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Familial Hypercholesterolemia: Real-World Data of 1236 Patients Attending a Czech Lipid Clinic. A Retrospective Analysis of Experience in More than 50 years. Part I: Genetics and Biochemical Parameters

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Introduction: The cause of familial hypercholesterolemia (FH) is defect in LDL receptor or familial defect of apolipoprotein B-100 (FDB) or, rarely, defect in proprotein convertase subtilisin/kexin type 9. Identification and treatment of patients with FH improves their prognosis. Our data represent retrospective analysis of 50 years of specialised care in our center.

Patients and Methods: A group of 1236 FH patients (841 women, 395 men; 993 study subjects and 243 relatives; mean age 44.8 ± 16.7 years) included 154 FDB patients followed at the Lipid Clinic of the General University Hospital in Prague since the mid-1960s to the present. Clinical diagnosis was based on the Dutch Lipid Clinic Network Criteria. Genetic analysis was performed using PCR-RFLP to detect FDB and apolipoprotein E (APOE) polymorphism. Biochemical data were collected and statistically analysed.

Results: At baseline, mean LDL-C and total cholesterol (TC) levels of all FH patients combined were 6.49 ± 1.92 mmol/L and 8.95 ± 1.95 mmol/L, respectively. Their LDL-C levels decreased to 3.26 ± 1.57 mmol/L and TC levels to 5.43 ± 1.69 mmol/L during follow-up. In the subgroup of LDL receptor-mediated FH (non-FDB) patients, baseline LDL-C and TC levels of 6.61 ± 1.95 mmol/L and 9.09 ± 1.97 mmol/L declined to 3.21 ± 1.60 mmol/L and 5.39 ± 1.72 mmol/L, respectively, during follow-up. In the FDB subgroup of patients, baseline levels of LDL-C and TC were 5.57 ± 1.46 mmol/L and 7.88 ± 1.58 mmol/L decreasing to 3.45 ± 0.24 mmol/L and 5.58 ± 1.37 mmol/L, respectively, during follow-up. Differences were also found in the effects of various APOE isoforms on lipid lowering. A significant decrease in lipid parameters was observed with the E2E2 isoform whereas a minimal decrease was seen with the E4E4 and E3E3 isoforms.

Conclusion: Whereas, overall, non-FDB patients had higher baseline lipid levels, these levels declined more appreciably compared with FDB patients during follow-up. Our retrospective analysis also found different effects of APOE isoforms on the decrease in lipid levels.

Keywords: familial hypercholesterolemia, familial defective apolipoprotein B-100, LDL-C, ApoB, ApoE isoform, Lp(a), statin, ASCVD

INTRODUCTION

Familial hypercholesterolemia (FH) is an autosomal dominant inherited disorder characterised by elevated levels of low-density lipoprotein cholesterol (LDL-C) whose accumulation leads to the development of atherosclerotic cardiovascular disease (ASCVD); moreover, if not treated properly, it may result in premature death (Watts et al., 2016). It is estimated that, while there are 30 million FH patients worldwide, most of them are unaware of their condition (The FH Foundation, 2021). The prevalence of heterozygous FH (HeFH) is 1 per 200 to 250, with their LDL-C levels ranging between 4 and 13 mmol/L (Cuchel et al., 2014; Benn et al., 2016). In homozygous FH patients, the levels of LDL-C are >13 mmol/L and the prevalence of this rare disease is approximately 1 per 160,000 to 300,000 (Cuchel et al., 2014). The diagnosis of FH is established based on the Dutch Lipid Clinic Network Criteria (DLCNC) categorizing patients into definite (>8 points), probable (6–8 points), possible (3–5 points) and unlikely (<3 points) FH groups. The patients are assigned to their respective categories based on each individual´s family history, clinical history, physical examination, levels of LDL-C and, possibly, genetic testing (Benn et al., 2016). The patients are first asked to change their eating habits and increase physical activity. However, lifestyle changes are not always enough and treatment has to be enhanced pharmacologically. Familial hypercholesterolemia patients are most often treated with statins, a class of drugs highly effective in lowering LDL-C levels, especially when combined with ezetimibe (Gagné et al., 2002). A breakthrough in the treatment of FH came with the discovery of PCSK9 inhibitors shown to decrease LDL-C by ≥50% (Watts et al., 2020).

Variants of three genes are a major cause of FH. The most common of these mutations occur in the LDL receptor (*LDLR*) gene and can lead to ligand-binding dysfunction, impaired LDL transport or internalization, recycling, or complete receptor deficiency (Soutar and Naoumova, 2007; Cuchel et al., 2014). Likewise, FH can be caused by mutations in the apolipoprotein B (*APOB*) and the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) (Vrablik et al., 2020). To the best of our knowledge, no mutation in the *PCSK9* gene in the Czech population has been reported to date. An important role is also played by the apolipoprotein E (*APOE*) gene, which affects the levels of LDL-C thus contributing to higher LDL-C levels in FH patients (Pirillo et al., 2017; Rashidi et al., 2017; Khalil et al., 2021).

A mutation in the *APOB* gene causes familial defective apolipoprotein B-100 (FDB), an autosomal dominant disease of lipid metabolism similar to LDL receptor-mediated FH (non-FDB) characterised by elevated plasma LDL-C levels (Vega and Grundy, 1986; Innerarity et al., 1987). The prevalence of FDB varies largely being, e.g., approximately 1 per 209 in Switzerland while the figure for Denmark is 1 per 883 (Miserez et al., 1994; Miserez and Muller, 2000; Benn et al., 2016). Familial defective apolipoprotein B-100 is caused by monogenic variants in the *APOB* gene where a single amino acid, arginine, at position 3527 is replaced, most frequently, by glutamine (p.R3527Q) and, rarely, by tryptophan (p.R3527W) or lysine (p.R3527L) or at position 3558 where arginine is replaced by cysteine (p. R3558C). This replacement leads to other protein conformations disrupting the binding of apolipoprotein B-100 (as a part of LDL particles) to LDLR (Brown and Goldstein, 1986; Whitfield et al., 2004).

The most common APOE isoform is E3E3 with the p.C112 and p.R158 variants (Ferrières et al., 1994; Eichner et al., 2002; Phillips, 2014). A less frequent isoform increasing LDL-C levels and contributing to the risk of developing Alzheimer's disease is E4E4, i.e., the p.C112R and p.R158 variants (Huebbe and Rimbach, 2017; Muñoz et al., 2019). A rare isoform is E2E2 determined by the p.C112 and p.R158C variants.

Familial defective apolipoprotein B-100 is clinically almost indistinguishable from FH; it is easier to identify FDB genetically as a common monogenic variant R3527Q. Unlike FDB, FH can be caused by monogenic variants as well as polygenic forms encountered in approximately 20% of FH patients (Trinder et al., 2019). Although many studies have focused on numerous aspects of FH, data in the relevant literature about the individual FH subgroups and the differences between them are relatively scarce. Still, it is most likely that the clinical features, effect of treatment and inherent risks of the disease are significantly different between the non-FDB and FDB subgroups of patients.

The aim of this retrospective analysis was to analyse data of a large homogeneous group of patients diagnosed to have FH and followed in a single lipid center and, also, to show the benefits of therapy and the results obtained over the course of half of a century in specialised care. This large group was followed and processed in 2 different perspectives. This article (Part I) is focused on differences in the lipid profiles in subgroups of FH patients, i.e. FDB versus non-FDB patients, and in FH patients with different *APOE* genotypes. Concurrent article (Part II) by Altschmiedova et al. (2022) is focused on clinical symptomatology, i.e., on differences between the parameters in patients whose FH is already complicated by overt ASCVD and those without ASCVD in order to identify factors contributing to a complicated course of the disease.

PATIENTS

Characteristics of Individuals Diagnosed With FH

A total of 1236 FH patients (841 women and 395 men; 993 study subjects, 243 relatives; mean age 44.8 ± 16.7 years) attending the Lipid Clinic of the General University Hospital in Prague, Czech Republic, were followed. The diagnosis of FH in our patients was based on the Dutch Lipid Clinic Network Criteria (DLCNC). Genetic analysis including FDB and APOE isoforms was performed in more than 76% of FH patients; however, a mutation in the *LDLR* gene was investigated in only ≥10% of these patients (**Supplementary Figure S1**). Risk factors and clinical complications are summarised in **Table 1** and they are in more detail described in the article about clinical symptomatology by Altschmiedova et al. (2022) Enrolled in the retrospective analysis were patients, both pharmacotherapy-naïve and those treated pharmacologically, and their relatives.

TABLE 1 | Clinical characteristics.

Risk factors, clinical complications	Percentage (%)
DM	6.47
Hypertension	26.70
Smokers	31.39
Arcus lipoides corneae	3.80
Xanthalesma	4.61
Tendon xanthomas	3.32
CAD (MI included)	9.63
Stroke	2.51
PAD	2.59
Death	2.83

DM, diabetes mellitus; CAD, coronary artery disease; MI, myocardial infarction; PAD, peripheral arterial disease.

The first patients of this retrospective analysis have been followed since the mid-1960s when diagnosed with FH based on their clinical symptoms; complete biochemical and genetic data have been available here since 1974. The follow-up has continued to date with the latest biochemical values recorded in late 2020.

MATERIALS AND METHODS

Biochemical Analysis

Blood samples were collected from study subjects. The serum levels of total cholesterol (TC), HDL-cholesterol (HDL-C) and triglycerides (TG) were measured enzymatically on automated analysers (Modular P800, Roche, Basel, Switzerland and UniCel DxC 880i Beckman Coulter, Brea, CA, United States). LDL-C was calculated using the Friedewald formula whereas apolipoprotein B (APOB) and lipoprotein (a) [Lp(a)] were measured by nephelometry and immunonephelometry.

Genetic Analysis

Genomic DNA was isolated from peripheral blood collected into EDTA-anticoagulated tubes using the salting out method proposed by Miller et al., 1998. The concentration and purity of DNA were determined using a spectrophotometer (A260/A280; BioPhotometer Eppendorf 6131, Eppendorf, Germany).

The p.R3527Q (*MluI*) and p.R3558C (*MspI*) variants in the *APOB* gene were detected by polymerase chain reaction (PCR). Oligonucleotides 5′-CTT ACT TGA ATT CCA AGA GCA CCC-3′ and 5′-TGT ACT CCC AGA GGG AAT ATA CGC-3′ were used as the primer set. *MluI* and *MspI* as restriction enzymes were used in PCR-restriction fragment length polymorphism (PCR-RFLP) analysis. Fragments were subsequently separated and visualised by electrophoresis on GelRed-stained 4% agarose gel (MetaPhor-agarose: agarose = 3:1).

The *E2*, *E3*, *E4* variants in the *APOE* gene were detected by PCR. Here, oligonucleotides 5′-TCC AAG GAG CTG CAG GCG GCG CA-3′ and 5′-ACA GAA TTC GCC CCG GCC TGG TAC ACT GCC A-3′ were used as the primer set. *CfoI* as a restriction enzyme was used in PCR-RFLP analysis. Fragments were subsequently separated and visualised by electrophoresis on GelRed-stained 10% polyacrylamide gel.

Variants in the *LDLR* gene were analysed by Sanger sequencing. The specific primers (**Supplementary Material S1**) were designed according to the sequence of 18 *LDLR* exons. The sequencing reaction was performed using the BigDye Terminator v3.1 Cycle Sequencing Kit.

Statistical Analysis of Baseline and Follow-Up Data

Statistical analysis was performed using STATISTICA 13 software (TIBCO Software Inc., Palo Alto, CA, United States). Values in the text, tables and figures are means ± standard deviation (SD). The level of statistical significance was set at 5%. Comparison of baseline vs. follow-up was done using the paired *t*-test. The lipid parameters of the two subgroups were compared using the two-sample *t*-test or, when comparing three groups, using analysis of variance (ANOVA).

RESULTS

An appreciable decrease in LDL-C levels, from 6.49 ± 1.92 mmol/L to 3.26 ± 1.57 mmol/L (~49.8%) was observed (**Figure 1**) in all patients. Likewise, a 39.3% decrease, from 8.95 ± 1.95 mmol/L to 5.43 ± 1.69 mmol/L, in TC levels was seen (**Supplementary Figure S2**). The mean levels of other 2 parameters (TG and HDL-C) are clearly shown in **Table 2**. Major reductions were noted in APOB (−38.1%) and TG (−23.8%) levels (**Supplementary Figure S3, S4**). The decrease in HDL-C levels was only a small one (−6.6%) (**Supplementary Figure S5**). The differences between the baseline and follow-up levels of the parameters investigated were statistically significant ($p < 0.001$) except for Lp(a) whose levels remained almost unaltered throughout the retrospective analysis (**Supplementary Figure S6**).

Based on the division of our patients into subgroups according to the use (Y) or non-use (N) of therapy, statistically significant differences ($p < 0.005$) were found between the subgroups in the baseline levels of LDL-C, TC, APOB, TG, and Lp(a). However, statistically significant differences ($p < 0.001$) between the subgroups were found during follow-up in their LDL-C, TC, APOB, and HDL-C levels. When comparing the on-therapy levels of these parameters with baseline, the biggest decreases—except for Lp(a)—were noted in the groups not initiating their therapy until the start of the analysis (N/Y). In this particular group, LDL-C, TC and APOB levels dropped by as much as 55.7, 44.8 and 45.4%, respectively (**Table 3**). A smaller decline (−26.4%) in the N/Y group occurred in TG levels. Decreases in the levels of lipid parameters were likewise observed in the group of patients on pre-existing therapy (Y/Y) whose LDL-C, TC and APOB levels decreased by 49.6, 38.2 and 37.0%, respectively. The Y/Y group showed a smaller decrease in TG levels (−23.4%). As to HDL-C levels, similar to all study parameters except for Lp(a), there was an obvious decrease in the group of patients not receiving therapy throughout the analysis (N/N). The decreases in the levels of LDL-C, TC, APOB, TG

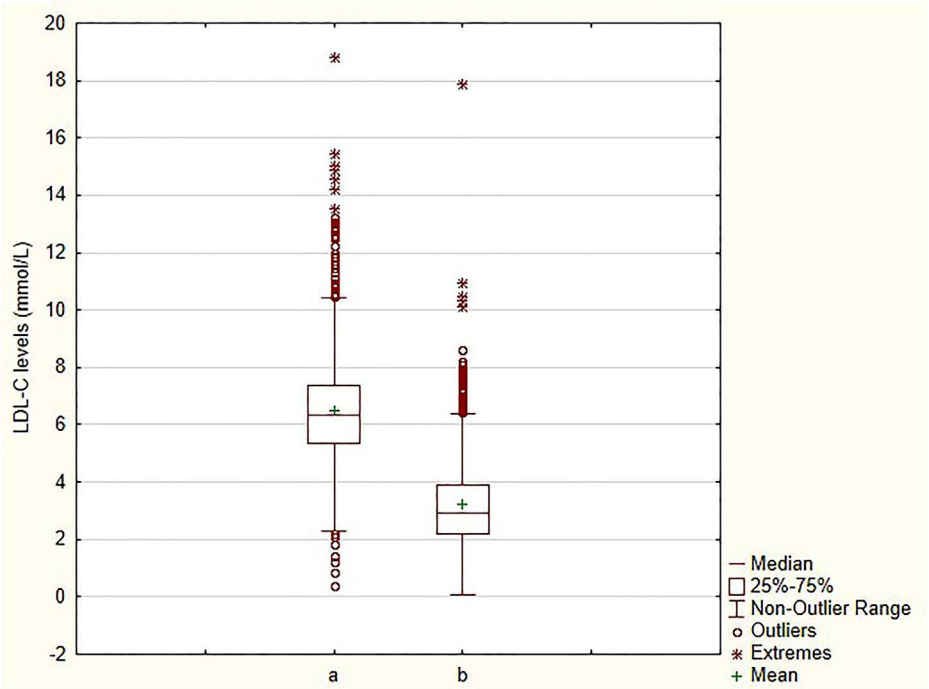

FIGURE 1 | Comparison of LDL-C levels at baseline and at follow-up a—baseline; b—follow-up; LDL-C—low-density lipoprotein cholesterol.

TABLE 2 | Baseline and follow-up lipid levels of FH cohort.

Parameter	Number of patients	Baseline	Follow-up	Difference (%)	*p*-value
		Mean ± SD	Mean ± SD		
LDL-C (mmol/L)	1,049	6.49 ± 1.92	3.26 ± 1.57	–49.8	$p < 0.001$
TC (mmol/L)	1,118	8.95 ± 1.95	5.43 ± 1.69	–39.3	$p < 0.001$
APOB (g/L)	184	1.76 ± 0.56	1.09 ± 0.56	–38.1	$p < 0.001$
TG (mmol/L)	1,108	1.81 ± 1.13	1.38 ± 0.78	–23.8	$p < 0.001$
HDL-C (mmol/L)	1,092	1.67 ± 0.46	1.56 ± 0.46	–6.6	$p < 0.001$
Lp(a) (g/L)	284	0.56 ± 0.74	0.59 ± 0.74	5.4	$p = 0.2706$

SD, standard deviation.

and HDL-C were significant in all groups of patients (categorised by their therapy) throughout the analysis ($p < 0.05$).

Another division of our population of FH patients was based on genetic analysis of *APOE* polymorphisms by individual APOE isoforms, where differences in the levels of individual lipid parameters throughout the analysis were compared (**Table 4**). The biggest decreases in the levels of lipid parameters were seen in patients with the E2E2 isoform, being 75.3, 59.0, 53.5, 56.2, 17.6, and 36.7% in LDL-C, TC, APOB, TG, HDL-C and Lp(a) levels, respectively. In patients with the other isoforms, the decreases in LDL-C, TC, APOB and TG levels were within the ranges of 49.0–57.8%, 36.8–43.9%, 32.3–57.3%, and 22.4–39.4%, respectively, while HDL-C levels remained almost unchanged. A decline in Lp(a) levels was only seen in patients with the E2E2 isoform. The decrease was significant ($p < 0.001$) in only TG levels during follow-up.

A total of 1008 FH patients were genetically tested. Familial defective apolipoprotein B-100 was detected in 154 patients (mean age 40.8 ± 18.1 years; 107 women and 47 men; 117 study subjects and 37 relatives). One of these patients was diagnosed as FDB homozygote. The rest of genetically tested FH patients group consisted of 854 patients (557 women and 277 men; 686 study subjects and 168 relatives) supposed to have mutation in *LDLR* gene (non-FDB) (mean age 44.8 ± 16.0 years). Five patients were diagnosed with homozygous FH due to a mutation in the *LDLR* gene. Overall, another 228 study subjects met the DLCN criteria for FH but genetic testing was not performed and, thus, these subjects were excluded from further analysis.

One of the main subgroups within our study participants was that of non-FDB patients where significant decreases in the levels of LDL-C were noted in 754 patients (–51.1%); TC, in 795

Table 3 | Distribution of FH patients by treatment and effect of treatment on lipid levels.

Parameter	Group	Baseline			Follow-up					
		N	Mean ± SD	*p*	N	Mean ± SD	*p*	N	Diference (%)	*p*
LDL-C (mmol/L)	Y/Y	167	5.76 ± 1.93	$p < 0.001$	166	2.89 ± 1.13	$p < 0.001$	160	−49.6	$p < 0.001$
	N/Y	678	6.83 ± 1.80		678	3.01 ± 1.37		660	−55.7	
	N/N	172	6.35 ± 2.07		97	5.48 ± 2.02		93	−10.7	
TC (mmol/L)	Y/Y	175	8.15 ± 1.98	$p < 0.001$	169	5.03 ± 1.31	$p < 0.001$	169	−38.2	$p < 0.001$
	N/Y	699	9.32 ± 1.83		689	5.16 ± 1.49		689	−44.8	
	N/N	177	8.72 ± 2.07		102	7.84 ± 1.90		102	−8.8	
APOB (g/L)	Y/Y	97	1.57 ± 0.51	$p < 0.001$	65	0.98 ± 0.34	$p < 0.001$	43	−37.0	$p < 0.001$
	N/Y	316	1.86 ± 0.51		190	0.99 ± 0.39		86	−45.4	
	N/N	89	1.85 ± 0.65		40	1.69 ± 0.79		14	−7.9	
TG (mmol/L)	Y/Y	174	1.86 ± 1.17	$p = 0.003$	169	1.41 ± 0.69	$p = 0.792$	168	−23.4	$p = 0.026$
	N/Y	690	1.85 ± 1.17		688	1.37 ± 0.80		680	−26.4	
	N/N	177	1.54 ± 0.84		102	1.38 ± 0.84		102	−11.8	
HDL-C (mmol/L)	Y/Y	174	1.63 ± 0.39	$p = 0.536$	169	1.51 ± 0.40	$p < 0.001$	168	−6.9	$p < 0.001$
	N/Y	687	1.67 ± 0.45		682	1.53 ± 0.44		671	−8.2	
	N/N	175	1.67 ± 0.50		102	1.77 ± 0.54		101	1.9	
Lp(a) (g/L)	Y/Y	154	0.61 ± 0.66	$p = 0.003$	47	0.74 ± 0.67	$p = 0.229$	47	7.7	$p = 0.921$
	N/Y	553	0.44 ± 0.60		181	0.57 ± 0.80		180	4.8	
	N/N	113	0.39 ± 0.62		14	0.38 ± 0.39		13	12.1	

Y/Y, on treatment at baseline and throughout the analysis; N/Y, no treatment at baseline/on treatment throughout the analysis; N/N, no treatment at baseline and throughout the analysis; N, number of patients; SD, standard deviation; p, p*-value.*

patients (−40.7%); APOB, in 132 patients (−40.7%) and TG, in 788 patients (−24.4%). In 781 non-FDB patients, HDL-C levels declined by a mere 7.6% whereas Lp(a) levels remained almost unaltered. The second subgroup consisted of 154 patients with FDB. Their mean values of lipid parameters, both baseline and follow-up, are given in more detail in **Table 5**. Among the FDB patients, appreciable decreases in LDL-C were seen in 120 patients (−37.7%), TC in 131 patients (−30.3%), APOB in 26 patients (−29.2%), and TG in 130 patients (−24.0%). In 127 patients, HDL-C levels decreased by 5.9% during follow-up.

A comparison of non-FDB and FDB patients revealed significant differences ($p < 0.001$) in their baseline levels of LDL-C, TC, APOB and TG whereas the difference versus follow-up levels was significant ($p < 0.001$) only in TG levels. Statistically significant differences ($p < 0.05$) were found between the baseline and follow-up levels of LDL-C, TC, APOB and Lp(a) in both, non-FDB and FDB, subgroups of patients.

At the end of the day, we would like to present the least favourable mean levels of Mr. and Mrs. FH and FDB in our analysis.

- Those of Mr. and Mrs. FH in our group are as follows: LDL-C 6.61 mmol/L, TC 9.09 mmol/L, APOB 1.83 g/L, TG 1.86 mmol/L, HDL-C 1.68 mmol/L and Lp(a) 0.46 g/L and
- Those of Mr. and Mrs. FDB in our group are as follows: LDL-C 5.57 mmol/L, TC 7.88 mmol/L, APOB 1.53 g/L, TG 1.40 mmol/L, HDL-C 1.68 mmol/L and Lp(a) 0.40 g/L.

DISCUSSION

What makes our retrospective analysis actually important is that our data were collected from a large group of more than 1,000 patients with FH attending a single lipid clinic. A positive finding of the long-term follow-up of patients in our center were decreases in the levels of LDL-C by more than 50%, which were not only statistically significant, but, also, clinically beneficial. Of no less importance was the decrease (by as much as 38%) in LDL-C levels in our FDB patients. As suggested by earlier reports, the levels of lipid parameters in FBD patients are generally lower than in those with LDL receptor-mediated FH (Gaffney et al., 2002; Vohnout et al., 2003; Fouchier et al., 2004). Similarly, the disorder diagnosed in our FDB homozygous patient was not as severe as that seen in homozygous individuals with receptor-mediated disorder. This may be explained by the APOE-regulated clearance of very low-density lipoprotein (VLDL) and intermediate-density lipoprotein (IDL) particles in FDB patients and the interaction between APOB and LDLR, important for the conversion of IDL to LDL-C (Gaffney et al., 2002; Vohnout et al., 2003).

Our analysis is a retrospective one whose first participants were receiving specialised care in a Prague-based clinic headed by Josef Šobra, with their data collection starting as early as 1960s (Šobra, 1970). Long-term care of these patients succeeded in reducing their cardiovascular risk and the clinic continues to provide individual care to each FH patient. While some of the patients have been taken care of for over 30 years, others have been attending the facility for less than 2 years; nonetheless, their personalised treatment plans have been shown to be beneficial in the long term. This explains the absence of statistically analysed data from the above period. This is partly due to the different numbers of patients and amount of analysed data in the individual subgroups of patients, with some of them referred to other physicians using different procedures, approaches and requirements for lipid parameter determination. However, the differences in the amount of data analysed and presented here are

TABLE 4 | Patients with specific APOE isoforms and effect of treatment on lipid levels.

Parameter	Group	Baseline			Follow-up					
		N	Mean ± SD	*p*	N	Mean ± SD	*p*	N	Diference (%)	*p*
LDL-C (mmol/L)	E3E4	219	6.40 ± 1.80	$p = 0.753$	212	3.09 ± 1.36	$p = 0.034$	202	−50.9	$p = 0.615$
	E2E3	63	6.58 ± 2.22		59	3.27 ± 1.45		58	−50.3	
	E3E3	651	6.49 ± 1.92		603	3.32 ± 1.65		588	−49.0	
	E2E4	9	6.03 ± 2.36		11	2.45 ± 0.77		9	−57.8	
	E4E4	22	5.94 ± 1.97		21	3.02 ± 1.26		21	−49.0	
	E2E2	4	6.51 ± 1.30		6	1.77 ± 0.50		4	−75.3	
TC (mmol/L)	E3E4	230	8.90 ± 1.86	$p = 0.644$	217	5.29 ± 1.46	$p = 0.126$	217	−40.5	$p = 0.202$
	E2E3	65	8.93 ± 2.22		61	5.42 ± 1.62		61	−39.6	
	E3E3	668	8.94 ± 1.97		619	5.48 ± 1.78		619	−38.9	
	E2E4	11	8.29 ± 2.07		11	4.65 ± 0.84		11	−43.9	
	E4E4	22	8.53 ± 1.98		21	5.38 ± 1.47		21	−36.8	
	E2E2	6	9.82 ± 1.98		6	4.02 ± 0.60		6	−59.0	
APOB (g/L)	E3E4	116	1.79 ± 0.53	$p = 0.129$	84	1.09 ± 0.46	$p = 0.361$	47	−37.7	$p = 0.933$
	E2E3	35	1.67 ± 0.62		24	1.13 ± 0.40		14	−32.3	
	E3E3	348	1.80 ± 0.54		182	1.08 ± 0.54		97	−39.8	
	E2E4	6	1.76 ± 0.68		3	0.81 ± 0.10		2	−45.5	
	E4E4	11	1.60 ± 0.34		7	0.78 ± 0.28		3	−57.3	
	E2E2	5	1.22 ± 0.24		2	0.60 ± 0.11		1	−53.5	
TG (mmol/L)	E3E4	228	1.86 ± 1.28	$p < 0.001$	217	1.44 ± 0.77	$p = 0.485$	215	−24.1	$p < 0.001$
	E2E3	65	1.77 ± 1.10		61	1.34 ± 0.54		61	−24.4	
	E3E3	662	1.73 ± 1.08		619	1.34 ± 0.83		613	−23.6	
	E2E4	11	2.30 ± 1.68		11	1.39 ± 0.76		11	−39.4	
	E4E4	22	1.76 ± 0.84		21	1.38 ± 0.72		21	−22.4	
	E2E2	6	4.20 ± 2.50		6	1.84 ± 0.53		6	−56.2	
HDL-C (mmol/L)	E3E4	225	1.71 ± 0.47	$p = 0.141$	214	1.58 ± 0.45	$p = 0.391$	209	−8.3	$p = 0.443$
	E2E3	65	1.59 ± 0.51		60	1.53 ± 0.50		60	−5.1	
	E3E3	662	1.67 ± 0.45		616	1.56 ± 0.47		610	−7.1	
	E2E4	11	1.52 ± 0.53		11	1.57 ± 0.45		11	3.3	
	E4E4	22	1.85 ± 0.49		21	1.78 ± 0.55		21	−4.5	
	E2E2	6	1.73 ± 0.41		6	1.43 ± 0.26		6	−17.6	
Lp(a) (g/L)	E3E4	199	0.52 ± 0.66	$p = 0.219$	61	0.66 ± 0.73	$p = 0.361$	60	−3.8	$p = 0.692$
	E2E3	51	0.32 ± 0.45		12	0.20 ± 0.18		12	10.0	
	E3E3	569	0.44 ± 0.56		173	0.53 ± 0.63		173	13.2	
	E2E4	9	0.42 ± 0.46		4	0.59 ± 0.95		4	51.3	
	E4E4	19	0.48 ± 0.62		5	0.94 ± 0.89		5	3.5	
	E2E2	6	0.16 ± 0.10		1	0.19 ± 0.00		1	−36.7	

N, number of patients; SD, standard deviation; p, p-*value.*

TABLE 5 | FDB and non-FDB patients and effect of treatment on lipid levels.

Parameter	FDB	Baseline			Follow-up					
		N	Mean ± SD	*p*	N	Mean ± SD	*p*	N	Diference (%)	*p*
LDL-C (mmol/L)	+	148	5.57 ± 1.46	$p < 0.001$	124	3.45 ± 0.24	$p = 0.117$	120	−37.7	$p < 0.001$
	−	812	6.61 ± 1.95		779	3.21 ± 1.60		754	−51.1	
TC (mmol/L)	+	153	7.88 ± 1.58	$p < 0.001$	131	5.58 ± 1.37	$p = 0.252$	131	−30.3	$p < 0.001$
	−	840	9.09 ± 1.97		795	5.39 ± 1.72		795	−40.7	
APOB (g/L)	+	85	1.53 ± 0.37	$p < 0.001$	43	1.13 ± 0.38	$p = 0.448$	26	−29.2	$p = 0.023$
	−	430	1.83 ± 0.56		255	1.07 ± 0.51		132	−40.7	
TG (mmol/L)	+	152	1.40 ± 0.98	$p < 0.001$	131	1.07 ± 0.51	$p < 0.001$	130	−24.0	$p = 0.251$
	−	833	1.86 ± 1.17		795	1.42 ± 0.83		788	−24.4	
HDL-C (mmol/L)	+	151	1.68 ± 0.47	$p = 0.960$	129	1.60 ± 0.46	$p = 0.316$	127	−5.9	$p = 0.455$
	−	831	1.68 ± 0.46		790	1.55 ± 0.47		781	−7.6	
Lp(a) (g/L)	+	133	0.40 ± 0.45	$p = 0.229$	33	0.65 ± 0.62	$p = 0.350$	33	61.2	$p < 0.001$
	−	715	0.46 ± 0.60		217	0.54 ± 0.66		216	1.0	

+, FDB; –, non-FDB; N – number of patients; SD, standard deviation; p, p-*value.*

mainly due to FH patients referred from general practitioners to specialised centers; the result is some patients had incomplete baseline data while baseline blood sampling had not been performed in others. Another reason for the incompleteness data of some patients is only one value of some of the lipid parameters was obtained before the patient decided to discontinue follow-up.

A limitation of our analysis is the composition of our entire FH group consisting predominantly of patients attending a lipid clinic with only a small proportion being their family members. While not usual in other countries (Bhatnagar et al., 2000; Jarauta et al., 2016), a small number of relatives is a typical feature in the Czech Republic.

As expected, the differences in the decreases in lipid parameters between the untreated versus treated groups seen during the analysis in our lipid clinic were statistically significant. Nonetheless, the group of patients receiving treatment from practitioners prior to initiation of therapy by a lipid specialist also showed an appreciable decrease in their lipid levels, similar to that seen in patients not starting therapy before admission to our center. The implication is that targeted and proper management of FH patients is of crucial importance and, despite the undeniable role of general practitioners, tailored and specific care provided in lipid clinics is more effective and beneficial.

While it is difficult to identify a specific therapeutic strategy for over more than 50 years, generally, the treatment copied the availability and development of pharmacotherapy. It can be clearly stated that, until 1990, the mainstay of therapy of FH were cholestyramine and colestipol. Since 1990, treatment of FH has been based on statins (always the most efficient statin available, i.e., lovastatin, simvastatin, atorvastatin and rosuvastatin). A combination with ezetimibe has been used since the beginning of the 21st century and the monoclonal antibodies evolocumab and alirocumab have been available since 2018.

Patients with the rare *APOE E2E2* genotype showed an obviously major drop in the levels of LDL-C, TC and TG corresponding the metabolic processing of the E2E2 isoform, where particle clearance does not occur through binding to LDLR but through the LDL-related receptor and heparin sulfate proteoglycans (Phillips, 2014). A similar major decrease was observed in patients with the E2E4 isoform, processed partly in the same way as the above E2E2 isoform. In FH patients, the decreases seen with the E3 and E4 isoforms were smaller, a fact possibly attributable to the clearance of APOE *via* LDLR where binding may be impaired due to the high frequency of *LDLR* gene mutations in FH patients.

Another parameter assessed in our study were Lp(a) levels not showing significant changes in some of our study subgroups. This may be partly explained by the fact that analysis of Lp(a) levels was undertaken in a period when no therapy to modify Lp(a) levels was available yet.

The relationship between high Lp(a) levels and proprotein convertase subtilisin/kexin type 9 (PCSK9) was not investigated until 2018, when Sun et al. reported their data obtained from patients with heterozygous FH; it has been shown only recently that Lp(a) levels can be decreased with the use of PCSK9 inhibitors (Sun et al., 2018). PCSK9 inhibitors were approved for clinical use in the Czech Republic in 2018; hence, the introduction of PCSK9 inhibitors is not significantly reflected in our analysis.

Limitation of Retrospective Analysis

Despite their long-term follow-up, a small group of patients has not had genetic testing, with their diagnosis established solely using the DLCNC. As a result, some of these patients could not be conclusively identified as actually being or not being FDB patients. The relatively small number of mutations detected in the *LDLR* gene is due to the fact that the sequencing technique developed by Sanger was adopted by our lipid clinic only recently. Besides, the technique is also more time-consuming than those of PCR restriction fragment length polymorphism (RFLP) or real-time PCR detecting point mutations. The analysed *LDLR* gene region contains 18 exons which have to be sequenced separately when using Sanger's technique. The proportion of *LDLR* gene mutation analyses is likely to increase in our clinic with the introduction of new generation sequencing (NGS) techniques in the years to come.

Our retrospective analysis provides initial data obtained from a large group of patients attending a single lipid clinic and analysed in terms of the biochemical and genetic characteristics.

CONCLUSION

Using a large group of patients with familial hypercholesterolemia, the present analysis reports data related to lipid and lipoprotein metabolism. The project was designed to assess changes in the levels of these parameters between baseline and follow-up in patients receiving personalised care admitted to our clinic. Our experience gained within the international ScreenPro FH project shows that patient surveillance and long-term follow-up are most beneficial as documented by Ceska et al., 2019. As an extension to the outcome of the present retrospective analysis, clinical data of our FH cohort are reported in Part II by Altschmiedova et al. (2022)

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusion of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Ethics Committee of General University Hospital, Prague. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

VT processed the data, wrote the article and participated in the interpretation of the analyzed data. TA contributed to the data

processing and TA and MV contributed to the writing and interpretation of the data. RC designed the project, interpreted the data and participated in the writing of the article.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.849008/full#supplementary-material

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Familial Hypercholesterolemia: Real-World Data of 1236 Patients Attending a Czech Lipid Clinic. A Retrospective Analysis of Experience in More than 50 years. Part II. Clinical Characteristics

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Introduction: Patients with familial hypercholesterolemia (FH) are at increased risk of premature atherosclerotic cardiovascular disease (ASCVD).

Aim of study: To perform a retrospective analysis of data to assess the effects of individual lipoproteins and other risk factors (RFs) on the development of ASCVD and to compare these parameters in individuals with versus without ASCVD.

Patients and methods: Our study group included a total of 1,236 patients with FH (395 men and 841 women with a mean age of 44.8 ± 16.7 years) attending a single lipid clinic. The diagnosis of FH was established using the Dutch Lipid Clinic Network score (DLCN). Among the 1236 FH patients, 1,008 of them [854 suspected with LDL receptor-mediated FH and 154 with familial defective apolipoprotein B-100 (FDB)] were genetically analysed. Their RFs were assessed based on the patients' clinical characteristics.

Results: While patients with ASCVD had higher baseline LDL-C, TC, TG and Lp(a) compared with patients without this diagnosis, this ratio was just the opposite by the follow-up. The highest statistically significant differences were seen in the baseline levels of Lp(a) and, quite surprisingly, TG. Except for Lp(a), the levels of all lipid parameters declined significantly over time. While the incidence of diabetes and arterial hypertension was not higher in our group compared with the general population, these patients were at a more significant risk of ASCVD.

Conclusion: Familial hypercholesterolemia is a major RF for the development of ASCVD. While our analysis confirmed the important role of LDL-C, it also corroborated a strong correlation between ASCVD and other lipid parameters, and Lp(a) and TG in particular. Familial hypercholesterolemia is not the only RF and, to reduce cardiovascular risk of their patients, physicians have to search for other potential RFs. Patients diagnosed to have FH benefit from attending a specialized lipid clinic perse.

Keywords: familial hypercholesterolemia, LDL-cholesterol, Lp(a), ASCVD, RWD

INTRODUCTION

With an estimated prevalence of 1 to 200–250, familial hypercholesterolemia (FH) ranks among the most frequent inherited metabolic diseases (Nordestgaard et al., 2013; Beheshti et al., 2020). The typical FH patient is predestined to have high LDL cholesterol (LDL-C) levels since childhood considerably raising the risk of premature atherosclerotic cardiovascular disease (ASCVD) (The Lipid Research Clinic, 1984; Watts et al., 2016; Ference et al., 2017). All patients diagnosed with FH are automatically at least at high risk of developing ASCVD (Visseren et al., 2021). However, we suppose there are differences between individual patients which will decide whether or not ASCVD will eventually develop. All FH patients require, in particular, an early diagnosis and initiation of lipid-lowering therapy as soon as possible. The class of drugs of choice are statins which, by effectively lowering LDL-C levels, significantly reduce cardiovascular morbidity and mortality (Versmissen et al., 2008). To achieve the target levels of LDL-C, combination lipid-lowering therapy is quite often necessary; most often a combination of a statin with ezetimibe or, alternatively, with a PCSK9 inhibitor, is used (Visseren et al., 2021).

AIM OF STUDY

One of the goals of our project was to present the baseline and follow-up clinical and biochemical findings in a large cohort of patients diagnosed to have FH and attending a single lipid center to show that patients do benefit from mere surveillance and highly specialized therapy. In addition to assessing the effects of therapy on pre-defined lipid parameters, we evaluated the effects of individual lipoproteins and other major risk factors on the development of complications associated with the atherosclerotic process. In particular, we focused our attention on differences between the parameters in patients whose FH is already complicated by overt ASCVD and those without ASCVD in order to identify factors contributing to a complicated course of the disease.

PATIENT CHARACTERISTICS AND METHODS

The submitted project is a retrospective analysis of data of a total of 1,236 patients (841 women and 395 men with a mean age of 44.8 ± 16.7 years) with FH on follow-up in a single lipid center.

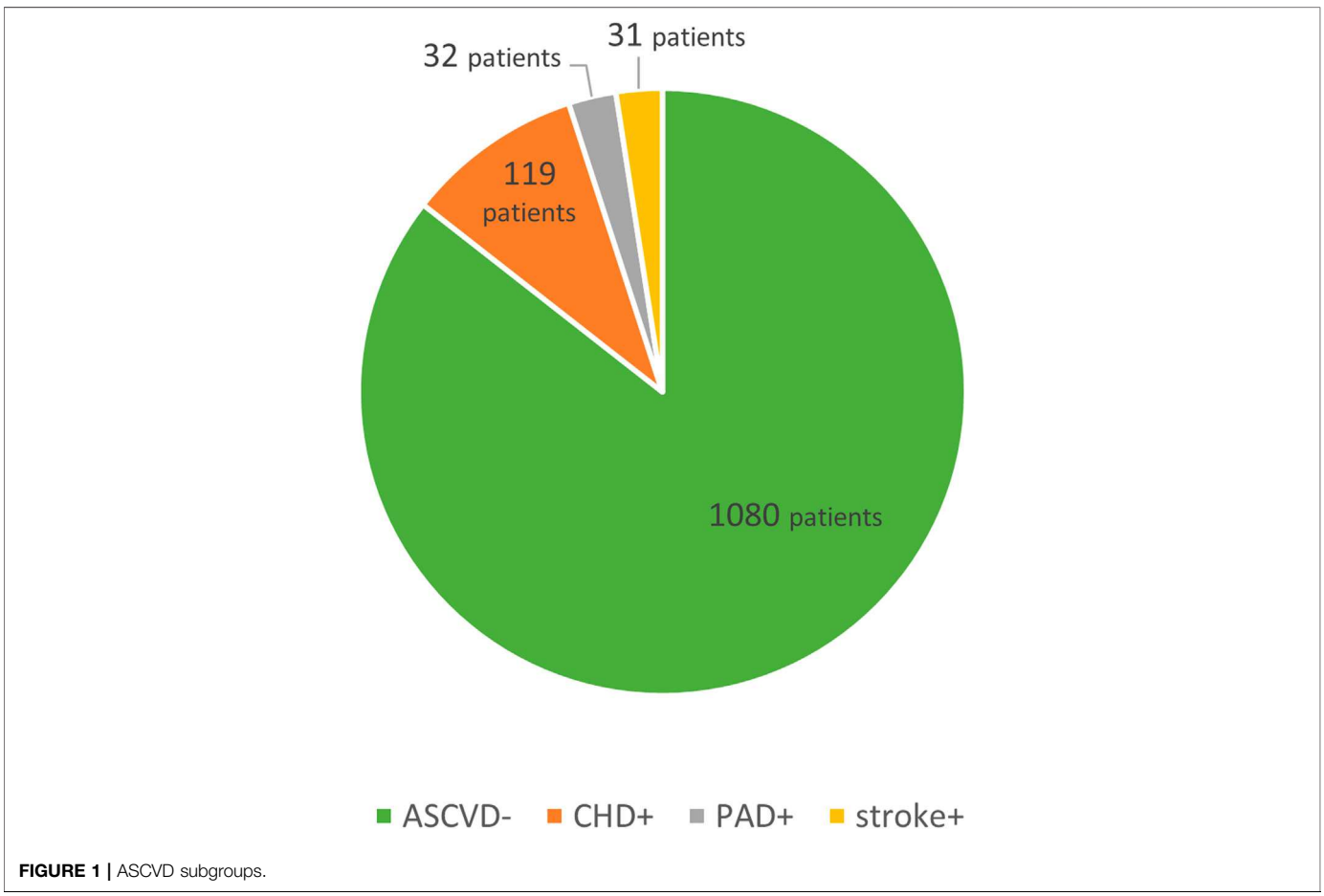

FIGURE 1 | ASCVD subgroups.

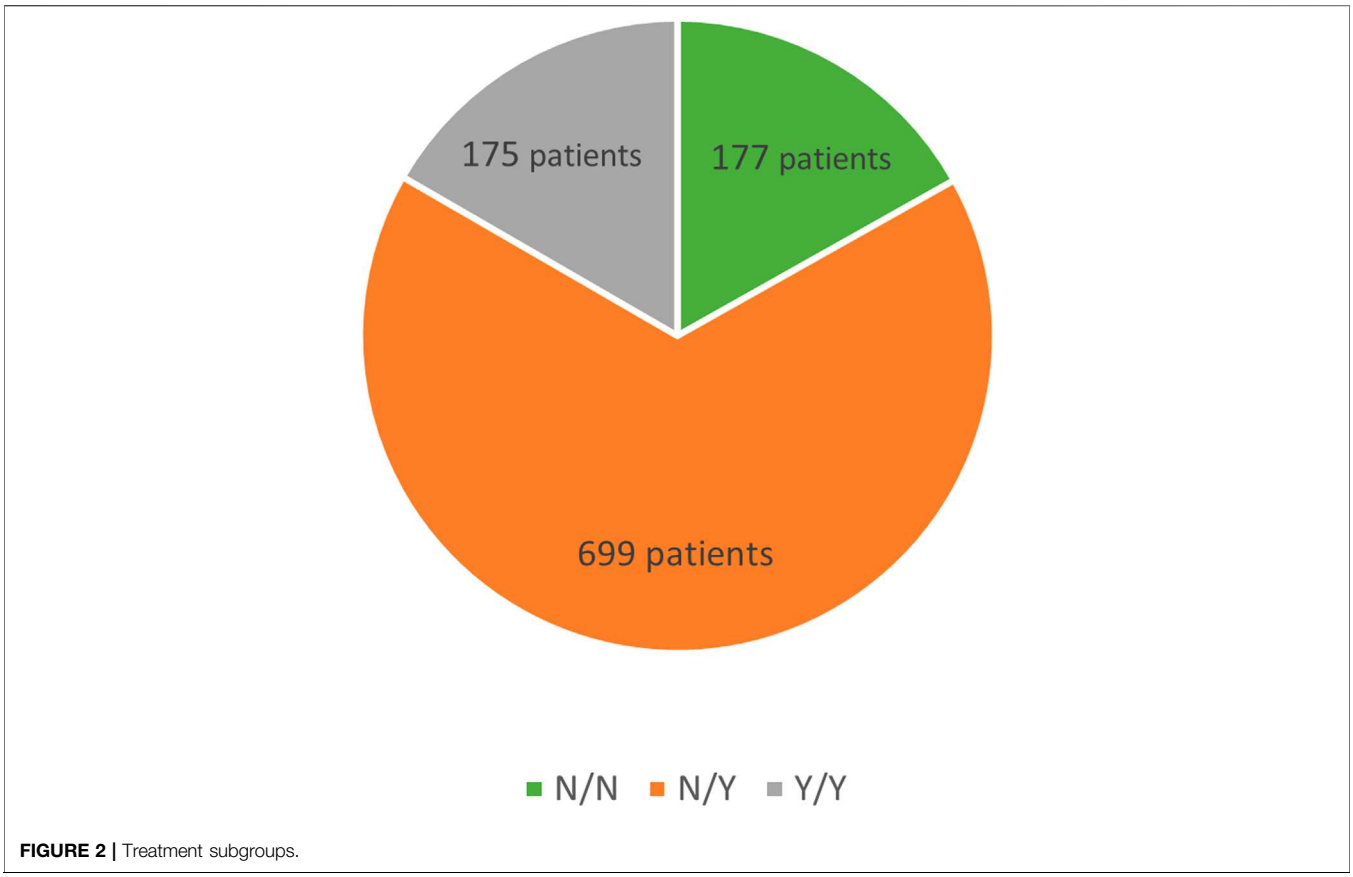

FIGURE 2 | Treatment subgroups.

The period of data collection started in the 1960s and last data were analysed in the 2020. The average follow-up time was not analysed.

Data of a large cohort of patients were analysed using multiple parameters. This article (Part II) focuses on FH clinical symptomatology. The principles of biochemical, statistical and genetic analyses of blood samples and classification of FH patients by the type of gene mutation are addressed in a Part I co-published by Todorovova et al. (2022) hence, they are not discussed more in detail in this article.

The diagnosis of FH was established using the Dutch Lipid Clinic Network score (DLCN). Among the 1,236 FH patients, 1,008 of them [854 supposed to have mutation in *LDLR* gene and 154 with familial defective apolipoprotein B-100 (FDB)] were genetically analysed (Todorovova et al., 2022).

The parameters of lipid and lipoprotein metabolism investigated in our analysis included LDL-cholesterol (LDL-C), total cholesterol (TC), apolipoprotein B (ApoB), HDL-cholesterol (HDL-C), triglycerides (TG) and apolipoprotein Lp(a). Their levels were recorded and analysed in patients at baseline in our clinic and compared with their current or latest available data. Also assessed was the presence of the other major risk factors for atherosclerosis, i.e., arterial hypertension, diabetes mellitus and smoking.

Our group of patients was further subdivided, into subgroups to be compared using several characteristics.

The first division was based on the presence/absence of ASCVD in their history, with patients showing overt complications of the atherosclerotic process further subdivided into three subgroups by the anatomical site involved, i.e., those with coronary heart disease (CHD), ischemic cerebrovascular event (stroke) and peripheral arterial disease (PAD). See **Figure 1**.

Another division, again into three subgroups, was based on differences in drug therapy. The first subgroup was made up of patients not taking any medications both prior to and during follow-up in our clinic, the second subgroup consisted of patients with pharmacotherapy not initiated until the start of follow-up whereas patients in the third subgroup had been on drug therapy already at baseline and continued their pharmacotherapy thereafter. See **Figure 2**.

Data were analyzed using STATISTICA 13 software (TIBCO Software Inc., Palo Alto, CA, United States). The baseline and follow-up levels were compared using the paired *t*-test. In univariate analysis, correlations between the lipid parameters and age were determined using Pearson's correlation coefficients. The tests used when comparing two

TABLE 1 | Patients with/without ASCVD and effect of treatment on lipid levels.

Parameter	ASCVD	Baseline			Follow-up			N	Diference (%)	*p*
		N	Mean ± SD	*p*	N	Mean ± SD	*p*			
LDL-C (mmol/L)	+	146	6.85 ± 2.05	$p = 0.011$	149	2.79 ± 1.54	$p < 0.001$	140	−59.72	$p < 0.001$
	-	1,035	6.42 ± 1.89		937	3.32 ± 1.57		909	−48.09	
TC (mmol/L)	+	156	9.28 ± 2.13	$p = 0.012$	153	4.83 ± 1.69	$p < 0.001$	153	−47.95	$p < 0.001$
	-	1,071	8.86 ± 1.93		965	5.53 ± 1.67		965	−37.84	
ApoB (g/L)	+	77	1.87 ± 0.62	$p = 0.137$	48	0.96 ± 0.43	$p = 0.05$	20	−48.03	$p = 0.219$
	-	525	1.77 ± 0.53		314	1.11 ± 0.50		164	−36.70	
TG (mmol/L)	+	153	2.11 ± 1.36	$p < 0.001$	153	1.39 ± 0.73	$p = 0.905$	150	−34.26	$p < 0.001$
	-	1,064	1.74 ± 1.06		964	1.38 ± 0.79		958	−21.48	
HDL-C (mmol/L)	+	153	1.55 ± 0.42	$p = 0.001$	152	1.43 ± 0.45	$p < 0.001$	149	−8.36	$p = 0.578$
	-	1,057	1.68 ± 0.46		955	1.58 ± 0.46		943	−6.57	
Lp(a) (g/L)	+	108	0.66 ± 0.79	$p < 0.001$	36	0.77 ± 1.08	$p = 0.107$	36	−8.35	$p = 0.123$
	-	844	0.44 ± 0.58		253	0.55 ± 0.67		248	7.62	

N—number of patients; SD, standard deviation; p—p-*value.*

TABLE 2 | Patients with/without CHD/stroke/PAD and effect of treatment on lipid levels.

Parametr	Group	Baseline			Follow-up			N	Diference (%)	*p*
		N	Mean ± SD	*p*	N	Mean ± SD	*p*			
LDL-C (mmol/L)	CHD+	110	6.90 ± 2.21	$p = 0.015$	113	2.82 ± 1.57	$p = 0.002$	105	−59.75	$p < 0.001$
	CHD-	1,071	6.43 ± 1.88		973	3.30 ± 1.57		944	−48.53	
	stroke+	31	6.84 ± 1.65	$p = 0.282$	30	2.57 ± 1.20	$p = 0.016$	30	−62.29	$p = 0.007$
	stroke-	1,150	6.47 ± 1.93		1,056	3.27 ± 1.58		1,019	−49.33	
	PAD+	31	7.14 ± 1.86	$p = 0.051$	32	2.99 ± 1.61	$p = 0.342$	31	−57.61	$p = 0.017$
	PAD-	1,150	6.46 ± 1.92		1,054	3.26 ± 1.58		1,018	−49.46	
TC (mmol/L)	CHD+	119	9.31 ± 2.29	$p = 0.020$	116	4.88 ± 1.73	$p < 0.001$	116	−47.57	$p < 0.001$
	CHD-	1,108	8.87 ± 1.92		1,002	5.50 ± 1.67		1,002	−38.27	
	stroke+	31	9.46 ± 1.78	$p = 0.115$	31	4.60 ± 1.33	$p = 0.005$	31	−51.40	$p < 0.001$
	stroke-	1,196	8.90 ± 1.97		1,087	5.46 ± 1.69		1,087	−38.91	
	PAD+	32	9.54 ± 1.97	$p = 0.069$	32	4.94 ± 1.75	$p = 0.096$	32	−48.16	$p = 0.006$
	PAD-	1,195	8.90 ± 1.96		1,086	5.45 ± 1.68		1,086	−39.00	
ApoB (g/L)	CHD+	57	1.88 ± 0.66	$p = 0.135$	34	0.98 ± 0.44	$p = 0.149$	15	−43.20	$p = 0.741$
	CHD-	545	1.77 ± 0.53		328	1.11 ± 0.50		169	−37.46	
	stroke+	16	1.78 ± 0.40	$p = 0.968$	11	0.79 ± 0.36	$p = 0.039$	4	−68.20	$p = 0.033$
	stroke-	586	1.78 ± 0.54		351	1.10 ± 0.50		180	−37.17	
	PAD+	17	2.01 ± 0.57	$p = 0.081$	9	1.10 ± 0.37	$p = 0.954$	4	−51.01	$p = 0.242$
	PAD-	585	1.77 ± 0.54		353	1.09 ± 0.50		180	−37.57	
TG (mmol/L)	CHD+	118	2.12 ± 1.47	$p < 0.001$	116	1.42 ± 0.78	$p = 0.623$	115	−33.55	$p = 0.002$
	CHD-	1,099	1.75 ± 1.05		1,001	1.38 ± 0.78		993	−22.10	
	stroke+	30	2.02 ± 0.85	$p = 0.235$	31	1.29 ± 0.46	$p = 0.523$	30	−35.74	$p = 0.119$
	stroke-	1,187	1.78 ± 1.11		1,086	1.39 ± 0.79		1,078	−23.13	
	PAD+	31	2.06 ± 0.83	$p = 0.155$	32	1.46 ± 0.67	$p = 0.562$	31	−28.87	$p = 0.365$
	PAD-	1,186	1.78 ± 1.11		1,085	1.38 ± 0.78		1,077	−23.33	
HDL-C (mmol/L)	CHD+	118	1.54 ± 0.43	$p = 0.002$	116	1.42 ± 0.45	$p < 0.001$	115	−7.87	$p = 0.825$
	CHD-	1,092	1.68 ± 0.46		991	1.57 ± 0.46		977	−6.68	
	stroke+	30	1.58 ± 0.44	$p = 0.304$	30	1.48 ± 0.52	$p = 0.355$	29	−7.39	$p = 0.947$
	stroke-	1,180	1.67 ± 0.46		1,077	1.56 ± 0.46		1,063	−6.78	
	PAD+	31	1.57 ± 0.41	$p = 0.236$	32	1.29 ± 0.34	$p = 0.001$	31	−17.22	$p = 0.023$
	PAD-	1,179	1.67 ± 0.46		1,075	1.56 ± 0.46		1,061	−6.51	
Lp(a) (g/L)	CHD+	80	0.72 ± 0.81	$p < 0.001$	25	0.96 ± 1.24	$p = 0.007$	25	0.71	$p = 0.801$
	CHD-	872	0.44 ± 0.58		264	0.54 ± 0.66		259	5.31	
	stroke+	17	0.53 ± 0.84	$p = 0.634$	6	0.46 ± 0.36	$p = 0.677$	6	−53.56	$p < 0.001$
	stroke-	935	0.46 ± 0.60		283	0.58 ± 0.74		278	6.85	
	PAD+	23	0.66 ± 0.65	$p = 0.112$	9	0.43 ± 0.44	$p = 0.521$	9	2.96	$p = 0.916$
	PAD-	929	0.46 ± 0.61		280	0.59 ± 0.74		275	4.67	

N—number of patients; SD, standard deviation; p—p-*value.*

TABLE 3 | Distribution of FH patients by treatment and effect of treatment on lipid levels.

Parameter	Group	Baseline			End of study			N	Diference (%)	p
		N	Mean ± SD	p	N	Mean ± SD	p			
LDL-C (mmol/L)	Y/Y	167	5.76 ± 1.93	$p < 0.001$	166	2.89 ± 1.13	$p < 0.001$	160	–49.6	$p < 0.001$
	N/Y	678	6.83 ± 1.80		678	3.01 ± 1.37		660	–55.7	
	N/N	172	6.35 ± 2.07		97	5.48 ± 2.02		93	–10.7	
TC (mmol/L)	Y/Y	175	8.15 ± 1.98	$p < 0.001$	169	5.03 ± 1.31	$p < 0.001$	169	–38.2	$p < 0.001$
	N/Y	699	9.32 ± 1.83		689	5.16 ± 1.49		689	–44.8	
	N/N	177	8.72 ± 2.07		102	7.84 ± 1.90		102	–8.8	
APOB (g/L)	Y/Y	97	1.57 ± 0.51	$p < 0.001$	65	0.98 ± 0.34	$p < 0.001$	43	–37.0	$p < 0.001$
	N/Y	316	1.86 ± 0.51		190	0.99 ± 0.39		86	–45.4	
	N/N	89	1.85 ± 0.65		40	1.69 ± 0.79		14	–7.9	
TG (mmol/L)	Y/Y	174	1.86 ± 1.17	$p = 0.003$	169	1.41 ± 0.69	$p = 0.792$	168	–23.4	$p = 0.026$
	N/Y	690	1.85 ± 1.17		688	1.37 ± 0.80		680	–26.4	
	N/N	177	1.54 ± 0.84		102	1.38 ± 0.84		102	–11.8	
HDL-C (mmol/L)	Y/Y	174	1.63 ± 0.39	$p = 0.536$	169	1.51 ± 0.40	$p < 0.001$	168	–6.9	$p < 0.001$
	N/Y	687	1.67 ± 0.45		682	1.53 ± 0.44		671	–8.2	
	N/N	175	1.67 ± 0.50		102	1.77 ± 0.54		101	1.9	
Lp(a) (g/L)	Y/Y	154	0.61 ± 0.66	$p = 0.003$	47	0.74 ± 0.67	$p = 0.229$	47	7.7	$p = 0.921$
	N/Y	553	0.44 ± 0.60		181	0.57 ± 0.80		180	4.8	
	N/N	113	0.39 ± 0.62		14	0.38 ± 0.39		13	12.1	

Y/Y—on treatment at baseline and throughout the study; N/Y—no treatment at baseline/on treatment throughout the study; N/N—no treatment at baseline and throughout the study; N—number of patients; SD, standard deviation; p—p-*value.*

TABLE 4 | Patients with/without arterial hypertension and effect of treatment on lipid levels.

Parameter	AH	Baseline			Follow-up			N	Diference (%)	p
		N	Mean ± SD	p	N	Mean ± SD	p			
LDL-C (mmol/L)	+	319	6.64 ± 1.90	$p = 0.073$	322	2.75 ± 1.21	$p < 0.001$	310	–58.17	$p < 0.001$
	-	862	6.41 ± 1.92		764	3.46 ± 1.66		739	–46.06	
TC (mmol/L)	+	332	9.12 ± 1.96	$p = 0.026$	328	4.89 ± 1.35	$p < 0.001$	328	–46.33	$p < 0.001$
	-	895	8.84 ± 1.96		790	5.66 ± 1.76		790	–36.27	
ApoB (g/L)	+	159	1.81 ± 0.57	$p = 0.455$	90	0.96 ± 0.37	$p = 0.003$	48	–46.83	$p = 0.122$
	-	443	1.77 ± 0.53		272	1.14 ± 0.52		136	–35.48	
TG (mmol/L)	+	331	2.09 ± 1.14	$p < 0.001$	328	1.52 ± 0.77	$p < 0.001$	327	–27.38	$p = 0.002$
	-	886	1.67 ± 1.07		789	1.32 ± 0.78		781	–21.49	
HDL-C (mmol/L)	+	327	1.61 ± 0.42	$p = 0.013$	327	1.46 ± 0.42	$p < 0.001$	322	–9.37	$p = 0.032$
	-	883	1.68 ± 0.47		780	1.59 ± 0.47		770	–5.74	
Lp(a) (g/L)	+	250	0.58 ± 0.75	$p < 0.001$	84	0.69 ± 0.92	$p = 0.112$	84	–5.95	$p = 0.056$
	-	702	0.42 ± 0.54		205	0.54 ± 0.64		200	11.20	

AH, arterial hypertension; N—number of patients; SD, standard deviation; p—p-*value.*

TABLE 5 | AH+/AH- patients developing ASCVD.

	AH	ASCVD +	ASCVD -	Total
Count	+	91	241	332
Row Percent (%)		27.41	72.59	
Count	-	65	839	904
Row Percent (%)		7.19	92.81	

and three subgroups in univariate analysis were the two-sample *t*-test and ANOVA test, respectively. We used multivariable logistic regression model to assess the effect of risk factors smoking, diabetes and arterial hypertension for total cardiovascular risk.

RESULTS

The present analysis compared the levels of lipid parameters obtained prior to start of follow-up and the most recent ones available. The primary endpoint LDL-C declined from a baseline mean of 6.49 ± 1.92 mmol/L to 3.26 ± 1.57 mmol/L (by 49.8%). A decrease by 39% was observed in TC levels

TABLE 6 | Patients with/without diabetes and effect of treatment on lipid levels.

Parameter	DM	Baseline			Follow-up			N	Diference (%)	p
		N	Mean ± SD	p	N	Mean ± SD	p			
LDL-C (mmol/L)	+	77	6.52 ± 1.78	$p = 0.827$	78	2.62 ± 1.35	$p < 0.001$	73	−58.95	$p = 0.008$
	-	1,104	6.47 ± 1.93		1,008	3.30 ± 1.58		976	−49.03	
TC (mmol/L)	+	83	9.07 ± 1.77	$p = 0.467$	82	4.74 ± 1.49	$p < 0.001$	82	−47.63	$p < 0.001$
	-	1,144	8.90 ± 1.98		1,036	5.49 ± 1.69		1,036	−38.60	
ApoB (g/L)	+	42	1.89 ± 0.55	$p = 0.166$	19	1.13 ± 0.49	$p = 0.755$	10	−40.21	$p = 0.122$
	-	560	1.77 ± 0.54		343	1.09 ± 0.50		174	−37.75	
TG (mmol/L)	+	83	2.40 ± 1.53	$p < 0.001$	82	1.73 ± 0.95	$p < 0.001$	82	−28.04	$p = 0.026$
	-	1,134	1.74 ± 1.05		1,035	1.36 ± 0.76		1,026	−23.01	
HDL-C (mmol/L)	+	81	1.55 ± 0.41	$p = 0.015$	82	1.37 ± 0.41	$p < 0.001$	80	−11.20	$p = 0.158$
	-	1,129	1.67 ± 0.46		1,025	1.57 ± 0.46		1,012	−6.48	
Lp(a) (g/L)	+	65	0.60 ± 0.84	$p = 0.059$	23	0.88 ± 1.33	$p = 0.042$	23	5.32	$p = 0.819$
	-	887	0.45 ± 0.59		266	0.55 ± 0.66		261	4.53	

DM, diabetes mellitus; N—number of patients; SD, standard deviation; p—p-*value.*

TABLE 7 | DM+/DM-patients developing ASCVD.

	DM	ASCVD +	ASCVD -	Total
Count	+	34	49	83
Row Percent (%)		40.96	59.04	
Count	-	122	1,031	1,153
Row Percent (%)		10.58	89.42	

falling from 8.95 ± 1.95 mmol/L to 5.43 ± 1.69 mmol/L. ApoB showed a decrease from a baseline mean of 1.76 ± 0.56 mmol/L to 1.09 ± 0.56 mmol/L. TG levels declined from a mean baseline of 1.81 ± 1.13 mmol/L to 1.38 ± 0.78 mmol/L. The change in HDL-C levels was 1.67 ± 0.46 mmol/L vs. follow-up levels of 1.56 ± 0.46 mmol/L. All the above differences were significant ($p < 0.001$). Lp(a) was unchanged (0.56 vs. 0.59 g/L, $p = 0.27$).

A total of 156 patients of the entire group (12.6%) had a history of ASCVD (ASCVD+ group; mean age 54.0 ± 12.5; 89 women, 67 men; 75 smokers) in the form of either CHD, stroke or PAD. As a total of 1,080 patients were in primary prevention of ASCVD, atherosclerosis had not yet manifested itself (ASCVD–group; mean age 43.5 ± 16.8; 752 women, 328 men; 313 smokers). The primary outcome was LDL-C declining, in ASCVD+ (ASCVD–) patients, from a baseline 6.85 ± 2.05 (6.42 ± 1.89) mmol/L to 2.79 ± 1.54 (3.23 ± 1.57) mmol/L during follow-up, which was 60% (48%) difference. This trend was seen in TC levels either, which fell in the ASCVD+ (ASCVD–) subgroups by 48% (38%). While the differences between the two subgroups in the baseline levels of ApoB were non-significant, follow-up difference reached statistical significance. The baseline TG levels of patients with a history of ASCVD were higher compared with patients without ASCVD. The TG levels decreased in either subgroup, 34% in ASCVD+, and 21% in ASCVD–patients. Statistically significant were the differences in baseline Lp(a) levels. In ASCVD+ subgroup, Lp(a) levels decreased, whereas in ASCVD-subgroup increased towards follow-up. HDL-C levels decreased over time, the overall change from baseline to follow-up was non-significant. For more details see **Table 1**.

Patients with ASCVD (ASCVD+; $n = 156$) were further subdivided into three subgroups by the anatomical site involved into those with CHD ($n = 119$), stroke ($n = 31$) and PAD ($n = 32$). Some patients were included in more than one subgroup. **Figure 1.**

All results are summarized in **Table 2**. In the CHD+ subgroup, LDL-C levels decreased by 60% from a baseline during follow-up compared with CHD–patients without a history of CHD (CHD–), whose baseline fell by 49%. In the CHD+ (CHD–) subgroups, TC levels decreased by 48% (38%). The differences in the levels of ApoB between the individual subgroups were non-significant both at the start and during follow-up. The baseline TG levels in the CHD+ (CHD–) subgroups were 2.12 ± 1.47 (1.75 ± 1.05) mmol/L to be non-significant during follow-up. Patients in the CHD+ subgroup had lower baseline levels of HDL-C compared with CHD–patients. Lp(a) levels were higher at baseline in CHD+ patients compared with CHD-. These levels rose in both subgroups over time, the changes were not significant ($p = 0.801$).

In patients with a history of stroke (stroke+), no significant differences in the baseline levels were found. The follow-up LDL-C (as well as TC or ApoB) levels in patients stroke+ were lower than in subgroup without this condition (stroke-).

While patients with PAD did not show significant differences in the lipid parameters at baseline, a significant difference was noted over time in HDL-C levels, being lower in PAD+ patients compared with PAD–subgroup.

Among the 1,236 patients, drug-status was available for 1,051 patients, and these were then subdivided into three subgroups based on whether or not the patients had been previously on lipid-lowering therapy and whether or not they were currently being treated with lipid-lowering agents.

The Y/Y subgroup ($n = 175$, lipid-lowering therapy at baseline and during follow-up) had baseline LDL-C levels of 5.76 ± 1.93 mmol/L decreasing to 2.89 ± 1.13 mmol/L (a 50% reduction; $p < 0.001$). The baseline TC levels of 8.15 ±

1.98 mmol/L declined to 5.03 ± 1.31 mmol/L (by 38%; $p < 0.001$) during follow-up, TG levels decreasing by 23% ($p = 0.026$).

The N/Y subgroup ($n = 699$, no therapy at baseline, therapy during follow-up) showed a decrease in LDL-C from baseline of 6.83 ± 1.80 mmol/L to 3.01 ± 1.37 mmol/L (a reduction by 56%; $p < 0.001$). TC levels dropped from 9.32 ± 1.83 mmol/L to 5.16 ± 1.49 mmol/L (down by 45%; $p < 0.001$). The levels of TG declined during follow-up by 26% ($p = 0.026$).

In the N/N subgroup ($n = 177$, therapy-naïve at baseline and no therapy during follow-up), the baseline LDL-C levels of 6.35 ± 2.07 mmol/L declined to 5.48 ± 2.02 mmol/L (11% reduction; $p < 0.001$), TC levels decreased from 8.72 ± 2.07 mmol/L to 7.84 ± 1.90 mmol/L (by 9%; $p < 0.001$) and TG levels reduction was 12% ($p = 0.026$).

More details in **Table 3**. When comparing the values between the three subgroups, the biggest decrease ($p < 0.001$) occurred in the LDL-C, TC, ApoB, HDL-C and TG levels in the N/Y subgroup ($p = 0.026$). The differences in Lp(a) levels were non-significant. The smallest changes were documented among N/N patients showing significantly ($p = 0.003$) lowest baseline TG levels compared with the Y/Y and N/Y subgroups. The follow-up levels of LDL-C, TC and ApoB were highest in the N/N subgroup ($p < 0.001$). All results summarized in a table are available in Todorovova et al., 2022

Our cohort comprised of 332 patients (27%) with arterial hypertension (AH). In the subgroup of patients with this diagnosis (AH+), the baseline levels of lipid parameters were significantly higher than in the subgroup without AH (AH–) such as in TC, TG, Lp(a) and lower in HDL-C. In the AH+ subgroup, the follow-up levels were significantly lower compared with the AH–subgroup in LDL-C (2.75 ± 1.21 vs 3.46 ± 1.66 mmol/L; $p < 0.001$), TC and ApoB, whereas TG levels in the AH+ subgroup showed poorer control (1.52 ± 0.77 mmol/L) than in AH–patients (1.32 ± 0.78 mmol/L). For more details see **Table 4**.

The number of AH+ patients developing ASCVD was significantly higher (27.4%) than of those without it (AH–) (7.2%). For details see **Table 5**. In group AH+ is 2.44 greater chance for KVO (OR = 2.44; CI0.95 = (1.65; 3.63)) than in AH-.

During follow-up, diabetes mellitus was diagnosed in a total of 83 patients (7% of the whole study group; $n = 1,236$). Patients with diabetes mellitus (DM+) showed worse control of lipid parameters than those without this diagnosis (DM–) as reflected in the levels of TG and HDL-C. The differences in the other parameters assessed were non-significant. Follow-up levels of LDL-C and TC in DM+ patients were lower compared with DM–patients. On the other hand, the follow-up levels of TG were higher in the DM+ subgroup than among DM–patients. The difference in ApoB levels was not significant. All pertinent data are shown in detail in **Table 6**.

In the DM+ subgroup ($n = 83$), 34 patients had a history of ASCVD (41%) whereas ASCVD was not present in 49 (59%). Among the DM–patients ($n = 1,153$), ASCVD was present in 122 (10.6%), with 1,031 patients (89.4%) without this diagnosis. The prevalence of ASCVD in DM+ vs. DM-was 41% vs 10,6%; $p < 0.001$ (see **Table 7**). In DM+ is 2.84 greater chance for KVO (OR = 2.84; CI0.95 = (1.67; 4.83)) than in DM-.

The baseline lipid profile in smokers ($n = 389$) differed significantly only in TG levels, which were higher (2.05 ± 1.37 mmol/L) compared with non-smokers (1.66 ± 0.93 mmol/L) and in HDL-C levels (1.60 ± 0.45 vs. 1.69 ± 0.46 mmol/L). During follow-up, TG levels in smokers remained higher (1.54 ± 0.99 vs. 1.3 ± 0.63 mmol/L), with the trend in HDL-C levels also unchanged (1.49 ± 0.47 vs. 1.59 ± 0.45 mmol/L). The follow-up levels of LDL-C and TC were lower in smokers (LDL 3.09 ± 1.47 vs. 3.3 ± 1.62 mmol/L, and TC 5.28 ± 1.65 vs. 5.51 ± 1.7 mmol/L, respectively). In smokers, their LDL-C levels declined by 3.44 mmol/L (3.13 mmol/L in non-smokers), with TC levels decreasing by 3.71 mmol/L (3.41 mmol/L in non-smokers).

Among smokers, 19.3% were classified as ASCVD+ and 80.7% as ASCVD–; the respective figures for non-smokers were 9.6 and 90.4%. In group of smokers is 1.87 greater chance for KVO (OR = 1.87; CI0.95 = (1.29; 2.71)) than in nonsmokers.

The whole group of our patients included 841 women and 395 men. Compared with men, women started follow-up with lower levels of TG (1.7 ± 1.05 mmol/L vs 1.95 ± 1.18 mmol/L; $p < 0.001$) and higher levels of HDL-C (1.77 ± 0.46 mmol/L vs 1.44 ± 0.34 mmol/L; $p < 0.001$). Over time, TG levels were higher in women, 1.35 ± 0.76 mmol/L (1.47 ± 0.83 mmol/L in men; $p = 0.016$) as were HDL-C levels, 1.67 ± 0.46 mmol/L (1.32 ± 0.36 mmol/L in men; $p < 0.001$). The follow-up TC levels were higher in women (5.57 ± 1.67 mmol/L) than in men (5.14 ± 1.68 mmol/L; $p < 0.001$). The changes between the baseline and follow-up levels of the other parameters assessed were non-significant.

Clinical presentation of FH was seen in a total of 145 (12%) patients, with xantelasma palpebrarum diagnosed in 57 cases (5%), arcus lipoides corneae in 47 patients (4%) and tendon xanthomas in 41 patients (3%).

DISCUSSION

The 1,236 patients with analyzed data attended a single Prague-based clinic with a history spanning more than 50 years. The period of data collection is not exactly defined as our project was a retrospective analysis with data of the first patients recorded as early as the 1960s when Šobra founded the Center of Preventive Cardiology (Center hereinafter) (Šobra, 1970). Over the decades, the Center was being attended by a large number of patients with familial hypercholesterolemia; however, the duration of their follow-up has varied substantially as, while some patients have been taken care of for decades, the follow-up period of other patients has not been longer than 2 years.

Needless to say, an ideal scenario would involve a patient referred to the Center by their general practitioner for assessment and subsequent follow-up. In practice, however, some patients presenting for follow-up do not have complete medical records, do not present for routine blood tests or are simply lost to follow-up. This explains the differences in the numbers of patients whose

data were available for analysis. Last but not least, an additional reason may be the different, or inconsistent, approach of individual physicians.

Recent studies have suggested that the only causal factor of ASCVD is dyslipidemia or, more exactly, LDL-C (Borén et al., 2020). In fact, the diagnosis of FH *per se* puts all our 1,236 patients into the category of at least high cardiovascular risk (Visseren et al., 2021); nonetheless, while some of them do develop ASCVD, others do not. This was why our project focused also on the differences between the two major groups (ASCVD+ vs ASCVD–) of FH patients.

During follow-up, all patient subgroups showed a significant decrease in the levels of LDL-C, TC, ApoB and TG. While the reason for the decrease in HDL-C levels over time remains unclear, its follow-up levels (1.56 mmol/L) were within the optimal range (van der Steeg et al., 2008). Until the advent of PCSK9 inhibitors, Lp(a) was traditionally seen as an important player in the atherosclerotic process independent of the other risk factors (O'Donoghue et al., 2019) and not modifiable by drug therapy (Sun et al., 2018). The levels of Lp(a) did not change significantly in our analysis of follow-up data. There is no doubt this is due to the fact that PCSK9 inhibitors were unavailable in the Czech Republic until the summer of 2018; hence, they could not have affected the outcomes of patients on follow-up. Other reasons include the small number of patients with baseline and follow-up data available and, also, the inconsistent approach by physicians many of whom simply failed to focus their attention on a parameter refractory to drug therapy.

As noted above, not all patients with FH develop premature ASCVD. We did suspect that the lipid profile of ASCVD+ patients would be associated with increased risk, which was eventually the case. Patients with ASCVD had higher baseline LDL-C and TC levels and lower HDL-C levels than ASCVD–patients. The most striking differences were observed in the baseline levels of TG and Lp(a), which were again higher in the ASCVD+ subgroup thus corroborating, together with lower HDL-C levels, the importance of residual cardiovascular risk (Hoogeveen and Ballantyne, 2021). The tide turned during follow-up with ASCVD+ patients showing significantly lower levels of LDL-C, TC and ApoB whereas the differences in TG and Lp(a) levels were non-significant. The reasons for the more favorable lipid profile in ASCVD+ patients are multiple. First and foremost, these at-risk patients (category of very high cardiovascular risk according to the guidelines (Visseren et al., 2021)) receive more attention by health care providers. Also, their target levels are more ambitious and, last but not least, patients with a history of cardiovascular disease are more likely to adhere to their recommended therapy and tend to comply with their physicians' advice (Jackevicius et al., 2002).

As in the ASCVD+ subgroup, patients assigned to the CHD subgroup had significantly higher baseline levels of LDL-C, TC, TG and Lp(a) a lower HDL-C levels compared with patients without a history of ASCVD. Except for TG and Lp(a), the follow-up levels in the CHD subgroup were lower (in analogy to ASCVD–vs ASCVD+). A similar trend was noted in the stroke (n = 31) and PAD subgroups (n = 32); however, the differences were non-significant due to the small number of patients on follow-up.

When comparing the subgroups with different therapeutic status (N/N, N/Y, Y/Y), it came as no surprise that the largest decrease in the levels of LDL-C, TC, ApoB and TG was seen in the subgroup with therapy not initiated prior to follow-up in the Center (N/Y). Nonetheless, a significant decrease in the above parameters was also seen in the (Y/Y) subgroup suggesting that patients benefit already from receiving therapy in a specialized center adopting the most recent therapeutic strategies combined with an effort to achieve target levels. Patients not currently on therapy and not treated at the time of starting outpatient follow-up showed minimal decreases in the investigated parameters. The most frequent reason for failure to initiate therapy in a specialized healthcare facility was statin intolerance. The number of patients not receiving therapy after the PCSK9 inhibitors had been approved for the Czech market is currently smaller (Altschmiedova et al., 2020); however, providing more details on this issue is outside the scope of this paper. Other reasons for not instituting therapy drug include the patients' unwillingness and/or reluctance to initiate therapy even after they had been informed about all the risks associated with untreated significant dyslipidemia.

A total of 27% of our patients had a history of arterial hypertension, a condition with a global prevalence estimated at 20–24% in years 1975–2015 (Zhou et al., 2017). In the Czech Republic, according to Cífková et al., the prevalence of hypertension declined from 47.1% in 1985 to 41.5% in 2016/17 (Cífková et al., 2020a). Diabetes mellitus was present in 7% of our cohort. The prevalence of diabetes in the Czech Republic was according to the same author about 8% in men and 5% in women (Cífková et al., 2020b). These results clearly show that familial hypercholesterolemia is a genetic disease whose incidence cannot be linked to a lifestyle. Patients with FH are not in higher risk of development of diabetes and AH. The lipid profile of them with AH and diabetes is worse because of higher TG and lower HDL and we assume that this trend is associated with the lifestyle of individuals.

Smokers totaling 389, i.e., 31% of our whole group of patients, initiated follow-up with higher baseline TG levels and lower levels of HDL-C than non-smokers; this fact remained unaltered during follow-up and is presumably associated with the lifestyle of these patients. However, the follow-up levels of LDL-C and TC were more favorable in smokers. Smokers also tended to respond better to therapy and showed greater decreases in LDL-C, TC and ApoB levels compared with non-smokers, likely due to their higher cardiovascular risk and' consequently, more ambitious LDL-C targets (Visseren et al., 2021).

Patients with FH and a history of arterial hypertension, diabetes or tobacco smoking, experienced more cardiovascular events than those without the above conditions.

At baseline and throughout follow-up, women had lower TG levels and higher HDL-C levels compared with men. These differences may be due to their more consistent adherence of women to a healthy lifestyle. We also assessed overall changes in the investigated parameters prior to and

during follow-up; however, no sex-related statistical significance was demonstrated.

Clinical presentations of FH such as tendon xanthomas, arcus lipoides corneae or xantelasma palpebrarum are currently less frequent than in the past. In a first-ever monograph on FH published in 1970, Šobra reported a 30% incidence of arcus lipoides corneae, 23% incidence of xantelasma palpebrarum and 10% of patients with some form of xanthomatosis (Šobra, 1970). By contrast, in a paper published in 2014 and reporting on patients currently treated in the same center, arcus lipoides corneae, xanthelasma palpebrarum and xanthomatosis diagnosed were in 3, 6, and 5% of patients, respectively (Ceska et al., 2014). The development of these clinical signs is associated not only with cholesterol levels but, also, with the period of time the body is exposed to these levels. Patients with a well-defined treatment plan initiated in a timely manner do not develop these clinical presentations or, in the opposite case, these regress or disappear completely (Ceska et al., 2014; Civeira et al., 2016). If comparing the current therapeutic options with those available more than 50 years ago, it comes as no surprise that tendon xanthomas, arcus lipoides corneae or xantelasma palpebrarum become less frequent. We consider our assessment of the clinical signs in the present paper only an estimate since the figures cover all patients treated since the 1960s and the final number is no doubt confounded by the above regression due to intensive lipid-lowering therapy.

During the 50 + years of follow-up, there have been some deaths; however, the exact numbers are unavailable as some of the deaths may not have been recorded.

CONCLUSION

The present analysis confirmed the well-known fact that, while LDL-C is a causal risk factor of ASCVD, every effort should be made to modulate all the known risk factors posing a residual risk, even after achieving target LDL-C levels. Therapeutic modification of Lp(a) by promising new agents still under development as well as by PCSK9 inhibitors already introduced into clinical practice may have the potential to further reduce cardiovascular risk in the near future. Results of this project have suggested that patients with the diagnosis of FH do benefit from receiving therapy in a specialized center which was confirmed by ScreenPro FH project (Ceska et al., 2019).

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethics Committee of the General University Hospital in Prague. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

TA interpreted data and wrote the article. VT analysed data of patients and contributed to writing the article MV contributed with interpretation of data and writing the article RC interpretated of data, paticipated on revision, proofreading, professional supervision.

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STATIN DRUGS (R. CESKA, SECTION EDITOR)

PCSK9 Inhibitors in Real-world Practice: Analysis of Data from 314 Patients and 2 Years of Experience in a Center of Preventive Cardiology

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Abstract

Purpose of Review PCSK9 inhibitors have been shown to be the most effective class of drugs modifying the levels of LDL-cholesterol as the main risk factor for atherosclerotic cardiovascular disease. The aim of this paper is to assess the effect of monoclonal antibodies on lipid and lipoprotein metabolism in real-world practice.

Recent Findings The outcome trials showed effective reduction of LDL-C by 56–62%. Landmark studies enrolling over a total of 46,000 patients with CHD in their medical history demonstrated the beneficial effect of both agents on cardiovascular morbidity and mortality. The data from real everyday clinical practice are very limited or missing.

Summary Even in real-world practice, PCSK9 inhibitors have been shown to be an effective, safe, and well-tolerated class of drugs with effects comparable with those reported from large randomized controlled trials.

Keywords Atherosclerotic cardiovascular disease (ASCVD) · LDL cholesterol · PCSK9 inhibitors · Alirocumab · Evolocumab · Real-world data (RWD)

Introduction

Despite the incessant advances in diagnostic and therapeutic technology and strategies, atherosclerotic cardiovascular disease (ASCVD) remains — not only in the Czech Republic — the leading cause of death responsible for almost 50% of total mortality [1]. A large body of evidence has accumulated about the role of low-density lipoprotein cholesterol (LDL-C) as the main risk factor for atherosclerosis as a springboard for the development of cardiovascular disease. Hence, LDL-C is the ultimate target in the management of dyslipidemias, with the current first-line class of drugs of choice being statins. In line with the guidelines developed by the respective European professional societies [2], statins are administered at their maximum tolerated doses, and, in patients failing to achieve LDL-C targets, it is recommended to add ezetimibe. In recent years, the arsenal of lipid-lowering drugs has expanded with the advent of proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors.

The pace of research in the field of PCSK9 inhibitors has been astonishingly fast. The proprotein convertase subtilisin kexin type 9 (PCSK9) protein was discovered in the early 2000s and was soon shown to bind to the LDL-C receptor. The discovery that individuals with hereditary loss-of-function mutations in the *PCSK9* gene have low LDL-C levels accelerated research with the first clinical trials launched in 2009 [3, 4]. Currently, 2 active substances — evolocumab and alirocumab — are available; both are administered subcutaneously every 2 weeks and were approved for the Czech market on June 1, 2018.

Aim of Study

Our study was designed to establish the extent to which the outcomes of therapy with PCSK9 inhibitors in real-world practice compare with those reported by large randomized

Between 1 October 2020 and 1 February 2021, the target LDL-levels for alirocumab and evolocumab, respectively, were further decreased, a fact not reflected in this manuscript given the time of patient enrolment.

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trials. The endpoints included a host of variables of lipid and lipoprotein metabolism as well as safety and tolerability of PCSK9 inhibitors.

We assessed the effect of PCSK9 inhibitor therapy on the entire study group and compared separately groups of patients with vs. without the diagnosis of familial hypercholesterolemia (FH), patients in primary and secondary ASCVD prevention and, also, those treated with a PCSK9 inhibitor in monotherapy vs. those receiving a PCSK9 inhibitor in combination with a statin.

Further, we were interested to know whether or not there is a correlation between baseline LDL-C levels and their decrease relative to therapy. Likewise, we focused on any potential differences in the effect and tolerability of both active substances — alirocumab and evolocumab. Given the size of our study group and study duration, the protocol did not include cardiovascular endpoints.

Patients and Methods

As the use of PCSK9 inhibitors in the Czech Republic is currently limited by the local healthcare reimbursement policies, study participants were enrolled based on the applicable criteria allowing to reimburse treatment in 2 indications.

The first major subgroup included FH patients (in primary or secondary ASCVD prevention) with LDL-C levels ≥ 4 mmol/l despite maximum tolerated statin doses. For therapy to be reimbursable, ezetimibe has also to be administered as add-on therapy except for cases where LDL-C levels are $> 50\%$ than the target for the respective the cardiovascular risk category (in statin-naïve patients) or $> 20\%$ (in patients already receiving a statin at a maximum tolerated dose). If the patient does not take a statin because of intolerance, this fact must be noted in their medical records. In our study participants, the diagnosis of FH was established using the Dutch Lipid Clinic Network Criteria (DLCNC) [5].

The second major subgroup included patients in secondary ASCVD prevention defined by the Czech healthcare reimbursement policies as the presence of coronary heart disease (CHD), peripheral arterial disease (PAD), or post-stroke status including transient ischemic attack (TIA) as well as status post-revascularization. These patients were indicated for therapy on condition that, despite maximum tolerated lipid-lowering therapy, their LDL-C levels were ≥ 3 mmol/l [5].

Patients not meeting the reimbursement criteria and self-payers were not eligible for inclusion.

Overall, the study group included 314 patients (138 men and 176 women) with a mean age of 63 years (range, 24–89 years), enrolled in a Prague-based hospital between 31 July 2018 and 30 September 2020. Data were collected until 31 December 2020. Study participants had laboratory tests before therapy initiation and, subsequently, at 12 and 24 weeks, and at 1 and 2 years to assess the trajectories of 6 pre-defined endpoints over time: LDL-cholesterol (LDL-C), total cholesterol (TC), lipoprotein (a) Lp(a), apolipoprotein B (apoB), high-density lipoprotein cholesterol (HDL-C), and triglycerides (Tg). Further, the study investigated the impact of therapy on variables of glucose metabolism (glycemia, glycated hemoglobin). The safety of therapy was evaluated using both physical examination and laboratory tests.

Complete lipid profile is available for all 314 patients at the beginning of the observation and in week 12. As the study progressed, depending on the time when the patient was included, the number of patients for whom we have complete data decreased gradually from 271 patients in week 24, 201 patients after 1 year to 73 patients after 2 years of treatment.

The subgroup of FH patients included 207 (65% of total) individuals, of which number 142 (69%) were in primary prevention and 65 (31%) in secondary prevention of ASCVD. On therapy initiation, secondary ASCVD prevention had been underway in 172 patients (55%) with FH diagnosed in 65 (38%); hence, secondary prevention (without FH) was indicated in 107 participants. The mean baseline LDL-C levels in FH and non-FH patients were 5.03 ± 1.43 (2.21–14.30) mmol/l and 3.75 ± 0.80 (2.02–6.28) mmol/l, respectively. Regarding cardiovascular prevention, baseline LDL-C levels in patients not diagnosed with ASCVD were 5.40 ± 1.40 (3.56–14.30) mmol/l whereas the values of patients in secondary ASCVD prevention were 3.93 ± 0.96 (2.02–9.30) mmol/l.

Patients were treated with evolocumab 140 mg or alirocumab 75 mg (150 mg if necessary).

They were not randomized to the treatment; therapy was selected according to the decision of indicating physicians in an effort to maintain an approximately equal representation of both of them, alirocumab as well as evolocumab. There were 156 patients receiving only evolocumab and 113 patients treated exclusively with alirocumab. The remaining 45 participants used both active substances (but separately) during the study, with therapy switched because of side effects or inadequate effect of therapy with one of the study drugs. To avoid any bias, data of the latter subgroup were put aside and will not be further discussed.

A total of 166 (53%) study participants were statin-intolerant patients thus taking a PCSK9 inhibitor either in monotherapy or in combination with ezetimibe. Conversely, 148 of those enrolled (47%) had a history of statin use, of whom 82 (26% of the entire group) were using statin even at a maximum dose. The mean baseline LDL-C levels in patients receiving a statin at a maximum dose were 4.18 mmol/l $\pm$ 1.62 mmol/l; in completely statin-intolerant patients, the levels were 4.95 ± 1.26 mmol/l.

Regarding smoking status, a greater proportion of the participants was made up of smokers and ex-smokers (157) vs. non-smokers (138). No information about smoking status was available in 19 patients.

Statistical Analysis

Statistical analysis was performed using STATISTICA 13 software (TIBCO Software Inc., Palo Alto, CA, USA). To assess the development of individual variables over time, their mean values at pre-defined time points were calculated and compared with baseline using the two sample *t*-test.

Results

The primary endpoint was change in LDL-C levels declining from a mean baseline of 4.59 ± 1.39 mmol/l to 1.87 ± 1.24 mmol/l at 12 weeks (–59.4%). The effect was persistent to become even stronger with LDL-C levels reaching a mean value of 1.72 ± 0.98 mmol/l at 2 years (–62.6%), statistically significant values.

The levels of TC decreased from a mean baseline of 6.88 mmol/l to 3.86 mmol/l at the end of the study showing a mean decrease of 41.7% at 12 weeks and 43.9% at 2 years, again a significant outcome.

Lipoprotein Lp(a) levels declined from a mean baseline of 0.79 to 0.59 g/l (–25.4%) at 12 weeks and further down to 0.51 g/l (–35.5%) at 24 weeks into the study. Results at 1 and 2 years were available in only a small number of patients and were statistically non-significant.

The changes in apolipoprotein B levels followed the pattern seen in LDL-C levels. While mean apoB levels fell by 54.2% at 12 weeks, the decrease vs. baseline was 58.1% at 2 years, again statistically significant improvement.

No major changes were noted in HDL-C levels, which rose slightly (by 4%) at 12 weeks and by 5.6% at 2 years; however, the differences were non-significant.

The decrease in Tg levels from 2.13 mmol/l at baseline to 1.62 mmol/l at 12 weeks and further down to 1.49 mmol/l at 2 years was statistically significant (–30.3% vs. baseline), with the levels reaching targets set by the 2019 ESC/EAS Guidelines for the management of dyslipidemias [2]; this variable, however, was not the primary focus of treatment with PCSK9 inhibitors.

Glucose metabolism was not affected by the therapy.

An overview of the development of all variables overtime is available in Table 1 and Fig. 1.

Apart from the courses of the above variables across the whole group, our study sought to identify any differences in the effect of therapy between individual patient subgroups. On entering the study, LDL-C levels of FH patients were higher (5.03 mmol/l) than those of non-FH patients (3.75 mmol/l).

However, the dynamics of decrease after therapy initiation were similar, with LDL-C dropping by 56.7% and 61.8% at 12 and 24 weeks in FH patients as against 67.4% and 68.8% in non-FH patients, respectively. In addition, while, in the FH group, LDL-C levels continued to decline steadily from 1 year onward, an opposite trend (a slight increase) was observed in non-FH patients. The LDL-C levels in FH patients (at 2 years) at the end of the study were 1.83 mmol/l (a drop by 3.19 mmol/l, i.e., –63.5% vs. baseline); the respective figures in non-FH patients were 1.42 mmol/l (a drop by 2.34 mmol/l, i.e., –62.3% vs. baseline). The differences were significant.

Patients in primary ASCVD prevention enrolled in the project with higher LDL-C levels (5.40 mmol/l) than those in secondary prevention (3.93 mmol/l). However, the dynamics of decrease after therapy initiation showed an almost similar pattern, with LDL-C levels falling by 55.7% and 61.5% at 12 and 24 weeks in patients in primary prevention and by 63.9% and 66.3% in those in secondary ASCVD prevention. In addition, while, in the primary prevention group, LDL-C levels continued to decline steadily from 1 year onward, an opposite trend (a slight increase) was observed in the secondary prevention group. At 2 years into the study, LDL-C levels in primary prevention participants reached 2.07 mmol/l (a decrease of 3.33 mmol/l, i.e., –61.7% vs. baseline), with the respective figures for the secondary prevention subgroup being 1.41 mmol/l (a decrease of 2.52 mmol/l, i.e., –64.2% vs. baseline).

On therapy initiation, completely statin-intolerant participants had higher LDL-C than those already being treated with a statin at a maximum (or lower-than-maximum) dose. However, the dynamics in response to therapy was already similar in all 3 main subgroups. While, by week 12, LDL-C levels in patients not on statin therapy decreased by 55% to further decline after week 24 onward, in patients receiving maximum (or lower-than-maximum) statin dose, the levels fell by 65.2% (or 65%, respectively) to start rising steadily from week 24 onward. The values at the end of the study differed significantly at 2 years being 59.3% in completely statin-intolerant patients, and 64% and 63% in patients receiving statins at maximum and lower-than-maximum doses, respectively.

Statistically, significant differences were also found between the groups based on the agents received. Here, our entire study population was divided into another 3 subgroups. The first two subgroups comprised patients treated throughout the study with either evolocumab or alirocumab. The third subgroup was made up of patients switched over from evolocumab to alirocumab or vice versa; data of this subgroup were not analyzed.

Table 1 An overview of the development of all variables over time

	Baseline	Week 12	Week 12 vs. baseline			Week 24	Week 24 vs. baseline			Year 1	Year 1 vs. baseline			Year 2	Year 2 vs. baseline		
	Mean	Mean	Difference	%	*p*	Mean	Difference	%	*p*	Mean	Difference	%	*p*	Mean	Difference	%	*p*
LDL-C (mmol/l)	4.59	1.87	−2.73	−59.35%	$p < 0.001$	1.67	−2.92	−63.54%	$p < 0.001$	1.73	−2.87	−62.42%	$p < 0.001$	1.72	−2.87	−62.46%	$p < 0.001$
TC (mmol/l)	6.88	4.01	−2.87	−41.68%	$p < 0.001$	3.83	−3.05	−44.34%	$p < 0.001$	3.88	−3.00	−43.66%	$p < 0.001$	3.86	−3.02	−43.91%	$p < 0.001$
Lp(a) (g/l)	0.79	0.59	−0.20	−25.38%	$p = 0.023$	0.51	−0.28	−35.49%	$p = 0.002$	0.62	−0.17	−21.81%	$p = 0.221$	0.56	−0.24	−29.85%	$p = 0.167$
ApoB (g/l)	1.59	0.73	−0.86	−54.15%	$p < 0.001$	0.69	−0.90	−56.57%	$p < 0.001$	0.72	−0.87	−54.57%	$p < 0.001$	0.67	−0.93	−58.09%	$p < 0.001$
HDL-C (mmol/l)	1.42	1.48	0.06	3.99%	$p = 0.127$	1.47	0.05	3.33%	$p = 0.207$	1.47	0.05	3.52%	$p = 0.231$	1.50	0.08	5.58%	$p = 0.155$
TG (mmol/l)	2.13	1.62	−0.52	−24.21%	$p < 0.001$	1.58	−0.55	−25.88%	$p < 0.001$	1.58	−0.55	−25.85%	$p < 0.001$	1.49	−0.64	−30.25%	$p < 0.001$
Glc (mmol/l)	5.65	5.72	0.06	1.10%	$p = 0.546$	5.72	0.07	1.16%	$p = 0.584$	5.72	0.07	1.22%	$p = 0.553$	5.52	−0.14	−2.42%	$p = 0.241$
HbA1c (mmol/mol)	42.93	41.55	−1.38	−3.21%	$p = 0.294$	42.64	−0.30	−0.69%	$p = 0.831$	42.78	−0.15	−0.35%	$p = 0.911$	40.36	−2.57	−5.99%	$p = 0.071$

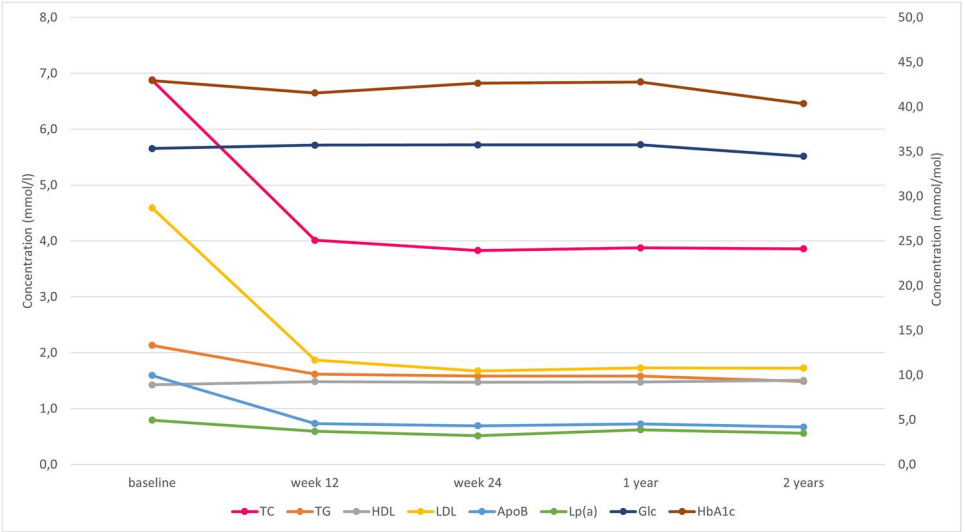

Fig. 1 An overview of the development of all variables over time

Patients in the two first subgroups had similar baseline LDL-C levels. By 12 weeks, the LDL-C levels of the alirocumab-only subgroup fell by 55.5% to continue decreasing steadily from week 24 onward while, in the evolocumab-only subgroup, the decrease at 12 weeks was 66.1% with the levels continuing to rise steadily from week 24 onward. At 2 years into the study, the decrease in the alirocumab-only and evolocumab-only subgroups was 64.7% and 61.0%, respectively, a statistically significant difference.

We also sought to determine whether or not there is a relationship between the absolute changes in LDL-C vs. baseline. Using Pearson's correlation coefficient (Pearson's *r*), the test showed a value of –0.7147, hence, a moderately strong inverse correlation, that is, the higher the baseline LDL-C levels, the less was the decrease in absolute numbers—again, a statistically significant difference.

To assess the rates of achieving LDL-C targets, we used participants' data available at 1 year of the study. Among 108 patients in primary ASCVD prevention, 71 (65.7%) were in the range of LDL-C ≤ 1.4 mmol/l thus achieving target LDL-C levels as defined by the guidelines of the 2019 ESC/EAS Guidelines for the management of dyslipidemias [2]. Among 92 FH patients in primary prevention, target LDL-C values (≤ 1.8 mmol/l) were found in 33 (35.9%).

Side effects were reported by a total of 28 study participants (9%), of which number 16 and 14 adverse reactions were considered evolocumab- and alirocumab-related, respectively. The most frequent side effect was flu-like syndrome (fatigue, malaise, and upper airways inflammation) reported by 13 patients whereas 5 study participants complained of pain at injection site and 5 of myalgia (a reason for their previous statin intolerance). Three patients experienced gastrointestinal intolerance of therapy and 2 reported various problems. Overall, 15 patients withdrew from the study for side effects.

During the 2 years of our study, PCSK9 inhibitor therapy was discontinued in a total of 36 study participants. Except for the 15 patients experiencing the above side effects, therapy was stopped in 8 for unsatisfactory effect of therapy defined as failure to reach LDL-C targets for the respective cardiovascular risk category and/or LDL-C reduction by a minimum of 40% vs. baseline; 6 patients were removed from the study for non-compliance, and 7 patients discontinued therapy for other reasons such as a condition not related to therapy or epidemiological situation related to COVID-19.

Discussion

Both molecules, evolocumab and alirocumab, have been evaluated in a number of clinical trials within the PROFICIO (evolocumab) and ODYSSEY projects (alirocumab). Early studies focused on the effect of the two agents on the levels of plasma lipids such as — most importantly — LDL-C followed by apoB or Lp(a). The outcomes were impressive with PCSK9 inhibitors effectively reducing LDL-C by 56–62% [6]. Landmark studies enrolling over a total of 46,000 patients with CHD in their medical history demonstrated the beneficial effect of both agents on cardiovascular morbidity and mortality. With both agents, cardiovascular death rates declined by 15–20% relative to placebo [7••, 8••].

Given the size of our study group and study duration, the aim of the present project was not to assess cardiovascular endpoints. Still, we were interested to know whether or not the new class of drugs has, in the real-world setting, an effect comparable to that reported by clinical trials since, in the above clinical trials, the percentage LDL-C reduction was

compelling, uniform across the individual subgroups and consistent over time.

Our study demonstrated that PCSK9 inhibitors offer an effective therapeutic option in statin-intolerant patients. While more encouraging outcomes were obtained in the statin-treated group (consistent with the finding that PCSK9 inhibition may enhance the LDL-C-lowering effect of statins [9]), the 55% LDL-C reduction seen in completely statin-intolerant patients as early as 12 weeks after initiation of therapy with a PCKS9 inhibition provides a long-awaited new hope to this patient population.

Furthermore, we sought to determine the proportion of patients achieving target values — also not optimal in the Czech Republic in the long run — an issue addressed also by major international studies. In the EUROASPIRE survey (enrolling 6648 patients with CHD in 24 European countries), LDL-C targets were achieved in only 19.3% of patients [10]. The results of the Czech participants ($n = 493$) were similar, with target LDL-C levels attained in 23.5% of CHD patients [11]. More optimistic data about dyslipidemia control were offered by a Czech observational study of Zlatohlávek et al. assessing, between June and December 2016, a total of 201 patients at high- and very-high cardiovascular risk in 11 centers across the country. In the high-risk and very-high risk subgroups, LDL-C targets were achieved in 46.4% and 56.1% of patients, respectively [12•]. This situation changed dramatically with the advent of PCSK9 inhibitors, with most encouraging outcomes reported in a study by Raal et al., where 80% of FH patients receiving standard therapy reached LDL-C targets when using a PCSK9 inhibitor as add-on therapy [13].

In the present study, target LDL-C levels were attained by 35.9% of patients in primary prevention (33 out of the 92 patients whose data were available at 1 year into the study). Among the 108 very high-risk patients in secondary ASCVD prevention, whose data were available at 1 year into the study, target LDL-C levels were reached by 71 (65.7%). The reason of this apparently less optimistic outcome should be first sought in the differently defined target LDL-C levels. While all the above studies [10–13] used the 2016 ESC/EAS Guidelines for the Management of Dyslipidaemias [14] defining target LDL-C values for high-risk and very high-risk patients as < 2.5 mmol/l and < 1.8 mmol/l, respectively, the present study had stricter limits of < 1.8 mmol/l and < 1.4 mmol/l, respectively [2]. If including patients with LDL-C levels of > 1.8 and > 2.6 mmol/l, the target values would have been achieved by 68.48% of FH patients, and by 79.6% of those in secondary prevention and at very high risk.

Nonetheless, we are in the year 2021 with more ambitious goals, so the fact that a "mere" 20.4% of patients in secondary ASCVD prevention have LDL-C levels ≥ 1.8 mmol/l is simply unsatisfactory and further causes must be identified. One of these — in relation to the present study — may be that our patients are being followed up and treated in 1 national center. There are only 2 national centers for the management of dyslipidemias with the implication being their patients are mostly those with generally more severe forms of dyslipidemia or those failing to respond to any therapeutic option currently available. The same may apply to the higher proportion of statin-intolerant patients. The pool of patients with failed therapeutic options was built long before PCSK9 inhibitors had been approved for use in the Czech Republic, and therapy was instituted soon after the respective healthcare reimbursement policy had been defined. By contrast, a definite plus in enrolling patients from only a single center is that we were able to eliminate potentially different approaches by various centers to the creation of and keeping patient medical records and data entry and, hence, to minimize the potential for data misinterpretation.

The effect of monoclonal antibodies on Lp(a) levels seems to be most encouraging. It is well known that this variable is yet another (and independent of other variables) risk factor of atherosclerosis, and, until the advent of PCSK9 inhibitors, no drugs were available to modify its levels. The reduction in Lp(a) levels by 24% vs. baseline seen in our study is consistent with data reported from large randomized studies [15].

Conclusion

Data from the first 314 patients treated with PCSK9 inhibitors in a Prague-based center of preventive cardiology confirm that PCSK9 inhibitors are a most effective, safe, and well-tolerated class of lipid-lowering agents. Their effect was uniform, sustained, clear-cut, and comparable with that reported by large randomized trials. Low-density lipoprotein cholesterol is generally recognized as a major risk factor for atherosclerosis and its complications. To succeed in our efforts to substantially reduce the incidence of atherosclerosis and its complications, LDL-C targets must be achieved in a greater proportion of patients. While LDL-C control across the subpopulations of our patients has not been satisfactory to date, the approval of PCSK9 inhibitors for the Czech market gives our healthcare providers a promising chance for reversing this unfavorable situation in the near future.

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Declarations

Conflict of Interest Tereza Altschmiedová, Michaela Šnejdrlová, Martin Šatný, and Richard Češka report grants and personal fees from Amgen and Sanofi, outside the submitted work.
Veronika Todorovová has nothing to disclose.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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•• Of major importance

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STATIN DRUGS (R. CESKA, SECTION EDITOR)

Statin Intolerance in Clinical Practice

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Abstract

Purpose of Review In our pilot study, we aimed to determine how many patients with the statin intolerance history referred to the specialized center for the diagnostics and treatment of lipoprotein metabolism disorders really suffer from a complete statin intolerance. The purpose of the study was to prove that complete statin intolerance is overestimated and overdiagnosed, and with the detailed knowledge of the issue and patient approach, it is possible to find an appropriate statin treatment for the most of patients.

Recent Findings With the increasing number of statin users worldwide, the issue of statin intolerance has been a frequently discussed topic in recent years. There are many factors that play a role in the manifestation of statin intolerance (predisposing factors as age, sex, and some diseases), genetic factors leading to a different metabolism, drug-drug interactions, psychological reasons, and the negative influence of the mass media. However, it is estimated that true complete statin intolerance, defined by an intolerance of at least three statins at their usual lowest daily doses, occurs in approximately 3–6% of all statin users.

Summary In our pilot study, we conducted a retrospective analysis of 300 patients who were referred to the Center of Preventive Cardiology with a history of statin intolerance. During the follow-up treatment, 222 patients (74%) were able to use some statin (rosu-, atorva-, simva-, fluvastatin), and in 21% of the cases (63 patient), the target values according their CV risk level were even achieved. Only 78 patients (26%) were confirmed as being complete statin intolerant following a thorough therapeutic effort. The most tolerated statin was rosuvastatin.

Keywords Complete statin intolerance · Partial statin intolerance · Statin associated muscle symptoms

Introduction

Statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) have been used for more than 30 years, not only for the treatment of dyslipidemia but, in particular, to reduce cardiovascular (CV) morbidity and mortality. Their effectiveness in reducing low-density lipoprotein cholesterol (LDL-C) level and CV risk in both primary and secondary prevention has been demonstrated in many randomized controlled trials (RCTs). Cholesterol Treatment Trialists' Collaboration meta-analysis, including 26 trials with 170,000 participants, followed up over a median of 5 years, demonstrated the 21% reduction in risk of major CV events (RR 0.79, 95% CI 0.77–0.81; $p < 0.001$ per 1 mmol/L in LDL-C reduction independent of baseline LDL-C levels). [1] However, the absolute benefit of statin therapy depends on the absolute risk of atherosclerotic CV event and the absolute reduction in LDL-C levels. The longer statin therapy is used, the greater the reduction of CV risk. [2]

Statins are generally safe and well tolerated, but the amount of information concerning statin intolerance has recently increased in the literature of both the scientific community and the general public. It is not surprising if we consider the rapidly increasing number of statin users. In USA, statin prescriptions have increased almost 80% over the past decade from 21.8 million individuals (17.9%) in 2002–2003 to 39.2 million individuals (27.8%) in 2012–2013 [3], the situation in all developed countries is similar.

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Statin intolerance can be defined as any adverse event (AEs) of statin treatment, considered unacceptable by the patient or some laboratory abnormalities, leading to its discontinuation [4]. In general, discontinuation of statin treatment due to laboratory abnormalities is less frequent, the most common manifestation of statin intolerance is so-called statin-associated muscle symptoms (SAMS) [5, 6]. Other side effects that may impair the patient's quality of life are nausea, dyspepsia, alopecia, erectile dysfunction, exanthema, or headache.

Myotoxicity of statins, like their pharmacological effect, is caused by HMG-CoA reductase inhibition and subsequently by reducing the production of mevalonate and other intermediates (genanyl-pyrophoshate, farnesyl-pyrophoshate, and squalene). Although the primary location of statin's effect is the liver, they can penetrate through the sarcolemma (especially lipophilic statins) and reduce the production of mevalonate and its metabolites in muscle fibers as well. Reduced squalene production leads to decreased levels of sarcolemmal cholesterol and to membranolysis. Decreased production of farnesyl-pyrophosphate leads to reduced amounts of ubiquinone (coenzyme Q10) and, thus, to mitochondrial dysfunction and attenuation of energy production, as well as decreased production of prenylated proteins, leading to altered gene expression, reduced protein synthesis, and apoptosis induction [7, 8].

Except of toxic muscle damage, in some rare cases the immunological muscle damage, called statin induced necrotizing autoimmune myopathy (SINAM), can occur. SINAM is characterized by creatine kinase (CK) elevation (CK levels between 10 and 100 times the upper normal limit), myopathic electromyography findings, and muscle biopsy usually showing necrosis with regeneration of muscle fibers and inflammation, mainly composed of macrophages. Muscle weakness and pain may develop even after prolonged administration of statins (months to years); in contrast with toxic damage, statin withdrawal does not usually lead to improvement in patient's symptoms, combination immunosuppressive treatment is required to obtain a clinical response. [9] However, SINAM is a very rare complication of statin therapy; according to recent trials, it develops in two to three cases per 100,000 patients treated with statins per year [10].

Statin intolerance is a very complex issue and many factors may be involved in its formation: age > 75 years, sex (more frequent in women), low body mass index, uncontrolled hypothyroidism, vitamin D deficiency, diabetes mellitus, acute infection, impaired renal and hepatic function, surgery with high metabolic demands, some neurological diseases (myasthenia gravis, primary myopathy), as well as rheumatological diseases (rheumatoid arthritis, polymyalgia rheumatica), nonspecific joint difficulties, excessive physical activity, and alcohol or drug abuse. In addition to these predisposing factors, also genetic factors leading to a different (slower) metabolism, drug-drug interactions, and, last but not least, psychological reasons and the negative influence of the mass media can play a major role in the development of statin intolerance.

Genetic Factors

The OATP1B1 (organic anion transport polypeptide 1B1) transport pump, encoded by the *SLCO1B1* gene, is used in the process of statin transporting to the hepatocyte. There is a loss of function variant C at rs4149056 (* 5) in the population which is associated with reduced activity of the hepatic OATP1B1 transporter and increased plasma concentrations of statin [11]. Heterozygous and homozygous carriers of the C variant of *SLCO1B1* gene had an OR for myopathy of 4.5 (95% CI, 4.7–61.1) and 17 (95% CI, 4.7–61.1) respectively, when compared with the TT homozygotes [12]. The transport dependence on OATP1B1 activity in the process of statins transport to hepatocyte differs between statins, the most is stimulated in the input of simvastatin, the least of fluva- and rosuvastatin (simva- > pitava- > atorva- > prava- > rosuva- > fluvastatin) [13, 14], so it is unknown whether prospective pharmacogenetic testing would improve outcomes for patients receiving statin treatment. By contrast, the importance of the P-gp pump activity based on the *ABCB1* gene polymorphism is very low, with atorvastatin or simvastatin exposure increasing by only 50% at low transport pump activity [15].

Drug-Drug Interactions

Drug-drug interactions play an important role in the statin intolerance issue. Lipophilic statins (in the order lova- > simva- > fluva- > atorva-) undergo transformation by cytochrome (CYP) isoenzymes during enterocyte absorption and liver passage, reducing their bioavailability and increasing the risk of drug-drug interactions. Likewise, they must be transformed again into the hydrophilic metabolite by the same oxidases before elimination. Rosuvastatin, pravastatin, and pitavastatin are hydrophilic; they are not significant substrates of metabolic systems, more than 95% of them are eliminated as the parent substance. The risk of drug-drug interactions is therefore much lower compared with lipophilic statins, the least with pitavastatin. Simvastatin, lovastatin, and atorvastatin are substrates of the glycoprotein P (P-gp) elimination pump and CYP 3A4 oxidase. P-gp and CYP3A4 form a functional whole, catalyzing oxidation in CYP3A4 lipophilic molecules, thereby increasing affinity for P-gp and allowing efflux from the cell (from the enterocyte to the intestine, from the hepatocyte to the biliary system, from the tubular nephron to urine). This binding provides a barrier against overexposure to xenobiotics—decreases absorption, increases elimination. A high affinity to the transport system reduces bioavailability and increases the risk of interactions. Simvastatin and lovastatin have low bioavailability (less than 5%); atorvastatin has around 15% bioavailability.

In clinical practice, co-administration of statins with verapamil, amiodarone, or propafenone, which are moderate inhibitors of both systems, is very common; exposure to simvastatin is then increased three times. If the potent inhibitor (clarithromycin or azole antifungals) is administered, exposure to simvastatin increases more than ten times. For atorvastatin, the increase in exposure is lower [16, 17]. These drug-drug interactions may play a very important role, in particular, when the statin treatment is initiated in secondary CV prevention at high doses (rosuvastatin 40 mg, atorvastatin 80 mg) immediately after myocardial infarction (MI) or stroke [18••, 19]. If the statins in this dose are given in combination with commonly used moderate CYP3A4 inhibitors, the actual exposure may be several times higher and the risk of side effects increased. The situation is different for fluvastatin. Fluvastatin is a light P-gp substrate and at the same time a moderate substrate and an inhibitor of the CYP2C9 isoenzyme. There is no evidence for drug-drug interactions at this level to significantly affect the incidence of myalgia, while co-administration of fluvastatin with CYP2C9 substrates (e.g., warfarin) may increase their exposure [20]. Significant drug-drug interactions may also occur at the OATP1B1 level, as many of the commonly used drugs (termisartan, candesartan, and non-steroidal anti-inflammatory drugs) significantly inhibit the activity of this pump and increase the statin plasma concentration, the most in the case of simvastatin (200–300%), and the least for fluvastatin and rosuvastatin (by 20–70%) [13, 14, 21].

In the list of drug-drug interactions, it is necessary to note the increased risk of myopathy, while concomitantly using statins with fibrates, the highest risk of myopathy is observed in the first 12 weeks of treatment [22]. The causes may be pharmacokinetic (fibrates, especially gemfibrozil, inhibits glucuronidation, which is important in the metabolism of lova- and simvastatin) as well as the influence on peroxisome proliferator-activated receptors (PPAR), which subsequently affect CYP regulation [22, 23]. Gemfibrozil also reduces the renal clearance of pravastatin and increases pravastatin and rosuvastatin concentrations by decreasing their biliary excretion [22]. Drug-drug interactions with gemfibrozil are the most serious; however, they can also be seen with other fibrates (bezafibrate, clofibrate, and fenofibrate); the incidence of rhabdomyolysis in combination with statin plus fenofibrate is 15 times lower compared with the combination statin plus gemfibrozil.

Mass Media Influence and Psychological Reasons

As the number of patients using statins increases, the absolute number of patients who do not tolerate this treatment increases. It is, therefore, not surprising that statin intolerance is the subject of many scientific and non-scientific articles published in newspapers and magazines. This information is then reflected in the patient's adherence to treatment. This issue was the subject of a Danish retrospective trial including almost 700, 000 patients aged 40 or older, who were initiated on statin therapy in 1995–2010 and followed them until the end of 2011. The prevalence of patients using statins increased from < 1% in 1995 to 11% in 2010, early statin discontinuation increased from 6% in 1995 to 18% in 2010. Focused on mass media influence—the OR for early statin discontinuation vs. continued use were 1.09 (95%CI, 1.06–1.12) for negative statin-related stories and 0.92 (95%CI, 0.90–0.94) for positive statin-related stories. Statin discontinuation led to more frequent cardiovascular events; during the follow-up, the hazard ratio for individuals with vs. without early statin discontinuation were 1.26 (1.21–1.30) for myocardial infarction and 1.18 (1.14–1.23) for death from cardiovascular disease [24].

The statin intolerance issue is very various, not only due to clinical manifestations, but to its severity as well. Some patients do not tolerate any statin, some of them do not tolerate only some statins, or statins at higher doses. From a clinical point of view, it has proved useful to differentiate statin intolerance into complete and partial. Complete statin intolerance means the inability to tolerate at least three different statins at the lowest daily doses (i.e., rosuvastatin 5 mg, atorvastatin 10 mg, simvastatin 20 mg, lovastatin 20 mg, pravastatin 40 mg, fluvastatin 20 mg, and pitavastatin 2 mg). Partial intolerance means a milder form; the patients tolerate statin therapy but do not tolerate it at doses needed to achieve LDL-C targets according to their CV risk level [4].

Methods and Results

At the Center of Preventive Cardiology of the General University Hospital in Prague, we performed a retrospective evaluation of anamnestic data and laboratory results of 300 patients who were referred to the center suspected to be statin intolerants with a request for further therapy recommendation. Statin intolerance was defined as an adverse event of statin therapy; patients were different in the number of tested statins, in their doses, and in the time period after which the adverse events occurred after statin administration. In the vast majority of cases, however, these data were not included in the documentation at all; necessary anamnestic data and time connections were obtained only during examination and follow-up at the Center of Preventive Cardiology.

In the group of 300 patients, women were more represented (190 women vs. 110 men), average age was 65 ± 12 years with 60 patients being over 75 years. A quarter of them (75 patients) were in secondary prevention of CVD, three-quarters in primary prevention of CVD; however, 79% of them (178 patients) were at high risk of CVD (SCORE > 5%). The most frequent side effect of statin treatment resulting in the discontinuation of statin therapy was muscle discomfort, which were

reported in 233 patients (77.6%), with 210 patients (70%) having muscle pain, 23 patients (7.6%) declaring muscle weakness and performance reduction, and half of these 23 (12 patients) following exercise and physical activity. Creatine kinase (CK) elevation was reported in 48 patients (16%). A relatively high percentage of patients (130 patients, 43.3%) experienced other side effects (nausea, dyspeptic problems, diarrhea, alopecia, rash, erectile dysfunction, and insomnia), separately or in combination with SAMS; in 36 patients (12%), these non-specific difficulties were the reason for discontinuation of treatment.

During the first examination, their medical histories were evaluated in great details; patients were asked about the circumstances of the adverse event occurrences and the timing to the initiation of treatment. Difficult issues of statin intolerance were explained to them, especially the principle of statin treatment. Many patients did not understand the association between lowering LDL-C and CV morbidity and mortality reduction. This step has already helped to elucidate a number of false cases of statin intolerance where muscle pain or other complaints occurred as a result of an acute infection or other concomitant disease (surgery), increased physical activity, etc.

During the follow-up treatment in the Center for Preventive Cardiology, 222 patients (74%) referred as statin intolerant patients were able to use some statin (rosuvastatin, atorvastatin, simvastatin, and fluvastatin) used in the Czech Republic; lovastatin and pravastatin were not used in our group of patients. In 21% (63) of these complicated patients, the target values according to their CV risk level were even achieved. Only in 78 patients (26%) was complete statin intolerance confirmed despite all therapeutic effort.

In the group of patients in secondary CV prevention (75 patients), thus at very high CV risk with a LDL-C target < 1.4 mmol/L, only 16 patients (21.3%) achieved the LDL-C target, 78.6% (59 patients) had LDL-C > 1.4 mmol/L. In the high-risk group (SCORE ≥ 5% and < 10%, 178 patients) with target value LDL-C < 1.8 mmol/L, 24 patients (13.5%) reached the target despite limited statin treatment options, and 154 patients (86.5%) did not achieve the LDL-C target. Best results were achieved in patients at moderate CV risk (SCORE ≥ 1% and < 5%, 47 patients), 23 patients (48.9%) achieved their LDL-C target value <2.6 mmol/L, and 24 patients (51.1%) had LDL-C > 2.6 mmol/ L. These patients were treated with statins because of other risk factors for CV disease, especially with a high-risk family history or adverse lipid profile (concurrent hypertriglyceridemia).

Of the 222 patients who were eligible for statin therapy, 124 patients (56%) received rosuvastatin, 56 patients (25%) atorvastatin, 27 patients (12%) fluvastatin, and only 15 patients (7%) simvastatin.

In the next step, we focused on a detailed evaluation of doses of two most tolerated and most efficient statins—rosuvastatin and atorvastatin (Fig. 1).

With the exception of common doses of rosuvastatin (10, 15, 20, 30, and 40 mg), 52 patients (42% of all patients taking rosuvastatin) received rosuvastatin at a dose lower than 10 mg per day, including atypical doses several times a week, two patients received 5 mg rosuvastatin once a week, and two patients 5 mg twice a week. Remarkably, although these patients were reported to be complete statin intolerants, four of

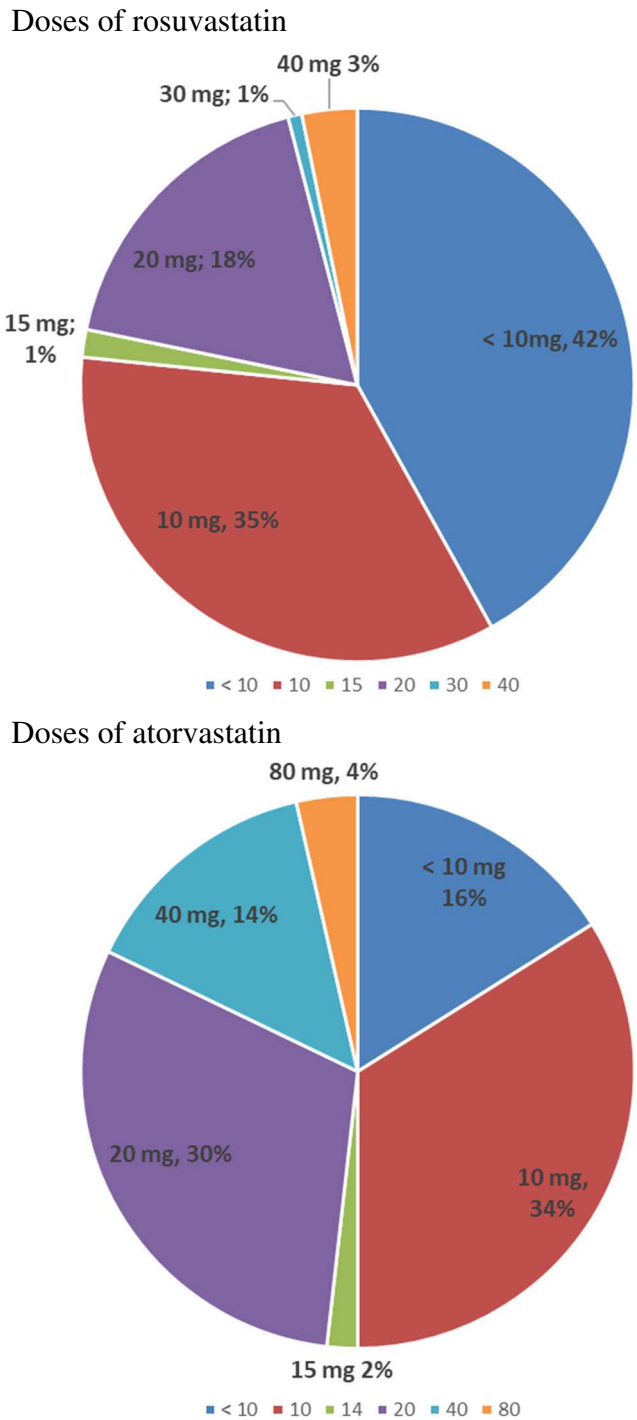

Fig. 1 Detailed evaluation of doses of two most tolerated and most efficient statins: **a** Doses of rosuvastatin. **b** Doses of atorvastatin

them received and have tolerated 40 mg of rosuvastatin, a dose corresponding to high-dose statins.

In the group of patients receiving atorvastatin, only nine of them (16% patients) used less than 10 mg per day. The remaining 86% (47 patients) received atorvastatin despite a baseline history of statin intolerance at common doses (10,15, 20, 40, and even 80 mg daily), a total of ten patients tolerated atorvastatin at dose ≥ 40 mg/day.

Retrospective processing of statin intolerant health data has included the period when PCSK9 inhibitors were already available in the Czech Republic; however, due to the discrepancy between recommended LDL-C target values according to the last European Society of Cardiology Guidelines and reimbursement conditions of health insurance companies for PCSK9 prescription in the Czech Republic (LDL-C > 3 mmol/L for patients in secondary CVD prevention, LDL-C > 4 mmol/L for patients with familial hypercholesterolaemia), patients receiving this highly efficient lipid-lowering therapy were excluded from our follow-up. However, we focused on the proportion of patients taking ezetimibe. Ezetimibe was used in less than a quarter patients (74 patients, 24.7%); the majority of these patients (51 patients) used it in combination therapy, 23 patients used it alone. When comparing efficacy in achieving the LDL-C target value, in the group of patients treated with combination therapy statin + ezetimibe ,41% of patients achieved the LDL-C target value (for all risk categories) compared with a 21% success rate in the whole group. Ezetimibe is a well-tolerated drug; however, in 50 patients (16%), intolerance manifested by gastrointestinal symptoms (flatulence, diarrhea, and nausea) or hepatopathy were reported.

Discussion

Statin intolerance has been a frequently discussed topic in recent years. Complications associated with statin treatment occur in 1–29% of users, according to data origin. In the randomized clinical trials, there is an incidence of statin intolerance about 1–5%, registers and observational studies are higher ranging from 11 to 29%. There are limitations to both of them—randomized trials generally exclude patients at risk of developing myopathy (elderly, patients with a history of muscle pain, patients with neurological and rheumatic diseases)—while observational studies usually lack control groups [25••].

In our pilot study of 300 patients suspected of being statin intolerant, we demonstrated that this is a very complex and difficult issue. With knowledge of all risk factors for statin intolerance and patient step-by-step treatment, we found a suitable statin treatment at the highest tolerated dose in 74% patients, whereas, only 26% of patients were confirmed as being complete statin intolerant. This number is relatively high compared with Canadian authors [26], where 20–30% patients were suspected of being statin intolerant, but diagnosis of statin intolerance was confirmed in about 5–6% patients. According to an evaluation by Banach and colleagues [27, 28], a very individual approach with the exclusion of all possible risk factors a diagnosis of complete statin intolerance was confirmed in only 2–3% of patients. However, our study population was not a general population, but patients are already suspected of being statin intolerants.

In accordance with the results of the USAGE trial [29], the majority of patients in our group were women (190 women, 63.3% of the group, vs. 110 men, 36.6% of the group;, however, the percentage of real statin intolerance were similar in each of them—complete statin intolerance was confirmed in 26.8% of women and 24.5% of men. Another reported risk factor for the development of SAMS is age over 75 years, but only 42 patients (14%) in our group were over 75 years confirming that the higher incidence of SAMS is not directly related to age. In RTCs, a higher frequency of muscle symptoms among elderly patients treated with statins compared with placebo was not reported, more frequent adverse events are caused by other factors (decrease in lean body mass, reduction in albumin level, etc.) [30] In old age, they are in etiopathogenesis of muscle symptoms more often involved impaired renal and hepatic function, drug-drug interactions, pre-existing joint and muscle pain from other causes, etc. Younger age categories (in our group 50 individuals younger 50 years, 16%) can play the role, with the exception of genetic predispositions, regular physical activity. It is known that muscle symptoms and CK elevation occur more frequently in physically active individuals during and after exercise. It is generally known that muscle pain is more common in active athletes using statins as shown in the Prediction of Muscular Risk in Observational conditions (PRIMO) study where more intense physical exercise increased the chance of myopathic symptoms. Furthermore, it was found that 14% of subjects participating in intensive forms of sports had statin-related muscular symptoms vs 10.8% of subjects performing less active physical exercise [31]. But not only muscle pain, muscle weakness and performance reduction can be a reason to discontinue statin therapy as well. In a randomized study of 1016 healthy individuals receiving simvastatin 20 mg or pravastatin 40 mg compared with placebo, a significant reduction in energy and higher exertional fatigue have been reported [32]. In our group, muscle weakness and performance reduction in the past were the cause of discontinuation of statin therapy in 23 (8%) patients with half of them occurring as a result of exercise and physical activity. According to the recommendation of International Lipid Expert Panel (ILEP), individuals with regular intense physical exertion should consider low to moderate intensity statin therapy. In patients on statin therapy, a reduction of dose or therapy discontinuation should be considered for at least 2 days before scheduled intense physical

exertion. Any such decision should be balanced with the risk of discontinuing statins. [27•].

A very specific sub-question concerns the issue of drug-drug interactions. According to some groups (ILEP [27•] and the Canadian Consensus Working Group [33]), in order to classify the muscle symptoms as SAMS, possible drug interactions must be first excluded. In our group of patients with statin intolerance, we did not change other cardiological medications (amiodarone, propafenone, verapamil, telmisartan, and candesartan), and when necessary, the statin doses were adjusted (decreased). The best tolerated statin in our group was rosuvastatin, which is not surprising due to its pharmacological profile (hydrophilic statin with minimal drug interactions). However, despite good tolerance, almost half of the users (42%) tolerated rosuvastatin in doses lower than 10 mg daily, including atypical dosing schedules several times a week. This approach is also supported by work in which the administration of rosuvastatin 1–2 times a week, at an average dose of 10 mg per week, resulted in a reduction of LDL-C of 23–29% and was well tolerated in up to 74–80% of patients [34, 35]. Despite lipophilicity and a high risk of drug interactions, atorvastatin was the second most frequently used statin, in 86% of the cases at common doses, 18% of atorvastatin users tolerated even higher doses (≥ 40 mg per day). This fact only confirms that the diagnosis of complete statin intolerance is often attributed to patients inaccurately after the questionable intolerance of another statin.

Conclusion

Statins are very effective drugs that significantly reduce cardiovascular morbidity and mortality; their efficacy as well as safety have been demonstrated in many randomized clinical trials. Statin intolerance has been increasingly discussed in recent years, but it should be noted that complete statin intolerance is extremely rare, overestimated, and overdiagnosed in clinical practice. The issue of statin intolerance is very complex, many predisposing factors can be applied, as well as genetic, pharmacological, and psychological factors, all of them must be taken into account during the start of the statin treatment and the next follow-up. The patient step-by-step approach can detect many removable risk factors, appropriately titrate the statin dose, prevent side effects, and improve the adherence to treatment.

The objective evaluation of statin intolerance is very difficult. The main drawback of this issue is the absence of any objective marker that would be useful when assessing the severity of statin intolerance. Except of CK elevation or hepatic transaminase alteration, which occur only occasionally, in most cases, it is possible to assess only the anamnestic data and patient's subjective evaluation. Moreover, most of these data were anamnestic, statin treatment was started by other physicians before the first examination in the Center of Preventive Cardiology, and the willingness to try other statins at a different dose in patients with experience of muscle pain during the statin treatment is significantly lower. The great advantage of our work is the experience of all participating physicians; patients with statin intolerance are very common clients in Center of Preventive Cardiology. The submitted work is a pilot study; evaluation of a larger number of patients will certainly be beneficial.

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Compliance with Ethical Standards

Conflict of Interest Dr. Snejdrlova reports grants from Ministry of Health, Czech Republic—conceptual development of research organization 64,165, General University Hospital in Prague—during the conduct of the study; personal fees from Amgen, personal fees and non-financial support from Sanofi, personal fees and non-financial support from Servier, and personal fees and non-financial support from Mylan, outside the submitted work.

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Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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EVIDENCE-BASED MEDICINE, CLINICAL TRIALS AND THEIR INTERPRETATIONS (L. ROEVER, SECTION EDITOR)

The Impact of the International Cooperation On Familial Hypercholesterolemia Screening and Treatment: Results from the ScreenPro FH Project

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Abstract

Purpose of Review Familial hypercholesterolemia (FH) is often perceived and described as underdiagnosed and undertreated, though effective treatment of FH is available. Owing to the mentioned facts, it is ever more imperative to screen and treat FH patients. Subsequent to the identification of patients, the project focuses on the improvement of their prognoses. The ScreenPro FH project was established as a functional international network for the diagnosis, screening, and treatment of FH. Individual countries were assigned goals, e.g., to define the actual situation and available treatment. With "central support," more centers and countries participated in the project. Subsequently, individual countries reported the results at the beginning and end of the project. Collected data were statistically evaluated.

Recent Findings The increasing number of patients in databases, from 7500 in 2014 to 25,347 in 2018, demonstrates the improvement in overall effectiveness, as well as an increase in the number of centers from 70 to 252. Before all, LDL-C decreased by 41.5% and total cholesterol by 32.3%. As data from all countries and patients were not available at the time of the analysis, only those results from 10 countries and 5585 patients at the beginning of the project and at the time of writing are included.

Summary Our data are quite positive. However, our results have only limited validity. Our patients are far from the target levels of LDL-C. The situation can be improved with the introduction of new therapy, PCSK9-i, evolocumab, and alirocumab. International cooperation improved the screening of FH and finally led to an improvement in cardiovascular risk.

This article is part of the Topical Collection on *Evidence-Based Medicine, Clinical Trials and Their Interpretations*

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Introduction

Familial hypercholesterolemia (FH) is still, despite great recent progress, underestimated, underdiagnosed, and undertreated, and it represents a significant problem as a common risk factor for the premature development of coronary heart disease (CHD) [1, 2••]. FH is a monogenic disease transmitted through autosomal dominant inheritance and stems from either an LDL-R defect, familial defective apolipoprotein B-100 (FDB), or PCSK9 gain-of-function mutations [3, 4•].

FH is an example of a disease that, by its very nature, allows us to study the relationship between lipid metabolism, especially LDL-cholesterol (LDL-C), and atherosclerosis, as well as the premature manifestation of cardiovascular disease (CVD) [1, 5]. FH occurs with a frequency of 1:250–1:500 and is one of the most common congenital metabolic disorders [6].

When we started to monitor and study FH, we primarily dealt with the mechanism of the development of the disease, and its genetic background and epidemiology [7–9]. As for the prognosis of patients, there was very little we could do. There were no options other than selecting the highest-risk patients with increased concentrations of lipoprotein/a/ (Lp/a/) [10] and trying to influence the most important risk factor beyond the scope of pharmacological treatment: the smoking of cigarettes [11]. However, the situation changed dramatically at the end of the 1980s, and especially in the 1990s, when statins were introduced to the market. Statins were widely used, and initially, their priority use was appropriate for the treatment of FH [12, 13]. In practice, treatment with statins has had an immediate impact as shown by a dramatic decrease in mortality, especially in FH patients under the age of 40 [14–16]. Later, the pharmacotherapy of FH was boosted by the introduction of ezetimibe [17, 18].

Though effective treatment was possible, interest in FH was relatively low; this situation led to numerous initiatives, both on the national and international level, such as the MedPed (Make Early Diagnosis in Medical Pedigree) project [19•], and the FH Foundation to name a few [20]. It should be noted that the Czech Republic, as the lead country of the described project, became one of the most successful countries in the identification of FH patients not only in Europe, but globally [21]. Although these activities were successful, most patients remained undiagnosed, were treated with low doses of medicaments, and did not receive the maximum therapy [22, 23]. In addition, even those patients cared for in specialized centers did not often reach the target values and LDL-C values in FH patients remained high above the upper limit of normal [24]. Of course, the cardiovascular (CV) risk also remained very high.

This is why both physician and patient communities involved in the field of FH appreciated the introduction of a novel generation of medicines into this field. Anti-PCSK9 monoclonal antibodies, also called the biological treatment for hypercholesterolemia or, simply, PCSK9-inhibitors (PCSK9-i), introduced the possibility of decreasing LDL-C levels by 40–60% when used additively to the maximum tolerated dose of lipid-lowering drugs [25]. They have been investigated in various populations, including FH [26]. Not only do they decrease the levels of LDL-C, but also Lp/a/ which is another independent risk factor for CV diseases [27, 28]. However, the most important fact in support of the use of these medicines in the treatment of FH is the results of randomized clinical studies in tens of thousands of enrolled patients with evolocumab (Fourier) [29, 30•], as well as those of alirocumab (Odyssey Outcomes) [31•], which show a reduction in CV event occurrence in a remarkably short time and, in one subanalysis, even a decrease of overall mortality.

Also, the other medications for LDL-C lowering are in development, e.g., bempedoic acid and others [32]. It means that potent and powerful therapy becomes available for FH patients. The identification of FH patients and other highest-risk patients subsequently became one of the priorities of current preventive cardiology and clinical lipidology.

Aim

Clearly, the aim of the project is to improve the identification, diagnostics, and treatment of FH patients in the regions of Central, Eastern, and Southern Europe (CESE). The standards of care for FH patients, as well as the identification of probands and affected members of families, greatly vary from country to country in the region. The awareness of FH among both experts and the general population varies as well. Therefore, when building the lipid center network and performing educational activities, exploiting the knowledge of more successful countries is a deciding factor. The ultimate objective of the project is the improvement of the lipid profile, the total CV risk and, finally, the improvement of the patient prognosis.

Methods

The ScreenPro FH Project is an international project dedicated to the improvement of complex care—screening, diagnosis, and treatment of FH in CESE. Originating in seven countries, it allowed us to identify enthusiastic country leaders and create national and international networks of lipid centers coordinated by the project leaders. Individual countries were then set goals, the first of which was to define the actual situation and to determine the available treatment. From this point on, the

project leaders regularly provided each country with information and instructions sent electronically or introduced during business meetings held in conjunction with major international congresses. With such "central support" (materials, education, web-based information), more and more centers and countries participated in the project.

Upon completion of the three-year project, each country reported the baseline results (from the beginning of the project) and results after the inclusion of the patients to the national database. Nowadays, the basic lipid parameters are available.

Data from individual countries included the number of FH patients, and averages and standard deviations of lipid parameters from the beginning of the project and after the inclusion of the patients to the national database. Average values of lipid parameters of individual countries were summed in relation to the number of patients, and the difference of the given lipid parameter values was evaluated. The results were evaluated using STATISTICA 13 software. All conducted tests were both-sided. The established level of significance was $\alpha = 0.05$ in all tests. It must also be stressed that all the data from participating countries are aggregate data, not individual patient data.

Description of the Situation in Countries

The countries involved in the ScreenPro FH project comprise about 500 million inhabitants in total. If we consider the prevalence of FH 1:250–500 [33], it constitutes approximately 1–2 million people suffering from this genetic disease. Although up-to-date results and analyses support the theory that the occurrence of FH in the overall population is 1:250, the participating countries have long estimated a prevalence of 1:500. The actual number of FH patients is, therefore, much higher in each individual country, and the rate of diagnosed cases is, on the contrary, much lower.

Bosnia and Herzegovina

Approximately 7000 people, in a country of 3.5 million, suffer from FH. Other than the National Centre in Zenica, there are two more centers operating in Bosnia and Herzegovina. The number of diagnosed FH patients, or, rather, FH patients registered in the database, is 1500; there is neither a lipid network nor patient organization in the country. Potential patients are selected from hospital databases based on an LDL-C level higher than 5 mmol/L. Diagnostics is based on the Dutch Lipid Clinic Network Criteria (DLCNC). The only treatment available to doctors is statins, with no option of combining them with ezetimibe or PCSK9-i. LDL apheresis is not an option in Bosnia and Herzegovina either. The FH program is focused on educational activities for general practitioners for children and adults, internists, and ophthalmologists.

Bulgaria

Bulgaria has 7.2 million inhabitants and the estimated number of FH patients is 14,000. The database compiles data from the national center, as well as from six other centers, and consists of 220 patients. The FH program commences at Intensive Cardiac Care Units, i.e., using patients with previous case histories of CVD. Diagnostics is based on the DLCNC. Therapy is based on statins which can be combined with ezetimibe, and upon achieving six or more points, based on the DLCNC, patients can also receive PCSK9-i therapy.

Croatia

In a country of 4.2 million inhabitants, the estimated number of FH patients is approximately 8500 with 150 patients included in the database. Croatia boasts a national center at the University Hospital in Zagreb, and four more centers are being planned. The existing lipid network is based on the MedPed project which is also the basis of the National MedPed program. The DLCNC are applied for the diagnostics of the disease, with treatment options including not only statins and ezetimibe but also PCSK9-i or LDL apheresis. Target LDL-C levels are based on the available recommended methods. No patient organization has been established in the country.

Czech Republic

The Czech Republic has 10.5 million inhabitants; thus, the estimated number of potential FH patients is more than 21,000. The country has a rich network of 69 centers including national centers in Prague at the General Faculty Hospital (VFN) and in Brno at St. Anne's Hospital. These centers are already cooperating with more than 8000 patients, and the FH program supported by the Czech Society for Atherosclerosis cooperates closely with this network of lipid centers. Coordinators help physicians to operate the centers and to enter patients into the database. Diagnostics is based on the modified MedPed criteria with genetic testing available. Statins, ezetimibe, and PCSK9-i are available for treatment. Two centers offer also LDL apheresis. A patient organization was established and operates in the country.

Georgia

There is one center in Georgia, however, whose number of registered patients is unknown. In this country of 3.7 million inhabitants, the estimated number of FH patients is 7500.

Greece

Greece has almost 11 million inhabitants, and this corresponds to an estimated 21,000 FH patients. More than 600 patients

have been diagnosed and are included in the database. The national center at the University Hospital in Ioannina cooperates with eight other centers. The FH program is based on a functioning network of lipid centers contributing to the national Hellas FH register. The DLCNC are used in diagnostics, and treatment options include statins, ezetimibe, and PCSK9-i. Four centers also perform LDL apheresis. A patient organization is also available for patients.

Hungary

In this country of 9.8 million inhabitants, the estimated occurrence of FH patients is 20,000 with 300 patients having been integrated into the ScreenPro FH database and monitored by two national centers in Debrecen and Budapest. Genetic analyses, sponsored by scientific grants, are also performed there, as well as in 18 regional centers. Hungary can be considered a country with a functioning lipid network. FH is diagnosed based on the DLCNC, and all the treatment modalities—statins, ezetimibe, PCSK9-i, and LDL apheresis—are available; the target levels of LDL-C are 1.8 mmol/L. An umbrella patient organization was also created for patients.

Kazakhstan

The Republic of Kazakhstan has 18.5 million inhabitants. The potential number of FH patients can thus be up to 40,000. Nevertheless, there is no information available on the number of patients included in the database.

Kyrgyzstan

In Kyrgyzstan, with a population of 5.8 million inhabitants, the occurrence of FH patients is estimated to be 11,700; these patients can be monitored in the national center in Bishkek or in 19 regional centers. The FH program deals with the analysis of the FH prevalence in patients with a premature manifestation of CVD, metabolic syndrome, and subsequent primary or secondary prophylaxis. Three hundred one patients, with diagnoses based on the DLCNC, are included in the database. Treatment options include statins, and LDL apheresis is also available. No patient organization has been founded yet.

Latvia

With a population of almost 2 million inhabitants, the occurrence of FH patients in Latvia is estimated to be approximately 4000, with an aggregate summary of data on 249 patients reported in the ScreenPro FH registry by the end of February 2019. Patients are monitored in the national center in Riga within the frames of the Latvian Registry of FH that was established in 2015 [34]. The registry currently is not financed by the government or any other organization, but it has effectively improved detection of cases from < 0.2% in early 2015 to more than 3% in early 2019. Index cases are diagnosed based on the DLCNC. The cascade screening is performed, and relatives are diagnosed based on 95th percentile of LDL-C. Statins, ezetimibe, and PCSK9-i are available, but only statins are 50% reimbursed for FH. LDL apheresis is not performed in this country. There is a working patient organization "ParSirdi.lv."

Lebanon

Lebanon's anticipated rate of FH incidence is 25 times higher than in Europe; i.e., in a land of 7.8 million inhabitants, the estimated occurrence of FH patients is 15,500, with only 38 patients included in the database. The higher incidence can be explained by the so-called founder effect and by the high number of marriages between blood relatives. A phenomenon called the Lebanese allele was described: qualifying FH in up to 81.5% of examined probands [35, 36]. There is one functioning national center in the country developing the FH program. Patients can be offered treatment with statins or ezetimibe; LDL apheresis is not available in this country, and no patient organization has been founded yet.

Lithuania

In the Lithuanian population of 2.9 million inhabitants, the occurrence of approximately 6000 FH patients is projected, with less than one-third of these patients included in the ScreenPro FH project. The national center was found in the capital, Vilnius, and four regional centers are being built. The Lithuanian High Cardiovascular Risk Primary Prevention Program (LiTHiR), started in 2006 and covered by health system, is the base for the functioning lipid network in the country. More than 250,000 middle-aged adults are screened every year and receiving primary prophylaxis. Data of > 92,000 individuals is currently included in the electronic database for detailed analysis. The prevalence of any dyslipidemia (DLP) among these patients is estimated to be 89%, and the prevalence of any type of severe DLP is 13.4%. The occurrence of patients with LDL-C levels ≥ 6 mmol/l in screened population is 3.2%, and in the subgroup of severe DLP, 24%. FH is diagnosed based on DLCNC. As far as treatment is concerned, patients can be offered statins, ezetimibe, and PCSK9-i, as well as LDL apheresis which is performed in one center. Genetic testing is available. The target is to achieve LDL-C levels in accordance with current European recommendations for the management of DLP treatment. Patient organization is being built under the umbrella of Lithuanian Heart Association.

Oman

Oman has a population of 5.2 million inhabitants, and the occurrence of FH patients is estimated to be approximately 10 thousand. Thirty-eight patients are included in the database.

Poland

Poland has a population of 38 million inhabitants, and 76,000 FH patients are to be expected, whereas fewer than 2000 have been diagnosed. Two national centers and seven other centers contribute to a functioning lipid network. The Polish national FH program is based on complex care for patients suffering from lipid metabolism disorders; diagnostics uses the DLCNC or Simon Broom Criteria. The program is focused on the selection of high-risk patients with the use of cascade screening in families with the option of genetic testing also available.

Romania

Almost 20 million people live in Romania, and taking into account the rates of occurrence we considered, up to 40,000 FH patients are assumed. The actual number of diagnosed patients is 69. The CardioPrevent Foundation Timisoara is the national center, and no other centers have been founded yet. No lipid network exists. Diagnostics is based on the DLCNC. Statins are available for treatment which can be combined with ezetimibe or PCSK9-i. LDL apheresis is not available. Target LDL-C levels depend on the degree of CV risk. No patient organization has been founded yet.

Russia

In a population of almost 147 million inhabitants, up to 300,000 patients suffering from FH are expected and 1400 FH patients have been successfully introduced to the database. The national Cardiology Research Center operates in Moscow, and 27 additional centers contribute to the lipid network. FH is normally diagnosed based on the DLCNC with DNA diagnostics also available. Treatment options available in Russia include statins and ezetimibe, as well as PCSK9-i; 10 centers also perform LDL apheresis. A patient organization is working in the country.

Serbia

Serbia has approximately 7.2 million inhabitants; thus more than 14,000 FH patients can be expected. Nine hundred of them have been included in the database. No specific FH program is available, there are eight regional centers and one national center functioning in the country with centralized screening, diagnostics, and treatment. Patients are referred to this site primarily by general practitioners. The DLCNC are used for diagnostics while statins, ezetimibe, and PCSK9-i are used for treatment, with LDL apheresis being available.

Slovakia

With a population of 5.4 million inhabitants, we expect up to 11,000 FH patients in Slovakia and more than 2500 of them are already included in the database. An extensive lipid network is established in the country; apart from the national center in Bratislava, there are 26 other centers (6 centers for pediatric patients). FH diagnostics can be performed based on the DLCNC, the Simon Broom Criteria, or the MedPed Criteria which is also the base of the Slovak FH program. Target LDL-C levels are < 2.5 mmol/L for patients in primary prophylaxis and < 1.8 mmol/L for patients in secondary prophylaxis. These levels can be achieved using statins, ezetimibe, PCSK9-i, and LDL apheresis. FH patients are united in a working patient organization.

Slovenia

In this country with a population of over 2 million people, more than 4000 FH patients can be expected. More than 50% of the considered number have been diagnosed and are included in the database. There are two University centers operating in Slovenia and a specialized network of lipid clinics. Unfortunately, no more information is available.

Turkey

There are approximately 168,000 FH patients in this land of 84 million people with 3159 patients already integrated into the ScreenPro FH project database. As far as the management of the treatment of patients is concerned, there is a national center in Izmir and 31 regional centers. The FH program is based on the Adult HoFH Apheresis Registry, and the creation of a functioning lipid network (which had not existed in the county until now) is being planned. The diagnosis of FH is made based on the DLCNC; treatment modalities are represented not only by statins, ezetimibe, or evolocumab (only for patients with homozygous FH) but also by LDL apheresis which is performed in 18 centers. Patients are treated to achieve target LDL-C levels of compliancy described by current European guidelines. There is also a working patient organization in the country.

Ukraine

In Ukraine, with a population of 43 million inhabitants, the occurrence of approximately 86,000 FH cases can be expected; 147 patients have been included in the ScreenPro FH database so far. A lipid network is being developed in the country to include a national center and four regional centers. The objective of the FH program in the country is to actively

search for patients with suspected FH; FH should be diagnosed with the use of the DLCNC or Simon Broom Criteria. Patients can be treated with statins and ezetimibe to achieve the target LDL-C levels of less than 1.8 mmol/L and 2.5 mmol/L, respectively, based on the category of CV risk. LDL apheresis is not available, and no patient organization has been founded in the country yet.

Uzbekistan

The occurrence of potential FH patients in Uzbekistan, with a population of 31 million inhabitants, is estimated to be 62,000, though there are only 106 cases in the ScreenPro FH project database. A lipid network has been created in the country, albeit at a slow pace, with the national center in Tashkent and four regional centers. The objective of the FH program is to introduce a personalized approach to the treatment of DLP patients. In these patients, the diagnosis is established based on the DLCNC; not only is treatment available with statins, but also with LDL apheresis which can be performed at two private clinics. Target LDL-C levels are set to 1.8 mmol/L. Patients with a diagnosis of FH can also register in a patient organization under the auspices of the Republican Specialized Center of Cardiology (RSCC).

Results

Ten of the 22 countries of the CESE region took part in the project to search for FH patients and investigated the effect of the care for these patients in specialized regional center networks on the levels of total cholesterol, triglycerides, LDL-C, and HDL-cholesterol (HDL-C). These countries included the Czech Republic, Bosnia and Herzegovina, Lithuania, Latvia, Hungary, Russia, Serbia, Slovakia, Uzbekistan, and Ukraine. During the project, levels of lipid parameters were subsequently obtained from 5585 of the 9065 monitored patients. In all countries, with the exception of Ukraine, the levels of total cholesterol, triglycerides, and LDL-C during the project were always statistically significantly lower ($p < 0.001$) than at their inclusion in the project. A substantial decrease of approximately 41.5% was noticed in LDL-C levels and by approximately 32% in total cholesterol levels (Table 1). The decrease in the triglyceride levels in the monitored countries was approximately 16% while there was almost no difference in the HDL-C levels.

Patient representation was markedly different in each of the 10 countries (Appendix Table 6). In the Czech Republic, only 3256 patients, from the original group of 4045 patients included in the project, were continuously monitored. This situation was similar in Russia and Slovakia, where 699 patients from the original group of 1200 patients and 200 patients from the original group of 2246 patients, respectively, were continuously monitored. In Bosnia and Herzegovina, 343 patients were included in the project and continuously monitored. Similarly in Serbia, Uzbekistan, and Ukraine, the number of patients monitored were 302, 106, and 147 respectively. Small differences between the numbers of monitored patients at the project entrance in comparison with the number of patients monitored during the project were seen in Lithuania, Latvia, and Hungary. In Lithuania, there were 98 patients included in the project and this number increased up to 100 patients in the course of the study. In Latvia, there were 249 patients included in the project and 105 patients had at least one follow-up visit. In Hungary, there were 329 patients included in the study and 327 patients continued; thus, a minimum decrease of patients was observed.

The levels of triglycerides and HDL-C were compared in only nine selected countries (Tables 2 and 3). Hungary was not included in the comparison of these two lipid parameters. However, the levels of total cholesterol and LDL-C were compared in all selected countries (Tables 4 and 5). The decreases in the levels of total cholesterol, LDL-C, and triglycerides were statistically significant in all selected countries with the exception of Ukraine ($p < 0.001$) where a statistically significant decrease was found only in the triglyceride levels ($p < 0.001$). The decreases in the levels of total cholesterol and LDL-C were not statistically significant ($p = 0.276$ and $p = 0.068$, respectively) in Ukraine, though a decrease in the values of these two lipid parameters was reported. As far as total cholesterol is concerned, a significant decrease in its levels was observed in Bosnia and Herzegovina, and Latvia (Fig. 2), in particular, where the values of total cholesterol decreased by more than 40%. Decreases in levels of total cholesterol by approximately

Table 1 Effect of screening and treatment on the lipid parameter levels of FH patients in 10 selected countries

	Start of the project	Follow-up	Difference (mmol/L)	Difference (%)
Cholesterol concentration (mmol/L)	8.640	5.850	2.790	32.30%
LDL-C concentration (mmol/L)	6.220	3.640	2.580	41.52%
Triglyceride concentration (mmol/L)	1.820	1.530	0.290	15.84%
HDL-C concentration (mmol/L)	1.438	1.437	0.001	0.04%

LDL-C LDL-cholesterol, *HDL-C* HDL-cholesterol. Average values of lipid parameters of individual countries from the beginning of the project and after the inclusion of the patients to the national database were summed and related to the number of patients, and the difference of the given lipid parameter values was evaluated; this difference was related to the total level of lipid parameter at the start of the project

Table 2 Comparison of triglyceride values at the project entrance and during the project in individual countries

Triglyceride concentration (mmol/L)	Start of the project		Follow-up		*p* value
	Mean	SD	Mean	SD	
Czech Republic	1.75	0.81	1.43	0.83	$p < 0.001$
Bosnia and Herzegovina	2.35	1.63	1.48	0.50	$p < 0.001$
Lithuania	2.30	1.80	1.70	1.00	$p < 0.001$
Latvia	2.10	1.46	1.24	0.60	$p < 0.001$
Hungary					
Russia	1.90	1.20	1.70	1.00	$p < 0.001$
Serbia	2.09	0.99	1.84	0.82	$p < 0.001$
Slovakia	1.60	0.70	1.40	0.70	$p < 0.001$
Uzbekistan	4.20	0.70	2.90	0.80	$p < 0.001$
Ukraine	2.30	0.93	1.83	0.93	$p < 0.001$

SD standard deviation

35% were also observed in the Czech Republic and Hungary, and by 25–30% in Slovakia and Uzbekistan. Decreases of approximately 20% were reported in Russia, Serbia, and Lithuania. A 3.6% decrease in the levels of total cholesterol was observed in Ukraine. Significant decreases in LDL-C levels were observed in the Czech Republic, Latvia, Hungary, and Uzbekistan where the levels of LDL-C decreased by more than 40% (Fig. 1). Decreases in LDL-C levels of approximately 25–35% were reported in Bosnia and Herzegovina, Lithuania, Russia, and Slovakia. Only in Serbia and Ukraine did the values of LDL-C decrease by approximately 20% and 7.8%, respectively. In Bosnia and Herzegovina as in Latvia, the triglyceride levels significantly decreased by more than 35%. In Lithuania and Uzbekistan, the triglyceride levels decreased by 26–31% (Fig. 3). Slight decreases, 18–21%, in the levels of triglycerides were observed in the Czech Republic and Ukraine, and by no more than 13% in Russia, Serbia, and Slovakia.

Table 3 Comparison of HDL-C values at the project entrance and during the project in individual countries

HDL-C concentration (mmol/L)	Start of the project		Follow-up		*p* value
	Mean	SD	Mean	SD	
Czech Republic	1.52	0.47	1.52	0.41	$p = 1.000$
Bosnia and Herzegovina	1.03	0.33	1.00	0.22	$p = 0.162$
Lithuania	1.30	0.40	1.20	0.20	$p = 0.027$
Latvia	1.69	0.65	1.49	0.42	$p = 0.004$
Hungary					
Russia	1.40	0.50	1.40	0.40	$p = 1.000$
Serbia	1.32	0.37	1.34	0.45	$p = 0.551$
Slovakia	1.40	0.50	1.50	0.60	$p = 0.008$
Uzbekistan	1.00	0.40	1.10	0.30	$p = 0.041$
Ukraine	1.23	0.33	1.28	0.33	$p = 0.195$

HDL-C HDL-cholesterol, *SD* standard deviation

Table 4 Comparison of total cholesterol values at the project entrance and during the project in individual countries

Cholesterol concentration (mmol/L)	Start of the project		Follow-up		*p* value
	Mean	SD	Mean	SD	
Czech Republic	8.77	1.57	5.54	1.43	$p < 0.001$
Bosnia and Herzegovina	7.60	1.43	3.95	0.86	$p < 0.001$
Lithuania	8.20	2.10	6.60	1.10	$p < 0.001$
Latvia	9.73	2.70	5.60	2.04	$p < 0.001$
Hungary	9.00	2.44	5.92	2.61	$p < 0.001$
Russia	9.20	2.00	7.40	2.30	$p < 0.001$
Serbia	6.87	1.93	5.91	1.39	$p < 0.001$
Slovakia	8.30	1.40	6.00	1.60	$p < 0.001$
Uzbekistan	9.20	2.70	6.30	1.80	$p < 0.001$
Ukraine	8.92	2.58	8.60	2.43	$p = 0.276$

SD standard deviation

In the case of HDL-C, statistically significant lower levels, when compared with the baseline values, were found in Lithuania (from 1.3 to 1.2, $p = 0.027$) and Latvia (a decrease from 1.69 to 1.49, $p = 0.004$), and statistically significant higher levels were found in Slovakia (an increase from 1.4 to 1.5, $p = 0.008$) and Uzbekistan (an increase from 1.0 to 1.1, $p = 0.041$). In the Czech Republic, Bosnia and Herzegovina, Russia, Serbia, and Ukraine, the baseline HDL-C levels remained almost the same during the project in comparison with the baseline (Fig. 4).

Discussion

The ScreenPro FH is not the only international activity in the field of FH. In the Pacific region, a similar project, the "Ten

Table 5 Comparison of LDL-C values at the project entrance and during the project in individual countries

LDL-C concentration (mmol/L)	Start of the project		Follow-up		*p* value
	Mean	SD	Mean	SD	
Czech Republic	6.46	1.53	3.37	1.33	$p < 0.001$
Bosnia and Herzegovina	3.33	1.15	2.40	0.84	$p < 0.001$
Lithuania	5.60	2.10	4.20	1.20	$p < 0.001$
Latvia	7.19	1.86	3.53	1.78	$p < 0.001$
Hungary	5.98	2.34	3.42	2.25	$p < 0.001$
Russia	6.90	1.70	4.90	2.40	$p < 0.001$
Serbia	4.51	1.69	3.61	1.19	$p < 0.001$
Slovakia	6.00	1.40	3.90	1.50	$p < 0.001$
Uzbekistan	6.50	1.70	3.70	1.10	$p < 0.001$
Ukraine	6.83	2.48	6.30	2.48	$p = 0.068$

LDL-C LDL-cholesterol, *SD* standard deviation

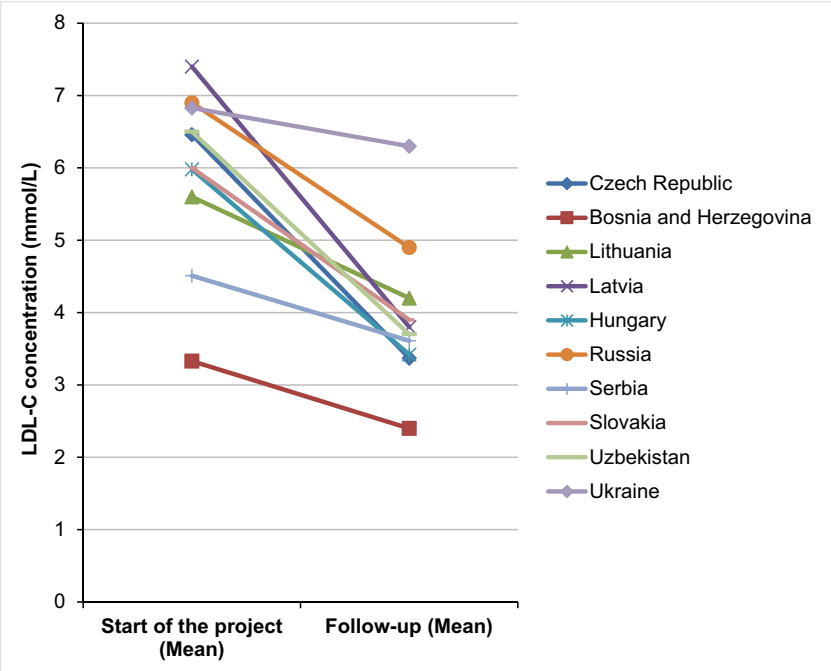

Fig. 1 Comparison of LDL-C Levels at the project entrance and during the project in individual countries

Countries Study," was carried out, with similar intentions and results [37, 38]. The biggest and only true global project is the FHSC [39]. It intends to create a global database which would be optimal both for data collection and their quality. On the other hand, there are mainly legal obstacles (of all kinds) in several countries which slow the recruitment of patients. Our study uses data summarized for each country. Consequently, the data of individual patients are not, for all intents and purposes, released from home countries. This explains why our group of FH patients is one of the biggest groups when compared with the global database.

The most valuable result of our project is considered to be the significant (not only statistically but mainly clinically) change in the lipid spectrum, in particular the decrease in LDL-C levels by more than 40% and the decrease of total cholesterol levels by more than 30% in patients from their inclusion to the database to post-intervention. Thus, we consider the results undoubtedly positive, despite the fact that we were not able to include results from all patients in the databases due to the lack of "before and after" data.

However, it must be stated that our results have only limited validity. In addition, it is necessary to mention that our patients are far from the target levels of LDL-C and total cholesterol. Nonetheless, this represents the first analysis; in many centers, physicians have a great opportunity to use higher doses of lipid-lowering drugs. Also, it will soon be possible, at least in some countries, to introduce monoclonal antibodies, evolocumab, and alirocumab. Regarding the change in triglycerides, although it is positive, we do not consider it significant. The change in HDL-C, which is minimal from a clinical point of view, is not considered substantial.

So far in the project, we have not paid much attention to treatment with LDL apheresis which is available in some countries; however, it can be considered highly selective and often aimed only at FH homozygotes.

Conclusions

As pointed out several times in the past, FH represents a significant CV risk. On the other hand, there are several treatment options: currently available standard treatment (statins + ezetimibe) and up-to-date treatment, MAB (PCSK9-i), as well as bempedoic acid [32], which is currently in development, or inclisiran. The search for patients and their early treatment is thus legitimate. The ScreenPro FH project exemplifies the benefits of the contributions of an international community to improving screening, diagnostics, and treatment of FH patients. It is further proof that sharing information, assisting in education, and increasing awareness can lead to positive changes in lipids, especially to a significant decrease in LDL-C in FH patients.

It can be generally concluded that the international cooperation in the ScreenPro FH project has led to a decrease in the CV risk in FH patients included in national databases. In further studies, we would like to focus on two issues in particular.

1. To increase the number of patients included in national databases

   (a) By increasing the number of cooperating centers

(b) By increasing awareness in both the general population and among medical experts
(c) By supporting patient organizations in individual countries

2. To improve FH patients' comprehensive treatment and improve the effects of the treatment with lipid-lowering drugs, so that, in optimal cases, the target values are achieved

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Compliance with Ethical Standards

Conflict of Interest Katarina Lalic, Olena Mitchenko, Zaneta Petrulioniene, Belma Pojskic, Aleksandr B. Shek, Tereza Altschmiedova, and Veronika Todorovova declare no conflicts of interest. Richard Ceska has been a consultant to Akcea Therapeutics, Amgen, Astra Zeneca, Bayer, Boehringer Ingelheim, Egis, MSD, NovoNordisk, Sanofi and board member of Amgen, Herbacos Recordati, Mylan, Novartis, Novatin, Pfizer, Promed, Roche, Sanofi, Servier, and he is paid speaker to Akcea Therapeutics, Amgen, Astra Zeneca, Bayer, Boehringer Ingelheim, Egis, Herbacos Recordati, MSD, Mylan, Novartis, Novatin, NovoNordisk, Pfizer, Promed, Roche, Sanofi, and Servier. Richard Ceska has received honoraria from Amgen, Esperion, Kowa, Regeneron, and Sanofi (PI, NC), and he reports grant support from IAS, Pfizer, and Teva. Gustavs Latkovskis declares speaker fees, honoraria, consultancy, and board membership to Astra Zeneca, Bayer, Berlin Chemie AG, Boehringer-Ingelheim, Servier, Mylan, Sanofi Aventis, Amgen, Novo-Nordisk, Krka Pharma, Roche Diagnostics, and Novartis, and he reports grant support from Latvian Council of Science. Marat V. Ezhov is a paid speaker from Amgen, Alexion, AZ, Berlin Chemie, Egis, KRKA, NovoNordisk, Pfizer, Recordati, and Sanofi, and he also reports travel fees. Tomas Freiberger has received honoraria from Amgen and Sanofi and grants from AZV, Ministry of Health, and CR for his institution, and he has been a consultant to Sanofi. Gyorgy Paragh has been a consultant and he declares travel fees from Richter Gedeon, fees for board membership from Sanofi Aventis and Amgen, and honoraria, and he is also a paid speaker. Katarina Raslova has received financial support from Sanofi, Amgen, NovoNordisk, and Mylan for consultancy, board membership, honoraria, and travel fees, and she also reports grant support from Sanofi for her institution. Branislav Vohnout has been a consultant to Sanofi, and he has received honoraria from Amgen, Sanofi, Mylan, Krka, and NovoNordisk. Branislav Vohnout has also received support from Amgen, Sanofi for board membership, and from Sanofi, Mylan, Amgen for traveling, and he is a paid speaker by Sanofi and Mylan.

Human and Animal Rights and Informed Consent The leaders in all countries involved in the ScreenPro FH project got acquainted with ethical statements of the country, and the project complies with ethical criteria of a non-interventional retrospective study in all participating countries.

Appendix

Table 6 Comparison of lipid parameter values at the project entrance and during the project in individual countries

Country	Start of the project		Follow-up		*p* value
	Mean	SD	Mean	SD	
Czech Republic	4045 patients		3256 patients		
Cholesterol concentration (mmol/L)	8.77	1.57	5.54	1.43	$p < 0.001$
LDL-C concentration (mmol/L)	6.46	1.53	3.37	1.33	$p < 0.001$
Triglyceride concentration (mmol/L)	1.75	0.81	1.43	0.83	$p < 0.001$
HDL-C concentration (mmol/L)	1.52	0.47	1.52	0.41	$p = 1.000$
Bosnia and Herzegovina	343 patients		343 patients		
Cholesterol concentration (mmol/L)	7.60	1.43	3.95	0.86	$p < 0.001$
LDL-C concentration (mmol/L)	3.33	1.15	2.40	0.84	$p < 0.001$
Triglyceride concentration (mmol/L)	2.35	1.63	1.48	0.50	$p < 0.001$
HDL-C concentration (mmol/L)	1.03	0.33	1.00	0.22	$p = 0.162$
Lithuania	98 patients		100 patients		
Cholesterol concentration (mmol/L)	8.2	2.1	6.6	1.1	$p < 0.001$
LDL-C concentration (mmol/L)	5.6	2.1	4.2	1.2	$p < 0.001$
Triglyceride concentration (mmol/L)	2.3	1.8	1.7	1.0	$p < 0.001$
HDL-C concentration (mmol/L)	1.3	0.4	1.2	0.2	$p = 0.027$
Latvia	249 patients		105 patients		
Cholesterol concentration (mmol/L)	9.73	2.70	5.60	2.04	$p < 0.001$
LDL-C concentration (mmol/L)	7.19	1.86	3.53	1.78	$p < 0.001$
Triglyceride concentration (mmol/L)	2.10	1.46	1.24	0.60	$p < 0.001$
HDL-C concentration (mmol/L)	1.69	0.65	1.49	0.42	$p = 0.004$
Hungary	329 patients		327 patients		
Cholesterol concentration (mmol/L)	9.00	2.44	5.92	2.61	$p < 0.001$
LDL-C concentration (mmol/L)	5.98	2.34	3.42	2.25	$p < 0.001$
Triglyceride concentration (mmol/L)					

Table 6 (continued)

Country	Start of the project		Follow-up		*p* value
	Mean	SD	Mean	SD	
HDL-C concentration (mmol/L)					
Russia	1200 patients		699 patients		
Cholesterol concentration (mmol/L)	9.2	2.0	7.4	2.3	$p < 0.001$
LDL-C concentration (mmol/L)	6.9	1.7	4.9	2.4	$p < 0.001$
Triglyceride concentration (mmol/L)	1.9	1.2	1.7	1.0	$p < 0.001$
HDL-C concentration (mmol/L)	1.4	0.5	1.4	0.4	$p = 1.000$
Serbia	302 patients		302 patients		
Cholesterol concentration (mmol/L)	6.87	1.93	5.91	1.39	$p < 0.001$
LDL-C concentration (mmol/L)	4.51	1.69	3.61	1.19	$p < 0.001$
Triglyceride concentration (mmol/L)	2.09	0.99	1.84	0.82	$p < 0.001$
HDL-C concentration (mmol/L)	1.32	0.37	1.34	0.45	$p = 0.551$
Slovakia	2246 patients		200 patients		
Cholesterol concentration (mmol/L)	8.3	1.4	6.0	1.6	$p < 0.001$
LDL-C concentration (mmol/L)	6.0	1.4	3.9	1.5	$p < 0.001$
Triglyceride concentration (mmol/L)	1.6	0.7	1.4	0.7	$p < 0.001$
HDL-C concentration (mmol/L)	1.4	0.5	1.5	0.6	$p = 0.008$
Uzbekistan	106 patients		106 patients		
Cholesterol concentration (mmol/L)	9.2	2.7	6.3	1.8	$p < 0.001$
LDL-C concentration (mmol/L)	6.5	1.7	3.7	1.1	$p < 0.001$
Triglyceride concentration (mmol/L)	4.2	0.7	2.9	0.8	$p < 0.001$
HDL-C concentration (mmol/L)	1.0	0.4	1.1	0.3	$p = 0.041$
Ukraine	147 patients		147 patients		
Cholesterol concentration (mmol/L)	8.92	2.58	8.60	2.43	$p = 0.276$
LDL-C concentration (mmol/L)	6.83	2.48	6.30	2.48	$p = 0.068$
Triglyceride concentration (mmol/L)	2.30	0.93	1.83	0.93	$p < 0.001$
HDL-C concentration (mmol/L)	1.23	0.33	1.28	0.33	$p = 0.195$

LDL-C LDL-cholesterol, *HDL-C* HDL-cholesterol, *SD* standard deviation

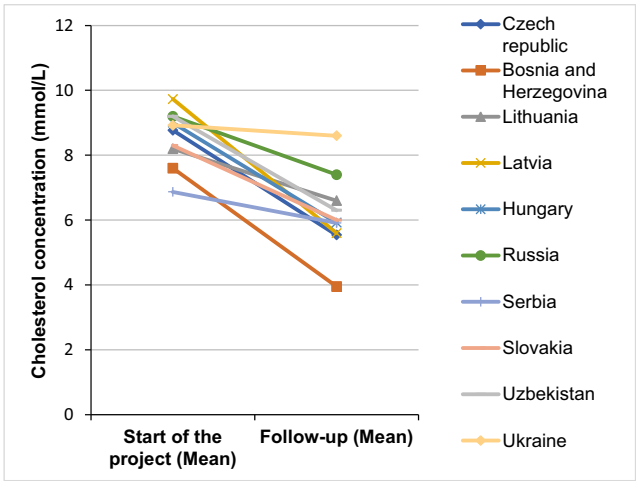

Fig. 2 Comparison of total cholesterol levels at the project entrance and during the project in individual countries

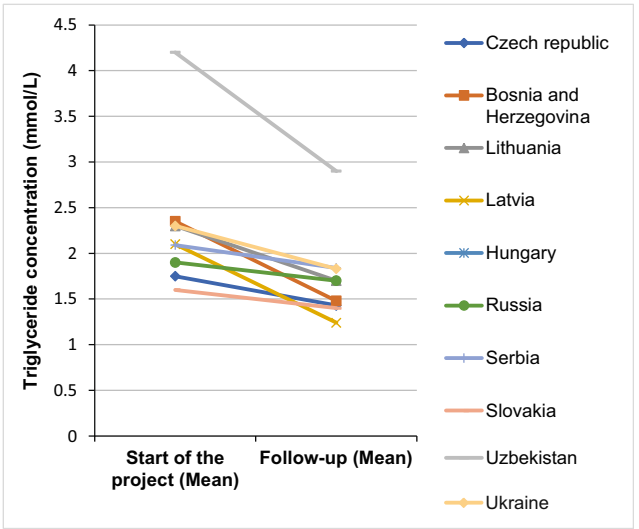

Fig. 3 Comparison of triglyceride levels at the project entrance and during the project in individual countries

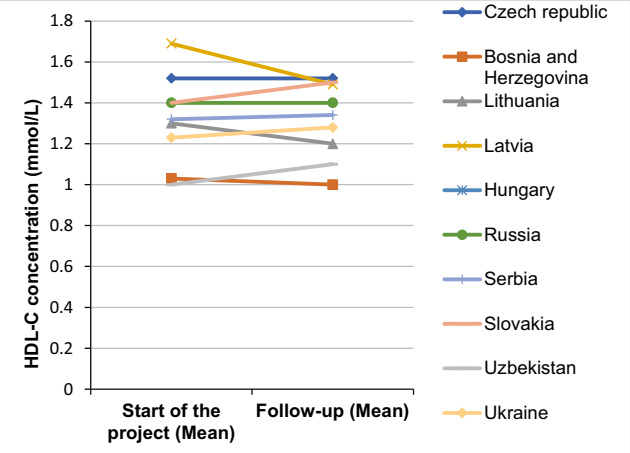

Fig. 4 Comparison of HDL-C levels at the project entrance and during the project in individual countries

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