Journal of Humanitarian Cardiovascular Medicine

Vol 1, No 1 (2022)

1st Issue

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doi: 10.12681/jhcvm.30181

To cite this article:

Polydorou, A., Alexopoulos, K., Polydorou, V., Skopelitis, E., Kougiali X. ., Liasis, N., Demesticha, T., Piagkou, M., Protogerou, V. ., Filippou, D. ., Velissariou, E. ., Dimakopulos, G., & Troupis, T. . (2022). Ten years follow-up under atorvastatin therapy of patients with carotid artery stenosis: the prognostic impact of oxidised low-density lipoprotein on carotid plaque progression and restenosis. : Atorvastatin and oxidized LDL in carotid artery stenosis . *Journal of Humanitarian Cardiovascular Medicine*, *1*(1). https://doi.org/10.12681/jhcvm.30181

Ten years follow-up under atorvastatin therapy of patients with carotid artery stenosis: the prognostic impact of oxidised low-density lipoprotein on carotid plaque progression and restenosis

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Keywords
Carotid stenosis, oxidize LDL, atorvastatin, angioplasty, atheromatosis

Acknowledgements:
We are grateful to Emeritus Professor Andrew Nicolaides for reviewing this manuscript and for his valuable comments.

Abstract

Background: Atorvastatin reduces oxidized low-density lipoprotein (oxLDL) levels and reduces the rate of plaque progression in patients with and without prior carotid angioplasty. The aim of our study was to investigate the durability of this effect and to explore the possible prognostic impact of oxLDL levels on carotid stenosis and plaque stability.

Methods: 106 patients (71 males, mean age 64.82±7.26 years) were studied. They were divided into two groups. Group 1 included patients with carotid stenosis >70% (n=50) who underwent carotid angioplasty prior to enrolment. Group 2 included those with <70% stenosis who were treated medically and were given atorvastatin with a dose adjusted to maintain LDL cholesterol <100mg/dl. Anthropometrics, complete lipid profile, oxLDL, and ultrasonography were performed at baseline, 1, 3, 6, 12 months, and yearly thereafter for 10 years.

Results: oxLDL levels significantly decreased from 53.3±10.91 mg/dl at baseline to 8.31±2.08 mg/dl at 12 months ($p<0.001$) and remained stable until the 10th year. In group 1, restenosis (>70%) was noticed in four patients, yet no further intervention was needed due to plaque morphology (echo-grade IV). In group 2, carotid stenosis was initially reduced (6th month - 4th year) and later relapsed (5th - 10th year), yet plaque morphology was recorded as type III or IV in almost all patients of group 2 (53=95%), indicating a significantly lower risk for stroke.

Conclusion: Atorvastatin treatment to a target of LDL <100 mg/dl in patients with carotid stenosis is associated with marked, and durable reduction of lipid levels, especially oxLDL and LDL and reduction in the rate of stenosis progression, improved plaque stability, and decreased stroke risk.

Introduction

Oxidized low-density lipoprotein (oxLDL) has a central role in the pathogenesis of atherosclerotic plaques. Circulating oxLDL is considered an indicator of atherosclerosis[1], coronary heart disease (CHD), and cardiovascular disease (CVD)[1-3]. Increased circulating oxLDL levels lead to the accumulation of lipids in the vessel wall, thus causing endothelial dysfunction[4] and contributing to atheromatous plaque instability[2,5,6]. LDL remains in the intima for a prolonged period of time, thus giving the opportunity to be oxidized by free radicals derived from the endothelial cells, the smooth muscle cells and the macrophages.[7]. Oxidized LDL then acts as a chemotactic agent for monocytes and smooth muscle cells through binding to scavenger receptors[8], leading to the formation of foam cells. Oxidized LDL also modifies the secretory activity of endothelial cells[8], inhibits the nitric oxide-mediated vasodilatation, induces adhesion of monocytes to intima through the expression of adhesion molecules on the endothelium[8], and enhaces the expression of inflammatory cytokines[9], a chronic inflammatory process which promotes atherosclerosis[10].

The statins (3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors) reduce mostly total cholesterol (TC), LDL cholesterol, and apolipoprotein B (apoB); they also have a minor effect on triglycerides (TG) and lipoprotein a (Lp-a) levels. Unquestionably, statins reduce the incidence of coronary events and are used for primary and secondary prevention of CHD[11]) Statins also have pleiotropic effects[12], among which remarkable is the modification of cell-mediated LDL oxidation[13,14]. These mechanisms contribute to the reversion of atherosclerosis. In a previous study we have shown that statin therapy reduces the oxLDL levels and reverses carotid artery stenosis independently from their lipid lowering effect during a follow-up period of one year.

The present study aimed to investigate the long-term efficacy of atorvastatin to slow down the progression of carotid stenosis and to provoke such plaque characteristics changes leading to stability of plaques. Also, to investigate the long-term effect of atorvastatin on oxLDL levels in such patients. We hypothesized that atorvastatin therapy would confer reduction of oxLDL levels in vivo, resulting in a significant change in the type of atherosclerotic plaque, delayed atherosclerotic plaque growth or even durable stenosis regression and therefore long-term prophylaxis against stroke.

Patients & Methods

This was a prospective open-label cohort study with a 10-year follow-up period. A total of 725 patients with hyperlipidemia and symptomatic and asymptomatic carotid atherosclerosis were screened, of which 127 fulfilled the inclusion criteria and were included in the study. The local and national ethics committee approved the study protocol.

Eligible were patients with hyperlipidemia, defined as total cholesterol levels ≥ 200 mg/dl, low-density lipoprotein (LDL) ≥ 150 mg/dl or 130 mg/dl in the presence of other cardiovascular risk factors and/or triglycerides ≥ 150 mg/dl up to 350 mg/dl as well as carotid artery stenosis defined as ≥ 50% restriction of the lumen diameter and/or ≥ 50% decrease of the vessel blood flow. Exclusion criteria included: acute coronary disease, unstable angina pectoris, clinically evident cardiac failure, severe arrhythmias, recent stroke, recent surgical procedures, inflammatory diseases, active liver disease or liver impairment, excessive alcohol consumption (>4 units/day), known allergic reaction to statins, poorly controlled diabetes mellitus as defined by a hemoglobin A1c (HbA1c) level of >7%, past history of thromboembolism, bleeding disorders, serum triglyceride levels above 350mg/dl, history of thyroid dysfunction, use of systemic corticosteroids, pernicious anemia, impaired vitamin B12 or folic acid levels, pregnancy or lactation, and end-stage renal disease.

Patients after signing informed consent were divided into two groups according to the degree of carotid artery stenosis: Patients with >70% stenosis (n=61) of common or internal carotid vessel (Group 1) underwent percutaneous carotid angioplasty and stenting with brain protection systems followed by statin administration. Those with stenosis <70% (n=62, Group 2) were treated conservatively with statins, without invasive pro-

cedures. In both groups, patients were encouraged to exercise and follow the American Heart Association step II diet. Clopidogrel or acetylsalicylic acid was additionally administered. Out of the 127 patients, four patients died soon after the initiation of the study due to reasons that did not relate to the study protocol, such as cancer, renal insufficiency, and accidental death. Of the remaining 123 patients, 17 (11 from group A and 6 from group 2) asked to be excluded or were lost to follow-up opting out of the study due to change of address or difficulty in commuting to the center. In total,106 patients completed the 10-year follow-up period and were included in the analysis.

Patients in both groups were placed on atorvastatin once daily at bedtime in individualized doses, with a goal to achieve and maintain serum LDL-cholesterol levels of <100mg/dl or <70mg/dl, if hypertension, renal impairment, smoking, symptomatic peripheral arterial obstructive disease, symptomatic coronary artery disease or diabetes mellitus were present. Usual doses were between 20 and 40 mg. Other medications that might cause elevation of CPK or frank rhabdomyolysis were discouraged during the time that patients were receiving statins. Possible adverse events were assessed during each visit along with evaluation by an expert in clinical biochemistry.

Past medical history, including smoking habits and anthropometrics were obtained during enrollment. Arterial blood pressure measurements, and laboratory investigations comprising of complete blood count, serum glucose and HbA1c levels, liver and kidney biochemistry, and detailed lipid profile including TC, low density lipoprotein (LDL) cholesterol, high density lipoprotein (HDL) cholesterol, serum TG, apoB, and apolipoprotein A, urate, B12 and folate, thyroid function, homocysteine, Lp-a, and oxLDL levels were obtaind at baseline and during follow-up visits at 1,3,6, 12 months and then yearly up to 10 years. Blood samples were collected after an at least a 12-hour fast; a light, low-fat meal was advised the night before sample collection. Standard biochemistry vacutainer tubes were used to collect blood samples, except for whole blood count, HbA1c and homocysteine, which were collected in ethylenediaminetetraacetic acid (EDTA) vacutainers. Serum for biochemistry analysis was centrifuged at 4000g at 4°C for 7 min and was immediately tested or stored in deep refrigeration.

Determination of TC, HDL, and TG was done by a Dade Behring analyser using commercially available enzymatic colourimetric methods (Dade Behring, Newark, USA). LDL was then calculated using Friedewald's equation[15]. Levels of circulating oxLDL were measured using a commercially available kit (Mercodia, Uppsala, Sweden). The method rely on a double antibody (4E6 and mouse monoclonal antiapoB)[16] capture ELISA, which primarily detects malondialdehyde LDL (MDA-LDL). The reference values range from 31 to 61mg/dl in our lab[17]. Apolipoprotein A, apoB, and Lp-a were measured using immunonephelometry with rabbit antisera (Dade Behring, Newark, USA) in a Dade Behring analyser.

Grading of stenosis was made by Duplex scanning according to the consensus statement of the society of radiologists in ultrasound[18] with the use of Apogee 800 plus scanner with a 7.5 MHz transducer (ATL Inc., Bothell WA, USA). Evaluation was carried out at baseline, at 12 months, and yearly thereafter. Internal (ICA) and common (CCA) carotid artery were evaluated on both sides for each patient by grayscale and color Doppler imaging. The vessels were imaged as completely as possible. Pulse wave Doppler spectral analysis and measurement of the blood-flow velocity of both vessels were additionally performed. Results were reported in a standardized format. Stenosis was defined as the presence of visible plaque in grayscale or color Doppler imaging. The degree of stenosis was calculated by the increase of peak systolic velocity. Other parameters such as the ICA/CCA peak systolic velocity ratio and the ICA end diastolic velocity were also calcutated in difficult cases. Stenosis >70% was characterized as severe and the patient was offered angioplasty. Stenosis 60-70% was characterized as substantial, 50-60% as moderate, and <50% as mild. The latter categories were managed conservatively. The value of the vessel with the greater degree of stenosis for each patient was used in the statistical analysis.

Table 1. Patients baseline characteristics and relevant comparisons between groups

Chacteristic	Total	Group 1	Group 2	P value
Males / females	71/35	34/16	37/19	0.833
Mean age in years ± SD	64.82 ± 7.26	65.66 ± 5.98	64.07 ± 8.22	0.263
Number of pts with DM (percentage)	32 (30,2%)	15 (30,0%)	17 (30,4%)	0.968
Number of pts with HTN (percentage)	91 (85,8%)	47 (94,0%)	44 (78,6%)	**0.023**
Number of smokers (percentage)	78 (73,6%)	42 (84,0%)	36 (64,3%)	**0.022**
Number of pts with CAD (percentage)	50 (47,2%)	26 (52,0%)	24 (42,9%)	0.347
Mean ± SD TC (mg/dl)	249.01 ± 24,7	254.8 ± 29.0	243.84 ± 18.94	**0.025**
Mean ± SD LDL cholesterol (mg/dl)	171.27 ± 21.92	174.76 ± 26.12	168.16 ± 16.98	0.132
Mean ± SD HDL cholesterol (mg/dl)	46.33 ± 9.24	46.08 ± 8.35	46.55 ± 10.04	0.794
Mean ± SD TG (mg/dl)	150.63 ± 29.22	154.78 ± 34.78	146.93 ± 22.87	0.179
Mean ± SD oxLDL (mU/l)	52.33 ± 10.91	62.06 ± 3.99	43.65 ± 7.09	**<0.001**
Mean ± SD homocysteine (mU/l)	15.06 ± 6.67	15.64 ± 5.55	14.55 ± 7.55	0.102*
*Mann Whitney test due to skewed data distribution				

SD=standard deviation, DM=diabetes mellitus, HTN=arterial hypertension, CAD=coronary artery disease, TC=total cholesterol, LDL=low-density lipoprotein, HDL=high-density lipoprotein, TG=triglycerides, oxLDL=oxidized low-density lipoprotein

Statistical analysis

Continuous variables were presented as mean values ± standard deviation, while qualitative variables were presented as absolute and relative frequencies. Normality tests were applied using the Shapiro-Wilk test and QQ plots were also considered. Univariate analysis was initially applied to test differences in baseline values in several patient characteristics between the two groups. Repeated measures analysis was applied to examine the changes in the patients' lipid profile during the follow up period. Correlations between skewed continuous or discrete variables were evaluated using Spearman's p-coefficient, whereas correlations of normally distributed variables were evaluated by calculating the Pearson's r-coefficient. Comparisons between normally distributed, continuous variables and categorical variables were made using the Student t-test. Analysis of categorical data was carried out with the chi square test or Fischer's exact test when appropriate. The effect of group and oxLDL on carotid stenosis was also tested through time dependent multiple Cox proportional hazard model. The results obtained were presented as Hazard Ratios (HR) and the 95% Confidence Intervals (CI). A backward elimination procedure was applied to all multivariate models (using P<5% as the threshold for removing a variable from the models). All models were adjusted for age, smoking, gender, HT, TC, BMI, homocysteine levels and DM diagnosis. Kaplan-Meier curves concerning stenosis over the study period were plotted. All reported P-values were based on two-sided tests and compared to a significance level of 5%. STATA 13.0 software (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP) was used for the analysis.

Results

Out of the 106 patients 71 were males and 35 females with a mean age of 64.82±7.26 years. Group 1 comprised of 50 patients and group 2 of 56. Diabetes mellitus was recorded in 32 patients (30%), 22 males (69%) and 10 females (31%). Hypertension had 91 patients (86%), 58 males (64%) and 33 females (36%). All patients had metabolic syndrome according to the national cholesterol education programme-adult treatment panel III (NCEP-ATP III) criteria[19]. Active smoking (defined as current or discontinued as far back as 5 years) was reported by 78 (74%) patients, 64 (82%) males and 14 females (18%). Of the 50 patients of group 1, 12 had transient ischemic attack, 8 had amaurosis fugax, and the remaining were asymptomatic. All patients of group 2 were asymptomatic.

The mean dose of atorvastatin at baseline was 20 mg in both groups. After 3 months the re-

spective doses were 20 mg in 71 patients (67%) and 40 mg in 35 patients (33%). Within Group 1, 28 patients (56%) received 20 mg and 22 patients (44%) 40 mg. In Group 2, 43 patents (77%) received 20 mg and the remaining 13 patients (23%) 40 mg. This difference was not statistically significant (p=0.877). The baseline characteristics of the patients in the two groups and their differences are depicted in **Table 1**. Group 1 had significantly more proportion of smokers and patients with hypertension, as well as significantly greater values of TC and oxLDL at baseline.

Lipid profile

TC, HDL, LDL, oxLDL, TG and apoB significantly improved soon after statin initiation in comparison to baseline in both groups (**Table 2**). Specifically, a statistically significant reduction of TC was observed in both groups after just one month of atorvastatin initiation, with further reduction recorded during the 3rd-month visit. The TC levels six months post treatment did not differ significantly compared to three months and remained approximately at 155mg/dl for both groups during the whole study period. Concern-

Table 2. Measurements of basic lipid profile values during the study period

			Tchol	TGL	HDL	LDL	ox LDL	ApoB-100
F-UP	Baseline	Mean	249,01	150,63	46,33	171,27	52,33	148,73
		St. D	24,70	29,22	9,24	21,92	10,91	19,23
	M1	Mean	195,12	135,68	48,50	119,09	27,45	118,49
		St. D	23,56	25,88	8,86	20,06	6,28	23,09
	M3	Mean	172,44	129,00	49,49	97,12	15,87	103,83
		St. D	17,86	23,72	8,46	15,16	4,87	20,29
	M6	Mean	161,71	122,45	51,54	86,49	11,66	91,92
		St. D	16,58	23,34	8,11	9,04	5,21	14,81
	Y01	Mean	159,87	121,89	51,59	83,99	8,31	88,15
		St. D	13,33	22,39	8,29	9,93	2,08	14,42
	Y02	Mean	157,30	119,85	51,33	82,38	7,97	85,36
		St. D	11,86	21,98	7,74	9,24	2,04	12,97
	Y03	Mean	156,68	118,38	51,63	81,75	7,83	84,17
		St. D	11,64	22,16	7,74	9,09	2,00	10,74
	Y04	Mean	155,76	117,28	51,99	80,57	7,58	84,62
		St. D	12,39	23,02	7,37	9,02	2,09	11,44
	Y05	Mean	155,33	114,71	52,35	80,36	7,53	83,60
		St. D	12,81	23,65	7,74	8,41	2,00	12,13
	Y06	Mean	153,92	114,41	52,75	78,69	7,36	81,95
		St. D	11,82	23,73	7,51	8,32	1,92	10,99
	Y07	Mean	154,79	113,72	53,24	79,27	7,18	80,75
		St. D	12,07	25,17	7,69	7,67	1,83	10,93
	Y08	Mean	154,89	113,69	53,10	79,48	6,97	81,68
		St. D	10,64	23,90	7,20	7,38	1,84	9,93
	Y09	Mean	154,44	114,13	53,41	78,64	6,79	81,31
		St. D	11,38	23,63	7,44	7,26	1,71	11,10
	Y10	Mean	154,66	113,75	53,72	78,80	6,60	81,31
		St. D	10,56	23,27	7,33	6,72	1,62	10,30

M=month, Y=year, F-UP=follow-up, St.D=standard deviation, Tchol=total cholesterol, TGL=triglycerides, HDL=high density lipoprotein, LDL=low density lipoprotein, oxLDL=oxidized low-density lipoprotein, apoB-100=apolipoprotein B 100.

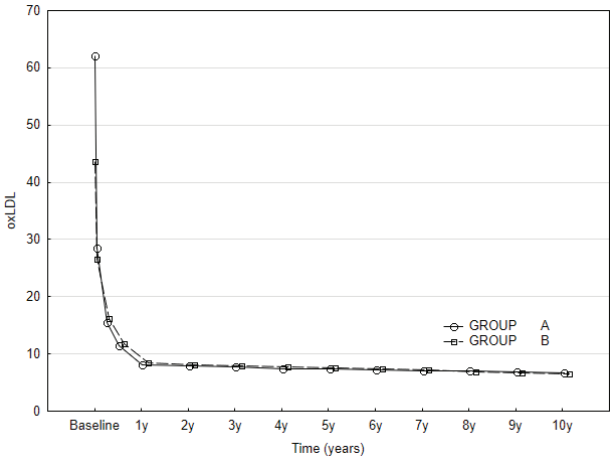

Figure 1A. oxLDL values through the 10 year follow up for groups 1 (curve A) and 2 (curve B)

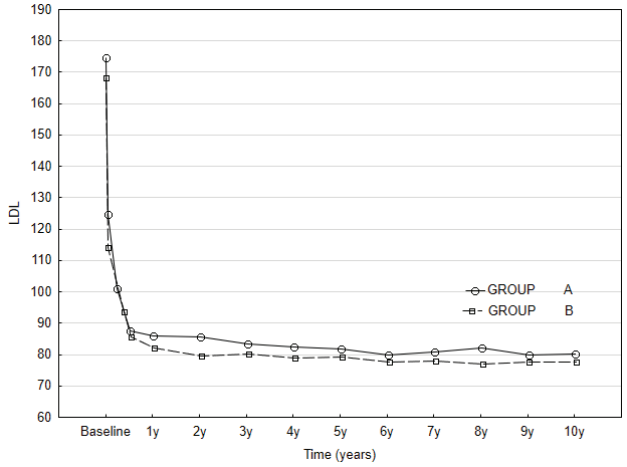

Figure 1B. LDL values through the 10 year follow up for groups 1 (curve A) and 2 (curve B)

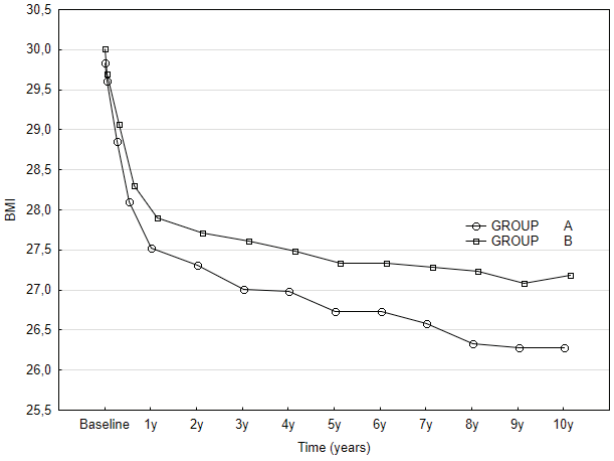

Figure 2. BMI values through the 10 year follow up for groups 1 (curve A) and 2 (curve B)

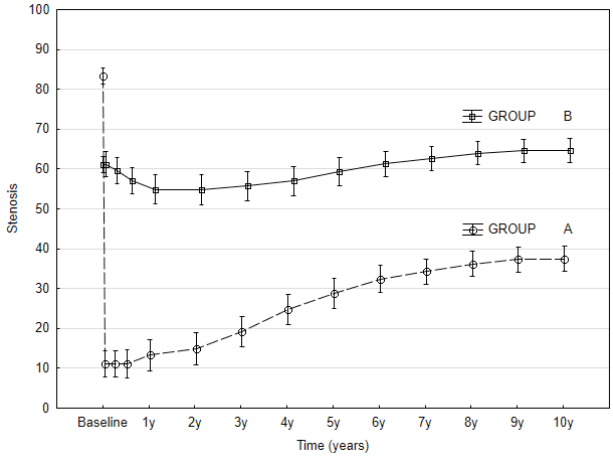

Figure 3. Stenosis values through the 10 year follow up for groups 1 (curve A) and 2 (curve B)

ing TG levels, a significant decrease was observed in both groups, reaching a nadir level after six months in group 1 and after three months in group 2. Regarding HDL values, a gradual increase was observed for both groups during the first six months, while after that timepoint no further changes were observed. There was no significant difference between group 1 and 2 regarding changes in the HDL levels at any time point. A marked reduction was observed regarding the oxLDL (**figure 1A**) as well as the LDL (**figure 1B**) levels in both groups. LDL and oxLDL values presented no statistically significant differences between group 1 and 2 at any time point except for the baseline values of oxLDL. For both parameters, a rather "flat line" was observed after the first six months (**figure 1A and B**). Specifically, mean oxLDL dropped from 52.33 +/- 10.91 mg/dl at baseline to 11.66 +/- 5.21 mg/dl at six months (p<0,001), and 8.31+/-2.08 at 12 months (p=NS). Finally regarding ApoB100, the observed changes are in parallel between the two groups, showing a marked reduction during the first six months and no further reduction for the remaining study period. The ApoB100 values of patients in group 1 were consistently higher compared to the values of group 2.

Within group 2, the subgroup of patients with mild stenosis (< 60%) had a significant reduction of oxLDL from 34.94±4.23 mg/dl at baseline to 12.12±7.53 mg/dl at 12months (p<0.001). It must be noted that a 10 unit statistically significant decrease of oxLDL levels was observed quite early – only after 3 months of atorvasta-

tin therapy. Within group 2, the subgroup of patients with substantial stenosis (≥ 60% and < 70%) had a significant reduction of oxLDL from 44.68±1.98 mg/dl at baseline to 11.79±4.21 mg/dl at 12months (p<0.001). It must be noted that a 17 unit statistically significant decrease was already demonstrable 3 months after the initiation of atorvastatin therapy.

Anthropometrics

During the first year of follow-up a marked reduction of body mass index (BMI) and net body weight was noticed, followed by a less prominent decline during the second year. There was no significant change beyond this timepoint until the end of the 10-year observation period. BMI reduction was significantly greater in group 1 compared to group 2 (**figure 2**). During the first three months no significant change was noticed in reference to waist circumference and waist:hip ratio, yet a significant decrease was observed after 6 months and continued until the second year of follow-up. The measurements beyond the second year indicate a rather steady profile for both groups. There was no significant difference in the reduction of waist circumference between the two groups.

Carotid stenosis and plaque stability.

In group 1 after 12 months of statin therapy, no case of restenosis was reported. At the end of the study period at 10 years 4 cases were observed. All patients in group 2 had significant stenosis at baseline (100%), which was reduced to 96.4% at 12 months and 80.4% the end of the observation period. The stenosis values and respected comparisons for the two groups are depicted in **figure 3**. Patients in group 2 who achieved LDL levels <70mg/dl during the observation period had a greater but not significant reduction of carotid stenosis compared to those with LDL levels between 70 and 100mg/dl.

Regarding plaque echogenicity, the 95% of patients (n=53) of group 2 had type III or IV plaque defined as stable by year two, while 100%

Table 3. Frequencies and percentages of echogenic plaques in group 1 (A) and group 2 (B) during the observation period and respective comparisons with p values

	GROUP								
	1				2				
	Echogenic		Echolucent		Echogenic		Echolucent		
	N	%	N	%	N	%	N	%	p-value
Baseline	9	18,0%	41	82,0%	12	21,4%	44	78,6%	0,658
1 month	50	100,0%	0	0,0%	13	23,2%	43	76,8%	<0,001
3 months	50	100,0%	0	0,0%	16	28,6%	40	71,4%	<0,001
6 months	50	100,0%	0	0,0%	29	51,8%	27	48,2%	<0,001
1 year	50	100,0%	0	0,0%	42	75,0%	14	25,0%	<0,001
2 years	50	100,0%	0	0,0%	53	94,6%	3	5,4%	0,245
3 years	50	100,0%	0	0,0%	55	98,2%	1	1,8%	1,000
4 years	50	100,0%	0	0,0%	56	100,0%	0	0,0%	-
5 years	50	100,0%	0	0,0%	56	100,0%	0	0,0%	-
6 years	50	100,0%	0	0,0%	56	100,0%	0	0,0%	-
7 years	50	100,0%	0	0,0%	56	100,0%	0	0,0%	-
8 years	50	100,0%	0	0,0%	56	100,0%	0	0,0%	-
9 years	50	100,0%	0	0,0%	56	100,0%	0	0,0%	-
10 years	50	100,0%	0	0,0%	56	100,0%	0	0,0%	-

of patients had stable plaque by year four. That means that no patient in either group had an "unstable plaque" after four years of statin therapy, while patients in group 2 had low plaque echogenicity during the first year of follow-up (**Table 3**).

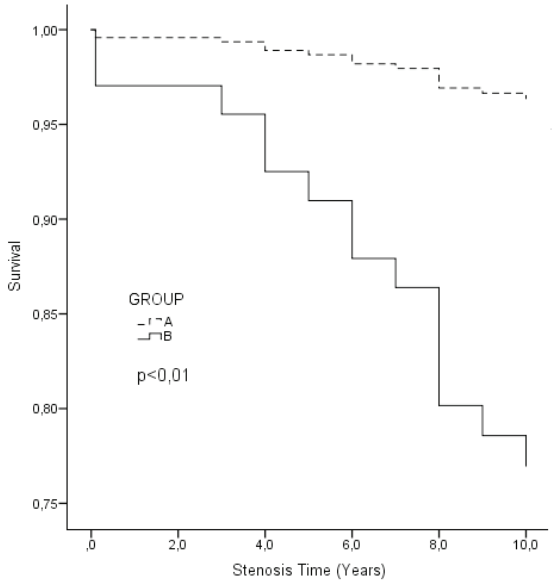

Figure 4. Cumulative stenosis rates using life table analysis 1 (curve A) and 2 (curve B)

The Cox regression analysis showed that there was a statistically significant difference between the two groups and a significant effect of the oxLDL levels only for group 2 (**figure 4 and table 4**). Specifically, the hazard of restenosis was significantly higher in group 2 where for every unit of greater baseline oxLDL value the hazard of restenosis increases by 56,7% (95% C.I.: 22,2% – 100,9%), p<0,001. The model was adjusted for the effect of age, diabetes mellitus, gender, hypertension, baseline cholesterol and baseline BMI for group 2, while for Group 1 HTN, diabetes and smoking were excluded as no convergence could be achieved. Time was not a statistically significant factor and therefore the interaction of time for the time dependent covariates (diabetes mellitus, hypertension, cholesterol, oxLDL and BMI) was in all cases and both groups, not significant.

Discussion

This study demonstrates that atorvastatin administered in individualised doses, titrated to maintain serum LDL cholesterol levels <100mg/dl, significantly improved lipid profile, decreased circulating oxLDL levels, reduced carotid artery stenosis and improves plaque stability in patients managed conservatively or prevented restenosis in patients with prior angioplasty. This effect was durable over a prolonged period , reaching ten years, thus indicating a possible survival benefit in such patients.

Oxidised LDL has been shown by multivariate analysis to represent an independent risk factor for carotid artery stenosis (**Table 3**). Specifically, oxLDL levels at baseline serve as a prognostic risk marker for future deterioration of carotid ar-

Table 4. Cox multivariate regression analysis for risk factors of restenosis

	GROUP 1				GROUP 2			
Baseline characteristics	p	HR	95,0% CI for HR		p	HR	95,0% CI for HR	
			Lower	Upper			Lower	Upper
Age	0,079	1,667	0,942	2,948	0,124	1,088	0,977	1,213
Homocysteine	0,793	0,520	0,004	68,556	0,917	1,089	0,219	5,418
GENDER	0,962	0,930	0,045	19,133	0,671	1,708	0,144	20,252
T chol	0,843	1,007	0,940	1,079	0,121	0,966	0,925	1,009
OxLDL	0,553	1,147	0,729	1,805	**0,000**	**1,567**	**1,222**	**2,009**
BMI	0,943	0,963	0,338	2,743	0,307	1,282	0,796	2,063
HTN	-	-	-	-	0,459	0,400	0,035	4,519
DIABETES	-	-	-	-	0,499	0,604	0,140	2,605
SMOKING	-	-	-	-	0,797	0,700	0,046	10,700

HTN=arterial hypertension, T chol=total cholesterol, BMI=body mass index, oxLDL=oxidized low-density lipoprotein.

tery stenosis under statin therapy. This was true for patients treated conservatively; in group 2, as the number of patients with restenosis in group 1 was rather small. Moreover, it is anticipated that patients that did not undergo invasive procedures would be at greater risk for long-term consequences and possible worsening of stenosis. Our study estimated a specific, significant risk for patients with greater oxLDL levels at baseline, showing that this target group might benefit more from statin therapy. Most importantly, the prognostic significance of oxLDL was not influenced by any other classic cardiovascular risk factor such as gender, aging, smoking, diabetes mellitus, or hypertension at any time point.

The two groups in our study did not serve the purpose of internal control but rather represented the two most common medical scenarios in carotid atherosclerotic disease. As expected, patients undergoing angioplasty (group 1) had worse atherogenic characteristics at baseline such as hypertension, smoking, and diabetes, yet the possibility for re-stenosis was alleviated with statin therapy used for secondary prophylaxis. Patients treated conservatively benefited from statin therapy as a measure of primary prophylaxis. Though such patients had a higher degree of restenosis between the 4th and 10th years of follow-up, yet the plaque was more echogenic indicating greater stability and by inference, posing a decreased overall risk for stroke.

This study aimed to investigate whether oxLDL could pose an independent risk marker of carotid artery stenosis in both types of patients, and this was shown by our results. To our knowledge, this study is among the few prospective studies with such a long observation period of ten years to investigate changes of oxLDL with statin therapy, yet among the first to report not only a clear, significant reduction of oxLDL levels following atorvastatin therapy but also, an association of the remission of oxLDL levels with the reduction of the degree of carotid stenosis. Noteworthy, this significant and long-standing reduction of oxLDL levels was achieved with everyday-life, usual doses of atorvastatin. Most of atorvastatin benefit was completed during the first six-month period. Practically no further reduction was noticed beyond this time point.

The mechanism by which statins alleviate oxLDL levels is still a matter of investigation in the literature. Moreover, a clear association of oxLDL level reduction with improvement of carotid atherosclerosis and a better overall survival benefit is not unequivocally established by large, double-blinded, randomised clinical trials. Under this perspective, the present observational cohort study with a 10-year observation period provides reasonable evidence that reducing oxLDL may independently improve carotid stenosis.

Carotid IMT is a validated measure of carotid atherosclerosis. Nevertheless, in the present study, a direct estimation of stenosis by ultrasonography was opted because it is readily available in most clinical settings and is apparently associated with clinical symptoms and signs. Besides, it is a reliable and reproducible method, and practically the one used for the pre-operative evaluation of patients eligible for endarterectomy. Carotid stenosis is a well-established surrogate marker for cardiovascular disease (CVD)[20]. Other parameters of the vessel wall status, such as the estimation of IMT and plaque morphology, although associated with CVD in the literature, require a more specific radiological evaluation and are not readily available in most real-life clinical settings. Future studies would ideally include such measurements. On the other hand, oxLDL has been recognized as a risk factor for carotid atherosclerosis in asymptomatic men[21] and has also been linked with CVD[21]. Increased oxLDL[2] and MDA-LDL levels[6] are related to plaque instability. It has also been reported that oxLDL is weakly associated with carotid IMT.[22]

Statins reduce the incidence of cardiovascular events not only due to their hypocholesterolemic properties[23], but also due to additional mechanisms of action, the so-called pleiotropic effects[12], such as the suppression of smooth muscle cell migration and proliferation[24], the reduction of monocyte adhesion to the vascular endothelium[25], the improvement of endothelial function[26], the inhibition of cell-mediated LDL oxidation[13,14], the immunomodulation of monocyte maturation and differentiation, and the modification of production of inflammatory cytokines[27]. Atorvastatin, in particular, suppresses cellular uptake of oxLDL from differentiating monocytes

by reducing the expression of LOX-1 and scavenger receptors[28] and accelerates the LDL-receptor-mediated removal of the non-oxidized LDL particles[29]. It has even been reported that active atorvastatin metabolites may have more significant anti-atherosclerotic effects than other statin molecules[30]. Atorvastatin hydroxy-metabolites have a protective action against LDL oxidation[22]. The established benefit from statin therapy after acute coronary events may be attributed to the stabilization of the plaque through the removal of oxLDL from the vessel wall[31], transient binding with apoB-100 particles and clearance from the circulation.

In the STAT trial[32], antibodies against oxLDL were equally decreased with aggressive and conventional lipid-lowering therapy. This indicates that statins reduce oxLDL in a non dose-dependent manner. The results of the present study confirm this as well. It may be attributed to their pleiotropic actions rather to their hypocholesterolemic potential. A study by Orem et al. using low doses of atorvastatin demonstrated a significant reduction of autoantibodies against oxLDL levels.[23]. Similarly in our study ox-LDL levels were reduced with low atorvastatin doses. This might explain why in the conservatively treated group 2, no further improvement of stenosis was noticed in patients achieving LDL levels lower than 100mg/dl. Alternatively, this could be attributed to the small sample size and the diversity of our population, although there are indications in the literature that intensification of statin therapy does not confer additional protection and only those with LDL >125mg/dl would benefit from a more aggressive statin therapy[34].

In acute coronary events statins have a dose-related action, but this has not been the case regarding oxidative stress[35]. This might also be explained by the hypothesis that statins achieve their uttermost benefit on oxLDL after a certain time point[33], beyond which, no additional benefit might be expected. Continuation of treatment in such cases serves only the purpose of maintenance. Atorvastatin reduces small dense LDL subfractions, remnant-like particles cholesterol and oxLDL, and improve endothelial function, after just a few weeks of therapy[36, 37]. This time-related effect may possibly explain the finding of our study that during the first six months, a sharp decline of oxLDL levels was noticed, followed by a milder reduction rate thereafter, and stabilization for the next ten years.

The concomitant reduction of both oxLDL and carotid stenosis in our study by statins may be explained by the fact that those drugs influence common pathways, responsible for the production of oxLDL and initiation of atherosclerosis; alternatively, the reduction of oxidative stress with statins may have resulted in remission of stenosis, as oxidation of LDL is the driving force of atherosclerosis. This study demonstrates that the association of oxLDL with carotid artery stenosis is independent of the LDL level changes. It could be assumed that oxLDL is reduced by statins as a consequence of the reduction of LDL levels. Nevertheless, the latter would not lead to less oxLDL, unless the oxidative capacity of the plasma was not simultaneously condensed. On the other hand, statins may decrease oxLDL independently of the changes of LDL levels in the context of their pleiotropic actions. Besides, ox-LDL triggers atherosclerosis by binding with different receptors than LDL. The above mean that the cutback of oxLDL might be more critical for the reversal of atherosclerosis than the drop of LDL levels.

The Mercodia oxLDL detects the MDA-modified apoB[16]. It has been proposed that oxLDL loses its predictive value for CVD when adjustment for apoB level is performed[34]. Significant reduction of Mercodia oxLDL with 10mg of atorvastatin-was demonstrated in several studies, even after adjustment for apoB levels[3, 21, 34]. In our studies the reduction of oxLDL was still significant after adjustment for apoB and LDL levels. In our study, the reduction of carotid stenosis was associated with the decrease of oxLDL levels.

Limitations of the present study were the relatively small size, the lack of a control group comprising of patients with carotid stenosis not on statin therapy, which would be unethical, the fact that researchers were not blinded to the patients' status, the lack of randomization of the dose-schedules and the use of only one method to detect oxLDL and carotid stenosis.

Conclusions

This prospective, observational study with a ten-year follow-up provides evidence of a favorable effect of usual-dose atorvastatin therapy on oxLDL, which was associated with a reduction in stenosis progression in patients with carotid atherosclerosis. Moreover, oxLDL level modifications were associated with conversion of the plaque type to a more stable form. These findings may lead to an amendment of our policy concerning the time of carotid endovascular treatment advising the patients with carotid artery disease to receive statin therapy 2-3 months pre-angioplasty, in order to avoid complications during and post-procedure. In conclusion, we postulate that oxLDL might represent a more sensitive risk marker for stenosis than LDL and apoB as shown by multivariate analysis.

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Authors' Contibution

Dr Adamantia Polydorou wrote the protocol and had the overall supervision, as the primary investigator. She discussed the protocol with professor of Anatomy in the Medical School of Athens, National and Kapodestrian University of Athens, prof. Theodore Troupis and make amendments before applying to the ethics committee. She also contributed to the data collection and entry and reviewing the paper prior to submission

Dr Konstantinos Alexopoulos and Dr. Victoria Polydorou gathered all the information from the patients' files and organized the data of the study, updated the spreadsheet, and contributed to the writing of the paper

Dr Skopelitits overviewed the data and contributed to the writing and editing of the paper, acting as the corresponding author as well

Dr Xanthoula Kougiali was responsible for the biochemistry samples collection and processing. She also contributed to the lab data entry and statistical analysis.

Dr. Nikolaos Liasis and Dr Maura Griffin were responsible for the carotid artery ultrasound evaluation of the patients

Dr. Theano Demesticha was responsible for collecting the anthropometric data of the patients and evaluation of the restenosis during the follow-up visits.

Dr. George Dimakopoulos and Dr. Maria Piagkou and Eleni Velissariou were responsible for the data corrections and statistical analysis and contributed to the editing of the paper.

Professor Dimitris Filippou, president of the Greek FDA, was responsible for the determination of the posology of atorvastatin and the evaluation of the impact on LDL, oxLDL and other laboratory parameters.

Dr Vasilios Protegerou overviewed the correct timing of the study population follow-up visits and the collection of the questionnaires, data entry and organization of the data to tables and spreadsheets. He also contributed to paper editing