European Atherosclerosis Journal
www.eathj.org
EAJ 2023;3:52-54

The XVII National Congress of the Società Italiana di Terapia Clinica e Sperimentale (SITeCS)

Circulating plasma exosomes reflect the severity of myocardial damage in STEMI patients

Marta Zarà[1], Andrea Baggiano[1,2], Patrizia Amadio[1], Jeness Campodonico[1], Sebastiano Gili[1], Andrea Annoni[1], Gianluca De Dona[1], Maria Ludovica Carerj[1], Francesco Cilia[1], Alberto Formenti[1], Laura Fusini[1,3], Cristina Banfi[1], Paola Gripari[1], Calogero Claudio Tedesco[1], Maria Elisabetta Mancini[1], Mattia Chiesa[1], Riccardo Maragna[1], Francesca Marchetti[1], Marco Penso[1], Luigi Tassetti[1], Alessandra Volpe[1], Alice Bonomi[1], Giancarlo Marenzi[1], Gianluca Pontone[1,4] and Silvia Stella Barbieri[1]

[1]Centro Cardiologico Monzino IRCCS, Milan, Italy
[2]Department of Clinical Sciences and Community Health, University of Milan, Italy
[3]Department of Electronics, Information and Bioengineering, Politecnico di Milano, Italy
[4]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy
https://doi.org/10.56095/eaj.v2i3.54
Marta Zarà: marta.zara@cardiologicomonzino.it

Exosomes are small extracellular vesicles involved in intercellular communication and they contribute to inflammation, coagulation and vascular injury. Exosomes have demonstrated a great potential as diagnostic markers of disease, however their ability to reflect myocardial damage assessed by Cardiac Magnetic Resonance (CMR) in ST-segment elevation myocardial infarction (STEMI) is still unknown. To fill this gap, plasma exosomes were isolated from 42 STEMI patients treated by primary percutaneous coronary intervention (pPCI) and evaluated by CMR between days 3 and 6. Exosome concentration and size were measured by Nanoparticle Tracking Analysis, surface epitopes by flow cytometry, and platelet marker expression by ELISA kit.
Exosome levels were greater in patients with anterior STEMI (p=0.0001), with the culprit lesion located in LAD (p=0.045), and in those who underwent late revascularization (p=0.038). A smaller exosome size was observed in patients with a low myocardial salvage index (MSI, p=0.014). Exosomes of patients with microvascular obstruction (MVO) had smaller dimension (p<0.002) and lower expression of the platelet marker CD41–CD61 (p=0.039). Exosome size and CD41–CD61 expression were independent predictors of MVO/MSI (OR [95% CI]: 0.93 [0.87–0.98] and 0.04 [0–0.61], respectively).
In conclusion, we reported for the first time the ability of exosomes isolated a few days after STEMI to reflect myocardial damage. In particular, the exosome size and expression of the platelet marker CD41–CD61, likely reflecting the level of circulating platelet-derived exosome, were independent predictors of MVO and low MSI that are both predictors of short-term prognosis of acute STEMI after pPCI treatment and are key variables for risk-stratification of patients after STEMI. This finding paves the way for the development of a new strategy for the timely identification of high-risk patients and their treatment optimization.

Plasma and cerebrospinal fluid cholesterol esterification is hampered in Alzheimer's disease

Marta Turri[1], Elisa Conti[2], Chiara Pavanello[1], Francesco Gastoldi[1], Marcella Palumbo[3], Franco Bernini[3], Vittoria Aprea[2], Francesca Re[4], Alberto Barbiroli[5], Davide Emide[5], Daniela Galimberti[6,7], Lucio Tremolizzo[2]*, Francesca Zimetti[3]*, Laura Calabresi[1]*

[1]Centro E. Grossi Paoletti, Dipartimento di Scienze Farmacologiche e Biomolecolari, Università degli Studi di Milano, Italy
[2]Neurology Unit, IRCCS "San Gerardo dei Tintori", Monza and University of Milano-Bicocca, Italy;
[3]Department of Food and Drug, University of Parma, Italy;
[4]School of Medicine and Surgery, University of Milano-Bicocca, Italy;
[5]Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente, Università degli Studi di Milano, Italy;
[6]Department of Biomedical, Surgical and Dental Sciences, University of Milan, Italy; 7Fondazione IRCCS Ca' Granda, Ospedale Policlinico, Milan, Italy
https://doi.org/10.56095/eaj.v2i3.53
Marta Turri: marta.turri@unimi.it

Introduction: Several epidemiological studies indicate a strong inverse association between the risk of developing Alzheimer's disease (AD) and plasma HDL-C levels. The mechanism by which plasma HDL influence the pathogenesis and progression of AD is still un-

solved and since cholesterol esterification is a crucial step in HDL metabolism it could be involved. The purpose of this study was to evaluate cholesterol esterification and HDL subclasses in plasma and cerebrospinal fluid (CSF) of Alzheimer's Disease (AD) patients.
Materials and Methods: The study enrolled 70 AD patients and 74 cognitively-normal controls comparable for age and sex. Lipids and lipoprotein profile, cholesterol esterification, and cholesterol efflux capacity (CEC) were evaluated in plasma and CSF using assays set for measurement in plasma, which were appropriately modified for CSF.
Results and Discussion: AD patients have normal plasma lipids, but significantly reduced unesterified cholesterol and unesterified/total cholesterol ratio. Lecithin:cholesterol acyltransferase (LCAT) activity and cholesterol esterification rate (CER), two measures of the efficiency of the esterification process, were reduced by 29% and 16%, respectively, in plasma of AD patients. Plasma HDL subclass distribution in AD patients was comparable to that of controls, but the content of small discoidal preβ-HDL particles was significantly reduced. In agreement with the reduced preβ-HDL particles, cholesterol efflux capacity mediated by the transporters ABCA1 and ABCG1 was reduced in AD patients' plasma. The CSF unesterified to total cholesterol ratio was increased in AD patients, and CSF CER and CEC from astrocytes were significantly reduced in AD patients. In the AD group, a significant positive correlation was observed between plasma unesterified cholesterol and unesterified/total cholesterol ratio with Aβ1-42 CSF content.
Conclusion: Taken together data indicate that cholesterol esterification is hampered in plasma and CSF of AD patients, and that plasma cholesterol esterification biomarkers (unesterified cholesterol and unesterified/total cholesterol ratio) are significantly associated to disease biomarkers (i.e., CSF Aβ1-42).

Different operational definitions of polypharmacy and their association with the risk of all-cause hospitalization: A conceptual framework using administrative databases

Stefano Scotti[1], Elena Olmastroni[1,2], Federica Galimberti[1], Lorenza Scotti[3], Manuela Casula[1,2]

[1]IRCCS MultiMedica, Laboratory of Cardiovascular Research, Sesto San Giovanni, Milan, Italy
[2]Epidemiology and Preventive Pharmacology Service (SEFAP), Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy
[3]Department of Translational Medicine, Università del Piemonte Orientale UPO, Novara, Italy
https://doi.org/10.56095/eaj.v2i3.52
Stefano Scotti: stefano.scottiultimedica.it

Background: As in all pharmacoepidemiology studies, also in the cardiovascular field it is essential to take into account the clinical complexity of patients, which is very frequently estimated with polypharmacy. We aimed at describing the current heterogeneity of polypharmacy definition, and assessing the association of polypharmacy with clinical outcomes.
Methods: Using administrative databases of the local health unit of Bergamo (Lombardy), all subjects aged ≥40 years with at least one reimbursed drug prescription during the year 2017 were identified. We selected from literature relevant operational definitions of polypharmacy. First, we applied World Health Organization (WHO) definition (at least ≥5 different medications, ATC 4th level code). Second, we excluded drug prescriptions associated with short-term treatment. Third, we considered only the prescriptions of drugs with a total annual defined daily doses (DDDs) ≥60. All the approaches were evaluated within one year, one quarter, and one month. A multivariate logistic regression model was performed to estimate odds ratios (OR) and 95% confidence intervals [95% CI] for the association between polypharmacy and the risk of hospitalization for all-causes.
Results: Overall, 431,620 subjects were included in our cohort. The DDD-based definition led to estimates with little variability depending on the time windows (range 20.47%-21.16%), while the WHO definition determined the greatest variability (range 39.98%-31.24%). The DDD-based definition identified an older (mean age [SD], 72.6 [10.9]) and more complex cohort of patients (average number [SD] of previous hospitalizations 1.2 [1.7], average number of dispensed drugs 9.7 [3.5]). A dose-dependent increase in risk was observed as the number of the dispensed drugs increases regardless of definitions.
Conclusions: Different definitions of polypharmacy led to different prevalence estimates. All definitions showed a dose-dependent association with hospitalization risk, with the definition based on DDDs being the least heterogeneous. However, only a patient-by-patient approach can determine whether or not polypharmacy is appropriate.

VLDL cholesterol associates with higher plasmatic expression of inflammatory proteins and atherosclerotic pathways compared to LDL cholesterol

Elisa Mattavelli[1,2], Elena Olmastroni[1,2], Nick Nurmohamed[3], Liliana Grigore[2], Fabio Pellegatta[2], Erik S.G. Stroes[3], Alberico L. Catapano[1,2], Andrea Baragetti[1,2]

[1]Department of Pharmacological and Biomolecular Sciences, University of Milan, Italy
[2]IRCCS MultiMedica Hospital, Milano, Italy
[3]Department of Vascular Medicine, Amsterdam University Medical Centers, University of Amsterdam
https://doi.org/10.56095/eaj.v2i3.51
Elisa Mattavelli: elisa.mattavelli@unimi.it

Background and Aim: High cholesterol in Low-Density Lipoproteins (LDL-C) is the key target of current pharmacological treatments aimed at reducing atherosclerotic cardiovascular disease (ACVD) risk. Increased cholesterol in Very low-density lipoproteins ("VLDL-C") is an independent predictor of ACVD. VLDL-C was previously associated with markers of inflammation (for instance C-reactive protein). We now tested the relationship between either VLDL-C or LDL-C with a large spectrum of inflammatory proteins in plasma collected from subjects at different ACVD risks.
Methods: We measured 276 proteins (Olink™) in plasma from a primary ACVD risk prevention cohort ("PLIC" in Milan; n=656 (8.2% on statins)) and a secondary ACVD risk prevention cohort (the Second Manifestations of ARTerial disease, "SMART", the Netherlands, n=630 (50.8% on statins)). Cohorts were divided into three

groups for VLDL-C ("Normal" VLDL-C<15 mg/dL, "High" VLDL-C 15-30 mg/dL, "Very high" VLDL-C >30 mg/dL) and LDL-C ("Normal" LDL-C <115 mg/dL, "High" LDL-C 115-155 mg/dL, "Very high" LDL-C>155 mg/dL). The expression (Normalized Protein eXpression, NPX) of each protein was compared among these groups by artificial intelligence. The performance to discriminate subjects with higher VLDL-C or LDL-C was evaluated by comparing the Areas Under the Curve (AUCs) of the Receiver Operating Characteristics curve (ROC) considering proteomics on top of ACVD risk factors ("CVRFs": age, body mass index, systolic blood pressure, glycemia, therapies), versus the AUC of the ROCs with CVRFs alone.

Results: The number of plasma proteins differentially expressed increased, as a function of higher VLDL-C in PLIC, as the NPXs of 84 were higher in "High" and the NPXs of 136 were higher in "Very high" vs "Normal" VLDL-C respectively. A similar trend was found in SMART, where the NPXs of 30 proteins were higher in "High" and the NPXs of 64 were higher in "Very high" vs "Normal" VLDL-C respectively. 26 proteins were shared between the two populations and recapitulated key atherosclerotic pathways (including chemotaxis of immune cells). The relationship between LDL-C was less marked; in PLIC, 14 proteins were more expressed in "High" and 33 in "Very high" vs "Normal" LDL-C respectively, while in SMART, the NPXs of 11 proteins were higher in "High" and the NPXs of 36 were higher in "Very high" vs "Normal" LDL-C respectively. Only 4 proteins were shared between high and very high LDL-C in the two populations. Finally, none of the proteins were shared between the groups of "High"/"Very high" VLDL-C and "High"/"Very high" LDL-C in the two cohorts.

Canonical CVRFs alone slightly improved the ability to identify subjects with increased VLDL-C both in PLIC and SMART (AUCs between 0.6 on average), but adding plasma proteomics markedly improved the performance to identify subjects with "High" VLDL-C, in PLIC (AUC=0.767 (0.709-0.837)) and in SMART (AUC=0.781 (0.681-0.873)), and with "Very high" VLDL-C (AUC=0.950 (0.899-0.976) in PLIC, and AUC=0.938 (0.894-0.971) in SMART).

The ROC of plasma proteomics with CVRFs was also superior to the ROC of the CVRFs alone to identify subjects with "High" and "Very high" LDL-C, but, as compared to the ROCs that discriminated subjects with "High" and "Very-high" VLDL-C, the AUCs were attenuated in both cohort (for "High" LDL-C: AUC=0.665 (0.558-0.774) in PLIC and AUC=0.775 (0.704-0.842) in SMART; for "Very high" LDL-C: AUC =0.776 (0.694-0.854) in PLIC and AUC=0.882 (0.825-0.931) in SMART).

Conclusion: High VLDL-C associates with a higher number of differentially expressed plasma proteins versus high LDL-C and none of the proteins were in common. Our data do not underestimate the value of LDL-C in ACVD but reinforce the concept that VLDL-C may also promote different atherosclerotic pathways involved in determining ACVD.