Atherosclerosis newsletter

Simona Negrini and Arnold von Eckardstein

Atherosclerosis 60th ANNIVERSARY

"BEST PAPER AWARD 2020"

Atherosclerosis was founded in 1960, hence, in 2020 the journal will turn 60.
The Editors and Publisher take this opportunity to announce a contest for "Best Paper Award 2020".

Eligible manuscripts are original research articles of young scientists (< 40 years) as first or corresponding authors, submitted between **July 1, 2019 and October 31, 2019,** either as first or revised version.

The Editor-in Chief and CoEditors will select the winner among the top rated papers accepted for publication before April 1, 2020. The winner will be invited to present the work in a lecture and will receive a certificate and an award of 1000 € during the **88th EAS (European Atherosclerosis Society) Congress** held May 31-June 03, 2020 in Geneva, Switzerland. Moreover, her/his Congress registration fees and travel/accommodations costs will be covered by EAS. The winning article will be published with promotional open access, free of charge for the authors.

First or corresponding authors younger than 40 years shall state in the covering letter that she/he applies for the "Best Paper Award 2020".

We look forward to the submission of your important and breakthrough research.

To submit your paper >>

Familial hypercholesterolemia is one of the most frequent monogenic diseases and the understanding of its etiology and pathogenesis played a pivotal role in the development of drugs that lower LDL-cholesterol and risk of atherosclerotic cardiovascular disease. Nevertheless, both FH and the LDL receptor continue to be in the interest of atherosclerosis research. The August and September issues of *Atherosclerosis* contain several articles describing diagnostic criteria and clinical consequences of FH, evaluating soluble versions of an LDL receptor related protein towards its relevance as a biomarker or exploiting the knock out of the LDL-receptor as a model for atherosclerosis research in mice.

Volume 287, Issue August 2019

Volume 288, Issue September 2019

Toward a new clinical classification of patients with familial hypercholesterolemia: One perspective from Spain

The introduction of singular therapies, such as proprotein convertase subtilisin/kexin type 9 inhibitors (PCSK9i), to lower high cholesterol levels requires better classification of patients eligible for intensive lipid lowering therapy. According to the European Medicines Administration, PCSK9i are recommended in primary prevention only in familial hypercholesterolemia (FH) patients. Therefore, an FH diagnosis is not simply an academic issue, because it has many clinical implications. The bases of a diagnosis of FH are not entirely clear. The availability of genetic testing, including large genome-wide association analyses and whole genome studies, has shown that some patients with a clinical diagnosis of definite FH have no mutations in the genes associated with the disease. This fact does not exclude the very high cardiovascular risk of these patients, and an early and intensive lipid lowering therapy is recommended in all FH patients. Because an FH diagnosis is a cornerstone for decisions about therapies, a precise definition of FH is urgently required. In this expert consensus document from the Spanish Atherosclerosis Society, Masana et al. propose the following classification: familial hypercholesterolemia syndrome integrated by (1) heterozygous familial hypercholesterolemia: patients with clinically definite FH and a functional mutation in one allele of the *LDLR, ApoB:100,* and *PCSK9* genes; (2) homozygous familial hypercholesterolemia: mutations affect both alleles; (3) polygenic familial hypercholesterolemia: patients with clinically definite FH but no mutations associated with FH are found (to be distinguished from non-familial, multifactorial hypercholesterolemia); (4) familial hypercholesterolemia combined with hypertriglyceridemia: a

subgroup of familial combined hyperlipidaemia patients fulfilling clinically definite FH with associated hypertriglyceridemia.

Risk of cardiovascular disease outcomes in primary care subjects with familial hypercholesterolaemia: A cohort study

Familial hypercholesterolemia (FH) is a common monogenic autosomal dominant disorder causing raised low-density lipoprotein (LDL) cholesterol from birth. Untreated FH is associated with a substantially higher risk of coronary heart disease (CHD) and premature death, but lipid-lowering therapy improves prognosis, reducing risk of coronary heart disease and all-cause mortality by at least 44% in patients with heterozygous FH treated with moderate-to high-intensity statins. While FH is a known major cause of premature heart disease, the risks of atherosclerotic disease in other vascular regions are less known. Iyen et al. determined the risk of major cardiovascular disease (CVD) outcomes associated with clinical FH.

The authors conducted a retrospective matched cohort study (1 January, 1999 - 22 July, 2016) using data from the Clinical Practice Research Datalink (CPRD), a large nationally representative electronic database of anonymised primary care data of subjects in the UK. They randomly-matched 14,097 subjects with clinical FH diagnoses or characteristics to 42,506 subjects without FH by age, sex, and general practice. They excluded those with CVD at baseline. Incident rates for coronary heart disease (CHD), stroke or transient ischaemic attack (TIA) and peripheral vascular disease (PVD) were estimated. Cox proportional hazards regression, stratified on matched-pairs, determined adjusted hazards ratios (HR) for incident CVD.

During follow-up, incidence rates of CVD were higher in FH than in non-FH subjects. The risk of CHD, stroke/TIA and PVD was also higher in FH compared to non-FH subjects. Undiagnosed FH subjects had much greater risks of all CVD outcomes than subjects with clinical FH diagnosis. Only 75% of the FH subjects were on lipid-lowering treatment, and only 38% of those on treatment were on high-potency statins.

The results show that, in addition to the recognised increased risk of CHD, subjects with FH have greatly elevated risk of stroke/TIA and PVD. This emphasises the need for early diagnosis and preventive interventions beyond CHD, to reduce CVD risk in these individuals.

The prevalence and management of familial hypercholesterolemia in patients with acute coronary syndrome in the Polish tertiary centre: Results from the TERCET registry with 19,781 individuals

The prevalence of familial hypercholesterolemia (FH) is high among patients with stable coronary artery disease (CAD). However, data on FH on admission among patients with acute coronary syndrome (ACS) are still relatively scarce. Dyrbu et al. aimed to assess the prevalence, lipid-lowering therapy and short- and long-term outcomes in patients with FH among ACS patients.

The investigation was performed in a cohort of 19,781 consecutive patients from the Hyperlipidaemia Therapy in tERtiary Cardiological cEnTer (TERCET) Registry (a multiannual observational cohort study started in 2006 assessing quality, safety and effectiveness of lipid-lowering treatment). There were 7319 patients admitted with ACS: 3085 due to ST-segment elevation myocardial infarction (STEMI), 2256 due to non-ST-segment elevation myocardial infarction (NSTEMI), and 1978 due to unstable angina (UA). The stable CAD group was considered the reference group. Based on the personal and familial history of premature cardiovascular disease and LDL cholesterol concentration, the Dutch Lipid Clinic Network (DLCN) algorithm was used for FH diagnosis.

The overall occurrence of probable/definite FH and possible FH was 1.2% and 13.5%, respectively. Among patients with ACS, 1.6% had probable/definite FH and 17.0% possible FH. The highest occurrence of FH was observed in the STEMI subgroup (20.6%). Patients with definite and probable FH had higher 30-day mortality than patients without FH. No significant differences were observed between the FH groups in the 12-, 36- and 60-month follow-up. Propensity-score matching analysis showed that definite/probable FH patients had significantly higher all-cause mortality at 36- and 60-month follow-up in comparison to non-FH subjects.

The prevalence of FH according to the DLCN criteria in the Polish very high-risk population is significantly higher in patients with ACS than in patients with stable coronary artery disease. FH is a cause of increased all-cause mortality in the long-term follow-up.

Economic evaluation of lipid lowering with PCSK9 inhibitors in patients with familial hypercholesterolemia: Methodological aspects

Familial hypercholesterolemia (FH) is characterized by increased plasma low density lipoprotein (LDL) cholesterol concentrations and severely increased risk of premature cardiovascular disease (CVD) [1]. FH is usually caused by mutations in genes encoding key proteins that clear serum of LDL cholesterol (LDL-C) such us *LDLR*, *ApoB*, and *PCSK9*. Since the cause of the clinical manifestations of FH lies in elevated LDL-C levels, reducing LDL-C is crucial for preventing CVD events. Statins in combination with ezetimibe represents the basis of current FH treatment. This treatment is

inexpensive and effective, but even with maximal dose it is often insufficient to achieve the treatment target in patients with FH, due to their particularly high LDL-C levels. Thus, proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors represent a new tool in those who do not reach treatment targets. They have proved to reduce low density lipoprotein cholesterol levels in numerous clinical trials. In two large clinical trials, PCSK9 inhibitor treatment reduced the risk of cardiovascular disease. The high price of PCSK9 inhibitors, however, raises questions about their cost-effectiveness. Wisløff et al. aimed to explore the impact of varying assumptions about clinical effectiveness on health and economic outcomes for patients with FH.

They used a previously published and validated Norwegian model for cardiovascular disease. The model was updated with recent data from the world's second largest registry of patients with genetically confirmed FH. They performed analyses for 24 different subgroups of patients based on age, gender, statin tolerance and previous history of cardiovascular disease.

PCSK9 inhibitors are cost-effective in only one subgroup of patients when assuming a similar effect regardless of LDL-C levels. PCSK9 inhibitors are cost-effective in 14 subgroups when assuming increased effect for patients with higher LDL-C levels.

In conclusion, cost-effectiveness of PCSK9 inhibitors depends highly on assumptions regarding effectiveness. Basing assumptions only on randomised controlled trials, and not taking into account varying effects based on baseline cholesterol level, results in much fewer groups being cost-effective.

Soluble low-density lipoprotein receptor-related protein 1 as a biomarker of coronary risk: Predictive capacity and association with clinical events

Low-density lipoprotein receptor-related protein 1 (LRP1) is a ubiquitously expressed cell-bound receptor of the low-density lipoprotein receptor family. It is involved in diverse biological processes, including lipoprotein metabolism and modulation of vascular integrity. Previous studies reported that the circulating soluble form of LRP1 (sLRP1) is a biomarker of atherosclerotic-related conditions and cardiometabolic disease. However, the association between sLRP1 and the risk of CAD and the predictive capacity of sLRP1 have not been addressed at the population level. Based on these, de Gonzalo-Calvo et al. examined whether circulating sLRP1 levels are associated with future CAD events and assessed the predictive capacity of sLRP1 beyond classical CAD risk functions in a population-based study. They also explored the causality of the association between sLRP1 and CAD using a Mendelian randomization approach.

The authors conducted a case-cohort study based on the follow-up of the REGICOR (Registre Gironí del Cor) population-based cohort survey that prospectively collected sociodemographic and

clinical data from all cases of myocardial infarction in the region of Girona (Catalonia, Spain). Of the 5404 participants (age between 35 and 74 years), without previous history of cardiovascular disease, 117 subjects with angina or fatal or non-fatal myocardial infarction were included, and 512 individuals were randomly selected as a subcohort (including 14 patients who presented coronary events). sLRP1 levels were measured in basal plasma samples by commercial ELISA. Hazard ratio (HR) was estimated with Cox models adjusted for potential confounding factors. Discrimination and reclassification were analyzed with the c-index and the net reclassification index (NRI), respectively. A Mendelian randomization approach was used to explore the causality of the association between sLRP1 and coronary artery disease (CAD).

The group of participants who presented a CAD event showed higher levels of sLRP1 than the subcohort. sLRP1 was significantly associated with CAD events even after adjustment for confounding factors. sLRP1 did not increase the predictive capacity or improve the cardiovascular risk stratification of the REGICOR risk function. The LRP1 genetic variants associated with CAD risk were not associated with plasma sLRP1 concentration.

The results show that plasma sLRP1 is independently associated with the incidence of coronary events, but it does not improve the predictive capacity of the REGICOR risk function.

Ultramorphological analysis of plaque advancement and cholesterol crystal formation in *Ldlr* knockout mouse atherosclerosis

The low-density lipoprotein receptor-deficient (*Ldlr*$^{-/-}$) mouse model has been utilized by cardiovascular researchers for more than two decades to study atherosclerosis. However, there has not yet been a systematic effort to document the ultrastructural changes that accompany the progression of atherosclerotic plaque in this model.

Employing several different staining and microscopic techniques, including immunohistochemistry, as well as electron and polarized microscopy, Baumer et al. analyzed atherosclerotic lesion development in *Ldlr*$^{-/-}$mice fed an atherogenic diet over time.

Lipid-like deposits occurred in the subendothelial space after only one week of atherogenic diet. At two weeks, cholesterol crystals (CC) formed and increased thereafter. Lipid, CC, vascular smooth muscles cells, and collagen progressively increased over time, while after 4 weeks, the relative macrophage content decreased. Accelerated accumulation of plate- and needle-shaped CC accompanied plaque core necrosis. Lastly, CC were surrounded by cholesterol microdomains, which co-localized with CC through all stages of atherosclerosis, indicating that the cholesterol microdomains may be a source of CC.

The authors documented atherosclerotic plaque morphology and composition from early to advanced stages in the *Ldlr*$^{-/-}$mouse, one of the most commonly used animal models utilized in atherosclerosis research.

Decreased M1 macrophage polarization in dabigatran-treated *Ldlr*-deficient mice: Implications for atherosclerosis and adipose tissue inflammation

The non-vitamin K oral anticoagulant dabigatran etexilate (dabigatran) is increasingly prescribed to patients with non-valvular atrial fibrillation and venous thromboembolism. Adipose tissue (AT) inflammation during obesity plays a crucial role in the development of insulin resistance, type II diabetes and atherogenesis. In this study, Feldmann et al. aimed to investigate the effects of thrombin inhibition by dabigatran in a combined model of diet-induced obesity and atherosclerosis.

To this purpose, female low density lipoprotein receptor knockout (*Lldr*$^{-/-}$) mice were fed a high-fat diet containing 5 mg/g dabigatran or matching control for 20 weeks.

Dabigatran-treated animals showed increased adipocyte hypertrophy, but reduced numbers of pro-inflammatory M1-polarized macrophages in the adipose tissue. Pro-inflammatory M1 macrophages also decreased in the aortic wall of dabigatran-fed mice. Multiple circulating cytokines were reduced, indicating an effect in systemically relevant secretory compartments such as the AT.

Dabigatran treatment reduces pro-inflammatory M1 macrophages in atherosclerotic lesions, thereby contributing to plaque stabilization and atheroprotective effects of the thrombin inhibitor. This finding is not restricted to the vascular wall but is also present in AT where dabigatran treatment reduces the release of pro-inflammatory cytokines and accumulation of M1 macrophages. Dabigatran effects on AT secretome may inhibit macrophage-driven inflammation during atheroprogression.