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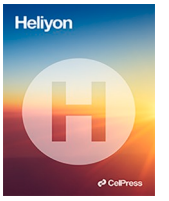

Research article

The influence of triglyceride and low-density-lipoprotein target levels on microcirculation: Is there a difference?

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ABSTRACT

Background and aims: This study aimed to validate the role of high low-density lipoprotein cholesterol [LDL-C] and triglyceride [TG] treatment target levels on the microcirculation in a very high and high cardiovascular risk group.
Methods: 119 patients with high or very high cardiovascular [CV] risk were included. We have registered the main co-morbidities, smoking habits, body mass index [BMI] and the lipid lowering medication. Hematocrit, whole blood viscosity [WBV] and plasma viscosity [PV], red blood cell [RBC] aggregation and deformability and fibrinogen, total cholesterol [TC], high-density lipoprotein cholesterol [HDL-C], LDL-C and TG levels were determined.
Results: The investigation found significantly higher PV values in patients with non-target LDL-C, associated with higher fibrinogen level. Non-target TG was related to deteriorated microcirculatory parameters, as significantly higher RBC aggregation, lower RBC deformability, and higher WBV and PV. The main microcirculatory benefit in diabetes could be gained from target level of TG, in chronic coronary syndrome [CCS] patients it is more advantageous to reach both LDL-C and TG target.
Conclusion: The results could highlight, that TG should play a role in failing microcirculation and cause potentially life-threatening complications, which would worsen the survival and quality of life of high or very high risk CV patients.

1. Introduction

Lipid particles are insoluble in water, therefore in blood they are transported covered by hydrophilic phospholipids and apoprotein molecules. There are six major lipoproteins: chylomicrons, very low-density lipoprotein [VLDL], intermediate-density lipoprotein [IDL], low-density lipoprotein [LDL], lipoprotein[a] [Lp[a]], and high-density lipoprotein [HDL]. These are essential in energy utilization, steroid hormone or bile acid production and lipid deposition.

Dyslipidemia is defined by elevated cholesterol [C] or triglyceride [TG] levels, resulting from rare genetic disorders, or secondary

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from high fat diet or alcohol consumption or diseases e.g. diabetes, hypothyroidism [1]. Based on large epidemiological and randomized clinical trials high LDL-C values are dose-dependently accompanied by high cardiovascular [CV] risk [3], which results from initiating and promoting chronic endothelial inflammation and atherosclerosis [AS] [4].

According to the ESC guideline from 2019 on dyslipidemia, measuring LDL-C and TG levels is I/C recommendation in every CV patient, for lipid profiling. As low LDL-C is associated with low CV risk, it is reasonable to decrease LDL-C level in every possible way [1,2]. In secondary prevention for patients at very high CV risk an LDL-C reduction of more than 50 % from baseline and an LDL-C goal of lower than 1.4 mmol/l are I/A recommendations [1,2]. In patients at high CV risk, an LDL-C reduction of more than 50 % from baseline and an LDL-C goal of lower than 1.8 mmol/l are I/A recommendations [1,2]. Lifestyle changes like physical activity [1], a Mediterranean diet [1], normal BMI [1] have pivotal role in reaching and maintaining LDL-C target levels. However, statins have the most dramatic effect on lipid levels, which are HMG-CoA reductase inhibitors. Statins reduce intracellular cholesterol production and increase the number of LDL receptors on the surface on hepatocytes, which leads to decreasing plasma levels of LDL-C and other ApoB containing lipoproteins like TG. Moderate-intensity statin therapy could reduce LDL-C level by 30–50 %, while high intensity by >50% [1]. Statin therapy also could reduce TG plasma level by 10–20 % [1]. The mechanism behind the TG lowering effect is not fully described, but presumably the higher uptake of VLDL particles from hepatocytes should have a role [5]. One recently introduced new drug is the proprotein convertase subtilisin/kexin type 9 [PCSK9] inhibitor evolocumab and alirocumab. In clinical trials [FOURIER, ODYSSEY] PCSK9 inhibitors reached dose dependently 60% LDL-C reduction alone, and even more than 80% as add-on therapy with statin and ezetimibe. This reduction was associated with a 15–20% CV risk reduction [6]. In phase II trials, evolocumab lowered TG levels by 26 %, and raised HDL-C by 9 % [7].

Although CV risk is increased when fasting TGs are higher than 1.7 mmol/l [8], the use of drugs (statin, fibrate, PCSK9 inhibitor) is only considered in high-risk patients, when TGs are higher than 2.3 mmol/l and if it cannot be lowered by lifestyle interventions [1]. Namely, lifestyle changes have more influence on TG level than medication [1]. If it comes to medication, fibrates are the most important TG lowering drugs. The peroxisome proliferator-activated receptor-a [PPAR-a] agonist could reduce TG level by 50 %, but the effect is highly dependent on the baseline TG value [1]. Previous studies like FIELD, LEADER or ACCORD failed to validate the CV mortality reducing effect of fibrates and lower TG levels. One recent study, the PROMINENT trial from 2022 was a double-blind, randomized, placebo-controlled trial of pemafibrate, which found that, although TG and VLDL levels were reduced in the pemafibrate group, the rate of CV events was not different from that of the placebo group [9]. Recently performed metaanalyses and Mendelian studies could also not prove the connection between hypertriglyceridemia and elevated CV risk. These assumed, that high TG values mean also high ApoB-containing lipoprotein levels, and high ApoB is the one which leads to increased CV risk, not hypertriglyceridemia [8].

Hemorheology studied blood flow and blood cell interactions. Blood is a two-phases (blood cells and plasma), thixotropic (time-dependently shear thinning), viscoelastic, non-Newtonian fluid. Macrorheological parameters as whole blood viscosity [WBV] and plasma viscosity [PV] are the main determiners of macro- and microcirculation. WBV is dependent on hematocrit and PV, but in the microcirculation, especially in the capillaries, WBV is highly dependent on the blood cell properties. Leukocytes and platelets have an obvious effect, but as the most abundant component, red blood cell [RBC] aggregation and deformability properties are the key factors in determining capillary blood flow and thus, WBV.

As human blood is a non-Newtonian fluid WBV decreases by increasing shear rate, meaning it is the lowest in capillaries. Increased WBV results in high endothelial wall shear stress, which could elevate endothelial nitric oxide [NO] production, cause vasodilatation and so maintain normal vascular resistance. This mechanism is mandatory to preserve normal blood flow properties in the arteries and capillaries, as well as in normal RBC deformability, which is also regulated by the shear dependent NO production [10]. However, in patients with impaired endothelial function due to atherosclerosis [AS], increasing WBV could not trigger this flow mediated vasodilatation to normalize wall shear stress, hence the vascular resistance and arterial pressure will rise. Pop et al. described, that increased blood viscosity decreases cardiac output and tissue perfusion, leading to ischemia and compromises tissue viability [11]. The increased vascular resistance and cardiac afterload caused by any kind of inflammation (e.g. AS or sepsis) further increase WBV and myocardial oxygen demand due to hemoconcentration (decreased fluid intake, increased insensible water losses due to fever, edema due to increased capillary permeability) [12]. Plasma is a Newtonian fluid hence it is independent from shear stress. PV is mostly determined by the amount of proteins e.g. acute phase proteins like fibrinogen, high sensitive C-reactive protein, inflammatory cytokines [13]. Thus, high PV values are associated with inflammatory, metabolic (e.g. diabetes) and cardiovascular diseases, and have a negative impact on outcomes in unstable angina or stroke [14]. Elevated WBV and PV are proven independent predictors of initial and recurrent myocardial infarction, CV and all-cause mortality [15].

Chylomicrons, VLDL and LDL added to plasma in vitro cause a dose-dependent and exponential rise in viscosity [16]. VLDL lead to a greater viscosity change than LDL. High LDL-C and TG levels do not only increase WBV or PV, they also contribute to a prothrombotic state. Hypertriglyceridemia facilitates thrombosis due to elevated WBV and PV thus slower blood flow, as well as elevate factor VII clotting activity and impair fibrinolysis mediated by plasminogen activator inhibitor-1 [PAI-1] [17]. The contribution of plasma triglycerides to viscosity is dependent on the size and concentration of the triglyceride enriched lipoprotein (chylomicron, VLDL). The effects of lipids and lipoproteins on hemorheology and hemostasis may contribute to the CV benefit of lipid lowering therapies [18].

Our research group intended to study hemorheological alterations in high and very high CV risk patients as well as in diabetes and chronic coronary syndrome patients, to show the influence of LDL-C and TG target levels on the microcirculation.

2. Material and methods

The study protocol was approved by the Regional and Local Research Ethics Committee at the Medical School, University of Pecs

(Reference number: 4378). The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki.

2.1. Population

119 patients with high or very high CV risk were included in our single-center, cross-section study. Risk stratification was applied according to the 2021. ECS guideline on cardiovascular prevention [19]. Exclusion criteria were unstable CV disorders, i.e. significant untreated arrhythmias, newly diagnosed acute coronary syndrome (defined by the 2023. ESC guideline on ACS), heart failure with reduced, preserved, mildly reduced or normal ejection fraction (defined by the 2023. focused update of the 2021. ESC guideline in heart failure), any hospitalization in the last 1 month and participation in another study. All patients gave their written informed consent.

Demographical data were collected using the electronic databases of the Clinical Center, University of Pecs (eMedSol and EESZT). We registered smoking habits, age, gender, body-mass-index [BMI], lipid-lowering medication (statin, ezetimibe, fibrate, PCSK-9 inhibitor) and the main co-morbidities: hypertension, diabetes, target-organ-damage [TOD] i.e. chronic kidney disease [CKD] (glomerular filtration rate under 60 ml/min/1.73 m^2 for longer than 3 months), stable cardiovascular diseases diagnosed more than 12 months ago (previous acute coronary syndrome [ACS], previous percutaneous coronary intervention [PCI] or coronary-artery bypass graft [CABG], chronic coronary syndrome [CCS]).

2.2. Blood sampling

Blood samples were obtained after 5-min rest from the antecubital vein, into three potassium EDTA-2Na coated (15 ml) and into one clot activator-coated and gel-containing (5 ml) Vacutainer tube with a 21-gauge Eclipse Blood Collection butterfly needle set, using a minimal tourniquet.

Clinical chemistry (fasting total cholesterol, TG, HDL-C, LDL-C, HbA1c, blood cell counts), fibrinogen and hemorheological parameters (hematocrit, WBV, corrected WBV for 40% hematocrit, PV, RBC aggregation and RBC deformability) were measured accordingly. LDL-C values were measured with direct LDL-assay and TG values with glycerol-blanked TG-assay [20–22].

2.3. Hemorheological measurements

Hematocrit [Htc] was measured by a micro-hematocrit centrifuge (Haemofuge Heraeus Instr., Germany) [23]. WBV and PV were determined by the Brookfield DV-III Ultra LV (Brookfield Engineering Labs, Middleboro, USA) programmable rotational viscometer at 37 °C at 90 s^{-1} shear rate [23]. Corrected WBV for 40% Htc was calculated for further analytic use according to the following equation: (PV*(POWER(WBV/PV; 40/Htc)). RBC aggregation was measured with Myrenne (MA-1 Aggregometer, Myrenne GmbH, Roentgen, Germany), which measures the infrared light, transmitted through the plasma gaps between RBC aggregates and provides two dimensionless indices at room temperature (M, aggregation at stasis, shear rate 0 s^{-1}, and M1, aggregation at low shear, 10s^{-1}) using the Schmid-Schönbein principle. The measurement required 30 μl whole blood [23]. RBC deformability was characterized with LORCA ektacytometer at 37 °C. 20 μl of whole blood was diluted in a viscous medium [polyvinylpyrrolidone] and injected between the cylinders of the instrument. The provided nine values of elongation indices [EI] at the shear stress ranging from 0.3 to 30 Pa refer to the RBC diffraction pattern changing from circular to elliptical shape [23].

2.4. Statistical evaluation

A sample size and power analysis was performed for the LDL-C and TG target and non-target patients using PS program version

Table 1
Demographic data in the study population. Continuous variables are shown as mean ± standard deviation, ordinal variables as total number and percentage. LDL-C: low-density lipoprotein cholesterol, TG: triglyceride.

	total number [N]	percentage [%]
gender [male/female]	71/48	59.66/40.34
age [year]	66.56 ± 8.71	–
body mass index [kg/m^2]	29.23 ± 4.71	–
smoker	17	15.45
hypertension	109	91.59
diabetes	69	57.98
chronic kidney disease	32	26.89
cerebrovascular disease	6	5.04
peripheral artery disease	3	2.52
chronic coronary syndrome	94	78.99
on lipid-lowering medication	91	76.47
LDL-C on target	47	39.49
LDL-C non-target	72	60.51
TG on target	53	44.54
TG non-target	66	55.46

3.1.2. For LDL-C a sample size of n = 39 patients is needed to detect a true difference of d = 0.6 in plasma viscosity with 90.49 % power, where type I error probability is α = 0.05. For TG a sample size of n = 55 patients is needed to detect a true difference of d = 0.5 in plasma viscosity with 90.12 % power, where type I error probability is α = 0.05.

Statistical analyses were evaluated by IBM SPSS Statistics® 27.0. Significance level was defined as $p < 0.05$. Continuous variables are reported as mean ± standard deviation [SD], categorical variables as frequencies and percentages. After testing the normality by the Kolmogorov-Smirnov test, one-way ANOVA statistical test and chi-square test were used to compare differences between the groups. Multiple linear logistic regression and stepwise analysis of the data were performed considering the principle of multi-collinearity to investigate LDL-C and TG levels as the dependent variable. In the analysis with LDL-C as dependent factor, BMI, PV and fibrinogen were the independent variables. In the TG regression, RBC aggregation (M and M1) and low shear stress RBC deformability parameters (EIs at 0.95, 0.53 and 0.3 Pa) were used as independent variables.

3. Results

Table 1 shows the descriptive demographic data of the total population of 119 patients. We found no significant differences in hemorheological data between the patients on any lipid-lowering medication or not on any (data not shown). Target levels for LDL-C and TG were considered according to the 2019 ESC guideline on dyslipidemia [1].

3.1. Hemorheological alterations considering LDL-C treatment goal

We observed no significant differences in demographic data or TG and HDL-C between patients on LDL-C target (<1.8 mmol/l) and not on target LDL-C (>1.8 mmol/l). There is a significant association between LDL-C target level and lower number of patients taking lipid-lowering medication in this group (Table 2).

Hemorheological parameters revealed significant differences between the two groups. We found significantly higher PV values in the non-target LDL-C group compared to target group. The difference may result from the higher fibrinogen value in the non-target level group. The other hemorheological parameters revealed no significant alterations (Table 2 and Fig. 1).

Multiple linear regression and stepwise analysis of the data were performed to predict LDL-C level concerning PV, fibrinogen and BMI. PV statistically significantly ($p = 0.017$) predicted the dependent variable, $F(3, 83) = 5.678$, $p < 0.001$, $R2 = 0.413$. BMI ($p = 0.055$) and fibrinogen ($p = 0.300$) were not significant, but increased the fitness of the model.

3.2. Hemorheological alterations considering TG treatment goal

We observed significantly higher prevalence of diabetes in the group with non-target TG values. No further significant differences were found in the basic demographic data between patients on target TG (<1.7 mmol/l) and patients not on target TG (>1.7 mmol/l) (Table 3).

Hemorheological parameters revealed significant differences between the two TG groups. We found significantly higher WBV, PV, M1 values in the non-target TG group compared to the target group. The EIs at middle shear stress showed significantly lower values in the non-target group compared to the target group, referring to impaired RBC deformability (Table 3 and Fig. 2).

Multinominal logistic regression and stepwise analysis of the data were performed to predict TG level concerning M, M1 and EIs at 0.3, 0.53 and 0.95 Pa. Each variable statistically significantly ($p < 0.040$) predicted the dependent variable, $F(5,111) = 9.767$, $p <$

Table 2
Demographic and hemorheological data regarding LDL-C target level. We observed higher number of patients on lipid-lowering medication in the non-target LDL-C group. Continuous variables are shown as mean ± standard deviation, ordinal variables as total number and as percentage, $p < 0.05$ refers to significant difference. TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, WBV: whole blood viscosity, PV: plasma viscosity, RBC: red blood cell.

	LDL-C on target	LDL-C not on target	p value
gender [male/female]	29 [61.70 %]/18 [38.30%]	42 [58.33 %]/30 [41.67 %]	0.714
age [year]	65.19 ± 9.38	67.46 ± 8.18	0.166
body mass index [kg/m^2]	29.79 ± 4.49	28.83 ± 4.85	0.332
smoker	7 [15.22 %]	10 [15.63 %]	0.953
hypertension	43 [91.49 %]	66 [91.66 %]	0.973
diabetes	32 [68.49 %]	37 [51.39 %]	0.071
chronic kidney disease	13 [27.66 %]	19 [26.39 %]	0.879
chronic coronary syndrome	39 [82.97 %]	55 [76.39 %]	0.388
on lipid-lowering medication	41 [45.05 %]	50 [54.95 %]	**0.025**
TG [mmol/l]	2.50 ± 3.27	2.36 ± 1.84	0.763
HDL-C [mmol/l]	1.22 ± 0.43	1.25 ± 0.33	0.711
hematocrit [%]	42.53 ± 5.11	43.08 ± 4.53	0.538
WBV [mPas]	4.22 ± 0.68	4.27 ± 0.52	0.624
PV [mPas]	1.23 ± 0.09	1.29 ± 0.08	**< 0.001**
RBC aggregation in M mode	7.20 ± 1.76	7.46 ± 1.84	0.448
RBC aggregation in M1 mode	14.03 ± 2.90	14.40 ± 2.56	0.471
fibrinogen [g/l]	3.17 ± 0.71	3.41 ± 0.72	0.077

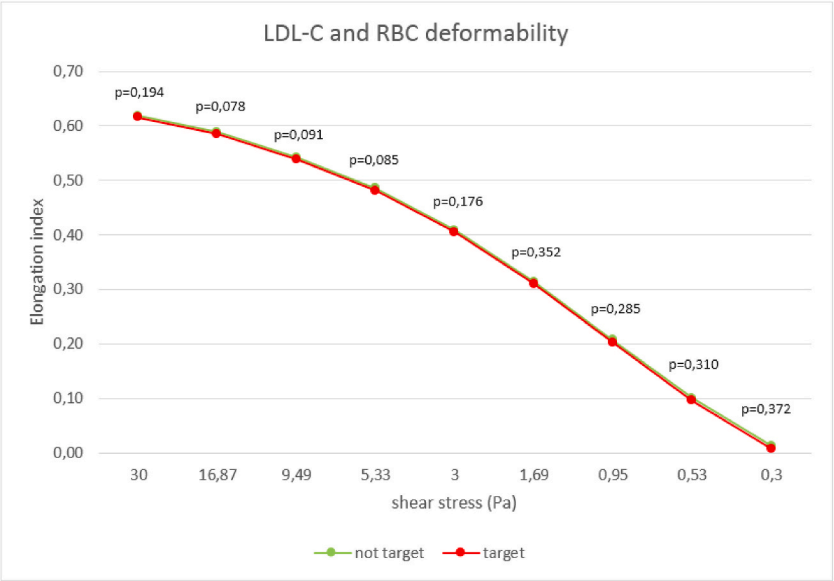

Fig. 1. LDL-C target has no influence on red blood cell deformability. No differences can be seen in the elongation indices measured at 9 different shear stress levels. LDL-C: low-density lipoprotein cholesterol, RBC: red blood cell. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 3
Demographic and hemorheological data regarding triglyceride target level. Continuous variables are shown as mean ± standard deviation, ordinal variables as total number and as percentage, $p < 0.05$ refers to significant difference. LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, WBV: whole blood viscosity, PV: plasma viscosity, RBC: red blood cell.

	TG on target	TG not on target	p value
gender [male/female]	30 [56.60 %]/23 [43.39%]	41 [62.12 %]/25 [37.88 %]	0.542
age [year]	67.47 ± 9.05	65.83 ± 8.42	0.310
body mass index [kg/m^2]	28.74 ± 4.96	29.66 ± 4.48	0.350
smoker	6 [11.76 %]	11 [18.64 %]	0.320
hypertension	47 [88.68 %]	62 [93.59 %]	0.304
diabetes	24 [45.28 %]	45 [68.18 %]	**0.012**
chronic kidney disease	12 [22.64 %]	20 [30.30 %]	0.349
chronic coronary syndrome	45 [84.91 %]	49 [74.24 %]	0.053
on lipid-lowering medication	37 [40.66 %]	54 [59.34 %]	0.125
LDL-C [mmol/l]	2.21 ± 0.78	2.61 ± 1.18	0.036
HDL-C [mmol/l]	1.39 ± 0.42	1.11 ± 0.26	**< 0.001**
hematocrit [%]	42.42 ± 4.91	43.23 ± 4.63	0.356
WBV [mPas]	4.13 ± 0.61	4.34 ± 0.55	**0.048**
PV [mPas]	1.24 ± 0.10	1.29 ± 0.08	**0.012**
RBC aggregation in M mode	7.00 ± 1.78	7.65 ± 1.78	0.053
RBC aggregation in M1 mode	13.36 ± 2.37	14.96 ± 2.75	**0.001**
fibrinogen [g/l]	3.35 ± 0.84	3.29 ± 0.63	0.660
elongation index at 1.69 Pa	0.317 ± 0.02	0.308 ± 0.022	**0.035**

0.001, R2 = 0.553.

3.3. Results of the diabetes subgroup analyses

We observed more diabetes patients in the high TG group, therefore a diabetes subgroup was built. In the analysis 69 diabetes patients were included. Diabetes patients do have more target organ diseases (CKD and CCS) and significantly higher BMI, TG and lower HDL-C values than not diabetes patients (Table 4). However, diabetes patients are more often on target LDL-C level (46.38 % vs. 30.00 %; p = 0.71), but significantly more diabetes patients have higher TG level than the goal (37.78 % vs. 58.00 %; p = 0.012). Results of hemorheological measurements showed significantly lower elongation indices along the whole nine shear stresses in diabetes patients (Table 4 and Fig. 3).

After the initial analysis we investigated the influence of LDL-C and TG target levels on the hemorheological parameters in diabetic patients. We revealed significantly higher PV values in diabetic patients not on target LDL-C (1,23 ± 0.09 vs. 1.29 ± 0.09; p = 0.015).

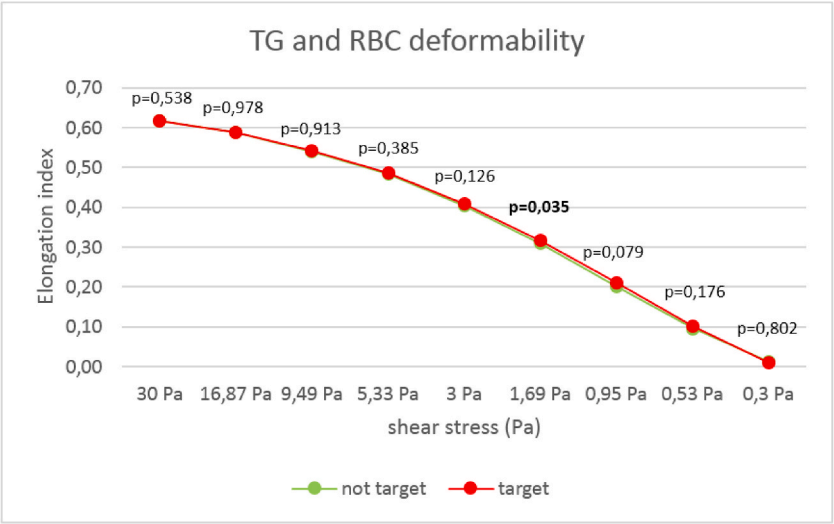

Fig. 2. Triglyceride target has a significant influence on red blood cell deformability, mainly at middle shear stress. TG: triglyceride, RBC: red blood cell. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 4
Demographic and hemorheological data regarding diabetes. Continuous variables are shown as mean ± standard deviation, ordinal variables as total number and as percentage, $p < 0.05$ refers to significant differences. LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, WBV: whole blood viscosity, PV: plasma viscosity, RBC: red blood cell.

	diabetes patients	non-diabetes patients	p value
gender [male/female]	41 [59.42 %]/28 [40.58%]	30 [60.00 %]/20 [40.00 %]	0.949
age [year]	65.87 ± 9.25	67.52 ± 7.89	0.310
body mass index [kg/m^2]	30.66 ± 4.93	27.60 ± 3.88	**0.001**
smoker	11 [18.03 %]	6 [12.24 %]	0.404
hypertension	47 [88.68 %]	62 [93.59 %]	0.304
chronic kidney disease	21 [30.43 %]	11 [22.00 %]	0.306
chronic coronary syndrome	50 [72.46 %]	44 [88.00 %]	**0.004**
on lipid-lowering medication	55 [60.44 %]	36 [39.56 %]	0.328
LDL-C [mmol/l]	2.34 ± 1.06	2.55 ± 0.99	0.274
HDL-C [mmol/l]	1.15 ± 0.32	1.36 ± 0.40	**0.001**
TG [mmol/l]	2.84 ± 3.09	1.82 ± 1.01	**0.027**
hematocrit [%]	42.48 ± 4.66	43.40 ± 4.87	0.298
WBV [mPas]	4.23 ± 0.59	4.28 ± 0.59	0.665
PV [mPas]	1.26 ± 0.09	1.28 ± 0.09	0.478
RBC aggregation in M mode	7.57 ± 1.74	7.06 ± 1.87	0.135
RBC aggregation in M1 mode	14.63 ± 2.76	13.72 ± 2.53	0.07
fibrinogen [g/l]	3.28 ± 0.64	3.38 ± 0.83	0.490

Our results showed significantly higher RBC aggregation indices M (6.85 ± 1.19 vs, 7.94 ± 1.86; $p = 0.014$) and M1 (13.15 ± 1.83 vs. 15.39 ± 2.87; $p = 0.001$) and corrected WBV value (3.79 ± 0.24 vs. 3.99 ± 0.32; $p = 0.010$) in diabetic patients not on target TG level.

3.4. Results of the chronic coronary syndrome subgroup analyses

In our study population we observed 94 CCS patients, with previous acute coronary syndrome and/or PCI and/or CABG. These patients build up the subgroup for the next evaluation. We observed significantly more diabetes among patients with CCS. Patients with CCS were in significantly higher number on lipid-lowering medication (Table 5). We could prove significantly higher EIs at high and middle shear stresses in the CCS patients (Fig. 4). The other hemorheological parameters were not different (Table 5).

Whether LDL-C was on target, CCS patients have significantly lower elongation indices at the high and middle shear stresses and significantly lower PV (1.23 ± 0.09 vs. 1.30 ± 0.08; $p = 0.001$) and lower fibrinogen value (3.14 ± 0.75 vs. 3.38 ± 0.66; $p = 0.118$). If TG values were on target, CCS patients showed significantly lower corrected WBV (3.82 ± 0.26 vs. 3.94 ± 0.26; $p = 0.047$), PV (1.25 ± 0.10 vs. 1.29 ± 0.08; $p = 0.024$) and RBC aggregation parameter M1 (13.47 ± 2.24 vs. 14.67 ± 2.6; $p = 0.020$).

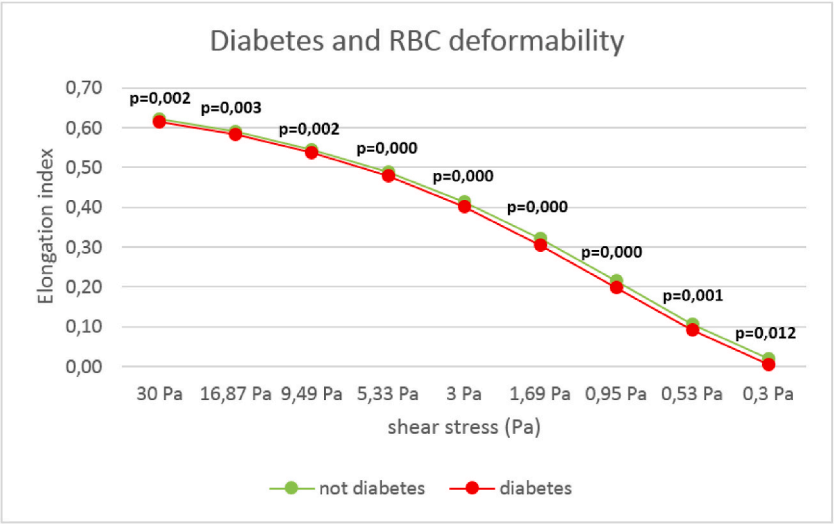

Fig. 3. Diabetes significantly impair red blood cell deformability, at all, but especially at low shear stresses. RBC: red blood cell. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 5
Demographic data regarding chronic coronary syndrome. Continuous variables are shown as mean ± standard deviation, ordinal variables as total number and as percentage, $p < 0.05$ refers to significant differences. LDL-C: low-density lipoprotein cholesterol, TG: triglyceride, HDL-C: high-density lipoprotein cholesterol, WBV: whole blood viscosity, PV: plasma viscosity, RBC: red blood cell.

	chronic coronary syndrome patients	non-chronic coronary syndrome patients	p value
gender [male/female]	59 [62.77 %]/35 [37.23 %]	12 [48.00 %]/13 [52.00 %]	0.181
age [year]	67.84 ± 8.52	61.76 ± 7.82	**0.002**
body mass index [kg/m^2]	28.85 ± 4.61	30.92 ± 4.89	0.102
smoker	15 [16.85 %]	2 [9.52 %]	0.403
hypertension	87 [92.55 %]	22 [88.00 %]	0.466
diabetes	50 [53.19 %]	19 [76.00 %]	**0.040**
chronic kidney disease	27 [28.72 %]	5 [20.00 %]	0.382
on lipid-lowering medication	49 [52.13 %]	6 [24.00 %]	**0.025**
LDL-C [mmol/l]	2.41 ± 1.04	2.52 ± 1.05	0.641
HDL-C [mmol/l]	1.23 ± 0.36	1.27 ± 0.42	0.647
TG [mmol/l]	2.25 ± 1.79	3.05 ± 4.18	0.155
hematocrit [%]	42.93 ± 4.79	42.64 ± 4.68	0.791
WBV [mPas]	4.23 ± 0.60	4.30 ± 0.55	0.593
PV [mPas]	1.27 ± 0.09	1.26 ± 0.09	0.485
RBC aggregation in M mode	7.27 ± 1.78	7.70 ± 1.91	0.296
RBC aggregation in M1 mode	14.11 ± 2.50	14.81 ± 3.35	0.252
fibrinogen [g/l]	3.29 ± 0.70	3.43 ± 0.79	0.373

4. Discussion

Dyslipidemia and its treatment are the focus of cardiovascular secondary prevention. There are I/A recommendations for target LDL-C levels, but no recommendation exists for TG, however, the 2019 ESC guideline for dyslipidemia suggests keeping TG values of high CV risk patients under 1.7 mmol/l [1]. It is a well-known fact, that lower LDL-C means lower CV risk. Beyond lifestyle changes the treatment involves medications as well. The significance of TG in CV risk estimation is not that simple, since the latest trials and metaanalyses could not validate the beneficial effect of low TG values on CV morbidity and mortality. But TG has an obviously negative effect on microcirculation, due to prothrombotic effect and impaired hemorheological factors.

Hemorheology is studying several parameters which are strongly connected to the microcirculation and could help to understand the contribution of different conditions and pathologies to impaired capillary blood flow and tissue damage. High WBV and PV values are related to several cardiovascular, cerebrovascular and metabolic disorders (e.g. diabetes). High WBV value leads to high vascular resistance, which can promote plaque rupture, but it is also associated with high thrombotic risk. Human whole blood is a non-Newtonian fluid, meaning, that WBV is minimal at high shear rates and high velocities, e.g. in capillaries. Low shear rates occur in veins and in arteries as well as in areas of flow separations i.e. dilatations, branches, and curves. At these places elevated WBV causes increased vascular resistance and wall shear stress according to Poiseuille's Law [23]. These factors aligned with impaired endothelial function in CV disease or diabetes could contribute to thrombosis at stenotic lesions or to atherosclerotic plaque ruptures. The findings

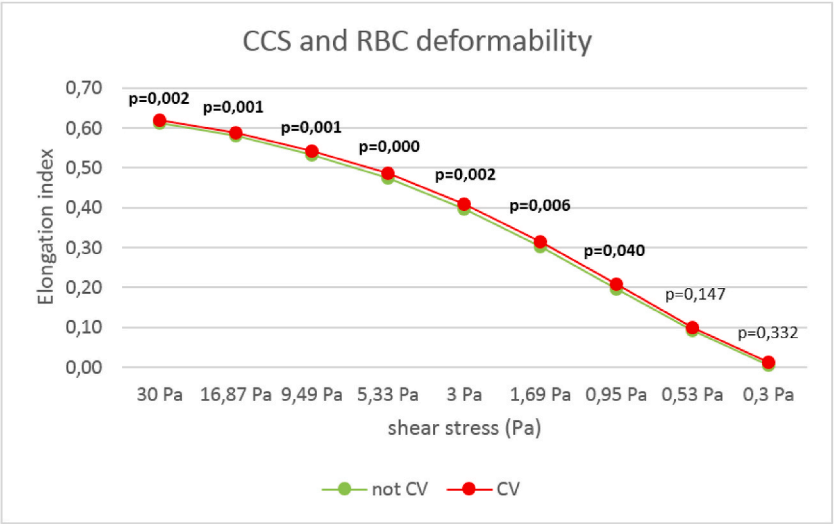

Fig. 4. Chronic coronary syndrome (CCS) patients have significantly better RBC deformability at high shear stresses, than non-cardiovascular patients. RBC: red blood cell. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

of our present study showed no difference in WBV regarding LDL-C target in patients with high or very-high CV risk. On the other hand, WBV was significantly higher in patients not on target TG. In a previous study, WBV raised the relative risk of a cardiovascular event by a factor of 1.2, and this was identical to the relative risk associated with LDL-cholesterol concentration [24]. This underlines the importance of high WBV in high CV risk patients with non-target TG values. Other studies have observed also the increase in blood viscosity with hyperlipoproteinemia [25]. These studies showed higher influence of TG on WBV than LDL, just as our findings suggest.

PV level was significantly higher in both non-target level groups. At least, high PV in the non-target LDL group was also related to higher fibrinogen values. Fibrinogen, as an acute phase protein is one of the major determinants of blood viscosity and plasma viscosity [26]. Fibrinogen levels could also be used to estimate CV risk in hyperlipidemia with and without established coronary heart disease [27]. In population studies (Framingham Offspring Study), fibrinogen was weakly, but positively associated with LDL cholesterol, Lp [a], triglycerides, and inversely associated with HDL cholesterol [28]. In our study high PV values associated with high fibrinogen eventually refer to high inflammation status in the case of high LDL-C values. High PV in not targeted TG group is mainly caused by the large amount of chylomicron and VLDL particles in the plasma, which are also associated with negative outcomes in different CV and cerebrovascular events [13,14].

Fibrinogen is an important factor in RBC aggregation as well. RBC aggregation is the interaction of RBCs, promoted by plasma proteins (mainly fibrinogen) or RBC membrane changes (e.g. sickle cells). At low shear rate i.e. in veins or in the bifurcation of arteries or at the lateral surface of stenotic lesions RBCs will form rouleaux-s (RBCs attached to each other one by one). At high shear rate this formation is mostly reversible, however, at permanently low shear rate (in CV diseases) rouleaux-s become clots and impossible for disaggregation [29]. Elevated RBC aggregation causes increased WBV. The fact, that these processes will take place mostly at low share rate parts of the circulation highlights the importance of irreversible clot formation by RBCs and the elevated WBV at least at pre-capillary level [29]. Any molecule large enough to broaden the intercellular distance and bind two RBCs can stimulate aggregation. These “bridging” particles include acute phase proteins i.e. fibrinogen, C-reactive protein, but also immunoglobulin M and LDL or TG particles [30]. Another process that causes RBC aggregation is the “depletion attraction”, when two RBC approach each other very closely and macromolecules are excluded from the area between them [31]. The increased TG levels could contribute to the elevated RBC aggregation by the bridging model, and thereby could increase WBV, which result in the already mentioned negative consequences on the microcirculation and tissue perfusion.

RBC deformability is the ability of the red blood cell to get through capillaries with a diameter of 4–5 μm although the cell itself has normally a biconcave disc shape and a diameter of 7 μm [32]. Depending on whether the RBC loses its capacity to deform and become rigid, WBV will deteriorate at low shear rates (i.e. in veins) and increase at high shear rates (i.e. arteries and capillaries) causing increased vascular resistance. Any negative changes in RBC deformability could increase flow resistance and deteriorate tissue oxygenation and nutritional supply [32]. We could observe significantly lower EIs at middle shear stress in patients not on target TG. In the background the RBC membrane damage could be assumed, caused by increased RBC aggregation and WBV [33].

In summary, if LDL-C values are at target, patients with high or very high CV risk have lower PV values, connected to lower fibrinogen level. Fibrinogen is an acute phase protein; hence inflammation is a causal factor to atherosclerosis, lower fibrinogen and lower PV could mean lower inflammation status. In fact, these results could contribute to lower CV risk. Based on our findings, TG target has more influence on the hemorheological parameters than LDL-C target. The significantly better WBV, PV and RBC aggregation and deformability values in patients with target TG values could refer to a healthier and more competent microcirculation in patients with CV risk even if, as according to population studies we assume, this is not influencing CV mortality. We found no

differences in hemorheological parameters between patients on or not on lipid-lowering medication, but obviously TC and LDL-C levels were lower in the group on lipid-lowering medication. In the LDL-C non-target group were more patients on lipid-lowering medication than in the targeted group (but in this group more patients were on target doses). These findings suggest, that the hemorheological benefits of target LDL-C and TG are present not only due to medication. LDL-C and TG levels based on the results of the two multilinear regression analyses are highly dependent on certain hemorheological factors. Hence, it is notable not just to use any kind and dose of lipid-lowering medication, but to aim the optimal doses and reach target values of lipoproteins. These could lead to take the advantage of the favorable hemorheological properties in the microcirculation.

4.1. Diabetes and target levels

Diabetes is an important metabolic disease with known high CV risk due to both macro-, and microvascular complications i.e. ACS, CKD, stroke, peripheral artery disease, major or minor amputation [29]. Previous studies already described impaired hemorheological parameters, mainly deteriorated RBC aggregation and deformability in diabetes [29]. Our diabetes subgroup showed significantly higher BMI values, and as expected more CKD and CCS patients than the not diabetes group. We could also observe lower RBC deformability values at all shear stresses in diabetes. Impaired RBC deformability is in connection with higher glucose, significantly higher TG level and the stimulated oxidative stress leading to RBC membrane injuries [33]. The decrease in RBC deformability could contribute to reduced tissue oxygenation and nutritional supply, which could lead to life-threatening microcirculatory complications like peripheral artery disease, ulcers, amputations as well as to limited quality of life [29]. This already detrimental microcirculation in diabetes could be further deteriorated by the non-target LDL-C, when, PV is significantly higher. On the other hand, if TG is high, an even more disadvantageous milieu could develop, accompanied by significantly higher RBC aggregation and corrected WBV level. Recently emerged agents such as SGLT-2 inhibitors (i.e. empagliflozin, dapagliflozin), GLP-1 receptor agonists (dulaglutide, liraglutide, semaglutide), and DPP-4 inhibitors (i.e. sitagliptin, vildagliptin), which represent a novel option in the complex treatment of diabetes especially in patients with high cardiovascular risk. These substances have shown a beneficial effect on major cardiovascular outcomes (mortality, hospitalization) in several multicenter clinical trials e.g. EMPA-REG OUTCOME, DAPA-HF, LEADER, SAVOR-TIMI 53. These effects appear not just because of the better diabetes control, but could also result from the improved lipid profile in addition. They are associated with reduced oxidative stress and inflammation and improved endothelial function, which properties may make their rheological investigation relevant in the future [34].

All in all, diabetes patients have impaired microcirculation, which could be further declined by non-target LDL-C or TG. Although, high TG levels could have more severe effect on the hemorheological status, which could highlight the thoughts, that not well controlled hypertriglyceridemia in diabetes could slowly and silently fail the microcirculation and result in potentially life-threatening complications like peripheral artery disease, amputation, retinopathy, renal failure or dementia.

4.1.1. CCS and target levels

In the CCS subgroup analysis, we found significantly higher PV and better RBC deformability data at the middle and high shear stress range in CCS patients compared to not CCS patients. Higher PV values are presumably associated, just like in the high and very high CV risk population with higher inflammation status. Surprisingly, RBC deformability seems to be better in CCS patients than in not CCS patients. This could originate from the higher number of CCS patients on lipid-lowering medication, mostly statins, compared to not CCS population. As statin lowers plasma LDL-C and TG, it could positively influence the RBC membrane lipid content and oxidative stress. In the last decades, the significance of the lipid content of RBC membranes on deformability was widely investigated. RBC display the unique property of being the cell with the highest cholesterol/phospholipid [C/PL] ratio (around 50 %) [35]. Because of their localization and the blood–brain barrier, only RBC cholesterol can freely exchange with plasma lipoproteins. Itel et al. [36] reported the first experiments showing that the permeability of cell membranes for carbon dioxide appears to be regulated by membrane cholesterol content, so there is an obvious effect on the tissue oxygen delivery. Cooper suggested: RBC membrane is sensitive to the C/PL ratio of the plasma, meaning, that increasing membrane C/PL by the exchange with plasma causes a decrease in membrane fluidity, and these changes are associated with a reduction in membrane permeability, a distortion of cell contour and deformability, as well as a shortening of the survival of RBCs in vivo [37]. Sagawa showed in his studies, that RBC osmotic fragility is highly correlated with the cholesterol content of the cell membrane and C/PL ratio [38]. Tziakas showed for the first time, that RBC membrane cholesterol is significantly higher in patients with acute coronary syndrome [ACS] compared with chronic stable angina patients [39]. Other clinical trials have demonstrated, that cholesterol-lowering therapy markedly reduces cardiovascular events associated with a modest regression of atherosclerotic stenosis [40]. During the cholesterol lowering therapy, angina symptoms are decreased within months of treatment, in which time regression of coronary atherosclerosis is unlikely to occur [40]. These observations may raise the hypothesis that a marked reduction in clinical cardiovascular events by decreasing serum cholesterol with statins is at least in part, due to an improvement in RBC deformability via better membrane fluidity. Our results are in concordance: RBC deformability in hyperlipidemia is at least in part related to plasma lipid levels and hence, it may be ameliorated by cholesterol-lowering therapy and target lipoprotein levels [41]. In our CCS population 58.5 % of the patients were not on target LDL-C level despite of lipid-lowering medication. Based on our data, if LDL-C is on target, PV is lower, but EIs at high and middle shear stresses are impaired (presumably because of the declined membrane fluidity). Higher PV could refer for higher plasma protein levels including acute phase proteins and inflammatory cytokines [42]. In case of target TG in CCS, we could detect significantly lower PV and corrected WBV, as well as significantly lower RBC aggregation values than in CCS patients not on target TG.

Even in CCS patients it is important to decrease not just LDL-C but TG values, because it goes along with better microcirculatory environment, leading to better tissue oxygen and nutritional supply.

As a single-centre study the quality of our results is limited by the relatively low number of participants, but the conclusions are worth for further investigation and confirmation, preferably by multi-centre observations. In conclusion, our study investigated the significance of LDL-C and triglyceride target values on hemorheological factors in high and very high CV risk patients, as well as in a diabetes and CCS subgroup. Our findings highlight the importance of effective treatment of hyperlipidemias regarding both high LDL-C and TG. However, in every subgroup the target lipid values could contribute to a better microcirculation, if triglyceride levels reached target, it resulted in more hemorheological benefit than LDL-C levels. Based on our data we should conclude, that hypertriglyceridemia may not influence CV mortality but definitely deteriorate microcirculation which could lead to long-term, quality-of-life declining complications like retinopathy, renal failure, dementia. Therefore, an effective treatment is univocally necessary not just for high LDL-C, but also for high TG levels, especially in patients with CCS and diabetes.

Data availability statement

The data underlying this article will be shared on reasonable request to the corresponding author, as these are part of an on-going scientific investigation and PhD thesis.

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CRediT authorship contribution statement

Gergely Galos: Conceptualization, Data curation, Investigation, Methodology, Project administration. **Miklos Rabai:** Conceptualization, Methodology. **Reka Szabo:** Data curation. **Rita Szalai:** Data curation. **Kalman Toth:** Conceptualization, Supervision. **Peter Hegyi:** Conceptualization, Supervision. **Barbara Sandor:** Conceptualization, Formal analysis, Investigation, Methodology, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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