SPRING 2023

Spring Meeting Giovani Ricercatori

SID SIIA SIMI SIPREC SISA

Basic and clinical research:
Until grant let us apart

Rimini, 16-18 Aprile 2023

Young investigators meeting

SPRING 2023

Carissime Colleghe e Colleghi,

Vi diamo il benvenuto alla VIII Edizione dello Spring Meeting Nazionale dei Giovani Ricercatori SID SIIA SIMI SIPREC SISA 2023, intitolato “Basic and clinical research: until grant let us apart”

Come suggerisce il titolo che abbiamo voluto dare all’evento, lo sforzo di questa edizione è quello di mostrare l’importanza della complementarità tra ricerca di base e clinica nella progressione della conoscenza scientifica in ambito cardiometabolico. A questo scopo, per il format dell’evento abbiamo pensato un confronto a due voci tra ricerca di base e clinica su tutte le principali tematiche che verranno trattate.

Anche quest’anno il meeting vedrà la partecipazione congiunta del gruppo giovani di SIIA (Società Italiana Ipertensione Arteriosa), SIMI (Società Italiana di Medicina Interna), SIPREC (Società Italiana per la Prevenzione Cardiovascolare) e SISA (Società Italiana per lo Studio dell'Aterosclerosi), a cui si aggiunge, novità di questa edizione, il gruppo giovani di SID (Società Italiana di Diabetologia). La partecipazione di quest’ultima, rappresenta un’ulteriore opportunità per ampliare il network tra società scientifiche e favorire le collaborazioni tra i giovani ricercatori, nel pieno spirito di questo evento.

La partecipazione congiunta di ben cinque società rappresenta per noi un motivo di grande entusiasmo, in quanto la possibilità di interazione e partecipazione attiva di tutti i giovani ricercatori, da sempre elemento caratterizzante dell’atmosfera e del successo dell’evento, ne risulta sicuramente amplificata.

Il Congresso cercherà di essere di nuovo un momento di incontro, non solo per la discussione dei risultati della ricerca scientifica in ambito cardiometabolico promossa dai giovani soci delle cinque Società Scientifiche coinvolte, ma anche un’occasione per promuovere lo scambio di idee tra i partecipanti, incoraggiare la nascita di collaborazioni intersocietarie e più in generale favorire la crescita professionale di molti giovani ricercatori.

Il format del congresso prevede sia sessioni dedicate a comunicazioni orali e a presentazioni poster sia workshop interattivi su vari aspetti critici e metodologici della ricerca biomedica e della comunicazione/informazione scientifica.

Cogliamo l’occasione per invitarvi a partecipare attivamente allo Spring Meeting 2023 per lavorare tutti insieme e condividere le nostre attività di ricerca.

Buon lavoro a tutti,

Il comitato organizzatore

Domenica - 16 aprile 2023

12.00-13.00 Arrivi e registrazione dei partecipanti

13.00-14.00 *Light Lunch*

14.00-14.30 **Apertura lavori**
Vanessa Bianconi (SISA), Massimiliano Cavallo (SID), Damiano D'Ardes (SIMI), Fabio Fimiani (SISA), Giovanna Gallo (SIPREC), Carla Greco (SID), Rosa Lombardi (SIMI), Alessandro Maloberti (SIIA), Chiara Pavanello (SISA), Francesco Perone (SIPREC), Francesco Spannella (SIIA), Giovanni Talerico (SIMI), Valeria Visco (SIIA)

Saluto dei Presidenti:
Angelo Avogaro (SID), Maria Lorenza Muiesan (SIIA), Giorgio Sesti (SIMI), Massimo Volpe (SIPREC), Alberico L. Catapano (SISA)

14.30-16.10 **Sessione 1 - SISA - Il lato oscuro del metabolismo delle lipoproteine**
Moderatori: Fabio Fimiani (SISA), Ilaria Rossi (SIMI)
Basic research - Verso la definizione del ruolo di PCSK9 oltre il fegato… • *Lorenzo Da Dalt (Milano)*
Clinical research - Lipoproteina(a) tra aterotrombosi e infiammazione: luci e ombre • *Vanessa Bianconi (Perugia)*
Discussione congiunta
Comunicazioni Orali (n.6)

16.10-18.10 **WORKSHOP (parte 1) - Come approcciarsi ai test statistici: building the foundation**
Coordinatore: Mario Luca Morieri (Padova)
Intervengono: • *Federica Galimberti (Milano)* • *Davide Bernasconi (Milano)*
WORKSHOP (parte 2) - Come approcciarsi ai test statistici: road to Mendelian randomization
Coordinatore: Mario Luca Morieri (Padova)
Intervengono: • *Federica Galimberti (Milano)* • *Davide Bernasconi (Milano)*

18.10-18.30 Coffee Break

18.30-19.30 **Sessioni Poster 1-4**
Sessione Poster 1: *Massimiliano Cavallo*
Sessione Poster 2: *Giovanni Talerico*
Sessione Poster 3: *Simonetta Lugari*
Sessione Poster 4: *Giovanna Gallo*

20.30 *Cena*

Lunedì, 17 aprile 2023

08.30-10.10 **Sessione 2 - SIIA - Come il cuore si scompensa: l'origine del male**
Moderatori: Damiano D'Ardes (SIMI), Francesco Spannella (SIIA)
Basic research - Meccanismi dell'insufficienza cardiaca: dalle basi molecolari ai modelli preclinici ● *Michele Ciccarelli (Salerno)*
Clinical research - Dall'ipertensione arteriosa all'HFpEF passando per il danno d'organo cardiaco ● *Costantino Mancusi (Napoli)*
Discussione congiunta
Comunicazioni Orali (n.6)

10.10-10.40 *Coffee Break*

10.40-11.40 **Sessioni Poster 5-8**
Sessione Poster 5: *Costantino Mancusi*
Sessione Poster 6: *Antonella Giammanco*
Sessione Poster 7: *Sebastiano Cicco*
Sessione Poster 8: *Manuela Casula*

11.40-13.00 **WORKSHOP (parte 3): Come approcciarsi ai test statistici: road to Propensity Score**
Coordinatore: Mario Luca Morieri (Padova)
Intervengono: ● *Federica Galimberti (Milano)* ● *Davide Bernasconi (Milano)*

13.00-14.30 *Lunch*

14.30-16.10 **Sessione 3 - SIPREC - Scompenso cardiaco: dai meccanismi molecolari alla sala di emodinamica**
Moderatori: Massimiliano Cavallo (SID), Giovanna Gallo (SIPREC)
Basic research - Meccanismi molecolari alla base dello scompenso cardiaco: focus sull'autofagia ● *Maurizio Forte (Napoli)*
Clinical research - Nuove frontiere del trattamento interventistico dello scompenso cardiaco ● *Beniamino Pagliaro (Milano)*
Discussione congiunta
Comunicazioni Orali (n.6)

16.10-16.40 *Coffee Break*

16.40-18.20 **Sessione 4 - SIMI - Il sistema immunitario: attore (non?) protagonista nella malattia metabolica nel fegato**
Moderatori: Francesco Baratta (SISA), Giulio Francesco Romiti (SIMI)
Basic research - …from bench ● *Moris Sangineto (Bari)*
Clinical research - ...to bedside ● *Andrea Dalbeni (Verona)*
Discussione congiunta
Comunicazioni Orali (n.6)

18.20-19.00 **Riunione societarie – Cosa bolle in pentola?**

20.30 *Cena*

Martedì 18 aprile 2023

08.30-10.10 **Sessione 5 - SID-Oltre la glicemia: effetti pleiotropici dei GLP1Ras**
Moderatori: Stefano Ciardullo (SID), Sara Coluzzi (SID)
Basic research - Evidenze precliniche di nuove possibili applicazioni terapeutiche dei GLP1Ras • *Nicola Marrano (Bari)*
Clinical research - GLP1Ras e NAFLD-NASH • *Alessandro Mantovani (Verona)*
Discussione congiunta
Comunicazioni Orali (n.6)

10.10-10.30 *Coffee Break*

10.30-11.30 **Sessioni Poster 9-12**
Sessione Poster 9: *Alessandro Maloberti*
Sessione Poster 10: *Alice Ossoli*
Sessione Poster 11: *Francesco Spannella*
Sessione Poster 12: *Arturo Cesaro*

11.30-13.00 **Tavola rotonda - comunicazione**
Il mito racconta (Ὁ μῦθος δηλοῖ ὅτι)…
Presentatori: Massimiliano Cavallo (SID), Giovanna Gallo (SIPREC), Rosa Lombardi (SIMI)
Il ricercatore di base contro tutti
Intervista con contraddittorio a *Marco Busnelli (Milano)*

13.00 ***Chiusura lavori e Lunch***

Comitato organizzatore

Vanessa Bianconi (SISA), Massimiliano Cavallo (SID), Damiano D'Ardes (SIMI), Fabio Fimiani (SISA), Giovanna Gallo (SIPREC), Carla Greco (SID), Rosa Lombardi (SIMI), Alessandro Maloberti (SIIA), Chiara Pavanello (SISA), Francesco Perone (SIPREC), Francesco Spannella (SIIA), Giovanni Talerico (SIMI), Valeria Visco (SIIA)

Moderatori e relatori

Francesco Baratta
Davide Bernasconi
Vanessa Bianconi
Marco Busnelli
Massimiliano Cavallo
Manuela Casula
Arturo Cesaro
Stefano Ciardullo
Michele Ciccarelli
Sebastiano Cicco
Sara Coluzzi
Lorenzo Da Dalt
Damiano D'Ardes
Andrea Dalbeni
Fabio Fimiani
Maurizio Forte
Federica Galimberti
Giovanna Gallo
Antonella Giammanco
Rosa Lombardi
Simonetta Lugari
Alessandro Maloberti
Costantino Mancusi
Alessandro Mantovani
Nicola Marrano
Mario Luca Morieri
Alice Ossoli
Beniamino Pagliaro
Giulio Francesco Romiti
Ilaria Rossi
Moris Sangineto
Francesco Spannella
Giovanni Talerico

SPRING 2023

Abstract

Sessione 1 - Il lato oscuro del metabolismo delle lipoproteine

Domenica 16 Aprile 2023 – ore 14.30-16.00

Moderatori: Fabio Fimiani, Ilaria Rossi

Sessione 2 - Come il cuore si scompensa: l'origine del male

Lunedì 17 Aprile 2023 – ore 08.30-10.10

Moderatori: Damiano D'Ardes, Francesco Spannella

Sessione 3 - Scompenso cardiaco: dai meccanismi molecolari alla sala di emodinamica

Lunedì 17 Aprile 2023 – ore 14.30-16.10

Moderatori: Massimiliano Cavallo, Giovanna Gallo

Sessione 4 - Il sistema immunitario: attore (non?) protagonista nella malattia metabolica nel fegato

Lunedì 17 Aprile 2023 – ore 16.40-18.20

Moderatori: Francesco Baratta, Giulio Francesco Romiti

Sessione 5 - Oltre la glicemia: effetti pleiotropici dei GLP1Ras

Martedì 18 Aprile 2023 - ore 08.30-10.10

Moderatori: Stefano Ciardullo, Sara Coluzzi

Sessione Poster 3 - Domenica 16 Aprile 2023 – ore 18.30-19.30
Infiammazione e immunità al crocevia tra trombosi, aterosclerosi e malattie metaboliche
Moderatore: Simonetta Lugari

Sessione Poster 4 - Domenica 16 Aprile 2023 – ore 18.30-19.30
Biomarcatori emergenti: significato diagnostico e prognostico
Moderatore: Giovanna Gallo

Sessione Poster 5 - Lunedì 17 Aprile 2023 – ore 10.40-11.40
Ruolo della dieta nel rischio cardiometabolico
Moderatore: Costantino Mancusi

Sessione Poster 6 - Lunedì 17 Aprile 2023 – ore 10.40-11.40
Dislipidemie primitive: dalla diagnosi alla terapia
Moderatore: Antonella Giammanco

Sessione Poster 7 - Lunedì 17 Aprile 2023 – ore 10.40-11.40

NAFLD & MAFLD

Moderatore: Sebastiano Cicco

Sessione Poster 8 - Lunedì 17 Aprile 2023 – ore 10.40-11.40

Trattamenti farmacologici in ambito cardiovascolare: from bench to bedside

Moderatore: Manuela Casula

Sessione Poster 9 - Martedì 18 Aprile 2023 – ore 10.30-11.30
Molecole chiave nella regolazione del metabolismo lipoproteico: aspetti emergenti
Moderatore: Alessandro Maloberti

Sessione Poster 10 - Martedì 18 Aprile 2023 – ore 10.30-11.30
Assi cuore-rene, cuore-fegato e cuore-intestino
Moderatore: Alice Ossoli

Sessione Poster 11 - Martedì 18 Aprile 2023 – ore 10.30-11.30
Malattia cardiovascolare: quando si verifica il raro e l'inatteso…
Moderatore: Francesco Spannella

Sessione Poster 12 - Martedì 18 Aprile 2023 – ore 10.30-11.30
Chiedi all'esperto…
Moderatore: Arturo Cesaro

Abstract n SO1_01 - Presenting author**: Bianca Papotti**

Serum and cerebrospinal fluid concentrations of PCSK9 and hydroxysterols in patients with cognitive impairment

Bianca Papotti[1], Marco Bertolotti[2], Cinzia Marchi[1], Maria Pia Adorni[3], Annalisa Chiari[2], Roberta Bedin[2], Maria Giovanna Lupo[4], Lisa Elviri[1], Giulia Remaggi[1], Enrica Baldelli[2], Giulia Lancellotti[2], Chiara Mussi[2], Franco Bernini[1], Nicola Ferri[4], Francesca Zimetti[1]

[1]Department of Food and Drug, University of Parma; Italy;
[2]Department of Biomedical, Metabolic and Neural Sciences, University of Modena and Reggio Emilia and University Hospital of Modena; Italy;
[3]Department of Medicine and Surgery, Unit of Neuroscience, University of Parma; Italy;
[4]Department of Medicine, University of Padua; Italy.

Aim: Altered cholesterol homeostasis has been described in cardiovascular and in several neurodegenerative diseases, including Alzheimer's Disease (AD). We previously demonstrated higher cerebrospinal fluid (CSF) PCSK9 levels in AD patients compared to controls. This study aimed to evaluate the possible association between PCSK9, cholesterol and its oxidative metabolites to investigate their potential as disease biomarkers in patients with different degrees of cognitive impairment.
Methods: We recruited 83 subjects divided as follows: patients with AD (AD; n=27), patients with mild cognitive impairment (MCI) that converted to AD at follow-up (MCI-AD; n=28) and patients with stable MCI (MCI; n=28). Serum/CSF PCSK9 was quantified through ELISA assay, cholesterol through a fluorometric assay, and 24-25- and 27-OHC through LC-MS/MS.
Results: PCSK9 CSF concentration was similar among the three groups, but higher if compared to those of control subjects previously analyzed ($p<0.0001$ vs all). Within AD patients, apoE Ɛ4 carriers presented higher PCSK9 concentration as compared to non-carriers (+58%; $p=0.04$). Moreover, CSF PCSK9 levels positively correlated with that in serum ($r=0.521$, $p=0.004$) and with CSF total-tau ($r=0.339$, $p=0.03$). Finally, again specifically in AD patients, CSF 24-OHC levels were significantly higher in apoE Ɛ4 carriers as compared to apoE Ɛ4 non-carriers (+34%; $p<0.05$), and inversely correlated with Mini-Mental State Examination test score ($r=-0.4$, $p=0.03$).
Conclusions: PCSK9 levels may be increased since the early phases of AD-related cognitive decline. The positive correlation between CSF/serum PCSK9 suggests a possible increase in blood-brain barrier permeability, specifically occurring in AD, and possibly linked to the apoE Ɛ4 genotype. CSF 24-OHC levels might be associated with AD-related alterations, and thus might be possibly considered for further investigations to unravel its potential as a specific disease biomarker.

Abstract n SO1_02 - Presenting author**: Daniele Tramontano**

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

Tramontano Daniele, Bini Simone, Di Costanzo Alessia, Minicocci Ilenia, Covino Stella, Arca Marcello, D'Erasmo Laura

Sapienza University of Rome, Department of Translational and Precision Medicine.

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.

Abstract n SO1_03 - Presenting author**: Rebecca Orsi**

Digital droplet PCR versus real-time PCR in genetic characterization of lipoprotein(a) kringle IV type 2 repeat polymorphism

R. Orsi[1], G. Barbieri[1], T. Capezzuoli[1], M. Giannini[1], G. Cassioli[1], F. Cesari[2], R. Marcucci[3], A.M. Gori[3], B. Giusti[3], E. Sticchi[3]

[1]University of Florence, Department of Experimental and Clinical Medicine, Florence;
[2]Atherothrombotic Diseases Center, Careggi Hospital, Florence;
[3]University of Florence, Department of Experimental and Clinical Medicine, Atherothrombotic Diseases Center, Careggi Hospital, Florence.

Aim: Evidence to support the role of lipoprotein(a) [Lp(a)] as a risk factor for atherosclerosis and thrombosis continue to increase. Lp(a) levels strongly differ among individuals, and this variability is mainly attributed to *LPA* gene, encoding apolipoprotein(a), with kringle IV type 2 (KIV2) copy number variation (CNV) representing the main genetic determinant. The peculiar structural characteristics of this variant constitute a significant challenge to the development of effective detection methods. Study aim was to compare quantitative real-time PCR (qPCR) and digital droplet PCR (ddPCR) in the evaluation of KIV2 repeat polymorphism.
Methods: One hundred subjects with suspected familial dyslipidemia, in which Lp(a) plasma levels were evaluated, were analysed. CNV values were obtained with qPCR and with ddPCR. To make CNV values comparable WWVwithin and between different plates, three internal controls (CNV: 41<C1<47, C2=50, 61<C3<68) were used.
Results: Correlation analysis between CNV values obtained with the two methods was slightly significant (R=0.413, p=0.00002), because of the wider data dispersion in qPCR compared with ddPCR. The greater stability of ddPCR approach emerged from internal controls C1, C2, C3 measurements throughout different experimental sessions, confirmed by a lower intra/inter-assay coefficient of variation with respect to qPCR. An inverse significant correlation between Lp(a) levels and CNV values was confirmed but higher when evaluated by ddPCR despite qPCR (R=-0.393, p=0.000053 vs R=-0.220, p=0.028, respectively). Dividing subjects in two groups according to 500 mg/L Lp(a) cut-off value, a significant lower number of KIV2 repeats emerged among subjects with greater Lp(a) levels, with stronger evidence in ddPCR despite qPCR (P=0.000013 and P=0.001, respectively).
Conclusion: Data obtained support a better performance of ddPCR in the evaluation of KIV2 repeat polymorphism. Further confirmation of data in larger cohort might be useful in the effort of identifying a more suitable method in order to improve genetic characterization of Lp(a) trait to better frame the cardiovascular risk profile of subjects.

Abstract n SO1_04 - Presenting author**: Federica Brucato**

Diagnosis of familial hypercholesterolemia in a large cohort of italian genotyped hypercholesterolemic patients

Federica Brucato[1], Chiara Scrimali[1], Davide Noto[1], Rossella Spina[1], Antonina Giammanco[1], Carlo M Barbagallo[1], Antonina Ganci[1], Gabriella Misiano[1], Marcello Ciaccio[2], Rosalia Caldarella[2], Angelo B Cefalù[1], Maurizio Averna[1].

[1]Department of Health Promotion, Mother and Child Care, Internal Medicine and Medical Specialties, University of Palermo, Italy.
[2]Department of Biomedicine, Neurosciences and Advanced Diagnostics, University of Palermo, Palermo, Italy.

Aim: Familial Hypercholesterolemia (FH) is the most relevant genetic cause of early cardiovascular disease (CVD). FH is suspected when LDL-C levels exceed the 95th percentile of the population distribution, but family and clinical history support the diagnosis. Different scoring systems have been developed, as the Dutch Lipid Clinic Network (DLCN) score, used worldwide to diagnose FH. The aim of the study is to describe the characteristics of FH patients of a large cohort of more than eight hundred genotyped subjects enrolled in an Italian Lipid Clinic and evaluated the DLCN score performance applied retrospectively to the case study.
Methods: 836 hypercholesterolemic patients with LDL-C > 4.88 mmol/L were genotyped for FH causative mutations in the LDLR, PCSK and APOB genes. Relatives of mutated patients were also analyzed by cascade screening.
Results: Mutation carriers were younger, presented higher LDL-C and DLCN score and lower HDL-C levels in comparison with hypercholesterolemic (HC) noncarriers and presented a five-fold higher prevalence of previous CV events. Carotid US data available in 490 subjects (FH n=195, HC n=295), showed that mutation carriers had an odds ratio of 3.66 (1.43 – 10.24) for atherosclerotic plaques in comparison with noncarriers. Scoring system were evaluated by ROC analysis in 203 subjects without missing DLCN items and with available pre-therapy LDL-C levels, and LDL-C levels (A.U.C.=0.737) resulted more performing than the DLCN score (A.U.C.=0.662), even including carotid US data (A.U.C.=0.641) in a modified DLCN score version.
Conclusions: The DLCN scoring systems failed to demonstrate a clear superiority in predicting FH mutations in comparison with the measure of LDL-C levels in a retrospective case study. The results enforce the need for more performant tools to detect FH.

Abstract n SO1_05 - Presenting author**: Matteo Pepa**

Plasma Levels of Proprotein Convertase Subtilisin/Kexin Type 9 Are Inversely Associated with N-Terminal Pro B-Type Natriuretic Peptide in Older Men and Women

Francesco Spannella [1,2]; Federico Giulietti [1,2]; Roberta Galeazzi [3]; Anna Passarelli [4]; Serena Re [1,2]; Matteo Pepa [1,2]; Chiara Di Pentima [1,2]; Massimiliano Allevi [1,2]; Paolo Magni [4,5]; Riccardo Sarzani [1,2]

1) Internal Medicine and Geriatrics, IRCCS INRCA, 60129 Ancona, Italy;
2) Department of Clinical and Molecular Sciences, Università Politecnica delle Marche, 60126 Ancona, Italy;
3) Clinical Laboratory and Molecular Diagnostic, IRCCS INRCA, 60129 Ancona, Italy;
4) Department of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, 20133 Milan, Italy;
5) IRCCS MultiMedica, Sesto S. Giovanni, 20099 Milan, Italy.

Aim: Cardiac natriuretic peptides (NPs) exert several metabolic effects, including some on lipid metabolism. Higher NPs levels are likely to be associated with a favorable lipid profile. In in vitro studies, NPs have been found to modulate low-density lipoprotein receptor (LDLR) trafficking by preventing proprotein convertase subtilisin/kexin type 9 (PCSK9) overexpression. The aim of our study is to investigate a possible association between plasma levels of PCSK9 and N-terminal pro B-type natriuretic peptide (NT-proBNP) in vivo.
Methods: We performed a cross-sectional study on 160 consecutive older male and female patients hospitalized for medical conditions. Patients taking lipid-lowering drugs and patients with an admission diagnosis of acute heart failure were excluded. Fasting blood samples were collected after clinical stabilization of the acute illness, the day before discharge.
Results: The mean age was 87.8±6.4 years with a female prevalence (62.5%). The median NT-proBNP was 2340 (814–5397) pg/mL. The mean plasma PCSK9 was 275.2±113.2 ng/mL. We found an inverse correlation between plasma PCSK9 and NT-proBNP (r= −0.280; p=0.001). This association was confirmed after taking into account NT-proBNP tertiles (plasma PCSK9 levels: 317.4±123.6 ng/mL in the first tertile, 283.3±101.8 ng/mL in the second tertile, 231.3±99.0 ng/mL in the third tertile, p=0.001) and even after an adjustment for confounding factors (beta= −0.361, p=0.001 for ln(NT-proBNP); beta = −0.330, p=0.001 for NT-proBNP tertiles). The strength of the correlation between plasma PCSK9 and NT-proBNP was likely greater in patients affected by type 2 diabetes mellitus (r= −0.483; p=0.006) and in male patients (r= −0.431, p=0.001).
Conclusions: The inverse association found between PCSK9 and NT-proBNP plasma levels in our real-life clinical study supports the hypothesis that NPs may play a role in cholesterol metabolism, possibly through an inhibitory action on circulating PCSK9 concentrations, thus increasing the availability of LDLR.

Abstract n SO1_06 - Presenting author**: Alessia Cipollone**

Effectiveness of siRNA on LDL-C and Lp(a) levels: emerging real-life data

A. Cipollone, R.M. Ricciardi, D. D'Ardes, I. Rossi, M.T. Guagnano, M. Bucci, F. Cipollone

Clinica Medica" Institute, Department of Medicine and Aging Science, "G. D'Annunzio" University of Chieti-Pescara.

Background: Reducing lipid plasma levels, as LDL-C, is the main strategy of CV events prevention. According to ESC/EAS 2019 guidelines, use of PCSK9i should be considered as a third level strategy after the highest tolerable statin dose + ezetimibe.
Methods: For this pilot study we enrolled 4 patients in basal treatment with statin+/- Ezetimibe, in which we added Inclisiran to reach the LDL target. Patient 1 was a 73yo man in secondary prevention. Patient 2 was a 68yo woman at high-cv risk due to diabetes and hypertension; patient 3 was a 51yo man with high levels of Lpa. Patient 4 was a 55yo man in primary prevention with BMI 29.5, affected by FH and severe arterial hypertension.
Results: Patient 1, on therapy with Rosuvastatin 20mg and N-PUFA N3 and intolerance to Ezetimibe started from LDL-C 100mg/dl (TC 214mg/dl; HDL 43mg/dl; TG 368mg/dl). After 3 months Alirocumab 150mg was added, soon switched to Inclisiran with a drastic improvement of LDL levels from 103 to 39mg/dl (-64.87%) (HDL 58mg/dl; TG 182mg/dl). Patient 2 had a basal LDL-C of 110.6mg/dl, that decreased after 2 months of Inclisiran to 46mg/dl. Patient 3 had LDL 88 mg/dl (from basal value of 110 mg/dl) and Lpa 125mg/d (from basal 373 mg/dl) after 8 months on therapy with Rosuvastatin/Ezetimibe 5/10mg and Ezetimibe 10mg every other day + Alirocumab 150mg; then he switched to Inclisiran reaching LDL=86mg/dl and Lpa=115mg/dl. Patient 4 on therapy with Ezetimibe/Simvastatin 10/40mg (basal LDL 123mg/dl), interrupted due to intolerance (CPK 871U/L), started Inclisiran with benefits (LDL 57mg/dl, at target).
Conclusions: Inclisiran was safe and effective in reaching LDL target (both in naive or previously treated with iPCSK9 patients) and in reducing Lp(a).

Abstract n SO2_01 - Presenting author**: Francesco Loria**

Mild cognitive impairment is associated with subclinical left ventricular dysfunction as assessed by global longitudinal strain in hypertensive patients

Francesco Loria[1], Germano Junior Ferruzzi [1], Valeria Visco[1], Gennaro Galasso[1], Guido Iaccarino[2], Carmine Vecchione[1] and Michele Ciccarelli[1]

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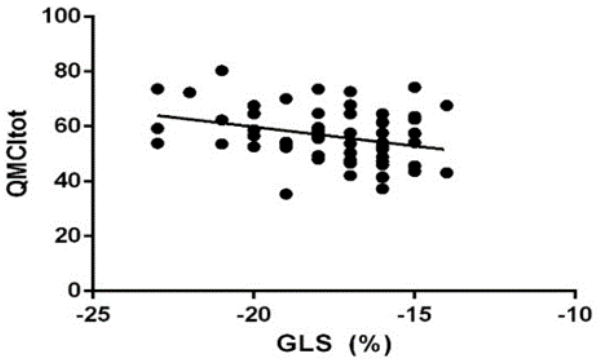
[2] Federico II University of Naples, Department of Advanced Biomedical Sciences, Naples, Italy.

Introduction: Global longitudinal strain (GLS) has emerged as a more precise myocardial function measure than left ventricular ejection fraction (LVEF). GLS detects subtle systolic abnormalities in a variety of conditions, such as hypertension, which represents a significant risk factor for cognitive impairment and cerebrovascular disease.
Aim: This study examines the relationship between subclinical cardiac dysfunction assessed by GLS and mild cognitive impairment (MCI) in hypertensive patients.
Methods: Complete clinical characteristics, laboratory analyses and echocardiographic data of 81 hypertensive patients were collected. Exclusion criteria were age < 50 or >80, severe carotid artery stenosis (> 50%), anticoagulation drugs, atrial fibrillation, cerebrovascular and/or neurodegenerative diseases. MCI was assessed by the Quick Mild Cognitive Impairment (QMCI) and final scores were corrected for age and education. This screening test evaluates spatial and temporal orientation, registration, delayed recall, clock design, logical memory and verbal fluency in a brief time (5 min - score 0-100).
Results: In our population of study mean LVEF was 50.47 ± 9.95% and mean GLS was -16.00 ± 3.66. MCI was detected in 21 patients (26%). When comparing the patients with MCI (QMCItot < 49.4) and without MCI (QMCItot > 49.4), a statistically significant difference of GLS values was detected (no MCI: -16.52 ± 3.66 vs MCI -14.18 ± 3.23; p 0.032); on the other hand, the two groups did not differ in LVEF (no MCI: 50.58 ± 9.70 vs MCI 48.86 ± 11.93; p 0.864). When patients with LVEF ≥ 45% were excluded from the analysis, a statistically significant linear regression was observed between the QMCI score (corrected for age and education) and the GLS (p 0.014), as shown in Figure 1.
Conclusion: Compromised GLS, but not LVEF, is associated to MCI in hypertensive patients. Furthermore, our study demonstrates for the first time the existence of a significant correlation between the QMCI and GLS.



Abstract n SO2_02 - Presenting author**: Andrea Busti**

Effects of PCSK-9 inhbitors on arterial structure and function: preliminary results

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Background: one of the latest chapters in the research for new and stronger lipid lowering therapies has led to the development of PCSK9 inhibitors. In addition to the benefit on lipid profile, a limited number of studies have been published showing a possible improvement in vascular function and structure following their administration.
Purpose: To evaluate the effects of PCSK9 inhibitors on structural (via carotid Intima Media Thickness – IMT) and functional (carotid-femoral Pulse Wave Velocity – cf-PWV - and brachial artery Flow Mediated Dilatation - FMD) arterial properties.
Methods: Twenty-two dyslipidemic patients with a clinical indication to PSCK9 inhibitors were prospectively recruited at the Cardiology Rehabilitation of ASST Grande Ospedale Metropolitano Niguarda Hospital (Milan, Italy). Anthropometric, clinical and therapeutic data were recorded. Each patient underwent instrumental examinations to assess cf-PWV, IMT and FMD at T0 (before the start of therapy), T1 (after 6 months) and T2 (after 12 months).
Results: in the 22 patients who reached 6 months of follow up there was a significant reduction in LDL cholesterol (from 127.8±31.7 to 47.4±29.1 mg/dl, p<0. 0001) while there was no significant difference in arterial parameters (cf-PWV: from 10.5±3.5 to 9.4±2.3 m/s, p=0.092; FMD: from 11.4±8.1 to 9.8±8.3%, p=0.407; IMT: from 775.6±192.8 to 768.7±144.7, microm, p=0.854). Similar results were also obtained in the 12 patients who reached one year of follow-up: LDL from 129.4±29.1 to 48.8±21.9 mg/dl, p<0.0001; cf-PWV from 10.4±3.3 to 10.5±2.9, p=0.874; FMD from 12.0±9.1 to 11.0±5.6%, p=0.930; IMT from 745.1±203.9 to 770.0±127.3 microm, p=0.710.
Conclusion: Our study confirm the strong LDL reduction with PCSK9 inhibitors that are needed in order to reach the targets set by the ESC 2019 guidelines. In contrast, they do not show a significant effect on arterial function and structure in terms of cf-PWV, FMD and carotid IMT.

Abstract n SO2_03 - Presenting author**: Elena Melchionda**

Effects of high glucose on PCSK9 expression in aortic vascular smooth muscle cells from insulin-sensitive and insulin-resistant Zucker rat: role of PCSK9 inhibitors

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Background and Aims: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is deeply involved in cholesterol homeostasis and associated to atherogenic processes including vascular smooth muscle cells (VSMCs) dysfunction. Aim of this study was to evaluate in VSMCs from insulin-sensitive (IS) and insulin-resistant (IR) animal models: i) the basal PCSK9 expression; ii) the expression of PCSK9 under normal glucose (NG) or HG conditions, iii) the role of PCSK9 in the HG-induced activation of MAPK/Erk-1/2 pathways, and iv)the influence of PCSK9 inhibitors on the above HG effects.
Methods: In cultured rat aortic VSMC from lean IS and obese IR Zucker rats incubated for 24 hours with 5-25mmol/L D-Glucose we measured PCSK9 expression and pErk-1/2 levels (by Western Blot), with or without the PCSK9 mAbs alirocumab or evolocumab (40 μg/mL) or the synthetic PCSK9-binding peptide PEP2-8(10 μm/L).
Results: In IS-VSMCs: HG increased PCSK9 expression ($n=6$, $p<0.0001$) with effects reduced by alirocumab ($n=6$, $p<0.001$), evolocumab ($n=6$, $p<0.005$), and PEP2-8 ($n=6$, $p<0.01$); if compared with HG alone, the coincubation with alirocumab or evolocumab increased HG effects on levels of pErk-1/2 ($n=8$, $p<0.001$ and $p<0.0001$, respectively); similar results were observed with HG+ PEP2-8 ($n=8$, $p<0.001$). IR-VSMC, if compared with IS-ones, showed a basal PCSK9 overexpression ($n=12$, $p<0.0001$) not furtherly influenced by HG ($n=8$, ns) but decreased by alirocumab or evolocumab ($p<0.001$ and $p<0.02$, respectively); basal pErk levels appeared significantly downregulated and not influenced by HG; however, alirocumab and evolocumab increased pERK-1/2 levels both in the absence ($p<0.005$ and $p<0.004$, respectively) and in the presence of HG ($p<0.001$ and $p<0.0001$, respectively).
Conclusions: Collectively, these findings indicate that in IS state the exposure to HG may impair VSMC response also through PCSK9-dependent mechanisms that can be improved by both external or internal PCSK9 inhibition. In IR state, VSMCs show a basal overexpression of PCSK9, not furtherly influenced by HG and, at least partially, corrected by PCSK9-inhibitors.

Abstract n SO2_04 - Presenting author**: Leonardo Bencivenga**

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

Leonardo Bencivenga, Mathilde Strumia, Yves Rolland, Sandrine Andrieu, Bruno Vellas, Philipe De Souto Barreto, Laure Rouch for the MAPT/D. S. A. group

Università degli Studi di Napoli Federico II, Dipartimento di Scienze Mediche Traslazionali (LB); Gerontopole de Toulouse, Institut du Vieillissement, CHU de Toulouse, France

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.

Abstract n SO2_05 - Presenting author**: Angelo Forte**

Circulating leptin concentration is associated with an increase in differential pressure values and development of arterial stiffness in adult male – the Olivetti Heart Study

Angelo Forte, Lanfranco D'Elia, Alfonso Giaquinto, Aquilino F. Zarrella, Anita Vergatti, Veronica Abate, Mariachiara Cardamone, Martina Bottiglieri, Roberto Iacone, Ornella Russo, Domenico Rendina, Pasquale Strazzullo, Ferruccio Galletti

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Introduction: Elevated circulating leptin concentrations are associated with an unfavorable cardiometabolic profile. A large number of studies have highlighted a direct correlation between leptin and vascular damage, but few observational studies have highlighted the potential predictive role of leptin on the development of arterial stiffness (RA).
Purpose: The purpose of this study was to determine the role of leptin on the incidence of RA and pulse pressure changes in a sample of adult males over an 8-year period (The Olivetti Heart Study).
Methods: The analysis included 460 men without RA (pulse pressure > 60 mmHg) at study entry and who were not taking antihypertensive therapy at entry and throughout the study (age: 50.0 years, BMI: 26.5 kg/m2).
Results: At the end of the follow-up period, the incidence of RA was 8%. Initial leptin levels were significantly higher in the RA group and strongly correlated with the changes in blood pressure observed over time ($p<0.05$). According to the mean serum leptin distribution in the whole population, the sample was stratified into two groups, one with leptin values below the median and the other with values above the median. The sample that had baseline leptin values above the median had a greater risk of developing arterial stiffness (Odds Ratio: 2.5, $p<0.05$) and a greater increase in differential pressure throughout the observation period (beta: 2.1, $p <0.05$), even after adjustment for main confounders.
Conclusions: The results of this prospective study indicate a predictive role of circulating leptin levels on the development of vascular damage, independent of body weight and blood pressure.

Abstract n SO2_06 - Presenting author**: Chiara Poliseno**

Effects of lifestyle advice associated with beetroot-based nutraceutical on 24-hour blood pressure in a population with high-normal blood pressure or grade 1 hypertension

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Aim: 2018 ESH hypertension guideline supports life-style changes as initial approach to be taken in both subjects with high-normal blood pressure (BP) and patients with grade 1 hypertension and low cardiovascular (CV) risk. Among many nutraceuticals, most with unproven or unlikely benefits, bioactive substances enriched with nitrates, sources of nitric oxide (i.e. red beets) exert proven vasodilator effects and may decrease BP levels. Aim: to evaluate the effect of lifestyle advice and beetroot-based nutraceutical on 24-h BP in a population with high-normal BP or grade 1 hypertension and low CV risk.
Methods: Longitudinal study on 43 consecutive subjects referred to two hypertension centers. A 24-h ambulatory BP monitoring (ABPM) was performed at baseline and after 3 month the introduction of lifestyle advice and beetroot-based nutraceutical [Cosmony, Servier, a nutraceutical based on Beta vulgaris L. (500 mg of dry beetroot extract)].
Results: Mean age: 49.9±10.7 years. Male prevalence: 53.5%. Median follow-up: 98 (92-121) days. Overweight/obese prevalence: 58.1%. The 62.8% of subjects had a grade 1 hypertension. At baseline, 24-h BP, daytime BP and night-time BP were 127.2±6.8/79.6±5.6 mmHg, 131.3±7.5/83.4±5.5 mmHg and 117.8±7.7/70.2±5.0 mmHg, respectively. At follow-up, all BPs showed a statistically significant decrease (all $p<0.005$), except for diastolic night-time BP ($p=0.074$) (-3.4±5.9/-2.2±3.7 mmHg for 24h BP; -3.9±6.0/-3.0±4.0 for daytime BP and -3.3±7.4/-1.3±4.7 for night-time BP). The percentage of subject with daytime BP <130/85 mmHg had increased from 25.6% at baseline to 48.8% at follow-up. The decrease in BP was independent of gender and overweight/obesity, whereas it was more evident in the group of grade 1 hypertensives compared with the group with high-normal BP.
Conclusions: Lifestyle advice in association with a nutraceutical, source of nitric oxide, may be a valid initial non-pharmacological therapy in subjects with high-normal BP or grade 1 hypertension and low CV risk.

Abstract n SO3_01 - Presenting author**: Lorenzo Airale**

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±11.8mg/m2 vs. 71.4±16.8mg/m2, p=0.001), lower LVEDV (84.6±14.6ml vs. 108±20.7ml, 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±15.4mmHg vs. 115±10.8mmHg, p=0.008) and diastolic (75.2±8.99mmHg vs. 69.8±7.16mmHg, 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.

Abstract n SO3_02 - Presenting author**: Emiliano Fiori**

Atrial natriuretic peptide stimulates autophagy/mitophagy and improves mitochondrial function in chronic heart failure

Emiliano Fiori[1], Giovanna Gallo[1], Salvatore Raffa[1], Maurizio Forte[2], Danilo Ranieri[1], Simona Marchitti[2], Damiano Magrì[1], Marco Testa[3], Rosita Stanzione[1], Franca Bianchi[1], Maria Cotugno[1], Vincenzo Visco[1], Sebastiano Sciarretta[4], Ludovica De Fazio[1], Chiara Pidone[1], Massimo Volpe[1], Speranza Rubattu [1,2]

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Background. Mitochondrial dysfunction, causing increased reactive oxygen species production, is a molecular feature of heart failure (HF). A defective antioxidant response and mitophagic flux, underlying mitochondrial dysfunction, have been reported in circulating leucocytes of patients with HF with reduced ejection fraction (HFrEF). Atrial natriuretic peptide (ANP) exerts many cardiac beneficial effects, including the ability to protect cardiomyocytes by promoting autophagy.
Objective. To test the impact of ANP on autophagy/mitophagy responses, altered mitochondrial structure and function and increased oxidative stress in chronic HFrEF patients.
Experimental design. The present study used both ex-vivo and in-vivo approaches.
We examined sixteen consecutive chronic HFrEF patients referring to the outpatient clinic of the Cardiology unit of Sant'Andrea Hospital in Rome from April 2019 to April 2022. Out of them, for the ex-vivo study we enrolled 10 patients matching the following inclusion criteria: age under 75 years and left ventricle ejection fraction below 40%. For the in-vivo study we recruited six patients and evaluated them before and after 2-month treatment with the Angiotensin Receptor Neprilysin inhibitor (ARNi) sacubitril/valsartan started at the dosage of 49/51 mg twice daily, rapidly uptitrated at the dosage of 97/103 mg twice daily, while maintaining unchanged the remaining therapy. Patients with recent hospitalizations for acute HF or other acute conditions within the last 3 months before the enrollment, with malignancy, inflammatory or infectious diseases, diabetes mellitus, history of cigarette smoking and alcohol abuse were excluded.
Results. The ex-vivo direct exposure to αANP caused an improvement of mitochondrial membrane potential with a restoration of the mitochondrial protonmotive force ($p<0.05$). The αANP treatment promoted the recovery of the IMM extension and determined a significant improvement in the IMM/OMM index ($p<0.05$). The production of ROS was significantly decreased after the exposure to αANP ($p<0.05$). At the same time, analyzing the mitochondrial stress, we observed that the treatment with αANP was able to induce a down-modulation of p66shc mRNA ($p<0.01$). The αANP treatment induced a rapid and significant autophagic response in the same samples described above. The cytofluorimetric analysis showed a significant increase of LysoTracker levels highlighting a rise of intracellular acidic compartments ($p<0.05$), with the up modulation of the LC3 and Beclin mRNA levels ($p<0.01$ and $p<0.05$ respectively). In addition, the significant reduction in mitochondrial mass observed in treated PBMCs evoked an increase of the mitophagic process related to αANP ($p<0.01$).
In vivo, the efficacy of the treatment with sacubitril/valsartan was proved by the increase in αANP levels ($p<0.01$) whereas levels of NT-proBNP decreased ($p<0.05$). PBMCs collected after treatment were characterized by lower mitochondrial oxidative stress levels and were provided of structurally more intact mitochondria characterized by better functional performances ($p<0.05$). PBMCs of the treated patients showed an increased level of acid compartments associated with an up-modulation of LC3 and Beclin mRNA levels and a reduction of mitochondrial mass ($p<0.05$). Also in this experimental context, the activation of autophagy was confirmed by ultrastructural morphometric evaluation ($p<0.05$).
Conclusions. αANP stimulates both autophagy and mitophagy responses, counteracts mitochondrial dysfunction and damage ultimately reducing mitochondrial oxidative stress generation in PBMCs from chronic HF patients. These properties were confirmed upon sacubitril/valsartan administration, a therapeutic approach indicated for HFrEF treatment.

Abstract n SO3_03 - Presenting author**: Giuseppe Palma**

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.

Abstract n SO3_04 - Presenting author**: Rosanna Di Fonzo**

The effect of dapagliflozin in a real-world population of HFrEF patients: symptoms, quality of life and echocardiographic parameters

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Aim: DAPA-HF trial demonstrated dapagliflozin's efficacy for reducing CV death/HF hospitalization in patients with HF with reduced ejection fraction (HFrEF) regardless of type 2 diabetes status. Nevertheless, there are still little real-world data; consequently, we aimed to evaluate the effect of dapagliflozin three months after its introduction in treatment in our real-world. population.
Methods: From February to September 2022, we introduced Dapagliflozin in 23 HFrEF patients' therapy, and we collected data from 11 patients (66.78±3.96 years; 89% men) at 3-months-FU. Specifically, on the first visit, we collected the clinical, laboratory, and echocardiographic parameters, and dapagliflozin was added to the optimal medical therapy of patients; then, the patients were evaluated after three months (follow-up).
Results: At follow-up, all patients were free from side effects, and we did not record statistically significant differences in laboratory parameters and/or blood pressure values. As regards the echocardiographic parameters, there was an improvement in FE (28.11±2.95vs37.00±5.71%, p 0.17), PAPS (46.89±3.94vs37.63±5.27mmHg, p0.17), and LVEDVind (75.34±10.58vs57.20±13.55ml/m2,p0.30), although not statistically significant. Moreover, we observed a statistically significant reduction in the diameter of the inferior vena cava (18.89±1.78vs11.5±1.15mm, p<0.01) in NYHA class (2.78±0.15vs 2±0, p<0.001), in basal SO2 (95.67±0.78 vs. 97.67±0.47%, p0.04) and an improvement of quality of life (EQ5Dtot73.33±4.71vs86.67±4.41,p0.04;pain/discomfort3.67±0.29vs4.78±0.22,p<0.01;anxiety/depression3.67±0.29vs4.67±0.24,p0.0163). Finally, we recorded a not statistically significant reduction in the amount of mineralocorticoid receptor antagonists (MRAs) at follow-up (62.5±12.5vs37.5±5.59mg eplerenone, p 0.09).
Conclusions: Dapagliflozin improved symptoms and quality of life in patients with HFrEF of our real-world population after 12 weeks, according to previous data of the DEFINE-HF trial. Moreover, already after three months was possible to record improvements in the echocardiographic parameters, even if they were not statistically significant. Indeed, it will be necessary to continue with the study to evaluate these results on a larger sample.

Abstract n SO3_05 - Presenting author**: Ludovica De Fazio**

Association of NDUFC2 polymorphic variants with left ventricular hypertrophy in human hypertension

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Aim: Previous experimental studies showed that a dysfunction of the NADH dehydrogenase (ubiquinone), the mitochondrial Complex I (CI), is associated with the development of left ventricular hypertrophy (LVH). A deficiency of Ndufc2 (a subunit of CI) impairs CI activity and causes severe mitochondrial dysfunction. The NDUFC2/rs11237379 polymorphic variant is associated with reduced gene expression and impaired mitochondrial function, contributing to increased susceptibility to vascular diseases.
We examined the association of NDUFC2/rs11237379 and another NDUFC2 polymorphic variant (rs641836) with the development of LVH in hypertensive patients. Two-hundred-fourty-six hypertensive subjects (147 male, 59.7%) with a mean age of 59±15 years were studied. Seventy-nine individuals (32%) presented LVH. The association analysis for both SNPs showed that hypertensive patients carrying the TT genotype at the NDUFC2/rs11237379 had a significant increase of echocardiographically assessed septal thickness (p=0.001), posterior wall thickness (p=0.003), relative wall thickness (RWT) (p=0.01), LV mass/ body surface area (BSA) (p=0.012) and LV mass/height2.(p=0.0033) compared to subjects carrying either CC or CT genotypes. To better dissect the genetic effect, a covariate ANOVA was performed for each cardiac variable, considering age, gender, body mass index (BMI), office blood pressure (BP), antihypertensive treatment with a combination of 2 or more drugs and the number of BP-lowering agents as covariates. The adjustment for covariates revealed significant differences for septal thickness (p=0.07), posterior wall thickness (p=0.008), RWT (p=0.021), LV mass/BSA (p=0.03). With regard to NDUFC2/rs641836, hypertensive subjects carrying the mutant A allele had a significant increase of septal thickness (p=0.001), posterior wall thickness (p=0.001), RWT (p=0.005), LV mass (p=0.001), LV mass/BSA(p=0.001), LV mass/height2.7(p=0.002) compared to wild-type homozygotes. After adjustment for covariates, the results were significant for septal thickness (p=0.017), posterior wall thickness (p=0.011), LV mass (p=0.003), LV mass/BSA (p=0.002) and LV mass/height2.7(p=0.010).
Our results demonstrate a significant association of NDUFC2 variants with LVH in hypertensives and highlight a novel role of CI dependent mitochondrial dysfunction on increased susceptibility to cardiac damage in human hypertension. This study paves the way of a new pathophysiological mechanism of LVH which may lead to new clinical strategies.

Abstract n SO3_06 - Presenting author**: Camilla Mazzanti**

Association between liver fibrosis and decreased myocardial mechano-energetic efficiency in individuals with different degree of glucose tolerance

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Introduction: Fibrosis is the most relevant prognostic factor in non-alcoholic fatty liver disease (NAFLD), and is associated with liver-related adverse outcomes and cardiovascular events. A decreased myocardial mechano-energetic efficiency (MEEi) was associated with NAFLD and poorer prognosis in liver cirrhosis. However, whether individuals at risk for advanced liver fibrosis have a compromised myocardial MEEi is still unsettled.
Aim: we determined whether liver fibrosis severity estimated by the fibrosis-4 index (FIB-4), is associated with MEE in a cohort of adults participating in the CATAnzaro MEtabolic RIsk factors (CATAMERI) study.
Methods: Myocardial MEEi was assessed by a validated echocardiography-derived measure in 2383
individuals with different degree of glucose tolerance. Participants were divided into four groups according to FIB-4 index values: lowest risk of fibrosis (<1.3); low risk of fibrosis (≥ 1.3 to <1.67); moderate risk of fibrosis (≥1.67 to < 2.67); high risk of fibrosis (≥2.67).
Results: Compared to subjects with the lowest risk of liver fibrosis, those with higher risk of liver fibrosis displayed a graded increase in myocardial MEEi. In a multivariable regression analysis, FIB-4 index was independently associated with MEEi (β =-0.080, P<0.001), along with total cholesterol (β =-0.067, P=0.01), hsCRP (β= -0.081, P<0.001), sex (β=-0.099, P<0.001), glucose tolerance status (β=-0.109, P<0.001) and HOMA-IR index on insulin-resistance (β =-0.143, P<0.001).
Conclusion: our finding that compromised myocardial MEE is already measured in individuals with high risk of hepatic fibrosis suggests that assessment of myocardial MEEi may help identify subjects with NAFLD having an unfavourable prognosis which requires a closer follow-up.

Abstract n SO4_01 - Presenting author**: Marta Garbin**

Glomerular Hyperfiltration: a new marker of fibrosis severity in non-cirrhotic NAFLD

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Background: Non-alcoholic fatty liver disease (NAFLD), mainly in its advanced form with hepatic fibrosis, represents a risk factor for the development of cardiovascular (CV) disease and chronic kidney failure, the latter early expressed by glomerular hyperfiltration (GH). GH shares the same CV and metabolic risk factors with NAFLD, and their association has been already evaluated in adults with metabolic syndrome. However, association between GH and NAFLD fibrosis has not been investigated yet.
Methods: 772 patients (mean age 47.3 ± 8.9 (range 40-65ys), 67.1% male) with non-cirrhotic NAFLD diagnosed by abdominal ultrasound were enrolled at three hepatology centers (Verona, Milan and London). Hepatic fibrosis was diagnosed by Fibroscan (echoSense) by a liver stiffness measurement (LSM) > 7.2 kPa. Glomerular filtration rate (GFR) was estimated using 2021CKD-EPI formula and GH was defined by GFR□110 mL/min, whereas normal filtration (nGFR) by GFR 60-110 mL/min. Anthropometric and biochemical data, as well as medical history and current therapy were recorded at enrollment.
Results: In the whole cohort, 152 (20%) belonged to the GH group. Compared to the nGFR, the GH group presented younger age (38.4 ± 8.3 vs 49.5 ± 7.7 years, p<0.001), higher prevalence of severe steatosis (39.9% vs 27.1%, p=0.03) and higher LSM values (8.04 ± 6.15 kPa vs to 7.47 ± 6.12 kPa, p=0.023). In multivariate analysis adjusted for age, sex, type 2 diabetes, hypertension and obesity, age (OR 0.83, CI 95% 0.77-0.89) and LSM (OR 6.6, CI 95% 2.2-19.9) were independent risk factors for GH. LSM remained independently associated with GH even when considered diagnostic for fibrosis (LSM >7.2 kPa) (OR 1.83, CI 95%, 1.10-3.03). These associations were maintained even adjusting for diuretic and ACE/ARBs therapy.
Conclusions: GH is associated with hepatic fibrosis by LSM independently of other metabolic alterations. Therefore, GH could be considered an early marker of liver fibrosis in non-cirrhotic NAFLD patients and calculation of a simple index as GFR could suggest the need for a Fibroscan assessment in NAFLD patient even in primary care.

Abstract n SO4_02 - Presenting author**: Monika Svecla**

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.

Abstract n SO4_03 - Presenting author**: Jaqueline Currà**

Improvement in hepatic fibrosis assested by Fibroscan in NAFLD patients with type 2 diabetes treated with SGLT2 inhibitors: a five years follow-up

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Introduction: Subjects with non-alcoholic fatty liver disease (NAFLD) and type 2 diabetes mellitus (T2DM) present high progression of liver disease to fibrosis, which is the main determinant of long-term adverse outcomes. Data are accumulating on the benefits of sodium glucose cotransporter 2 inhibitors (SGLT-2i) on hepatic fibrosis mainly in pharmacologic or retrospective studies.
Aim: to prospectively evaluate change in metabolic alterations and hepatic disease in patients with NAFLD and T2DM and predisposing factors.
Materials and Methods and Results: 237 patients with NAFLD (mean age 67 ±9 years, 54% male) were enrolled at the diabetology outpatient clinics and re-evaluated after 5 years. Information about diabetic control, metabolic comorbidities and medications were collected at baseline and follow-up. Additionally, NAFLD was assessed by liver ultrasonography, whereas LSM was detected by Fibroscan® at baseline and after 5 years. During follow-up no change in the prevalence of metabolic alterations except for hypertension (81% vs 73%, p<0.001) was observed, whereas an increase in LSM values (6.0 ± 2.8 vs 5.8± 2.7 kPa, p=0.02) was registered, despite stability of diabetic control. In particular, LSM worsened in 133(56%) subjects, with 92 (39%) having a worsening of >10% from baseline and 20 (8%) of at least 1 fibrosis stage at Fibroscan from baseline. Moreover, a higher prescription of SGLT2i was seen (21% vs 6%, p<0.001). Compared with those with no worsening of LSM, patients with worsening of LSM had a higher prevalence of increase in BMI during follow-up (45% vs 32%, p=0.06). In multivariate analysis, after adjustment for age, sex, liver enzymes and HbA1c, the use of SGLT2-inhibitors at follow-up (adjusted-hazard ratio 0.34, 95% CI 0.13-0.91) was associated with a reduced risk of worsening of LSM by Fibroscan, even if considered>10% from baseline. However, this association was markedly attenuated after further adjusting for change in BMI over time.
Conclusions: Despite a high prevalence of fibrosis progression in NAFLD subjects with T2DM, we showed a potential effect of SGLT2-inhibitors in reducing the risk of worsening of liver stiffness, possibly also mediated by weight loss. Therefore, our data suggest that using this category of antidiabetic drug in NAFLD patients may prevent the progression of fibrosis, especially if weight control is obtained in these patients.

Abstract n SO4_04 - Presenting author**: Michela D'Avino**

The spectrum of fatty liver disease and the role of genetic, metabolic and lifestyle factors associated with its severity in heterozygous apolipoprotein B-related familial hypobetalipoproteinemia

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Introduction and Aims. Heterozygous apolipoprotein B (APOB)-related familial hypobetalipoproteinemia (He-APOB-FHBL) is a genetic disorder characterized by LDL-c and apoB low levels. Patients with He-APOB-FHBL are prone to develop fatty liver disease (FLD) that has been anecdotally associated with risk of progression toward cirrhosis. Our study aims to characterize the FLD spectrum and determine the association of genetic, metabolic and lifestyle factors with the severity of FLD in a single cohort of He-APOB-FHBL patients.
Methods. 21 adults with He-APOB-FHBL were consecutively enrolled in the Lipid Clinic in Modena. FLD and liver stiffness were assessed by liver ultrasound and elastography techniques (Fibroscan® and 2D-SWE). 6 patients underwent liver biopsy. Lifestyle habits were evaluated by self-administration of three-day food diary, Sofi's Mediterranean diet adherence score and international physical activity questionnaires.
Results. 4 patients had APOB missense or intronic splice-site variants and 17 patients were carriers of APOB truncating mutations, of whom 12 had a protein shorter than ApoB-48. The main factors significantly associated with the presence of severe steatosis were indices of adiposity (BMI, p=0.036; waist circumference, p=0.006), fasting glucose, p=0.008; HbA1c, p=0.045, HOMA-IR, p=0.018 and markers of liver injury (GOT, p=0.036; GPT, p=0.011). Of note, the presence of severe steatosis was significantly associated with significant fibrosis (p=0.031). 28.6% patients had significant fibrosis according to liver stiffness and/or histology. Significant fibrosis was not associated with APOB length, but was significantly associated with metabolic comorbidities (abdominal obesity, p=0.019; insulin resistance, p=0.004; T2DM, p=0.015), platelet count (p=0.003) and markers of liver injury (GPT, p=0.029; GGT, p=0.045). With regards to lifestyle, few patients were adherent to Mediterranean diet (16.7%) and most of them reported unhealthy dietary habits with excessive fat intake (80%). Moreover, 23.8% of patients were inactive.
Conclusions. Efforts to promote healthy lifestyles and prevent obesity and diabetes should be made in order to avoid liver disease progression in He-APOB-FHBL patients.

Abstract n SO4_05 - Presenting author**: Giosiana Bosco**

Analysis of steatosis biomarkers and inflammatory profile after adding on PCSK9 inhibitor treatment in familial hypercholesterolemia subjects with nonalcoholic fatty liver disease: A single lipid center real-world experience

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Aims: Nonalcoholic fatty liver disease (NAFLD) may be crucial in subjects with familial hypercholesterolemia (FH). We aimed to evaluate the effect of the inhibitors of proprotein convertase subtilisin/kexin type 9 (PCSK9-i) on steatosis biomarkers such as triglyceride-glucose index (TyG) and hepatic steatosis index (HSI) and analyse the role of TG/ HDL in this population before and after adding-on PCSK9-i.
Methods: In this observational study, we evaluated 26 genetically confirmed FH patients with NAFLD and an LDL-C off-target despite high-intensity statins plus ezetimibe. All patients added PCSK9-i treatment and obtained biochemical analysis and TyG and HSI evaluation at baseline and after six months of PCSK9-i.
Results: No difference of steatosis biomarkers was found after adding-on PCSK9-i therapy. In a secondary analysis, we divided the study population in two groups according to TG/HDL median value: high TG/HDL group (H-TG/HDL) and low TG/HDL group (L-TG/HDL). TyG and HSI were significantly lower in the L-TG/HDL than H-TG/HDL group (for TyG 9.05 ± 0.34 vs 9.51 ± 0.32; for HSI 38.43 ± 1.35 vs 41.35 ± 1.83, p value for both < 0.05). After six months of PCSK9-i therapy, TyG and HSI were significantly reduced in the L-TG/HDL group after PCSK9-i therapy (7.5% and 8.4% respectively, p value for both < 0.05) and these biomarkers were lower compared to H-TG/HDL group (for TyG 8.37 ± 0.14 vs 9.19 ± 0.12; for HSI 35.19 ± 1.32 vs 39.48 ± 1.33, p value for both < 0.05).
Conclusion: In conclusion, PCSK9-i therapy significantly ameliorate steatosis biomarkers in FH patients with low TG/HDL; our results appear to be consistent with a beneficial role of PCSK9-i on steatosis biomarkers in FH subjects with NAFLD.

Abstract n SO4_06 - Presenting author**: Samantha Maurotti**

A novel endogenous hormone regulates lipolysis in adipose tissue and lipid accumulation in the Liver and skeletal Muscle

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Aim: Untreated type 1 diabetes (T1DM) is a wasting disease characterized by complications on muscles (myosteatosis), adipose (fat catabolism) and liver tissues (steatosis), as well as altering lipid profile which contributes to cardiovascular diseases onset. Despite the growing body of literature highlighting various aspects of insulin signaling impairment leading to health consequences, the fact that insulin therapy did not prevent the common complications of diabetes is yet not fully understood. Several studies had shown that C-peptide replacement therapy exerting positive actions on nerves, vasculature, muscle and kidney, but nobody investigated its role in lipid metabolism and fat accumulation in liver and skeletal muscles.
Our aim was to evaluate if C-peptide replacement therapy improve hyperglycemia, hypertriglyceridemia and fatty acid metabolism in muscle and liver diabetic rats.
Methods: Twenty-three male Wistar rats were randomized in normal control (CTR), diabetic (D-CTR) and C-peptide treated diabetic group (C-PEP). Diabetes was induced by streptozotocin injection and C-peptide was administered subcutaneously for 6 weeks.
Hematological parameters, gene involved in lipid metabolism and markers of autophagy were assessed on muscle, as well as in white adipose tissue mass and liver intracellular lipid content.
Results: We found a lower Srebp-1c, Ppar-α and Cpt-1 mRNA expression in C-PEP rats compared to the D-CTR group ($p=0.002$; $p=0.01$ and $p<0.001$, respectively) in gastrocnemius muscle. Interestingly, Cpt-1 activation, probably caused by excessive triglycerides, was reverted by C-peptide. Accordingly, C-peptide administration reversed hyperglycemia and hypertriglyceridemia compared to diabetic group ($p=0.007$; $p=0.01$, respectively). In this regard, p-Ampk expression was higher in C-PEP group than the D-CTR($p=0.002$), while p62 and Traf-6 (autophagy markers) had opposite trend ($p=0.002$ and $p=0.01$; respectively). Finally, C-PEP group showed a decrease in liver steatosis compared to the D-CTR group, which was confirmed by further in vitro study on HepG2 cell ($p<0.05$).
Conclusion: Our results, for first time, suggest C-peptide involvement on lipid metabolism, autophagy and lipid accumulation in liver and muscles, thus preventing T1DM complications.

Abstract n SO5_01 - Presenting author**: Giuseppina Biondi**

GLP-1 receptor agonists counteract the deleterious effects of pasireotide on pancreatic beta-cell survival and function

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Aim: Pasireotide is a new generation somatostatin analogue used for the treatment of acromegaly, Cushing disease, and neuroendocrine tumors. Due to its high affinity for somatostatin receptors 5 and 2, which are highly expressed in pancreatic beta-cells and enteroendocrine cells, pasireotide may reduce the secretion of insulin, GLP-1, and GIP, causing hyperglycemia in some patients. Of note, the diabetogenic effects of pasireotide are reduced *in vivo* by co-administration of liraglutide or vildagliptin, two incretin-based drugs. The objective of this work was to evaluate the direct effects of pasireotide on beta-cell function and survival, the ability of different incretin-based drugs to prevent pasireotide action, and the molecular mechanisms underlying these effects.
Methods: INS-1E cells and human pancreatic islets were stimulated with pasireotide or DMSO as a control; in another set of experiments, INS-1E cells were pretreated with a GLP-1R agonist (GLP-1RA, i.e., exendin-4, lixisenatide, or liraglutide) prior to stimulation with pasireotide. Glucose-induced insulin secretion (GSIS), insulin content, and apoptosis levels were assessed through specific ELISA assays, while signaling proteins and calcium levels were evaluated by immunoblotting and fluorimetric assay, respectively.
Results: Pasireotide induced apoptosis and reduced GSIS, without altering insulin content in both INS-1E cells and human islets. All the GLP-1RAs tested were able to prevent the pasireotide-induced apoptosis in INS-1E cells, although the molecular mechanism remains unidentified. Furthermore, exendin-4 and liraglutide prevented pasireotide-induced secretory dysfunction in INS-1E cells, by interfering with PKA and cytoplasmic calcium pathways.
Conclusions: Pasireotide reduces beta-cell survival and GSIS, possibly contributing to the onset of hyperglycemia in some patients. GLP-1RAs can prevent these effects, by interfering with PKA and calcium pathways. These results obtained *in vitro* and *ex vivo* support the co-administration of incretin-based drugs and pasireotide, whose therapeutic efficacy has already been demonstrated *in vivo*.

Abstract n SO5_02 - Presenting author**: Miriam Longo**

Circulating levels of endothelial progenitor cells are associated with better cognitive function in older adults with glucagon-like peptide 1 receptor agonist-treated type 2 diabetes

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Aim: To evaluate cognitive function in subjects with type 2 diabetes (T2D) treated with glucagon-like peptide 1 receptor agonist (GLP-1RA) plus metformin or metformin alone and its association with endothelial progenitor cells (EPCs).
Methods: Adults with T2D treated with GLP-1RA plus metformin (GLP-1RA + MET) or MET alone for at least 12 months were included. Montreal Cognitive Assessment test (MoCA), Mini-Mental State Examination (MMSE), Mini Nutritional Assessment (MNA) tests were administered. Circulating levels of seven EPCs phenotypes were measured by flow cytometry.
Results: A total of 154 elderly patients were included, of whom 78 in GLP-1RA + MET group and 76 in MET group. The GLP-1RA + MET group showed better cognitive function as indicated by a significant higher MoCA, MMSE and MNA score, and higher levels of CD34+ CD133+, CD133+ KDR+, and CD34+ CD133+ KDR+ as compared with MET group. The number of CD34+ CD133+ KDR+ cells was an independent predictor of higher MoCA, MMSE and MNA scores.
Conclusions: People with T2D on GLP-1RA + MET treatment had better cognitive function and higher circulating levels of EPCs as compared with those on MET alone warranting further studies to understand the interrelationship between EPCs, GLP-RA treatment and cognitive health.

Abstract n SO5_03 - Presenting author**: Angelo Di Vincenzo**

Short-term treatment with dapagliflozin increases the hepatic expression and synthesis of FGF-21 in lean but not obese mice

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Aim: the underlying mechanisms explaining the cardio-renal protection presented by SGLT2 inhibitors (SGLT2i) still remain unclear. Their systemic action is probably mediated by the release of signal molecules, and the fibroblast growth factor-21 (FGF-21) has been identified as the main responsible for SGLT2i pleiotropic effects. However, high levels of FGF-21 are observed in obesity and diabetes, questioning the benefits of this endocrine factor. In this study, we aimed to evaluate the effects of dapagliflozin on FGF-21 gene expression in both genetically obese and lean animal models to identify if a different metabolic status could modulate the therapeutic potential of SGLT2i.
Methods: C57BL/6J WT and B6.V-LEP (ob/ob) mice were randomly assigned to the control or treatment group (dapagliflozin 0.15 mg/kg/day for 4 weeks). FGF-21 gene expression was evaluated in the liver, subcutaneous adipose tissue (SAT), and visceral adipose tissue (VAT) of both ob/ob and lean mice, at baseline and after treatment. Being the liver the main site of FGF-21 synthesis, we also evaluated the effect of dapagliflozin on hepatic FGF-21 protein concentration in control and treated groups.
Results: as expected, basal expression of FGF-21 was higher in the liver, SAT, and VAT of ob/ob mice with respect to lean animals ($p<0.05$). After treatment, the FGF-21 gene expression significantly increased in the liver ($p<0.001$), SAT ($p<0.05$), and VAT ($p<0.05$) of lean mice, while remaining unaffected in ob/ob mice. Similarly, after SGLT2i administration, a significant increase of FGF-21 hepatic protein was observed in lean but not ob/ob mice ($p<0.05$).
Conclusions: dapagliflozin treatment increases FGF-21 gene expression and hepatic protein concentration in lean but not ob/ob mice. These results suggest a less sensitive response to SGLT2i in obesity, probably due to chronic stimulation of the FGF-21 axis. Future studies are needed to understand the therapeutic potential and clinical applications of this endocrine regulator.

Abstract n SO5_04 - Presenting author**: Umberto Capece**

Dapagliflozin treatment is associated with a reduction of epicardial adipose tissue thickness and glucose uptake in human type 2 diabetes

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Background: It is widely accepted that SGLT2 inhibitors decrease cardiovascular mortality and hospitalization for heart failure in patients with type 2 diabetes. Many mechanisms could be responsible for this protective effect, including a reduction in the volume of epicardial adipose tissue, whose increase through the release of cytokines negatively affects coronary atherogenesis and cardiac function.
Methods: 16 patients with type 2 diabetes and chronic ischaemic heart disease were randomized to dapagliflozin or placebo. Systemic and epicardial glucose uptake were analyzed by FDG PET during hyperinsulinemic euglycemic clamp before and after one month of treatment.
Results: Treatment with dapagliflozin resulted in a numerical increase in insulin sensitivity (p=0.06), a significant reduction in epicardial adipose tissue thickness (p=0.03) associated with a significant decrease in glucose uptake (p=0.01).
Conclusions: These data suggest that SGLT2 inhibitors reduce atherogenic epicardial adipose tissue thickness, validating the cardioprotective effects of these drugs. In addition, the decrease in glucose uptake documented by FDG-PET may indicate a reduction of epicardial adipose tissue inflammation, providing a new explanation of cardio-protective effects of SGLT-2i treatment.

Abstract n SO5_05 - Presenting author**: Francesca De Vito**

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.

Abstract n SO5_06 - Presenting author**: Maristella Donato**

Hyperglycemic conditions promote early progression of calcific aortic valve disease in animal models and isolated valve interstitial cells

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Background and aim: Calcific aortic valve disease (CAVD) is the most common valvulopathy in the Western world. Diabetes contributes to the progression of CAVD, but the pathophysiological mechanisms are still not completely understood. Therefore, we aimed to assess the molecular processes leading to diabetic CAVD. In particular, we investigated the effects of a high glucose treatment in isolated VICs and of hyperglycemic conditions in two animal models of CAVD.
Methods: LDLr$^{-/-}$ and LDLr$^{-/-}$:ApoB$^{100/100}$ mice were fed a diabetogenic (HFD) or control diet (NC) for 6, 12 and 26 weeks; then, the aortic valves were collected for bulk RNA sequencing analyses. For the *in vitro* systems, non-human primate VICs and human VICs were treated with low- or high-glucose media (5.5 mM or 25 mM) for 5 days and pro-calcific media to induce their osteogenic differentiation. Total RNA and proteins were extracted for gene expression analysis and enzymatic activity assays (ALP); calcium deposition was quantified through a colorimetric assay.
Results: The transcriptomic analyses detected an overexpression of inflammatory, immune, and diabetes-related pathways in HFD-fed LDLr$^{-/-}$:ApoB$^{100/100}$ mice. Moreover, HFD downregulated cardioprotective genes (such as TBX5, GATA5, and NKX2-5) in both animal models of CAVD. In our *in vitro* systems, high glucose treatment did not affect calcium deposition, but it modified ALP activity, osteogenic markers and cardioprotective genes.
Conclusions: Diabetogenic diet induces inflammatory and immune processes, accelerating early progression of CAVD. Moreover, hyperglycemic conditions downregulate cardioprotective genes in our *in vivo* and *in vitro* systems. These preliminary findings give new insights on the pathophysiology of diabetic CAVD and may lead to the discovery of novel therapeutic targets.

Abstract n SP1_01 - Presenting author**: Emanuele Guerrieri**

Accuracy of CHADS2, CHA2DS2-VASC and HAS-BLED and Machine Learning models in predicting stroke/TIA and major bleedings, in critically-ill patients with non-valvular atrial fibrillation

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Aim: Non-valvular atrial fibrillation (NVAF) is common among critically-ill patients, being associated to both markedly raised thrombo-embolic events (TEE) and major bleeding (MB) risk: antithrombotic management of these patients is diffcult, since the stratification of haemorragic and cardioembolic risk is currently performed with scores which are not validated for critically-ill patients. We have observed that CHADS2, CHA2DS2-VASc and HAS-BLED scores were not helpful stratifying TEE and MB in a previous cohort, the “Atrial fibrillation in critically-ill patients 1.0”, and suggested newer methods based on topological data analysis (TDA) and machine learning (ML). With this paper, we aimed to validate the ML system on larger cohort.
Methods: We retrospectively enrolled all the consecutive patients in a 10 year time frame admitted to our Subintensive Medicine unit for a critical illness and affected by NVAF. For each patient we calculated CHADS2, CHA2DS2-VASc and HAS-BLED. We also calculated stroke/TIA and MB occurrence during the admission. We assessed the accuracy of each score with ROC curve analysis. The AFICILL 1.0 ML algorithm was tested and trained with the new cohort.
Results: After removing trauma and non-critical patients, we obtained 4196 consecutive, critically-ill patients (age: 78,5±8,82 years; 52,8% females). We observed 508 (14,6%) in-hospital deaths or ICU transfers, 339 (9,7% stroke/TIA and 368 (10,6%) MB. CHADS2 (AUC: 0,54; 95% CI: 0,50-0,57; p<0,0001), CHA2DS2-VASc (AUC: 0,54; 95% CI: 0,51-0,58; p=0,006) and HAS-BLED (AUC: 0,51; 95% CI: 0,48-0,54; p=0,184) had a very poor performance in predicting respectively TEE and MB in this setting. We trained the ML with 2870 novel patients (1326 were discarded since used to generate AFICILL 1.0 ML) and obtained an AUC in predicting TEE of 0,96 (95% CI: 0,94-0,96) and an AUC in predicting MB of 0,97 (95% CI: 0,95-0,98).
Conclusions: Among elderly, critically-ill patients, the rate of death, TEE and MB is high. ML is more accurate than classical scores in predicting events and should be considered to guide a successful antithrombotic therapy in this setting.

Abstract n SP1_02 - Presenting author**: Annalisa Cespiati**

The diagnostic accuracy of non-invasive tests of fibrosis in patients with metabolic associated fatty liver disease. A multicentric comparative study between liver biopsy and non-invasive scores or Fibroscan

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Background and Aims: Metabolic dysfunction-associated fatty liver disease (MAFLD) is characterized by liver steatosis and at least one metabolic comorbidity. Due to limitations of liver biopsy, non-invasive tests of fibrosis (NITs) (FIB4 and NFS) and liver stiffness measurement (LSM) by Fibroscan are used to diagnose fibrosis. Aims 1) to evaluate the diagnostic accuracy of NITs and LSM in MAFLD 2) to evaluate their performance specifically in diabetic and obese subjects 3) to identify new thresholds for scores NITs and LSM in MAFLD.
Method: We enrolled 164 biopsy-proven MAFLD (mean age 56±12 ys, 62% males) in Milan and Lisbon. Clinical, laboratory and Fibroscan data were collected within 6 months from biopsy. FIB-4<1.3, NFS<-1.455 and LSM<8 kPa ruled out advanced fibrosis (≥F3), FIB-4>3.25, NFS>0.675 and LSM≥8 kPa suggested advanced fibrosis.
Results: Prevalence of fibrosis ≥F3 was 24% at histology. FIB4 and NFS ruled out advanced fibrosis in 49% and 44% of cases, diagnosed it in 7% and 8% and had indeterminate values in 43% and 49% of cases, respectively. LSM ≥8 kPa in 62% of subjects. All NITs showed a lower accuracy for both identification (AUROCs FIB-4 0.62; NFS 0.57; LSM 0.72) and exclusion (AUROCs FIB-4 0.65; NFS 0.68; LSM 0.72) of advanced fibrosis at biopsy compared to those for non-alcoholic fatty liver disease (NAFLD). The 59% of the cohort was obese, 47% diabetic. For ruling-in advanced fibrosis, FIB-4 and LSM performed worst in diabetic vs non-diabetic (AUROCs FIB-4 0.58 vs 0.69 p<0.001; LSM 0.66 vs 0.71 p<0.001) and in obese vs non-obese MAFLD (AUROCs FIB-4 0.59 vs 0.65 p<0.001; LSM 0.68 vs 0.75 p<0.001). NFS accuracy did not significantly differ between diabetic and non-diabetic (AUROCs 0.58 vs 0.50 p=0.06), whereas it seemed to perform better in obese vs non-obese (AUROCs 0.58 vs 0.55 p=0.01). For the exclusion of fibrosis, all NITs performed worst in diabetic vs non-diabetic (AUROCs FIB-4 0.59 vs 0.67 p=0.001; NFS 0.58 vs 0.63 p=0.003; LSM 0.68 vs 0.69 p=0.06) and in obese vs non-obese (AUROCs FIB-4 0.66 vs 0.69 p=0.002; NFS 0.65 vs 0.70 p=0.003; LSM 0.68 vs 0.74 p<0.001). The Youden indexes of current cut-offs for FIB4 and NFS were <0.5 for both ruling in and ruling out advanced fibrosis, whereas new thresholds as FIB-4>1.63/NFS>-1.09 and FIB-4<1.22/NFS<-1.23 had the best Youden indexes. As for Fibroscan, LSM>10.4 kPa seemed to better identify MAFLD patients with advanced fibrosis, whereas a cut-off of 8.4 kPa to exclude it.
Conclusion: In MAFLD, both FIB4 and NFS and Fibroscan performed worse compared to NAFLD, with the latter having the higher accuracy. The presence of diabetes and obesity impairs the performance of score and LSM. In MAFLD lower cut-off of both FIB-4 and NFS are warranted, whereas no change seems to be needed for LSM. Nevertheless, more accurate NITs should be developed specifically for MAFLD.

Abstract n SP1_03 - Presenting author**: Stefano Pezzoli**

Reclassification with ABI and carotid ultrasound of multivascular involvement in Cardiological Rehabilitation

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Introduction: polyvascular atherosclerotic involvement is one of the definitions of extreme CV risk. For this reason, the search for carotid or lower limb asyntomatic atherosclerotic pathology can be useful to guarantee more intensive treatments for these individuals, who have already had a heart attack
Purpose: the aim was to understand how much the polyvascular patients can improve in functional terms after Cardiological Rehabilitation, comparing them with monovasculars. Besides, the study purpose was to evaluate how many patients are reclassified with an active research of asyntomatic atherosclerotic pathology with carotid ultrasound and Ankle Brachial Index (ABI).
Methods: the study sample was composed by 87 patients who underwent a cardiological rehabilitation cycle at the Niguarda hospital in Milan from March 2021 to April 2022. Of these, personal, medical, clinical, laboratory and instrumental data were collected. Functional improvement was assested as the difference in meters walked on the 6-minutes walking test (6MWT) on the start day (6MWT-1) and on the end day of rehabilitation (6MWT-2). All patients performed an ABI (to evaluate asyntomatic PAD) and a carotid ultrasound (to evaluate asyntomatic cerebrovascular disease).
Results: pre-riclassification, polyvascolar patients (13) compared to monovascular (74), in addition to being on average older (70 years vs 59 years, p=0.01), males (100% vs 73%, p<0.001) and having had more previous recurrent myocardial infarctions (46% vs 8%, p=0.002), are less performing in terms of 6MWT-1 (428m vs 514m, p=0.002) and 6MWT-2 (517m vs 597m, p=0.008). About absolute functional improvement from the beginning to the end of rehabilitation, there are no statistically significant differences (81m vs 82m, p=0.919). Following reclassification, 7 patients switched from monovascular (87) to polyvascular (20).
Conclusions: our data showed that polyvascular patients can improve as much as monovasculars after Cardiological Rehabilitation. Furthermore, following ABI and carotid ultrasound, 8% of patients were reclassified. Polyvascular patients may receive more targeted and intensive therapies if properly diagnosed.

Abstract n SP1_04 - Presenting author**: Giulia Cassioli**

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 FoldChange=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.

Abstract n SP1_05 - Presenting author**: Perillo Andrea**

Clinical usefulness and prognostic value of lung ultrasound in heart failure

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Aim: Lung ultrasound (LUS) has been demonstrated to be a valid tool for the assessment and monitoring of pulmonary congestion through B-line evaluation. The aim of this study was to point out, in patients presented with heart failure, the presence of a possible correlation between the grade of residual pulmonary congestion at hospital discharge, assessed by LUS, and short-term prognosis.
Methods: A prospective observational study was conducted on a sample of 87 patients, presented with heart failure during hospitalization in the Department of Rehabilitation Cardiology Unit of the Federico II University Hospital of Naples since March 2021 until September 2022.
LUS was performed at hospital discharge, using LUS Score as ultrasound indicator of pulmonary congestion. Data relating to clinical and echocardiographic parameters, and laboratory values were also collected.
90 days after hospital discharge, patients were interviewed by telephone about their health status, considering as main endpoints the new onset/worsening of dyspnea and any major complications, such as rehospitalization and/or death.
Results: Results of the analysis revealed a statistically significant direct correlation between LUS score and NT-pro BNP (p= 0.019) and a statistically significant reverse correlation between LUS score and eGFR (p= 0.008). As far as main endpoints were concerned, a statistically significant correlation between LUS score and major complications at 90 days was not proved (p = 0.798). A statistically significant correlation between LUS score and the new onset/worsening of dyspnea at 90 days emerged (p = 0.021). Additionally, the LUS score values in the subgroup of patients with dyspnea was significantly higher than the LUS score values in the subgroup of patients without dyspnea.
Conclusions: The LUS score has proved to be a reliable parameter for the evaluation of pulmonary congestion in patients with heart failure and can boast a high short-term prognostic value, in this category of patients.

Abstract n SP1_06 - Presenting author**: Bernadette Corica**

Sex-based analysis of the efficacy of a mobile health technology integrated care in patients with atrial fibrillation: insights from a cluster randomised trial

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Aim: The Mobile Health Technology for Improved Screening and Optimized Integrated Care in AF (mAFA-II) randomized trial showed that a mobile Health (mHealth)-implemented 'Atrial fibrillation Better Care' (ABC) pathway approach (mAFA intervention) was able to improve prognosis in patients with atrial fibrillation (AF). Nonetheless, women patients with AF have a suboptimal management and poorer cardiovascular outcomes compared to males, and whether the benefit of mAFA intervention can be applied in both sexes is unclear. We sought to perform a sex-stratified analysis of the mAFA-II trial.
Methods: In the mAFA-II trial, adults AF patients were enrolled across 40 centres between June 2018 and August 2019; centers were randomized to mAFA intervention (based on a smartphone application for both doctors and patients) or usual care. Primary outcome was the composite of all-cause death, ischemic stroke (IS), thromboembolism, and rehospitalization; in this analysis, we evaluated the effect of mAFA intervention according to sex through adjusted Cox-regression models.
Results: 3,324 patients enrolled in the trial: 2,062 (62.0%) patients were males (mean age: 67.5±14.3; 1,021 allocated to mAFA intervention) and 1,262 (38.0%) were females (mean age: 70.2±13.0; 625 allocated to mAFA intervention). Among patients allocated to mAFA intervention, males showed lower prevalence of prior IS and coronary artery disease, while females were mostly affected by heart failure and prior IS. A significant risk reduction of the primary composite outcome in patients allocated to mAFA intervention was observed in both males (adjusted Hazard Ratio (aHR) 0.30, 95% Confidence Interval (CI) 0.17-0.52) and females (aHR 0.50, 95%CI 0.27-0.92, Figure 1), without a statistically significant interaction (p_{int}=0.225). Sex-based interactions were observed for other exploratory secondary outcomes, including all-cause death (p_{int}=0.026) and bleeding events (p_{int}=0.032).
Conclusion: A mHealth-technology implemented ABC pathway was effective in improving prognosis in both sexes; greater benefit on secondary outcomes was observed in males.

Abstract n SP1_07 - Presenting author**: Christian Basile**

Normalization of ascending aorta dimension for body size influences pathophysiologic correlation in hypertensive patients: the Campania Salute Network

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Aim: Dilatation of the sinus of Valsalva is associated with increased cardiovascular risk (CV) but less is known about remodelling of ascending aorta (AscAo). In the present study, we assessed correlates and their consistency of AscAo measurement in treated hypertensive patients.
Methods: 1634 patients ≥ 18 years old with available AscAo ultrasound were included. AscAo was measured at end-diastole with leading edge to leading edge method, perpendicular to the long-axis of the aorta in parasternal long-axis view at its maximal identifiable dimension. Correlations of AscAo and AscAo normalized for height (AscAo/HT) or body surface area (AscAo/BSA) with demographics and metabolic profile were explored. Multivariable regression was also used to identify potential confounders influencing univariate correlations. Sensitivity analysis was performed using CV outcome.
Results: Correlations with age, eGFR, systolic BP and HR were similar among the 3 aortic measures. Women exhibited smaller AscAo, but larger AscAo/BSA than men with AscAo/HT offsetting the sex difference. Obesity and diabetes were associated with greater AscAo and AscAo/HT but with smaller AscAo/BSA (all $p<0.001$). In multivariable regression model all aortic measure confirmed the sign of their relations with sex and metabolic profile independently of age, BP and HR. In Kaplan Mayer analysis only dilated AscAo and AscAo/HT were significantly associated with increased risk of CV events (both $p<0.008$).
Conclusions: Among patients with long standing controlled systemic hypertension, magnitude of aortic remodelling is influenced by the type of the measure adopted, with physiological consistency only for AscAo and AscAo/HT, but not for AscAo/BSA.

Abstract n SP2_01 - Presenting author**: Federica Ricciutelli**

Treatment with allopurinol attenuates the detrimental impact of systemic inflammation on endothelial function in patients with COVID-19

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Background. Impaired endothelial function (EF) is frequently found in patients with COVID-19 and participates in the deterioration of their prognosis. Allopurinol, a xanthine oxidase inhibitor with hypouricemic and anti-oxidative effects, has been reported to have a variable impact on EF, with some studies reporting protective and other neutral effects.
Methods. The proportion of patients taking Allopurinol at hospital admission was recorded in 665 patients hospitalized for COVID-19. Brachial artery flow-mediated dilation (baFMD), an instrumental measure of EF, has been measured at hospital admission in the entire study cohort. The association between Allopurinol treatment, inflammation (C-reactive protein, CRP) and EF has been evaluated.
Results. Thirteen percent of COVID-19 patients were taking Allopurinol treatment at hospital admission. BaFMD (median, IQR: 4.3%, 2.7-3.5) was inversely associated with diabetes, hypertension, CRP and COVID-19 severity. Allopurinol-treated patients had higher baFMD than Allopurinol-untreated patients (5.30%, 3.42-8.20 vs 4.15%, 2.64-6.21, p<0.001) and were less likely to have depressed (<3.78%, 33rd percentile in patients with lower COVID-19 severity) baFMD (30% vs 45%, p=0.002). Allopurinol treatment was associated with a lower probability to have depressed baFMD (OR and 95th IC: 0.46, 0.22-0.98, p=0.043), irrespective of confounders. The age- and sex-adjusted inverse association between plasma CRP and baFMD (beta -0.11, p=0.007) in Allopurinol-untreated patients was abolished in Allopurinol-treated patients (beta -0.092, p=0.412).
Conclusions. Allopurinol treatment, preceding hospital admission for COVID-19, is associated with a lower likelihood to have depressed baFMD. In addition, the detrimental impact of systemic inflammation on endothelial function appears to be halted in Allopurinol-treated patients.

Abstract n SP2_02 - Presenting author**: Rosa Curcio**

NLRP3 inflammasome-related exosomal miRNAs are associated with radiologic sequelae in survivors of COVID-associated acute respiratory distress syndrome

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Aim: There is limited understanding of the pathophysiology of post-acute pulmonary sequelae in COVID-19-associated acute respiratory distress syndrome (ARDS). The aim of the present study was to investigate 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 five months from clinical recovery. Patients were selected based on imaging pattern evolution according to chest HRCT findings into "fully recovered" (FR), corresponding to those who showed complete disease remission, and into "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, miR146a-5p), a NLRP4 inflammasome miRNA (miR-141-3p), and other miRNAs involved in post-transcriptional regulation of acute phase cytokines (miR128-3p, miR3168, miR125b-2-3p) were evaluated.
Results: 33 patients (69% men, mean age 61±7 years, mean BMI 31,2±7 Kg/m^2) were selected for the present study. No statistically significant differences between FR, PO and FL groups were observed according to clinical features. NLRP3 inflammasome-related miRNAs, miR17-5p, miR223-3p and miR146a-5p were significantly up-regulated in patients with PO when compared to patients with FL.
Conclusions: In patients with long-term pulmonary radiological sequelae following COVID-19-associated ARDS, a down-regulation of miRNAs inhibiting NLRP3 (mir-17-5p, mir-146a-3p and miR223-3p) correlates 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.

Abstract n SP2_03 - Presenting author**: Andrea Boccatonda**

Correlation between coagulation activation and pulmonary changes detected by lung ultrasound in COVID-19 patient

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Aim: to evaluate the correlation between the degree of pulmonary compromise assessed by lung ultrasound and the activation of the coagulation cascade by dosing d-dimer levels in COVID-19 patients.
Methods: each patient underwent an ultrasound examination of the chest by using a 12-scan protocol, with calculation of the LUS SCORE (0-3 for each pulmonary field). Moreover, blood tests for d-dimer dosage and arterial blood gas analysis were performed.
Results: 47 patients with COVID-19 pneumonia were examined. The LUS score correlated directly with the D-dimer value ($p=0.019$; rho:0.342); the LUS score correlated directly with C-reactive protein ($p<0.001$;rho:0.647); the LUS score correlated directly with the oxygen alveolar-arterial gradient ($p<0.001$;rho:0.640); the LUS score indirectly correlated with the P/F ratio ($p<0.001$;rho:-0.614).
Conclusions: the results of our work demonstrate that the activation of the coagulation cascade is a fundamental mechanism in COVID-19 pneumonia. The degree of pulmonary alteration, evaluated by lung ultrasound, correlates directly with the values of d-dimer and therefore with the degree of coagulation activation.

Abstract n SP2_04 - Presenting author**: Marco Giannini**

Identification of rare coagulation variants and screening of polymorphisms F2 rs1799963 and F5 rs6025 in COVID-19 patients

Marco Giannini, Elena Sticchi, Tommaso Capezzuoli, Rosina De Cario, Lapo Squillantini, Rebecca Orsi, Samuele Suraci, Giulia Cassioli, Sara Neroni, Angela Antonietta Rogolino, Martina Berteotti, Anna Maria Gori, Rossella Marcucci, Betti Giusti

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Aim: Severe SARS-CoV-2 infections are characterized by perturbation of physiological coagulation mechanisms, and are associated with a high incidence of thrombotic complications. An association between thrombophilia and the most severe clinical course of COVID-19 has also been suggested. Thrombophilia may be due to hereditary factors, and the most frequent causes are factor V Leiden polymorphism (rs6025) in *F5* gene and the G20210A polymorphism (rs1799963) in *F2* gene. Aim of the study was to identify genetic variants and profiles associated with severity of the disease and thrombotic events susceptibility.
Methods: An NGS analysis was conducted on a cohort of n=40 patients with COVID-19; genetic analysis included a sequencing panel of 11 genes (*PROC, PROS1, FGA, FGB, FGG, SERPINC1, F2, F5, F10, PLAT, PLG*) known to be involved in the coagulation process. Moreover, a genotyping analysis of rs6025 and rs1799963 polymorphisms has been conducted through Real Time PCR on the whole cohort of n=994 patients hospitalized at the AOU Careggi with COVID-19.
Results: As regards NGS analysis, 29 rare variants (MAF≤1%) have been identified at the heterozygous state in 24 of the 40 patients studied: 7 missense, 13 synonymous, 5 non-deep intronic and 4 variants concerning zone 3'-5' UTR/downstream. A higher prevalence of rare missense variants with potential pathogenic prediction in ICU or dead patients (26.7%) was observed than in ordinary ward patients (8%). Concerning common genetic thrombophilia, in the whole n=994 patients cohort, n=45 were heterozygous for the rs1799963 polymorphism and n=31 were heterozygous for rs6025 polymorphism. MAF for *F2* rs1799963 and *F5* rs6025 was comparable to that observed in the tuscan population.
Conclusions: The presence of common genetic factors of hereditary thrombophilia does not seem to indicate a significant contribution in modulating the risk of developing thromboembolic complications in SARS-CoV-2 patients; on the other hand, NGS results show that genetic variability, due to rare variants, might modulate clinical severity of COVID-19 disease in patients.

Abstract n SP2_05 - Presenting author**: Silvia Santini**

Cardiovascular risk and outcomes in the post-covid syndrome

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Aim: Post-acute COVID-19 is defined by signs and symptoms that develop after COVID-19 and continue for more than 12 weeks and are not explained by an alternative diagnosis. Understanding the possible risk factors associated with the post- COVID-19 is extremely helpful in identifying high-risk patients who will benefit of an evaluation after the resolution of acute phase. The present study aims to evaluate whether the cardiovascular risk (CVR) of patients with previous COVID-19 infection correlates with the incidence of symptoms and alterations in respiratory function parameters in post-COVID19. The association between estimated cardiovascular risk and acute-illness severity was also considered.
Methods: Between 21/04/21 and 01/09/21 we enrolled 1782 consecutive patients with RTPCR-confirmed COVID-19. These subjects underwent a complete clinical/instrumental follow-up at 3 months after COVID-19 and were divided into: 4-levels according to COVID-19 severity (home care; hospital no oxygen; hospital oxygen; hospital NIV/ICU); 3-levels according to CVR calculated according to SCORE charts. We assessed at 3 months after COVID-19: arterial blood gas analysis, persistent symptoms correlated with post-covid syndrome, and spirometric data (P/F and DLCO values).
Results: In patients with previous COVID-19, a high CVR was associated to (i) an increased risk of COVID-19 hospitalization ($p < 0.0001$), (ii) an increased prevalence of severe clinical COVID-19 manifestations and ICU hospitalization ($p < 0.0001$), (iii) development of several post-COVID-19 symptoms including fever ($p=0.002$), exhaustion/asthenia/fatigue ($p=0.002$), diarrhea ($p=0.0001$), headache ($p=0.0001$), anosmia ($p=0.0001$), dysgeusia ($p=0.002$), red eyes ($p=0.007$), decreased vision ($p=0.017$), dizziness ($p=0.035$), arthralgias/arthritis ($p=0.025$) and chest pain ($p=0.003$). Persistent dyspnea ($p=0.049$) was found to be significantly associated to CVR. We also observed a significant trend between P/F and DLCO and increasing CVR ($p<0.001$).
Conclusions: we found a statistically significant association between CVR, COVID-19 severity, post-COVID-19 symptoms, P/F ratio and DLCO at 3 months after the end of acute illness.

Abstract n SP2_06 - Presenting author**: Lapo Squillantini**

Individual vulnerability to SARS-COV-2 infection: genetic profiles and the role of the host's virus entry machine

Lapo Squillantini, Elena Sticchi[1], Tommaso Capezzuoli[1], Marco Giannini[1], Giulia Cassioli[1], Giulia Barbieri[1], Samuele Suraci[1], Anna Maria Gori[1], Martina Berteotti[2], Angela Antonietta Rogolino[1], Rosina De Cario[1], Ada Kura[1], Rossella Marcucci[1], Betti Giusti[1]

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Aim: Since the 2019 SARS-CoV-2 pandemic outbreak, hundreds of studies reported evidences of a host genetic background's role. As regards the virus entry process, several factors are involved both at the cell surface and intracellular levels. Various genetic variants (mutations and polymorphisms) were found to be associated with the severity of the disease in a detrimental or beneficial way, their identification potentially representing a useful prognostic/therapeutic tool. In this study, genetic characterization of 40 Covid19 patients referred to the Advanced Molecular Genetics Laboratory, Atherothrombotic Diseases Center, Careggi Hospital-University of Florence, was made by Next Generation Sequencing (NGS) to identify genetic profiles potentially representing prognostic factors modulating the susceptibility to virus infection.
Methods: NGS was performed by Illumina MiSeq and Haloplex HS enrichment protocol targeting 7 virus entry-related genes (*ACE2*, *TMPRSS2*, *CTSL*, *CTSB*, *HSPA5*, *IL6*, *FURIN*).
Results: Eighteen heterozygous rare variants [Minor Allele Frequency (MAF)<0.01] were identified in 16/40 patients involving all 7 genes included in the panel. Potentially functional mutations (10/18) involved *ACE2*, *HSPA5*, *CTSL* with *TMPRSS2* being the most affected gene. With regards to the disease progression in terms of death/hospitalization in intensive care unit (ICU, 15 subjects, 3 variants) or early discharge (25 subjects, 7 variants), statistically significant difference was not observed among the 2 rare variants carriers' groups. About the polymorphic burden, the most affected genes were *FURIN*, *CTSB*, and *TMPRSS2*. Considering the total number of polymorphisms per gene, the only slight difference among the abovementioned subgroups was observed regarding the *FURIN* gene as 37 polymorphisms affected the early discharge group, while 18 (67% vs 83%) were carried by the death/ICU group.
Conclusions: Our data suggest how the genetic variants involving the host's virus entry machinery are not likely to exert a significant effect in modulating the severity of the COVID-19 progression when considering the prognosis parameters of death, intensive care unit hospitalization and early discharge from the hospital.

Abstract n SP2_07 - Presenting author**: Marialuisa Sveva Marozzi**

Block of the angiotensin pathways effects flow-volume spirometry in patients with SARS-CoV-2 infection

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Aim: ACE2 pathways inhibitors have been first evaluated in COVID-19 patients for the risk of poor prognosis. Pulmonary function tests (PFTs) are the main diagnostic tools for most respiratory diseases. We evaluated patients that are hypertensives and not and the role of ACE2i/ARB through flow-volume spirometry in follow-up after infection recovery.
Methods: We evaluated 112 Caucasian patients 3-6 months after Covid-19 disease. The series of patients showed a great variability due to a wide spectrum of age, the severity of disease manifestations, hospitalization, invasive/non-invasive ventilation, comorbidities, the presence/absence of a previous pneumological diagnosis, and the variants of the virus. Patients were divided into those who were hypertensives (Group1, 18 females, aged 63.47±14.24), and who were not (Group2, 32 females, aged 53.03±16.66). Group1 was further analysed according to treatment: patients in ACEi/ARB treatment (Group1A, 23 females, aged 63.63±10.40) and those who not (Group1B, 6 females, aged 53.03±16.66). Distal airflow obstruction (DAO) was evaluate as forced expiratory flow (FEF) at 25%, 50% and 75% of total flow. PFTs values were related to age, sex and BMI before comparison.
Results: Group1 presented lower peripheral oxygen saturation percentage (SpO2) vs Group2 ($p<0.05$). Spirometry data were worst in Group1: Forced expiratory volume at first minute (FEV1)($p<0.05$), Forced vital capacity (FVC)($p<0.05$), and Tiffenau Index ($p<0.05$). There was a DAO in Group1. In Group1, we found also a reduction in FEF25 ($p<0.05$), FEF50 ($p<0.05$), and FEF25-75 ($p<0.05$), and in FEF75 ($p<0.05$). There was no difference in those parameters comparing Group1A vs Group1B
Discussion: In hypertensives the indexes of respiratory function were shifted towards the lower limits (albeit within normal limits). These parameters were significantly reduced compared to controls. Treatment with ACEi/ARB did not affect PFTs at follow-up evaluation after recovery. Thus, COVID-19 is not only a pulmonary, but also a vascular disease worsening when a previous CV comorbidity.

Abstract n SP3_01 - Presenting author**: Alessia Bertelli**

Vaccine-induced thrombotic thrombocytopenia (VITT): evaluation of immunologic and functional tests on an Italian case series

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Aims: Several cases of vaccine-induced thrombotic thrombocytopenia (VITT), have been developed after vaccination with adenoviral vectors encoding the spike protein of severe acute respiratory syndrome coronavirus2.
Methods: We assessed the clinical and laboratory features of 8 suspected VITT patients. We used three different immunologic tests to detect PF4–heparin antibodies: two ELISA, a latex immunoturbidimetric assay (LIA) and a chemiluminescence immunoassay (CLIA). To detect the ability of the antibodies in inducing platelet activation, we performed two functional tests: modified Heparin-Induced Platelet Activation test (HIPA) on washed platelets and flow cytometry (FC) on platelet rich plasma.
Results: All patients [2M/6F; median age: 66 (41-78)] had a confirmed diagnosis of VITT based on clinical and laboratory features. Blood samples were obtained, except cases#1, #2, #6, #8 before Immunoglobulin(IG) or anticoagulant administration. There was a significant correlation between the two different ELISA tests (Table rho=0.683, p=0.042). According to the ELISA cut-off values, all patients were positive for the presence of anti-PF4 antibodies. None of the patients had LIA and CLIA values above the cut-off (> 1 U/mL). By performing functional tests, all patients resulted positive to the modified HIPA test, except for VITT#6 and VITT#2, who resulted negative and weakly positive, probably due to the concomitant IG therapy. By performing FC, patients with the concomitant IG therapy showed a significant lower percentage of activated platelets with respect to the patients without [MFI of CD62P platelets: 4458 (4320-4629) vs 11170 (6716-17492), p=0.03]. Patients#4F, #5F, showed a great platelet activation with VITT serum and buffer, and a decreased CD62P expression with low dose of unfractionated heparin (0.3IU/mL), by showing a typical positive VITT pattern. In patients #7, we found an enhanced CD62P expression in the tube with unfractioned heparin 0.3IU/mL, with a typical HIT pattern.
Conclusions: ELISA test in association with functional test are able to detect platelet activating antibodies against PF4 in VITT. HIPA test remains the gold standard but, the FC seems a sensitive and reliable method with respect to HIPA. Patients treated with IG might be associated with false negative results and in some cases FC results less sensitive with respect to HIPA in detecting platelet activating antibodies.

VITT case	Lifecode PF4-IgG (OD)	Zymutest HIA IgG (OD)	Hemosil Acustar IgG (U/mL)	Hemosil HIT-Ab PF4 (U/mL)	HIPA	Flow Cytometry
VITT #1	2.47	1.94	0.04	0.4	POSITIVE	NEGATIVE
VITT #2	3.13	2.26	0.06	0.1	WEAKLY POS.	NEGATIVE
VITT #3	2.89	3.32	0.03	0	POSITIVE	WEAKLY POS.
VITT #4	3.34	3.74	0.01	0.2	POSITIVE	POSITIVE
VITT #5	1.47	3.63	0.15	0	POSITIVE	POSITIVE
VITT #6	1.91	0.24	0.15	0.3	NEGATIVE	NEGATIVE
VITT #7	3.26	3.69	0.51	0	POSITIVE	HIT PATTERN
VITT #8	3.02	2.54	0.19	0	POSITIVE	WEAKLY POS.

Abstract n SP3_02 - Presenting author**: Tommaso Capezzuoli**

Vaccine induced thrombotic thrombocytopenia (VITT): evaluation of genetic susceptibility throught WES

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Background: VITT syndrome, characterized by unusual thrombotic events and thrombocytopenia (associated in some cases with hemorrhagic manifestations), has been observed in a small number of individuals after vaccination with adenoviral vectors encoding the spike protein antigen of SARS-CoV-2. Evidences suggest that this rare syndrome is caused by platelet-activating antibodies directed against platelet factor 4 (PF4), a chemokine stored in the platelets' alpha granules necessary for platelet aggregation, also known to be involved in the atherosclerotic plaque formation.
Methods and Aims: Due to the referral role of the Center for Atherothrombotic Diseases (University of Florence/AOU Careggi) for the management of COVID-19 patients and diagnosis of VITT, fifty patients were examined. Eight out of fifty patients were diagnosed with VITT and six of them underwent genetic testing. We performed a WES analysis approach by Illumina NextSeq500 platform for the identification of possible genetic predisposition profiles underlying VITT in 6 females (mean age 64,2±13,8), who received an adenoviral vector-based vaccine.
Results: WES analysis revealed a total of 140,563 variants. We focused on rare variants (MAF <1%) in gene involved the molecular processes of platelets. We found a total of 89 rare variants in genes involved in integrin signaling pathways, in thrombocytopenia, and other genes inducing/inhibiting platelet aggregation/activation processes. Two subjects with the worst clinical outcome reported a significantly higher number of rare genetic variants than the other patients. Interestingly, both report a rare variant in GP6, a gene associated with thrombotic generation and hemorrhagic diathesis.
Conclusions: WES analysis exhibit a considerable number of variants in platelet molecular pathways. Patients with the worst clinical outcome presented a significantly higher number of suggestive rare variants with respect to other patients; consequently, it is not possible to exclude the potential contribution of a greater number of rare suggestive variants in the modulation of the phenotype of patients with worse clinical course.

Abstract n SP3_03 - Presenting author**: Nour Jasmine**

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.

Abstract n SP3_04 - Presenting author**: Chiara Pidone**

Impact of a NDUFC2 variant on the occurrence of acute coronary syndromes

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Background. Among several potential mechanisms, mitochondrial dysfunction has been proposed to be involved in the pathogenesis of coronary artery disease (CAD). A mitochondrial complex I deficiency severely impairs cardiovascular health and contributes to CAD development. Previous evidence highlighted a key role of NDUFC2, a subunit of complex I, deficiency in the increased occurrence of renal and cerebrovascular damage in an animal model of hypertension, and of juvenile ischemic stroke occurrence in humans. Furthermore, a significant decrease of NDUFC2 mRNA was detected in peripheral blood mononuclear cells from patients experiencing acute coronary syndrome (ACS). The T allele at NDUFC2/rs23117379 variant is known to associate with reduced gene expression and mitochondrial dysfunction.
Objective. In the present study we evaluated 1) the impact of the carrier status of the T vs C allele at NDUFC2/rs11237379 variant on the occurrence of a first ACS episode, and 2) the long-term prognostic impact of T vs C allele on the incidence of recurrent ACS episodes in a prospective cohort of Caucasian CAD patients.
Methods. We enrolled 260 patients admitted at the Cardiology Unit of Sant'Andrea Hospital in Rome for a first ACS episode. The inclusion criteria were ACS episodes [unstable angina, non-ST-segment elevation MI (NSTEMI), ST-segment elevation MI (STEMI)] as first manifestation of CAD; evidence of critical coronary artery stenosis in at least one vessel (70%), as documented by coronary angiography; availability of a well-documented clinical follow-up after the first ACS episode. Exclusion criteria were lack of coronary angiography, of a well-documented clinical follow-up, patients with neoplasia and short life expectancy (<6 months).
Results. Hypertension, smoking habit, diabetes and hypercholesterolemia were present in a large proportion of patients. NSTEMI represented the most frequent type of ACS (44%, n=115), followed by STEMI (34%, n=88) and unstable angina (22%, n=57).
Regarding the T/C alleles distribution at NDUFC2/rs11237379 variant, 45.4% of the enrolled subjects were carrier of the C allele and 54.6% carried the T allele. The frequency of the observed genotypes was 22.3% for CC genotype (n=58), 46.5% for CT genotype (n=121) and 31.2% for TT genotype (n=81); chi-square test: p>0.05 respecting the HWE.
The alleles/genotypes distribution for T/C at NDUFC2/rs23117379 revealed that the TT genotype was associated with a trend toward the development of ACS at an earlier age (TT 61±12, CT 65±12 and CC 66±11 years; p=0.051 after adjustment for gender, hypertension, smoking habit, diabetes and hypercholesterolemia) and with a significant predictive role for ACS recurrence (hazard ratio [HR]1.671; 95% confidence interval [CI], 1.138-2.472; p = 0.009).
Conclusions. Our findings are consistent with a deleterious effect of NDUFC2 deficiency on acute coronary events predisposition and further support a role of the NDUFC2/rs23117379 variant as a genetic cardiovascular risk factor.

Abstract n SP3_05 - Presenting author**: Jennifer Vanoli**

Impact of the increase in left ventricular mass on the risk of long-term cardiovascular mortality: a prospective cohort study

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Michele Bombelli and Jennifer Vanoli contributed equally to this work.

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Background. Left ventricular hypertrophy (LVH) is an independent predictor of cardiovascular (CV) events and death and in several high CV risk conditions or diseases evidence has been obtained that an increase of a normal LVM or new onset LVH over time increases CV outcomes. We addressed this issue in a sample of a general population at relatively low CV risk.
Methods. We analysed subjects with normal echocardiographic LVM enrolled in the PAMELA study (Pressioni Arteriose Monitorate E Loro Associazioni) to follow the increase of LVM over more than 10 years and assess the prognostic impact of this change on the subsequent incidence and risk of CV and all-cause mortality (mean follow-up 18.5 years).
Results. In 990 subjects with no LVH at baseline there was a significant average increase of LVM (21.2%), $LVMI_{BSA}$ (18.9%) and $LVMI_{HT}$ (22.3%) more than 10 years later. About a quarter developed LVH and thus the large majority remained with a normal LVM. The $LVMI_{BSA}$ change exhibited an association with the risk of CV mortality during the following 18.5 years as assessed by the Cox model (hazard ratio or HR for 1 SD increase: 1.9 ,95% confidence intervals or CI 1.6-2.3, $p<0.0001$) and the association remained significant after adjustment for demographic and clinical confounders including baseline and time-related changes in office and 24h blood pressure (HR: 1.2 ,1.0-1.5). Similar findings were obtained for LVM in absolute values or indexed for height. The association between the increase in LVM exhibited an increase both in males and in females but their association with the risk of CV mortality was statistically significantly in males only. There were similar qualitative changes for the risk of all-cause mortality, which, however, did not reach statistical significance.
Conclusions. Our data provide evidence on the relationship between increases in echocardiographic LVMI or LVM indices and CV mortality in people with normal baseline LVMI in a low CV risk sample of the population. The evidence is that, although over 10 years the LVM increase does not reach a LVH status, the LVM increase is associated with an augmented CV mortality risk. This suggests that it might be important to consider periodical LVM assessment, even when LVM is within the normal range, to timely detect its increase and cope with the need of CV risk re-stratification.

Abstract n SP3_06 - Presenting author**: Nadia Di Pietrantonio**

Epigenetic regulation of NF-κB p65 in women with gestational diabetes and their offspring

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Aim: Gestational diabetes (GD) is characterized by chronic hyperglycemia during pregnancy. Both GD women and their offspring are predisposed to atherosclerotic cardiovascular disease (ASCVD). Recently, putative association between maternal GD and offspring's epigenome is emerging, thus GD might be considered an interesting model to elucidate molecular mechanisms possibly involved in the transmission of abnormal phenotypes to GD offspring. Therefore, we investigated the role of histone modifications, such as H3K27acetylation and H3K4monomethylation, in the onset of inflammatory phenotype and whether they are transmitted to the offspring, focusing on the role of histone acetyltransferase p300.
Methods: We employed peripheral blood mononuclear cells (PBMC) from GD and control women as well as human umbilical vein endothelial cells (HUVEC) and cord blood mononuclear cells (CBMC) from newborn's umbilical cords. H3K27ac and H3K4me1 on a putative enhancer region of NF-κB p65 gene were assessed by ChIP, RT-qPCR and Luciferase assay. Protein expression of histone acetyltransferase p300 and NF-κB p65 were evaluated by flow-cytometry following p300 gene silencing.
Results: We found higher NF-κB p65 expression in HUVEC, PBMC and CBMC from GD women compared to controls. Most importantly, we detected increased levels of H3K4me1 and H3K27ac at a region proved to be a putative enhancer of NF-κB p65 in all GD cells. Additionally, protein expression of p300 was significantly higher in GD-HUVEC compared to control cells and its gene silencing was able to significantly inhibit NF-κB p65 protein expression.
Conclusion: Our results suggest that a complex interplay of histone modifications may be responsible for the inflammation associated to chronic hyperglycemia and this provides the basis for demonstrating the transmission of these modifications to the offspring. Although further elucidations are needed, our results offer the way for pharmacological reprogramming of adverse histone modifications to dampen early abnormal phenotypes in GD offspring which may accelerate ASCVD burden.

Abstract n SP3_07 - Presenting author**: Mirko Zoncapè**

Platelets behavior in metabolic patients affected by hepatic steatosis/steatohepatitis and liver cirrhosis

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Aim: NAFLD is the most common liver disease worldwide. NASH usually develops in patients with metabolic syndrome, which is per-se a pro-thrombotic and pro-atherogenic condition in which platelets could play an important role. Few studies until now explored platelets' role in NAFLD and its evolution in cirrhosis. Our aim was to verify the possible role of platelets in the NAFLD/NASH genesis and evolution in cirrhosis, compared to healthy subjects, and their possible interaction with coagulation factors.
Methods: Microfluidic and Flow Cytometry, were used to investigate the possible presence of a pre-activation state and the in vitro level of activation in NAFLD/NASH and cirrhotic patients, compared to healthy subjects. We also checked for the possible procoagulant and profibrotic alterations of platelets, studying their interaction with coagulant factors: experimental combinations were performed using the platelet-poor-plasma of healthy controls, and adding washed platelets of patients (NAFLD/NASH or cirrhotic), and vice versa.
Results: In NAFLD group, platelets adhere significantly more compared to controls and cirrhotic subjects ($p<0.01$) and seem more prone to react to stimuli. Cirrhotic patients have a lower number of circulating platelets, but with a normal adhesion capacity (compared to controls). Furthermore, even if high concentration of von-Willebrand-Factor (vWF) is found in cirrhosis (cirrhotic vs controls and NAFLD/NASH subjects: $p<0.01$), the efficiency of collagen binding vWF-mediated is quite low. Flow cytometry highlights that cirrhotic patients have a lower capacity in forming heteroaggregates and expressing PAC-1 and P-selectin, compared to controls ($p<0.05$, $p<0.01$ and $p<0.01$, respectively). Moreover, we found no significant platelets contribution in their interaction with coagulation factors.
Conclusions: Our study defines the pivotal role of platelets in NAFLD patients and its evolution up to cirrhosis: the NAFLD subjects present more "reactive" platelets, suggesting an inflammatory and pro-fibrotic role, while in metabolic cirrhosis platelets are less prone to react, "exhausted".

Abstract n SP4_01 - Presenting author**: Federica Catalano**

The adipokine, asprosin, as modulator of obesity metabolic complications

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Aim: The aim of the present study was to clarify the mechanisms underlying the release of the novel adipokine, asprosin from white adipose cells, in vivo and in vitro, and to obtain evidence of its role as a modulator of obesity metabolic complications.
Methods: Adipose tissue biopsies were obtained from 21 (7 males, 14 females) subjects undergoing bariatric surgery (mean age= 41±9; mean BMI= 47.2±6.8). For in vitro experiments, differentiated 3T3L1 adipocytes were exposed to hypoxia (O2 2%) or incubated for 24 hrs in the presence of glucose (25/50 mM, HG) or TNFa (2.5 nM). Total RNA was obtained from frozen biopsies and 3T3L1 cells with TRIzol reagent, reverse transcribed and analyzed by SYBR Green qPCR to assess asprosin mRNA levels. Asprosin content in cell media was analyzed by ELISA assay.
Results: Asprosin mRNA expression was higher in male than in female patients and significantly greater in subjects with a family history of diabetes (p=0.014 by T-test). Furthermore, asprosin mRNA levels showed a direct correlation with inflammatory parameters (Fibrinogen, Pearson’s r=0.36, p=0.081; C-reactive protein, Pearson’s r=0.40, p=0.044) as well as with fasting plasma glucose concentration (Pearson’s r=0.39, p=0.060). By contrast we did not observe any correlation with body weight, BMI or fat mass percentage. In vitro experiments showed that glucose 50 mM and TNFa for 24 hrs significantly increased asprosin mRNA expression levels in 3T3L1 cells (HG= +70±3.1%; TNFa= +50±3.1%, p=0.05 by T-test, n=3). Accordingly, HG (25/50 mM) for 24 hours increased asprosin release in the cell medium as compared to control cells (+50±12%, +45±22% respectively; p=0.05 by T-test, n=3). By contrast, asprosin mRNA levels decreased (-45±5.3%, p=0.05 by T-test, n=3) when 3T3L1 were exposed to hypoxia to mimic a condition of inflammation-free obesity.
Conclusions: Our data suggest that asprosin represents a putative player mediating the metabolic effects of obesity.

Abstract n SP4_02 - Presenting author**: Cecilia Colangelo**

Visit-to-visit variability of LDL-C predicts carotid intima-media thickness

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Aim: A higher visit-to-visit variability in LDL cholesterol (LDL-C) has been demonstrated to be associated with an increased frequency of cardiovascular events, including ischemic stroke. However, its impact on carotid atherosclerotic burden remains unexplored. This study aimed to assess the association between visit-to-visit variability of LDL-C and carotid intima-media thickness (IMT) as a measure of early atherosclerosis.
Methods: We consecutively enrolled a cohort of patients referring to our Lipid Clinic with at least three antecedent lipid profiles which were all taken during the same therapeutic regimen and separated by at least 1 year. Carotid IMT was assessed through carotid Doppler ultrasound and patients with any carotid atherosclerotic plaque were excluded. The mean of IMT values detected at common and internal carotids was marked as mean-IMT whereas the mean of maximum IMT values detected at common and internal carotids was marked as mean-max-IMT. Visit-to-visit variability of LDL-C was measured as standard deviation of the mean (SD), variation coefficient (CV), and average real variability (ARV). The association between either LDL-C SD, CV, or ARV and either mean-IMT or mean-max-IMT was assessed through univariable and multivariable analyses.
Results: Among 385 patients (mean age 61 ± 4 years, 21% males), the median LDL-C SD, CV, and ARV were 27.9 (19.4-37.7), 0.21 (0.15-0.30), and 3.7 (1.4-7.1), respectively. A positive association was observed between LDL-C SD and mean-IMT (rho=0.110, p=0.032) as well as between LDL-C SD and mean-max IMT (rho=0.110, p=0.03). Also, a positive association emerged between, LDL-C CV and mean-IMT (rho=0.128, p=0.012) as well as between LDL-C CV and mean-max IMT (rho=0.126, p=0.014). Instead, no significant correlation was observed between LDL-C ARV and either mean-IMT or mean-max-IMT. The positive association between LDL-C SD and mean-IMT/mean-max-IMT as well as that between LDL-C CV and mean-IMT/mean-max-IMT were significant even after adjusting for the potential confounding effect of other cardiovascular risk factors beyond LDL-C and concomitant therapies for cardiovascular prevention (β=0.141 p=0.004, β=0.143 p=0.004, β=0.129 p=0.008, β=0.125 p=0.012).
Conclusions: Intraindividual visit-to-visit LDL-C variability measured as LDL-C SD and LDL-C CV is independently associated with carotid IMT. As to whether LDL-C variability may be an additional therapeutic target for cardiovascular prevention is worthy to be explored.

Abstract n SP4_03 - Presenting author**: Martina Chiriacò**

Inappropriately elevated plasma leptin levels identify individuals with more severe metabolic adiposity-related complications

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Aim: Leptin regulates energy balance and metabolism, its plasma levels are proportional to fat mass, but with a wide interindividual variability. We tested whether a different chronic exposure to leptin affects the metabolic consequences of obesity.
Methods: We analyzed 1372 healthy adults from the Relationship Between Insulin Sensitivity and Cardiovascular Disease (RISC) study cohort (30-60 years, M/F 595/777, BMI 26±4 kg/m^2) followed-up after 3.5-years and characterized for body composition and metabolic variables with a 75-g OGTT, euglycemic-hyperinsulinemic clamp, intravenous glucose bolus, β-cell function, insulin clearance and hepatic and adipose insulin sensitivity.
Results: Individuals were divided into 3 groups of leptin levels (Hyperleptinemic, Normoleptinemic and Hypoleptinemic) on the bases of the residuals' tertiles of the gender-specific leptin vs fat mass correlation. HyperL showed ~60-70% and ~120-140% higher leptin levels compared to NormoL and HypoL, respectively. The 3 phenotypes showed similar fasting and OGTT glucose levels, but HyperL had markedly higher fasting and OGTT insulin levels (+53% and +41% vs HypoL, respectively, $p<0.0001$) and also showed an average 27% reduction in insulin sensitivity ($p<0.0001$). Also, fasting and OGTT insulin clearance both showed a progressive reduction from HypoL to HyperL (-13% and -22%, respectively, $p<0.0001$), independently of insulin secretion and insulin sensitivity. Additionally, glucose sensitivity and acute insulin response to iv glucose, representing β-cell function, were respectively +14% and +12% higher in HyperL vs HypoL individuals ($p<0.05$), along with reduced liver (-22%, $p<0.05$) and adipose tissue (-63%, $p<0.0001$) insulin sensitivity. Overall, the associations were stronger in men and in lean individuals.
At 3.5-year follow-up, changes in weight and metabolic parameters were similar in the 3 phenotypes.
Conclusion: Inappropriately elevated leptin levels are associated with more severe adiposity-related metabolic complications including whole-body insulin resistance, marked hyperinsulinemia (also sustained by increased β-cell function and reduced insulin clearance) and by adipose tissue and hepatic insulin resistance.

Abstract n SP4_04 - Presenting author**: Anna Borrelli**

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.

Abstract n SP4_05 - Presenting author**: Claudio Mario Ciampi**

Uric acid significantly correlates with the presence of low-voltage areas at the endocardial mapping in patients with non-valvular atrial fibrillation

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Background: Interest in the role of atrial substrate in maintaining Atrial Fibrillation (AF) is growing. Fibrosis is the culprit in the electrical derangement of the myocites. Many cardiovascular risk factors are known to be linked to atrial scarring; among them Uric Acid (UA) is emerging. The purpose of our study is to evaluate whether UA is associated with atrial fibrosis in AF patients.
Methods: 81 patients who underwent radiofrequency transcatheter ablation for nonvalvular AF at the cardiological department of the Niguarda Hospital were enrolled. UA levels were analysed before the procedure as well as known predictors of atrial fibrosis. High density electroanatomic mapping of the left atrium was performed and patients were divided according to the presence or not of areas of pathological substrate (bipolar voltage < 0.5mV in sinus rhythm).
Results: 19 patients showed a pathological atrial substrate. The population of patients with pathological atrial substrate was older (64.7±1.6 vs 58.2±10.9 years, p=0.032) and had more often a persistent phenotype of AF (84.3 vs 35.8%, p<0.001). UA levels were signifi-cantly higher in the pathological group (6.8±1.9 vs 5.3±1.4, p<0.001) as well as the preva-lence of hyperuricemia (26.5 vs 6.5%, p=0.021). The association between uric acid and pathological atrial substrate remains significant even after correction for confounding fac-tors (age, left ventricular dysfunction, valvular disease, AF phenotype and furosemide use).
Conclusions: In a population of patients who underwent atrial fibrillation's ablation, higher uric acid's levels were significantly associated with pathological left atrium's substrate at electro-anatomical mapping.

Abstract n SP4_06 - Presenting author**: Rossana Matarrese**

Uric acid relationships with lipid profile and adiposity indices: impact of different hyperuricemic tresholds

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Background and aim: previous studies focused on the relationships between Serum Uric Acid (SUA) and lipids have found an association mainly with triglycerides. Furthermore, previous studies on adiposity indices have been focused on the evaluation of the Visceral Adiposity Index (VAI). The present study was aimed at providing within the same population a systematic evaluation of lipids and adiposity indices with SUA, employing both the classic cutoff for hyperuricemia and the newly one identified by the Uric Acid Right for Heart Health (URRAH) study.
Methods: we analyzed data collected in 1892 subjects of the Pressioni Arteriose Monitorate E loro Associazioni (PAMELA) study with available SUA, lipid profile and variables necessary to calculate VAI, Cardio-Metabolic Index (CMI) and Lipid Accumulation Product (LAP).
Results: at linear regression model (corrected for confounders) SUA correlated with all the lipids values (with the strongest b for triglycerides) and adiposity indices. When the two different cutoffs were compared, the URRAH one was significantly related to atherogenic lipids profile (OR 1.207 for LDL and 1.33 for non-HDL, $P<0.001$) while this was not the case for the classic one. Regarding adiposity indices the classic cutoff displays highest OR as compared to the URRAH one.
Conclusions: newly reported URRAH cutoff for hyperuricemia better relate to atherogenic lipoprotein (LDL and non-HDL) when compared to the classic one. The opposite has been found for adiposity indexes where the classic cut-off seems to present highest performance. Among adiposity indexes, LAP present the highest OR for the relationship with hyperuricemia.

Abstract n SP4_07 - Presenting author**: Francesco Di Giacomo Barbagallo**

Evaluation of HDL-bound long non-coding RNAs in subjects with familial hypercholesterolemia

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Aims: Long non-coding RNAs (lncRNAs) could be attractive circulating biomarkers for cardiovascular risk stratification in subjects at high ASCVD risk such as familial hypercholesterolemia (FH). Our aim was to investigate the presence of lncRNAs carried by HDL in FH subjects and to evaluate the associations of HDL-lncRNAs with lipoproteins and mechanical vascular impairment assessed by pulse wave velocity (PWV).
Methods: This was a retrospective observational study involving 94 FH subjects on statin treatment. Biochemical assays, HDL purification, lncRNA and PWV analyses were performed in all subjects.
Results: LncRNA HIF1A-AS2, LASER and LEXIS were expressed in HDL; moreover, HDL- lncRNA LEXIS was associated with Lp(a) plasma levels ($p < 0.01$). In a secondary analysis, the study population was stratified into two groups based on the Lp(a) median value. The High-Lp(a) group exhibited a significant increase of PWV compared to the Low-Lp(a) group (9.23 ± 0.61 vs 7.67 ± 0.56, $p < 0.01$). While similar expressions of HDL-lncRNA HIFIA-AS2 and LASER were found in the two groups, the High-Lp(a) group exhibited a significant downregulation of HDL-lncRNA LEXIS compared to the Low-Lp(a) group (fold change -4.4, $p < 0.0001$).
Finally, Lp(a) and HDL-lncRNA LEXIS were associated with PWV (for Lp(a) $p < 0.01$; for HDL-lncRNA LEXIS $p < 0.05$).
Conclusions: LncRNA HIFIA-AS2, LASER and LEXIS were expressed in HDL; moreover, significant relationships of HDL-lncRNA LEXIS with Lp(a) levels and PWV were found. Our study suggests that HDL-lncRNA LEXIS may be useful to better identify FH subjects with more pronounced vascular damage.

Abstract n SP5_01 - Presenting author**: Mattavelli Elisa**

Long-term dietary fats reduction attenuates the inflammatory potential of postprandial lipoproteins

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Aim: Fat-enriched meals are daily consumed and are supposed to iteratively exert known acute pro-inflammatory effects. However, no data clearly affirmed if and how long-term decrease of dietary fats abrogates their inflammatory potential.
Methods: The intake of fats was assessed in the dietary habits of ten subjects (58±7 y-old, 6 men) at basal visit (T0) and four weeks later (T4), following a dietary intervention to reduce fats intake. The effectiveness of the dietary intervention was checked with the Dietary Inflammatory Index (DII). At T0 and at T4, the inflammatory potential of fats was tested by four-hours stimulating heterologous monocytes (from healthy donors) with the autologous Very Low-Density Lipoprotein (VLDL) of the subjects isolated by ultracentrifugation in fasting after an oral fat load challenge (OFL) (640 Kcal/body surface; 92% from fats). Then, after six days' culture, monocytes were re-stimulated in acute with LPS.
Results: The daily intake of fats reduced over follow-up (from 61.00±4.48 to 48.75±3.09 g/day; p=0.020), together with DII (by 1.02±0.35 to 0.24±0.46; p=0.190). Postprandial increase in immune cells counts were not affected by the dietary intervention. Then, the over-expression of CD11b, NFkβ, TNFα and NRLP3 in monocytes, induced by postprandial VLDL at T0, was attenuated after exposure with VLDL isolated from T4 upon re-stimulation with LPS.
Conclusions: Long-term dietary intervention impacts on inflammatory potential of the iterative postprandial response. We need to study the molecular aspects of these long-term effects.

Abstract n SP5_02 - Presenting author**: Rosario Mare**

Micellar Lycopene as Natural Treatment for Liver Steatosis

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Aim: Since understanding molecular mechanisms of liver steatosis is extremely important for preventing fibrosis and cirrhosis, we focused on the activity of lycopene extracted from a patented tomato sauce on ACE-I and HMGCoA reductase enzymatic activities, as well as intrahepatic fat accumulation in McA-RH7777cell. ACE-I is a component of the renin–angiotensin system producing angiotensin-II, an endogenous peptide promoting vasoconstriction, oxidative stress, fibrosis, and inflammation, while HMGCoA reductase is the rate-controlling enzyme in production of cholesterol. Delivery of lycopene was assured by an innovative and nano-sized formulation also aiming to protect this carotenoid from degradation and oxidation phenomena, as well as assure intracellular uptake.
Because lycopene might be inversely correlated with induction genes related to lipid metabolism, were tested whether different doses of lycopene affect Srebp-1c and Srebp-2 expression.
Methods: Lycopene extracted from a patented tomato sauce was quantified and encapsulated into Tween80® micelles. McA-RH7777 cells were treated with 0.1 and 0.5 µM micellar lycopene. The intracellular lipid content was evaluated by Red Oil Staining, the mRNA expression of Srebp-1c and Srebp-2 by rt-PCR, as well as ACE-I and HMGCoA enzymatic activity by specific assay kit.
Results: In McA-RH7777, the incubation with micellar lycopene at doses 0.1 and 0.5 µM decreased intracellular lipid content ($p=0.007$ and $p=0.002$, respectively). In addition, we show a statistically significant reduction in mRNA expression of genes involved in triglycerides (Srebp-1c: $p=0.008$ and $p=0.01$, respectively) and cholesterol metabolism (Srebp-2: $p<0.001$ and $p<0.001$, respectively). Finally, lycopene clearly suppressed HMG-CoA reductase ($p=0.01$) and ACE-I enzymatic activity ($p<0.001$ and $p=0.02$, respectively at 0.1 and 0.5 µM treatments).
Conclusions: We demonstrated that nano-delivery of lycopene reduces liver fat accumulation thought genes and pathways involved in hepatic lipid metabolism, as well as reducing HMGCoA reductase and ACE-I enzymatic activities. Therefore, supplementation with micellar lycopene might exert helpful effects in coadjuvant treatments of NAFLD.

Abstract n SP5_03 - Presenting author**: Antonio Vacca**

Coffee consumption relates to a reduction of aortic stiffness in well-controlled hypertensives

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Aim: Nutritional interventions potentially preventing hypertension-related organ damage are far to be fully defined. Coffee is one of the most used beverages all over the world. Many studies tried to define the optimal amount of coffee in order to understand whether coffee has a role in cardiovascular prevention. Thus, we evaluated vascular stiffness in well controlled hypertensives according to coffee consumption.
Methods: We evaluated (well-treated: SBP/DBP?) 449 patients (225F, 224M, aged 62.56±11.49) with essential hypertension. All patients were checked for organ damage screening. The number of coffee cups per day was asked during the visit. The median coffee consumption was 2 cups per day.
Results: patients were subdivided into three groups according to tertiles of cups consumed: Group 1, 0-1 cups 148 patients (87F, 61M; 63.66±12.94), Group 2, 2 cups, 159 patients (77F; 82M; 64.75±10.09), Group 3, >2 cups, 142 patients (60F; 82M; 58.96±10.54). No differences highlighted in BMI, in SBP/DBP, in carotid-femoral pulse wave velocity (cfPWV) and in ankle-brachial Index (ABI). On the contrary, aortic stiffness evaluated as Augmentation index (AI) results significantly decreased in high coffee consumers compared to other groups (ANOVA $p<0.0001$). In particular, Group 3 was lower than Group 1 (Group 3 9.54±15.48 vs Group 1 17.49 ± 19.56, $p<0.05$) and (Group 3 9.54±15.48 vs Group 2 15.88 ± 16.57, $p<0.05$). To evaluate microcirculation, amydriatic retinography was performed. Arteriolar-to-Venular diameter Ratio (AVR) was higher in less coffee consumers compared to higher (Group 1 0.91 ± 0.10 vs group 3 0.87 ± 0.11, $p<0.05$), but no difference resulted between Group 2 and 3 or Group 1 and 2. Moreover, AVR directly did not correlate to Augmentation Index in any group.
Conclusions: Coffee consumption may have a role in prevention of aortic remodelling and stiffness. Consumption of at least 3 cups of coffee per day reduces vascular stiffness and increase central vascular compliance, despite this effect is reduced in distal vascular circulation. Our findings need further analysis, mainly to the effects in distal/central hemodynamics due to different drug treatment.

Abstract n SP5_04 - Presenting author**: Giulia Nesti**

Metabolic effects of early Time-Restricted Carbohydrate consumption

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Background/Aims: Early time-restricted feeding (eTRF) is a form of intermittent fasting that involves restricting food consumption early in the day to align with the circadian rhythm and promote ketosis. We hypothesized that restricting carbohydrate-rich food in the morning may provide the same cardiometabolic benefits of eTRF while not requiring an absolute daily fast of 16-18 hours, which is often unfeasible and poorly accepted. In this proof-of-concept study, we examined the efficacy of early time-restricted carbohydrate consumption (eTRC) on weight loss, glucose homeostasis, and □-cell function in subjects with type 2 diabetes (T2D).
Methods: In this parallel-arm, randomized clinical trial, 27 patients with T2D were randomized to a 12-week eTCR diet or a Mediterranean-style control diet with matched calorie restriction and macronutrient distribution. Body composition, continuous glucose monitoring (CGM), and food diary analysis were performed every 4 weeks. Mixed meal tests (MMT) and routine biochemical analyses were performed at baseline and at 12 weeks.
Results: Twelve (85.7%) patients in the eTCR arm and 11 (84.6%) patients in the control arm completed the study. The two groups experienced significant and comparable reductions in body weight (-3.1 kg [-3.4,-2.7] eTRC vs -2.6 kg [-8.4,-1.3] Control, p=0.498), fat mass (-6.4% [-8.2,-4.3] eTRC vs -3.3% [-5.0,-1.6] Control, p=0.538), fasting plasma glucose (-12.5 mg/dL [-18.5,-6.5] eTRC vs -13 mg/dL [-29.0,-3.0] Control, p=0,758), and HbA1c (-3 mmol/mol [-6,0] eTRC vs -4 mmol/mol [-6,-2] Control, p=0.488), as well as similar improvements in free-living glucose excursions assessed by CGM (glucose CV% 23 [19,26] eTRC vs 23 [20,23] Control, p=0.999) and MMT-derived glucose tolerance (glucose AUC 2479 mg/dL [180;5149] eTRC vs 3473 mg/dL [1898;5918] Control, p=0.762), insulin resistance (Matsuda Index 1.1 [-0.1;2.9] eTRC vs 1.5 [-0.5; 4.5] Control, p=0.450), and β-cell function (β-cell glucose sensitivity -8.2 [-28.2;24.1] eTRC vs -10.4 [-17.0;-3.3] Control, p=0.762).
Conclusions: The proposed eTRC diet provides a feasible and effective option for weight loss and glucose control in patients with T2D, without additional metabolic benefits compared with standard-of-care dietary regimens.

Abstract n SP5_05 - Presenting author**: Rossella Bianco**

PUFA and MUFA-rich food deficiency in patients with nonalcoholic fatty liver disease

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Aim: Dietary monounsaturated (MUFAs) and polyunsaturated fatty acids (PUFAs) constitute a weapon against cardiovascular diseases. However, to date, there are no relevant data on non-alcoholic fatty liver disease (NAFLD), a pathological condition that is considered a cardiovascular risk-factor. Here, we investigated the alimentary habits of a cohort of patients affected by NAFLD (including nonalcoholic steatohepatitis, NASH), with a particular focus on MUFAs and PUFAs-rich foods.
Methods: Sixty (60) NAFLD patients were enrolled at the Liver Unit of University of Foggia, from November 2022 to February 2023, and invited to complete a food frequency self-administered questionnaire. In particular, the weekly assumption of PUFA and MUFA-rich food was recorded.
Results: Stratifying patients from simple steatosis to NASH, we observed that the mean weekly consumption of MUFA and PUFA-rich foods progressively decreased along with steatosis severity.
In particular, comparing low steatosis patients (L) with NASH subjects (N), we found a significant difference in weekly PUFA consumption (L:9 vs N:6.5). Overall, the mean omega-3-rich food consumption was 2.46, hence a little more than twice a week. In detail, comparing L with N we observed a significant lower assumption of omega-3-rich food in N (L:3.1 vs N:1.28). The Mediterranean Diet (MD) promotes daily consumption of seeds and oily fruits and fish consumption more than twice a week. Therefore, NAFLD patients and especially NASH subjects assume a quantity of PUFA much lower than MD recommendations. In addition, DM promotes the use of extra-virgin olive oil (EVO) as the main source of fats, especially MUFA. However, the intake of MUFA-rich foods (EVO oil and olives) decreased by 34% in N subjects compared to L subjects (L:9.1 vs 5.93), with a daily consumption of EVO of about 30g in L vs about 15g in N patients.
Conclusion: These findings suggest that MUFAs and PUFAs-poor diets may be involved in the progression of NAFLD. In particular, the introit of omega-3 and EVO is very low in NASH patients.

Abstract n SP5_06 - Presenting author**: Isabella Calderoni**

Insulin Prevents Palmitate-Induced Stress Kinase Activation, Autophagy and Apoptosis in Human Cardiac Progenitor Cells

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Aim: Abnormal accumulation of saturated fatty acids in the heart results in insulin resistance, stress kinase activation, and increased cardiovascular risk in humans. The viability of human cardiac progenitor cells (CPC) is essential for myocardium homeostasis. This study investigates the ability of palmitate, a saturated fatty acid, to induce apoptosis, autophagy, and stress kinase activation in human CPC, and the potential protective effects of insulin on palmitate-induced damage.
Methods: human CPC were obtained from non-diabetic and non-obese subjects undergoing cardiac surgery. Human CPC were pretreated with 100 nM insulin for 1 h and then exposed to 0.25 mM palmitate for 16 h. Expression of insulin receptor (IR) isoforms, A (IR-A) and B (IR-B), was evaluated by quantitative Real Time PCR. IR and LC3II protein levels, as well as Akt (S473), p44/p42 MAPK (T202/Y204), and c-Jun (S63) phosphorylation levels were assessed by immunoblotting. JNK and autophagy inhibition were achieved pretreating cells with 20 μM SP600125 and 10 mM 3-methyladenine for 1 h, respectively. Apoptosis was assessed by caspase-3 cleavage by immunoblotting and ELISA assay.
Results: Human CPC expressed both IR-A and IR-B, and IR-A was more expressed than IR-B. Exposure of human CPC to insulin for 15 min induced Akt (S473) and p44/p42 MAPK (T202/Y204) phosphorylation ($p<0.05$). Treatment of human CPC with palmitate induced apoptosis, autophagy and increased S63-phosphorylation of c-Jun ($p<0.05$). Pretreatment with SP600125 inhibited palmitate-induced apoptosis ($p<0.05$), but not autophagy. Similarly, pretreatment with 3-methyladenine decreased palmitate-induced apoptosis ($p<0.05$). Interestingly, palmitate effects on apoptosis, autophagy, and stress kinase activation were prevented when human CPC were pretreated with insulin ($p<0.05$).
Conclusions: Insulin prevents palmitate-induced apoptosis by inhibition of JNK signaling and autophagy in human CPC. Hence, preserving insulin signaling in human CPC might protect from lipotoxicity-induced metabolic alterations in the heart.

Abstract n SP6_01 - Presenting author**: Carosi Martina**

The complexity of diagnosis of a rare disease: the case of a suspected Fabry disease

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Aim: Rare diseases (RD) occur globally, affecting 6-8% of the population worldwide; they include 6000-8000 different conditions of either genetic (80%) or multifactorial (20%) origin. RD may involve any organ or system, often are multisystemic, arising at any age. Severe knowledge gaps still exist on many conditions, due to the combined effects of low prevalence, scarce awareness and weak commitment by funding bodies and enterprises. Moreover, undiagnosed conditions are estimated to affect around 10%-30% RD patients thus highlighting the need to improve the awareness of RD in routine clinical practice.
Methods: We discuss the case of a 57-year-old Italian male came to the Lipid Clinic of Policlinico Umberto I in Rome for the management of mixed hyperlipidemia.
Results: At visit patient was showing an LDL-C and non-HDL respectively of 105 and 149 mg/dl on ezetimibe only. He was presenting an history of hypertrophic cardiomyopathy (HCM), clinically significant coronary atherosclerosis (30-40% and 80% in the medium and apical trait of DA respectively, 20-30% diagonal arm and 60% Cdx), herniated discs and history of testicular seminoma (10 years before). Laboratory data were showing iperCPkemia (CPK>5 ULN), marked hypertransaminasemia, hyperferritinemia with normocromic normocitic anemia. Kidney function and proteinuria were normal. To explain HCM and iperCPKemia, the patient had previously undergone specific genetic and autoimmune analyses with negative results and CMR which was showing non-specific sign (late gadolinium enhancement in interventricular septum and posterior wall). The clinical examination showed obesity (BMI 32 kg/cm2), bronze skin, large erythematous lesions in the legs, muscular fatigability in the lower limbs. Basing to this phenotype, main diseases included in the differential diagnosis were hemochromatosis, Fabry disease and amyloidosis. Therefore, at visit dismission, the patient was requested with several tests: 1) fibroscan and hepatology evaluation 2) electromyography to characterize the neuropathy 3) enzymatic activity through dried blood spot for Fabry disease. For lipid management he was prescribed with PCSK9 inhibitors (PCSK9i) fixing an ideal target of LDL-C below 55 mg/dl. At the second visit, we observed that the PCSK9i in association with Ezetimibe was effective in reducing LDL-C allowing the achievement of an LDL-C of 13 with an estimated percent reduction from baseline of 80%. Noteworthy, patient did not refer any safety concern after iPCSK9 start. The fibroscan detected initial fibrosis (8.5 kPa) and for this reason he underwent hepatic biopsy (in progress). In the meanwhile, patient developed a worsening of gait disturbance and acroparesthesias and the electromyography of the limb documented reduced recruitment pattern of motor units. During the second visit, he underwent α-galactosidase A activity measurement through dried blood spot to exclude Fabry disease (currently in progress) and was prescribed with dermatologist evaluation to evaluate the need for a skin biopsy.
Conclusions: This clinical case clearly points out on the potential difficulties in the diagnosis of a rare disease. Diagnosis and care usually require multidisciplinary expertise, but prompt diagnosis might be crucial for reducing the severity of outcomes, including morbidity and early mortality. PCSK9 inhibitors was found to be safe and effective in this patient.

Abstract n SP6_02 - Presenting author**: Alessia Cavicchioli**

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.

Abstract n SP6_03 - Presenting author**: Riccardo Mattia Ricciardi**

A border-line clinical expression in a patient with compound heterozygous mutation (LDLR/PCSK9): what is the best management?

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Background: Heterozygous mutations of LDLR, APOB or PCSK9 genes cause a severe increase in cholesterolemia ("Familial Hypercholesterolemia") and raise the risk of early cardiovascular events. Statin and ezetimibe reduce LDL-c by up to >60%, but it is often insufficient to reach the target. Today, PCSK9-inhibitors can further lower LDL-cholesterolemia and allow therapeutic targets to be reached even in the most difficult cases, even if their prescription is still strictly regulated.
Material and methods: We visited a 62 y female with high-cholesterol resistant to traditional therapies. Latest exams on treatment with rosuvastatin 40 mg/ezetimibe 10 mg were: Total cholesterol=236 mg/dL, HDL-c=45, triglycerides=121, LDL-c=167. Her DLS was 9. We performed a genetic study that revealed a compound mutation: LDLR (c.418G>T; p.Glu119*; null allele) and PCSK9 (c.60_65dupGCTGCT; p.Leu22_Leu23dup). Although "null allele" mutations are usually associated with severe phenotypes and patients could be considered to have homozygous FH-like phenotype, our patient was in primary prevention and her clinical characteristics were borderline (repeated LDL-c was exactly=130). As inclusion criteria required LDL-C ≥130, patient could not undergo treatment with PCSK9i, preventing the possibility to reach the LDL target.
Conclusions: Our patient is undoubtedly at high-risk and has not reached the LDL-goal. Treatment with PCSK9-i would significantly reduce LDL and consequently her cardiovascular risk. This case represent a paradigmatic example of the limits of AIFA criteria to access the PCSK9i treatment. It would be desirable that if there is a genetic diagnosis (currently not required) and patient is not at LDL-target, therapeutic plan for PCSK9i could be activated anyway.

Abstract n SP6_04 - Presenting author**: Martina Ferrandino**

Two sisters with severe hypertriglyceridemia caused by APOA5 biallelic variants

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Aim: Severe hypertriglyceridemia (HTG) is an autosomal recessive disease characterized by increased plasma levels of triglycerides (TG) (>886 mg/dL) and eruptive xanthomas, lipaemia retinalis, hepatosplenomegaly and pancreatitis. HTG is due to 2 pathogenic variants in 5 known genes involved in metabolism of the TG-rich lipoproteins (*LPL*, *APOA5, APOC2, GPIHBP1* and *LMF1*).
Methods: We analyzed two sisters with a clinical diagnosis of Familial Chylomicronaemia Syndrome (FCS) with multiple measurements of TG levels > 1000 mg/dL and maximum levels, respectively, > 6500 mg/dL and > 5000 mg/dL. Patients were analyzed by Next-generation sequencing (NGS) to detect variants in a large panel of 35 lipid related-genes.
Results: In both the sisters we have identified two heterozygous variants in the *APOA5* gene. According to ACMG guidelines the variant c.427delC was classified as Pathogenic, while the second one, c.49+5G>A, was classified as Uncertain significance variant (VUS). Genetic analysis of healthy parents reveled that each parent carried one of the two variants at the heterozygous state, indicating that in probands the variants were on the two different alleles (compound heterozygote). Based on this datum the variant c.49+5G>A received an additional moderate pathogenicity criterium, allowing to reclassify the variant as Likely Pathogenic.
Conclusions: This is the first evidence that the variant c.49+5G>A is Likely Pathogenic. We have identified two rare severe cases of HTG due to compound heterozygosity for *APOA5* variants. The patients with *APOA5* biallelic variants reported so far in literature are very few.

Abstract n SP6_05 - Presenting author**: Teresa Maria Grazia Fasciana**

Novel missense variants in the lmf1 gene: identification by next generation sequencing and functional characterization

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Aim: Hypertriglyceridemia (HTG) is a common form of dyslipidemia associated with an increased risk of cardiovascular disease and pancreatitis. The severe forms are characterized by very high plasma levels of triglycerides (TG) (> 1000 mg/dL -11.2 mmol/l). Monogenic autosomal recessive forms are characterized by homozygous or compound heterozygous loss-of-function mutations of genes involved in the intravascular lipolysis of the triglyceride-rich lipoproteins, namely lipoprotein lipase (LPL), apolipoprotein C2 (APOC2), apolipoprotein A5 (APOA5), glycophosphatidylinositol (GPI)-anchored high-density lipoprotein-binding protein 1 (GPIHBP1), lipase maturation factor 1 (LMF1), and glycerol-3-phosphate dehydrogenase 1 (GPD1). LMF1 has been shown to be essential for the maturation of both LPL and hepatic lipase (HL) to their fully functional forms.
Methods: We performed Next Generation Sequencing (NGS) analysis on Ion GeneStudio S5 Plus to study the coding exons and intron/exon boundaries of genes affecting the main pathways of triglyceride synthesis and metabolism.
Results: In the majority of subjects no functionally relevant mutations in the LPL, APOC2, APOA5, GPIHBP1 genes were detected. Four patients were found to be carriers of unknown missense variants in LMF1 gene: a) one compound heterozygous carrier for c.787C>T (p.His263Tyr) and c.1381C>T (p.Arg461Cys); b) one homozygous carrier for c.874 G>A (p.Gly292Arg). The other two were heterozygous carriers for c.1351 C/T (p.Arg451Trp) and c.428 C/T (p.Thr143Met) respectively. A functional analysis was carried out to assay LMF1 activity, protein expression and specific activity.
Conclusions: The results showed that the Arg461Cys and Gly292Arg dramatically impair LMF1 function, the Arg451Trp does not have an impact, whereas His263Tyr and Thr143Met exhibit moderate effects.

Abstract n SP6_06 - Presenting author**: Marina Lanza**

Identification of a novel nonsense mutation in the apob gene by next generation sequencing

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Aim: Familial hypobetalipoproteinemia (FHBL) is an autosomal codominant disorder of lipoprotein metabolism characterized by low plasma levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) and apolipoprotein B (apoB) below the 5th percentile of the distribution in the population. It may be due to loss-of-function mutations in APOB or, less frequently, in PCSK9 genes. The most frequent dominant monogenic form of HBL is the Familial Hypobetalipoproteinemia type-1 (FHBL-1, OMIM # 615558). The 50% of FHBL-1 cases is caused by mutations in APOB gene which result in assembly defects and secretion of lipoproteins containing apoB. Most of the FHBL-1 subjects are heterozygous carriers of nonsense pathogenetic variants and frameshift of the APOB gene which interfere with the complete translation of the mRNA coding the apoB protein, determining the formation of truncated forms of apoB. FHBL heterozygotes are generally asymptomatic but often develop fatty liver.
Methods: We designed a custom panel for Next Generation Sequencing (NGS) in order to analyze known genes involved in FHBL by Ion Torrent GeneStudio S5 Plus. We sequenced the FHBL candidate genes in 10 patients presenting LDL-C and ApoB levels below the 5th percentile.
Results: In the majority of subjects no functionally relevant mutations in candidate genes were detected. Two unrelated patients was found to be carrier of a novel heterozygous nonsense mutation in the exon 26 of the APOB gene (c.10324C>T, p.Gln3442Ter). The mutation lead to the formation of a premature stop codon and an apoB truncated protein of an expected size of 75.8% of wild type apoB (apoB-75.8).
Conclusions: In this work we describe a novel nonsense mutation of the APOB gene responsible for FHBL identified by a Next generation sequencing approach.

Abstract n SP7_01 - Presenting author**: Santo Colosimo**

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.

Abstract n SP7_02 - Presenting author**: Gramegna Davide**

Risk of atherosclerotic diseases in hypertensive patients with NAFLD and sleeping disorders

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Aim: Nonalcoholic Fatty Liver Disease (NAFLD) defines liver conditions caused by a build-up of fat in the liver in subject with no addition to alcohol. TYG score is a risk score for NAFLD. In hypertensive patients, NAFLD, as well as sleep apnoea might relate to an increased risk of atherosclerotic diseases. Then, our aim was to highlight if OSAS and NAFLD might be associated to induce ASCVD.
Methods: We evaluated 121 caucasian patients affected by arterial hypertension with a negative AUDIT score for alcohol addiction. Patients were evaluated by physical examination (BMI, waist circumference) and routine lab tests.
Lausanne NoSAS Score, ASCVD (Atherosclerotic Cardiovascular Disease) Risk Score and TYG (triglycerides × glucose) index were calculated for each patient. Patients were divided in higher (Group 1, 82 patients, 47 males, aged 66.72±8.67, NoSAS Score ≥8) and lower risk of OSAS (Group 2, 48 patients, 14 males, 60.72±13.43, NoSAS Score <8).
Results: Group 1 presented an increased BMI and waist circumference ($p<0.0001$ both). An increased rate of diabetes was also present (group 1 21.95% vs Group 2 2.56%, $p=0.006$). HDL was significantly decreased in group 1 ($p=0.027$). No difference there was in blood glucose, triglycerides, total and LDL cholesterol and, in particular, TYG score was similar. SBP, DBP and HR were also similar. On the contrary, a significant increase was found in ASCVD risk in group 1 (Group1 9.04±10.70% vs Group 2 15.65±10.49%, $p=0.008$). A significant direct correlation highlighted between ASCVD and both TYG and NoSAS ($p=0.0003$ and <0.0001 respectively). Moreover, NoSAS was significant direct correlated to diabetes rate ($p=0.0011$).
Conclusion: All patients with an increased OSAS risk have a tight correlation with a high risk of NAFLD and, in particular with an enhanced ASCVD score. This result shows the close correlation between sleeping disorders and NAFLD which participate to the development of ASCVD disease. Further studies will probably enhance primary and secondary prevention of cardiovascular events.

Abstract n SP7_03 - Presenting author**: Floriana Santomenna**

Metabolic associated fatty liver disease (MAFLD) and its features: different impact on the hepatic and cardiovascular complications

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Background and aims: Metabolic associated fatty liver disease (MAFLD) is defined by the presence of hepatic steatosis and one criteria among: (1) body mass index (BMI)>25 kg/m2; (2) type 2 diabetes (DM); (3) metabolic dysregulation in lean subjects (BMI<25). MAFLD exposes to hepatic and cardiovascular (CV) disease. Aim: to evaluate the different impact of each of the three features of MAFLD on the hepatic and CV disease.
Methods: 688 subjects (69% males, mean age 53+12 ys) were classified as MAFLD and enrolled in two Italian liver units. Both ultrasound (US) and a Fibroscan were performed to evaluate liver desease: the former, to detect and grade hepatic steatosis, the latter to diagnose advanced fibrosis (defined by liver stiffness measurement, LSM>8.2 kPa). CV disease was evaluated by carotid Doppler US and radiofrequency (carotid plaques; carotid stiffness as pulse wave velocity(PWV)), echocardiography (increased epicardial adipose tissue: EAT>9.5/7.5 mm M/F).
Results: Among the enrolled patients, 80% had BMI>25 and no other metabolic alterations (obese, group 1), 2% had DM without other metabolic alterations (group 2), 13% had BMI>25+DM (group 3) and 5% had BMI<25 with metabolic dysregulation (lean, group 4). Because of the small number of pure DM, we considered group 2 and 3 together (BMI>25+DM, group 2a). By comparing group 1 and 4, obese and lean patients had the same severity of liver (severe steatosis 16% vs 12%, p=0.63; advanced fibrosis 8% vs 3%, p=0.49) and CV disease (plaques 30% vs 44%, p=0.129; increases EAT 27% vs 33%, p=0.53; PWV 7.8+1.9 vs 7.9+1.9 m/s, p=0.77). When comparing patients with BMI>25+DM with simple obese or lean, an increased prevalence of severe steatosis was evident in this group vs the other two (30% vs 16%, p=0.006; 30% vs 12%, p=0.06) and >F3 (31% vs 8%, p<0.001; 31% vs 3%, p=0.001). As for CV disease, a higher prevalence of increased EAT (40%, p=0.02), carotid plaques (61% vs 30%, p<0.001) and increased PWV values (8.7+2 m/s vs 7.8+1.9, p<0.001) was seen in group BMI>25+DM compared only to pure obese, with superimposable results compared to lean subjects. In multivariate analysis (adjusted for age, sex, smoking and statins use), BMI>25+DM remained an independent risk factor for severe steatosis (OR 2.4, CI 95 1.5-4.1), >F3 (OR 3.6, CI 95 1.9-6.6) and carotid plaques (OR 1.8, CI 95 1.1-3.0).
Conclusions: Among all features of MAFLD, lean subjects with metabolic dysregulation present the same hepatic and cardiovascular alterations of obese subjects without DM and even the same CV alterations of patients with coexistence of obesity and DM. As expected, the coexistence of obesity and DM seems to play the major role in the onset of hepatic and CV damage This points out the need of a careful screening for complications and metabolic alterations in MAFLD patients regardless of the BMI.

Abstract n SP7_04 - Presenting author**: Giovanni Petralli**

Insulin resistance predicts advanced liver fibrosis in subjects with obesity and Metabolic dysfunction-Associated Fatty Liver Disease (MAFLD)

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Aim: Insulin resistance (IR) plays a pivotal role in the pathogenesis of Metabolic dysfunction-Associated Fatty Liver Disease (MAFLD), which can progress to liver fibrosis. We compared the performance of different markers of IR in predicting the severity of MAFLD-associated liver fibrosis and steatosis in obese non-diabetic individuals.
Methods: In 346 overweight/obese subjects with newly-diagnosed MAFLD (age 50.2±13.3 yrs, 34% females, BMI 30.8±4.4 kg/m2), liver stiffness (LS) and controlled attenuation parameter (CAP) were measured by Fibroscan® to assess liver fibrosis and steatosis. Anthropometric and biochemical data were collected to calculate surrogate markers of IR (HOMA-IR, TyG-I, TG/HDL ratio), liver fibrosis (NFS, FIB-4, APRI) and steatosis (FLI, HSI).
Results: HOMA-IR positively correlated with LS ($r=0.275$, $p<0.0001$), even after adjustment for BMI ($b=0.203$, $p<0.0001$) and liver-specific fibrosis markers, including NFS ($b=0.185$, $p<0.0001$), FIB-4 ($b=0.192$, $p<0.0001$), and APRI ($b=0.137$, $p=0.0002$), while TyG-I and TG/HDL ratio were not associated with LS. All three scores positively correlated with CAP. Insulin resistant individuals (HOMA-IR>2.5, n=165) had higher liver enzymes (ALT +15 (8,21) U/L, $p<0.0001$; AST +6 (2,9) U/L, $p<0.0001$), steatosis (CAP +28 (16,41) dB/m, $p<0.0001$), and fibrosis (LS +2.4 (1.3,3.6) kPa, $p<0.0001$), with a 4-fold increased risk for advanced fibrosis (LS >9.7 kPa, OR 4.42 (1.95-10.01), $p=0.0002$), compared with insulin-sensitive subjects. Among the two components of HOMA-IR, fasting insulin was independently associated with LS ($r=0.270$, $p<0.0001$), but not fasting glucose ($r=0.080$, $p=0.139$). ROC AUC for HOMA-IR and fasting insulin to predict advanced fibrosis were virtually identical (0.748 and 0.758, respectively).
Conclusions: In non-diabetic obese individuals with MAFLD, HOMA-IR provides a simple, independent marker of liver fibrosis and advanced liver disease. The relationship between IR and LS is largely mediated by hyperinsulinemia, while mild fasting hyperglycemia has a marginal role. Measuring insulin levels in MAFLD subjects might be useful to identify those more prone to liver fibrosis.

Abstract n SP7_05 - Presenting author**: Roberta Stupia**

Prevalence of portal vein thrombosis in non-alcoholic fatty liver disease: a meta-analysis of observational studies

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Background: Portal vein thrombosis (PVT) is a common complication of cirrhosis as a result of portal hypertension and modification in the hemostatic balance. Accumulating evidence now suggests that patients with non-alcoholic fatty liver disease (NAFLD), especially those with advanced forms, have an increased risk of PVT. Hence, we performed a meta-analysis of observational studies to estimate the overall prevalence of PVT in patients with NAFLD and its advanced forms compared with patients with advanced liver diseases from other etiologies.
Methods: We systematically searched PubMed, Scopus and Web of Science databases from the inception date to December 30 th 2022, using predefined keywords, to identify observational studies. Meta-analysis was performed using random-effects modeling.
Results: We included five observational studies for a total of 225,571 patients. Of these, 26,840 (11.9%) patients had NAFLD, whereas the PVT prevalence was 8.5% (n=2,280). When compared with patients with advanced liver diseases from other etiologies, patient with NAFLD and its advanced forms had a higher risk of prevalent PVT (OR 1.36, 100% CI 1.14-1.62 p < 0,01). The between-study heterogeneity was substantial (I 2 = 88%).
Conclusions: This meta-analysis suggests that compared with patients with advanced liver diseases from other etiologies, patient with NAFLD and its advanced forms had a higher risk of prevalent PVT. Further research is required to understand the complex link between NAFLD/NASH and PVT development.

Abstract n SP7_06 - Presenting author**: Giuseppe De Girolamo**

Misperception of dietary regimens in patients with nonalcoholic fatty liver disease

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Aim: Nonalcoholic fatty liver disease (NAFLD) encompasses a spectrum of histopathological features linked with fat accumulation in the liver, due to genetic and environmental factors, including excessive energetic introit and micronutrients deficiencies. It is widely known that Mediterranean diet (Md) exerts a protective role on cardiovascular and metabolic diseases. Although the South Italy culture assumes the knowledge of Md principles, metabolic diseases like NAFLD represent a healthcare growing burden. Here, we questioned the alimentary habits of NAFLD patients.
Methods: We enrolled 60 patients with NAFLD, referring to Centro Cure (Centre for diagnosis and treatment of hepatic diseases) of University of Foggia. The diagnosis of NAFLD and NASH was assessed by abdominal ultrasound and histological examination respectively. Every patient filled a self-administered survey reporting information regarding red or white meat, fish, wholegrain cereals, and olive oil consumption.
Results: Interestingly, 73% of patients defined their dietary regimen as “Mediterranean”, while only 22% reported an “Omnivorous” diet. However, 87% of subjects reported to eat red meat at least twice a week, and 18% consumes white meat more than three times a week. 54% assumes two or fewer portions of raw extra virgin olive oil (EVO) per day.
80.5% of patients, who reported a Mediterranean regimen, declared to consume red meat between 1 to 3 times per week, and more importantly, 44.6% and 80.8% of them reported to eat fish and wholegrain cereals less than once a week, respectively. Therefore, only 5% of patients declaring a Md regimen was truly adherent to Md principles.
Conclusions: Although most of NAFLD patients declared to follow a Md, the analysis of survey revealed unhealthy alimentary habits as it was reported a high assumption of meat, while wholegrain cereals, fish and EVO were underrepresented.
Clinicians should not underestimate patient misperception of alimentary regimens, and a multidisciplinary intervention is eagerly needed.

Abstract n SP7_07 - Presenting author**: Laura Vero**

Liver fibrosis is associated with an increased risk of non-fatal acute myocardial infarction

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Introduction: Compelling studies suggest that liver fibrosis is a risk factor not only for adverse liver outcomes but also for cardiovascular events. Percutaneous liver biopsy is the gold standard method for the assessment of hepatic fibrosis but has several limitations, including invasiveness, complications, and costs. To overcome these problems, several noninvasive scoring indexes have been developed by combining clinical and serological variables that are capable to discriminate the presence or the absence of advanced fibrosis. Recently, it has been developed and validated the Hepamet Fibrosis Score (HFS) which identified patients with advanced fibrosis with greater accuracy than the FIB-4 and the NAFLD fibrosis score. Whether HFS is able to identify patients with non-fatal acute myocardial infarction (MI) is unknown.
Aim: To evaluate the association between advanced liver fibrosis and prevalent MI in a cohort of adults participating in the CATAnzaro MEtabolic RIsk factors (CATAMERI) study.
Methods: Participants (n=2844) were divided into three groups according to HFS: low risk of fibrosis (<0.12); intermediate risk of fibrosis (≥ 0.12 to <0.47); high risk of fibrosis (≥0.47). The association between the risk of liver fibrosis and MI was analysed by a logistic regression model.
Results: Of the whole group, 24% subjects at high risk of liver fibrosis had MI in comparison to those with low risk (5.3 %) and a moderate risk (12.9%). In a logistic regression analysis, individuals at increased risk of liver fibrosis exhibited a 3.2-fold increased risk of having MI as compared with those with low risk (OR 3.18; 95% CI 1.31-7.70) independently of a number of confounders including smoking habit, total cholesterol, triglycerides, lipid-lowering therapies, and glucose lowering therapies.
Conclusion: In this cross-sectional study, individuals with higher values of HFS show a higher risk of MI, suggesting that cardiovascular screening are imperative in adults at risk for advanced liver fibrosis.

Abstract n SP8_01 - Presenting author**: Sining Xie**

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.

Abstract n SP8_02 - Presenting author**: Talerico Rosa**

Safety and effectiveness of oral anticoagulants in patients with atrial fibrillation and severe renal failure

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Aim: Patients with non-valvular atrial fibrillation (NVAF) and chronic kidney disease (CKD) have both an increased thromboembolic and bleeding risk. The aim of the study was to compare safety and effectiveness of direct oral anticoagulants (DOACs) versus vitamin K antagonists (VKAs) in patients with NVAF and severe CKD.
Methods: Patients on DOACs or VKAs for NVAF and CKD (eGFR ≤30-≥15 mL/min/1.73 m2 with Cockcroft–Gault or MDRD formula) referred to University Hospital of Perugia and Fondazione Policlinico Universitario A. Gemelli IRCCS of Rome from March 2013 to March 2022 were included. Primary study outcome: major bleeding (MB) according to ISTH criteria. Secondary outcomes: ischemic stroke/systemic embolism, clinically relevant non-major bleeding (CRNMB) according to ISTH criteria, death.
Results: 176 patients were retrospectively analyzed, 102 on DOACs and 74 on AVKs (Table 1). Overall, 29 MB events occurred. During a total observation period of 222 patient-years (PY) in the DOAC group and 208 PY in the AVK group, 18 and 11 patients developed MBs (8,1 and 5,3 per 100 PY, respectively) (Table 2). Concerning DOACs at MB time, 2 patients were taking an off-label dosage (apixaban 5 mg BID), 3 an inappropriate therapy according to CKD stage (dabigatran 110 mg BID). Cumulative rates of MBs did not differ significantly by log-rank test (p=0,460) between two groups (Figure 1). At Cox regression analysis, DOAC versus AVK use resulted in a not significant association with MBs (HR 1,63, 95%CI 0,75-3,54; p=0,21, whereas HAS-BLED (HR 1,61; 95%CI 1,10-2,35; p=0,013) and haemoglobin value (HR 0,71; 95%CI 0,57-0,90; p=0,004) resulted significantly associated with MBs, directly and inversely respectively.
Conclusions: In patients with AF and severe renal failure, anticoagulation with DOACs appeared to result in a higher risk of MBs in comparison to AVKs. Data from randomized studies are needed to clarify this issue.

Table 1. Baseline characteristics of the study population	DOAC (n=102)	AVK (n=74)	p value
Demographic			
Age (years)			
Mean ± SD	85,1 ± 7,1	83,7 ± 6,5	0,177
>75, n (%)	98 (96,1%)	69 (93,2%)	0,639
Range	57-101	66-98	NA
Female gender, n (%)	57 (55,9%)	44 (59,5%)	0,341
Laboratory tests			
Hemoglobin (g/dL), mean ± SD	11,9 ± 1,7	11,6 ± 1,6	0,141
Platelet (1,000/mm3), mean ± SD	208 ± 74	215 ± 96	0,59
Female gender, n (%)	57 (55,9%)	44 (59,5%)	0,341
Baseline eGFR, mean ± SD	26,4 ± 3,8	24,3 ± 4,7	0,002
Creatinine (mg/dL), mean ± SD	1,7 ± 0,6	2,2 ± 0,7	<0,001
Clinical characteristics			
Congestive heart failure, n (%)	54 (52,9%)	59 (79,7%)	<0,001
Hypertension, n (%)	97 (95,1%)	68 (91,9%)	0,085
Diabetes, n (%)	24 (23,5%)	29 (39,2%)	<0,001
Previous stroke/TIA, n (%)	20 (19,6%)	15 (20,3%)	0,829
Vascular diseases, n (%)			
History of MI/angina	26 (25,5%)	19 (25,7%)	0,956
Peripheral artery disease	13 (12,7%)	18 (24,3%)	<0,001
Liver disease, n (%)	4 (3,9%)	2 (2,7%)	0,381
Previous bleeding or predisposition, n (%)	12 (11,8%)	6 (8,1%)	0,112
Medication use predisposing to bleeding, n (%)	8 (7,8%)	12 (16,22%)	<0,001
Alcohol use, n (%)	0 (0%)	0 (0%)	NA
HAS-BLED score, mean ± SD	2,7 ± 0,9	3,1 ± 1,0	0,005
CHA_2DS_2-VASc score, mean ± SD	4,9 ± 1,4	5,5 ± 1,4	0,007
DOAC, n (%)			
Apixaban 5 mg BID	4 (3,9%)	NA	NA
Apixaban 2.5 mg BID	49 (48%)	NA	NA
Dabigatran 150 mg BID	0 (0%)	NA	NA
Dabigatran 110 mg BID	9 (8,8%)	NA	NA
Edoxaban 60 mg OD	1 (1%)	NA	NA
Edoxaban 30 mg OD	16 (15,7%)	NA	NA
Rivaroxaban 20 mg OD	1 (1%)	NA	NA
Rivaroxaban 15 mg OD	22 (21,6%)	NA	NA

Table 1. Baseline characteristics of the study population.
Values are presented as number (%), or mean ± standard deviation.
aspirin, clopidogrel, NSAIDs. BID: two times a day. OD: once daily. NA: not available.

Table 2. Study outcome events	N. of patients (Patient years *100)	
	DOAC (n=102)	AVK (n=74)
PRIMARY STUDY OUTCOME		
MAJOR BLEEDING (MB)	18 (8,1)	11 (5,3)
Type of MB		
Fatal bleeding	1 (0,4)	2 (1,0)
Fall in Hb level of 2 g/dL or transfusion (2 or more U)	12 (5,4)	3 (1,4)
Symptomatic bleeding in a critical area	5 (2,3)	6 (2,9)
Site of MB		
GI	8 (3,6)	4 (1,9)
ICH	3 (1,5)	4 (1,9)
Others	7 (3,2)	3 (1,5)
SECONDARY STUDY OUTCOMES		
ISCHEMIC STROKE OR SYSTEMIC EMBOLISM	2 (0,9)	0 (0,0)
CLINICALLY RELEVANT NON-MAJOR BLEEDING	5 (2,3)	6 (2,9)
DEATH	17 (7,7)	31 (14,9)

Table 2. Study outcome events.

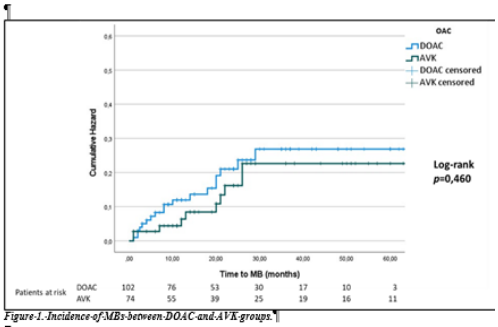

Figure 1. Incidence of MBs between DOAC and AVK groups.

Abstract n SP8_03 - Presenting author**: Giulia Barbieri**

Edoxaban effects on platelet function: in vitro and ex vivo studies

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Aim: All anticoagulants are expected to have an indirect effect on platelet function since they interfere with the generation or activity of thrombin, but the impact of Direct Oral Anticoagulants (DOACs) is largely unknown. Aim of this study was to evaluate Edoxaban effects on platelet function by in vitro and ex vivo studies.
Methods: We evaluated platelet aggregation (PA), thrombin generation (TG) and thromboxane B2 (TXB2) levels in 20 healthy donors: samples were incubated in vitro with increasing concentrations of Edoxaban [E50, E150, E250 (ng/mL)] or vehicle as control. We also investigated the same parameters in 12 patients with Atrial fibrillation treated with Edoxaban (ex vivo study).
Results: The incubation of PRP with different Edoxaban concentrations significantly reduced TF-induced PA with respect to vehicle. TF-induced PA was significantly lower in patients treated with Edoxaban than in controls. ADP and TRAP-6-induced PA was not inhibited by any Edoxaban concentrations in vitro, and also in patients versus controls in ex vivo experiments. THR-induced aggregation in E150 group showed a trend towards a reduction, though not significant. Among the parameters related to TG, Lag Time was significantly and positively related to Edoxaban concentrations. Patients showed more prolonged Lag Time values versus controls and ETP and Peak were significantly reduced in vitro. Moreover, ETP ratio values were significantly reduced according to increasing Edoxaban concentrations and also patients showed reduced levels of ETP ratio with respect to controls. We found a 24% decrease in serum TXB2 concentration in the E250 group versus control, while the reduction is not significant in other Edoxaban concentrations.
Conclusions: Edoxaban significantly reduces TF-induced PA in a dose-dependent manner, and it is also able of reducing TG and TXB2 levels. These data suggest its antiplatelet effect, which may, in turn, lead to the delayed/reduced formation of coagulation complexes reinforcing its antithrombotic potential.

Abstract n SP8_04 - Presenting author**: Romina Alessandroni**

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.

Abstract n SP8_05 - Presenting author**: Alfredo Caturano**

Impact of glycaemic control on cardiovascular outcomes and mortality in a cohort of patients with type 2 diabetes mellitus in a multifactorial randomized controlled trial

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Aim: The alteration of glucose metabolism significantly influences the natural history of cardiovascular disease in diabetic patients. Several studies have evaluated the role of glycemic control in cardiovascular risk, although its role in the context of a multifactorial intervention has not been assessed yet. The study aims to determine the impact of glycemic control on Major Adverse Cardiovascular Events (MACE) in a multifactorial randomized controlled trial.
Methods: Nephropathy In Diabetes type 2 (NID-2) is an open randomized clinical trial conducted on a population of type 2 diabetic patients followed in 14 Italian diabetes referral centers. Patients were center randomized to intensified treatment (MT) and Standard of Care (SoC). Of the 395 randomized patients, 368 completed the intervention phase (deaths=27) and of these 321 (SoC n.139; MT n.182) did not suffer from MACE during the intervention. They were analyzed according to the achievement of HbA1c<7% and according to treatment (MT vs. SoC).
Results: During the post-intervention follow-up (median 7.9 years, IQR 6.6-10.4) 183 MACE occurred (56.7%), 92 in the SoC group (33 in the HbA1c <7% and 59 in the ≥7% group), and 91 in the MT group (64 HbA1c events <7% and 27 in the ≥7% group). Kaplan Meier's analysis showed a statistically significant difference between the two arms of the SoC (p=0.0256), but not in the two arms of the MT group (p=0.198). Using the MT subgroup with HbA1c<7% as a reference, by Kaplan-Meier analysis there is no statistically significant difference with the MT subgroup and HbA1c≥7% (p=0.251) and with the SoC subgroup HbA1c<7% (p =0.472), while the difference with the SoC group and HbA1c≥7% is significant (p=0.01).
Conclusions: In the context of MT, obtaining good glycemic control has little impact on MACE compared to the control of other risk factors, but it is important if MT is contraindicated.

Abstract n SP8_06 - Presenting author**: Alessandro Croce**

Use of combination treatment in hypertension management and its association with hypertension control

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Aim: Achievement of blood pressure (BP) targets in hypertension is crucial for cardiovascular prevention and, in most cases, requires combined pharmacological treatment. Current ESC/ESH Guidelines for hypertension management recommend initiating treatment with combination therapy in most hypertensives, except low-risk individuals with mildly elevated BP. Our aim was to assess how this recommendation is applied in practice and whether it is associated with differences in hypertension control.
Methods: In the setting of an ongoing cardiovascular prevention trial (CV-PREVITAL) we prospectively recruited 278 hypertensive patients with no history of cardiovascular disease, among individuals who responded to the advertisement campaign in outpatient facilities of Istituto Auxologico Italiano. Physical examination, standard routine blood tests, office blood pressure recording and standard 24h ABPM has been performed at baseline. We analyzed the office and ambulatory BP control in relationship to the number of antihypertensive drugs and cardiovascular risk assessed by SCORE risk countries algorithm.
Results: Median age of our sample was 61 years, with balanced gender distribution. Ninety-nine (36%) participants were not receiving any pharmacological treatment, 106 (41%) were on monotherapy and 73 (23%) on combined treatment. Comparison of principal characteristics among the three groups is shown in Table 1.
Among study participants 44 (16%) had low to moderate CV risk and 227 (84%) had high or very high estimated risk. The percentage of participants receiving combined treatment in these groups was 4.1% and 22.5%, respectively, and the percentage of patients with well controlled office/ambulatory BP was 50%/41% in low to moderate risk and 35%/65% in high to very high risk participants, respectively.
Conclusions: In this sample of patients with known hypertension, a considerable proportion received substandard treatment (no treatment or monotherapy), even among those at high risk. This was associated with worse BP control in these individuals. Implementation of current hypertension guidelines in practice remains largely suboptimal.

	Overall (N= 278)	No treatment (N= 99)	Monotherapy (N= 106)	Combination (N= 73)	P value
Age (years)	61.9±9.2	59.7±9.3	63.5±9.5	62.4±8.1	**0.0106**
Male sex (N, %)	139(50%)	49(49%)	49(46%)	41(56%)	0.4224
BMI (kg/m2)	27.4±5	26.6±5.2	27.4±4.4	28.3±5.3	0.0822
24-hour SBP (mmHg)	133.3±12.7	137.8±12.9	132.8±11.9	128±11.6	**<0.001**
24-hour DBP (mmHg)	79.2±8.1	81.8±8.9	79.1±7.5	75.6±6.4	**<0.001**
24-hour HR (bpm)	70.4±8.6	70.1±8.8	71±7.5	70±10	0.6407
Office SBP (mmHg)	142.2±16.7	149.5±14.1	139.3±16.2	136.7±17.5	**<0.001**
Office DBP (mmHg)	86.7±9.9	91.1±8.8	85.4±10.1	82.8±8.9	**<0.001**
BP Category (N, %)					
Sustained NT	55 (20%)	0 (0%)	27 (25%)	28 (38%)	**<0.001**
White coat HT	44 (16%)	24 (24%)	12 (11%)	8 (11%)	
Masked HT	40 (14%)	0 (0%)	26 (25%)	14 (19%)	
Sustained HT	139 (50%)	75 (76%)	41 (39%)	23 (32%)	

Table 1. Participants' characteristics according to number of antihypertensive drugs. Data shown as means±SD or counts(proportions)

Abstract n SP8_07 - Presenting author**: Stefano Scotti**

Role of concomitant chronic therapies on adherence to cardiovascular treatments

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Background: Adherence to pharmacological therapies is of paramount importance in achieving therapeutic goals. Among influencing factors, concomitant therapies could play a key role. We aimed at assessing adherence to three chronic cardiovascular (CV) therapies, focusing on patients with concomitant treatments.
Methods: Using administrative data from 3 Local Health Units (LHUs) in Lombardy, three cohorts of new users of antihypertensive, lipid-lowering (statins), and antidiabetic treatments were identified. Within each cohort, subjects were stratified according to the concomitant presence of the other CV therapies. Adherence was calculated with the proportion of days covered (PDC) method. Through a logistic regression analysis, the association between concomitant CV therapies and the level of adherence was estimated, as well as the effect of influencing covariates.
Results: In the antihypertensive cohort, 76.4% of patients were taking only antihypertensives, while 3.3% were taking all three CV therapies. These values were 26.9% and 13.0% in the statin cohort, and 24.8% and 30.40% in the antidiabetic cohort, respectively. Overall, the mean PDC always remained below 0.80 (standard cut-off for optimal adherence); however, PDC level (mean±DS) increased when concomitant CV therapies were present in the antihypertensive cohort (from 0.70±0.29 with monotherapy to 0.81±0.25 with triple therapy) and in the statin cohort (from 0.73±0.21 to 0.80±0.20). Instead, this value remained almost unchanged in the diabetic cohort (from 0.60±0.27 to 0.59±0.26). Being female and older than 80 years were associated with a lower probability of being adherent; however, while an increase in adherence was observed in both sexes with concomitant CV therapies, an increase in age seemed to attenuate this effect.
Conclusions: In the cluster of CV therapies, the presence of other CV treatments was mostly associated with higher adherence, suggesting that awareness of increased CV risk is a powerful driver of therapy adherence.

Abstract n SP9_01 - Presenting author**: Elsa Franchi**

ApoA-I deletion or overexpression in apoE deficient mice alters lipid deposition in peripheral tissues

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The reverse cholesterol transport is a multistep process whereby excess cholesterol is conveyed by HDL from the peripheral tissues to the liver for excretion. In this study, the impact of the genetic manipulation of HDL/apoA-I levels on lipid deposition in intestine, liver, kidney and adrenals was investigated.
Mice with extremely low plasma HDL levels, deficient for both murine apoA-I and apoE (DKO), were compared with mice characterized by elevated HDL, deficient for both apoA-I/apoE, but overexpressing human apoA-I (DKO/hA-I). Mice, both female and male, were fed a standard rodent diet until one year of age. Plasma lipids were quantified by enzymatic methods. Intestine, liver, kidney and adrenal morphology was evaluated by light microscopy on frozen sections.
Plasma total cholesterol concentration in DKO mice was comparable with that of wild-type mice and 3-fold lower than that observed in DKO/hA-I mice. Plasma HDL-C was almost absent in DKO mice and strongly elevated in DKO/hA-I mice. The H&E-stained sections did not reveal the presence of morphological alterations in the tissues analyzed: intestinal villi and crypts were regular, steatosis in liver parenchyma, as well as foam cells in renal glomeruli were absent and adrenal size was comparable in both genotypes. The neutral lipid-specific staining with Oil Red O showed instead interesting differences. The intestine did not exhibit HDL-mediated effects on lipid deposition. On the contrary, in the hepatic parenchyma, an increased accumulation of lipids around the centrilobular vein was observed in DKO/hA-I mice only. In addition, within the glomeruli and the adrenal cortex of DKO/hA-I mice, lipid accumulation was significantly higher than in DKO.
On summary, although DKO mice are almost completely devoid of HDL and prone to atherosclerosis development, they do not exhibit signs of abnormal lipid accumulation in liver, kidney and adrenals, as in DKO/hA-I mice, characterized by elevated HDL levels.

Abstract n SP9_02 - Presenting author**: Martina Ugolotti**

Development of novel PCSK9 inhibitors as pharmacological approaches for the treatment of alzheimer's disease: in vitro and in vivo studies

Martina Ugolotti, Bianca Papotti[1], Francesca Zimetti[1], Ilaria Zanotti[1], Martina Bodria[2], Antonietta Vilella[2], Daniela Giuliani[2], Lisa Giannessi[1], Marco Radi[1], Maria Giovanna Lupo[3], Nicola Ferri[3], Franco Bernini[1]

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Aim: Impairment in 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 ELISA kit; cholesterol uptake was measured with radioisotopic techniques. 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 liver 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, MR-533 exhibited good *in vivo* tolerability; MR-3 and MR-532 were detected both in plasma and in brain tissue.
Conclusions: Preliminary *in vitro* screening allows 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.

Abstract n SP9_03 - Presenting author**: Luana Orlando**

PCSK9: A New Marker of Cardiovascular Risk in Post-menopausal Diabetic Women in Primary Prevention

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Introduction: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a circulating serine protease involved in low-density lipoprotein receptor (LDLr) degradation, inducing increase of LDL-c concentrations. Furthermore, PCSK9 may be related to glycemic profile dysregulation affected by estrogens. We aim to assess its role in CVD, evaluate PCSK9 predictive factor and correlation with CV parameters in post-menopausal diabetic women in primary prevention.
Methods: An Italian multicentric observational study was conducted on post-menopausal diabetic women. Descriptive statistics were adopted to evaluate clinical and demographics characteristics of patients stratified according to PCSK9 concentration quartile ranges. Generalized linear models were adopted to investigate predictors of PCSK9 concentration, including PCSK9 levels, related to the following CV outcomes: pulse wave velocity (PWV), pulse pressure (PP), augmentation index (AI).
Results: A total of 157 post-menopausal diabetic women in primary prevention, with a median (Q1-Q3) age of 65 (59-75) years, were enrolled in the study. Median (Q1-Q3) serum PCSK9 levels were 370.5 (344.0-410.0) ng/ml. Significant differences on diastolic blood pressure and triglycerides levels were observed among PCSK9 quartile ranges (p = 0.043 and p = 0.049, respectively). The analysis of CV outcomes showed significant differences in AI values only (p = 0.034). ApoB values resulted an independent predictor of PCSK9 concentration (B = 0.002; p = 0.005). However, LDL values were inversely related to PCSK9 concentration (B = -0.001; p = 0.001). Moreover, PCSK9 levels and systolic pressure influenced PWV (B = 0001; p = 0.009 and B = 0.004; p < 0.001, respectively), although PCSK9 did not influence other CV outcomes (PP: B = 0.000, p = 0.519; AI: B = 0.000; p = 0.860).
Conclusion(s): Our data suggest that PCSK9 concentrations directly influence PWV and are influenced by apoB and LDL-c levels. Further studies are needed to establish circulating PCSK9 levels as a CV risk biomarker.

Abstract n SP9_04 - Presenting author**: Simone Bini**

ANGPTL3 and PCSK9 interact and show coordinated metabolic regulation in vitro

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Background and Aims: ANGPTL3 and PCSK9 are known regulators of lipoprotein metabolism. Patients harboring homozygous loss of function mutations in the *ANGPTL3* gene, show reduced levels of circulating PCSK9, indicating a possible coordinate regulation of these two proteins. This study aimed to establish whether the two proteins can cross-regulate in different conditions of nutritional availability.
Material and Methods: *ANGPTL3*, *PCSK9,* or both genes were overexpressed in HepG2 cells grown in glucose rich (*Feeding*) and glucose poor (*Fasting*) conditions. We performed Real-time qPCR to study ANGPTL3 and PCSK9 mRNA levels, Co-immunoprecipitations (Co-Ip) to verify protein-protein interaction and western-blotting to quantify the produced proteins and ApoB secretion both intracellularly and extracellularly in the culture medium.
Results: Glucose determines a 5-fold increase in the ANGPTL3 mRNA levels and a 1.5-fold the PCSK9 mRNA levels in HepG2 cells. The Co-immunoprecipitation in baseline growth conditions highlighted a direct protein-protein interaction of PCSK9 and ANGPTL3 intracellularly. The western blot analysis showed that the two proteins have a similar secretion pattern dependent from glucose availability in the culture medium. ANGPTL3 overexpression determines PCSK9 intracellular accumulation in *fasting* conditions, the opposite is observed in the overexpression of PCSK9. The double overexpression determines a consensual secretion increase of both proteins, more evident in *feeding* conditions. In addition, also ApoB secretion appears to be tightly dependent on glucose levels showing a substantial increase in case of ANGPTL3 and PCSK9 overexpression (Fold-change x2,8).
Conclusion: ANGPTL3 and PCSK9 are transcriptionally cross-regulated, they respond to changes in glucose availability and show a co-secretion pattern *in vitro*. The two proteins are in intracellular close interaction, they are finely regulated in the same direction in response to metabolic stimuli and both promote ApoB secretion and accumulation in culture media.

Abstract n SP9_05 - Presenting author**: Sara Matteucci**

PCSK9 interacts with a subspecies of LDL with IDL-like characteristics

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Introduction: Circulating PCSK9 is known to interact with the LDL-R thus promoting its degradation, reducing its recycling and blunting the uptake of LDL from the circulation. In this context, two anti-PCSK9 monoclonal antibodies are approved for the treatment of hypercholesterolemia. Previous studies have demonstrated that a significant portion of circulating PCSK9 associates to LDL. The purpose of our research is to better understand the PCSK9-lipoprotein interaction.
Methods: A three-layered iodixanol gradient was used to isolate lipoproteins fractions from plasma of patients before and after mAbs treatment. Lipoproteins components were studied by ELISA, lipidomic and proteomic analysis.
Results: After therapy, LDL-C levels decreased (115±52 to 46±27 mg/dL), while plasma PCSK9 levels increased 10-fold ($p<0.05$) of which on average 15% was associated with LDL, with no apparent correlation to LDL-C (n=27). Our analyses demonstrated that PCSK9 is associated to a LDL subfraction that has a lower density than average LDL, due to its percent composition: 5% proteins, 23% phospholipids, 23% triglycerides, 39% cholesterol ester and 15% free cholesterol. By LC-MS, this subfraction showed a higher amount of ApoE, ApoCI, ApoCII, ApoCIII than LDL fraction (89±10 and 11±1; 64±8 and 6±1; 76±10 and 15±2; 53±5 and 6±1 attomoles/femtomoles ApoB respectively; $p<0.05$).
Conclusions: Our studies identified a LDL subfraction more buoyant than the classical LDL, an IDL-like lipoprotein involved in PCSK9 binding. MAbs therapy significantly reduced the LDL-C levels while the total amount of PCSK9 increased. The biological activity and the nature of the PCSK9 bound to LDL is under investigation.

Abstract n SP9_06 - Presenting author**: Ilaria Rossi**

In vitro silencing of ANGPTL3 increases lipid accumulation in hepatic Huh7 cell line

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Aim: ANGPTL3 is an hepatokine acting as negative regulator of lipoprotein lipase (LPL) and targeted by multiple therapies. Vupanorsen, ANGPTL3 directed antisense oligonucleotide, has been discontinued from phase 2b clinical trial due to an unexpected increase in liver fat fraction. The aim of this project is to shed new insights on the intracellular mechanism causing fat accumulation.
Methods: We utilized hepatocarcinoma Huh7 cells treated with siRNA-ANGPTL3, human recombinant ANGPTL3 (hrecANGPTL3) or the combination of the two (siRNA+hrec).
By western blot, Oil red-O, biochemical assays and ELISA assays, we analysed the expression of genes and proteins involved in lipid metabolism.
Results: Oil red-O analysis demonstrated that lipid content increased after ANGPTL3 silencing (5.89±0.33 fold), hrecANGPTL3 administration (4.08±0.35 fold) and the combination of both (8.56±0.18 fold) compared to untreated cells.
We observed an increase in pro-SREBP1 and fatty acid synthase, respectively by 100% and by 45% after siRNA-ANGPTL3 and combined treatment.
Cellular LPL activity doubled with siRNA-ANGPTL3 treatment as expected. No differences in secreted ApoB and total cholesterol were found in the different conditions.
Conclusions: efficient lipid accumulation following gene silencing emerged from our experiments, mirroring what was seen in patients treated with vupanorsen. The investigation led us to hypothesize an increase in triglyceride synthesis and lower secretion rate when ANGPTL3 is silenced. Moreover, silencing and administration appear to behave in an addictive manner, following two different pathways that are currently under analysis. These results suggest a possible hepatic role of ANGPTL3 which is still unknown and must be further studied.

Abstract n SP9_07 - Presenting author**: Angelica Di Agostini**

Injection site reaction to Alirocumab and Evolocumab in a young woman with familial hypercholesterolemia: a possible cell-mediated hypersensitivity to polysorbate and an effective therapeutic alternative based on Inclisiran

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Aim: Proprotein convertase subtilisin/kexin type 9 (PCSK9)-inhibitor is recommended in patients with familial hypercholesterolemia (FH), if low-density lipoprotein cholesterol (LDL-C) goal is not achieved with high intensity statin+ezetimibe. Currently, only two fully human monoclonal antibodies (mAbs) with subcutaneous route, alirocumab and evolocumab, are available in clinical practice.
Methods: We describe a young female patient with heterozygous FH developing injection site reaction (ISR) to both mAbs with a subsequent exposure to an increased risk of future cardiovascular disease.
Results: a 28-year-old woman was referred to our centre for LDL-C 364 mg/dl and Lp(a) 178 mg/dl. Given the family history of hypercholesterolemia and premature coronary heart disease, and the bilateral Achilles tendon xanthomas, genetic testing was performed resulting in a positivity for a variant c.1374_1375del, p.(Arg458SerfsTer8) in the LDLR gene in heterozygosis (null allele). After 3 months of treatment with rosuvastatin 40 mg + ezetimibe 10 mg, LDL-C was still 198 mg/dl. Therefore, the patient started treatment with alirocumab 150 mg SQ every 2 weeks. After 48 hours following the second and third SQ injections in left arm and in contralateral arm, respectively, the patient developed a painful ISR with an erythematous nodule of about 5 cm in diameter, compatible with cell-mediated hypersensitivity reaction, resolved after few days of topical application of betamethasone. Similar ISR appeared with evolocumab 140 mg, despite pretreatment with oral prednisone. A ISR for polysorbate, an excipient contained in both mAbs, was suspected. Therefore, the small interfering RNA (siRNA), inclisiran, which does not contain polysorbate, was started without adverse reaction. Three months after the first injection of inclisiran, LDL-C was 103 mg/dl and Lp(a) was 113 mg/dl.
Conclusions: Inclisiran is an efficacious therapeutic alternative in patients at high and very high CV risk who cannot achieve LDL-C goal despite maximal dose of statin+ezetimibe and are intolerant to mAbs.

Abstract n SP10_01 - Presenting author**: Lara Ponsa**

Renal denervation in patients with resistant hypertension: follow up 2011-2021 of the series of the ESH center of excellence in Turin

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Aim. Renal denervation (DRN) is one of the invasive therapeutic approaches of resistant arterial hypertension. The aim of this study was to re-evaluate patients undergoing DRN, in terms of safety and efficacy.
Methods. A retrospective evaluation of patients undergoing DRN between 01/01/2011 and 31/12/2021 in the Arterial Hypertension Center of Turin.
Results. The series included 14 subjects, of which 8 (57%) were female and with an average age 54±10 years. The number of antihypertensive drugs before renal denervation was 5.7±1.0 and at baseline the blood pressure was 188/113±22±18 mmHg. 28% of subjects had a history of ischemic heart disease and 27% of previous stroke and/or transient ischemic event. 9 subjects were denervated with Symplicity device, 1 with Vessix, 4 with Symplicity Spyral. The mean duration of follow-up was 5.2 years. From a safety point of view, there were no short-term or long-term complications. In terms of efficacy, the number of responders at 6 months, defined as a reduction of at least 10 mmHg in systolic blood pressure (PAS) at 24h Blood Pressure Monitoring, was 9 subjects (64%). Two subjects reported systolic blood pressure values below 135 mmHg.
Regarding the type of device used, 75% of subjects treated with Spiral devices were responders, compared with 60% of subjects treated with other catheters. Among the non-responders, 2 presented events one year after the DRN. Only 1 responder had high level of renin at baseline.
Conclusions. The data relating to the case studies of the Arterial Hypertension Center of Turin highlight the safety of the renal denervation procedure and a blood pressure response in 60% of patients. The latest generation catheters seems to be more effective. However, the small number of cases did not allow to identify predictive factors of response to DRN.

Abstract n SP10_02 - Presenting author**: Di Gioia Giuseppe**

Association of kidney function impairment and coronary artery disease severity

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Aim: We explored the association of kidney function with the magnitude of coronary artery disease (CAD) in terms of the number of coronary arteries involved.
Methods: We recruited 150 patients affected by CAD from the Cardiology Unit of Policlinico di Foggia between March 2018 and November 2018 who underwent coronary angiography. Participants were stratified based on the number of coronary arteries affected by the atherosclerotic disease (single vessel disease; two-vessel disease; three-vessel disease). Measurements of glomerular filtration rate (eGFR) were obtained from blood tests. Stages of CKD were defined according to eGFR levels (ml/min) (G1, >90; G2, 89-60; G3a, 59-45; G3b, 44-30; G4, 29-15; G5, <14). Data about age, sex, anthropometrics, lifestyle, comorbidities were collected. Ordinal regression model was used to assess the odds ratio between groups and CKD cut-offs. Analysis was controlled for gender, age, smoking status, BMI, diabetes status and hypertension.
Results: Of the 150 participants (mean age 65.84 ± 9.94, range 45 – 87 years), 111 (74%) were male. Patients with at least two cardiometabolic diseases (heart disease, hypertension, stroke, type 2 diabetes, obesity) were more likely to be male and smokers. A one-point increase in eGFR levels was associated with a decreasing number of coronary arteries involvement (OR 0.97, p-value <0.05). Interestingly, analysis by eGFR cut-offs showed increasing or decreasing odds trends according to the severity of CAD. Among patients with three-vessel disease, the odds were highest at the G5 stage and decreased with lower stages until they were lowest at the G1 stage. Similarly, the two-vessel group showed lower odds at the G5 stage and higher odds at the G1 stage. In addition, the probability of being in the one-vessel group was the highest at the G1 stage and the lowest at the G5 stage.
Conclusions: According to our study, the number of coronary arteries affected in CAD patients is associated with kidney function.

Abstract n SP10_03 - Presenting author**: Giammarco Camedda**

Factor associated with hyperuricemia development during a 25 years follow-up: the PAMELA Study

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Background and aim: HyperUricemia (HU) has been associated with future fatal and non fatal CV events as well as with hypertension and metabolic syndrome development. However, only few data exist regarding the change in Uric Acid (UA) and the development of HU during a population follow-up. In particular, no study analyzed the variations during a very long follow-up. The present study was aimed at evaluating the factors associated with UA changes and HU development during a 25-years follow-up in the PAMELA study. Both the classic cut-off for hyperuricemia and the newly one identified by the Uric Acid Right for Heart Health (URRAH) study were used to define HU.
Methods: we analyzed data collected in 561 subjects of the Pressioni Arteriose Monitorate E loro Associazioni (PAMELA) study with available SUA who complete the 3rd wave study (mean follow-up time 25.2±0.5 years). The classic cut-off of 6/7 mg/dL (females and males respectively) and URRAH one (5.1/5.6 mg/dL for females and males) were used for HU definitions.
Results: mean UA value during follow-up increase from 4.7±1.1 to 5.0±1.2 mg/dL ($p<0.001$) with a mean increase of 0.3±1.1 mg/dL. UA changes significanlty correlated with baseline UA ($r=-0.39$, $p<0.001$) and creatinine ($r=-0-21$, $p<0.001$). With the classic cut-off (6/7 mg/dL) 9.1% of the subjects develop HU and this was associated at the multivariate model with age, sex (male), baseline diastolic Blood Pressure (BP) (OR 1.038, $p=0.02$) and UA (OR 2.62, $p<0.001$). With the new URRAH cut-off 19.1% of the subjects develop HU. At the multivariate model variables associated were gender (male) and baseline 24h systolic BP (OR 1.05, $p<0.001$), tryglicerides (OR 1.01, $p=0.01$) and UA (4.99, $p<0.001$).
Conclusions: The present study provide evidence that in the PAMELA study during the 25 year follow-up there is a progressive increase in UA. Baseline UA is the most important factor associated with HU development. However, also age, gender, triglycerides and BP could help to identify subjects that most probably will develop HU.

Abstract n SP10_04 - Presenting author**: Elisa Sconfienza**

Prevalence of functioning adrenal incidentalomas: a systematic review and meta-analysis

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Aim. Adrenal hyperfunction is associated with an increased risk of cardio-metabolic complications in subjects with adrenal incidentaloma. The aim of our study was to assess the prevalence of autonomous/possible autonomous cortisol secretion, primary aldosteronism, pheochromocytoma and Cushing syndrome in patients with adrenal incidentaloma.
Methods. We performed a comprehensive search of multiple databases (PubMed, Ovid MEDLINE, Web of Science) for potentially relevant studies without language restriction, up to February 2022. Studies were excluded if the diagnostic criteria used for diagnosing the hormonal alteration were not available or not in agreement with the available guidelines, in case of duplicate reports or if the studies were performed on selected cohorts of patients/ specific populations.
Results. Of the 1,661 publications evaluated at title and abstract levels, 161 were examined as full text and 36 were included. The overall prevalence of functioning adrenal incidentalomas was 27.5%. Autonomous cortisol secretion/possible autonomous cortisol secretion, with a prevalence of 11.7% (95% C.I. 8.6, 15.7), was the most frequent hormonal alteration, while primary aldosteronism occurred in 4.4% of the patients (95% C.I. 3.1, 6.2). Subgroup analysis showed that primary aldosteronism was more prevalent in patients from Asia compared with patients from Europe/America, on the contrary, autonomous cortisol secretion/possible autonomous cortisol secretion had a lower prevalence in Asian countries. At meta-regression analysis, the prevalence of autonomous cortisol secretion/possible autonomous cortisol secretion was influenced by the proportion of female patients, while the prevalence of primary aldosteronism was positively associated with the proportion of patients with hypertension and the publication year. Finally, pheochromocytoma and Cushing syndrome prevalence were 3.8% (95% C.I. 2.8, 5.0) and 3.1% (95% C.I. 2.3, 4.3), respectively.
Conclusions. This meta-analysis provides extensive data on the prevalence of functioning adrenal incidentalomas and the factors affecting heterogeneity in prevalence estimates.

Abstract n SP10_05 - Presenting author**: Ilaria L. Pizzulo**

Metabolic syndrome and its components predict the development of arterial stiffening in a sample of adult men – the Olivetti Heart Study

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Aim. Metabolic Syndrome and its components are associated with greater cardiovascular risk. A number of studies found a positive association between Metabolic Syndrome and vascular damage, but few observational studies evaluated the predictive role of Metabolic Syndrome on Arterial Stiffening. Therefore, the aim of this study was to estimate the ability of Metabolic Syndrome and its components to predict the risk of Arterial Stiffening, in an 8-year follow-up of a sample of adult men (Olivetti Heart Study).
Methods. The analysis included 778 men without Arterial Stiffening (pulse pressure >60 mmHg) at baseline. A positive diagnosis of Metabolic Syndrome was made by recognized criteria, if at least three components were present.
Results. At the end of follow-up period, there was an incidence of 11% in Arterial Stiffening. The percentage of participants that developed Arterial Stiffening was greater in the Metabolic Syndrome group than those without Metabolic Syndrome, also after adjustment for main confounders (OR: 2.5, 95%CI:1.3-4.9). The risk of Arterial Stiffening also increased with increase in numbers of Metabolic Syndrome elements (p for trend<0.01). In addition, the analysis of the predictive role of the single Metabolic Syndrome component showed that high blood pressure was the strongest predictor.
Conclusions. The results of this prospective study indicate a predictive role of Metabolic Syndrome on Arterial Stiffening, independently of main confounders. In addition, high blood pressure seems the strongest predictor of Arterial Stiffening, among Metabolic Syndrome components.

Abstract n SP10_06 - Presenting author**: Jacopo Burrello**

Cardiovascular risk, metabolic profile and inflammatory fingerprint of obese subjects who underwent bariatric surgery

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Aim: Bariatric surgery has emerged as effective treatment for obesity not only for the substantial and durable weight loss, but also by improving several obesity-related comorbidities. Extracellular vesicles (EVs; membrane nanoparticles released by cells) reflect endothelial dysfunction and inflammation, and may predict post-surgical outcomes in these patients. The aim of our study was to exploit an EV signature to assess cardiovascular (CV) risk, metabolic profile, and inflammatory fingerprint before and after bariatric surgery.
Methods: The study cohort was composed by 62 patients; for each subject, clinical/biochemical parameters, disease status (including diabetes, dyslipidemia, hypertension), medications, and EV profiling were evaluated at baseline (T0) and 1-/3-years (T1/T2, respectively), after surgery (sleeve gastrectomy or Roux-en-Y-gastric-bypass). EVs were isolated from serum by beads-based immuno-capture and analyzed for the expression of 37 membrane-associated antigens.
Results: After bariatric surgery, patients gradually lost weight from a median of 110 at T0 down to 80Kg at T2 (cumulative weight loss 31Kg; 29.9% BMI reduction). Accordingly, the overall CV risk and metabolic-inflammatory profile of patients improved: blood pressure, HbA1c, total cholesterol, triglycerides, LDL, uric acid, white blood cells, and C-reactive protein decreased, while HDL and renal function (eGFR) increased at follow-up; prevalence of hypertension, dyslipidemia, and diabetes decreased together with the number of assumed drugs (anti-hypertensives, statins, hypoglycemic, and anti-platelets agents). Consistently, levels of expression of EV specific markers (CD9-CD63-CD81) and 11 out of the 37 evaluated antigens (mainly from endothelium, platelets, and inflammatory cells) decreased at T1/T2, reflecting changes of main CV risk indicators. Interestingly, lower baseline levels of CD4-CD31-CD40-CD42a-CD62P were associated to a complete post-surgical outcome, defined as no residual disease without medications, with a BMI at T2 lower than 30Kg/sqm.
Conclusions: EV-derived biomarkers reflect the improvement of CV profile in patients who underwent bariatric surgery and may become a new tool to predict post-surgical outcome.

Abstract n SP10_07 - Presenting author**: Domenico Tricò**

Metabolic crosstalk between adipose tissue and β-cells in youths with primary insulin hypersecretion

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Aim: Excessive insulin secretion independent of insulin resistance, defined as primary hypersecretion, is associated with obesity and type 2 diabetes (T2D) progression. The mechanisms and metabolic effects of chronic β-cell overstimulation in the early disease course remain elusive. In this study, we examined whether the adipocyte-insular axis can promote the development and maintenance of insulin hypersecretion and the progression to glucose intolerance in youths with obesity.
Methods: In 100 non-diabetic adolescents with obesity, primary insulin hypersecretion was defined as the upper tertile of the residuals' distribution of the oral glucose tolerance test (OGTT)-derived insulin secretion rate (ISR) by insulin sensitivity fit curve. Hepatic fat fraction (HFF) and visceral (VAT) and subcutaneous adipose tissues (SAT) were measured by abdominal MRI. Adipocyte cell size distribution was assessed in abdominal SAT biopsies. Subsets of subjects underwent a two-step euglycemic-hyperinsulinemic clamp, a long-term 2H2O labeling protocol to assess lipid turnover, and a second OGTT after a 2.3-year follow up.
Results: Compared with normosecretors, hypersecretors had enhanced model-derived β-cell glucose sensitivity and rate sensitivity, but worse glucose tolerance, despite similar demographics, adiposity, and insulin resistance. Hypersecretors also had higher HFF and VAT proportion, enlarged adipocytes, higher FFA and leptin levels per fat mass, and faster lipid turnover. At follow up, hypersecretors had increased fat mass and a 3-fold higher risk for altered glucose tolerance, while individuals with larger adipocytes or higher leptin levels showed increased β-cell glucose sensitivity.
Conclusion: In youths with overweight/obesity, primary insulin hypersecretion is associated with alterations in adipose tissue distribution, cell morphology, and lipid fluxes, independent of whole-body adiposity and insulin resistance. The complex metabolic crosstalk between adipose tissue and β-cell may influence the trajectory of glucose tolerance and β-cell function over time.

Abstract n SP11_01 - Presenting author**: Lorenzo De Censi**

Cardiological hypertensive emergencies: data from clinical practice and guidelines recommendations

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Introdution: Since the end of August 2018, new guidelines issued jointly by the European Society of Cardiology (ESC) and the European Society of Hypertension (ESH) have been available online for all physicians involved in the management of hypertension. The turning point of 2018 ESC / ESH guidelines is the recommendation of immediate reduction of systolic blood pressure under 140 mmHg in acute coronary syndrome and in acute cardiogenic pulmonary edema.
Aim: The first aim of our single-centre retrospective study is to analyze the prevalence and clinical characteristics of patients accessing Emergency Department with severe elevation of blood pressure during the calendar year 2019. The second aim of the study is to compare cardiological hypertensive emergencies management before and after the release of the 2018 ESC/ESH guidelines on arterial hypertension, verifying adherence to the latter.
Methods: This single center retrospective study was conducted at ASST Grande Ospedale Metropolitano Niguarda, Milan. All Emergency Department reports recorded in our electronic system between 1st January and 31st December in 2017 and 1st January and 31st December in 2019 were analyzed. Inclusion criteria consisted in age ≥ 18 years and systolic blood pressure ≥ 180 mmHg and/or a diastolic blood pressure ≥ 120 mmHg at the first measurement. The access of enrolled patients was then classified as emergency hypertension or uncontrolled hypertension, depending on the presence or absence of organ damage. After the construction of a database a statistical analysis was performed: T-test was used for continuous variables, while chi-squared test was use for categorical variables.
Results: Presentation for uncontrolled hypertension and hypertensive emergencies in 2017 and 2019 were respectively 706 (0,96%) and 601(0,89%). The reduction in blood pressure obtained in emergency department was significantly greater in 2017 (44.7 ± 31.4 mmHg) compared to 2019 (35.4 ± 24.5 mmHg), with p = 0.011. Patients who received drug therapy in emergency department were 75.7% and 77.8% respectively in 2017 and 2019. Comparing cardiological hypertensive emergencies of the year 2019 with those of 2017 it emerged that the achievement of the pressure goals as defined by ESC/ESC 2018 guidelines was achieved in 51.4% of patients in 2017 vs 28.9% of patients in 2019.
Conclusion: Our study demonstrates the lack of implementation of 2018 ESC/ESH guidelines in clinical practice, at least in the analyzed center. Further studies are needed to understand if these aggressive targets proposed by the guidelines determine in all respect a reduction in negative outcomes in patients with cardiological hypertensive emergencies and if their lack of application derives from ineffective information or from the conviction of physicians of poor effectiveness if not even a possible damage.

Abstract n SP11_02 - Presenting author**: Ilaria Garofani**

Hypertensive emergencies and urgencies: blood pressure management and its relationship with short and medium term outcome

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Introduction: Data regarding prevalence and clinical management of hypertensive emergencies and urgencies are lacking and heterogeneous. Our goal is to characterize patients with hypertensive emergencies and urgencies admitted to the emergency department (ED) of Niguarda hospital and Pio XI Hospital of Desio. In this population we also want to evaluate factors associated with organ damage, adherence to guidelines and the impact of blood pressure (BP) management on hospital mortality.
Method: We performed a multi-centre retrospective study collecting data about all adult patients with systolic blood pressure ≥ 180 mmHg and/or diastolic blood pressure ≥120 mmHg admitted to our hospitals' ED during 2017 and 2019.
Results: Admission to ED for BP elevation were 1838 (0.95% of total admission to ED), of whom 38% were hypertensive emergencies and 62% were hypertensive urgencies. Patients with hypertensive emergencies were older, mainly male, with more comorbidities and more symptomatic at ED admission. In the emergencies group, we observe a SBP mean reduction of 39.50 mmHg (±26.35) and a DBP mean lowering of 16.28 mmHg (±17.57); the most used drugs were furosemide, nitroglycerin and parenteral labetalol. In the urgencies group, the mean reduction was 39.09 mmHg (±22.46) for SBP and 15.34 mmHg (±16.07) for DBP. The most used drug was short-acting nifedipine benzodiazepine and amlodipine in this group. Age, sex, clinical history of heart failure and chronic obstructive pulmonary disease, symptoms at ED admission ad eGFR have been recognised as factors associated with organ damage. Instead, BP at ED admission and its management didn’t appear to have a significant impact on outcomes.
Conclusions: Our study demonstrated better adherence to guidelines in the treatment of hypertensive emergency than of hypertensive urgencies. On the other hand, no significant association were found between the BP management in the ED and in-hospital mortality.

Abstract n SP11_03 - Presenting author**: Alfonso Riccio**

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.

Abstract n SP11_04 - Presenting author**: Andrea Caccia**

Acupuncture in arterial hypertension: evaluation of its efficacy with both office and ambulatory blood pressure measurements

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Introduction: a possible alternative to pharmacological antihypertensive therapies in grade 1 low risk hypertensive patients or in those who experienced drugs adverse effects could be acupuncture.
Aim: we focused on its possible effects on BP both as Office BP (OBP) and as Ambulatory BP Monitoring (ABPM) evaluating it before starting a 6 weeks twice weekly (total 12 session) acupuncture cycle and after 2 months from its completion.
Methods: in this prospective study we treated with acupuncture 45 patients: 24 of them presented high-normal BP values and low cardiovascular risk while 21 patients were on anti-hypertensive drug with slightly uncontrolled BP values (from 140 to 145 mmHg for Systolic BP – SBP – and/or from 90 to 95 mmHg for Diastolic BP – DBP).
Results: regarding SBP, a significant reduction has been observed for office values (from 134.2±15.7 to 125.1±12.2, p=0.03), and for ABPM 24h (from 131.1±10.7 to 126.0±10.1, p=0.01) and day-time values (from 134.7±10.5 to 127.1±18.4, p=0.02). For DBP, only ABPM 24h and day-time values showed significant changes (from 85.3±9.1 to 82.1±7.5, p=0.03; and from 88.5±9.3 to 85.7±7.8, p=0.02). Within session SBP decrease was -5.8 mmHg (-3.75%) during the first session while it falls to -2.1 mmHg (-1.25%) and stands firmly under 2 mmHg for all the next session. At the last session SBP reduction was -1.9 mmHg (-1.6%).
Conclusions: we found a significant reduction in office, 24h and day-time ABPM SBP determined by a 6-weeks twice weekly acupuncture cycle that lasts at least for the first two months after its completion.

Abstract n SP11_05 - Presenting author**: Martina Morelli**

Natural history and clinical burden of moderate aortic stenosis: a systematic review and meta-analysis

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Aim: The mortality risk of patients with moderate aortic stenosis is not well known but recent studies suggested that it might negatively affect prognosis. The aim of this study was to assess the natural history and clinical burden of moderate aortic stenosis and to investigate the interaction of left ventricular ejection fraction and of age with prognosis.
Methods: A systematic research was conducted on PubMed. The inclusion criteria were: 1) inclusion of patients with moderate aortic stenosis; 2) report of the survival at 1-year follow-up (minimum). Incidence ratios related to all-cause mortality in patients and controls of each study were estimated and then pooled using a fixed effects model. Meta-regression analysis was performed to assess the impact of left ventricular ejection fraction and age on the prognosis of patients with moderate aortic stenosis.
Results: Fifteen studies and 11,596 patients with moderate aortic stenosis were included. All-cause mortality was significantly higher among patients with moderate aortic stenosis than among controls: 10.7% (95% CI: 0.1010-0.1136) vs 4.5% (95% CI: 0.0438-0.0454) at 1-year ($p < 0.0001$), 17.7% (95% CI: 0.1692-0.1854) vs 7.6% (95% CI: 0.0754-0.0775) at 2-year, 23.0% (95% CI: 0.2204-0.2387) vs 9.9% (95% CI: 0.0973-0.0997) at 3-year, 27.9% (95% CI: 0.2685-0.2899) vs 11.9% (95% CI: 0.1177-0.1203) at 4-year, and 32.4% (95% CI: 0.3125-0.3360) vs 14.2% (95% CI: 0.1402-0.1430) at 5-year follow-up. Left ventricular ejection fraction did not significantly impact on the prognosis of patients with moderate aortic stenosis (estimate = -0.0020; 95% CI: -0.0078-0.0038; $p = 0.4584$), unlike of age (estimate = 0.0067; 95% CI: 0.0007-0.0127; $p = 0.0323$).
Conclusions: Moderate aortic stenosis is not a benign disease. Further studies are necessary to confirm the prognostic impact of this valvulopathy and the possible benefit of the aortic valve replacement.

Abstract n SP11_06 - Presenting author**: Davide Bonadies**

A not-so-rare complication of connective tissue disease

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The frequency of this complication in connective tissue disease, the need for screening tests and different treatment approaches of pre and post capillary pulmonary hypertension is discussed.
Pulmonary hypertension is a not–so–rare complication of connective tissue diseases and is usually related with an inauspicious prognosis. We present the case of a 67–years–old woman hospitalized for progressive dyspnea. Two–dimensional echocardiography showed numerous features related to pulmonary hypertension (PH) with moderate Mitral Regurgitation. The right cardiac catheterization confirmed mixed PH (pre and post capillary) without chest angio–TC evidence of pulmonary thromboembolism. Thanks to optimized medical therapy (B–Blocker, ACE–i and diuretics), there was a significant clinical improvement documented at discharge. After 1 year, due to recurrence of dyspnea, she was admitted to our medical unit. Her clinical examination showed some of the characteristics of a connective tissue disease. Compared to previous echocardiography, a moderate to mild reduction of MR was documented. Chest CT scan revealed interstitial lung disease likely related to patient's underlying pathology. Immunological screening tests detected the presence of antinuclear (ANA) and anti–topoisomerase (ATA) antibodies. Right heart catheterization documented a worsening of PH with only pre–capillary component. On the basis of the above findings, a diagnosis of CREST syndrome complicated with PH was made, and the patient was started on oral combination of macitentan and riociguat.

Abstract n SP12_01 - Presenting author**: Sebastiano Cicco**

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.

Abstract n SP12_02 - Presenting author**: Manuela Casula**

Does the lipoprotein(a) genotype influence the diagnosis of familial hypercholesterolemia?

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Background: Evidence suggests that LPA genotypes associated with elevated lipoprotein(a) [Lp(a)] levels may result in a severe phenotype suggestive of clinical familial hypercholesterolemia (FH). We aimed at determining the prevalence of two Lp(a) raising variants in FH individuals enrolled in the Italian LIPIGEN study.
Methods: We selected adults (aged≥18 years) with a clinical diagnosis of FH. We defined FH subjects as mutation-positive (FH/M+), with at least one causative variant on the gene encoding for LDL receptor (LDLR), or mutation-negative (FH/M−). For each subject, the genetic predisposition to high Lp(a) levels was evaluated, calculating an Lp(a) genetic score by summing the number of risk-increasing alleles inherited at rs3798220 and rs10455872 variants. In addition, we also calculated a polygenic score including 12 common LDL-C–raising single nucleotide polymorphisms in order to evaluate for each subjects the probability of having polygenic hypercholesterolemia.
Results: Overall, in the 4.6% of 1695 clinically diagnosed FH patients, the phenotype could not be explained by a monogenic or polygenic aetiology, but only by genotype associated with high Lp(a) levels. Among 765 FH/M− subjects and 930 FH/M+ patients, 21.0% and 10.2% were characterized by at least one of the variants associated with higher Lp(a), respectively. We found that FH/M− subjects had higher levels of Lp(a) than patients in the FH/M+ group (median values 41 mg/dL [9-103] vs 19 mg/dL [8-41], p-value <0.0001). Overall, the adjustment of LDL-C levels based on Lp(a) concentrations reduced from 68% to 42% the proportion of subjects with LDL-C level ≥190 mg/dL, which is one of the main criteria considered for the clinical diagnosis of FH.
Conclusion: Our study supports the importance of measuring Lp(a) to appropriately perform the diagnosis of FH.

Abstract n SP12_03 - Presenting author**: Giulio Francesco Romiti**

The Impact of Clinical Complexity and integrated care management in patients with Atrial Fibrillation: insights from an European-wide prospective registry

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Aim: Clinical complexity is an increasing concern in patients with atrial fibrillation (AF). Indeed, the 'Atrial fibrillation Better Care' (ABC) pathway has been previously proposed to streamline an integrated care approach to AF, but its efficacy in clinically complex patients is unclear. We sought to analyse the epidemiology of clinical complexity, and the impact of the ABC pathway, in a contemporary cohort of AF patients.
Methods: From a large, contemporary European-wide registry of AF patients, we defined clinical complexity as the presence of frailty, multimorbidity and/or polypharmacy. A K-medoids cluster analysis was also performed, to identify subpopulations of patients based on their level of complexity. The efficacy of the ABC pathway on the risk of all-cause death and major adverse cardiovascular events (MACEs) was assessed using Cox-regressions. Number needed to treat (NNT) were estimated based on 1-year absolute risk reduction.
Results: 9,966 AF patients were included. Clinical complexity was found in 8,289 (83.2%) patients, and was associated with higher risk of both all-cause death and MACE. In the clinically complex group, adherence to the ABC pathway was associated with lower risk of all-cause death (adjusted HR [aHR]: 0.71, 95%CI 0.57-0.89), MACE (aHR: 0.68, 95%CI 0.53-0.87) and the composite outcome of all-cause death and MACE (aHR: 0.69, 95%CI: 0.57-0.84; Figure 1). We identified a cluster of patients with high clinical complexity, in which adherence to the ABC pathway was associated with lower risk of death (aHR: 0.73, 95%CI 0.55-0.96) and composite outcome (aHR: 0.69, 95%CI 0.57-0.84). 1-year NNTs for ABC pathway adherence was 24 for all-cause death, 31 for MACEs and 20 for the composite outcome in clinically complex patients.
Conclusions: Clinical complexity is common in AF patients, and the ABC pathway is useful to improve outcomes in these subjects. Integrated care approaches should be routinely implemented in AF patients.

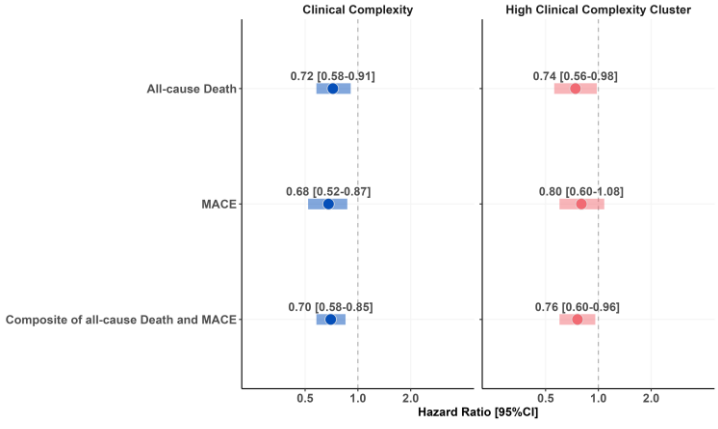

Figure 1 – Risk of Major Outcomes in patients with clinically complex and high complexity cluster patients treated according to the ABC pathway

Abstract n SP12_04 - Presenting author**: Fabrizia Bonacina**

Lysosomal acid lipase (LAL) deficiency alters immune cells distribution in humans and mice

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Background. Lysosomal acid lipase deficiency (LAL-D) is a rare recessive disorder of intracellular lipid metabolism leading to liver disease and dyslipidemia. Despite LAL is ubiquitously expressed, the available enzyme replacement therapy (ERT) has been shown to correct liver disease, while the effect in other cells or tissues has never been investigated; indeed, abnormal proliferation and activation of hematopoietic cells has reported in severe LAL-D. We aimed to unveil the consequence of LAL-D on immune response.
Methods. 8 ERT-naïve and 3 ERT-treated LAL-D patients (aged 11-80 years) were compared to age/sex matched healthy controls. Flow cytometry analysis of circulating immune cells was performed and paralleled in *Lipa*$^{-/-}$ mice.
Results. LAL-D showed a trend in reduction in circulating levels of leukocytes, irrespective of ERT compared to controls. No difference was observed in the frequency of Tcells, despite increased Bcells was reported in LAL-D on ERT compared to naïve, possibly as an immune reaction to the therapy. No main differences were reported in the levels of monocytes and their subsets, while neutrophils were significantly increase in LAL-D naïve compared to controls and normalized by ERT. Instead, we reported a significant and robust decrease of NK cells in LAL-D subjects, which persisted even in ERT-treated ones. In parallel, LAL KO mice showed an expansion of granulocytes resulting in reduced monocytes, T and B cells compared to WT. Consistently with LAL-D patients, the frequency of NK was significantly reduced in LAL-KO mice compared to WT, with a trend in reduction of CD11b^{+}/NK and increased CD27^{+}/NK that resemble the phenotype observed in patients.
Conclusions. LAL-D affects the distribution of immune cells and particularly that of NK cells; furthermore, while ERT rescues most of these immune alterations, the reduction of NK cells is not reversed by ERT, thus suggesting that LAL could act as an immunometabolic regulator beyond hepatic dysfunction.

Young
investigators
meeting

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