atherosclerosis

Atherosclerosis newsletter

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The two issues contain several articles that describe clinical, biochemical or genetic prognostic markers of cardiovascular events.

Comparative mortality according to peripheral artery disease and coronary heart disease/stroke in the United States

Lower-extremity peripheral artery disease (PAD) is often recognized as the third major atherosclerotic disease following coronary heart disease and stroke. A recent trial reported that patients with PAD without coronary heart disease or stroke (CHD/stroke) had worse prognosis than those with CHD/stroke without PAD. However, community-based data are lacking. Matsushita et al. study compared mortality according to the status of PAD and CHD/stroke in the general population.

In 6780 participants from the National Health and Nutrition Examination Surveys 1999–2004, mortality risk according to PAD (ankle-brachial index ≤0.9) and CHD/stroke (self-report) at baseline was compared using the Kaplan-Meier method and multivariable Cox models accounting for sampling weights.

The prevalence of having both PAD and CHD/stroke was 1.6%. The prevalence of PAD without CHD/stroke and CHD/stroke without PAD was 4.1% and 8.5%, respectively. Over a median follow-up of 12.8 years, 21.2% of patients died. Individuals with both PAD and CHD/stroke had the worst survival. Those with PAD without CHD/stroke had the second worst prognosis, followed by those with CHD/stroke without PAD and those without CHD/stroke or PAD. Adjusted hazard ratio of mortality was 2.70 for PAD with CHD/stroke, 1.81 in CHD/stroke without PAD, and 1.68 in PAD without CHD/stroke *vs.* no CHD/stroke or PAD.

In the US adults, PAD contribute to increased mortality in persons with and without CHD/stroke. The prognosis of PAD without CHD/stroke is not better than that of CHD/stroke without PAD. These results suggest the importance of recognizing the presence of PAD in the community.

Early statin use and cardiovascular outcomes after myocardial infarction: A population-based case-control study

Clinical practice guidelines give statins a class IA recommendation after myocardial infarction (MI) and recommend their use in all patients irrespective of low-density lipoprotein (LDL) levels. However, a number of patients do not use statins for secondary prevention, and this is mainly due to suspected adverse events. Kytö et al. investigated the association of not using statins early after MI with adverse outcomes.

Consecutive MI patients admitted to 20 Finnish hospitals were retrospectively studied. Statin was not used by 17.1% within 90 days after MI discharge (exposure). Differences in baseline features, comorbidities, revascularization, and other evidence-based medications were balanced with propensity score matching, resulting in 10,051 pairs of patients with and without statin. Median follow-up was 5.9 years.

Patients not using statin early after MI had higher all-cause mortality in 1-year and 10-year follow-up in the matched cohort. The cumulative incidence of major adverse cardiovascular event was higher at 1- and 10-years in matched patients not using statins. Cardiovascular death, new MI, and ischemic stroke were more frequent without early statin. A lack of statin was associated with outcomes regardless of sex, age, atrial fibrillation, dementia, diabetes, heart failure, revascularization, or usage of other evidence-based secondary preventive medications in subgroup analyses.

Lack of statin therapy early after MI is associated with adverse outcomes across the spectrum of MI patients. Results underline the importance of timely statin use after MI.

Oxidized phospholipids on apolipoprotein B-100 *versus* plasminogen and risk of coronary heart disease in the PROCARDIS study

Following a concentration-dependent uptake of atherogenic lipoproteins in the vessel wall, oxidative modification of lipids generates a variety of pro-inflammatory oxidation-specific epitopes, including oxidized phospholipids (OxPL) that trigger the initiation of atherosclerosis. Oxidized phospholipids carried on the apolipoprotein B-100 (OxPL-apoB) component of Lp(a) are predictive of coronary heart disease (CHD), but the role of oxidized phospholipids carried on plasminogen (OxPL-PLG) is unknown. Clarke et al. examined the independent effects of OxPL-apoB and OxPL-PLG for risk of CHD before and after adjustment for Lp(a).

Plasma levels of OxPL-apoB, OxPL-PLG, plasminogen and Lp(a) were measured in the PROCARDIS study of early-onset CHD (906 cases/858 controls). Multivariable logistic regression was used to estimate the odds ratios (OR) for each biomarker with CHD after adjustment for established risk factors.

Mean levels of OxPL-apoB were higher in cases than controls, but levels of OxPL-PLG and plasminogen were similar. For OxPL-apoB, individuals in the top *vs* bottom fifth had 2-fold higher age and sex-adjusted OR of CHD, which were partially attenuated after adjustment for established risk factors. The findings for OxPL-apoB and CHD in PROCARDIS were comparable with those of a meta-analysis of all such studies. However, the associations of OxPL-apoB with CHD were fully attenuated by additional adjustment for Lp(a). Neither OxPL-PLG nor plasminogen were associated with CHD. Overall, there were no differences in the predictive value for CHD of high *vs* normal levels of OxPL-apoB, OxPL-PLG, plasminogen or Lp(a) after stratifying for each other.

These results highlight the context-dependency of OxPL in plasma and suggest that their associated risk of CHD is mainly mediated by their carriage on Lp(a).

Omega-3 fatty acids, subclinical atherosclerosis, and cardiovascular events: Implications for primary prevention

High-dose eicosapentaenoic acid (EPA) therapy reduces event rates in high-risk patients without clinical cardiovascular disease (CVD). THe association of plasma levels of EPA and docosahexaenoic acid (DHA) with risk of CVD events in humans without subclinical CVD is unclear. Alfaddagh et al. evaluated the interactions between plasma omega-3 fatty acids and coronary artery calcium (CAC) in relation to CVD events.

The authors examined 6568 participants from the Multi-Ethnic Study of Atherosclerosis (MESA) with baseline measurements of plasma EPA and DHA levels and CAC. The primary outcome was incident CVD events (myocardial infarction, angina, cardiac arrest, stroke, CVD death). The median follow-up time was 15.6 years.

Higher EPA and DHA levels were independently associated with fewer CVD events. The difference in absolute CVD event rates between lowest *vs.* highest EPA tertile increased at higher CAC levels. A similar association was seen in tertiles of DHA by CAC category.

In an ethnically diverse population free of clinical CVD, higher plasma omega-3 fatty acid levels were associated with fewer long-term CVD events. When coronary artery calcium (CAC) is present, higher plasma omega-3 fatty acids are associated with less CVD events. Significant residual CVD risk exists in patients with elevated CAC even when plasma omega-3 fatty acid levels are high.

De novo DNA methylation induced by circulating extracellular vesicles from acute coronary syndrome patients

In the last years, the interest in extracellular vesicles (EVs), including exosomes, microvesicles (MVs) and apoptotic bodies, has increased. Recent evidence has suggested a key role for EVs in cardiovascular diseases (CVDs), however, their use as biomarkers in clinical research is still limited.

DNA methylation is associated with gene silencing, but its clinical role in cardiovascular diseases (CVDs) remains to be elucidated. Schiano et al. aimed to elucidate the potential role of circulating EVs in acute coronary syndrome (ACS) patients in the modulation of gene expression through epigenetic-sensitive mechanisms. They hypothesized that EVs may carry epigenetic changes, and could represent an ideal sample for "liquid biopsy" as their content reflects changes in the condition of the cell of origin. Therefore, they isolated and characterized circulating EVs of ACS patients and assessed their role on DNA methylation in epigenetic modifications.

EVs were recovered from plasma of 19 ACS patients and 50 healthy subjects (HS). Flow cytometry, qRT-PCR, and Western blot were performed to evaluate intra-vesicular and intra-cellular signals. ShinyGO, PANTHER, and STRING tools were used to perform gene ontology (GO) and protein-protein interaction (PPI) network analyses.

ACS-derived EVs showed increased levels of DNA methyltransferases (DNMTs). Specifically, *de novo* methylation transcripts, as *DNMT3A* and *DNMT3B*, were significantly increased in plasma ACS-EVs. DNA methylation analysis on peripheral blood mononuclear cells (PBMCs) from healthy donors treated with HS- and ACS-derived EVs showed an important role of DNMTs carried by EVs. PPI network analysis evidenced that ACS-EVs induced changes in PBMC methylome.

Extracellular vesicles modulate gene expression through *de novo* DNA methylation. The results show an important role for ACS-derived EVs in gene expression modulation through *de novo* DNA methylation signals.

The association between mitochondrial DNA abundance and stroke: A combination of multivariable-adjusted survival and Mendelian randomization analyses

Stroke is the second leading cause of death and loss of disability-adjusted life years worldwide. Oxidative stress has been hypothesized to play an important role in the pathophysiology of stroke. As a result of direct or indirect reactive oxygen species (ROS)-induced damage to the (cerebral) vascular wall, multiple aspects of the vascular system are affected including platelet aggregation, endothelial function, vascular permeability and vasodilation. Mitochondrial dysfunction is associated with increased ROS that are thought to drive disease risk, including stroke. Martens et al. investigated the association between mtDNA abundance, as a proxy measure of mitochondrial function, and incident stroke, using multivariable-adjusted survival and Mendelian Randomization (MR) analyses.

Cox-proportional hazard model analyses were conducted to assess the association between mtDNA abundance, and incident ischemic and hemorrhagic stroke over a maximum of a 14-year follow-up in European-ancestry participants from the UK Biobank. MR was conducted using independent lead variants for mtDNA abundance as instrumental variables. Single-nucleotide polymorphism (SNP)-ischemic stroke associations were derived from three published open source

European-ancestry results databases (cases/controls): MEGASTROKE, UK Biobank and FinnGen. MR was performed per study, and results were subsequently meta-analyzed.

In total, 288,572 unrelated participants were included in the Cox-proportional hazard analyses. After correction for considered confounders, no association was found between low *versus* high mtDNA abundance and ischemic or hemorrhagic stroke. However, in the MR analyses after removal of platelet count-associated SNPs, evidence for an association between genetically-influenced mtDNA abundance and ischemic stroke was found.

Although the results from both multivariable-adjusted prospective and basis MR analyses did not show an association between low mtDNA and increased risk of ischemic stroke, in-depth MR sensitivity analyses may suggest evidence for a causal relationship.