, LIPC, SCARB1) and associated with both high risk of coronary heart disease and high concentrations of HDL-C.[23-26] Subsequently, an altered HDL-C particle in individuals with extreme high HDL-C might no longer function normally but rather cause harm.[21] Conclusively, plasma HDL-C concentration is a relatively simple measure of a more complex picture. HDL-C particles might be highly heterogenous as well as functionally diverse due to different structure, metabolism, and function providing different atheroprotective functions toward endothelium.[27]

In a previous study, we found that elevated HDL-C > 70 mg/dL may moderately predict the absence of carotid atherosclerosis in middle-aged women with untreated essential hypertension and consequently contribute to total CV risk estimation and treatment planning.[28] In the present study, we provided evidence that HDL-C ≥ 71 mg/dL protects endothelial function in older hypertensives with no other comorbidities. We chose to study a population

at risk since the majority of our patients had at least two risk factors, treated arterial hypertension under treatment and advanced age (90% of men aged ≥55 years and 61% of women aged ≥65 years). In hypertensive patients, the age >55 years for men and >65 years for women counts as a non-modifiable factor for total CV risk stratification since it can influence prognosis according to ESH guidelines.[1]

Most observational studies investigating the association between HDL-C and mortality have categorized individuals in larger groups; focus has been on low concentrations of HDL-C cholesterol, while the few individuals with extremely high HDL-C often are grouped together with individuals with only modestly high concentrations, thereby failing to elucidate associations at extremely high concentrations.[29] Our population consisted of 120 hypertensive patients, while one-third of them, mostly females (85%), had HDL-C ranging between 71 and 101 mg/dL. Indeed, people living in Mediterranean areas exhibit higher concentrations of HDL-C probably related to their dietary habits.[30] It is also known that women have higher plasma HDL-C concentrations than men,[31] while there are different lipoprotein changes with age in both sexes regarding the HDL-C protective role. Indeed, HDL-C seems protective against progression of carotid atherosclerosis in middle-aged men; anti-atherogenic effects of HDL-C may diminish in women around the age of menopause.[32]

Endothelial dysfunction predicts outcome in patients with a variety of CVDs, although data on hypertension are still rather scant.[33] In arterial hypertension, endothelial dysfunction evaluation is valuable, as an index of subclinical organ damage.[1] Several in vitro and in vivo studies have shown that HDL-C can contribute to endothelial cell homeostasis by (a) inducing the release of vasoactive molecules, particularly nitric oxide (NO) and thus promoting vasorelaxation, (b) inhibiting the expression of cell adhesion and pro-inflammatory molecules, induced by different stimuli in endothelial cells thus preventing blood cell adhesion and their consequent extravasation and favoring the integrity of the endothelium, and (c) repairing the endothelial layer.[34,35]

Endothelial function can be assessed by various techniques, including flow-mediated vasodilation, carotid intima-media thickness, and through measurement of serum biomarkers. However, the techniques available for investigating endothelial responsiveness to various stimuli are laborious, time-consuming, and often invasive.[36] EG acting as an interface between the blood and the vascular wall is increasingly recognized as a novel biomarker of endothelial damage. It is a dynamic structure; playing a key dynamic role in vascular homeostasis by transmitting shear stress forces to endothelial cells and regulating vessel wall permeability due to its mesh-like structure and charge and size selectivity.[37] Under physiological conditions, the overall net negative charge of the EG acts as a barrier to inhibit leukocyte adhesion by acting as an electrorepulsive shield, but also aids with leukocyte recruitment when necessary, through chemokine presentation and adhesion molecule activity.[38] Additionally, it acts as a sodium buffer system and a reservoir for sodium storage.[39] However, impaired glycocalyx barrier properties may contribute to transendothelial leakage of atherogenic LDL at lesion-prone arterial sites.[40] Fragile, and unable to be visualized or its complex mesh easily measured, the properties of the EG have traditionally proven difficult to study. Investigative techniques include in vivo, ex vivo, and in vitro studies, all with limited success and utility. New techniques to visualize the EG have emerged in the field of optics, using a handheld video microscope by means of a probe placed sublingually to capture recordings of the microcirculation. This technique incorporates Sidestream Darkfield (SDF) imaging. The EG width is calculated by measuring PBR, a cell-poor layer, which results from the phase separation between the flowing red blood cells (RBC) and plasma on the surface of the microvessel lumen. The PBR includes the most luminal part of glycocalyx that does allow cell penetration. Thus, an increased PBR is consistent with deeper penetration of erythrocytes into glycocalyx, indicating a loss of glycocalyx barrier properties and is a marker of reduced glycocalyx thickness and subsequently endothelial dysfunction.[41] This technique has been used in numerous clinical scenarios with variable interpretative success; however, there is no established PBR normal range yet.

In our study, we chose to estimate endothelial function using this new SDF imaging technique. Despite any challenges regarding the role of extremely elevated HDL-C, we found that HDL-C levels ranging between 23 and 101 mg/dL were inversely and moderately related to PBR 5-9, an index of endothelial dysfunction. Our results support the role of HDL-C as a CV risk factor since lower HDL-C was found in patients with endothelial dysfunction (higher PBR 5-9), and higher HDL-C was measured in patients with better endothelial function (lower PBR 5-9). Moreover, using the cutoff of 71 mg/dL for HDL-C, we proved that patients with HDL-C ranging between 71 and 101 mg/dL had significantly better endothelial function than those with HDL-C ≤ 71 mg/dL, and this also was evident across the HDL-C tertiles. Of course, our results underline the significance of extremely elevated HDL-C in older hypertensive patients but they cannot predict the role of HDL-C above 101 mg/dL regarding endothelial function. The relationship between HDL-C and PBR 5-9 was moderate and not strong, meaning that probably a number of patients with extremely elevated HDL-C might have dysfunctional HDL-C particles.

Weight loss resulting from gastric surgery has increased the ability of HDL-C to induce the release of nitric oxide from endothelial cells.[42] In our study, patients in the HDL_H group were less obese than those patients in the HDL_L group. We proved that both HDL-C (inversely) and PBR 5-9 (positively) are related to BMI. However, the relationship between HDL-C and PBR 5-9 was not proved independent in the whole hypertensive population. Indeed, when this relationship was investigated in a multivariate regression analysis model inserting age, smoking habit, LDL-C levels, and BMI as independent variables (factors that can influence endothelial integrity), we found that BMI and not HDL-C independently predicted PBR and endothelial function ($\beta = 0.25$, $P = 0.006$). It seems that increased BMI plays a significant negative role in endothelial function separately from HDL-C levels. In case vascular bed is under stress (increasing CV risk profile, as reflected by higher age, body mass index, arterial hypertension, total cholesterol, and Framingham risk score), functional

recruitment of "reserve" capillaries with preserved glycocalyx happens.[43] However, in most vascular beds, not all microvessels are perfused simultaneously, and probably, the fraction of healthy capillaries might progressively decrease over time if CV risk remains.[44]

Cigarette smoking is a major modifiable risk factor for cardiovascular disease. Increased arterial stiffness,[45] endothelial dysfunction,[46] and oxidative stress accentuation[45] represent significant pathophysiologic substrates of its toxic effects. In a previous study, we showed that a smoking cessation program using varenicline or nicotine replacement therapy for 3 months resulted in EG restoration.[47] In this study, a trend toward an inverse relationship between HDL-C and PBR 5-9 ($P = 0.06$) was revealed only in the small group of smokers (n = 32). We hypothesize that smoking could not overcome the beneficial role of extremely elevated HDL-C levels in endothelial function in our hypertensive population.

Acute and long-term hyperglycemia is associated with the profound thinning of the glycocalyx which in turn facilitates the attachment and rolling of circulating inflammatory cells in the subendothelial space and increases endothelial permeability to oxidized lipids and proteins.[48] In our study, we did not reveal any differences regarding PBR 5-9 in diabetics and non-diabetics. However, patients in our small diabetic group (n = 26) were significantly older, more obese with lower levels of both HDL-C and LDL-C but they had similar central and peripheral BP. Subsequently, neither the number of the patients in each group nor their clinical and laboratory characteristics were matched in order to compare endothelial function within groups. EG partially recovered after statin therapy in patients with heterozygous familial hypercholesterolemia.[49] When we tried to explore the role of the treatment with statins in our hypertensive population, we found that an inverse relationship between HDL-C and PBR 5-9 was present only in the non-treated with statin group as it was expected. We hypothesize that statins increasing HDL-C levels and probably improving endothelial function in a different proportion disrupt the relationship between HDL-C and PBR 5-9 in our hypertensive patients under treatment with statins.

It is not known whether successful antihypertensive therapy leads to endothelial recovery regarding EG levels. We found that only the patients with controlled hypertension had an inverse relationship between HDL-C and PBR 5-9. However, since our study is a cross-sectional one, our data can only hypothesize but they cannot support any theory regarding PBR lowering and EG restoration after BP control.

4.1 | Study limitations

Several limitations should be considered concerning the present study since its cross-sectional design prevented us from inferring definite cause-effect relationships while the moderate number of patients, overall and in each study group, does not support us to generalize our results in both untreated and treated hypertensives. Since our results are based on a single measurement of PBR in a relatively small group of patients, they might reflect a regression to the mean phenomenon. On the other hand, the recruitment of consecutive participants supports those results. Since hypertension has a high prevalence in population, a greater number of patients are needed in future studies in order to expand our results. Additionally, our study was just based on a single HDL-C assessment, while we were not able to access the size of HDL-C particles, especially the large ones, which might not be protective against atherosclerosis and endothelial function. However, one of the strengths of our study is that all HDL-C measurements were performed in a core laboratory and were referred to a relatively homogenous hypertensive group since all patients were older than 50 years with no other comorbidities except for diabetes mellitus diagnosed in the 22% of our population. We have to admit that the two study groups differed considerably with regard to the presence of diabetes mellitus ($P = 0.02$), smoking status ($P = 0.08$), and sex ($P < 0.001$ for male gender). However, in order to minimize those differences, we added the following parameters: sex, diabetes mellitus, and smoking habit in the multivariate regression analysis as independent variables. It is noteworthy that in everyday clinical practice, patients with increased HDL-C levels are mostly non-smoker women, less obese with a minor incidence of diabetes mellitus. Since HDL-C levels in our study ranged between 23 and 101 mg/dL, it needs to be further investigated in future studies if any beneficial association between high HDL-C levels and PBR 5-9 still exists in levels well above HDL-C > 100 mg/dL. The last limitation might be the use of a non-invasive imaging technique, the Sideview Dark Field imaging, which indirectly assess the EG thickness. Limitations with this technique include the fact that the sublingual circulation may not be representative of all other microcirculatory beds and that the technique is operator-dependent and requires substantial training due to a learning curve. Instead, the non-invasive nature of this technique, the semi-automated analyses, and the good reproducibility stand as important advantages.

4.2 | Clinical implications

There are clinical implications for individuals with extremely high concentrations of HDL-C. First, when HDL-C is used for risk assessment, clinicians should be aware that those with extremely high HDL-C might also be a high-risk group for all-cause mortality. This seems to be more pronounced in men, and for cardiovascular mortality.[22] Moreover, increased HDL-C levels might mislead physicians to undergo diagnosis of cardiovascular events especially in women. When HDL-C is elevated and the patient refers to atypical symptoms, physicians might underestimate a female's CV risk based on the expected HDL-C protection for CV disease.[50] Subsequently, careful evaluation of both symptoms and laboratory results taking also into account any favorable cardiovascular risk markers should lead to correct diagnosis.

In conclusion, in hypertensive patients, older than 50 years, HDL-C ranging between 71 and 101 mg/dL might moderately protect EG and subsequently endothelial function. Future studies in several groups of low- or high-risk hypertensive patients are needed in order to evaluate the beneficial role of extremely elevated HDL-C

regarding cardiovascular risk as well as the EG as a novel index of target organ damage in essential hypertension.

CONFLICT OF INTEREST

The authors state that they have no conflict of interest to declare.

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