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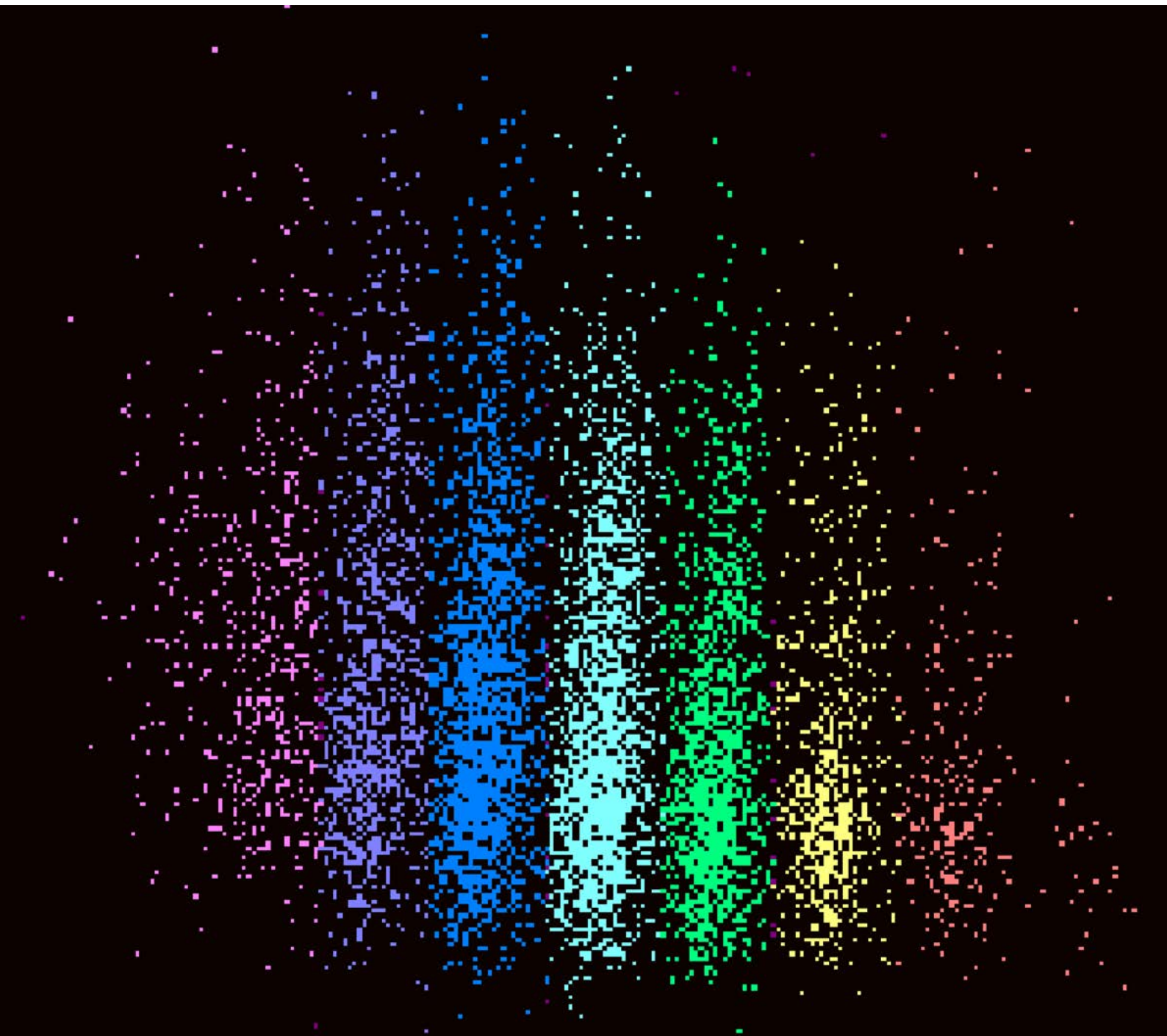

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Contents

European Atherosclerosis Journal is an international, peer-reviewed, fully open access, four-monthly journal covering all topics within atherosclerosis and cardiovascular disease areas. The European Atherosclerosis Journal is an official journal of SITeCS (Società Italiana di Terapia Clinica e Sperimentale - Italian Society for Experimental and Clinical Therapeutics).

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Figure/Table number	6	5	3	2
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Abstract. The abstract should not exceed 250 words (150 words for Methodology papers).

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Main text. The manuscript must contain the following sections: Introduction, Methods, Results, Discussion, Conflict of interest (mandatory), Funding (if applicable), Author contribution (mandatory), Acknowledgements (if applicable), References. Abbreviations must be defined when first used in the text.

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Lipid-lowering treatment and LDL-C goal attainment in high and very high cardiovascular risk patients: Evidence from the SANTORINI study-The Italian experience

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ABSTRACT

The SANTORINI study is an observational study that enrolled 9602 adult individuals at high or very high cardiovascular (CV) risk across Europe, aimed at providing information on the current status of the management of dyslipidaemias, in light of the most recent 2019 ESC/EAS guidelines.
Italy participated in the study with 1977 patients, 1531 (77.4%) of whom were classified at very high CV risk and 446 (22.6%) at high CV risk. Overall, in the Italian population, 79.31% of the patients had a history of atherosclerotic cardiovascular disease (ASCVD). At enrolment, the mean level of LDL-C in the total population was 98.4 mg/dL. LDL-C levels were lower in the very high-risk group (94.6 mg/dL) than in the high-risk group (111.4 mg/dL). Considering the therapeutic goals recommended by the most recent 2019 ESC/EAS guidelines (LDL-C <55 mg/dL or <70 mg/dL in very high or high-risk patients, respectively), only 20.3% of the overall study population achieved such goals (19.9% of very high-risk patients and 21.8% of high-risk patients). About one-third of the patients included in the study (32.6%) were not prescribed any therapy, one-third received statin monotherapy (34.4%), and only one-third (33%) were taking combination therapy; these percentages were comparable in the two risk subgroups.
Based on the most recent 2019 ESC/EAS guidelines, the use of cholesterol-lowering therapies is not always optimal to achieve the therapeutic goals even in patients with very high CV risk. This means that about 80% of patients are far from the recommended therapeutic goals for their risk category.

Keywords
Atherosclerotic cardiovascular disease; Lipid lowering therapy; LDL-cholesterol; Cardiovascular risk assessment; Guidelines

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Introduction

The causal role of low-density lipoprotein cholesterol (LDL-C) in atherosclerotic-related cardiovascular diseases (ASCVD) has been unequivocally established and a vast amount of studies have indisputably shown that reducing LDL-C levels reduces the risk of ASCVD (1, 2). Treating with statins has been for long the best approach for primary and secondary prevention of CVD, and, based on the considerable evidence, guidelines for the treatment of dyslipidaemias recommend statins as the first-line approach (3, 4). However, several new hypolipidaemic drugs have been developed and approved in the

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last few years, thus expanding the pharmacological armamentarium available for efficient control of circulating LDL-C levels.

It is also clear that the reduction of CV risk is proportional to the magnitude of LDL-C level reduction, independently of the drug used to achieve such a reduction (1). These last observations imply that combination therapy may represent an excellent chance to safely achieve larger LDL-C reductions, taking advantage of complementary mechanisms of action of different drugs.

Based on these considerations, it is expected that patients may also take advantage of this opportunity in everyday clinical practice. Nevertheless, several studies have shown that this is not the case. The most recent DA VINCI study reported relevant gaps in Europe between clinical practice and 2016 ESC/EAS guidelines, with only 54% of enrolled patients achieving the LDL-C goal, a percentage even lower (39%) among those at very high-risk (5). This observation validates the results of previous observational studies reporting less-than-optimal management of LDL-C levels in patients at high CV risk (6-8). Since the last 2019 ESC/EAS guidelines have introduced substantial downward adjustments to the LDL-C goals (3), the gap between recommendations and clinical practice is bound to grow.

The SANTORINI study is an observational study that enrolled patients at high and very high CV risk to evaluate the management of dyslipidaemia in a real-world setting and assess the gaps in clinical practice (9). In this paper we have analysed the data deriving from the Italian patients recruited in the SANTORINI study.

Methods

Study design

The Treatment of high and very high-risk dyslipidemic pAtients for the preveNTion of cardiovascular events in Europe - a multInatioNal observatIonal (SANTORINI) study is a multinational, multicentre, prospective observational, non-interventional study that enrolled 9602 patients (9044 with complete data) at high and very high CV risk requiring lipid-lowering therapies from 14 European countries between March 2020 to February 2021 (NCT04271280) (9, 10). The methodology and rationale for this study have been described previously (10). Italy participates in the study with 1977 patients; data were obtained from each patient at enrolment and included baseline biochemical parameters, current lipid-lowering therapies, and medical history.

Eligibility criteria

Patients were eligible if they were ≥18 years old, had high or very high CV risk and required lipid-lowering therapy. The CV risk was defined at enrolment by the investigators; the Systematic Coronary Risk Estimation (SCORE) system was used centrally to assess CV risk in the primary prevention population. All participants provided written informed consent. No specific exclusion criteria were defined.

Data source

As an observational descriptive study, the sample size of the whole study was based on the assumption that data from approximately 8000 patients would provide sufficient precision (95% confidence interval) to show the rates of CV events during one-year follow-up. Therefore, all adult patients deemed by the physician as being at high or very high CV risk, and who would be eligible for lipid-lowering therapy (LLT) as per 2019 ESC/EAS guidelines were included in this study. CV risk was assigned by physicians at enrolment, and the basis for risk category was documented. CV risk was also assessed centrally based on the information present in the study database according to SMART, Framingham or Systematic Coronary Risk Estimation [SCORE] risk score systems per 2019 ESC/EAS guideline criteria (11). When inconsistencies were found between the CV risk as assessed by the physician and the CV risk category recalculated centrally, a medical query was raised and the physicians were given the possibility to confirm their classification. The results presented here are from the Baseline Analysis Set, which consisted of all patients from the All-Documented Patients Set with adequate baseline information, including completing a medical review of all open queries.

Objectives of the study

This study's primary objectives were to evaluate the use of lipid-lowering therapies and the effectiveness of these treatment approaches in achieving the recommended goals in high and very high-risk patients requiring lipid-lowering therapies in a real-world setting. Furthermore, a comparative analysis has been performed between Italy and the rest of Europe enrolled patients.

Statistical analysis

Baseline characteristics are presented as means (standard deviation [SD]) or median (interquartile range [IQR]) of continuous variables and as percentages of categorical variables. Results are reported by CV risk classification as assessed by physicians (high-risk, and very high-risk), ASCVD status (with ASCVD, and without ASCVD), LLT received, and proportion of patients achieving LDL-C goals.

CV risk was calculated using patient data and applying the CV risk classification of 2019 ESC/EAS guidelines (3, 12). ASCVD was considered present if any of the following was reported in the medical history: coronary ASCVD (myocardial infarction; unstable angina; angina pectoris; coronary artery bypass graft surgery; percutaneous transluminal coronary angioplasty; coronary artery disease [CAD]; CAD unequivocal on imaging); cerebral ASCVD (stroke; transient ischemic attack; cerebrovascular disease; cerebrovascular disease unequivocal on imaging; carotid artery disease); peripheral/other ASCVD (peripheral arterial disease [PAD]; lower extremity artery disease; PAD unequivocal on imaging; retinal vascular disease; abdominal aortic aneurysm; renovascular disease); polyvascular ASCVD (if affecting more than one vascular bed).

Ethics approval and consent to participate

The SANTORINI study has been performed in accordance with the Declaration of Helsinki and Good Clinical Practice. All patients provided written informed consent before participating in the study.

Results

Patient characteristics

A total of 1977 patients were enrolled in Italy from 125 sites (**Appendix 1**); 1531 (77.4%) were classified by the investigators as very high CV risk and 446 (22.6%) as high CV risk patients. The overall population included 73.5% men and 26.5% women; the percentage of women was much higher in the high-risk group than in the very high-risk group (44.8% and 21.1%, respectively) (**Table 1**). Baseline characteristics of the overall population and CV risk subgroups are presented in **Tables 1 and 2** and **Table S1**. Renal insufficiency was present in 15.1% of the enrolled individuals, most of whom showed mild-to-moderate renal insufficiency (**Table S1**).

The majority of enrolled patients had a previous diagnosis of dyslipidaemia (>4 weeks) (77.3%) (**Table 2**). The mean LDL-C level was 98.4±49.7 mg/dL; high-risk patients had higher LDL-C levels than very high-risk patients (111.4±55.3 mg/dL vs 94.6±47.3 mg/dL) (**Table 2**). ApoB and Lp(a) were measured in a very limited

Table 1 | Baseline characteristics of the overall population and cardiovascular risk subgroups at enrolment visit – Italy.

Baseline characteristic	Overall (N=1977)	Risk classification as reported by the investigators	
		Very high-risk (N=1531)	High-risk (N=446)
Gender Male, n (%) Female, n (%)	 1454 (73.5%) 523 (26.5%)	 1208 (78.9%) 323 (21.1%)	 246 (55.2%) 200 (44.8%)
Age, years, mean (SD)	64.5 (11.1)	65.3 (10.6)	61.9 (12.2)
Smoking history, n (%) Current Former Never	 393 (19.9%) 752 (38.0%) 832 (42.1%)	 332 (21.7%) 650 (42.5%) 549 (35.9%)	 61 (13.7%) 102 (22.9%) 283 (63.5%)
Hypertension, n (%)	1409 (71.3%)	1154 (75.4%)	255 (57.2%)
Familial hypercholesterolaemia, n (%)	254 (12.9%)	126 (8.2%)	128 (28.7%)
Diabetes, n (%) Diabetes with target organ damage, n (%)	569 (28.8%) 131 (6.6%)	457 (29.9%) 113 (7.4%)	112 (25.1%) 18 (4.0%)
BMI, kg/m², mean (SD)	27.4 (4.2)	27.5 (4.2)	26.9 (4.2)
BP systolic, mean (SD) *BP diastolic, mean (SD)*	129.5 (16.5) 76.6 (9.5)	129.5 (16.9) 76.3 (9.8)	129.4 (15.0) 77.8 (8.5)

SD: standard deviation; BMI: body mass index; BP: blood pressure.

Table 2 | Laboratory values in the overall population and cardiovascular risk subgroups at enrolment visit – Italy.

Baseline characteristic		Overall (N=1977)	Risk classification as reported by the investigators	
			Very high-risk (N=1531)	High-risk (N=446)
Newly diagnosed with dyslipidaemia, n (%) Newly diagnosed (<4 weeks) Previously diagnosed (> 4 weeks)		 448 (22.7%) 1529 (77.3%)	 373 (24.4%) 1158 (75.6%)	 75 (16.8%) 371 (83.2%)
LDL-C [mg/dL]	*n*	1964	1519	445
	Mean (SD)	98.4 (49.7)	94.6 (47.3)	111.4 (55.3)
HDL-C [mg/dL]	*n*	1963	1519	444
	Mean (SD)	47.9 (15.5)	46.0 (14.9)	46.0 (14.9)
non-HDL-C [mg/dL]	*n*	1962	1518	444
	Mean (SD)	120.2 (54.4)	116 (51.4)	134.6 (61.7)
TC [mg/dL]	*n*	1968	1522	446
	Mean (SD)	169.7 (57.6)	163.3 (54.5)	191.4 (62.4)
ApoB [g/L]	*n*	57	51	6
	Mean (SD)	0.9 (0.4)	0.9 (0.3)	1.1 (0.4)
TG [mg/dL]	*n*	1725	1351	374
	Mean (SD)	135.6 (91.9)	134.8 (92.4)	138.5 (90.1)
Lp(a) [mg/dL]	*n*	108	94	14
	Median (IQR)	31.0 (10.0, 79.1)	28.2 (10.0, 71.7)	60.8 (13.0, 102.0)
HbA1c [%]	*n*	567	457	110
	Mean (SD)	6.56 (1.30)	6.56 (1.33)	6.57 (1.13)
Fasting glucose [mmol/L]	*n*	1179	966	213
	Mean (SD)	6.35 (2.08)	6.41 (2.16)	6.07 (1.69)
Hs-CRP [mg/L]	*n*	223	195	28
	Median (IQR)	3.0 (0.7, 10.2)	3.30 (0.98, 12.2)	0.90 (0.35, 2.75)

LDL-C: low-density lipoprotein cholesterol; HDL-C: high-density lipoprotein cholesterol; TC: total cholesterol; apoB: apolipoprotein B; TG: triglycerides; Lp(a): lipoprotein(a); HbA1c: glycated haemoglobin; hs-CRP: high sensitivity C reactive protein.

number of patients (**Table 2**). Lp(a) levels were higher in the high-risk group than in the very high-risk group (median: 60.8 [13.0-102.0] mg/dl vs 28.2 [10.0-71.7] mg/dL). Overall, the high-risk patient subgroup exhibited a worse lipid profile than the very high-risk patient subgroup. Hs-CRP was much higher in the very high-risk subgroup (**Table 2**).

Cardiovascular risk assessment

Almost all individuals were enrolled from hospitals (97.7%), with cardiologists being the major specialty involved in the recruitment (64.2%) (particularly for very high-risk patients), followed by internists/internal medicine specialists (24.2%) (**Table 3**)**.** General practitioners only contributed with 10 out of 1977 enrolled patients.

At enrolment, individuals' CV risk was assessed by investigators; 1531 patients (77.4%) were classified as very high CV risk and 446 (22.6%) were classified as high CV risk. The majority of patients were classified based on the 2019 ESC/EAS guidelines (72.8% in the overall population), 22.8% were classified based on the clinical experience of the investigators, and a small percentage were classified using other criteria (**Table 3**). Similar percentages were reported in the very high-risk and high-risk subgroups.

Patients whose risk was calculated by the investigators according to the 2019 ESC/EAS guidelines (N=1439, very high-risk 1164, high-risk 275) were further evaluated centrally, again according to the 2019 ESC/EAS guidelines. Among patients classified by the investigators as "very high-risk", the central determination of the CV risk confirmed this classification in 99% of individuals (only 9 out of 1164 were reclassified as "high-risk") (**Figure 1A**). On the contrary, among patients classified as "high-risk" by the investigators, the central assessment of the CV risk provided a reclassification as "very

Table 3 | Specialty of investigators by cardiovascular risk factors at enrolment visit-Italy.

	Overall (N=1977)	Risk classification as reported by the investigators	
		Very high-risk (N=1531)	High-risk (N=446)
Site setting, n (%)			
Hospital	1931 (97.7%)	1521 (99.4%)	410 (91.9%)
Medical practice	46 (2.3%)	10 (0.6%)	36 (8.1%)
Specialty, n (%)			
Cardiologist	1269 (64.2%)	1102 (72.0%)	167 (37.4%)
Diabetologist	125 (6.3%)	63 (4.1%)	62 (13.9%)
General practitioner	10 (0.5%)	5 (0.3%)	5 (1.1%)
Internal medicine specialist/ internist	479 (24.2%)	282 (18.4%)	197 (44.2%)
Lipidologist	135 (6.8%)	100 (6.5%)	35 (7.8%)
Basis for risk classification, n (%)			
Clinical experience	451 (22.8%)	327 (21.4%)	124 (27.8%)
Institutional practice and/or considerations	17 (0.9%)	10 (0.7%)	7 (1.6%)
Institutional guidelines	34 (1.7%)	13 (0.9%)	21 (4.7%)
Regional guidelines	0 (0.0%)	0 (0.0%)	0 (0.0%)
National guidelines	19 (1.0%)	14 (0.9%)	5 (1.1%)
ESC/EAS guidelines	1439 (72.8%)	1164 (76.0%)	275 (61.7%)
Other	17 (0.9%)	3 (0.2%)	14 (3.1%)

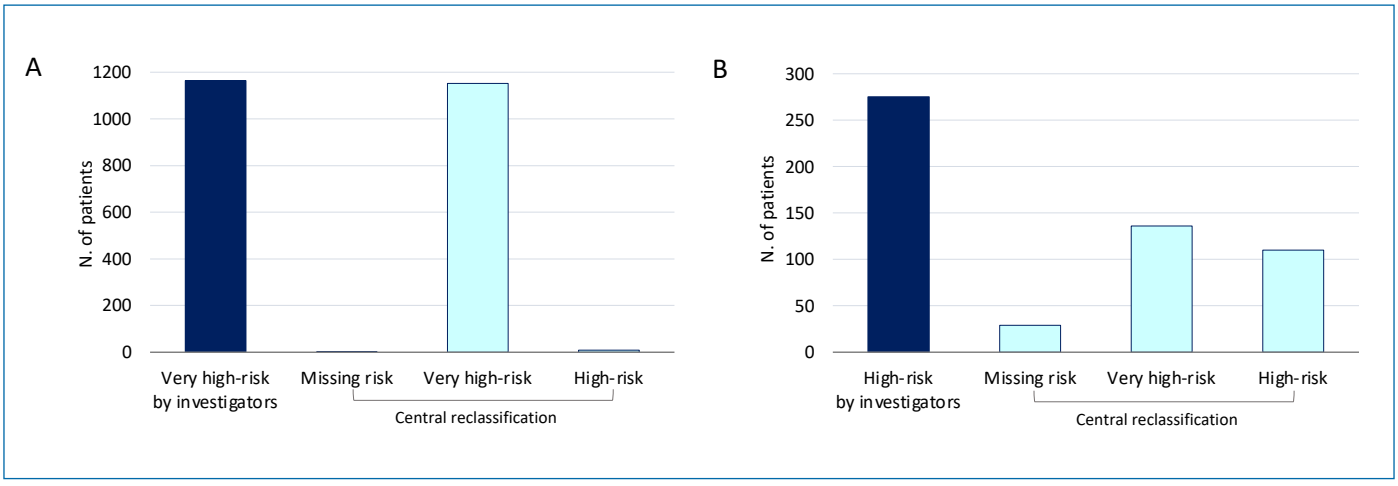

Figure 1 | Recalculated risk classification by ESC/EAS criteria. *Patients classified by investigators as very-high-risk (A) or high-risk (B) by ESC/EAS criteria were reclassified centrally by ESC/EAS criteria. Blue bars represent the number of patients classified as very-high-risk (A) or high-risk by investigators based on ESC/EAS criteria; grey bars represent the same patients whose risk was recalculated centrally based on ESC/EAS criteria.*

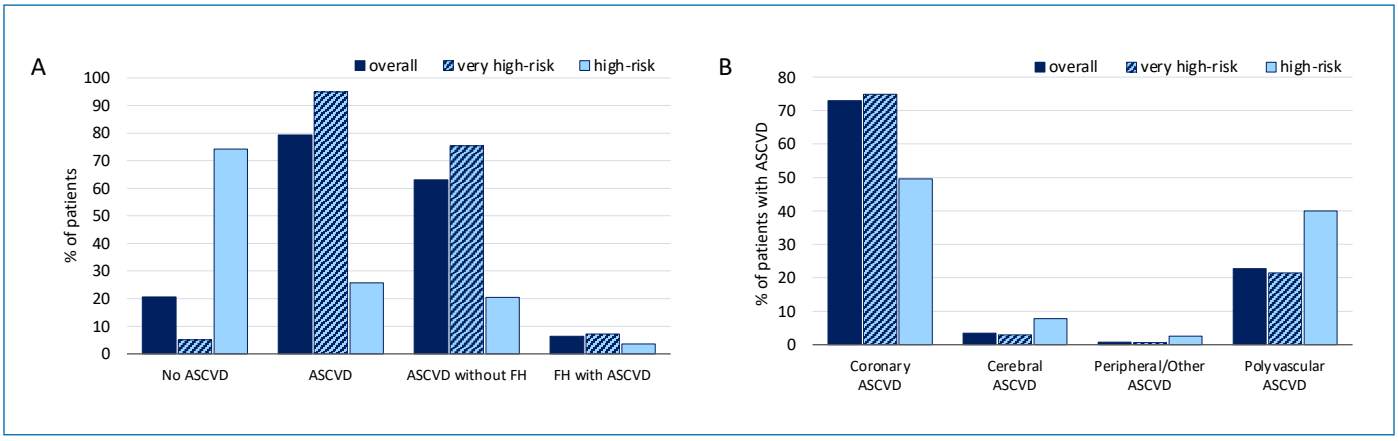

Figure 2 | ASCVD status at baseline. *(A) percentage of patients with or without ASCVD and with or without familial hypercholesterolemia. (B) ASCVD subtypes among patients with ASCVD.*

high-risk" in 136 out of 275 (49.5% of patients) (**Figure 1B**). Overall, the risk classification based on 2019 ESC/EAS criteria identified 1288 patients at very high-risk and 119 at high-risk.

Cardiovascular history at enrolment

Overall, 79.3% of the enrolled patients had ASCVD (**Figure 2A**); this percentage was higher in the very high-risk subgroup and much lower in the high-risk group (94.9% and 25.8%, respectively). Most patients had ASCVD without having FH. Coronary ASCVD was the most common type of ASCVD, representing 74.9% in the very high-risk subgroup and 49.6% in the high-risk subgroup; polyvascular ASCVD was more common among high-risk patients (**Figure 2B**).

About half of the enrolled patients had a history of myocardial infarction, most of whom were in the very high-risk subgroup (936 out of 978) (**Table 4**). Similarly, most of the patients having a history of angina pectoris and unstable angina were part of the very high-risk subgroup (**Table 4**). Overall, 58.6% of individuals (73.9% of those having ASCVD) have had a percutaneous transluminal coronary angioplasty (PTCA) procedure and 9.3% (11.7% of those with ASCVD) had undergone a coronary artery bypass graft (CABG) (**Table 4**). Coronary artery disease was identified in 70.2% of the enrolled patients (86.0% in the very high-risk subgroup); peripheral artery disease and carotid artery disease were present in 16.4% and 12.5% of enrolled patients, respectively (**Table 4**).

Table 4 | Relevant cardiovascular history by cardiovascular risk at enrolment visit.

	Overall (N=1977)	Risk classification as reported by the investigators	
		Very high-risk (N=1531)	High-risk (N=446)
ASCVD, n (%)			
MI	978 (49.5%)	936 (61.1%)	42 (9.4%)
Angina pectoris	461 (23.3%)	436 (28.5%)	25 (5.6%)
Unstable angina	216 (10.9%)	208 (13.6%)	8 (1.8%)
Cardiac arrhythmia	279 (14.1%)	227 (14.8%)	52 (11.7%)
PTCA	1158 (58.6%)	1110 (72.5%)	48 (10.8%)
CABG	183 (9.3%)	176 (11.5%)	7 (1.6%)
CAD	1388 (70.2%)	1316 (86.0%)	72 (16.1%)
CAD unequivocal on imaging	925 (46.8%)	889 (58.1%)	36 (8.1%)
Stroke	78 (3.9%)	72 (4.7%)	6 (1.4%)
TIA	72 (3.6%)	63 (4.1%)	9 (2.0%)
PAD	325 (16.4%)	279 (18.2%)	46 (10.3%)
PAD unequivocal on imaging	132 (6.7%)	114 (7.4%)	18 (4.0%)
Cerebrovascular disease	155 (7.8%)	137 (8.9%)	18 (4.0%)
Cerebrovascular disease unequivocal on imaging	67 (3.4%)	59 (3.8%)	8 (1.8%)
Carotid artery disease	247 (12.5%)	207 (13.5%)	40 (9.0 %)

ASCVD: atherosclerotic cardiovascular disease; MI: myocardial infarction; PTCA: percutaneous transluminal coronary angioplasty; CABG: coronary artery bypass graft; CAD: coronary artery disease; TIA: transient ischemic attack; PAD: peripheral artery disease.

Table 5 | Subgroups by cardiovascular risk factors.

	Overall (N=1977)	No ASCVD (N=409)	Confirmed ASCVD (N=1568)				
			Total (N=1568)	Coronary ASCVD (N=1145)	Cerebral ASCVD (N=53)	Peripheral/ Other ASCVD (N=12)	Polyvascular ASCVD (N=358)
Female, n (%)	523 (26.5%)	190 (46.5%)	333 (21.2%)	214 (18.7%)	23 (43.4%)	3 (25.0%)	93 (26.0%)
Age, years, mean (SD)	64.5 (11.1)	61.6 (13.0)	65.3 (10.4)	64.1 (10.5)	66.6 (9.0)	67.8 (12.9)	68.6 (9.5)
Hypertension, n (%)	1409 (71.3%)	237 (58.0%)	1172 (74.7%)	817 (71.4%)	44 (83.0%)	7 (58.3%)	304 (84.9%)
FH	254 (12.9%)	128 (31.3%)	126 (8.0%)	81 (7.1%)	1 (1.9%)	0 (0.0%)	44 (12.3%)
Diabetes, n (%)	569 (28.8%)	127 (31.1%)	442 (28.2%)	285 (24.9%)	18 (34.0%)	3 (25.0%)	136 (38.0%)
Diabetes with target organ damage, n (%)	131 (6.6%)	28 (6.9%)	103 (6.6%)	57 (5.0%)	3 (5.7%)	1 (8.3%)	42 (11.7%)
BMI, kg/m², mean (SD)	27.4 (4.2)	26.85 (4.3)	27.48 (4.2)	27.61 (4.37)	27.03 (4.1)	26.22 (2.73)	27.19 (3.83)
LDL-C, mg/dL, mean (SD)	98.2 (39.8)	114.5 (56.1)	94.4 (47.1)	96.7 (47.1)	107.9 (55.8)	83.1 (40.7)	85.1 (43.9)
Very high-risk patients, n (%)*	1531 (77.4%)	78 (19.1%)	1453 (92.7%)	1088 (95.0%)	44 (83.0%)	9 (75.0%)	312 (87.2%)

ASCVD: atherosclerotic cardiovascular disease; FH: familial hypercholesterolemia; BMI: body mass index; LDL-C: low-density lipoprotein cholesterol. *Risk classification as reported by the investigators.

Table 5 lists the main baseline characteristics of patients according to the absence or presence of ASCVD (N=409 and N=1568, respectively) and the type of ASCVD.

Use of lipid-lowering therapy

Overall, 67.4% of the enrolled participants were taking a lipid-lowering therapy; half of them were taking a combination therapy (**Figure 3**). Among very high-risk patients, 34.2% were not under lipid-lowering treatment.

Of the patients taking LLT (N=1332), 44.7% were taking statin monotherapy, in most cases a moderate- or high-intensity statin (**Table 6, Figure 4A**). Ezetimibe alone or PCSK9 inhibitors alone were administered in only 2.6% and 3.1%, respectively. Patients treated with combination therapy mainly received a combination of statin and ezetimibe (23.6%), while a combination with a PCSK9 inhibitor was administered in 14% of patients (**Table 6, Figure 4B**). Interestingly, a higher percentage of the high-risk patients were treated with a PCSK9 inhibitor in combination with ezetimibe or ezetimibe+statin than the very high-risk patients (**Figure 4B**).

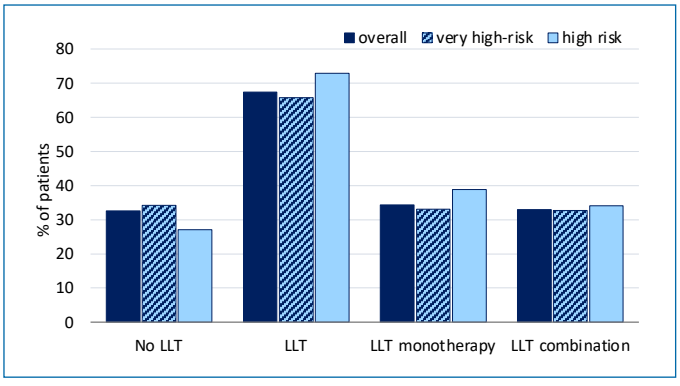

Figure 3 | Lipid-lowering therapy at baseline.

The analysis according to the ASCVD status showed that a high percentage of patients with ASCVD were not taking LLT (34.1% versus 27.1% in patients without ASCVD) (**Table 6**).

Attainment of 2019 ESC/EAS guideline LDL-C goals

Overall, 79% of study participants (1562 out of 1977) were not at goal; only 19.9% of the very high-risk patients and 21.8% of high-risk patients achieved LDL-C goals recommended by current 2019 ESC/EAS guidelines (**Table 7**). Among patients with ASCVD, 78.1% were not at LDL-C goal (**Table 7**), a percentage similar to that reported among patients without ASCVD (82.4%), very high-risk with ASCVD (79%), or very high-risk without ASCVD (84.6%). Only 4% of individuals not taking LLT were at LDL-C goal; among those taking a lipid-lowering therapy, 21.6% of patients taking a monotherapy and 35.1% of patients taking a combination therapy were at LDL-C goal (**Table 7**).

European (without Italy) and Italian data comparison (descriptive analysis)

Then, we compared the results obtained in the Italian subgroup with those obtained in the rest of the enrolled European patients ("Europe w/o Italy" group). Baseline characteristics of the "Europe

Table 6 | Lipid-modifying therapy in the overall population and according to cardiovascular risk or ASCVD status.

	Overall (N=1977)	Risk classification as reported by the investigators*		ASCVD status	
		Very high-risk (N=1531)	High-risk (N=446)	ASCVD (N=1568)	No ASCVD (N=409)
LLT, n (%)					
No LLT	645 (32.6%)	524 (34.2%)	121 (27.1%)	534 (34.1%)	111 (27.1%)
LLT	1332 (67.4%)	1007 (65.8%)	325 (65.8%)	1034 (65.8%)	298 (65.8%)
Monotherapies	680 (34.4%)	507 (33.1%)	173 (38.8%)	518 (33.0%)	162 (39.6%)
Statin alone	595 (30.1%)	446 (29.1%)	149 (33.4%)	458 (29.2%)	137 (33.5%)
Ezetimibe alone	34 (1.7%)	26 (1.7%)	8 (1.8%)	27 (1.7%)	7 (1.7%)
PCSK9i alone	43 (2.2%)	31 (2.0%)	12 (2.7%)	30 (1.9%)	13 (3.2%)
Any other oral LLT alone	8 (0.4%)	4 (0.3%)	4 (0.9%)	3 (0.2%)	5 (1.2%)
Combination therapies	652 (33.0)	500 (32.7%)	152 (34.1%)	516 (32.9%)	136 (33.3%)
Combination statin+ezetimibe	315 (15.9%)	256 (16.7%)	59 (13.2%)	267 (17.0%)	48 (11.7%)
PCSK9i combination	187 (9.5%)	128 (8.4%)	59 (13.2%)	134 (8.6%)	53 (13.0%)
Any other oral combination therapy	150 (7.6%)	116 (7.6%)	34 (7.6%)	115 (7.3%)	35 (8.6%)

ASCVD: atherosclerotic cardiovascular disease; LLT: lipid-lowering therapy; PCSK9i: proprotein convertase subtilisin/kexin 9 inhibitors.

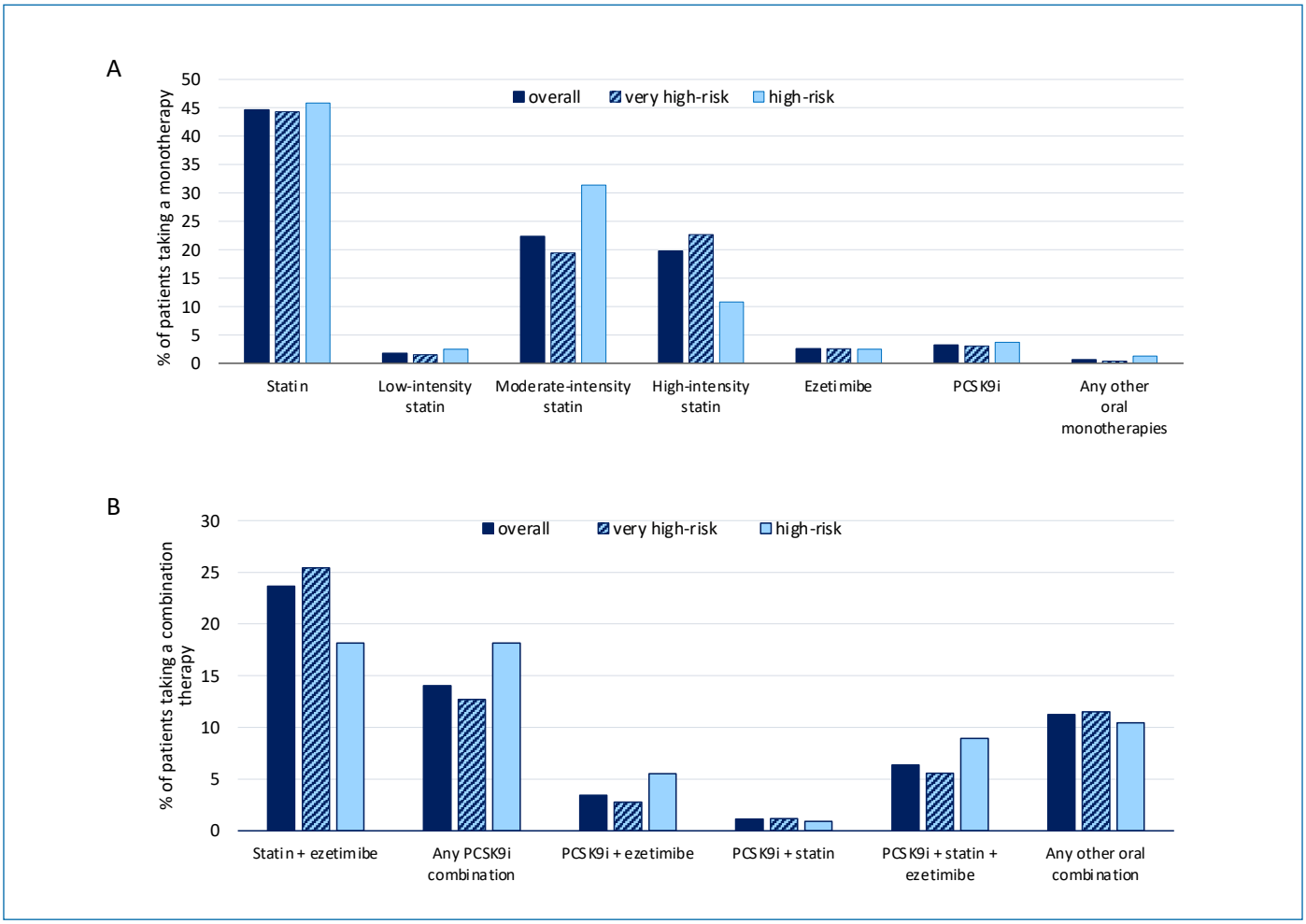

Figure 4 | Details on the lipid-lowering therapies used in Italy. *(A) Monotherapies, (B) combination therapies.*

without Italy" group are listed in **Tables S2** and **S3.** No major differences were observed compared with the Italy subgroup in most baseline characteristics. The prevalence of FH was lower than in the Italy group (9.0% vs 12.9%), whereas diabetes was more prevalent (34.9% vs 28.8%).

Over 90% of enrolled patients had a previous diagnosis of dyslipidaemia, compared with 77% in Italy (**Table S3**). LDL-C levels were 98.4 mg/dL and 91.7 mg/dL in Italy and Europe w/o Italy subgroups, with 79% and 71.7% of individuals not at goal, respectively. Median hs-CRP levels were comparable (**Tables 2 and S3**); interestingly, no differences were observed between very high-risk and high-risk subgroups in the "Europe w/o Italy" population (**Table S3**), contrarily to what observed in the Italian patients (3.30 mg/L [0.70-10.20] and 0.90 mg/L [0.35-2.75], respectively).

Hospitals represented the major centre for patient recruitment in Italy (97.7%), whereas in the group Europe w/o Italy medical practice contributed significantly (43.1%) (**Table S4**). Similarly, while in Italy the contribution of general practitioners is almost irrelevant (0.5%), in the Europe w/o Italy subgroup 16.5% of patients were enrolled by the general practitioners, who contributed more specifically to the recruitment of high-risk patients (27.9%) (**Table S4**). Clinical experience appears to be more relevant for the risk classification in the Europe w/o Italy group (37.3% vs 22.8% in Italy), whereas 2019 ESC/EAS guidelines were less applied (46.2% vs 72.8% in Italy) (**Table S4**).

Furthermore, the application of 2019 ESC/EAS guidelines for risk classification performed very well for the "very high-risk" subgroup in both Italy and the Europe w/o Italy groups, where 99% and 98.2% of patients received the same risk classification by investigators and centrally. However, among the patients classified as "high-risk" by investigators according to 2019 ESC/EAS guidelines, a larger percentage would have been reclassified as "very high-risk" by central assessment (65.1% in the Europe w/o Italy group compared with 49.5% in the Italy group) (**Figure 5**).

Overall, the incidence of ASCVD did not differ between Italy and the Europe w/o Italy groups (**Figure 6**). A higher percentage of high-

Table 7 | LDL-C goal attainment using investigator-reported risk.

	LDL-C (mg/dL) Mean (SD)	Patients at LDL-C goal, N (%)	Patients not at LDL-C goal, N (%)	Unknown, N (%)
Overall	98.2 (49.7)	402 (20.3%)	1562 (79.0%)	13 (0.7%)
Very high-risk (Investigator-reported)	94.7 (47.3)	305 (19.9%)	1214 (79.3%)	12 (0.8%)
High-risk (Investigator-reported)	111.4 (55.3)	97 (21.8%)	348 (78.0%)	1 (0.2%)
ASCVD	94.3 (47.0)	332 (21.2%)	1225 (78.1%)	11 (0.7%)
No ASCVD	114.5 (56.2)	70 (17.1%)	337 (82.4%)	2 (0.5%)
Very high-risk with ASCVD	94.0 (46.8)	294 (20.2%)	1148 (79.0%)	11 (0.8%)
Very high-risk without ASCVD	104.8 (54.6)	11 (14.1%)	66 (84.6%)	1 (1.3%)
ASCVD (excluding FH)	84.0 (45.6)	260 (20.9%)	978 (78.5%)	8 (0.6%)
No LLT	131.1 (45.9)	26 (4.0%)	615 (95.4%)	4 (0.6%)
Monotherapy	86.6 (39.8)	147 (21.6%)	527 (77.5%)	6 (0.9%)
Combination therapy	78.1 (46.1)	229 (35.1%)	420 (64.4%)	3 (0.5%)

ASCVD: atherosclerotic cardiovascular disease; LDL-C: low-density lipoprotein cholesterol; FH: familial hypercholesterolemia; LLT: lipid-lowering therapy,

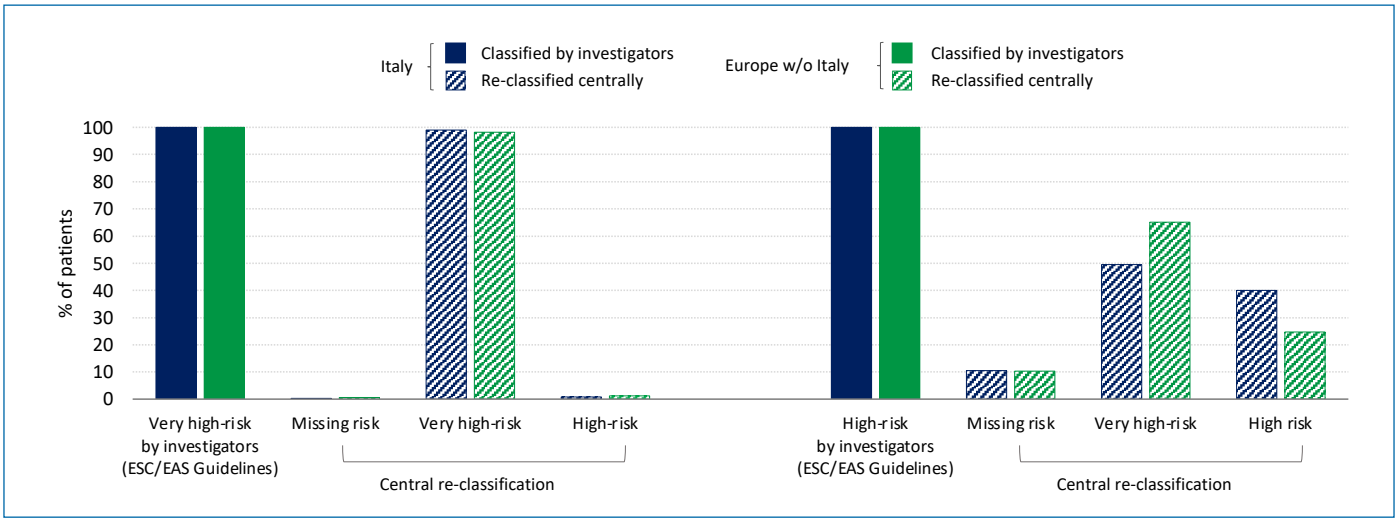

Figure 5 | Risk classification details: comparison between Italy and Europe w/o Italy groups.

risk individuals in the Europe w/o Italy group had coronary ASCVD (29% vs 12.8%) and had a previous myocardial infarction (17.7% vs 9.4), but overall a lower percentage of patients had experienced an MI. Overall, high-risk patients in the Europe w/o Italy group have a greater history of cardiovascular disease compared with the Italy group (**Figure 6**), which can at least in part explain the higher percentage of patients that would have been reclassified as very high-risk patients when re-evaluated centrally (**Figure 5**).

More individuals were taking an LLT (81.2% vs 67.4%), but most were treated with monotherapy (59.7% vs 34.4% in Italy), with statin

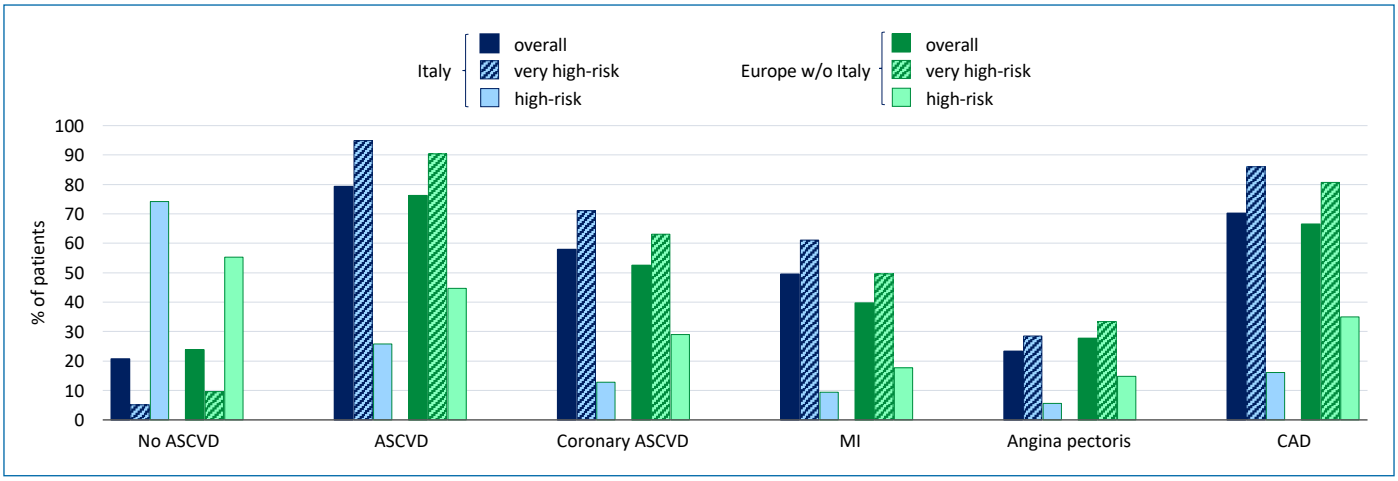

Figure 6 | Detailed cardiovascular history at baseline: comparison between Italy and Europe w/o Italy.

Figure 7 | Lipid-lowering therapy at baseline: comparison between Italy and Europe w/o Italy. *(A) percentage of patients without therapy or taking a lipid-lowering therapy (monotherapy or combination) overall and by CV risk in Italy and Europe w/o Italy groups. (B) Details on the type of lipid-lowering therapies used in Italy and Europe w/o Italy.*

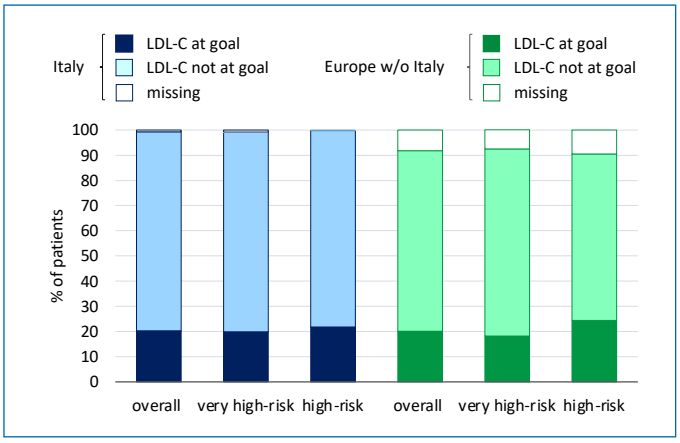

Figure 8 | LDL-C goal attainment in Italy and Europe w/o Italy.

being used in almost twice as many patients in the Europe w/o Italy subgroup than in Italy (moderate- or high-intensity) (**Figure 7A and 7B**). Combination therapy with PCSK9i was more frequently used in Italy.

Overall, 79% and 71.7% of enrolled patients were not at LDL-C goal in Italy and Europe w/o Italy, respectively; a major difference was observed between the high-risk groups, with 78% and 66.1% of patients being not at LDL-C goal, respectively (**Figure 8**).

Discussion

Recognizing that LDL-C has a causal role in ASCVD has greatly pushed research in developing new and more efficient lipid-lowering drugs so that physicians have adequate pharmacological tools to manage efficiently hypercholesterolemia and reduce the CV risk. Based on the observation that reducing LDL-C reduces the CV risk proportionally to the absolute reduction in LDL-C, even at the very low LDL-C levels that can be achieved by combining the most effective cholesterol-lowering drugs, the 2019 ESC/EAS guidelines have further reduced the LDL-C goals, particularly for high-risk and very high-risk patients (3).

Indeed, several observational studies have unequivocally shown that, in clinical practice, patients with high and very high CV risk are substantially undertreated, far from the recommended goals, and thus retain an elevated risk of experiencing a CV event. Relevant gaps were reported between observational studies in real-world settings and the recommendations contained in 2016 ESC/EAS guidelines (5-8); due to the tightening of LDL-C goals contained in 2019 ESC/EAS guidelines (3), it is expected that these gaps may further make things worse. The SANTORINI study was thus set to answer this question (10), and the present study provides information about the management of high and very high CV risk patients in clinical practice in Italy, assessing the quality of the treatment and the attainment of LDL-C goals according to the 2019 ESC/EAS guidelines.

The majority of patients enrolled in this observational study were classified as very high-risk patients. Guidelines recommend that these patients have an LDL-C <55 mg/dL together with an LDL-C reduction of ≥50% from baseline when treated; here we found that very high-risk patients had an LDL-C level very far from optimal and only one-fifth of them were at goal. Analysing the lipid-lowering treatment status, we observed that not all patients had a prescribed therapy, and only half of those taking an LLT were given combination therapy. These represent relevant issues. Moreover, if on the one hand, there is a too high percentage of patients at very high-risk who are not treated, on the other hand, there is a substantial underutilization of combination therapies. Combination therapy represents the most effective approach to reduce substantially LDL-C levels and CV risk in these patients (13). Such an inadequate pharmacological approach implies that a large proportion of individuals at high or very high CV risk are not able to meet the goals recommended by current guidelines. As highlighted in this analysis, only an irrelevant percentage of untreated patients were at goal, and, among those taking an LLT, those treated with combination therapy had more chance to be at LDL-C goal.

We must also underline that, in the Italian setting, the contribution of general practitioners in the recruitment of patients at high or very high CV risk is neglectable while being more relevant in the rest of Europe. This represents a major gap that needs to be filled shortly.

An interesting observation is that overall 2019 ESC/EAS guidelines drive the risk classification by investigators; however, in Italy 2019 ESC/EAS guidelines are followed by a higher percentage of investigators compared with the Europe w/o Italy subgroup. This might, at least in part, explain a higher use of combination therapy in Italy in both high-risk and very high-risk; despite that, LDL-C level is far from optimal in both settings.

The analysis of baseline characteristics of patients involved in the Santorini study, and specifically those recruited in Italy, suggest that high and very high CV risk patients are still undertreated, with LDL-C levels much higher than guidelines recommended goals and underutilization of effective lipid-lowering combination therapies.

Although the guidelines provide clear evidence that treating dyslipidaemias is crucial for the prevention of cardiovascular disease, several observational studies have unequivocally demonstrated that, in real-world clinical practice, individuals at high/very high CV risk are generally not adequately treated. Underestimation of risk and underutilisation of combination therapies are major factors contributing to this. In most cases, monotherapies are insufficient to achieve the recommended goals in these patients, but they are still widely prescribed. Clinicians should bear in mind that high-intensity statin monotherapies can provide an average 50% reduction in LDL-C; oral combination therapies and, where appropriate, treatment with monoclonal antibodies against PCSK9 allow to achieve ≥80% reduction in LDL-C (13). The current availability of cholesterol-lowering therapies with different mechanisms of action should help physicians to personalize treatment based on individual needs. A tailored therapy might represent the right tool to reduce side effects while increasing adherence and compliance, resulting in a higher chance of achieving LDL-C goals and consequently reducing CV risk.

The therapy algorithm of the ESC/EAS guidelines suggests a stepwise therapy strategy in which combination therapy is the second step of intervention. While this approach may be useful for patients at moderate CV risk or with LDL-C levels not far from the goal, patients at very high CV risk who are distant from the goal need therapies that can substantially lower their LDL-C levels regardless of their baseline. Lowering LDL-C log-linearly reduces the risk of CV events without reaching a plateau, suggesting that patients at very high-risk may benefit greatly from an early intervention based on combination therapy. It is expected that a maximised therapy strategy with a combination of high-intensity statin therapy, ezetimibe, a PCSK9 inhibitor (and possibly bempedoic acid) could effectively lower LDL-C levels, increase adherence and consequently reduce CV risk in very high-risk patients.

Conflict of interest

MA Arca has received payments for the provision of grants and consulting services from Akcea/Ionis, Alfasigma, Amgen, Amryt, Amarin, Daiichi-Sankyo, Pfizer, Regeneron, Sanofi, SOBI, Viatris

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Authorship and Author Contributions

MA, PC, AS, RG, KR, and ALC contributed to the study conceptualization and methodology; all authors contributed to the design and writing of the manuscript, and revising it critically.

Permissions information

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Appendix 1

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CONFERENCE REPORT

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The VIII Spring Meeting of Young Researchers from the Italian Society of Diabetology (SID), the Italian Society of Arterial Hypertension (SIIA), the Italian Society of Internal Medicine (SIMI), the Italian Society of Cardiovascular Prevention (SIPREC) and the Italian Society for the Study of Atherosclerosis (SISA) "*Basic and clinical research: Until grant let us apart*" was held in Rimini on April 16-18, 2023. As is usual, the Congress was organized by the young members of the above scientific societies working in the field of cardiometabolism. The Congress included five sessions discussing the latest findings in basic and clinical research on the treatment and prevention of cardiometabolic diseases. Many young researchers had the opportunity to present their scientific work in dedicated oral and poster sessions. In this report, we provide a summary of the most important topics discussed during the meeting lectures.

The meeting opened with a session organized by SISA that was focused on the role of lipoprotein metabolism beyond atherosclerosis and addressed two hot topics in the field of lipidology, namely the biological functions of proprotein convertase subtilisin/kexin type 9 (PCSK9) beyond lipid metabolism and the role of lipoprotein(a) in thrombosis and inflammation.

The role of PCSK9 in regulating low-density lipoprotein (LDL) cholesterol is well established, and it is currently the target of novel lipid-lowering therapies. However, little is known about the role of PCSK9 in other biological processes. Dr. Lorenzo Da Dalt presented available and new evidence on the role of PCSK9 beyond its known involvement in lipid metabolism, focusing on glucose metabolism and cardiomyocyte function. First, he showed that clinical studies on the role of PCSK9 in glucose metabolism are contradictory. In fact, Mendelian randomization studies suggest that loss-of-function polymorphisms of the *PCSK9* gene are associated with an increased risk of developing diabetes, whereas clinical trials do not show an increased risk of diabetes with drugs that target circulating PCSK9. Secondly, he discussed data from his preclinical studies suggesting that local expression of PCSK9 by pancreatic beta cells, but not circulating PCSK9, may be involved in the pathophysiology of diabetes. Indeed, he showed that reduced expression of PCSK9 in the pancreas promotes the accumulation of cholesterol and subsequent toxic effects in pancreatic beta cells (1). Finally, he showed new evidence that local expression of PCSK9 by cardiomyocytes, but not circulating PCSK9, is involved in the pathophysiology of heart failure (HF). He reported that selective deletion of the *PCSK9* gene in cardiomyocytes is associated with cholesterol accumulation in cardiomyocytes, leading to a rewiring of cardiac metabolism towards an anaerobic pathway (2).

Lipoprotein(a) is a pro-atherogenic lipoprotein with putative pro-inflammatory and pro-thrombotic properties (3). However, there is considerable uncertainty about the pathogenic involvement of lipoprotein(a) in venous thromboembolism and inflammatory diseases. Dr Vanessa Bianconi discussed this issue starting from the available evidence from preclinical and clinical studies on the effects of lipoprotein(a) in thrombosis and inflammation. She then reviewed the available data on the role of lipoprotein(a) in thrombo-inflammation in the context of coronavirus disease 2019 (COVID-19). Despite the lack of clear evidence on the latter issue, she showed original data from her retrospective study of a large population of patients hospitalized for COVID-19 and described the lack of any correlation between lipoprotein(a) levels and biomarkers of thrombo-inflammation, as well as the non-significant predictive role of lipoprotein(a) levels at hospital admission for the occurrence of in-hospital thrombotic events and the risk of the composite endpoint of intensive care unit admission/in-hospital death (4).

The following two sessions, organized by SIPREC and SIIA, were dedicated to the emerging evidence on pathophysiological pathways involved in the development of HF and new therapies for the treatment of HF.

Prof. Maurizio Forte discussed the molecular mechanism underlying the development of HF, with a special focus on autophagy and its stimulation by atrial natriuretic peptide (ANP), a cardiac hormone belonging to the family of natriuretic peptides, which is secreted mainly by atrial cardiomyocytes in response to mechanical stress, such as pressure or volume overload (5). In detail, he showed that ANP exerts critical pleiotropic effects in the cardiovascular system by limiting cardiomyocyte hypertrophy and death, reducing cardiac fibrosis and promoting vascular integrity. In addition, he discussed evidence that stimulation of autophagy by ANP is a protective mechanism that may underlie these beneficial effects and counteract the progression of heart disease towards HF (6).

Prof. Beniamino Pagliaro then discussed interventional treatment options for HF, illustrating the most recent advances in interventional technologies and strategies for the treatment of HF and emphasizing the importance of multidisciplinary treatment including surgery, catheter interventions and mechanical circulatory support devices (7,8).

Prof. Michele Ciccarelli described new insights into molecular mechanisms involved in cardiac remodeling and HF progression. Cardiac remodeling is characterized by several processes, including cardiomyocyte growth, neoangiogenesis, and immune system activation, which are perfectly coordinated under physiological conditions but become aberrant under pathological conditions such as hypertension and diabetes (9). The molecular mechanisms involved in adverse cardiac remodeling were reviewed, focusing on cardiomyocyte metabolic alterations that play a crucial role in the progression of HF and represent potential therapeutic targets. In this context, attention was paid to serine-threonine kinase G protein-coupled receptor kinase 2 (GRK2), a molecule involved in desensitization and downregulation of cardiac beta-adrenergic receptors and the modulation of the metabolic signature of cardiomyocytes (10).

Prof. Costantino Mancusi discussed the main mechanisms associated with the transition from hypertensive heart disease to the development of HF with preserved ejection fraction (HFpEF) and the recommended clinical approach for this condition. First, he reported epidemiological data from the Framingham Heart Study cohort (5,143 subjects), which showed that 1) hypertension precedes the development of HF in 91% of all newly diagnosed HF over a 20-year follow-up, 2) the risk of developing HF is increased 2-fold in men and 3-fold in women in hypertensive compared with normotensive subjects, and 3) the absence of hypertension, obesity, and diabetes from age 45 to 55 years is associated with up to 86% lower risk of incident HF (11). Second, he remarked that, in agreement with international guidelines, the diagnosis of HFpEF might be made using a specific approach that includes the assessment of cardiac morphological remodeling and dysfunction along with circulating levels of specific biomarkers (12). In this context, he discussed the importance of the clinical evaluation of hypertension-induced damage to target organs (in particular, left ventricular hypertrophy and decline of renal function biomarkers), as the main determinants of HFpEF. He also pointed out that in patients with hypertensive heart disease, several comorbidities, including obesity and diabetes, act synergistically to promote the development of hypertension-induced target organ damage and subsequent overt HFpEF.

The role of the immune system in metabolic liver disease was the topic of the session organized by SIMI.

Dr. Moris Sangineto showed preclinical evidence supporting

the role of immunometabolism (a link between metabolic processes and immune cell responses) as a potential therapeutic target in metabolic liver disease (13,14). In particular, he described recent findings showing that in non-alcoholic steatohepatitis (NASH) the bioenergetic profile of monocytes is profoundly altered and characterized by increased levels of glycolysis and oxygen consumption along with mitochondrial dysfunction; furthermore, the activity of complex II (succinate dehydrogenase, SDH) is high and associated with increased production of hydrogen peroxide. In addition, he reported that inhibition of hydrogen peroxide production by SDH through dimethyl malonate normalizes monocyte bioenergetics and reduces hepatic infiltration by immune cells in a preclinical model of NASH.

Dr. Andrea Dalbeni analyzed the role of the immune system in the setting of non-alcoholic fatty liver disease (NAFLD). First, he discussed literature data showing that the immune system plays a crucial role in the development of NAFLD and its progression to NASH and hepatocellular carcinoma (HCC) (15). Second, he highlighted that understanding the intricate relationship between the immune system and NAFLD/NASH/HCC is critical for developing targeted therapies that modulate the immune response to prevent the progression of metabolic liver disease. In this regard, he reported that in 2021, the combination of programmed cell death ligand 1 (PD-L1) inhibitors with vascular endothelial growth factor (VEGF) inhibitors was approved as a new first-line therapeutic strategy for HCC, providing a significant improvement in overall survival (>17 months). However, he also reported a recent meta-analysis by Pfister et al. suggesting that treatment with immune checkpoint inhibitors, either as monotherapy or in combination with bevacizumab, is associated with a significant increase in overall survival only in patients with HCC caused by viral hepatitis. Thus, he concluded that future research efforts are warranted to unravel the specific mechanisms underlying immune system involvement in NAFLD and to identify novel therapeutic targets to mitigate liver inflammation and prevent the progression of NAFLD towards NASH and HCC (16).

The last session, organized by SID, was dedicated to glucagon-like peptide 1 receptor agonists (GLP-1RAs).

Dr. Nicola Marrano discussed the pleiotropic effects of these antidiabetic drugs beyond their glucose-lowering effects. First, he showed that GLP-1RAs are characterized by pronounced anti-lipotoxic effects not only in different peripheral organs (skeletal muscle, heart, liver, adipose tissue, and pancreas) but also in the brain, where they may be crucially involved in neuroregulation and neuroprotection (17). Second, he reported that GLP-1 counteracts palmitate-induced apoptosis by inhibiting ceramide generation in human cardiac progenitor cells. Finally, he discussed the ability of GLP-1RAs to prevent lipotoxicity-induced beta-cell failure by targeting numerous dysfunctional pathways, including inflammation, oxidative stress, endoplasmic reticulum stress, and, to a lesser extent, autophagy and amyloid accumulation (18).

Finally, Prof. Alessandro Mantovani discussed the available clinical data supporting the beneficial role of GLP1RAs in metabolic liver diseases, including NAFLD and NASH. In detail, he showed that GLP-1RAs can exert direct and indirect beneficial effects on NAFLD and NASH by attenuating underlying comorbidities and additional risk factors beyond type 2 diabetes (19). Accordingly, he remarked that given the multiple pathways involved in the pathophysiology of metabolic liver disease, combining a GLP-1RA with other therapeutic approaches may be the best approach to treat these conditions (20).

The congress, traditionally, hosted an unconventional session, that in this edition was dedicated to a debate on the relationship between basic and clinical research. Dr. Marco Busnelli highlighted the need for a common language between clinicians and basic researchers by presenting virtuous and successful examples of research achieved thanks to a profitable link between the two fronts. He concluded by remarking that the strict collaboration between these two sides of science is essential to promote real progress.

This issue of *Eur Ath J* publishes the award-winning abstracts selected from the many high-profile studies presented during the congress.

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SELECTED ABSTRACTS

How age and gender affect hemodynamic forces in healthy subjects

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Aim: noninvasive echocardiographic analysis of blood-tissue interaction has recently been made possible by a sophisticated mathematical model. This model uses speckle-tracking technology to estimate instantaneous intraventricular gradients (IVPGs), which are represented as hemodynamic forces (HDFs). The aim of the present study is to examine how HDFs are affected by gender and age, providing reference value.

Methods: 85 healthy subjects were recruited and underwent transthoracic echocardiography. Speckle-tracking analysis was performed from the three apical views, and the mitral annulus and left ventricular outflow tract were measured to compute HDFs. Longitudinal HDFs have been examined, decomposing them in amplitude and time parameters.

Results: study population showed a median age of 47[25-60] years and 53% were female.

Female patients showed lower LVMi (60.1±11.8 mg/m^2 vs. 71.4±16.8 mg/m^2, p=0.001), lower LVEDV (84.6±14.6 ml vs. 108±20.7 ml, p<0.001), and a lower E/e' (7.26[6.47;7.78] vs. 5.31 [4.77;6.30], p<0.001). Nor systolic nor diastolic blood pressure differed between male and female patients (p NS for both). Several time parameters differed between gender: female subjects had a later systolic deceleration peak (38.7±4.21% vs. 34.8±4.31%, p<0.001) and a later diastolic deceleration peak (60.9±7.64% vs. 56.5±8.39%, p=0.015). No amplitude HDFs parameter was found to differ between gender (p NS for all).

Regarding age, patients over50 years showed higher systolic (124±15.4 mmHg vs. 115±10.8 mmHg, p=0.008) and diastolic (75.2±8.99 mmHg vs. 69.8±7.16 mmHg, p=0.005) blood pressure, and higher E/e' (7.41[6.91;8.58] vs. 5.58[4.81;6.56], p<0.001). HDFs time variables differed between patients under and over50 years: systolic ejection duration was longer in over50-group (27.8±3.44% vs. 25.6±3.48%, p=0.005), systolic deceleration duration was shorter in over50-group (7.69±1.48% vs. 8.54±1.97%, p=0.025) and systolic acceleration peak was earlier in over50-group (13.5±2.46% vs. 14.8±2.59%, p=0.020).

Conclusion: Among healthy subjects, female patients showed later systolic deceleration peak and later diastolic deceleration peak. Subjects over50 years showed longer systolic ejection duration, shorter systolic deceleration duration and earlier systolic acceleration peak.

Statin-Associated Muscle Symptoms – Clinical Index in a hypertensive population candidated to lipid-lowering therapy but not taking statins

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Aim: Statin-associated muscle symptoms (SAMS) are claimed to be frequent in clinical practice. The SAMS-clinical index (SAMS-CI) assesses the likelihood that muscle symptoms are related to statin use. We evaluated the prevalence and characteristics of muscle symptoms in hypertensive patients eligible for statin therapy according to their individual cardiovascular risk.
Methods: Observational study on 390 consecutive outpatients referred to our Centre. All patients were asked the following question: "Have you ever taken a drug/nutraceutical that you think gave you muscle symptoms?". Patients who answered "yes" were evaluated with SAMS-CI.
Results: Mean age: 60.5±13.5 years. Male prevalence: 53.8%. Patients who have ever taken a statin ("statin+" group): 250. Patients who have never taken a statin but have taken at least one other drug ("statin-" group): 140. Prevalence of muscle symptoms did not differ between the groups (p=0.217). Age and number of drugs taken were significantly associated with muscle symptoms at multivariate analysis. A not clinically significant higher SAMS-CI score emerged in the "statin+" group (p=0.004). Localization and pattern of muscle symptoms did not differ between the groups (p=0.170). Timing of muscle symptoms onset after starting the drug (p=0.036) and timing of symptom improvement after withdrawal (p=0.002) were associated with statin therapy.
Conclusions: Prevalence of patient-reported muscle symptoms was not associated with statin therapy in our real life clinical study, confirming the growing evidence that subjective muscle-related symptoms are often misattributed to statins, while they may more likely be related to the nocebo/drucebo effect or other common undiagnosed conditions.

Biomarkers of mitochondrial dysfunction and inflammaging in older adults and blood pressure variability

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Aim: Increased Blood Pressure (BP) Variability (BPV) may represent an alteration in BP physiological homeostatic patterns. Most physiopathological mechanisms underlying BPV are implicated in aging. Vascular aging is associated with chronic low-grade inflammation occurring in late life, known as "inflammaging", and the hallmark "mitochondrial dysfunction" associated to stress due to age-related disorders, which in turn might contribute to higher BPV and risk of cardiovascular disease. We aimed to determine whether plasma levels of the pleiotropic stress-related mitokine Growth/Differentiation Factor 15 (GDF-15) and two inflammatory biomarkers, Interleukin 6 (IL-6) and Tumor necrosis factor receptor 1 (TNFR-1), are associated with visit-to-visit BPV in a population of community-dwelling older adults.
Methods: The study population consisted of 1,096 participants [median age 75 (72-78) years; 699 females, 63.7%] selected among community-dwelling participants aged ≥70 years from the MAPT study. Plasma blood sample was collected 12 months after enrolment and BP was assessed up to seven times over a subsequent 4-year period. Systolic BPV (SBPV) and diastolic BPV (DBPV) were determined through several indicators including the coefficient of variation (CV%) and taking into account BP change over time, the order of measurements and formulas independent of mean BP levels.
Results: Higher values of GDF-15 were significantly associated with increased SBPV (all indicators) after adjustment for demographics, body mass index, MAPT randomization group, baseline systolic BP, antihypertensive drugs, diabetes mellitus, cardiovascular and non-cardiovascular comorbidities [adjusted 1-SD increase in GDF-15: β (SE)= 0.07 (0.04), p< 0.044, for CV%]. GDF-15 levels were not associated with DBPV. No significant associations were found between IL-6 and BPV, whereas TNFR1 was only partially related to DBPV.
Conclusions: Unlike inflammation biomarkers, higher GDF-15 levels were associated with greater SBPV. Our findings support the age-related process of mitochondrial dysfunction underlying BP instability, suggesting that BPV might be a potential marker of aging.

Reference

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Irisin administration restores beta-cell functional mass in a mouse model of type 2 diabetes

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Aim: Irisin is a hormone secreted by skeletal muscle able to improve metabolic homeostasis. Serum irisin levels are reduced in type 2 diabetes (T2D), while exogenous irisin administration improves glycemic control in diabetic mice. We have previously demonstrated that irisin promotes beta-cell survival and function both in vitro and in vivo in healthy wild type mice. We have also demonstrated that irisin restores the defective glucose-stimulated insulin secretion (GSIS) and reduces apoptosis in human pancreatic islets from patients with T2D. Nevertheless, the beta-cellular effects of in vivo irisin administration to T2D mice are still unknown.
Methods: C57Bl/6 mice (n = 8) were fed a high-fat diet (HFD, 60% of energy deriving from fat) for 10 weeks and then intraperitoneally injected with streptozotocin (STZ, 100 mg/kg) to induce diabetes. Four standard diet (SD)-fed mice were used as control. HFD/STZ mice were treated with 0.5 µg/g irisin (n = 4) or vehicle (n = 4), for 14 days. Fasting glycemia, insulinemia, body weight, glucose tolerance, and pancreatic islet function were assessed. Pancreatic islet architecture was also evaluated through immunofluorescence analyses.
Results: Compared to SD mice, HFD/STZ mice showed higher fasting glycemia and body weight, glucose intolerance, and reduced GSIS; in addition, HFD/STZ mice showed reduced islet volume (-78%), beta-cell area (-35%), and insulin content (-60%), and increased alpha-cell area (+54%). Irisin administration significantly restored glycemia (-31%), body weight (-13%), glucose tolerance (-27%), GSIS (+23%), islet volume (+61%), beta-cell area (+34%) and alpha-cell area (-49%), and insulin content (+36%). Of note, irisin induced a 9-fold increase in beta-cell proliferation rate.
Conclusions: These results show that irisin improves glycemic homeostasis and restores the functional beta-cell mass when administered in vivo to diabetic mice, probably by promoting beta-cell proliferation.

Acute ischemic stroke: how to investigate the association between disease etiology and gene expression profiles

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Background: Acute ischemic stroke (AIS) represents one of the principal causes of neurological morbidity and mortality worldwide. For a prompt and efficient cerebral blood restoration, intravenous treatment with rt-PA is often combined with mechanical thrombectomy (MT) which provides cerebral thrombi (CT) as study material, allowing the investigation of its cellular composition, morphological and histopathological features. Indeed, the determination of stroke etiology, typically defined by the TOAST classification, is paramount for prognostic factors, outcome, and management of the event. Aim of the study is therefore to highlight and analyze gene expression profiles in thrombotic tissue and peripheral blood (PB) in the comparison between strokes of cardioembolic (CE) and atherosclerotic (LAA) origin.
Methods: We performed gene expression profiles of 92 patients. CT were stored in RNA later and RNA was extracted by PAX gene blood miRNA kit. The global gene expression profile was assessed by Affymetrix technology using GeneChip Human Transcriptome Array 2.0 combined with Affymetrix Transcriptome Analysis Console (TAC) Software.
Results: Currently, we focused our attention on CT data analysis. The analysis revealed a significant difference (p-value<0.05 and Fold-Change=2 as threshold) in gene expression when comparing LAA and CE stroke. In particular, from CT of atherosclerotic origin emerges an overexpression of 1766 genes. Prominent among them are genes such as MMP-9, TGFB, TGFBR and CXCL1, primarily involved in neutrophil-mediated immunity, Blood-Brain Barrier (BBB) disruption processes, and associated with atherosclerotic plaque instability and related to poor neurological outcome, suggesting a deleterious role in human brain injury. As concerns CE patients, 57 genes mainly involved in transcriptional regulatory processes turn out to be significantly overexpressed.
Conclusions: Transcriptome profiling is a powerful weapon for revealing expression patterns associated with complex disorders. The variation of gene expression profiles confirmed and extended several known pathophysiological mechanisms and may be one way of delineating different stroke etiology.

Successful treatment with lomitapide in a patient with homozygous familial hypercholesterolemia and severe fatty liver disease

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Introduction and Aims: Homozygous-familial hypercholesterolemia (Ho-FH) is a rare condition due to biallelic mutations in low-density lipoprotein-receptor (LDL-R) genes characterized by high level of LDL-cholesterol (LDL-c) and huge risk of premature atherosclerotic cardiovascular disease (ASCVD), determining low quality of life and life expectancy.
Lomitapide represents a therapeutic option for Ho-FH, but caution should be observed when used in fatty liver disease (FLD) and hypertransaminasemia since it is associated with onset/worsening of liver steatosis. We present a case of safe lomitapide therapy in an adult Ho-FH patient with pre-existing FLD.
Case presentation: A 39-year-old man with severe hypercholesterolemia since childhood (LDL-c 405 mg/dl) and premature coronary heart disease history, was referred to our Modena Lipid Clinic. He presented an overt metabolic syndrome, FLD with hypertransaminasemia and elastosonographic significant liver fibrosis. Lipid-lowering-therapy (LLT) included rosuvastatin 20 mg, ezetimibe and evolocumab 140 mg twice a month without reaching LDL-c goal. Genetic analysis revealed homozygous pathogenic LDL-R gene mutation. Evolocumab was increased up to 420 mg twice a month and LDL-apheresis was started with quality of life worsening. Therefore, lomitapide 5 mg daily and low-fat diet were started, obtaining weight loss and lipid profile improvement. However, liver enzymes elevation higher than 5-fold was observed, leading to lomitapide discontinuation and baseline liver enzymes values restoration. After one-month wash-out, lomitapide was gradually reintroduced up to 5 mg daily without significant hypertransaminasemia recurrence, leading to LDL-c target achievement and LDL-apheresis discontinuation. Adherence to low-fat diet and weight loss resulted in FLD and fibrosis improvement.
Conclusion: Ho-FH requires complex, combined treatment. Metabolic comorbidities co-existence makes Ho-FH management more difficult. Lomitapide can be safely used in Ho-FH patients with FLD and hypertransaminasemia, but strict follow-up of liver disease and a multidisciplinary approach are needed. Before lomitapide introduction, low-fat diet should be started advantageously and weight stabilization should be obtained.

Optimization of glucose control drives improvement of NAFLD independent of weight loss in people with T2D

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Aim: The mainstays for the treatment of non-alcoholic fatty liver disease (NAFLD) are lifestyle intervention with the aim of significant weight loss alongside aggressive cardiovascular risk reduction. NAFLD is tightly associated with both obesity and type 2 diabetes (T2D). In people with T2D, glucose lowering agents that promote weight loss have shown a beneficial impact on NAFLD based on histological features. However, it remains unclear as to whether glucose lowering can improve NALFD in patients with T2D, independent of weight loss.
Methods: In a consecutively recruited population of 637 patients with T2D with HbA1c levels above treatment targets, DPP-IV inhibition, GLP-1RA therapy or SGLT2 inhibition was initiated, alongside lifestyle education with maintenance of exiting background glucose lowering treatment. We examined the longitudinal impact of the optimization of glycaemic control on fatty liver index (FLI) and Fibrosis score 4 (Fib-4) adjusting for changes in BMI and choice of glucose lowering regimen over a 12-month period.
Results: Change in HbA1c and change in FLI correlated significantly in a linear regression analysis after adjustment for change in BMI, age, sex, and drug class ($R=0.467$, $p=0.031$). The greatest reduction in FLI was observed in patients with the largest reduction in HbA1c ($p<0.0001$). The probability of improvements in FLI with optimization of glycaemic control was similar with all 3 glucose lowering agents, despite differences in weight reduction. Similar relationships were observed examining the changes in glycaemic control and Fib-4.
Conclusions: Significant reductions of HbA1c are associated with improvement in NAFLD independently from weight loss. These results suggest a prominent role for the optimization of glucose control in the management of coexistent NAFLD and T2D, especially in the 'lean' NAFLD and where significant weight loss may not be achieved.

Exosomal miRNAs targeting NLRP3 inflammasome platform are associated with radiologic sequelae in survivors of COVID-19-associated acute respiratory distress syndrome

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Background: There is limited understanding of the pathophysiology of post-acute pulmonary sequelae in COVID-19-associated acute respiratory distress syndrome (ARDS). We aimed at investigating the association of circulating microRNAs (miRNAs) involved in post-transcriptional regulation of NLRP3-inflammasome pathways and lung radiological features among COVID-19- associated ARDS survivors.
Methods: We evaluated COVID-19-associated ARDS survivors at 4±2 months from clinical recovery. Patients were selected based on imaging pattern evolution according to chest high-resolution computerized tomography (HRCT) findings into "fully recovered" (FR), "pulmonary opacities" (PO) and "fibrosis-like lesions" (FL) according to radiological appearance. Plasma miRNA profiling was performed using real time quantitative polymerase chain reaction (RT-qPCR). The exosomal expression of NLRP3 inflammasome related miRNAs (miR-17-5p, miR-223-3p, miR-146a-5p) was evaluated.
Results: 31 patients (33% men, mean age 60±6 years, mean BMI 31.1±6.6 Kg/m2) were selected for the present study. No statistically significant differences between FR, PO and FL patterns were observed according to clinical features. NLRP3-inflammasome-related miRNAs such as miR-17-5p, miR-223-3p and miR-146a-5p were significantly up-regulated in patients with PO when compared to patients with FL. miR-146a-5p was also up-regulated in patients with FL than in FR.
Conclusions: In patients with long-term pulmonary radiological sequelae following COVID71 19- associated ARDS, a down-regulation of miRNAs inhibiting NLRP3 (miR-17-5p, miR-146a72 3p and miR-223-3p) correlated to fibrosis development in patients showing persistent hyper-reactivity to inflammatory stimulation. NLRP3-Inflammasome-related miRNAs could be a possible therapeutic target to prevent the fibrotic evolution of COVID-19-associated ARDS.

Exploring the role of FXR signaling in maintaining ileal mucosa integrity in subjects with altered glucose tolerance conditions

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Aim: Treatment with the FXR agonist obeticholic acid (OCA) has been found to improve glucose metabolism in type 2 diabetes (T2DM) subjects with mechanisms not completely elucidated. In the gut, FXR is mainly expressed in the ileum where promotes transcription of fibroblast growth factor-19 (FGF19) having positive effects on glucose homeostasis, and maintains gut barrier integrity by regulating tight-junction (TJ) proteins expression. Herein, we evaluate whether subjects with dysglycemic conditions exhibit a down-regulation of the intestinal FXR-FGF19-TJ axis and whether treatment with OCA may revert this aberration.
Methods: Levels of FXR, FGF19 and TJ proteins and pro-inflammatory cytokines were assessed in ileal mucosa specimens collected during colonoscopy from 53 subjects subdivided according to their glucose tolerance in: NGT (n=26), prediabetes (n=12) and T2DM (n=15). Effects of OCA treatment was assessed on ileal mucosa specimens of subjects with prediabetes or T2DM cultured in absence or presence of OCA for 6h.
Results: Ileal FXR protein and mRNA levels were progressively decreased in prediabetes (-26%) and T2DM (-34%) as compared to the NGT group (both P<0.05). Ileal FXR downregulation was paralleled by lower FGF19 expression and circulating levels (both P<0.05). Additionally, we observed a progressive decrease of proteins and mRNA levels of the TJ zonulin (ZO)-1, occludin and claudin-1 (P<0.05 for all) with an activation of pro-inflammatory pathways in the ileal mucosa of subjects with prediabetes and T2DM as compared to the NGT group. OCA treatment resulted in an up-regulation of FGF19 expression and release (both P<0.01), mRNA and protein levels of the TJ ZO-1, occludin and claudin-1 and in reduced pro-inflammatory cytokines synthesis and release (P<0.05 for all).
Conclusion: FXR stimulation by OCA treatment reverts the altered FGF-19/TJ axis in subjects with prediabetes and T2DM, indicating intestinal FXR signaling as a novel target for prevention and/or treatment of T2DM.

Change over time of lipid profile relates to steroid treatment but not to an inflammatory state in Granulomatosis with poliangioitis polyangiitis (GPA)

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Aim: Granulomatosis with polyangiitis (GPA) is a small vessel vasculitis. Inflammation of the vessel wall may induce multiple vascular damages. Atherosclerosis is accelerated during vasa inflammation. Metabolic profile and cardiovascular risk are far to be determined in these patients. Thus, Cardiovascular atherosclerotic disease (ASCVD) may represent a risk for patients' outcomes. The purpose is to evaluate ASCVD risk in GPA over time during disease follow-up.
Methods: We retrospectively evaluated 37 patients (22 Females, aged 51.45±17.15) who received a diagnosis of GPA (T0). Patients were evaluated at 1 (T1) and 2 (T2) year follow-up. All patients were treated with high steroid dose followed by a one-year tapering, associated to another immunosuppressant. Lipid profile included total cholesterol, HDL, LDL and Triacylglycerol. To evaluate inflammatory activity, we evaluate erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) and neutrophil to lymphocyte ratio (NLR) at the same time points. ANOVA for repeated values was used to evaluate the trend over time and Tukey's multiple comparisons test was a second step evaluation.
Results: At T1 there was an increase in total cholesterol compared to baseline (T1vsT0, $p<0.05$) and T2 (T1vs T2, $p<0.05$). Similarly, LDL (T1vsT0, $p<0.05$) presents the same trend, while Triacylglycerol increased in T1 compared to baseline (T1vsT0, $p<0.05$), but no difference there was in T2 compared to T1 or T0. No difference was found in HDL between the different time points. CRP was no different, despite a reduction being noticed. On the contrary, we found a reduction at T2 but not in T1 in ESR (T1vsT0, $p<0.05$) and NLR (T1vsT0, $p<0.05$).
Conclusion: Our data suggest that a change in lipid profile may not relate to better control of inflammation. On the contrary, the increase in the first year of follow-up should be a consequence of steroid treatment needed to spread disease control. These data may be helpful in the evaluation of both cardiovascular disease and lipid metabolism due to the connection between the two parameters with vessel inflammation. Further studies are needed to better evaluate the cardiovascular effect of vasculitis and consequent treatment.

Impact of immune system humanization on atherosclerosis in dyslipidemic Rag2-KO/IL2rg-KO/CD47-KO/ LDL-R KO mice

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Aim: Given the key role of the immune response during atherosclerosis and the therapeutic interest of biologics targeting human immune cells, the need of experimental models to translate molecular mechanisms and to test therapeutic approaches for atherosclerosis is continuously increasing. Here we describe the characteristics of an innovative immunodeficient mouse humanized with hCD34+ cells on an atheroprone background.
Methods: LDLR-KO mice were crossed with the immunodeficient C57BL/6J strain Rag2-KO/IL2rg-KO/CD47-KO (TKO, IMSR_JAX: 025730) to generate an immunocompromised dyslipidemic mouse TKO-LDLR KO recipient of human hematopoietic stem cells (hCD34+).
Results: TKO-LDLR KO were first characterized for their immune and metabolic profile. TKO mice are deficient in mature lymphocytes and NK cells and this profile was conserved in TKO-LDLR KO mice. Under high cholesterol diet for 8 weeks, both males and females TKO-LDLR KO present monocytosis with increased levels of Ly6Chi monocytes compared to TKO-LDLR KO at standard diet, develop marked dyslipidemia (total cholesterol 870.9 and 890.1 mg/dL male and females respectively), steatosis and atherosclerosis. This profile confirms the suitability of TKO-LDLR KO mice for atherosclerosis studies. Next, we tested the impact of immune system humanization. TKO-LDLR KO pups received a low-dose irradiation (150-200 cGy) and thereafter 1,5-2 x 10^5 hCD34+ were injected with in the liver. Engraftment of human leukocytes (hCD45+) was evaluated after two months by flow cytometry analysis from tail blood. This approach allows to reconstitute between 10-30% of hCD45+, mainly B and T cells.
Conclusions: We have generated and characterized for the first time a humanized dyslipidemic TKO-LDLR KO mouse. This mouse model presents human B and T cells and could represent an important tool to investigate the impact of biologics targeted toward human targets in the context of atherosclerosis.

Dapagliflozin counteracts the pro-apoptotic effects of the secretome of visceral adipose cells from obese subjects in human cardiac progenitor cells via the SGLT2 co-transporter

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Aim: Dapagliflozin (DAPA), an SGLT2 inhibitor, has been shown to counteract heart failure outcomes in subjects with obesity and diabetes. We investigated the protective mechanisms of DAPA in human cardiac progenitor cells (hCPC) exposed to the conditioned medium (CM) from abdominal visceral (AV) and epicardial (E) adipose stem cells (ASC) and from AV mature adipocytes from obese subjects.
Methods: ASC and mature adipocytes were isolated from AV adipose tissue biopsies of 27 obese (Ob) and 19 non-Ob subjects (n-Ob), and from E adipose tissue biopsies of 9 Ob and 10 non-Ob subjects, respectively. hCPC were isolated from right auricle biopsies of 10 healthy non-Ob donors.
Results: Exposure of hCPC to the CM of adipose cells from Ob, but not from non-Ob subjects, induced apoptosis, c-Jun phosphorylation, and impairment of actin filaments, while these effects were not observed when hCPC were pretreated with DAPA. The CM of adipose cells from Ob compared to n-Ob subjects displayed a different pattern of cytokines. The levels of pro-inflammatory cytokines RANTES and MIP1β were increased in the CM from AV-ASC with higher BMI ($p<0.05$), while the levels of the cardioprotective factor GCSF in the CM of E-ASC were inversely correlated with BMI ($p<0.05$). SGLT2 was found to be expressed as both mRNA and protein in hCPC, and silencing of SGLT2 with a specific siRNA abrogated the capacity of DAPA to counteract the pro-apoptotic effects of the CM.
Conclusions: In human obesity, the CM of both AV- and E-ASC and mature adipocytes is characterized by pro-inflammatory cytokines that induce stress kinase activation and apoptosis in hCPC. DAPA prevents the hCPC damage induced by the CM through an SGLT2-dependent mechanism.

Extreme cardiovascular risk in cardiological rehabilitation: prevalence and impact on patient's functional improvement

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Background and Aims: Among patients at very high cardiovascular risk, some are more likely to experience recurrent cardiovascular events. In May 2022, an article was published in the European Heart Journal proposing different definitions of patients at extreme cardiovascular risk. However, the process of defining such patient is still ongoing and more data on its prevalence are needed. Our aims consisted in assessing the prevalence of patients at extreme cardiovascular risk in cardiological rehabilitation and in evaluating the clinical features of such patients. Furthermore, we wanted to establish how the extreme cardiovascular risk condition correlates with the functional improvement obtained during cardiac rehabilitation.
Methods: The study included 938 patients suffering from atherosclerosis who attended the cardiological rehabilitation of Niguarda Hospital in Milan. Patients classified as at extreme cardiovascular risk were compared with the remaining patients and a multivariate linear regression was performed with absolute functional improvement as the dependent variable.
Results: Among 938 patients, 26.9% belong to the category of extreme cardiovascular risk. Patients at extreme cardiovascular risk showed a higher average age (67.8±10.4 vs 64.1±11.1 years; $p \leq 0.001$), a higher prevalence of significant comorbidities (peripheral arterial disease, cerebrovascular disease, dyslipidemia, diabetes, chronic kidney disease, hypertension) and a lower functional improvement during cardiac rehabilitation (102.9±68.6 vs 138.1±86.5 m; $p \leq 0.001$). At multivariate analysis extreme cardiovascular risk remains a significant determinant of the absolute functional improvement at Six-Minute Walking Test obtained during cardiac rehabilitation with $b = -0.137$ and $p = 0.035$, together with female sex ($b = -0.136$; $p = 0.035$).
Conclusions: Extreme cardiovascular risk is a widespread condition among patients with chronic coronary syndrome and adversely affects the patient's functional improvement during cardiac rehabilitation. The identification of patients at extreme cardiovascular risk is a goal to be pursued in order to intensify secondary prevention strategies.

Role of ASGR1 on obesity and metabolic syndrome

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Background: Obesity-related fat accumulation is linked to the metabolic syndrome and increases the risk of CVD by involving FFA, insulin resistance, and inflammation. Taking into account the findings from the third chapter, our goal was to assess the potential role of ASGR1 in metabolic reprogramming and immunoinflammatory state during obesity.
Methods: After 20 weeks of high fat diet, flow cytometry, proteomics, lipid profile, glucose tolerance, and insulin tolerance were assessed in WT and ASGR1-/- mice (HFD). Additionally, metabolic parameters such as oxygen consumption, CO2 production, and food intake were measured during the diet.
Results: After 20 weeks of HFD, the ASGR1−/− mice displayed a significant reduction in the circulating monocytes compared to WT. The body weight and food intake were comparable in between two groups. The adipose tissue VAT was significantly increased in ASGR1-/- compared to WT mice (WT 3.2%±0.8%, ASGR1-/- 4.7%±1.2%, P-value<0.001). The proteomics revealed, n=3412 proteins were aligned from which 624 proteins were significantly differentially expressed on the liver of ASGR1-/- and WT mice under HFD. From prediction analysis the significant proteins that were increase in the liver of ASGR1-/- mice were necrosis, apoptosis, and inflammation compared to the WT. Additionally, a significant downregulation in proteins protein expression involved in fatty acid synthesis and fatty acid uptake, except the increased expression of fatty acid coenzyme A ligase (FATP5), which belongs to very long chain acyl-CoA synthetases, capable mediation the transport of long chain fatty acids.
Conclusion: Our findings indicate that ASGR1 deficiency causes increased inflammation and changes in metabolic pathways when subjected to HFD. This can also have an impact on the synthesis of apolipoproteins secreted in plasma.

Thrombocytopenia and Kidney disease, two possible hallmark of FCS phenotype: preliminary evidence from a cohort study

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Background and Aim: Familial Chylomicronemia Syndrome (FCS) is a rare monogenic autosomal recessive disorder of lipid metabolism determining severe hypertriglyceridemia (HTG). As the use of Volanesorsen, a novel FCS treating drug, has been associated with thrombocytopenia, the relationship between FCS and low platelets counts should be firmly established. It has been reported also kidney complication in FCS, but the data are sparse. To this aim, we have retrospectively evaluated the spontaneous variation of platelet counts and Kidney impairment in a cohort of patients with FCS.
Methods: Single-center retrospective cohort study on 20 FCS patients included in the LIPIGEN. Medical charts have been revised to collect retrospectively information on kidney function in a cohort of patients with FCS.
Results: Across the study population, the median PLT count was 187,225 platelet/mcL. The median on treatment TG levels in the whole cohort was 1309 mg/dl. During follow-up, 8 (44.4%) patients experienced at least one episode of mild and 1 (5%) of moderate thrombocytopenia. None had severe thrombocytopenia. Mean triglycerides do not significantly predict mean platelet values. However, when considering a multivariate model including mean triglycerides, sex, the presence of hepatic steatosis and age we found that male sex and the presence of ultrasound estimated hepatic steatosis were associated with significantly lower platelet (respectively β-0,473, P=0,044 and β-0,469, P=0,048). Age was of borderline statistical significance (β-0,388, P=0,087). Across the study population, the median GFR values was 99.5 ml/min. Median eGFR was significantly associated with history of hypertension (β-0,508, P=0,031). Overall, proteinuria occurred in 5 (25%) patients, and it did not associate with hypertension, diabetes, age, sex nor triglyceride levels. Four (20.0%) patients meet the criteria of hyperfiltration whereas 3 (15.0%) were exhibiting an eGFR below 90 ml/min. Among hyperfiltrating, two had also proteinuria in at least one occasion during life. One patient with eGFR below 90 ml/min and proteinuria had a biopsy-proven diagnosis of glomerulonephritis. Overall, the impairment in kidney function was independent from age, diabetes, hypertension, median TGs, AP, sex.
Conclusions: The present analysis confirmed that thrombocytopenia and kidney impairment might be a clinical characteristics of FCS phenotype. Further studies in larger cohort are needed to better clarify if kidney disease and thrombocytopenia might be a hallmark of FCS in broader population and understand the potential patho-physiological mechanism.

In vitro and *in vivo* studies on novel pcsk9 inhibitors as pharmacological approach for the treatment of alzheimer's disease

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Aim: Impairment of cholesterol homeostasis is one of the multiple etiopathological mechanisms at the origin of both cardiovascular and neurodegenerative diseases. The PCSK9 protein, known for its role in the degradation of hepatic LDLR and plasma cholesterol regulation, is expressed also in the CNS, where it exacerbates β-amyloid neurotoxicity and reduces neuronal cholesterol uptake, suggesting an involvement in AD. This study proposes an *in vitro* screening of molecules (MR) with inhibitory activity on PCSK9, selecting the best compounds to test their activity on cerebral cell models and their *in vivo* tolerability.
Methods: 30 newly synthesized compounds were tested at increasing concentrations on human hepatoma cells (HepG2) to evaluate their cytotoxicity and efficacy in inhibiting PCSK9. MR-3 was tested on human neuroblastoma cells (SH-SY5Y) overexpressing PCSK9 to assess neurotoxicity and cholesterol uptake. Cytotoxicity was determined through MTT assay; PCSK9 secretion was quantified with an ELISA kit; and radioisotopic techniques measured cholesterol uptake . Three compounds were selected to be tested *in vivo* on C57BL/6 mice at a dose of 40 mg/Kg for 7 days to evaluate: tolerability with SHIRPA test; plasma lipid profile by ELISA assay; biodistribution in plasma and brain through LC-MS/MS.
Results: Among the tested compounds, MR-3, MR-532, MR-533 demonstrated no sign of cytotoxicity and the greatest efficacy on HepG2 cells (IC_{50}=1.7μM; 5.7μM; 6.1μM). Neuronal cholesterol uptake was restored after treatment with MR-3 at 10μM ($p<0,05$). MR-3, MR-532, and MR-533 exhibited good in vivo tolerability; MR-3 and MR-532 were detected in plasma and brain tissue.
Conclusions: Preliminary *in vitro* screening allowed the identification of MR-3, MR-532, MR-533 as promising PCSK9 inhibitors. The outcome of MR-3 on neuronal cholesterol uptake may suggest a neuroprotective effect to be further investigated. *In vivo* treatment with selected inhibitors shown absence of toxicity, however, it is necessary to bring proof of efficacy.

Effect of lipid-lowering therapies on lipoprotein(a) levels: a meta-analysis of randomized controlled trials

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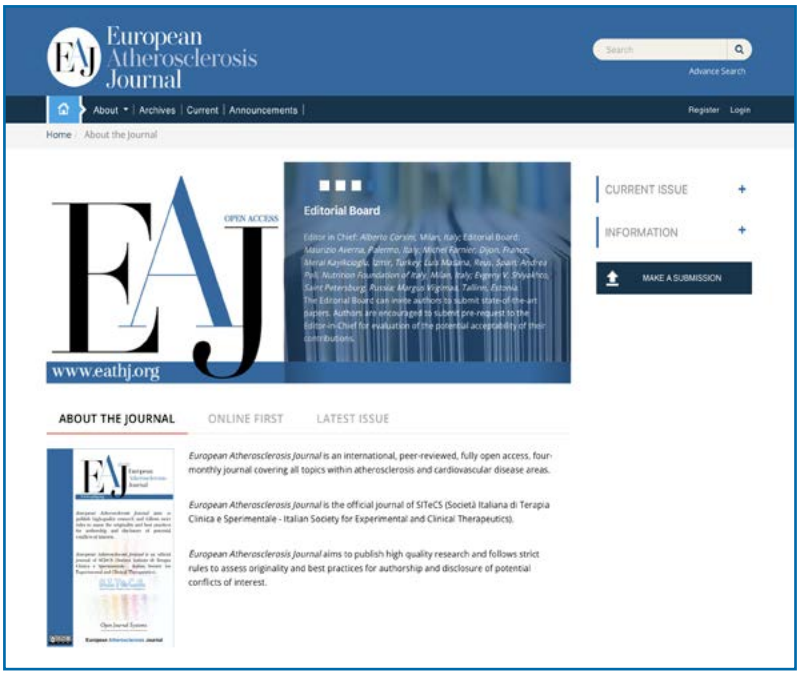
Aim: Epidemiological studies, Mendelian randomized studies, and genome-wide association studies confirmed that elevated lipoprotein(a) [Lp(a)] concentration is an independent risk factor for cardiovascular diseases. However, no approved therapy for patients with elevated Lp(a) levels is available. Our aim is to investigate to what extent PCSK9 inhibitors (PCSK9i), statins, and ezetimibe affect Lp(a) level.
Methods: This meta-analysis was conducted according to the PRISMA guidelines. Databases were searched from inception to February 2023. Inclusion criteria were: (1) randomized controlled trials (RCTs) in adults (≥18 years), phase II, III or IV; (2) English language; (3) reporting the effects on Lp(a) levels; (4) with intervention duration more than 3 weeks. Pooled estimates were assessed by a random-effects model. Between-study heterogeneity was tested and measured by Cochrane's Q test and I^2 statistics.
Results: Overall, 51 RCTs were included for PCSK9i (39,271 participants), 35 RCTs for statins (15,425 participants), and 14 RCTs for ezetimibe (5,607 participants). Starting from a baseline Lp(a) level of 33.12 mg/dL, participants treated with PCSK9i compared to placebo experienced an additional reduction in Lp(a) levels of -26.34% (95%CI -28.83 to -23.85). Lp(a) levels were marginally reduced by statins by -3.43% (95%CI -9.09 to 2.23) from a baseline Lp(a) level of 15.87 mg/dL, although this reduction was not statistically significant. Finally, ezetimibe had a negligible and still not statistically significant effect on Lp(a) levels (0.51% [95%CI -1.67 to 2.70]), from a baseline Lp(a) level of 20.80 mg/dL.
Conclusions: Among the lipid-lowering approaches evaluated, only PCSK9i seemed to lower Lp(a) levels. Further research is requested to understand whether it translates into a clinically relevant cardiovascular benefit.



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