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Oral Communications

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Allergology and Clinical Immunology

Hepcidin regulates duodenal expression of genes involved in iron absorption

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Iron absorption is negatively regulated by hepcidin, a peptide hormone secreted by hepatocytes. Through its binding to the iron exporter ferroportin (FPN1), at the basolateral membrane of duodenum enterocytes, hepcidin causes FPN1 degradation and inhibits the release of iron from enterocytes to plasma. Iron retention within enterocytes, in turn, reduces the expression of DMT1, the iron transporter mediating enterocyte uptake of inorganic iron form the intestinal lumen. Since hepcidin has been recently described as having transcriptional effects on macrophages, regulating mRNA expression levels of a wide variety of genes, we investigated the effects of hepcidin treatment on the expression of several genes involved in iron absorption/metabolism. For this purpose, we used in vitro organ cultures derived from macroscopically normal duodenal biopsies obtained from subjects undergoing esophageal-gastro-duodenoscopy for dyspesia. Biopsy samples were cultured for 6 h with or without synthetic hepcidin, 1.4 μg/ml. Total RNA was isolated from cultured biopsies and, following reverse transcription, was analysed by real-time PCR. Table 1 shows the genes that were analysed. Expression levels were expressed relative to the housekeeping gene GAPD.

Table 1 Genes analysed in this study

Gene name	Function
FPT1 (total)	Iron exporter ferroportin, mediates cellular iron release
DMT1 (total)	Divalent metal transporter, mediates the enterocyte uptake of inorganic iron from the lumen of the gut
DMT1 (non-IRE)	DMT1 mRNA devoid of the Iron responsive element
DMT1 (IRE)	DMT1 mRNA with the iron responsive element
Dcytb	Cytochrome b, is an iron reductase necessary for iron uptake through DMT1
Heph	Hephaestin, a ferroxidase required for enterocyte iron release to plasma through FPT1
HCP1	Heme carrier protein 1, the hypothetical intestinal heme transporter
HO-1	Heme oxygenase 1, required for the release of iron from heme
FLVCR1	Cytoplasmic heme exporter
TfRc-1	Transferrin receptor 1
FTN-H	H-ferritin
FTN-L	L-ferritin

Treatment with hepcidin induced a mild but significant reduction in the levels of HCP-1, Hephestin and FTN-H mRNAs. Our results suggest that hepcidin modulation (inhibition) of iron absorption occurs at different levels; in addition to the hepcidin-induced degradation of FPT1, these may include the down-regulation of the enterocyte uptake of heme iron (through the effect on HCP-1 mRNA) and the inhibition of iron release to plasma (through the effect on hephaestin mRNA).
This contribution has been awarded as Best Communication.

Acyl-coenzyme a-cholesterol acyltransferase (ACAT) inhibitor avasimibe and the 3-hydroxyl-3-methylglutaryl coenzyme a (HMG-COA) reductase inhibitor fluvastatin can differently regulate lymphocyte activation and endothelial cell adhesion to substrate

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Lipid-lowering drugs as inhibitors of 3-hydroxyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase, also known as statins, are commonly used in the treatment of atherosclerosis. Statins can modulate intracellular signalling pathways, inhibit inflammation and regulate some activities of immunocompetent cells. This effect is dependent on a) the depletion and/or disruption of cholesterol rich membrane micro-domains and b) the inhibition of functional activities of small guanosine-triphosphate (GTP) binding protein of Rho and Ras family involved in cytoskeleton rearrangement and cell proliferation, respectively. Recently, other drugs interfering with cholesterol metabolism have been proposed for the treatment of atherosclerosis. Among them, avasimibe is an acyl-coenzyme A- cholesterol acyltransferase (ACAT) inhibitor which catalyses the formation of cholesterol esters from cholesterol and fatty acyl-coenzyme. Two different ACAT enzymes play a key role in cholesterol matabolism. Indeed, the development of atherosclerotic plaque is associated with the accumulation of cholesteryl ester via ACAT1 in macrophage-derived foam cells of the arterial wall. On the other hand, ACAT2 plays a key role in very low density lipoprotein (VLDL) secretion from the liver and in dietary cholesterol absorption in the form of chylomicron from the gut. Avasimibe is an inhibitor of both ACAT1 and ACAT2. Herein, we have analysed the effect of avasimibe on a) the phenotype and proliferative response of human T lymphocytes, b) the triggering of cytolytic activity of natural killer (NK) cells by antibody dependent cellular cytotoxicity (ADCC) and tumor necrosis factor-a (TNF-a) production c) the phenotype and substrate adhesion of endothelial cells. This to determine whether this

ACAT inhibitor could play a role in the regulation of the interaction between lymphocytes and endothelial cells during the inflammation processes associated to atherosclerosis in autoimmune diseases. We have found that avasimibe did not significantly affect the surface expression of CD3, CD4 and CD8 antigens on T cells. On the other hand, avasimibe can markedly down-regulate the adenosine triphosphate (ATP) content and proliferation of peripheral blood lymphocytes to different mitogenic stimuli including phytohemagglutinin, anti-CD3 monoclonal antibodies and bacterial derived toxin as staphylotoxin B. This effect was detected at 10 mM of avasimibe and it was not accompanied by an evident apoptotic effect of the treated cells. In addition, proliferation of CD8 and CD4 T cells in mixed lymphocyte cultures (MLC) was strongly decreased (by 90%) at lower concentration of avasimibe (2 mM). Avasimibe reduced the NK cell-mediated cytolytic activity against NK sensitive target cells; however, the ADCC mediated by NK cells triggered by humanized antibodies to the CD20 antigen (Rituximab) against CD20+ cells was not significantly affected. In addition, avasimbe almost blocked the production of TNF-a by NK cells incubated with target cells. Finally, avasimibe did not affect endothelial cell morphology and expression of CD31 endothelial cell marker, relevant in the formation of the aptotactic gradient responsible for lymphocyte transendothelial migration. On the contrary, the HMG-CoA reductase inhibitor fluvastatin strongly altered both adhesive properties and morphologic features of endothelial cells. Indeed, endothelial cells incubated with 1 mM of fluvastatin detached from the substrate and died in 72 h. In addition, fluvastatin did not inhibit TNF-a release from T or NK cells at doses efficient in blocking proliferation and triggering of cytolysis (10-1 mM). Altogether these findings would indicate that the ACAT inhibitor avasimibe can downregulate efficiently both innate and specific immune responses, also reducing the production of pro-inflammatory cytokines without affecting endothelial cell integrity, at variance with HMG-CoA reductase inhibitor fluvastatin. Experiments are in progress to determine whether the interaction between lymphocyte and endothelial cells are regulated by avasimibe as well.

Depletion of circulating memory B cells in splenectomized and common variable immune deficiency patients is associated with decreased IGA plasma cells in the gut mucosa

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Background & Aims: Memory B cells preserve and recall previous antigenic experience in order to prevent or limit re-infection. The common immunological disorder observed in patients with a reduced frequency of memory B cells is the increased susceptibility to bacterial infections at parenchimal and mucosal sites. Secretory IgA (sIgA) is the tool used by B cells to protect the mucosa of the intestine and respiratory tract.
Patients & Methods: We studied two groups of patients with a reduced number of memory B cells: 7 individuals splenectomized because of trauma, and 34 patients with common variable immune deficiency (CVID). Diagnosis of CVID was done according to the ESID/PAGID criteria: marked decrease (at least 2 SD below the mean for age) in serum IgG and IgA, onset age >2 years, poor response to vaccines and exclusion of other causes of hypogammaglobulinaemia. All CVID patients were on intravenous or subcutaneous immunoglobulins according to the national guidelines of the Italian Association of Pediatric Hematology and Oncology. Fifty healthy donors were enrolled as controls for blood values, and 15 for intestinal biopsies. Peripheral blood mononuclear cells were isolated and stained with the appropriated combination of monoclonal antibodies to recognize memory B cells. Duodenal biopsies were collected and immediately frozen in liquid nitrogen, and kept at −80°C until the time of the study. Multiple 5 μm cryostat sections obtained from the frozen samples, included in cryostat embedding medium were fixed in cold acetone, washed with PBS and incubated for 45 min with Phalloidin-TRITC, anti-human-IgA, anti-human Ig kappa light chain, goat anti-human IgM, μ chain specific, anti-human secretory component and anti-human J-chain (Mc19-9). Duodenal sections were analysed at a confocal microscope to analyze mucosal IgA plasma cells and sIgA.
Results: We found that the reduction of memory B cells in the peripheral blood of splenectomized patients is associated to a significant diminution of IgA plasma cells and the disruption of the film of sIgA on the luminal side of epithelial cells. In patients with CVID, the absence of sIgA in the gut and IgA in the serum were associated to a significant reduction of memory B cells in the peripheral blood and high risk of respiratory infections. The subset of CVID patients with sIgA at mucosal sites showed a normal number of circulating memory B cells, detectable serum IgA and have a low risk of respiratory infections.
Conclusions: Memory B cells are indispensable for the production of sIgA at mucosal sites. The disruption of the sIgA film in the gut impairs the local defense against invading pathogens. New tools should be developed in order to replace the function of sIgA in immune-deficient and splenectomized patients.
This contribution has been awarded as Best Communication.

Low peripheral voltages in patients with al amyloidosis: only a clue to the diagnosis of cardiac involvement?

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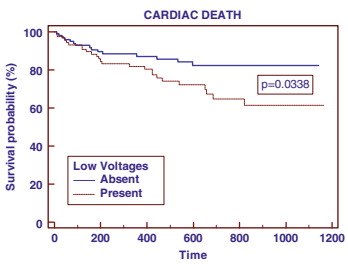
Introduction and Aim: The term amyloidosis refers to a group of diseases characterized by extracellular deposition of insoluble fibrils in various tissues. Clinical presentation of amyloidosis is variable, depending on the extension of deposits and on the extent of organ dysfunction, and it often manifests as a systemic disease. In AL amyloidosis, the amyloidogenic protein is an immunoglobulin light chain or a fragment of an Ig light chain, more frequently of the lambda isotype, synthesized by clonal plasma cells in bone marrow. Cardiac involvement is not only frequent but it is also the most common cause of death. The 12-leads electrocardiogram (EKG) reflects the generalized infiltrative nature of this disease with low voltages in the limb leads, pseudoinfarction patterns in the anterior precordial or inferior limb leads, or both, and abnormalities of conduction such as fascicular block or varying degree atrioventricular block. In particular the presence of "low voltages" was defined as QRS voltage amplitude $\leq$0.5 mV in all limb leads or $\leq$1 mV in all precordial leads. Beyond confirming the characteristic high prevalence of peripheral low voltages in a large cohort of patients, aim of the present study was to assess the prognostic implications of this EKG abnormality and evaluate its possible correlation with other commonly-used electrocardiographic, echocardiographic and biochemical parameters.

Methods: We enrolled 295 consecutive never-treated subjects, in whom a first diagnosis of primary AL amyloidosis was concluded between 2008 and 2009, according to the International Society of Amyloidosis criteria. The cohort was divided into two groups depending on the presence (n = 193) or the absence (n = 102) of cardiac involvement. Standard 12-leads EKG and cardiac echo-colorDoppler data were evaluated at diagnosis, and prognosis was assessed after a median follow up of 477 days.
Results: When compared with patients without myocardial involvement, cardiac AL patients showed a higher prevalence of low voltages (63.9% vs. 22%; $p < 0.0001$), whereas the prevalence of strain pattern and of pseudonecrosis was 38.5% and 52.2%, respectively. Moreover, when compared with patients without cardiac amyloidosis, the presence of myocardial involvement was associated with prolonged PQ, QRS and QT intervals ($p < 0.05$ for all comparisons). Overt first degree atrio-ventricular block was present in 24.8%, and intraventricular conduction delays were evident in 28% of patients with cardiac AL. After a median follow-up of 477 days, Kaplan–Meier survival analysis revealed a significantly higher mortality in the peripheral low voltage group when compared with the "normal" voltages group ($p = 0.0338$). The same trend wasn't confirmed in the group with cardiac involvement ($p = 0.5337$). This is most likely due to the fact that the cardiac involvement in this disease is itself the most important and robust prognostic factor in amyloidosis. In patients with cardiac AL amyloidosis the presence of peripheral low voltages was significantly associated with PQ duration ($p = 0.008$) and interventricular septum thickness ($p = 0.032$). No association was found with the duration of QRS, and QTc intervals, or the presence of pseudonecrosis or a strain pattern. Moreover, in patients with cardiac involvement no association was found between the presence of low voltages and NT-proBNP or troponin I levels.



Conclusion: Beyond confirming the diagnostic value of the presence of peripheral low voltages, these data demonstrate the prognostic value of such a simple parameter in the population of patients affected by AL amyloidosis. This confirms that EKG is a simple and cheap diagnostic tool that can help in stratifying the prognosis of patients with AL amyloidosis.

Clinical Case for the Gymnasium Session

An unusual case of syncope

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A 76-year old-man was admitted to the Emergency Department with a syncopal episode. He denied palpitations, chest pain and dyspnea, but referred falling to the ground with mild cervical injury.

The physical examination revealed a hypotension (blood pressure (BP), 80/50 mmHg) in clinostatic position. Laboratory tests were normal except for a mildly increased creatinine (1,31 mg/dL) and hemoconcentration (Hb 14 g/dL, Ht 41,5%) possibly due to dehydratation. ECG, Chest X-ray, cervical X-ray, cranial CT-scan were negative.
While the patient was waiting in the emergency department in a seated position, he had a pre-syncopal episode characterized by dizziness with hypotension and was then admitted to the hospital for further investigations.
His past medical history was negative and he did not take any medications. He reported to be on an autoprescribed hypocaloric diet since 4 months, because of mildly-elevated glycemic level found in routine blood test. He also reported a weight loss (30 kg in the last four months) associated with lack of appetite and progressive asthenia. Family history was positive for colon cancer.
On admission in our department the patient was asthenic, hypotensive (BP 90/60 mmHg) and pulse rate was 64 beats/minute; physical examination was negative and he did not show clinical signs of heart failure.
For investigating the pre-syncopal episodes, suspecting a cardiac origin, we performed long-term ECG monitoring that was negative for arrhythmias and heart blocks. Cardiac Ultrasound did not show any structural heart abnormalities with normal ejection fraction. Additionally, 24 h-blood-pressure-monitoring showed BP values persistently around lower normal limits (systolic BP 80-90 mmHg, diastolic BP 40-50 mmHg) and the physiological circadian rhythm was absent. Moreover, BP did not change between clinostatic and orthostatic position, so the possible hypotensive ethiology of the episodes was less likely.
In order to explore the possible cause of significant weight loss associated with lack of appetite and asthenia in patient with family history of colon cancer, we evaluated the presence of gastrointestinal neoplasia. Thoracic and abdominal CT-Scan, esophagogastroduodenoscopy and colonscopy did not reveal any neoplastic lesions. Neoplastic markers (CEA, CA 19.9, PSA) were also negative. During in-hospital routine blood test, we found a mild hyponatremia (130 mEq/L). Considering the symptoms still present, especially hypotension and weakfullness, all the tests performed negative and the mild hyponatriemia we suspected an endocrine disorder, namely a cortico-adrenal insufficiency. Surprisingly, the plasmatic hormonal tests showed a markedly reduced morning cortisol (3 ng/mL). To differentiate between central or peripheral hypoadrenalism, we assessed ACTH plasma levels and found that it was significantly decreased (10 pg/mL). To evaluate the presence of panhypopituitarism, pituitary hormones were tested as well: prolactin, LH, fT4, urinary free cortisol, GH and free testosterone were significantly decreased (prolactin 2 ng/mL, LH 1,5 mlU/mL, fT4 3,8 pg/mL, urinary free cortisol 1,7 mcg/24 h, GH 0,3 ng/mL, free testosterone 0,1 pg/mL). Based on the presence of reduced plasma cortisol, ACTH and other pituitary hormones, the patient was diagnosed with central panhypopituitarism.
Panhypopituitarism in elderly could be a consequence of pituitary adenoma, empty-sella, autoimmune process and vascular injury. Brain-MR with intravenous contrast showed a pituitary gland increased in volume, in relation to patient age, and hyperplasia of left posterior half gland, but it was negative for the presence of adenomas as well as empty sella. The autoimmune process was reasonably excluded with a negative autoimmune profile and the absence of any other organ involvement.
The conclusive diagnosis was panhypopituitarism due to vascular injury, supported by the brain MR results and the clinical presentation. A therapy with cortone acetate (25 mg + 12,5 mg die) was immediately started with rapid improvement of general conditions.

Endocrinology

Familial and sporadic papillary thyroid carcinoma: face off!

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Context: Even if no specific genetic determinants have been identified, it is known that the risk for developing thyroid carcinoma of follicular origin is elevated in individuals with 1st-degree relatives harboring such kind of malignancy. However, it remains controversial whether the behavior of familial differentiated thyroid carcinoma (FDTC) is more aggressive in comparison with sporadic forms. Several studies have been conducted to analyze the phenotype of FDTC, but most of those had not an adequate case series nor a prospective setting. To get over these limits a collaboration with several international thyroid cancer referral centers has started with the aim to create an international register for FDTC.
Objectives: In this prospective multicentric study, we investigated the prevalence of FDTC, tumor characteristics at baseline and patients clinical outcome, compared to sporadic forms.
Methods: A preliminary analysis of this study was carried over two consecutive series of 198 FDTC and 202 sporadic differentiated thyroid carcinomas. Sex, age at diagnosis, histological features and persistence-recurrence rate were collected. Statistical analysis was performed using independent samples t test and Fisher exact test (p value significance <0.05) as appropriate.
Results: FDTCs occur earlier than sporadic form ($p = 0.02$), with a higher rate of multifocality ($p < 0.0001$) and bilaterality ($p < 0.001$); moreover, the percentage of affected males was significantly higher in the FDTC series ($p = 0.02$). Disease recurrence-persistence rates of familial carcinoma did not differ from those of sporadic forms.
Conclusion: Preliminary data of our study do not support a more aggressive behavior of FDTC as compared to the sporadic counterpart. Longer prospective case series are needed, and the creation of a World Wide Thyroid Cancer Registry could give a great contribution to a correct prognostic assessment and appropriate management of this clinical entity.

Effects of transdermal testosterone treatment on inflammatory markers in older men

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During aging in men there is a progressive reduction in testosterone levels with an increase in inflammatory markers. It has been hypothesized a possible relationship between these two phenomena whereas the *primum movens* is still unclear. Recent observational studies and pivotal clinical trials in a small number of subjects let suggest a potential anti-inflammatory role for testosterone. However this hypothesis has never been tested in randomized clinical trials.
Aim of the Study: In older subjects with low-normal testosterone levels we tested the effects of transdermal preparation of testosterone on traditional and new inflammatory markers.
Method: 108 older subjects selected according to baseline testosterone levels (1 sd or more below the mean for normal young men, <475 ng/dL) were randomized to receive testosterone patch or placebo in a double blind fashion for 36 months. 96 subjects completed 36 months follow-up period. The present analysis was restricted to 70 men, 42 in the treatment and 28 in placebo group, with available data on testosterone, sex hormone binding globulin, c-reactive protein, interleukin-6, soluble Interleukin-6 receptors (sIL6r and sgp130), TNF-alpha, soluble TNF-alpha receptor 1, and body composition. Outcomes were examined using random-effect regression analyses, modelling an unstructured covariance matrix with slope and intercept as random effects. Interaction term between treatment status and time was entered in the models to test the change in inflammatory markers according to treatment.
Results: At baseline, testosterone and placebo groups had similar age (71.9 ± 4.7 years in treatment and 71.5 ± 5.3 years in placebo group), inflammatory markers and fat mass. The testosterone treated group had lower levels of testosterone and BMI but the difference between T and placebo-controlled was not statistically significant (p values of 0.06 and 0.07, respectively). As expected, 36 months testosterone treatment was associated with an increase in testosterone levels (from 490.15 ± 232.97 to 682.12 ± 307.56) that was statistically significant ($p = 0.001$). The delta change in testosterone levels was 192.41 ± 282.41 and $6{,}86 \pm 83.34$ in testosterone and placebo group, respectively. CRP levels were 1.58 ± 3.33 and 1.48 ± 2.75 at baseline in T and placebo-group, respectively. After 36 months of treatment CRP levels increased in both T (2.79 ± 4.10) and placebo ($9{,}97 \pm 24.77$) groups. Similar trends were observed for the other inflammatory markers. A significant treatment $\times$ time interaction term, indicating a less steeper increase in inflammatory molecules in T treated patients compared to those on placebo, was found only for CRP ($p = 0.03$) and TNFR1 ($p = 0.02$). No significant difference was found for TNF-alpha, IL-6, sIL6r and sgp130. After adjusting for body fat the results did not change.
Conclusion: In older men, transdermal testosterone treatment induced a significant lower increase of CRP and TNFR1 during 36 month period. Further studied are needed to address whether these findings have clinical implications in aging-related phenomena and diseases.

Tumor angiogenesis andexpression of proangiogenic molecular markers in papillary thyroid carcinomas: a limited role for the braf v600e mutation?

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Context: Gain of function mutations in the *BRAF* oncogene are common in papillary thyroid carcinomas (PTCs) and seems to maintain and promote the tumor progression and aggressiveness. The proangiogenic effects have been proposed to play a key role in determining the outcome of *BRAF* mutated PTC.
Objective: Our objective was to assess the role of *BRAF* mutation in promoting thyroid cancer angiogenesis.
Patients and Methods: A large series of PTCs patients characterized for the presence ($n = 55$) or absence ($n = 35$) of $BRAF^{V600E}$ mutation were analyzed for the expression level of the major proangiogenic factors. Quantitative real-time PCR was used to measure mRNA levels for vascular endothelial growth factor A (VEGF-A) and VEGF receptor family [i.e., VEGFR-1/Flt-1, VEGFR-2/KDR, VEGFR-3/Flt-4, neuropilin-1]. The analysis was extended to the platelet derived growth factor receptor-beta (PDGFR-β), a non-VEGF angiogenic factor frequently expressed in tumors. VEGF and VEGF receptor expression and localization was also analyzed by immunohistochemistry in a sub-group of patients ($n = 22$). Microvessel

density (MVD) and lymphatic vessel density (LVD) were assessed to depict angiogenic and lymphangiogenic phenotype, respectively. Finally, an in vitro model with enhanced/silenced *BRAF* mutant was exploited to further evaluate the in vivo findings.
Results: The mRNA levels for all proangiogenic factors were significantly lower in patients carrying the $BRAF^{V600E}$ mutation than in wild-type cases ($P < 0.0001$). In the subgroup of PTCs investigated also by immunohistochemistry (11 *BRAF* wild-type and 11 *BRAF* mutated tumors), there were no difference in VEGF and VEGF-receptor expression. Based on in vitro experiments, the induction of *BRAF* mutation in PCCL3 cell line led to a significant reduction of *VEGF-A* mRNA level ($P = 0.01$), while the silencing of *BRAF* mutation in 8505C cell line caused a significant increase of *VEGF-A* mRNA level ($P = 0.004$). Despite the differential expression of angiogenic factors, no significant difference were observed in MVD and LVD values with respect to *BRAF* mutational status.
Conclusions: The *VEGF* family and *PDGFR-β* gene expressions are down-regulated in PTC patients carrying $BRAF^{V600E}$ mutation. However, tumor angiogenesis is not affected, suggesting a pivotal role of other signalling pathways.
This contribution has been awarded as Best Communication.

A case of thyrotoxic periodic paralysis with serious cardiac complications

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Introduction: Thyrotoxyc periodic paralysis (TPP) is a rare complication of hyperthyroidism in caucasian populations, while is more frequent in Asians. Muscle paralysis occurs due to a massive intracellular shift of potassium and consequent hypokalemia, rather than an absolute potassium deficiency.
Case report: a 29-year-old caucasian man came to our emergency department with a complete flaccid paralysis involving lower and upper limbs. This symptom had abruptly begun at night, soon after an abundant and carbohydrate-rich meal. In the last months he complained profuse night sweating, tachycardia and state of anxiety. He had no family history of periodic paralysis. On physical examination, he had mild exophthalmos but not evidenced thyroid enlargement. His potassium level was initially 1.3 mEq/L (3.5 – 5.3), with normal acid–base status. Serum magnesium level was 1.8 mEq/L (1.7 – 2.6). Urine potassium was 68.5 mEq/L (25 – 125). Electrocardiogram showed sinus tachycardia. Six hours later, in spite of intravenous potassium supplementation, he started to have dyspnea and ventricular sustained tachycardia, which suddenly evolved into torsades-de-pointes tachycardia. We traited this arrhythmia with magnesium sulphate, with ready reversion, as well as improved dyspnea. Paralysis persisted during the first day of admission. Intravenous potassium supplementation went on till serum level increased to 4.4 mEq/L, on the second day of admission. Limbs paralysis started to regress, but sinus tachycardia persisted even after correction of hypokalemia. Thyroid-stimulating hormone was 0.01 μUI/mL (0.35 – 5.50) and the free thyroxine (T4) level was high 4.81 ng/dl (0.89 – 1.76), so we diagnosed thyrotoxicosis. Methimazole 30 mg per day and Propranolol 60 mg per day he started, with a gradual improvement in clinical condition (tachycardia slowly reversed, as well as muscle weakness and sweating). Thyroid ultrasonography evidenced enlargement of all glandular diameters, with hypoechoic tissue, and hypervascularity classically seen in Graves disease. This diagnosis was finally confirmed by 99 m-technetium scintigraphy. Patient was discharged eight days after admission, in optimal state, with chronic antithyroid and nonselective beta-blockers therapy.
Discussion: attack in TPP is characterized by a high variability, from very mild weakness to severe flaccid paralysis. In our case complete muscle paralysis involving all limbs has been accompanied by serious cardiac complications: life-threatening ventricular arrhythmias. Hyperthyroidism in TPP may even be clinically silent, and severity of paralysis do not correlate with serum thyroid hormone levels, but with degree of hypokalemia. Our patient had very low serum potassium level and very high thyroid hormone levels. Potassium supplementation is very effective to promote recovery of paralysis and to prevent cardiopulmonary complications, but not to prevent recurrence of attacks. Nonspecific beta-adrenergic blocker Propranolol is also effective to prevent recurrence of paralysis and to ameliorate symptoms during attack. Thyroid hormone replacement is essential during attack as well as in order to prevent paralysis: TPP does not occur in euthyroid patient.
Conclusion: a rare case of hypokalemic flaccid paralysis occurred in a young patient with Graves disease, accompanied by life-threatening cardiac complications.

Cardiovascular Diseases

The low IGF-1 syndrome in chronic heart failure: preliminary data from the T.O.S.CA. italian multicenter study

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Background: Extensive evidence supports the concept that multiple hormonal and anabolic deficiencies are common in Chronic Heart Failure (CHF) and identify subgroups of patients with higher mortality. Chief among these is the reduced activity of the GH/IGF-1 axis.
Patients and Methods: Ninety-nine patients with CHF, selected from a larger cohort participating in a multicenter trial, were divided according to their IGF-1 levels. Low IGF-1 syndrome was defined in CHF patients with IGF-1 levels below the 25th percentile of an age and sex-matched population.
Results: Results are shown in the table below. Patients with low IGF-1 levels displayed higher depression and anxiety scores, reduced indexes of quality of life as well as of cardiopulmonary performance compared with CHF patients with normal IGF-1 levels. Moreover, LV volumes tended to be higher in low IGF-1 patients with significantly higher wall stress and larger mitral regurgitation jets.
Conclusion: low IGF-1 syndrome defines a subgroup of CHF patients characterized by worse clinical status, cardiopulmonary performance, and LV dynamics.

Clinical status, echo and CPET

	Patients with normal IGF-1	Patients with low IGF-1	p
	n = 76	n = 23	
Age	62 ± 1	64 ± 3	ns
IGF-1, ng/ml	142 ± 5	68 ± 3	<0.0001
MLHFQ	47 ± 3	38 ± 3	<0.05
Anxiety score	38 ± 2	31 ± 2	<0.05
Depression score	43 ± 3	32 ± 2	<0.01
Peak VO2, ml/kg·min	17.5 ± .8	12.3 ± 1	0.002

Table b continued

	Patients with normal IGF-1	Patients with low IGF-1	p
Peak workload, W	92 ± 6	66 ± 6	<0.05
LV EDV, ml	205 ± 29	236 ± 22	ns
LV ESV, ml	141 ± 23	168 ± 22	ns
EF, %	33 ± 2	27 ± 1	ns
MR area, cm^2	4.3 ± 2	6.2 ± 2	0.04
ESS, kidynes·cm^2	544 ± 33	419 ± 25	0.04

Data are expressed as mean ± SEM; MLHFQ: Minnesota Living with Heart Failure Questionnaire; EDV: end-diastolic volume; ESV: end-systolic volume; MR: mitral regurgitation; ESS: end-systolic stress

Usefulness of satisfactory control of low-density-lipoprotein cholesterol to predict left ventricular remodeling after a first st-elevation myocardial infarction successfully reperfused

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Background: Left ventricular (LV) remodeling represents an important determinant in the progression to heart failure in patients after myocardial infarction (MI). Development of LV remodelling is a complex phenomenon that involves a constellation of factors including a) the characteristics of the infarct, such as size, location, and transmural- extension; b) the functional consequences of infarct like presence and severity of mitral regurgitation; c) the effects of reperfusion therapy such as the restoration of patency of the infarct-related artery and microvascular dysfunction; d) the effects of pharmacological and non pharmacologic (i.e. exercise-based cardiac rehabilitation) interventions. Although in the modern clinical practice therapeutic strategies are available that, on one hand, promptly allow the restoration of patency of the infarct-related artery, and, on the other hand, exert an "antiremodeling" effect, LV remodeling still affects almost 30% of patients. This suggests that other factors can affect development of LV remodelling. In particular, it is reasonable to hypothesize that the conventional cardiovascular risk factors, in particular hypercholesterolemia may be involved in the development of postinfarction LV remodelling.
Aim: The present study was performed to evaluate whether the control of cholesterol is predictive of left ventricular (LV) remodelling in patients with first ST-elevation acute myocardial infarction (STEMI) who were successfully and completely reperfused.
Methods: We retrospectively analysed 109 patients referred to coronary care unit (CCU) for a first STEMI receiving successful reperfusion therapy. Inclusion criteria were: 1) confirmed first STEMI; 2) successful percutaneous coronary intervention (PCI) within 6 h from the onset of symptoms; 3) successful rescue PCI; 4) successful PCI within 24 h from onset of symptoms in case of effective thrombolysis; 5) adequate echocardiographic image quality recorded within the first 24 h of admission in CCU. All patients that met the inclusion criteria were asked to return to the outpatient clinic of our Department to perform exercise testing and laboratory tests, and to undergo Doppler echocardiography at least 6 months after discharge. LV volumes and LV ejection fraction were calculated from 4- and 2-chamber views using the modified biplane Simpson method. According to the change of indexed left ventricular end-diastolic volume (LVEDVi) detected at follow-up visit, patients were divided in non-remodelling and remodelling. LV remodelling was defined by an increase of LVEDVi ≥20%. Target levels of LDL-C in postinfarction patients were considered those recommended by the Adult Treatment Panel III (<100 mg/dl).
Results: Among 714 subjects that consecutively referred to CCU for STEMI from May 2005 to May 2009 we analysed 109 patients. Forty-six patients died in CCU and 28 died after hospital discharge. The reasons for the exclusion from the analysis were: 432 patients did not satisfy the inclusion criteria, 55 patients refused the follow-up visit, 12 and 32 patients had incomplete medical history and poor quality of ultrasound evaluation, respectively. Patients were categorised in two groups: non-remodelling (n = 79) and remodelling (n = 30). At CCU admission, the prevalence of cardiovascular risk factors resulted to be similar in the two groups. Follow-up visits were performed 17 ± 10 (range 6-44 months) and 17 ± 8 (range 6-48 months) months after hospital discharge in the remodelling and non-remodelling group, respectively. At follow-up visit, no differences were found in the prevalence of patients with target levels of blood pressure between remodeling (76%) and non-remodelling group (71%). The prevalence of patients with target levels of plasma LDL-cholesterol (LDL-C) was lower in remodelling compared with non-remodelling group (67% and 91%; respectively, $p < 0.01$). A further analysis showed that patients who achieved target level of LDL-C at follow-up had a lower increase of LVEDVi compared with those did not. Similar trend was also detected in the sub-group of patients taking statins. Moreover, no difference were detected in changes of LVEDVi, between patients taking (10 ± 27%) and not taking (17 ± 28%. $p = 0.18$) statins. Aiming at testing the hypothesis that non-target LDL-C were predictors of LV remodeling, patients were categorized according to LDL-C (target or non-target LDL-C values) control. After adjusting for age, gender, baseline LV ejection fraction, baseline LVEDVi, presence of hypertension, diabetes, obesity, smoking status, time from acute event and drug therapy (beta-blockers, angiotensin-converting enzyme-inhibitors, angiotensin-receptor blockers, calcium channel blockers and statins) wall motion score index and troponin levels, logistic regression analysis showed that patients with non-target LDL-C values at were significantly more likely to show cardiac remodeling (OR 22.3, 95% CI: 2.91–171.9, $p = 0.003$). In addition, patients with target LDL-C values were significantly less likely to show cardiac remodeling (OR 0.04, 95% CI: 0.006 – 0.34, $p = 0.003$).
Conclusions: The present study shows that unsatisfactory control of LDL-C independently predicts LV remodelling in patients with first STEMI. Therefore, the present study extends the spectrum of beneficial effects that can be obtained by the achievement of target levels of LDL-C. This finding strengthens the need of achievement and maintaining target LDL-C levels though secondary prevention for reducing LV remodelling.

Platelet function in diabetic patients treated with aspirin: role of platelet isoprostanes

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Background: Aspirin modestly influences cardiovascular events in type 2 diabetic (T2DM) patients but the reason is unclear. We investigated whether in T2DM patients aspirin enhances platelet isoprostanes, which are eicosanoids with pro-aggregating properties derived from arachidonic acid oxidation by platelet NADPH oxidase.

Methods and Results: A cross-sectional study was performed in T2DM patients, treated (n = 50) or not (n = 50) with 100 mg/d aspirin. One hundred non-diabetic patients, matched for age, sex, atherosclerosis risk factors and aspirin treatment were enrolled as controls. A short-term (7 days) treatment with 100 mg/day aspirin was performed in 36 aspirin-free diabetic and non-diabetic patients, matched for sex, age, and atherosclerotic risk factors. Platelet recruitment, which mimics the propagation of aggregation and is dependent upon isoprostanes, platelet thromboxane (Tx) A2, platelet isoprostanes and platelet NOX2, the catalytic subunit of NADPH oxidase, were determined.
Patients with diabetes had higher platelet recruitment, isoprostane levels, and NOX2 activation compared to non-diabetic patients ($p < 0.001$). Aspirin-treated diabetic patients showed greater TxA2 inhibition, higher platelet recruitment, increased isoprostane and greater NOX2 activation compared to non-treated diabetic patients ($p < 0.001$). In non-diabetic patients, aspirin inhibited TxA2 ($p < 0.001$) without affecting recruitment, isoprostane level, or NOX2 activation compared to aspirin-untreated controls.
In the interventional study, aspirin similarly inhibited platelet TxA2 in diabetic and non-diabetic patients ($p < 0.001$). Platelet recruitment, isoprostane level and NOX2 activation showed a parallel increase in diabetic patients ($p < 0.001$) but no change in non-diabetics.
Conclusion: In aspirin-treated diabetic patients, oxidative stress-mediated platelet isoprostane over-production is associated with enhanced platelet recruitment, an effect that mitigates TxA2 inhibition. This contribution has been awarded as Best Communication.

Genetic risk score from GWA studies is strongly associated with angiographically-defined coronary artery disease, but not with mortality in the setting of secondary prevention

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Background: Genome-wide association studies (GWAS) have identified several polymorphisms consistently associated with coronary artery disease (CAD) and myocardial infarction (MI). However, the clinical usefulness provided by this genetic information is controversial.
Methods: we calculated a weighted genetic risk score (GRS) on the basis of 10 GWAS-based single nucleotide polymorphisms (rs 4977574 in locus 9p21, rs646776 in locus 1p13, rs9982601 in locus 21q22, rs17465637 in locus 1q41, rs1746048 in locus 10q11, rs12526453 in locus 6p24, rs1122608 in locus 19p13, rs6725887 in locus 2q33, rs11206510 in locus 1p32, rs3184504 in locus 12q24) in 1,312 subjects of Verona Heart Study (338 CAD-free and 974 with angiographically-defined CAD). Within CAD population, for 643 subjects prospective data on total and cardiovascular mortality during a 5-years follow-up were available.
Results: the weighted GRS was strongly associated with CAD in the case–control arm. The risk of CAD increased progressively from the lowest to the highest GRS quintile ($P = 1.33 \times 10^{-7}$ by χ^2 for linear trend). When compared with the bottom quintile, subjects in the top quintile presented a marked increase of CAD risk in a model adjusting for all the traditional risk factors (OR 4.72 with 95% CI 2.48-8.98). On the other hand, GRS was not useful for discriminating CAD subjects with or without MI. During the follow-up period 115 CAD patients died, 84 of whom from cardiovascular cause. GRS was not significantly associated with either total (19.3% and 19.4% in the bottom and in the top quintile, respectively), or cardiovascular mortality (12.3% and 12.0%, respectively).
Conclusions: in our study GRS was strongly associated with CAD, but did not discriminate MI events within CAD population. In the setting of CAD secondary prevention, the GRS did not predict mortality. The clinical significance of GRS in this setting remains uncertain.

Hematology

Four proteins governing overangiogenic endothelial cell phenotype in patients with multiple myeloma are plausible therapeutic targets

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Bone marrow (BM) angiogenesis plays an important role in the initiation and progression of multiple myeloma (MM). We looked at novel mechanisms of vessel formation in patients with MM through a comparative proteomic analysis between BM endothelial cells (ECs) of patients with active MM (MMECs) and ECs of patients with monoclonal gammopathy of undetermined significance (MGUS) (MGECs) and of subjects with benign anemia (normal ECs). Four proteins were found overexpressed in MMECs: filamin A, vimentin, α-crystallin B, and 14-3-3ζ/δ protein, not yet linked to overangiogenic phenotype. These proteins gave a typical distribution in the bone marrow (BM) of MM patients and in MMECs *vs.* MGECs, plausibly according to a different functional state. Their expression was enhanced by vascular endothelial growth factor (VEGF), fibroblast growth factor 2 (FGF2), hepatocyte growth factor (HGF) and by the MM plasma cell conditioned medium (CM) in step with enhancement of MMEC angiogenesis. Their siRNA knockdown affected critical MMECs angiogenesis-related functions, such as spreading, migration and tubular morphogenesis. A gradual stabilization of 14-3-3ζ/δ protein was observed with transition from normal ECs to MGECs and MMECs that may be a critical step for the angiogenic switch in MMECs and maintenance of the cell overangiogenic phenotype. Results suggest that these four proteins could be new targets for the antiangiogenic management of MM patients.

Patients with essential thrombocythemia and pregnancies complications are prone to develop thrombo-embolic events

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Background: Thrombotic complications are the main causes of morbidity and mortality in essential thrombocythemia (ET). Fetal loss, mainly represented by first trimester abortions, occurs in about 1/3 of pregnancies of females affected by ET. Thrombotic occlusion

of chorionic or placental circulation is considered an important mechanism of miscarriage.
We retrospectively investigated a large cohort of young females affected by ET to evaluate if thrombotic and pregnancy complications occur in the same patients.
Patients and methods: Main data of our patients are summarized in the following table:

Patients number	34
Mean age at ET diagnosis (y)	29.4 ± 5.8 (18 – 41)
Mean platelets x 10^9/L at diagnosis	851 ± 350 (251 – 1831)
Patients with thrombotic complications	9
Mean age at first thrombosis (y)	35.9 ± 6 (30 – 47)
Patients with multiple thrombotic complications	3
Pregnancies number	57
Mean age at conception (y)	32.5 ± 5.4 (23 – 41)

Statistical analysis was performed with X^2 test with Yates correction.
Results: In our cohort of ET, we observed 13 first trimester abortions and 8 later fetal loss. Eight females had one normal baby after 1 (6 cases) or 2 (2 cases) abortions.
Thrombotic events occurred in 9 females. One patient had a myocardial infarctions and 1 a stroke, in both cases long time after their pregnancies. In contrast, unusual veins thrombosis (1 cerebral sinuses, 4 Budd-Chiari and 2 portal vein) occurred post-partum or no more than two years after delivery.
The correlation between pregnancy outcome and thrombosis is summarized in the following table:

	Uneventful deliveries	Miscarriages	TOT
Thrombosis	7	11	18
No thrombosis	29	10	39
TOT	36 (64%)	21 (36%)	57

Patients with thrombotic events have a higher rate of miscarriages ($p < 0.01$).
Discussion: Our data show that thrombosis, mainly in unusual sites, occur frequently in females with negative pregnancy outcomes. Knowing that a previous thrombosis is a validated risk factor for a second cardiovascular event and that placental thromboses have a basic role in pregnancy failures, we suggest that miscarriage should be considered a risk factor for thrombotic risk stratification of patients with ET. This contribution has been awarded as Best Communication.

Hypoxia-inducible factor-1 alpha (hif-1α) in multiple myeloma angiogenesis and progression

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Multiple Myeloma (MM) is a malignancy of immunoglobulin (Ig)-synthesizing plasma cells, that home to and expand in the bone marrow. Similarly to solid tumors its development is correlated to the formation of regions of hypoxia, whose degree is positively associated with the expression of the transcription factor Hypoxia-Inducible Factor 1 (HIF-1). The production of HIF-1 has been identified as a key element in allowing cells to adapt and survive in a hostile hypoxic environment via a variety of pathways. In hypoxia conditions HIF-1α subunit becomes stable and regulates the expression of target genes required for angiogenesis and tumor growth, such as the Vascular Endothelial Growth Factor (VEGF) Receptor-2 (VEGFR2); the Fibroblast Growth Factor (FGF) Receptor (FGFR); the Mesenchymal-epithelial Transition Factor (c-Met) and its ligand the Hepatocyte Growth Factor (HGF). Here we demonstrated the role of HIF-1α in MM angiogenesis and progression. Western Blot and ELISA analysis show the overexpression of HIF-1α and its target genes in endothelial cells (ECs) of patients with relapsed/refractory MM, but not in patients with nonactive MM (avascular phase), neither in patients with Monoclonal Gammopathies of Undetermined Significance (MGECs) or in healthy Human Umbilical Vein ECs (HUVECs) used as controls. Immunofluorescent staining and immunohistochemistry confirm the nuclear stabilization of HIF-1α in MMECs of relapsed patients. Moreover, we demonstrated no differences in the amount of HIF-1α mRNA (indicating that in MMECs the post-transcriptional control is affected). However the signaling pathway that in normoxia stabilizes HIF-1α in MMECs remains unknown. Finally, we show that HIF-1α knockdown by siRNA affects critical MMECs angiogenesis-related functions, (adhesion, spreading, migration and tubular morphogenesis in vitro) and makes MMECs refractory to Bortezomib, more sensitive to its action.
Our findings indicate that HIF-1α is correlated to the progression and to the angiogenic switch from nonactive to active MM. HIF-1α stabilization may represent a mechanism of resistance to therapy and a potential target for MM antiangiogenic treatment.

Low insulin-like growth factor-1 levels are associated with anemia in adult nondiabetic subjects

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Background: Increasing evidence suggests that anemia is a risk factor for cardiovascular morbidity and mortality. Several studies have reported the importance of anemia as a risk factor for adverse outcomes in a number of clinical conditions. Different factors may contribute to anemia including failure of appropriate erythropoietin production, damage to erythropoietin-producing peri-tubular cells, chronic systemic inflammation, iron deficiency, and use of angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs). Among these, insulin-like growth factor-1 (IGF-1) is a plausible candidate for several reasons.
Aims: In this study, we evaluated the association of IGF-1 with hemoglobin (Hb) concentration and anemia in a cohort of 1039 Caucasians subjects participating to the CATAnzaro MEtabolic RIsk factors Study (CATAMERIS), a cross-sectional study for assessment of cardio-metabolic risk factors in individuals carrying, at least, one risk

factor including elevated blood pressure, dyslipidemia, dysglycemia, overweight/obesity, and family history for diabetes or cardiovascular disease.

Methods: After a 12-h overnight fast, subjects underwent anthropometrical evaluation and a venous blood sample was drawn for laboratory determinations. Hemoglobin, mean corpuscular hemoglobin (MCH), and mean corpuscular volume (MCV) were determined using an automated particle counter and total serum IGF-1 concentrations were determined by chemiluminescent immunoassay

Results: Subjects with anemia exhibited lower IGF-1 ($P = 0.006$), and higher hsCRP levels ($P = 0.003$). To estimate the independent contribution of variables to Hb concentration, a multivariable regression analysis was modeled including age, gender, body mass index (BMI), waist circumference, blood pressure, fasting glucose, fasting insulin, IGF-1, fibrinogen, hsCRP, mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), serum iron, estimated glomerular filtration rate (eGFR), and treatment with angiotensin-converting enzyme (ACE) inhibitors or angiotensin II receptor blockers (ARBs). The variables significantly associated with Hb concentration were gender ($P < 0.0001$), IGF-1 ($P < 0.0001$), waist circumference ($P = 0.02$), hsCRP ($P < 0.04$), MCH ($P < 0.0001$), MCV ($P < 0.0001$), serum iron ($P = 0.001$), IGF-1 ($P = 0.003$), hsCRP ($P = 0.008$), and waist circumference ($P = 0.01$), accounting for 54.0% of its variation. Hb concentration was significant lower in subjects in the lowest IGF-1 quartile as compared with those in the third ($P = 0.02$) and fourth ($P = 0.001$). In a logistic regression model adjusted for age, gender, BMI, waist circumference, blood pressure, fasting glucose, fasting insulin, fibrinogen, hsCRP, MCH, MCV, serum iron, eGFR, and treatment with ACE inhibitors or ARBs, subjects in the first quartile of IGF-1 had a 2.49-fold higher risk of having anemia as compared with those in the fourth (OR 2.70, 95% CI 1.02–7.16).

Discussion and Conclusion: In our study, we provide evidence that circulating IGF-1 is an independent determinant of Hb and anemia. We found that IGF-1 levels were associated with Hb concentration even after adjustment for several confounders including gender, age, BMI, waist circumference, blood pressure, fasting glucose, fasting insulin, IGF-1, fibrinogen, hsCRP, MCH, MCV, serum iron, eGFR, and treatment with ACE inhibitors or ARBs. Subjects with anemia exhibited a significant reduction in IGF-1 levels, and low levels of IGF-1 were associated with an increased risk of anemia after adjustment for several confounders. Thus, low IGF-1 concentration appears to be an important contributor to anemia in adult nondiabetic subjects. Mechanistically, a reduction in IGF-1 levels may contribute to anemia by reducing erythropoiesis as a consequence of impaired IGF-1-stimulated erythroid cell growth and differentiation from bone marrow or peripheral blood. Alternatively, because IGF-1 has anti-inflammatory effects, and decreases expression of pro-inflammatory cytokines, a decrease in IGF-1 concentration may results in increased levels of inflammatory molecules, which are known to affect erythropoietin secretion, and red cell precursors survival.

Gastroenterology and Hepatology I

Increased apolipoprotein B (APOB) related RET/GDNF pathway indicates impaired enteric neuron development/differentiation in patients with chronic intestinal pseudo-obstruction (CIPO)

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Background: CIPO is a severe dysmotility syndrome characterized by recurrent intestinal sub-occlusive episodes with no detectable mechanical causes. CIPO is secondary to a variety of diseases or it can be idiopathic in origin. In this latter group, genetic abnormalities may play a pathogenetic role in altering the enteric neuro-muscular integrity. In this line, the RET/GDNF gene pathway regulates cell survival, differentiation, migration and proliferation of neural crest-derived cells/mature neurons. RET-dependant signalling molecules, including the downstream gene *ApoB*, have been shown to exert a critical role in enteric neuron differentiation processes in the nematode model *Caenorhabditis elegans:* Although RET mutations play a pathogenic role in Hirschsprung's disease (HSCR), but not in CIPO, the involvement of RET signalling downstream mediator *APO-B* gene remains largely unknown.

Objective: As an index of RET/GDNF impaired pathway, this study was designed to establish *ApoB* mRNA expression in a neuroenteric cell line (Neuro2A), in a knock-in mouse carrying the HSCR-ret^{C620R} mutation as well as in CIPO patients.

Methods: Neuro2A cells (ATCC, UK) were cultured according to previously established and validated protocols for neural cell lines. RET mRNA silencing was obtained by transfecting Neuro2A cells (5×10^5) with pRS-shRNA vector specific for mouse *ret*. ret^{C620R} knock-in mouse were commercially available. DNA and RNA were extracted from blood samples (10 cc) from 9 consecutive CIPO patients (8 F, age range: 18-55 yrs) defined by clinical, radiologic and manometric criteria (Gut 1987;28:5-12), and from 6 age-sex-matched asymptomatic controls. *ApoB* mRNA expression was assessed by quantitative real time PCR in the cell line, mouse RET knock-in model and CIPO patients.

Results: *ApoB* expression in Neuro2a is specifically activated by the RET/GDNF signalling pathway, since Ret mRNA silencing abolished *ApoB* increase. Further analysis showed that this mechanism is dependent on MAP P38 kinase activation. *ApoB* expression is downregulated in mouse embryos homozygous for the mutation ret^{C620R} and presenting a severe HSCR phenotype, whereas heterozygous mice with an apparently normal phenotype showed a significant increase in *Apob* expression. In line with this results, patients with CIPO showed an abnormal ApoB mRNA expression as compared to healthy subjects.

Conclusions: ApoB mRNA upregulation is a relevant mechanism in neuronal development/differentiation as demonstrated by Neuro2A cells and the ret^{C620R} mouse model. The evidence that *ApoB* has an aberrant expression in CIPO patients indicates an altered neuronal development/differentiation underlying such severe dysmotility syndrome.

Hepatic veins doppler profile alterations in cirrhosis: the role of intrahepatic shunts

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Background: In cirrhosis changes of hepatic veins Doppler waveform occur, but the cause is not well established. At the same time, derangement of intrahepatic structure and portal hypertension cause the opening of intrahepatic shunts.

Purpose: 1) verifying the presence of hepatic veins Doppler waveforms abnormalities in cirrhotic patients; 2) correlating these changes with the degree of portal hypertension and the severity of the disease; 3) evaluating if changes in hepatic veins Doppler waveform depends on abnormalities of intrahepatic circulation.

Methods: Fifty-eight cirrhotic patients without heart diseases, portal vein thrombosis, inversion of portal blood flow and 24 normal subjects were studied. An abdominal eco-color Doppler examination was performed in all of them. In 16 cirrhotic patients and in 4 normal subjects the arrival times of a contrast agent (Sonovue) in hepatic artery, portal vein and hepatic vein were measured together with the following parameters: hepatic veins: peak velocity of A and S waves, A/S ratio; portal vein: diameter, mean velocity, blood flow, congestion index, calculated portal pressure, pulsatility indexes of hepatic and splenic arteries, spleen diameter, Child-Pugh score, degree of oesophageal varices.
Results: The hepatic veins Doppler waveform was triphasic in 41% of cirrhotic patients, biphasic in 33%, monophasic in 26% and triphasic in all normal subjects. Cirrhotic patients had a significant reduction in portal blood velocity ($p < 0.0001$), and a significant increase ($p < 0.0001$) in portal vein diameter, congestion index and calculated portal pressure. Portal blood flow did not differ between patients and normal subjects. No significant differences of A and S waves peak velocities were registered at intrahepatic level and two cm from the inferior vena cava. No differences in hepatic veins pulsatility among patients with normal and irregular hepatic veins were observed. Cirrhotic patients were divided into three groups following the A/S ratio: the first one with negative A/S ratio (triphasic hepatic vein Doppler waveform), the second with $0 < A/S < 0,6$ (biphasic waveform), the third with $A/S \geq 0,6$ (monophasic waveform). The arrival time of Sonovue in hepatic veins was shorter ($25,4 \pm 5,8$ s vs $32,9 \pm 4,4$ s; $p < 0.05$) in cirrhotic patients than in controls and the portal-hepatic vein transit time too ($4,3 \pm 2,5$ s vs $9,0 \pm 2,4$ s; $p < 0.01$). The mean portal-hepatic vein transit time was $5,6 \pm 2,0$ s in cirrhotic patients with $A/S < 0$; $4,8 \pm 3,2$ s with $0 < A/S < 0,6$; $2,3 \pm 1,1$ s with $A/S \geq 0,6$. Controls had mean portal-hepatic vein transit time of $9,0 \pm 2,4$ s. An inverse correlation between portal-hepatic vein transit time and A/S ratio (Spearman $r = -0,57$; $p < 0,01$) was present. Patients with a monophasic hepatic vein Doppler waveform had a significantly higher Child Pugh score than the other cirrhotic patients ($p < 0.05$). A higher prevalence of oesophageal varices was present in patients with changes of hepatic vein Doppler waveform ($p < 0.05$).
Conclusions: The data of this study suggest that portal-hepatic shunts are responsible for hepatic veins Doppler waveform abnormalities. In cirrhosis the increase of intrahepatic shunts corresponds to a lower hepatic veins pulsatility. The high pressure present in the portal system prevents the positive A wave formation through these shunts. Changes in hepatic veins Doppler waveform seem to be associated with a more serious disease and to a higher prevalence of oesophageal varices.

Balloon-occluded rfa plus tace: a new combined single-step therapy for treatment of multinodular unresectable hepatocellular carcinoma

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AIM: To evaluate the feasibility and safety of combined single-step therapy with balloon-occluded RFA followed by TACE in patients with multi-nodular unresectable hepatocellular carcinoma.

Materials & methods: 10 consecutive patients with multinodular (2-6 nodules) unilobar unresectable HCC and with a target main lesion larger than 3 cm (range: 3.5-6 cm) were enrolled in our single-center multidisciplinary pilot study. The schedule consisted of: percutaneous RFA (single 3-cm monopolar needle insertion) of the target lesion during occlusion of the hepatic artery supplying the tumor followed by lobar TACE (450 mg carboplatin and lipiodol plus temporary embolization with spongostan). Adverse events as well as intra/peri-procedural complications were clinically assessed. Early local efficacy was evaluated on 1-month follow-up multiphasic CT based on RECIST criteria. A separated evaluation of target lesions in terms of enhancement, necrotic diameter, presence and distribution of lipiodol uptake was also performed.
Results: No major complications occurred. Overall technical success, defined as complete devascularization during the arterial phase of all nodules, was achieved in 7/10 patients with 3 partial response (persistence of hypervascular small nodules). When considering only target lesions, technical success was obtained in all patients, with a nonenhancing area corresponding in shape to the previously identified HCC (necrotic diameter: 3.5-5 cm) always obtained, and with a circonferential peripheral lipiodol uptake, as safety margin of lesion, of at least 0.5 cm (0.5-1.3 cm).
Conclusion: Balloon-occluded-RFA plus TACE seems to be a safe and effective combined therapy for the treatment of advanced unresectable HCC lesions, allowing to obtain a high complete local response rate also in large lesions.

Bone marrow-derived mesenchymal stromal cells locally injected for refractory fistulizing chron's disease modulate serum immunoglobulins, secretory iga, and antibody production

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Background: Mesenchymal stromal cells (MSC) are multipotent non-haematopoietic progenitor cells capable of mesenchymal lineage differentiation and even transdifferentiation into other cell lineages. They also display the ability to homing to sites of inflammation and injury, thus contributing to tissue regeneration, and exhibit robust immune-suppressive and modulatory functions towards almost all cells involved in the immune response, i.e., T- and B-cells, dendritic cells, monocytes/macrophages and natural killer cells. Up to date, only a few in vitro studies have focused the attention on the effects of MSC on B-cell function with conflicting results. Specifically, it has been shown that B-cell development is partly dependent on the close interaction of B-cell progenitors with MSC, and that unidentified soluble factors produced by MSC can interfere with B-cell activation, proliferation and differentiation into immunoglobulin (Ig)-producing plasma cells. By consequence, MSC seem exhibit an inhibitory effect on Ig secretion. On the other side, a favouring effect on auto-antibody production has been demonstrated in a peculiar experimental condition. However, data related to the effects of MSC therapy on alloantigen-specific humoral response in humans are still lacking.
Aim: We aimed, therefore, to investigate the in vivo potential capacity of MSC therapy as compassionate use for refractory fistulizing Crohn's disease (CD) in modulating Ig production, including secretory IgA, anti-Saccharomyces cerevisiae antibodies (ASCA),

and anti-neutrophil cytoplasmatic antibodies (ANCA), the latter specifically correlated with CD.

Patients and Methods: Ten CD patients (7 males, median age 32 years) were successfully treated with intrafistular injections (median 4) of autologous bone marrow-derived MSC. Injections were scheduled at 4-week intervals, with a median of 20×10^6 cells each time (range 15-30). The white blood cell count, the lymphocyte population characterization and the levels of circulating Ig were quantified by applying standard laboratory assays, while the secretory IgA were determined by immunoelectrophoresis on patients' saliva samples and expressed as mg/dl. ASCA and ANCA were quantified by ELISA and indirect immunofluorescence, respectively. All determinations were performed before, after each MSC treatment and 12 months after the last one. Statistical comparisons between mean values were performed with the Mann–Whitney *U* test for parametric data. A p value less than 0.05 was considered statistically significant.

Results: The white blood cell count and the number of B cells were unchanged throughout the duration of the study. By contrary, the mean value of secretory IgA detected after the first MSC infusion resulted statistically decreased ($p < 0,01$) in comparison to the pre-infusion level, although it returned to baseline after 12 months from the last MSC injection. At this time, a statistically significant reduction of both serum IgG and IgA was also observed ($p = 0,05$), whilst no change of IgM at anytime was found. Moreover, two patients who were ANCA positive before the first MSC injection, resulted negative at the end of the study, while no significant change for ASCA was detected.

Conclusions: MSC, even when locally injected, may display potent systemic immune-modulatory properties in vivo which could be exploited clinically, especially in those conditions in which B-cells play a major role.

This contribution has been awarded as Best Communication.

Infective Diseases

Evaluating the response to different therapeutic protocols defined for health-care associated pneumonia

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Different etiological forms of pneumonia are classified as CAP (Community-acquired pneumonia), HAP (Hospital-acquired pneumonia) and VAP (Ventilator-acquired pneumonia). A more recent nosological form was formulated by Friedman and collaborators in 2002, defined as a "Health-care associated infection" in the sense that they found that patients undergoing in-home medical care are exposed to a higher risk of infection by multidrug resistant (MDR) pathogens. The risk factors for HCAP (Health-care associated pneumonia) include: hospitalization for 2 or more days in the last 3 months; residence in a nursing home, long-term hospital care facility or sanatorium; in-home infusion treatment; chronic dialytic treatment in the last 30 days; in-home medication of surgical wounds and/or decubitis sores; a family member with a MDR pathogen. As regards therapeutic aspects, there are some differences between the protocols recommended by the ATS-IDSA guidelines in 2005 (that distinguish patients at risk or not of infection by multi-resistant pathogens) and by the SIMI (that consider all patients at risk of MDR infection). The aim of this work was to make a retrospective analysis of the prevalence of HCAP in an Internal Medicine ward, assessing etiologies, clinical pictures and therapeutic approaches, as well as whether the administration of the therapies recommended by the international guidelines is efficacious in reducing mortality and hospital stay. All patients with a HCAP admitted to the Internal Medicine Operative Unit "C. Frugoni" of Bari University Hospital between January 2008 and March 2010 were enrolled. Reviewing the chosen therapy, level of compliance to the guidelines, mortality and length of hospital stay, it was found that 32% of the patients with HCAP had been hospitalized for more than 2 days in the last 6 months, and more than 70% had been treated with polytherapy regimens for a mean duration of 15 days. Patients were subdivided into 2 groups according to whether they were treated in accordance with the ATS-IDSA 2005 guidelines or not: in patients treated with a single drug, 46% belonged to the former group versus 54% to the latter. The mean duration of therapy was 12 days in both groups; mean hospital stay was 22 days in the first group and 18 days in the second; mortality was 8.3% in the group treated in compliance with the guidelines versus 13.6% in the other group. Failure to comply with the guidelines consisted in 86% of cases of administration of a drug with no efficacious activity against Meticillino-Resistent-Staphylococcus Aureus (MRSA), i.e. vancomycin or linezolid, in patients at risk: in 9% of cases an anti-MRSA drug had been administered but not a second anti-Pseudomonas; in 27% the anti-MRSA drug used was teicoplanin, not included in the guidelines. Among the patients undergoing polytherapy, compliance to the guidelines was 26%, versus a different antibiotic therapy in 74%; the duration of therapy was 13 versus 15 days in the two groups; hospital stay was 21 days versus 17 days; mortality was 11% in the former group versus 12% in the latter, in patients with comparable disease severity (PORT class III-IV). In conclusion, HCAP are very frequent in an Internal Medicine ward. In this comparison of groups treated in accordance with the international guidelines or not, the lack of a significant difference in terms of hospital stay and mortality demonstrates the need for further study, as pointed out in the national protocol SIMI, to reach statistical significance. Should our results be confirmed in further studies, this would demonstrate the need to establish more restrictive criteria for the definition of HCAP.

Current clinical features of infective endocarditis on cardiac implantable electronic devices: a prospective cohort study

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Introduction: Infective endocarditis (IE) is a life-threatening disorder that may affect both native and prosthetic heart structures and can have either a spontaneous or a health-care related acquisition. An emerging form of health-care related IE is represented by infection of intracardiac electronic devices, such as pacemakers and defibrillators. Despite of this, over the last ten years, the features of cardiac device IE have been the subject of very few studies, mostly on a small number of patients. In this report, we aimed at presenting our prospective cohort of cardiac electronic device IE with a special focus on the current microbiology and antibiotic treatment and the outcome of percutaneous catheter extraction.

Design: Included in this study were 64 consecutive patients (50 males and 14 females), with a median age of 65 years (range 17-90), admitted to our unit from December 1999 through August 2010 with a diagnosis of definite IE on a pacemaker or a defibrillator according to the modified Duke criteria. Only patients with a staphylococcal etiology were included in this report.

Results: IE involved a pacemaker in 52 cases (81%) and a defibrillator in 12. The estimated average time span from clinical onset to diagnosis was lower than one month in 15 cases (23%), between 1 and 6 months in 33 (52%) and greater than 6 months in 16 (25%). Among presenting symptoms, fever was recorded in 89% of cases, pulmonary embolism in 37% and an increase of C-reactive protein in 92%. Comorbidities such as obstructive lung disease, heart failure, diabetes and chronic renal failure were each present in more than 20% of patients.

Blood cultures and/or intracardiac lead cultures grew Staphylococcus epidermis in 37 cases (58%), coagulase-negative non epidermis staphylococci in 15 (23%) and Staphylococcus aureus in 12 (19%). Methicillin-resistant (MR) staphylococci were involved in 54.7% of cases. IE acquisition was consistent, as only 25% of cases were deemed 'community acquired', while 70% were nosocomial and 5% non-nosocomial, health-care related.

The trans-esophageal echocardiogram displayed right heart vegetations in 98% of cases whereas the trans-thoracic study was positive in only 80% of them. Upon echocardiography, endocardial lead vegetations, with a median of 19.5 mm (range 2-33 mm), were observed in 58 patients (90%), while a tricuspid involvement with valve vegetations and regurgitation was present in 15 (22%).

The treatment of these 64 patients was with antibiotics alone in 13 cases (20%) and with a combined medical and surgical approach in 51 cases (80%). Seventeen patients (26%) underwent open-heart surgery, while 34 (53%) underwent percutaneous catheter extraction. The antimicrobial treatment course was based on daptomycin (at a median dose of 8 mg/kg/day) in 26 cases, amoxicillin-clavulanic acid (average dose 8.8 gr/day) in 21, and on vancomycin, teicoplanin or linezolid in the remaining 17 cases. The total duration of antimicrobial treatment was at least 6 weeks, and was prolonged in most cases up to 8 weeks. On average, in-hospital treatment lasted 25 days. The most common adverse events, possibly related to the antimicrobial treatment, were renal functional impairment (19%), liver function test abnormalities (8%) and muscle toxicity (3%). There were 2 cases of drug fever and 1 case of hypersensitivity to vancomycin.

Percutaneous lead extraction was complicated by pulmonary embolism in 3 patients while leads or vegetations persisted in the heart in 3 patients, 2 of whom subsequently underwent open-heart surgery.

Overall, 8 patients died in hospital (12.5%): 2 died in the medical treatment group (mortality 15%), 3 in the open-heart surgery group (mortality 18%) and 3 in the percutaneous lead extraction group (mortality 9%).

Discussion and Conclusions: Staphylococcal device IE mostly affects elderly patients showing a high rate of comorbid conditions, and may present without fever or other ill-defining signs or symptoms. It is often associated, however, with signs of pulmonary involvement. Delayed diagnosis is common and even transesophageal echocardiography may be falsely negative. These factors most likely affect the mortality rate, that in our experience appears to overlap with that of left sided valve IE, despite the lower incidence of heart failure in such right sided IE cases.

As the prevalence of methicillin-resistance is higher than 50%, the initial empirical antimicrobial treatment must cover for both MS and MR strains. In this respect, an adequate option may be represented by daptomycin, that indeed was used in our center in a substantial proportion of these patients.

This contribution has been awarded as Best Communication.

Diagnostic performance of multiple real-time polymerase chain reaction in patients with suspected sepsis hospitalized in an internal medicine department

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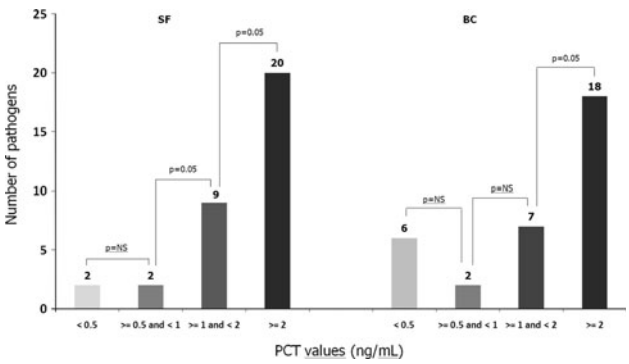
Sepsis is a potentially deadly condition frequently found in patients hospitalized in tertiary care hospitals. Early pathogen detection and prompt administration of appropriate antimicrobials is pivotal to decrease high morbidity and mortality rates and improve patients outcome. The diagnosis of sepsis relies heavily on blood cultures (BCs) however, sensitivity of BC is limited, particularly when antibiotics have been already administered; moreover BC often cannot provide time-critical results that can impact on early management. New molecular methods allow rapid pathogen detection and should help clinicians to diagnose sepsis and to initiate earlier more-specific antimicrobial therapy. SeptiFast, a commercially PCR based system (SF), has recently been used in the molecular diagnosis of sepsis in patients with endocarditis, neutropenic and immunocompromised febrile patients and in those hospitalized in emergency room and intensive care units. There are still limited data about the impact of SF on etiological diagnosis in routine clinical management of patients with presumed sepsis. We evaluated the diagnostic accuracy and clinical usefulness of SF, compared to BC, in combination with serum Procalcitonin (PCT) determination, in 260 patients hospitalized in a department of internal medicine and suspected of having systemic inflammatory response syndrome (SIRS), caused by bacterial or fungal infection. SIRS was defined as a condition that fulfilled two or more of the following criteria: temperature $> 38°C$ or $< 36°C$; heart rate > 90 beats per minute; respiratory rate > 20 breaths per minute; white blood cell count $> 12{,}000$ cells/μL or $< 4{,}000$ cells/μL. One hundred and sixty one of the enrolled patients (62%), were affected by SIRS and a causative pathogen was identified in 58 cases (36%) with either the use of BC or SF test. SF identified a significant higher number of pathogens compared to BC in patients in which blood samples were drawn at least 24 h after starting antibiotic therapy (19 vs 11; $p = 0.05$) and among the 178 samples from 161 SIRS patients (42 vs 31, $p = 0.027$). In the latter concordant results between BC and SF were obtained in 87% of the cases: 118 samples were concordant negative and 26 samples concordant positive: sixteen pathogens were detected only by SF, while 12 pathogens only by BC; among these, seven were not included in the SF master list. The combination of the two methods significantly improved the number of pathogens detected in comparison to BC or SF alone. Both SF and BC, identified a significant greater number of pathogens in patients with PCT plasma values ≥ 2 ng/mL; while SF pathogen detection was improved also for PCT values between 1 ng/mL and 2 ng/mL. ($p = 0.05$) (Figure). Our results suggest that *SF* served as a highly valuable adjunct to conventional BC in detecting pathogens in SIRS patients hospitalized in an internal medicine ward, particularly if pretreated from more than 24 h with antibiotics and if their PCT levels were higher than 1 ng/mL.



Difficult differential diagnosis of multiple nodular pulmonary lesions: A case report

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Case presentation: A 78 years old man presented with cough and weight loss. In his past medical history he referred right mastectomy for invasive breast cancer followed by adjuvant radiotherapy on internal mammalian nodes, femoral fracture and alcohol abuse; poor social conditions were also reported. In September 2010 he performed a chest x-ray and a thorax CT-scan with evidence of a 6 cm nodular, parahilar, lung lesion and multiple, bilateral centimetric nodular lesions. Between October and November, 2010 three fibrobronchoscopies with broncho-alveolar lavage and biopsy were performed with no evidence of neoplastic cells; microscopic acid-alcohol fast bacilli (AAFB) research resulted negative. He was admitted in our Service in December, 2010 with diagnosis of suspected lung cancer.

At admission he complained dyspnoea, cough with purulent sputum, malaise. A sample of sputum for culture and AAFB research was collected; an empirical antimicrobial therapy with piperacillin-tazobactam was administered. Culture resulted positive for *Acinetobacter baumannii* and ciprofloxacin was administered according to antibiogram. PCR assay on sputum for *Mycobacterium spp* research resulted positive and typization confirmed a *Mycobacterium intracellulare*. A total body CT-scan resulted in unmodified pulmonary lesions and no evidence of other metastatic sites. HIV test was negative.

To better characterize pulmonary lesions we further performed a [18F]-Fluoro-2-Deoxy-D-Glucose (FDG)-PET/CT, that noticed high metabolic activity of known pulmonary lesions consistent primarily with heteroplastic lesions but also suggestive for inflammatory disease. Therefore, lacking a proven diagnosis of cancer, we decided to start antimycobacterial therapy with rifampin 600 mg *qd*, ethambutol 1500 mg *qd*, clarithromycin 500 mg *bid* and amikacin 1000 mg three times per week. Patient was then discharged in January, 2011. However, he was soon readmitted for development of anorexia, nausea, vomiting and fatigue with weigh loss and scarce adherence to therapy. Amikacin was stopped for evidence of mild renal insufficiency, clarithromycin was changed wit azithromycin 500 mg iv *qd*; other drugs were temporarily administered iv. General conditions improved and switch to oral therapy was possible. A second FDG-PET/CT performed in March, 2011 revealed a metabolic reduction of the parahilar lesion but a metabolic and dimensional increase of the other lesions. Considering the short course of antimycobacterial therapy and the initial scarce compliance we decided to continue with oral rifampin, ethambutol and azithromycin. Clinical conditions further improved; a third FDG-PET/CT in May, 2011 showed a metabolic and dimensional reduction of all lesions.

Discussion: isolation of *Mycobacterium intracellulare* is not typical in patients with no known risk factors for immunodeficiency; in this case alcoholism, malnutrition, poor social conditions and previous radiotherapy could have decreased host defences. Differential diagnosis between neoplastic and benign lesions including mycobacteriosis may be difficult, especially without certain histological findings. PET/CT cannot distinguish between infection and cancer, especially when radiologic imaging of flogistic lesions has a nodular pattern. We used FDG-PET/CT to follow up response to antimycobacterial therapy and to exclude further radiologic worsening mostly compatible with neoplastic progressive disease. Further studies are warranted to improve differential diagnosis between mycobacterial infections and cancer in immunocompetent patients, especially when imaging and histology are not conclusive.

Thrombosis and Hemostatis I

Statins discontinuation in compliant chronic users induce pro-atherothrombotic profile despite baseline clinical setting and associated treatments

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Statins offer important benefits for the large populations of individuals at high risk for cardiovascular and cerebrovascular events in both primary and secondary prevention. Statins primary mechanism of action is the reduction of LDL-C, that is a relevant risk factor for atherothrombotic events, via HMG-CoA reductase inhibition. Due to the positive clinical impact, the concern of statins withdrawal on putative rebound vascular events has been suggested and evaluated both in terms of biological mechanisms or clinical settings. In fact several studies have suggested that statins discontinuation was associated with negative impact on the majority of above reported anti-atherothrombotic mechanisms as extensively reviewed.

The aim of the present study was to determine whether statins discontinuation could induce a pro-atherothrombotic profile in different clinical settings.

Ninety-six subjects treated for familial dyslipidaemia (group A) and one-hundred-forty subjects treated for dyslipidaemia and hypertension (group B) and/or clinical atherosclerosis (including ASA 100 mg/day, group C) were evaluated at seven (T7), fourteen (T14), thirty (T30) and sixty (T60) days after statin withdrawal for total cholesterol, LDL-cholesterol, HDL-C, triglycerides (enzymatic-colorimetric methods), oxidized-LDL (ox-LDL) (ELISA), apolipoprotein B/A ratio (apo B/A) (immunodiffusion), hs-CRP (ELISA), soluble CD40 ligand (sCD40L) (ELISA), platelet P-selectin (CD62 +) (FACScan) and endogenous thrombin potential (ETP®) (chromogenic-coagulative method).

Group A: sCD40L and CD62 + were significantly increased ($p < 0.05$) from T14 in relation with hs-CRP and ox-LDL changes (each $r > 0.31$, $p < 0.05$). ETP was significantly changed ($p < 0.05$) from T30 in relation with apo B/A and LDL-C (each $r > 0.31$ and $p < 0.05$ or higher). Group B: sCD40L, CD62 + and ETP were significantly changed from T30 (each $p < 0.05$ or higher) in relation with LDL-C, apo B/A and ox-LDL modifications (each p $r > 0.31$ and $p < 0.05$ or higher). Group C: sCD40 L and CD62 + were increased from T30 whereas ETP at T60 (each $p < 0.05$) in relation with LDL-C, apo B/A and ox-LDL changes (each $r > 0.31$ and $p < 0.05$ or higher). When percent differences of each variable were analyzed, the multivariate analysis was confirmatory; in particular O.R. for categorized percent increase of each variable showed an association for sCD40L (>30% increase), CD62 + (>20% increase) with ETP (>20% increase) in relation with LDL-C (>30% increase), ApoB/A ratio (>25% increase), and ox-LDL (>25% increase) in groups A and B.In group C the multivariate analysis confirmed a relation between sCD40L (>25% increase) and CD62 (>15% increase) with LDL-C (>35% increase), apoB/A ratio (>30% increase) and ox-LDL (>30% increase) (Fig. 1).

Our results confirm a pro-atherothrombotic profile, despite going-on treatments with anti-hypertensive drugs and ASA, that occurs globally but at different timings in subjects discontinuing statins. In particular the increased sCD40L in relation with CD62 + and ETP suggest a complete inflammatory/thrombotic rebound. Furthermore the evidence that such changes occur at different times in relation with lipid profile changes indicate that various mechanisms occurs in statins withdrawal rebound according also to associated treatments and could have a specific impact in different clinical settings.

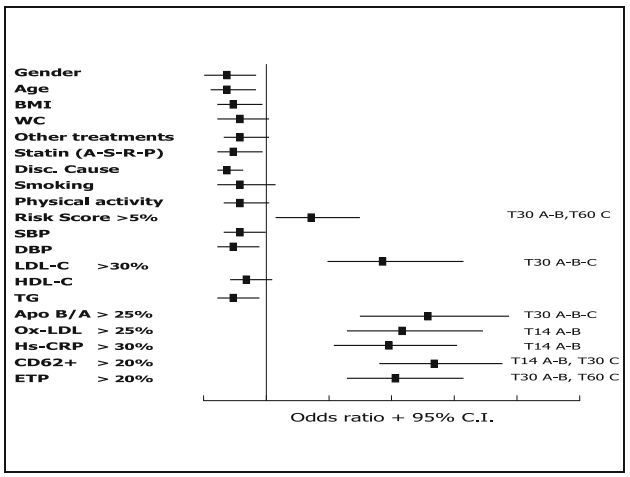

Fig. 1

Association between the presence of factor V leiden or prothrombin G20210A mutations and location of venous thromboembolism: a systematic review and a meta-analysis of the literature

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Venous thromboembolism (VTE) is a common vascular disease that results in two major clinical manifestations: deep venous thrombosis (DVT) and pulmonary embolism (PE). Factor V Leiden (FVLeiden) and prothrombin G20210A are the most common genetic causes of thrombophilia and established risk factors for different clinical manifestations of VTE. However, It is still uncertain whether the presence of inherited thrombophilia influences the risk of developing symptomatic PE and whether different thrombophilic alterations are associated with different risks of symptomatic PE. To investigate such issue we perform a systematic review and a meta-analysis of the literature.

Methods: The MEDLINE, EMBASE, Cochrane Library databases, reference lists of retrieved articles and contact with content experts were used. Studies comparing the prevalence of prothrombotic abnormalities in patients with PE and in patients with DVT without PE disease were included. Two reviewers independently selected studies and extracted study characteristics, quality and outcomes. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated for each trial and pooled using a random-effects model. Statistical heterogeneity was evaluated using the I^2 statistic. Sensitivity analyses were performed examining separately studies according to the etiology of VTE.

Results: Twelve studies for a total of more than 5000 patients were included in our systematic review. Presence of factor V Leiden was associated with an increase risk of presenting with DVT in VTE patients (OR 1.51, 95% CI 1.18, 2.02; $p = 0.02$). Heterogeneity among the trials was not negligible ($I^2 = 67\%$).

On the other hand, presence of G20210A mutation of the prothrombin was not associated with an increase risk of presenting with DVT or symptomatic PE in VTE patients (OR 0.86, 95% CI 0.70, 1.06; $p = 0.18$). There was no heterogeneity among the trials was not negligible ($I^2 = 0\%$). Sensitivity analyses including only patients with idiopathic VTE gave similar results (data not shown).

Conclusion: Results of our systematic review and meta-analysis of the literature suggest that presentation as DVT occurs more frequently in VTE patients with factor V Leiden mutation whereas the presence of G20210A mutation did not seem to influence the VTE location at the presentation.

Clinical history of patients with cerebral vein thrombosis: results of a large multicenter international cohort study

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Background: Little information is available on the long term clinical outcome of cerebral vein thrombosis (CVT).

Aims and Methods: In an international, cohort study, involving 27 centers, we assessed the long term rates of mortality and recurrent venous thrombosis in a large cohort of patients with a first episode of CVT. The role of potential risk factors for thrombosis recurrence (thrombophilic abnormalities, cancer, infections, trauma, oral contraceptives, pregnancy, puerperium, neurosurgery and myeloproliferative neoplasms) was evaluated.

Results: 706 patients (73.7% female) with CVT were included. Patients were followed for a total of 3171 patient-years. Median follow up was 40 months (range 6 to 297 months). At the end of follow up 20 patients died (2.8%). About 84% of patients were treated with oral anticoagulants, the mean duration of treatment was 12 months. CVT recurred in 31 patients (4.4%), and 46 patients (6.5%) had a venous thrombosis in a different site, for an overall incidence of recurrence of 23.6 events per 1000 patient-years (95% Confidence.

Interval [CI] 17.8, 28.7). Incidence of recurrence after anticoagulant therapy was stopped was 35.1 events/1000 patient-years (95% CI, 27.7, 44.4). In the univariate model, personal history of VTE (Hazard Ratio [HR] 2.733, 95% CI 1.528-4.887), Recent head trauma (HR 4.197, 95% CI 1.926-9.146) and cancer (HR 2.565, 95% CI 0.906, 3.945) were associated with recurrent VTE whereas indefinite OAT was not associated with improved event free survival. When all potential risk variables were included in a multivariate model, only personal history of VTE remained significantly associated with recurrent CVT or VTE in other sites (HR 2.700; 95% CI 1.251, 5.830; p 0.011).

Conclusions: The long term risk of mortality and recurrent venous thrombosis appears to be low in patients with a first episode of CVT. A previous venous thrombosis was found as the only independent predictor of recurrent events.

This contribution has been awarded as Best Communication.

Which role for hyperhomocysteinemia in patients with early-onset ischemic stroke?

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The pathogenic mechanisms of ischemic stroke in young adults are still poorly understood. In this setting the impact conventional cardiovascular risk factors is thought to be lower than in elderly patients and data not entirely consistent are available concerning the contribution of thrombophilic conditions, including hyperhomocysteinemia (HHcy). We compared 225 patients (104 men, 121 women) with a history of early onset (< 50 yrs) ischemic stroke (mean age at the event 36.5 ± 9 yrs) with a population of 258 sex- and age-matched healthy controls. A significantly higher prevalence of arterial hypertension (34.9 vs. 11.6%, $p < 0.0001$; odds ratio [OR], 95% confidence interval [CI]: 4.08, 2.60-6.40) and of smoking habit (59.7 vs. 30.6%, $p < 0.0001$, OR, 95% CI: 3.36, 2.36-4.78) was found in stroke patients than in controls. Diabetes mellitus and hypercholesterolemia were also more prevalent in the patient group ($p < 0.05$), whereas no statistically significant difference was found in the prevalence of Factor V Leiden, prothrombin G20210A, and C677T 5,10-methylenetetrahydrofolate reductase (MTHFR) gene mutations. Fasting total homocysteine levels (tHcy) differed significantly between patients and controls (14.7 ± 10.4 vs. 12.6 ± 7.1 μmol/L; $p = 0.003$), HHcy being associated to a 1.8-fold increase of risk of ischemic stroke (OR, 95% CI: 1.83, 1.23-2.73). Interestingly, this increase of risk was mainly found in female patients, in which both HHcy and tHcy data showed stronger statistical significance (28.9% vs. 5.1%; $p < 0.0001$; OR, 95% CI: 7.53, 3.34–16.97 and 13.2 ± 7.8 vs. 9.8 ± 3.6 μmol/L, $p < 0.0001$, respectively). Moreover, a synergistic effect of HHCy with other risk factors was found, being the increase of risk further elevated when smoking habit (9-fold), arterial hypertension (7-fold) or hypercholesterolemia (4.8-fold) were associated with high tHcy levels. Consistent with current literature data, HHcy is a mild risk factor in our cohort of patients with early-onset ischemic stroke. However, its contribution is likely to be higher in women and in patients with other cardiovascular risk factors.

Emergency Medicine

Role of copeptin in patients with chest pain: the rossini trial

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Background and Aim: Copeptin is the C-terminal fragment of the pro-hormone AVP and it is a marker, stable and sensitive, of the releasing of AVP in the body. Our study aim is to evaluate if initial levels of copeptin <14 pmol/l combined with a negative Tn-T can correctly rule out the diagnosis of acute myocardial infarction, as suggested by recent studies, as well as of other life-threatening causes of chest pain.
Materials and Methods: This is an observational, prospectic, multicentric study. The study is ongoing in several hospitals of Milan. We present the results of the first 178 patients of two of the hospitals, referring to the Emergency Room with the leading symptom chest pain started in the previous eight hours. Mean age of the patients (68% males) was 57 ± 16 yrs. Clinical evaluation of patients included ECG and blood exams with Tn-T (cut off <0.03 μmol/l).
Results: Patients were divided into three groups according to the final diagnosis: SCA (Acute Coronary Syndrome - STEMI, NSTEMI, unstable angina); Potentially lethal diseases not SCA (aortic dissection, pulmonary embolism, pulmonary edema, acute heart failure, sepsis); Non- potentially lethal diseases. Copeptin levels were significantly higher in patients with STEMI and NSTEMI than in patients with other diagnosis, while in patients with unstable angina values of copeptin were similar to those of patients with other diagnosis. The combination of copeptin and troponin-T reached a negative predictive value of 99.1% (CI 95.3-100) for SCA, of 98.2% (CI 93.9-99.8) for other potentially lethal diseases non-SCA and of 96.6% (CI 91.5-99.1) for all potentially lethal diseases (SCA + others). Age, sex and BMI did not influence copeptin values, while there was a positive correlation with the values of glucose, urea and the presence of artery disease.
Conclusions: In our study, values of copeptin were significantly higher in patients with STEMI and NSTEMI and other potentially lethal diseases than in patients with other diagnosis; in patients with unstable angina, values of copeptin were similar to those of patients with non potentially lethal diseases. The combined use of troponin and copeptin significantly improved diagnostic accuracy of troponin alone, both in SCA (STEMI and NSTEMI) and in all potentially lethal diseases.

Recognizing oligoanalgesia in the emergency department: a first step towards the implementation of new clinical pathways for acute pain management

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Introduction: acute pain is the most common presenting symptom in the Emergency Department (ED); neverthless, in this setting, oligoanalgesia is known to be a very frequent problem.
Objective: to recognize the presence of oligoanalgesia in our ED.
Design: retrospective study.
Setting: ED of a community-based, 700-bed hospital.
Patients and methods: retrospective analysis of the first 3000 ED visits in 2011 (software AIRO, *Area Informativa Ricoveri Ospedalieri*), with regard to the following indicators: A) assessment of pain intensity at triage using pain scales; B) number of patients with moderate/severe pain who received analgesics; C) analgesic drugs used in the ED; D) average door-to-drug time in patients with moderate/severe pain; E) number of patients with severe pain with door-to-drug time >20 min.; F) number of patients with moderate pain with door-to-drug time >60 min.; G) number of patients who received a reassessment of pain; H) number of patients who received a home prescription of analgesic drugs at discharge.
Patients with age <12 years, chest or abdominal pain, severe headache (yellow code) and major trauma were excluded. We identified 606/3000 patients (20.2%) with potentially treatable pain (68/606 yellow code 11.3%, 538/606 green code 88.7%). In patients with severe pain, diagnoses were the following: minor trauma 56 (82.5%); renal colic 9 (13.3%); biliary colic 1 (1.4%); low back pain 1 (1.4%); other kind of pain 1 (1.4%). In patients with moderate pain, diagnoses were the following: minor trauma 410 (76.3%); renal colic 16 (2.9%); biliary colic 11 (2.1%); low back pain 23 (4.2%); headache 26 (4.9%); other kind of pain 52 (9.6%)
Results: A) all the patients received an assessment of pain intensity with verbal rating scale (mild, moderate, severe pain); B) 23/68

(33.8%) patients with yellow code and 97/538 (18.9%) patients with green code received analgesics; C) the drugs used were the following (single doses): acetaminophen IV (45), ketoprofen IV (35), tramadol IV (3), diclofenac IM (35), miorelaxants IM (20), antispastics IV (20), lorazepam OS (6), betamethasone IV (3), acetylsalicylic acid IV (1), methylprednisolon IV (2), Oxygen (2); D) average door-to-drug time was 90.2 min. for yellow code (range: 7-679 min) and 93.7 min. for green code (range: 9-908 min.); E) 39% of the patients with yellow code who received analgesics (9/23) had a door-to-drug time > 20 min.; F) 50.5% of the patients with green code who received analgesics (49/97) had a door-to-drug time > 60 min.; G) none of patients received a reassessment of pain intensity; H) 22/37 (59.4%) patients with yellow code discharged home received a clear prescription of analgesics. 314/490 (64%) patients with green code discharged home received a clear prescription of analgesics.

Conclusions: as reported in previous studies, acute pain is undertreated also in our ED. Recognizing this problem could be the first step to develop clinical pathways for pain management in this setting. This contribution has been awarded as Best Communication.

Gerontology and Geriatric Medicine I

Subclinical hypothyroidism and cognitive dysfunction in the elderly

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While overt hypothyroidism is associated with reversible dementia in the elderly, the relationship of subclinical hypothyroidism with cognition remains a controversial issue. Our aim was to investigate the correlation between subclinical hypothyroidism and cognition in the elderly, with particular reference to long term memory and selective attention.

We selected 337 outpatients (177 men and 160 women), aged 64 to 86 (mean 74.3 years), excluding patients already affected by thyroid dysfunction and/or already treated with drugs influencing thyroid function.

Comorbid conditions, medication use and socio-demographic profiles were recorded. Thyroid function was evaluated by measuring serum concentrations of thyrotropin (TSH), free thyroxine (FT4), free triiodothyronine (FT3) and antibodies to thyroid peroxidase (TPO-Abs) and to thyroglobulin (TG-Abs). Cognitive functions was established by using the Mini Mental State Examination (MMSE), the Prose Memory Test (PMT) and the Matrix Test (MT).

The score of MMSE was significantly lower in the group of patients with subclinical hypothyroidism than in euthyroid subjects (22.0 ± 4.1 vs 23.3 ± 3.7, $p < 0.03$).

By means of a logistic regression model it was observed that patients with subclinical hypothyroidism have a probability about 2 times greater ($RR = 2.028$, $p < 0.05$) of developing cognitive impairment. PMT score was significantly lower in subjects with subclinical hypothyroidism compared with the euthyroid group (6.4 ± 4.1 vs. 7.9 ± 4.8, $p < 0.04$). Considering the MT, the performance was slightly reduced in subclinical hypothyroidism (36.1 ± 10.8 vs. 38.4 ± 11.1, NS). Furthermore, TSH was negatively correlated with MMSE ($p < 0.04$), PMT ($p < 0.05$) and MT score (NS). No correlation was found between FT4 and FT3 and MMSE, PMT and MT score.

In the elderly, subclinical hypothyroidism is associated with cognitive impairment, and its impact on specific aspects of cognition (long term memory and selective attention) is less evident.

Stem cells transplantation into an animal model of glucocorticoid-induced bone damage

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Background: Glucocorticoid-induced osteoporosis is the most common type of secondary osteoporosis. Glucocorticoid treatment is a well known method to induce osteoporosis in animal models. The aim of this work is to investigate whether preosteoblasts could repopulate injured bone in an animal model treated with glucocorticoids.

Materials and methods: Preosteoblasts were isolated from calvariae partially digested with type IV collagenase of newborn GFP mice with an isolation method based on the capability of these cells to migrate onto plastic surface. Their osteogenic potential, induced with osteogenic medium containing ascorbic acid and β-glycerophosphate, has been analysed by cytochemistry assays to detect alkaline phosphatase and mineralized bone nodules (Alizarin Red and Von Kossa staining) and by Real time PCR to assess expression of the osteogenic marker Runx2.

To realize the in vivo model, C57BL/6 three months aged mice have been divided into three groups [group I ($n = 4$): mice not treated with drug and not infused with cells, group II ($n = 4$): mice treated with drug and not infused with cells, group III ($n = 4$): mice treated with drug and infused with cells]. Drug (methylprednisolone) has been administered for one month with a dose of 75 mg/Kg/week. In mice of group III, 5×10^5 GFP preosteoblasts, previously expanded in vitro, have been infused with injection into the tail vein. Mice have been sacrificed, tibial and femoral bones have been harvested, processed and analysed by histomorphometry and immunohistochemistry. Expression of osteogenic markers Runx2, osteonectin (SPARC) and alkaline phosphatase (ALP) has been detected with Real time PCR in these tissues.

Results: In vitro preosteoblasts produce alkaline phosphatase during early time in culture with normal medium, while the level decreases in differentiating conditions. Preosteoblasts maintained in differentiation medium for 30 days are positive to Alizarin and Von Kossa staining, hence they are able to produce mineralized extracellular matrix that is a feature of functional mature osteoblasts. Runx2 expression increases during differentiating conditions; in cells maintained in differentiation medium for 30 days there is an increase of 50% compared to cells maintained in normal medium ($p < 0.05$).

In mice of group III an increased level of parameters concerning osteoid has been detected (Osteoid Thickness, Osteoid Surface/Bone Surface, Osteoid Volume/Bone Volume) and an increased number of active osteoblasts (during synthetic activity) has been observed compared to group II.

Real time PCR analysis revealed a reduction in osteogenic gene expression in group II compared to group I (ALP: -50%, $p < 0.01$; Runx2: -56.75%, $p < 0.01$; SPARC: -44.5%, $p < 0.05$).

In group III there is a recovery of expression of osteogenic markers (ALP: $+40\%$, $p < 0.05$; Runx2: $+66.28\%$, $p < 0.001$; SPARC: $+55\%$; $p < 0.01$) compared to group II.

Conclusion: These preliminary data show that our model induces the engraftment of preosteoblasts in injured bone.

Further studies with a longer observation time are needed to investigate if preosteoblasts are able not only to graft onto host tissue, but also to proliferate in vivo and to differentiate in full mature and functional osteoblasts.

Relationship between sex hormones, sex hormone binding globulin (SHBG) and peripheral arterial disease in older persons

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Lower extremity peripheral arterial disease (PAD) is widely accepted as an indicator of generalized atherosclerosis (1). Men and women with PAD have faster functional decline and higher mortality risk compared with those without (2). In older population the prevalence of PAD is similar to or even slightly higher in women as compared with men. Moreover, women with PAD have greater mobility loss than men (3). These data suggest that PAD might be affected by sex hormones and Sex Hormone Binding Globulin (SHBG) levels. This hypothesis is supported by the evidence of metabolic actions of sex hormones and SHBG. We have previously demonstrated that low SHBG is an independent risk factor of metabolic syndrome in both sexes (4,5), whereas low testosterone and high estradiol levels are risk factors of metabolic syndrome in men (6). It is reasonable to hypothesize a similar relationship between sex hormones and PAD, but data at this regard are scant (7,8).

Aim of the Study: Therefore, the aim of our study is to test the relationship between testosterone (T), estradiol (E2), SHBG and lower extremity PAD and to identify potential differences in two sexes.

Methods: We selected 921 men and women aged $\geq$65 (65-102 yr) from the InCHIANTI study. T was measured by RIA (Inter- and intra-assay CVs were 9.6 and 9.1%, MDC 0.08 nmol/l). SHBG was measured with IRMA (MCD 3.00 nmol/l, Inter- intra-CVs were <3.7 and 11.5). E2 was measured with ultrasensitive RIA (MCD 2.2 pg/ml and intra- inter-assay CV 8% and 10%). PAD was defined by an ABI <0.90. Means ($\pm$DS) were compared using t-test and percentages using Chi-square. Factors correlated with PAD were identified using age-adjusted partial correlation coefficient and Spearman partial rank-order correlation coefficients. Logistic regression models were used to identify independent factors of PAD : in MODEL 1 the analysis was adjusted only for age; MODEL 2 included also BMI, interleukin-6, physical activity, smoking, hypertension, chronic heart failure. MODEL 3 included covariates of Model 2, and T, SHBG, E2.

Results: Mean Age ($\pm$ SD) al baseline was 74.2 $\pm$ 6.5 yrs in men and 5.6 $\pm$ 7.0 in women. 62 (41 men, 21 women) had ABI $<$ 0.90 and 859 ABI $>$ 0.90. Men with ABI $<$ 0.90 had SHBG mean values of 96.9 ($\pm$ 56.1), significantly lower than those with ABI $>$ 0.90 (109.3 $\pm$ 54.4) ($p = 0.03$); no significant trend was found in women ($p = 0.22$). An inverse and significant relationship was found between SHBG and PAD only in men, in Models 1 and 2 ($p = 0.004$ and $p = 0.028$, respectively), but not in Model 3 ($p = 0.07$). In women we found a positive and significant relationship between testosterone and PAD in Models 1 and 2 (OR 5.44, CI 1.13-26.06 and OR 1.64, CI 1.01-2.67, respectively) association that was significant also in Model 3 ($p = 0.01$). No significant association was found between testosterone and PAD in men (OR 1.23, CI 0.95-1.58), and between E2 and PAD in both sexes.

Conclusions: Peripheral arterial disease is associated with low SHBG levels in older men, and with high total testosterone levels in women. Further longitudinal studies are needed to verify the potential role of SHBG and testosterone in PAD.

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This contribution has been awarded as Best Communication.

Long term effects of low protein diet on depressive symptoms and quality of life in elderly type II diabetic patients

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Objectives: Long term effects of a low protein diet (LPD) on depressive symptoms and the quality of life in elderly type 2 diabetic are unclear.

Methods: Thirty-eight elderly type 2 diabetic patients with CRD (stage 3-4) were enrolled in the study. After 4 weeks on a normal protein diet regimen (NPD) providing 1.0 g/kg per day, all participants were assigned, randomly, a LPD (0.7 g/kg per day), either seven days a week (LPD 7/7) or six days a week (LPD 6/7) for 30 months. Mini mental state examination (MMSE), activities daily living (ADL), cumulative illness severity (CIRS-IS), geriatric depression scale (GDS-15) and short-form healthy survey (SF-36) were evaluated every 3 months.

Results: Before the LPD regimen creatinine clearance (CrCl), MMSE, ADL, CIRS-IS, GDS-15 and SF-36 were similar in both LPD 7/7 and LPD 6/7 groups. During 30 months, the mean GDS-15 increased significantly more in LPD 7/7 group in comparison to LPD 6/7 group (7.0 $\pm$ 0.0 vs 4.8 $\pm$ 0.4) while, both mean SF-36 MCS (36.8 $\pm$ 0.0 vs 49.0 $\pm$ 0.0) and SF-36 PCS (37.0 $\pm$ 0.0 vs 48.0 $\pm$ 0.0) were significantly decreased in LPD 7/7 group in comparison to LPD 6/7 group ($p < 0.05$). After 30 months, the decline in CrCl observed was similar in LPD 7/7 and LPD 6/7 groups (2.77 $\pm$ 0.3 and 2.84 $\pm$ 0.3 ml/min/yr, respectively).

Conclusion: In elderly type 2 diabetic patients, long term effects of LPD 6/7 regimen in comparison to LPD 7/7, are associated with a similar decline in CrCl, but with a decreased depressive symptoms and a better quality of life.

Metabolism, Diabetes and Clinical Nutrition I

Euthyroid women with thyroid-stimulating hormone within the normal reference range are at increased risk of metabolic syndrome

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Overt hypothyroidism is associated with increased risk of cardiovascular disease. Some reports, but not others, suggest that an increase in cardiovascular risk occurs also in subclinical hypothyroidism, defined as an elevated serum thyroid-stimulating hormone (TSH) with normal free T_4 and T_3, possibly due to abnormalities in lipoprotein pattern, blood sugar (BG), insulin sensitivity and blood pressure, often fulfilling the diagnostic criteria of metabolic syndrome (MetS). Less known is the relationship of TSH within the normal reference range with MetS.

In this study we have investigated the association of TSH levels with the prevalence of the components of the MetS in a large cohort of euthyroid women.

The study was carried out in 744 non diabetic women with normal thyroid function. Of them, 353 (47%) were obese, 288 (39%) overweight and 103 (14%) normal weight. Central obesity (waist girth $\geq$ 88 cm) was present in 61% of women and hypertension in 52% (45% of them was on antihypertensive drug therapy). TSH ranged from 0.3 to 4.9 μU/mL, with a mean of 2.19 (SD 1.06) and a median of 2.01 μU/mL.

Patients with TSH above the median were more obese, had greater waist girth, were more hypertensive and had higher levels of serum triglycerides (TG), total cholesterol (TC) and BG and lower levels of HDL cholesterol (HDL-C) than patients with TSH below the median (Table).

	TSH below median ≤2.1 μU/mL	TSH above median >2.1 μU/mL	P value
Number	372	372	
Age (years)	45.4 ± 15.92	47.2 ± 14.27	NS
Body mass index (kg/m^2)	29.6 ± 6.16	32.2 ± 5.44	<0.001
Waist girth (cm)	90.3 ± 13.13	95.2 ± 11.61	<0.001
TSH (μU/mL)	1.30 ± 0.46	3.09 ± 0.68	<0.001
Total cholesterol (mg/dL)	200.3 ± 39.95	206.4 ± 36.81	<0.035
Serum triglycerides (mg/dL)§	84 (65-124)	102 (75-148)	<0.001
HDL-cholesterol (mg/dL)	62.2 ± 15.04	58.3 ± 13.10	<0.001
Blood glucose (mg/dL)	88.3 ± 11.66	91.3 ± 12.51	<0.001
Hypertension (%)*	45.7%	58.9%	<0.001

Simple regression analysis showed that TSH was significantly associated with body mass index (BMI), waist circumference, TC, BG, TG, HDL-C (inverse) and hypertension. BMI and waist circumference showed a strong relationship with BG, TG, HDL-C (inverse) and hypertension, suggesting that the association of TSH with metabolic variables and hypertension could be accounted for by obesity and, in particular, by central obesity. However, multiple backward stepwise regression analysis with age, waist circumference and TSH as independent variables showed that TSH remained positively associated with TG, BG and hypertension (dummy variable) and inversely associated with HDL-C, but not with TC that was positively related only with age. In summary, TSH resulted to be strongly related with the determinants of the MetS.

A total of 205 patients (28%) fulfilled the definition criteria of the MetS and the prevalence of MetS was significantly greater in patients with TSH above than in patients with TSH below the median (34.9% vs 20.2%, $\chi^2 = 21.116$, $P < 0.001$). Results of logistic analysis, including age and TSH as predictor variables, confirmed the association of TSH with MetS (odds ratio 1.382, 95% CI 1.175-1.625).

In conclusion TSH in the upper normal reference range is suggestive of increased cardiovascular risk due to the association with several well known risk factors fulfilling the diagnostic criteria of the MetS, whereas TSH values below 2.1 μg/mL are associated with a more favorable metabolic profile.

This contribution has been awarded as Best Communication.

Osteoprotegerin and metabolic syndrome

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Introduction: Metabolic syndrome (MS) is commonly defined as the cluster of glucose metabolism abnormalities, central adipose tissue accumulation, dyslipidemia, and abnormal blood pressure regulation. Due to this detrimental combination of cardiovascular risk factors, MS is combined to an increased cardiovascular risk. MS also includes the presence of a low-grade inflammatory state that is known to up-regulate the receptor activator of nuclear factor-kB (RANK) ligand (RANKL). This up-regulation might increase bone reabsorption and then leads to osteoporosis in MS patients. Physiologically, bone reabsorption is inhibited by the binding of RANKL with osteoprotegerin (OPG), a glycoprotein that acts as false receptors reducing the amount of RANKL available for interaction with RANK. Concordantly, the elevated serum levels of OPG observed in women with osteopenia and osteoporosis are often interpreted as a compensatory mechanism to a condition favouring bone reabsorption. Since OPG production is influenced by several factors, including proinflammatory cytokines, it is reasonable to assume that the state of low-grade inflammation that characterizes MS may increase the synthesis and release into the circulation of the glycoprotein, regardless of the presence of bone rearrangement.

Objective: To evaluate the association between MS and bone metabolism in premenopausal women.

Methods: We enrolled 17 premenopausal women affected by MS, according to the Adult Treatment Panel III (ATP-III) criteria, 17 women affected by osteopenia and 17 healthy women. We evaluated anthropometric parameters, standard laboratory tests, and circulating parameters of bone metabolism including serum levels of OPG (ELISA Kit). Instrumental evaluation of bone tissue was performed using calcaneal quantitative ultrasonometry (GE Lunar Achilles Express).

Results: Women with MS and women with osteopenia showed higher circulating levels of OPG compared to healthy women (23.53 ± 2.02 pg/ml and 27.91 ± 3.80 pg/ml vs 15.23 ± 1.48 pg/ml,

respectively, $p = 0.002$ and $p = 0.003$), whereas OPG levels were not significantly different between the three groups. The comparison of instrumental parameters of bone metabolism and blood chemistry also showed no significant difference between groups. Moreover, in the MS group there was no correlation between serum OPG and parameters that define the classically SM, excepting a significant positive correlation between OPG and serum levels of LDL cholesterol ($r = 0.73$, $p < 0.001$). Finally, osteopenic women manifested with an inverse correlation between OPG and bone density indices: Stiffness Index (SI) ($r = -0,794$, $p < 0.001$), T-score ($r = -0,832$, $p < 0.001$) and Z-Score ($r = -0,672$, $p = 0.004$); and a direct correlation between OPG and serum calcium concentrations ($r = 0,506$, $p = 0.04$).

Discussion: Our study shows increased circulating levels of OPG in women with MS but no alteration of bone metabolism and women with osteopenia but no MS. In addition, women with MS presented a direct correlation between OPG serum levels and LDL cholesterol, while osteopenic women showed an inverse correlations between serum OPG levels and various parameters of bone densitometry (i.e. SI, T -Score, Z-Score). Taken together, these results suggest that MS represents a condition favouring the secretion of OPG, probably due to underlying low-grade inflammation. Of note, for the first time we demonstrated a direct association between OPG serum levels and LDL cholesterol in women with MS, thereby suggesting OPG might represent an early marker of vascular damage.

Autonomic profile in patients with lada diabetes

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Background: Latent autoimmune diabetes of adults (LADA) is an adult-onset form of autoimmune diabetes (it accounts for $\sim$ 10% of all cases of diabetes), whose nosological entity and pathophysiology are still discussed, that genetically and immunologically resembles adult-onset type 1 diabetes (similar HLA association and presence of serum autoantibodies) but metabolically is more similar to type 2 diabetes (significant association with metabolic syndrome and insulin resistance).

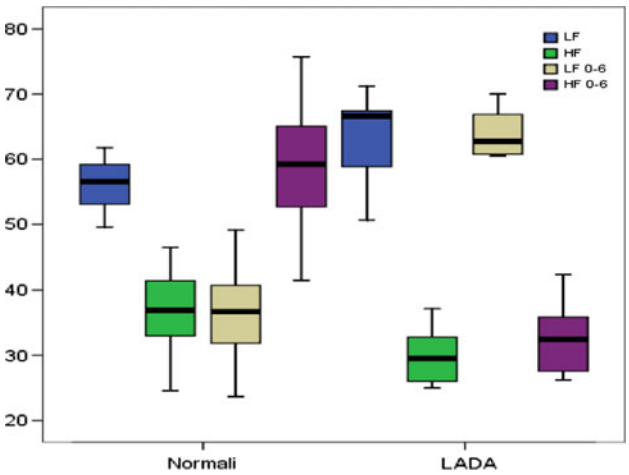
Complications' pattern, in LADA, shares some features with both type 1 and type 2 diabetes.

Autonomic neuropathy (CAN) is a common consequence of diabetes disease progression, resulting from microvascular damage to parasympathetic and sympathetic fibers, it predicts increased risks for cardiovascular arrhythmias, sudden death, and myocardial infarction in adults with diabetes.

Object of the study: Our aims were to assess, in patients with LADA, the autonomic nervous activity/function by measuring the Heart Rate Variability (HRV) with 24 h Holter ECG recordings

Materials and Methods: We enrolled 7 patients suffering from LADA diabetes. We have defined LADA as follows: age of onset above 35 years; ICA and/or GAD positivity. Were excluded from the study: patients suffering from diseases of autonomic activity; taking drugs that act on autonomic nervous system smokers; who developed macro- and micro-vascular complications. For each of the subjects enrolled was performed a 24 h electrocardiographic dynamic monitoring with analysis of HRV. The group was matched with a control group of similar age and sex.

Results: In Time domain we observed a reduced global autonomic activity (SDNN) in LADA patients compared with controls, with statistically significant difference between the two groups. (SDNN 24 h respectively: LADA 121,74 ± 32,53; controls 159,84 ± 45,45). Consensually parasympathetic activity (RMSSD) was reduced in LADA patients, especially during the night compared with controls, with statistically significant difference between the two groups. (RMMSD 24 h LADA 27,64 ± 9,86; controls 38,83 ± 13,45)Analysis of frequency domain showed in LADA patients during the night an altered simpatho-vagal balance due to significant reduction of parasympathetic activity (HF) associated to sympathetic hyperactivity(LF) (see Fig.)



Conclusions: In the time domain, values of SDNN and RMSSD highlight autonomic nervous system dysfunction. The pattern observed in patients in our study seems similar to that observed in patients with type 2 diabetes. The analysis in the frequency domain confirms the greater impairment of parasympathetic component at night, associated with a constant sympathetic hyperactivity during the entire 24 h. We hypothesize that insulin resistance plays a more important role than autoimmunity in involvement of autonomic nervous system in LADA diabetes.

Liraglutide promoted more weight loss with improved glycemic control than sitagliptin

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Most glucose lowering therapies are associated with increases in body weight (BW). In contrast, incretin therapies promote weight loss (GLP-1 agonists) or are weight-neutral (DPP-4 inhibitors). The relationship between BW loss and improved HbA1c, if any, is not well understood.

We assessed this association in a 26-week randomized, open-label study comparing the once-daily human GLP-1 analog liraglutide with sitagliptin, a once-daily DPP-4 inhibitor, in patients with type 2 diabetes who were not in control on metformin monotherapy at baseline.

Results showed that liraglutide 1.8 mg (LIRA) reduced mean HbA1c from baseline (8.5%) more than sitagliptin 100 mg (SITA): 1.5% vs. 0.9%, respectively ($p < 0.0001$). BW was reduced 3.4 kg with LIRA vs. 1.0 kg with SITA: ($p < 0.0001$). A post hoc ANCOVA of the LOCF ITT population including treatment, BW loss and their interaction with baseline HbA1c as a covariate was conducted to examine the impact of >3% BW reduction on the decrease in HbA1c with LIRA ($n = 214$) and SITA ($n = 215$). More patients lost >3% BW with LIRA than SITA (51% vs. 21%, OR = 3.92, $p < 0.0001$). BW changes in the >3% BW change group for LIRA and SITA were –6.3 kg and –5.3 kg ($p = 0.04$), respectively and correspondingly –0.2 kg and +0.4 kg (ns) in the ≤3% BW loss category.
Patients who had >3% BW loss with LIRA, had larger reductions in HbA1c (−1.8% vs. −1.2%, $p < 0.0001$), but the same was not true for those treated with SITA (−1.0% vs. −0.8%, ns). However, within each weight loss category LIRA reduced HbA1c more than SITA.
In conclusion, greater improvements in HbA1c were seen amongst patients also experiencing substantial weight loss with LIRA. However, HbA1c reduction was better with LIRA in both BW loss categories.
Beneficial effects associated with weight loss, such as increased insulin sensitivity may be associated with the incremental glycemic effect of BW reduction seen with LIRA.

Sunday, October 23rd 2011

Thrombosis and Hemostatis I

HPA-3 gene polymorphisms and cardiovascular risk in coronary artery by-pass surgery patients. A prospective study

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Several polymorphisms in endothelial and platelet receptor genes have been shown to impair response to antiplatelet drugs in subjects with coronary artery disease (CHD). In particular, platelet GPIIb/IIIa receptor polymorphisms can affect platelet aggregation and may represent an inheritable risk factor for atherothrombosis. Whereas most studies agree that mutations in the GPIIIa represent an inherited risk predisposing to acute coronary syndromes, there are only few studies on the influence of GPIIb HPA-3 mutation on the cardiovascular risk. In a sample of 44 CHD male subjects under aspirin treatment after Coronary Artery By-pass Graft (CABG) surgery, after performing platelet functional studies by light transmission aggregometry, collagen-induced TXB2 generation, PFA-100 and cytofluorimetric analysis, we found that, following stimulation with collagen 4 μg/ml, HPA-3a/a GPIIb genotype subjects exhibited a residual platelet aggregation and a TXB_2 synthesis significantly higher as compared to that of subjects with the HPA-3b/b genotype. Based on these data, we prospectively evaluated the incidence of cardiovascular events in a sample of CABG male subjects, stratified according to the presence of GPIIb HPA-3 polymorphism during a 8 year follow-up. After excluding subjects with other mutations affecting platelet function, and those that were heterozygotes for HPA-3 polymorphism, 238 subjects [181 (43.0%) with the HPA-3a/a and 57 (13.5%) with the HPA-3b/b genotype] were enrolled in this study. All patients were under continuative aspirin therapy (100 mg/day) and have been followed-up every 6 months. No significant differences were found in demographic characteristics and cardiovascular risk factors distribution between the two HPA-3 genotype groups. Age at CHD onset and the type of CHD at onset (acute coronary syndrome/instable angina vs stable angina) did not show a significant difference between the two groups (p always > 0.05).
During an mean follow-up of 7.41 ± 6.47 years, 48 subjects [34 (18.8%) HPA-3a/a and 14 (24.6%) HPA-3b/b subjects, $p = 0.349$] developed an acute coronary syndrome event (ACS). The mean time elapsed before ACS occurrence was similar between the two groups ($p = 0.966$) and a Kaplan–Meier survival analysis, showed no significant difference between the two genotypes as to the maximal survival time before ACS occurrence (24.21 vs 16.28, lok-rank $p = 0.470$).
Conclusions: this is the first prospective study evaluating the impact of HPA-3 polymorphisms on clinical outcome in CABG subjects. Our findings are in line with previous findings in other clinical settings (stroke and acute coronary syndromes with stent implantation).

Functional antithrombin levels and risk of a first episode of venous thromboembolism (VTE). A cross-sectional study

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Although present in < 10% of VTE patients, inherited deficiencies of antithrombin (AT), Protein C and Protein S, have been recognized as very important inherited thrombophilic conditions, being associated with an extremely high risk of first and of recurrent VTE. Furthermore, relatives of patients with such deficiencies show a significant incidence of first VTE as compared with relatives of those without. Whereas most studies document the risk of thrombotic events in subjects with AT deficiency, no data are available as to whether a progressive increase in the VTE risk is present for decreasing AT levels or a clear cut-off effect is identifiable.
Among those referred between March 1993 and March 2003 to the Regional Reference Center for Coagulation Disorders of the Federico II University, 1228 subjects experiencing their first VTE event have been screened for this study. After excluding those unwilling to give informed consent; with allergy to warfarin; with a history of more than one VTE; with an indication for continuous oral anticoagulation (e.g., an artificial heart valve or chronic atrial fibrillation); with events occurring during pregnancy, malignancy, puerperium, oral contraceptive intake, hormone replacement therapy or with a current or previous venous ulcer; with paresis/paralysis of the affected leg; with arterial insufficiency graded at functional class III (pain at rest) or worse; those with deficiencies of Prot. C and Prot S or with combined inhibitor deficiencies, the remaining 918 VTE patients (case group) were included in the present study and compared with 859 individuals (control group) that were chosen among those referred during the same period in the frame of a primary prevention program. Cases and controls were entirely comparable for age, gender and vascular risk

factors. In each case, AT levels were evaluated at least twice by a functional method (Berichrom ATII (Behringwerke, Marburg, Germany) and both groups were stratified into 5 subgroups according to AT levels (< 70%; 70-80%; 80-90%; 90-100% and > 100%). Among 51 subjects with < 70 AT%, 41(80.4%) were in the VTE group and 10 (19.6%) were in the control group. Similarly, among 69 subjects with 70-80 AT%, 46(66.7%) were in the VTE group and 23(33.3%) were in the control group. No difference was found in the prevalence of subjects with > 80 AT% in VTE and control group.
Subjects with < 70 AT%(mean age 33.44 + 13.62 yrs) and those with 70-80 AT% (mean age 35.63 + 9.52 yrs) showed a significantly younger age at VTE onset as compared with subjects with 90-100 AT% (42.36 + 13.96 yrs, p = 0.002 and 0.027, respectively). The same differences were confirmed comparing 70-80% AT and 90-100% AT subjects with 100-130 AT% (41.85 + 14.07 yrs, p = 0.002 and 0.043, respectively). A significant correlation was found between age at VTE occurrence and AT levels (r = 0.090, p = 0.006).
Cox regression odds ratios for the risk of VTE in the population stratified according to AT levels showed that, as compared to the first AT% level group (101-130%), subjects with AT% levels < 70, or those with AT levels of 70-80% showed a significantly increased risk of VTE (OR:4.37 and 2.13, respectively). A slightly increased risk of VTE (OR:1.44) was found in those with AT levels of 81-90% as compared to the first AT% level group (101-130%). Based on the results of this cross-sectional study, we conclude that a progressive increase in the risk of VTE is present for AT levels below 90%.
This contribution has been awarded as Best Communication.

In hospital outcome of warfarin or antiplatelet-related intracranial hemorrhage

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Background: Intracranial hemorrhage is one of the most feared complications of long-term anticoagulation or antiplatelet therapy.
Objective: Among patients with warfarin or antiplatelet-related intracranial hemorrhage we examined mortality during hospitalization and functional outcome at the hospital discharge. We delineated factors associated with mortality, and variables responsible for poor functional outcome at hospital discharge.
Methods: We retrospectively identified all patients hospitalized with warfarin or antiplatelet associated intracranial hemorrhage in the ASO S. Croce e Carle Cuneo from 1 January 2005 through February 2010. Every case were matched with control (intracranial hemorrhage not warfarin or antiplatelet related), who had same age, hemorrhage and year of happen.
We collected clinical, anamnestic and radiological informations, related to intracranial hemorrhage.
Intracerebral volumes were measured on the first available brain scan by using the abc/2 method.
Statistical evaluation was performed by Kaplan–Meier survival analysis, univariate and multivariate analysis.
Results: We identified 170 patients eligible for the study (74 patients taking warfarin and 96 taking antiplatelet therapy) and 166 controls. Mortality during hospitalization was double in patients with warfarin-related intracranial hemorrhage than control *(32,4% vs 16,9% $p = 0.02$)*; and higher than control in patients with antiplatelet related intracranial hemorrhage *(25% vs 16,9% $p = 0.02$)* (Fig. 1).

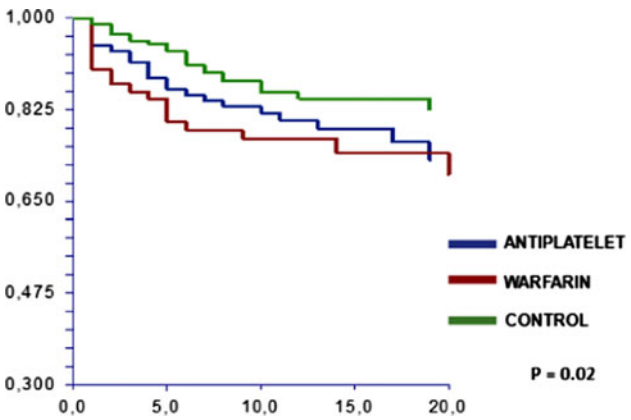

Fig. 1: Survival analysis during hospitalization

Functional outcome at hospital discharge, measured with Rankin scale, in patients with warfarin or antiplatelet therapy, was poorer than control *(4.1 ± 1.8; 4.1 ± 1.5 vs 3.4 ± 1.9; $p = 0,005$)*.
Multivariate analysis showed significant association between mortality and initial GCS < 13 *($p = 0.04$)*, and glucose concentration > 160 mg/dl *($p = 0.02$)*, in patients with warfarin-related intracranial hemorrhage. In the same group, at multivariate analysis, intracerebral localization *($p = 0.02$)* was significantly associated with long-term outcome. Only univariate analysis revealed that a larger initial intracerebral volume was significantly associated with mortality *($p = 0.02$)*. INR at the presentation, time to INR reversal and treatment taken to reverse INR < 1.5 were not associated with mortality and functional outcome at discharge.
In antiplatelet-related intracranial hemorrhage mortality was associated with age > 75 *($p < 0.005$)*, and initial glucose concentration > 160 mg/dl *($p = 0.02$)*, at multivariate logistic regression analysis.
Multivariate logistic regression analysis indicated that age > 75 *($p = 0.005$)* and initial GCS < 13 *($p < 0.001$)* were independently associated with poor long-term outcome.
Conclusion: Warfarin or antiplatelet-related intracranial hemorrhage had a higher mortality and poor functional outcome than intracranial hemorrhage in non users. Further studies are needed to verify if a more aggressive management of acute phase of intracranial hemorrhage in such patients could improve their poor short and long time outcome.

Thrombophilia in infertile women with repeated implantation failure: can low molecular weight heparin improve the outcome?

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Introduction: Implantation failure in patients submitted to ART assisted reproduction techniques is since many years a matter of debate. Low molecular weight heparin (LMWH) role is emerging as a possible factor improving the early phases of implantation.
Study design and population: Retrospective observational analysis of patients with at least two implantation ART failures, screened for the presence of thrombophilic factors and submitted to successive ART cycles with or without administration of LMWH.
Aim: Main aim of the study was to evaluate the pregnancy rate in patients with or without heparin administration.

Materials and Methods: 265 patients fulfilled the enrolling criteria. 149 (56%) were primary infertile and 116 (4%) were secondary infertile. Their mean age was 36,3 ± 3,6 years and the basal FSH level was 7,73 ± 3,03. 81/265 (?) were positive for at least 1 thrombophilic mutation (G1691A FV, G20210A FII, homozygous C677T- MTHFR); 25/265 (9.4%) if we consider only G20210A FII e G1691AFV. They were submitted to 569 new ART cycles 512 (90%) non supported and 57 (10%) supported by heparin administration.
Results: 105 clinical pregnancies were observed in 569 cycles (18.80%). Stratified by age group, in term of previous pregnancies, previous ART cycles, basal FSH, number of retrieved oocytes, embryo transferred, BMI and smoking were not significantly different between the group with or without heparin administration; 17% (88/512) was the pregnancy rate in patients not treated with heparin and 33% (19/57) in the heparin treated group ($p = 0.006$).
Discussion: A significant higher pregnancy rate in ART implantation failures was observed in patients supported by heparin administration. These interesting findings from our observational study should be confirmed by further randomized controlled trials before routine application of LMWH for ART cycles.

Miscellanea

High prevalence of splenic hypofunction in patients with an incidental finding of small sized spleen at abdominal ultrasound

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Background and Aims: Hyposlenism is an acquired condition, accompanied or not by a reduction in spleen size, potentially associated with different disorders, and characterized by depletion of circulating IgM memory B cells which predisposes to a higher risk of infections caused by encapsulated bacteria. Neverthless, the relationship between splenic function and size in hyposplenic individuals is unknown. We here evaluated splenic function through pitted erythrocyte counting, that is erythrocytes bearing membrane abnormalities visible under interference phase microscopy as 'pit', in patients with an incidental finding of a small sized spleen at abdominal ultrasound.
Patients and Methods: Between October 2009 and January 2011, we examined 4585 patients who referred to the Ultrasound Unit of our Department of Internal Medicine to undergo an abdominal ultrasound for the following indications: abdominal pain (46%), malignancies (27%), liver and biliary disorders (10%), intestinal diseases (8%), nephrolitiasis (5%), others (4%). All images were obtained by an experienced sonographer, using an Esaote model MyLab 70 XVG, with a 1-8 MHz convex probe and an ATL model HDI 3500 a 5.2 MHz convex probe. Spleen length was defined as the maximum distance between the dome and the tip of the spleen. Patients with reduced spleen length (Ø ≤ 8 cm in men and Ø ≤ 7.5 cm in women) underwent peripheral blood collection for the evaluation of spleen function by counting the number of pitted erythrocytes (upper limit of normal = 4%). As positive control for the measurement of splenic function, we also enrolled 52 patients who had undergone splenectomy.
Results: We identified 203 patients with a small sized spleen. Only 128 of them (mean age 61.5 years; 90 females and 38 males) gave the consent for the collection of a peripheral blood sample. Eighty-five of the 128 patients (66%) had pitted erythrocyte value higher than 4% and then were diagnosed as hyposplenic. Among them, 54 patients had mild-to-moderate hyposplenism (pitted erythrocytes < 8%), while 31 patients had moderate-to-severe hyposplenism (pitted erythrocytes ≥ 8%). As expected, all the 52 splenectomized patients showed high pitted erythrocyte values (mean 14.5%). A significantly ($p < 0.001$) higher percentage of pitted erythrocytes was found in the moderate-to-severe hyposplenic group (mean 10.5%) in comparison to mild-to-moderate hyposplenic patients (mean 5.7%). The most frequent disorders found in the 85 hyposplenic patients were malignancies, i.e. colon cancer (n = 11), breast cancer (n = 3), gastric cancer (n = 2), gynecologic tumors (n = 2), chronic myeloproliferative disorders (n = 2); intestinal diseases, i.e. inflammatory bowel diseases (n = 13), Whipple's disease (n = 3), refractory coeliac disease (n = 2); and autoimmune disorders, i.e. thyroiditis (n = 2), systemic lupus erythematosus (n = 1), rheumatoid arthritis (n = 1). No significant correlation was found between the spleen size and the degree of splenic hypofunction ($r_s = 0.97$; $p = 0.78$). However, patients with pitted erythrocyte values ≥ 8% had a spleen length (mean 4.9 cm) significantly ($p < 0.05$) lower than that of patients with pitted erythrocyte values < 8% (mean 6.9 cm).
Conclusions: We here showed that 2/3 of individuals with a small sized spleen incidentally detected at abdominal ultrasound are affected by splenic hypofunction. Of note, more that 1/3 of these hyposplenic patients showed moderate-to-severe hyposplenism, with pitted erythrocyte levels comparable to those of splenectomized patients. On the basis of our data, we suggest that the incidental finding of a small sized spleen at routinary abdominal ultrasound should lead the internist to measure the spleen function and exclude the major hyposplenism-associated disorders. As our study is burdened by a referral bias, further studies on larger reference populations are needed in order to better clarify the real incidence of splenic hypofunction in individuals with a small sized spleen, and ascertain the clinical relevance of this incidental finding. Whether prophylactic measures recommended in splenectomized patients should be adopted at least in patients affected by moderate-to-severe hyposplenism, and whether traditional polysaccharide T-independent vaccines should be replaced by T-dependent protein-conjugated vaccines, which bypass the hyposplenism-associated defect of IgM-memory B cells, are still of concern.

Adherence to osteoporosis treatment: role of spot therapy and causes of interruption of therapy

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Osteoporosis is a chronic widespread disease: it affects 4.7 million people in Italy, 80% of whom are women. The prerequisite for a successful therapy is the right execution in terms of continuity (persistence) and number of doses (compliance). The adherence to osteoporotic treatment is estimated at 40%: this value is still far from what might be considered sufficient for an actual reduction in fracture risk, that is in fact 80%. The aim of our study is to examine the values of adherence in a sample group of patients with osteoporosis who live in Rome, assessing any changes deriving from the inclusion or the exclusion of spot therapy (a therapy of less than 30 days duration), and analyze any causes of discontinuation of therapy, considering variables such as age, the pathology causing the prescription, the prescribed drug and the doctor who makes the prescription. The data are collected from database of general practitioners, who record at

least 90% of prescriptions. Our study is based on the observation of 6721 antiosteoporotic therapy, started January 2001 till December 2009. Among these treatments the ones selected are those in which adherence has been below 50%. The average adherence is estimated at 29%, and the treatment is not applied for more than three years. The spot therapy (less than 30 days) accounted for 50% of the total number, 63% in the case of calcium, and 40% in the case of alendronate. The adherence's values tend to increase by 20-30% if we exclude spot therapies from our analysis. The adherence appears to be influenced by variables such as age, in fact patients who are older than 85 years have a higher rate of adherence, and prescribed medications: the adherence is less than 30% for raloxifen and calcium and more than 30% for vitamin D and bisphosphonates. Usually the causes of discontinuity of therapy are associated to adverse reactions (43%) and wrong information given by the physician (28%). An analysis in a subgroup of patients with low adherence, indicates as the main factors that influence adherence: side effects in 23% of patients, which are gastrointestinal in 78% of cases, lack of motivation in the 12%, which is associated with a lower severity of illness that caused the prescription and high co-morbidity, and wrong information in 17% of the study sample; in the latter case the prescription of the drug is made by a general practitioner in 30% of times. In our study, in 17% of cases the therapy has been interrupted by the physician, who gives indications that turn out to be not in accordance with guidelines in 56% of cases. The causes of interruption of therapy are mainly side effects in patients younger than 65, and insufficient motivation and misinformation in older patients. The analysis of causes of low adherence by drug type, dosage and regimen, shows that in patient on treatment with daily and weekly bisphosphonates, with calcium and with vitamin D, poor adherence is due to side effects(25%) and wrong information(25%), while in monthly regimens low adherence is due to adverse reaction(50%) and insufficient motivation(37%), and within intramuscular therapy is due to lack of motivation(50%) and fear of side effect(45%). Adherence is also influenced by the severity of Osteoporosis: when the condition is more serious, especially when vertebral fractures occur, the treatment is mainly stopped because of side effects (70%). In conclusion, in our sample factors influencing adherence are connected to this type of disease, which is silent in less severe stages so patients underestimate its potential consequences, to the anti-osteoporotic drugs for their side effects, to the doctor-patient relationship, which is defective(far from being optimal). The physician should collaborate with patient in choosing the appropriate type of treatment, he should be clearer when he gives information about both efficacy and side effects of anti-osteoporosis treatment, and should have a better knowledge about osteoporosis and anti osteoporotic drugs.

A novel role for the sonic hedgehog pathway in skeletal muscle regeneration and dystrophy

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Introduction: Duchenne Muscular Dystrophy (DMD) is an X-linked recessive disease that affects primarily skeletal and cardiac muscle and is the most common deadly genetic disorder in children, affecting about 1 in 3,500 males worldwide. In this pathologic condition, cardiac and skeletal muscles lack the protein dystrophin, which serves as a link between cytoskeletal actin and the extracellular matrix. As a result of this, DMD patients have extremely fragile myofibers, which are unable to tolerate the repeated mechanical stress brought on by recurring contractions. The consequence is progressive muscle degeneration. Therefore, all the cellular and molecular mechanisms involved in muscle regeneration are constantly activated, as they are required to respond to the continuous ongoing trauma and to replenish injured skeletal muscle. In this scenario, muscle satellite cells (MSCs) play an essential role. These cells are a population of undifferentiated mononuclear myogenic cells, which, in the course of muscle regeneration, exit their normal quiescent state to start proliferating. After several rounds of proliferation, the majority of MSCs differentiates and fuses to form new myofibers or repair damaged ones. After proliferation, quiescent satellite cells are restored underneath the basal lamina for subsequent rounds of regeneration. In DMD, MSCs are forced to undergo multiple cycles of activation, proliferation, and differentiation and ultimately this exhausts the satellite cell population. Consistent with this, MSCs from 9-year-old DMD subjects have only one-third of the proliferative potentials exhibited by MSCs of age-matched healthy individuals. In this study, we investigated the role of the Sonic hedgehog (Shh) pathway during muscle injury and regeneration, both in normal and dystrophic conditions.
Methods: We used *mdx* mice, an established experimental model of DMD, and wild-type littermates. Injury of the skeletal muscle was induced both using a toxic agent – cardiotoxin – and creating ischemia of the hindlimb. The activation of the Shh pathway was assessed by RT-PCR and also using transgenic mice which express the lacZ reporter gene under the control of the promoter of ptc1, which is a Shh-target gene. Inhibition of the Shh pathway was achieved by using the inhibitor cyclopamine. Treatment with Shh was performed by using a plasmid encoding the Shh gene.
Results: We discovered that injury of the adult skeletal muscle induces significant upregulation of the Shh developmental pathway. However, this upregulation is significantly reduced in the muscles of dystrophic mice, compared to wild-type controls. Activated MSCs respond to Shh in vivo, by expressing the Shh-target gene ptc1. Shh inhibition results in reduced regeneration and functional recovery after injury. In contrast, treatment with Shh significantly improves muscle repair in dystrophic mice. In particular, Shh treatment improves 1) muscle weight, 2) number of regenerating myofibers, 3) capillary density, and 4) muscle strength. In addition, Shh treatment reduces in a significant manner 1) muscle fibrosis and 2) myofiber permeability.
Conclusion: This is a novel study that breaks new ground in the field of muscle pathobiology. Our findings demonstrate that the Shh signaling pathway is important for the regeneration of the adult skeletal muscle and Shh is a myogenic factor that acts directly on MSCs. Impaired activation of the Shh signal might contribute to the inability of dystrophic mice to respond properly to skeletal muscle injury, while Shh treatment may have pleiotropic beneficial effects on dystrophic diseases of the skeletal muscle.
This contribution has been awarded as Best Communication.

Unexpected rare disease in a young hypertensive woman

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A 36-year-old woman was referred to our unit because of refractory hypertension, joints and abdominal pain. She had chronic pain and stiffness in the knees, shoulders, and hands, as well as occasional numbness and tingling in the hands. Weight was 51 kg, the height 175 cm, and the body-mass 16.6.

Her medical history was remarkable for smoking 5 sigarets/day since 20 years, for occasional alcohol intake, regular cannabis use. She had a long history of amenorrhea due to polycystic ovary syndrome diagnosed at the age of 17 and treated with drospirenone-ethinylestradiol. She had undergone multiple surgery events because of injuries due to a car accident seven years back; since than, thin, gaping scars developed on her skin. At that time, blood hypertension was found. At admission to our unit her blood pressure was 220/130 mmHg, having a constant 100% saturation on room air. We primarely focused on this problem. Her cardiac and neurological examinations were unremarkable. Electrocardiogram was within normal limits. Blood and urine routine investigations did not reveal any abnormality but hypokalemia. The measurement of basal cortisol level and over night suppression, serum TSH, plasma levels of catecholamines, MRI scanner for adrenal glands and brain extended into the suprasellar region were performed and all resulted negative. Basal concentrations of serum aldosterone were 238 pg/mL supine (7.5-150) and 1230 pg/ml standing (35-300) and basal concentrations of active renin were 2 pg/mL (0.2-2). The renal artery evaluated by ultrasound sonography were normal. Based on these evaluations, we concluded that she suffered from essential hypertension and that the hyperaldosteronism was probably secondary to the use of drospirenone-ethinylestradiol. She was treated with alpha-adrenergic blockade, angiotensin II receptor antagonists and spironolactone with partial benefit. At family history she recorded that her sister had dislocatable joints, history of arthritis and frequent nose bleeds. She reported first steps at the age of 24 months, characterized by clumsiness and coordination difficulties. For these reasons, the patient underwent further evaluations taking into account her daily joints pain. Physical examination revealed peripheral joints and spine hypermobility suggestive of generalized hypermobility syndrome. Dorsal kyphosis, flat feet in standing, hallux valgus and hypermobility of knee and shoulders were all evident. She had a velvety skin all over the body which was hyperelastic over the back of the hand. White, enlarged, flat, cigarette paper scars were present on the skin of the elbows, the hands and the knees. The X-ray showed dystrophic cysts in the trochanteric region and cervical spondylo-arthrosis. Ocular anterior chamber abnormalities were detected. A thoracic CT evaluation revealed a slight dilatation of the proximal aortic root (approximately 3.6 cm) and a mild mitral regurgitation. A genetic counselling was performed in the suspicion of a Ehlers-Danlos syndrome (EDS). The medical history, the patient's symptoms and the generalized joint hypermobility were suggestive for diagnosis of the Classic type of the Ehlers-Danlos syndrome (ex EDS I-II). EDS is a genetic syndrome affecting primarily joints, skin, and blood vessel walls. It is a heterogeneous group of generalized connective tissue disorders associated with multiple modes of inheritance and collagen abnormalities. The classical-type EDS has an autosomic dominant inheritance with unknown etiology.

Gastroenterology and Hepatology II

Immunosuppressive effect of bone marrow-derived mesenchymal stromal cells on gliadin-specific t cell lines

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Background: Thanks to their ability to modulate immune response and promote tissue repair, mesenchymal stromal cells (MSC) represent an attractive novel source for cellular therapy in immune-mediated diseases, including celiac disease (CD), an autoimmune enteropathy. Specifically, the MSC-mediated immunosuppression is exerted non-selectively on virtually all the cells involved in immune responses. The best characterized effect is on T lymphocytes. These are considered the main player in CD pathogenesis and responsible for tissue damage, i.e., villous atrophy and inflammatory infiltrate of small intestinal mucosa which follows gluten ingestion, by producing pro-inflammatory cytokines, such as interferon (IFN)-γ. Indeed, the latter is able to induce the characteristic lesions in experiments of organ culture.

Aim: We aimed, therefore, to exploit the immunosuppressant properties of bone marrow-derived MSC on gliadin-specific T cells for future clinical application.

Patients and methods: Mononuclear cells were isolated from bone marrow of two adult donors, plated and expanded ex vivo until passage 3, when the adherent cells underwent morphological and immunophenotypical characterization by microscopy and flow cytometry, respectively. The ability of MSC to modulate in vitro proliferation and secretion of IFN-γ and interleukin (IL)-10 upon antigen stimulation of already established gliadin-specific T cell lines was evaluated in an allogeneic co-culture setting at different MSC:T ratio (1:2, 1:20, 1:200). The antigen was prepared by digestion of gliadin powder (Sigma) with pepsin-trypsin and subsequent treatment with tissue transglutaminase (Sigma) to obtain an immunostimulatory antigen preparation. T cell lines were obtained from perendoscopic duodenal biopsies of 6 CD patients (F/M 4/2, mean age: 32 years, range 8-56) through weekly cycles of stimulation with the antigen preparation, IL-2 and IL-15, and stored at -80°C until required. After the co-cultures were settled in triplicate, proliferative T cell response to antigen preparation (50 μg/ml) was detected by ^{3}H-thymidine incorporation, while IFN-γ and IL-10 expression was evaluated on cell supernatants by ELISA assay (anti-cytokine monoclonal antibodies supplied by MabTech, Nacka Strand, Sweden). All data were expressed as fold increase (FI): ratio between the value upon antigen stimulation and medium alone. A value ≥ 2 was considered as a positive response. A Student T test was used for statistical analysis and a p value less than 0.05 was considered statistically significant.

Results: We found a significant reduction of IFN-γ production in response to gliadin (median value 14.7, range 1.0 – 258.0,) when T cells were co-cultured with allogeneic MSC at 1:200 ratio in comparison to the value observed when T cells were cultured with gliadin alone (median value of FI: 35.2, range 5.2 – 277.1, $p < 0.03$). A robust, even though not statistically significant, inhibition was detected even at 1:1 and 1:20 ratios (median FI and range: 9.3 (1 - 194) and 17.8 (1.0 – 258) at ratio 1:1 and 1:20, respectively. In parallel, no modification of IL-10 production was registered at any MSC:T cell ratio tested with respect to the value found in the absence of MSC. Finally, a weak reduction of T cell proliferation following antigen stimulation was found mainly at MSC:T cell 1:20 ratio.

Conclusions: Bone marrow-derived allogeneic MSC seem to exert in vitro immunological effects on gladin-specific T cells isolated from CD patients mainly in reducing IFN-γ production. This evidence may open a new era for the use of MSC as alternative therapeutic strategy in CD. This contribution has been awarded as Best Communication.

Effects of infliximab on circulating dendritic cell populations in crohn's disease

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Background: Crohn's disease (CD) is a chronic inflammatory enteropathy caused by a dysregulation of peripheral immune tolerance towards intestinal bacteria in genetically susceptible individuals. Dendritic cells (DC) are professional antigen presenting cells having an amazing capacity to orchestrate both innate and adaptive immune response, as well as to maintain immune tolerance. Their progenitors in the bone marrow give rise to circulating precursors that home to peripheral tissues where they recognize antigens and undergo transition from immature antigen capturing cells to mature antigen presenting cells which are able to re-circulate in the peripheral blood. Two main subsets of DC are distinguished on the basis of a different expression of surface molecules: the myeloid- (mDC, $CD11c^+$) and the plasmacytoid-derived DC (pDC, $CD123^+$). In CD, therefore, an imbalance of DC subsets may play a role in the initiation and/or maintenance of chronic inflammation, and a correlation between changes of circulating DC subsets and disease activity has been previously shown. Infliximab, a biological agent blocking both soluble and membrane tumour necrosis factor-α, has been proven successful in treating steroid-dependent/refractory and fistulizing CD, although the mechanisms underlying its effects are not fully understood.
Aim: We aimed to evaluate the modifications occurring on circulating DC subsets in CD patients who underwent Infliximab treatment.
Patients and Methods: Peripheral blood samples were collected from 12 patients (6 males) before and at 24th week of Infliximab therapy (I-CD), 18 patients (9 males) following traditional therapy, i.e. mesalazine, steroids, antibiotics (T-CD), and 27 healthy volunteers (11 males). Quantification and phenotype characterization of DC populations were carried out by multidimensional flow-cytometric analysis (EPICS-XL, Coulter) by using two approaches, one based on the expression of CD11c and CD123 on lineage$^-$/HLA-DR$^+$ cells and the other on that of three specific markers BDCA-1, -2 and –3 (Miltenyi). Clinical activity was determined by evaluation of CD activity index (CDAI), white blood cell count and C-reactive protein (CRP) levels. Comparisons between mean values were performed by the Mann–Whitney *U* test and correlations by applying the Spearman rank correlation test. A p value less than 0.05 was considered statistically significant.
Results: In I-CD patients before starting Infliximab therapy (mean CDAI value: 239 ± 76) a significant higher number of mDC in comparison to T-CD patients ($p = 0.006$) and controls ($p = 0.04$) was found, together with a positive correlation between CDAI score and the number of pDC ($p = 0.04$, $r = 0.60$). After 24 weeks of therapy, 92% of patients showed clinical remission (mean CDAI value: 124 ± 70; $p = 0.0007$) together with a significant reduction of the pDC subset ($p = 0.04$). Finally, a statistically significant difference of the count of white blood cells in I-CD (both pre- and post-therapy) and T-CD groups in comparison to healthy controls ($p < 0.001$) was invariably found.
Conclusions: In the present study, we demonstrate the presence of a complete modification of both the number and distribution of circulating DC populations in those patients with active disease who are candidate to more aggressive therapy, and the efficacy of biological therapy in inducing clinical remission together with a restoration of DC balance in peripheral blood. These results suggest a robust immunomodulatory action of Infliximab in CD that could explain a more favourable rate of early clinical remission in comparison to conventional therapies. Finally, the characterization of circulating immune cells could represent an additional useful diagnostic tool in the management of CD patients.

Assessment of the risk of local recurrence after radiofrequency ablation for small (≤ 3.0 CM) Hepatocellular Carcinoma (HCC): analysis of a multicentre series of 363 patients

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Aims and Background: The aim of the present study was to determine the rate and the risk factors of local recurrence (LR) in patients affected by treatment-naive HCC smaller than 3 cm undergoing radiofrequency ablation (RFA).
Methods and Materials: We performed a multicentre retrospective analysis of 363 patients (215 men and 148 women; mean age 67 years), Child-Pugh A/B, with single HCC in very early and early stage (107 patients with HCC smaller than 2 cm and 256 patients with HCC between 2-3 cm), observed between 1998 and 2010 in 7 Italian Centers. All patients had undergone RFA using internally cooled or expandable electrodes, showed complete short-term HCC necrosis, and had a follow up period $\geq$ 12 months. Nodule size ranged from 1.0 to 3.0 cm (mean 2.3 ± 0.5). The mean follow-up period was 39.8 months (range 12-132). After ablation, the patients underwent periodic follow up evaluations. LR was diagnosed using imaging methods. The risk factors for LR were analysed using the Kaplan Meyer method, and Cox regression models.
Results: LR was observed in 81 (22.3%) patients, was associated to distant intrahepatic recurrence in 41 cases, and did not affect overall survival. Local recurrence was diagnosed within 1 year from treatment in 38% of cases, within 2 years in 80% of cases, and within 3 years in 95% of cases. In particular, only 4 cases of LR were diagnosed after the 3rd follow up year. The local 1-, 3 and 5-year cumulative recurrence rate in the whole series was 8%, 28% and 36% and no statistical difference was found between patients with HCC < 2 cm and HCC 2-3 cm. At multivariate analysis alpha-fetoprotein serum level ($p = 0.029$) and higher age ($p < 0.001$) were linked to LR but at multivariate analysis only age was confirmed as an independent predictor ($p < 0.001$, OR $= 1.06$).
Conclusions: Our study shows that in patients with single HCC < 3 cm submitted to RFA the risk of local recurrence is higher than 30% within 3 years from ablation; in our opinion this datum does not contrast with the available data concerning the effectiveness of RFA at explant analysis in HCC patients submitted to liver transplantation. Furthermore, the detection of LR is very unlikely after the 3rd year of follow up, and does not differ significantly between nodules up to 2 cm, and 2-3 cm large.

Cardiovascular Diseases II

Clues to detect tumor necrosis factor receptor-associated periodic syndrome (TRAPS) among patients with idiopathic recurrent pericarditis: results of a multicentre study

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Background: Idiopathic recurrent acute pericarditis (IRAP) represent the most troublesome complication of acute pericarditis. Although IRAP is generally presumed to derive from autoimmune process, increasing interest in currently being to autoinflammatory diseases (AID). Among AID, tumor necrosis factor receptor-associated periodic syndrome (TRAPS) is the most common autosomal disorder and it has been hypothesized that the low-penetrance TNFRSF1A variants may contribute to the development of atypical inflammatory responses, including cardiac diseases such as myocarditis and pericarditis. Therefore, subjects carrying low-penetrance alleles may also display recurrent pericarditis as the only clinical manifestation, hindering differential diagnosis between TRAPS and IRAP. The potential clinical expression of TRAPS, in the form of IRAP, has not been widely explored in the medical literature.
Objective: 1)To evaluate the incidence of TRAPS mutations in patients with recurrent pericarditis. 2) To identify a set of clues for detecting among IRAP patients, those at high risk of carrying TRAPS mutations, for whom genetic testing would thus be indicated.
Methods and Results: 131 consecutive Caucasian patients (50F/81M, mean age 42.21 years) with IRAP were investigated for mutations of the TRAPS gene and prospectively evaluated. Each patient underwent detailed examinations in order to rule out underlying diseases such as infections, connective tissue disorders and malignancies. Eight of the 131 patients (6.1%) carried a mutation in the TNFRSF1A gene. The two groups of genetically-negative (n.123) and positive (n.8) patients were homogeneous with regard to gender ($p = 0.258$) and age ($p = 0.087$), although the median age was higher in the genetically-negative ones (42 vs 30.5). Compared with those without genetic mutations, patients with TRAPS mutations had more frequently a positive family history for pericarditis and periodic fever syndromes (respectively 75% vs. 8%; $p < 0.001$), a higher mean number of recurrences after the first year (respectively 4 vs. 0.75; $p < 0.001$), a higher mean number of recurrences on colchicine treatment (respectively 4 vs. 0.16; $p < 0.001$), and a higher need of immunosuppressive therapies (respectively 88% vs. 21%; $p < 0.001$).
No significant differences were found in the comparison between the two groups for attack duration/year ($p = 0.53$), average duration of recurrences after the first attack ($p = 0.964$) or time between recurrences($p = 0.575$), involvement of other serous membranes ($p = 0.483$) or arthralgia/myalgia ($p = 1.000$), and corticosteroid administration ($p = 0.104$).
Conclusion: TRAPS is a cause of recurrent pericarditis in 6% of unselected cases with recurrent pericarditis. A positive family history for pericarditis or periodic fever syndromes, a poor response to colchicine, recurrences after the first year from the index attack or on colchicine treatment, as well as the need of immunosuppressive agents are clues of the possible presence of TNFRSF1A gene mutations in patients with recurrent pericarditis. The identification of genetically-positive IRAP patients would also allow – in the case of poor response to conventional therapies – to prescribe more specific treatments, based on the recent experience acquired in the management of AID, such as anti-TNF or anti-IL1 agents.

Role of hyperglycemia on myocyte precursor cells during acute myocardial infarction: effects of tight glycemic control

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Stress hyperglycemia during acute myocardial infarction (AMI) is associated with an increased risk of mortality in patients with and without diabetes. Moreover, although some evidences suggest that the use of insulin to lower glucose concentrations decreases mortality in diabetic patients who have AMI, the role of tight glycemic control in the AMI outcomes remains unclear. The latest studies highlight that the heart possesses a compartment of multipotent progenitor cells that can differentiate into cardiomyocytes. In this context, Anversa and coworkers propose a classification of cardiac immature cells into 4 classes: cardiac stem cells (CSCs), progenitors, precursors, and amplifying cells. These cell types can be viewed as subsequent steps in the progressive evolution from a more primitive to a more differentiated phenotype. CSCs differentiate into a daughter cardiac progenitor cells (CPCs). CPCs give rise to myocyte progenitor cells and precursor cells (MPCs). MPCs become transient amplifying cells, which divide and differentiate into mature myocytes. MPCs can populate acutely damaged regions of the ischemic myocardium, refurbishing functional units and reversing remodeling. There is evidence that diabetes plays a central role in the dramatic loss of the MPCs viability and function in animal models. The high quantities of reactive oxygen species produced by hyperglycemia persist for a long time within the cells and results in inhibition of cell replication and differentiation, favoring the development of a cardiac myopathy characterized by a decrease in muscle mass and impaired ventricular function.
Objectives: We analyzed the effects of tight glycemic control on regenerative potential of myocardium during acute myocardial infarction. Our study evaluated whether hyperglycemia during AMI was associated with increased levels of oxidized and senescent MPCs and reduced levels of cycling myocytes in myocardial biopsies, obtained from patients who were admitted to intensive care unit (ICU) for first AMI, and had to undergo coronary bypass surgery (CABG). We also sought to determine whether the early tight glycemic control during AMI reducing myocardial levels of oxidized and senescent MPCs, increases the expansion of cycling myocytes. Finally, to discriminate between the effects of insulin treatment to achieve glycemic control and the glycemic control per se on the regenerative potential of the ischemic myocardium, we evaluated the effects of glucose-insulin-potassium infusion (GIK group) on histological findings.
Methods: Eighty-one patients with their first AMI undergoing coronary bypass surgery were studied: 25 patients with glycemia < 140 mg/dl served as control group; hyperglycemic patients (glycemia > 140 mg/dl) were randomized to intensive glycemic control (IGC, $n = 20$; glucose-goal 80–140 mg/dl), or conventional glycemic control (CGC, $n = 20$; glucose-goal 180-200 mg/dl) or glucose-insulin-potassium (GIK, $n = 16$; glucose-goal 180-200 mg/dl), for almost 3 days before surgery, using insulin infusion followed by subcutaneous insulin treatment. During surgery, MPCs (c-kit/MEFC2/GATA4-positive-cells), oxidation of MPCs DNA (c-kit/8-OH-deoxyguanosine-positive-cells), senescent MPCs (c-kit/p16INK4a-positive-cells) and cycling cardiomyocytes (Ki-67-positive-cells) were analysed in biopsy specimens taken from the peri-infarcted area.
Results: Before surgery, plasma glucose reduction was greater in the IGC than in CGC and GIK groups ($p < 0.001$ for both). IGC patients had higher MPCs ($p < 0.01$) and cycling myocytes ($p < 0.01$), as well as less oxidized ($p < 0.01$) and senescent MPCs ($p < 0.01$) in peri-infarcted specimens compared to both CGC patients and GIK patients.
Conclusions: We hypothesized that tight glycemic control, by reducing oxidative stress, may inhibit cellular senescence and increase the regenerative potential of the ischemic myocardium.

Endothelial, angiogenic, and vasculogenic defects in muscolar distrophy

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Background: Recent work demonstrates that vascular abnormalities are required for, and an essential cause of, muscle damage in Duchenne muscular dystrophy (DMD), the most common fatal genetic disorder in children. In particular, it has been shown that dystrophin is physiologically expressed in endothelial cells (ECs) and plays a specific role in arterial shear-stress mechano-transduction. More recently, it has been demonstrated that mdx mice, the murine equivalent of DMD in humans, have reduced expression of endothelial nitric oxide synthase (eNOS) and defects in endothelial-dependent blood flow modulation in response to muscle contractile workload. Also, Flt-1 haploinsufficiency ameliorates the dystrophic phenotype in mdx mice. Indeed, mdx:Flt-1(±) adult mice show improved muscle histology compared with the mdx mice, with decreased fibrosis, calcification, and membrane permeability.
Aim of the Study: In the present study, we evaluated whether additional important biological functions of the EC compartment, such as angiogenesis and vasculogenesis, are impaired in muscular dystrophy.
Methods and Results: First, we used the **hindlimb ischemic model of angiogenesis** and found that mdx mice are unable to successfully revascularize the ischemic hindlimb over a of follow-up period of 4 weeks, while age-matched wild-type mice have this capability. Next, we used the **corneal model of angiogenesis**, in which single pellets containing the angiogenic agent VEGF were implanted in the eyes of mdx mice and wild-type controls. As expected, a strong angiogenic response was observed in VEGF-treated eyes of wild-type mice. In contrast, VEGF-induced corneal angiogenesis was significantly compromised in mdx mice. Then, we used the **in vivo matrigel angiogenic assay** and found that the number of new vessels was significantly lower in mdx mice, compared to controls. Finally, we used a **tumor implantation angiogenic assay**, in which human lung cancer cells were injected subcutaneously into mdx mice and wild-type controls. Tumor growth and tumor vascularization were significantly reduced in mdx mice. We also isolated ECs from the inguinal adipose tissues of mdx mice and found that these cells have significantly reduced proliferative and migratory capacities, compared to the ECs isolated from the adipose tissue of wild-type controls. Mdx-derived Ecs also have significantly reduced expression levels of VEGFR-2, eNOS, and Sirt1 – three prototypica angiogenic genes - compared to ECs isolated from wild-type mice. Finally, we found that endothelial progenitor cells (EPCs) derived from the bone marrow (BM) of wild-type mice were able to rescue the angiogenic deficits observed in mdx mice, while EPCs derived from the BM of dystrophic mice did not have this capability.
Conclusions: Our data strongly indicate that, in mdx mice, angiogenesis is significantly and systemically impaired. Such impairment depends, at least in part, on abnormalities of the EC compartment. Also vasculogenesis is compromised in mdx mice, thus suggesting that muscular dystrophy is also characterized by abnormalities of the EPC compartment. These data indicate that the vascular endothelium is an important player in muscular dystrophy and suggest that vascular ECs should be taken into consideration as potential therapeutic target in this disease, with important biological and clinical implications.
This contribution has been awarded as Best Communication.

PM10-Induced nadph oxidase activation: a new link between air pollution and oxidative stress

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Background: Ambient and occupational exposure to Particulate Matter (PM) has been linked with increased hospitalization and mortality from cardiovascular disease, respiratory disease, and lung cancer. Extensive evidence indicates that generation of oxidative stress contributes to the health effects associated with ambient and occupational PM exposure. Aim of the present study is to investigate whether PM exposure determine changes of oxidative stress markers in workers of a steel plant with well-characterized exposure to air particles.
Methods and Results: We recruited 113 male healthy workers (mean age 42 years) who had been working in a steel production plant in Brescia, Northern Italy for at least one year.. Individual exposures to ambient PM_{10} were estimated based on measurements in the 15 work areas and time spent by the study subjects in each area; exposure to metals was evaluated by urinary metal levels.
We analysed plasma levels of soluble NOX2-derived peptide (sNOX2-dp), a marker of activation of NOX2 (the catalytic core of NAPDH oxidase) and two validated markers of oxidative stress, namely plasma levels of 8OH-dG and urinary isoprostanes.
Workers were exposed to a wide range of PM_{10} levels (between 44-2270 μg/m3).
The three markers sNOX2-dp, 8OH-dG and urinary isoprostanes showed an high and positive statistically significant correlation (r sNOX2-dp/8OH-dG = 0.73, $p < 0001$; r sNOX2-dp/urin isoprost = 0.72, $p < 0001$; r 8OH-dg/urin isoprost = 0.73, $p < 0001$).
sNOX2-dp and urinary isoprostanes were associated with levels of PM_{10} (sNOX2-dp: $\beta_{std} = 2.48$, $p = 0.05$; urinary isoprostanes: $\beta_{std} = 101.24$, $p = 0.02$) in multivariable regression models adjusted for age, body mass index and smoking. Metal components of PM_{10} were not associated with the oxidative markers analysed.
Conclusion: This study demonstrated for the first time a positive association between PM_{10} and NADPH oxidase activation in a population of highly PM exposed subjects. These findings, suggesting a specific role for PM_{10} in inducing reactive oxygen species (ROS) formation, deserve further investigation on the role of PM in oxidative stress.

Rheumatology

Screening of rheumatoid arthritis patients by means of echocardiograhy

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Background: Cardiovascular (CV) disease represents one of the leading causes of morbidity and mortality in rheumatoid arthritis (RA). Enhanced atherosclerosis and impaired endothelial function develop early after the onset of the disease and generally remain clinically silent for long-term. Assessing preclinical atherosclerosis before structural abnormalities occur both in large arteries and in the coronary bed should be the main challenge for both cardiologists and

rheumatologists who want try to change the clinical outcomes of RA patients. Our goal was to assess the most sensitive and feasible diagnostic tools able to detect preclinical atherosclerotic damage in RA patients without overt CV disease.
Methods: 120 adult patients with RA who fulfilled the ACR classification criteria [M 20 (16.6%), F 100 (83.4%)], mean age 61 ± 13 years) without clinical evidence of CV disease and 80 healthy controls matched for age and sex were recruited.
All patients underwent a dypiridamole stress echo with evaluation of coronary flow reserve (CFR) in the left descending coronary artery.
Results: 72/120 patients (60%) had CFR < 2.5. All patients had normal ejection fraction and left ventricular dimensions, wall thickness and wall motion. Performing a stepwise regression, the predictive variables of decreased CFR were the isovolumetric relaxation time ($P < 0.0001$), deceleration time ($P < 0.0001$), E/A ratio ($P < 0.0001$), E wave ($P < 0.0001$), A wave ($P < 0.0001$) and left ventricular mass ($P < 0.0001$).
Conclusions: In our study RA patients without clinical evidence of CV diseases showed an early impairment of coronary microcirculation and endothelial dysfunction. This suggests that reduced CFR is an early marker of enhanced atherosclerosis in a preclinical stage and it is associated with endothelial dysfunction. Moreover, isovolumetric relaxation time, deceleration time, E/A ratio, E and A wave, ejection fraction and left ventricular mass resulted predictors of coronary microvascular integrity.

Refractory Wegener's granulomatosis: report of six cases treated successfully with rituximab

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Introduction and objective of the study: Wegener's Granulomatosis (WG) is a primary systemic ANCA associated vasculitis with a predilection for the respiratory tract and kidneys. The combination of glucocorticoids and cyclophosphamide remains the standard therapy of severe WG: remission can be induced in about 70% of patients. WG has a high relapse rate and some patients do not respond adequately to this treatment. Moreover, standard therapy is associated with substantial toxicity. B lymphocytes have been implicated in the pathogenesis of WG: their depletion has been proposed as innovative treatment for refractory disease. We describe six patients with WG refractory to first line standard therapy treated successfully with Rituximab (RTX).
Patients and methods: We enrolled six male patients (median age 40 yr). All had ANCA positivity (five reacting with PR3, one with MPO), and resistance to cyclophosphamide. The mean activity score, calculated with BVAS/WG, was 6. Treatment consisted of four weekly infusions of RTX (375 mg/m^2).
Results: All patients tolerated well RTX infusion and have been evaluated at 6 and 12 months from first infusion. Five of them achieved a complete clinical remission, with the BVAS/WG activity score of 0. One patient presented a clinical flare, concurrently with B-cells reconstitution, at the 11th month of follow up, retreated successfully.
Conclusions: RTX was well tolerated without serious adverse effects and induced an effective remission for severe refractory WG. RTX represents a promising alternative therapy when cyclophosphamide fails or is contraindicated.
This contribution has been awarded as Best Communication.

Allergology and Clinical Immunology II

Best matching for HCV genotype 1 liver transplant recipients is predicted by HCV-STAR. A study from aisf recolt-c database

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Background: Hepatitis C virus (HCV) infection is the most common indication for liver transplantation (LT) in the US and Europe. It is well-known that the combination of liver graft and recipient features may have a great impact on the long-term post-LT outcome.
Objectives: We retrospectively analyzed data from 12 Italian transplant centers, to determine how donors and HCV-genotype 1 recipients' characteristics may affect the survival of patients treated for post-LT hepatitis C recurrence.
Methods: 326 patients (230 males, 96 females, 19-68 yrs) transplanted from deceased donors between 1989 and 2008 for genotype 1 HCV-related cirrhosis and treated for hepatitis C recurrence were included in the retrospective analysis. The impact on survival of several donor and recipient related clinical and biochemical variables assessed at the moment of transplantation was evaluated.
Results: The cumulative survival rates after LT were 96% at one year and 84% at 5 years. Among the analyzed variables, MELD > 22, ($p < 0.001$), recipient BMI < 23 ($p < 0.001$), donor age > 60 yrs ($p < 0.01$), donor BMI > 25 ($p < 0.05$), absence of HCC ($p < 0.10$) and donor/recipient gender mismatch (male donor, female recipient; $p < 0.10$) were recognized to be associated to a worse outcome and were, therefore, included in the multivariate analysis. We provided a prognostic Score for liver Transplantation Allocation Risk (HCV1-STAR; Fig. 1), ranging from 0 (favorable outcome) to 7 (unfavorable outcome), which was able to predict post-LT survival in genotype 1 HCV recipients with a high accuracy (chi2 = 31.8, $p < 0.0001$; Fig. 1). Moreover, the predictive power of HCV1-STAR was independent from the achievement of a sustained virological response (SVR) after antiviral treatment.
Conclusions: In our retrospective series, MELD > 22, low recipient and high donor BMI, donor age > 60, absence of HCC and donor/recipient gender mismatch were associated to a worse overall survival. A prognostic index combining these six variables (HCV1-STAR) was statistically able to stratify post-LT survival in genotype 1 HCV recipients. Further studies are needed to prospectively validate the HCV1-STAR and to assess its effectiveness even in genotypes 2, 3, and 4 HCV recipients.

Figure 1 Star Point

VARIABLE	STAR POINT	POINT FOR MISSING DATA
MELD > 22	2	0.5
R BMI < 23	2	0.5
NO HCC	1	0.3
D AGE > 60	2	0.5
D BMI > 25	1	0.3
D/R gender mismatch	1	0.3

d: donor

r: recipient

Increased cardiovascular risk in patients subjected to lung transplantation

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Introduction and Aim: Cardiovascular disease (CVD) is a common cause of morbidity and mortality after solid organ transplantation, due to a combination of pre-existing cardiovascular risk factors in recipients and immunosuppressive drug toxicity. Aim of the present study was to describe the prevalence of new-onset hypertension, hypercholesterolemia and diabetes mellitus in lung transplant recipients.
Methods: Sixty-seven patients (mean age: 47.9 ± 14.5 years) were followed for at least 3 years after lung transplantation in the Pavia center. Cumulative prevalence of new-onset hypertension, dyslipidemia, and diabetes were calculated. To this aim, either the values of the relevant parameters (i.e. blood pressure, plasma lipids, and glucose) or a new prescription of any antihypertensive, lipid lowering, or antidiabetic drug therapy, were assessed at each follow-up visit.
Results: When compared to the time of lung transplantation, the prevalence of hypertension increased from 11.9% to 70.1% (from 8 patients before transplantation to 47/67 patients at the 3-year follow-up visit; $p < 0.01$). The concomitant prevalence of diabetes and dyslipidemia raised from 13.4% to 29.9%, and from 47.8 to 85.1%, respectively (from 9 to 20/67, and from 32 to 57/67 patients; $p < 0.01$ for both). During the 3-year follow-up body weight and body mass index increased from 65.4 ± 10.8 to 74.5 ± 12.1 kg, and from 22.4 ± 3.7 to 26.1 ± 3.9 kg/m^2, respectively ($p < 0.01$ for both), further contributing to the increased cardiovascular risk already over this relatively short follow-up after lung transplantation.
Conclusions: A large number of lung transplant recipients develop new-onset hypertension, diabetes or dyslipidemia after transplantation. The increased cardiovascular risk of these patients should be taken into account during follow-up, to better define a proper and timely cardiovascular prevention.
This contribution has been awarded as Best Communication.

CD4 + CD28 Null t cells presence in peripheral blood of patients with acute ischemic stroke: relationship with toast subtype neurological deficit of the acute phase

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Introduction: CD4 + CD28null T cells differ from conventional CD4 + CD28 + helper T lymphocytes in both phenotype and function. CD4 + CD28 + T cells are terminally differentiated and have pro-inflammatory functions characterized by the production of high levels of interferon-γ (IFN-γ), tumour necrosis factor-a (TNF-a), and IL-2. In addition, CD4 + CD28null T cells are cytotoxic and effectively kill endothelial cells in vitro. Killer cell immunoglobulin-like receptors (KIRs) on natural killer cells (NK) and on a subset of T cells recognize allelic determinants that are shared by groups of HLA class I alleles. KIRs belong to a multigenic family consisting of 13 genes encoded within the leukocyte receptor complex (LRC) on chromosome 19q13.414–16 and are expressed on NK cells and infrequently on CD8 + T cells. Patients with acute coronary syndrome can be distinguished from age-matched healthy controls and patients with stable angina by the increased frequency of a subset of CD4 + T cells that are oligoclonally expanded in the peripheral blood and have lost the expression of the costimulatory molecule CD28.
Nevertheless no study, to our knowledge, evaluated peripheral expression of subset of CD4 + CD28null T cells, KIR receptor differential expression and KIR gene aplotypes in subjects with acute cerebrovascular events.
Aim of the study: To evaluate in patients with ischemic stroke the frequency of peripheral CD4 + subset of foam-based oligoclonale, the peripheral percentage of CD4 + CD28null T cells, the expression of signaling protein DAP12 by by peripheral CD4 + CD28null T cells, the expression of KIR receptors and the distribution of KIR haplotype in relation to the TOAST subtype of stroke, clinical severity of clinical presentation of stroke at admission and functional outcome at discharge and at follow-up.
Materials and Methods: We enrolled patients with a diagnosis of acute ischemic stroke admitted to the Internal Medicine Department of the "Policlinico P. Giaccone" at the University of Palermo and to the Neurology Division of "Azienda Ospedaliera Civico" of Palermo between November 2009 and January 2012. As control group will be also enrolledpatients admitted, in the same period, to our Internal Medicine Department, atched for cardiovascular risk factors and without acute ischemic stroke.
Blood samples were obtained in the nonfasting state. After 10 min of rest in the supine position, vital signs were recorded and blood samples were collected from the antecubital vein.
EDTA-anticoagulated peripheral blood was drawn from each patient within 12 h from symptoms onset. Serum and plasma were immediately separated by centrifugation and stored in aliquots at −80°C until analysis. Peripheral blood mononuclear cells (PBMCs) and T-cell clones were stained with PE-labeled anti-CD158b/j (Beckman Coulter, Miami, Fla), PerCP-labeled anti-CD4, and FITC-labeled anti-CD28 mAbs (both Becton–Dickinson, San Jose, Calif). The cells were analyzed on a FACSCalibur flow cytometer (Becton–Dickinson), and the surface expression of these molecules was calculated using WinMDI software (Joseph Trotter, Scripps Research Institute, La Jolla, Calif).
Results: Patients with acute ischemic stroke showed a higher frequence of CD4 + CD28null T cells in peripheral blood in comparison with control group. In relationship of TOAST subtype patients with cardioembolic stroke showed the highest frequence of CD4-CD28 null T cells. We observed a positive correlation between NIHSS score and CD4 + CD28 + T cells. With regard of KIR expression on CD4 + CD28null of activating types KIR2DS2 and a lowe expression of inhibiting KIR2DL2 e KIR2DL3 ones.
Discussion: These findings confirm our previous findings concerning the higher acute immunoinflammatory activation of stroke also with regard of T cell activation and the highest degree of inflammatory markers of cardioembolic strokes with a positive correlation with seriousness of clinical presentation of brain ischemia.

Is the primary immunosuppressive drug (cyclosporin a or tacrolimus) playing a role on the response to antiviral treatment for post-transplant HCV Recurrence?

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Background: HCV-related cirrhosis is the most common indication for liver transplantation. Standard immunosuppression, based on Calcineurin inhibitors (CNI) may also affect HCV replication and response to antiviral therapy.
Aim: to evaluate the impact of CNI on SVR in a population of HCV transplanted patients undergoing antiviral therapy for HCV recurrence.
Patients and methods: A multicenter database of 12 Italian Centres was set up to carry on a retrospective analysis of 464 liver transplant recipients, treated for HCV recurrence, from 1992 to 2008. Patients were considered eligible for combination interferon plus ribavirin-based therapy according to defined criteria. Antiviral treatment was aimed for 48 weeks regardless of viral genotype (73,9% genotype 1); median follow up was 87 ± 45 months. Immunosuppressive therapy was based on cyclosporine in 39% of cases, on tacrolimus in 56,9%.
Results: SVR rate was 34,1%. EOT was significantly higher in the Cyclosporine group (64%) compared with the Tacrolimus (54,5%) ($p = 0,04$): a longer interval between OLT and starting of antiviral therapy (32,7 vs 19,2 months), higher daily dose of Ribavirin (659,9 versus 561,9 mg) were associated with virological response in the Cyclosporin group**.** Acute and chronic rejection rate ($p = 0,536$ and $p = 0,585$ respectively) and pre-treatment staging score, were no different between the two groups. No difference in SVR rate and in patients survival was observed (88% survival in Cyclosporin group vs 87%).
At multivariate analysis Cyclosporine was confirmed as an independent significant predictor of EOT ($p = 0,04$), regardless of viremia, donor and recipient features, genotype, distance from OLT, olt-recurrence interval, fibrosis stage and treatment dose and duration.
Conclusions: EOT response to antiviral treatment for post-OLT HCV recurrence is significantly higher among Cyclosporin treated recipients, however no differences in SVR and patient survival was observed.

Gerontology and Geriatric Medicine II

A circadian variation for in-hospital patients falls? A preliminary study in Ferrara

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Background: Falls represent common and serious problems among older people. One-third of subjects aged 65 years and older fall at least one a year, and risk increases with age. Moreover, falls represent a social and economic burden: in the US, in 2006 more than 2.6 million older adults experienced a fall-related injury, and health costs exceeded $19 billion (1). It is known that acute diseases do not occur randomly over time, but exhibit circadian variations. In particular, morning hours are characterized by higher frequency of myocardial infarction, stroke, and aortic diseases (2-4). As for in-hospital falls, chronobiological studies are not available. This study was aimed to verify whether they occur randomly throughout the day or exhibit a rhythmic pattern.
Patients and Methods: The region Emilia-Romagna, total population of ~ 4,3 million people, is divided into nine Provinces. As for health service, the Province of Ferrara is organized into one Local Health Authority (Azienda Unità Sanitaria Locale–AUSL) with 6 non-teaching hospitals (distributed around the territory, with limited number of beds) and one Teaching Hospital (Azienda Ospedaliera-Universitaria S.Anna). In the AUSL hospitals, a program of falls surveillance has been started. Each event is recorded into a database, containing anagraphic data (name, surname, age, gender), detailed information on modalities (when, where, why, how), and outcome. We considered all falls occurred from January 1 to December 31, 2009. Based on time of event, total sample and subgroups: gender, age (< 65, 65-75, > 75 yrs), modality (witnessed, alone, upright, sitting, bed with or without bedrails, slipped on dry or wet floor, stumbled, loss of strength, equilibrium or consciousness; bathroom, room), outcome (no bruise, minor bruise, fracture), were analyzed for circadian rhythmicity by means of a validated chronobiological software (ChronoLab). The program permits the selection of the harmonic(s) that best explain the variance of data. The percentage of rhythm (PR, percentage of overall variability of data about the arithmetic mean of the fitted rhythmic function) and the probability value resulting from the F statistic (hypothesis of zero amplitude) are representative parameters of goodness of fit and statistical significance of each fitted function, respectively. The program also calculates the peak time of each harmonic. Significance levels were assumed for $p < 0.05$.
Results: During 2009, 371 consecutive falls were recorded (mean age 73.8 ± 17.6 years, men 55.8%). A circadian variation was found for the entire population, with a biphasic pattern characterized by significant peaks in morning and late-evening hours (main peak 10:28 AM). A significant morning main peak was found for men (10:44 AM), subjects falling alone (11:00 AM), loss of consciousness (10:28 AM), falls in the bathroom (9:16 AM), subjects wearing footwear (10:52 AM), and falls with fracture (1:04 PM). The evening-night main peak was found for patients with beds with bedrails (12:28 AM), slipped on wet floor (3:48 AM), subjects not wearing footwear (12:28 AM), falls with minor trauma and no significant bruise (11:08 PM).
Discussion: Falls events are one of the most common and severe adverse events for hospitalized older patients. This preliminary study shows that falls exhibits a temporal pattern, characterized by two peaks of highest risk of occurrence, nighttime and late-morning hours. Moreover, morning hours are characterized by higher frequency of fractures. The only available study, conducted in a setting of patients with dementia, showed that most cases occurred between 9 PM and 6 AM (5). To the best of our knowledge, this is the first chronobiologic study. Identification of rhythmic patterns of certain events means predicibility of events itself, and when an event is predicible, adequate strategies of prevention may be attempted. On one hand, further studies are needed to verify whether studies addressing patient's most important risk factors for falls could help in reducing the number of falls. Moreover, the identification of temporal frames of highest risk during the day could help to customize preventive strategies, optimize patient assistance, and reduce economic burden. The region Emilia-Romagna is now planning a study aimed to the identification of time of risk of in-hospital falls (Studio CAOS – Cadute in Ospedale).

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Effects of therapy with sitagliptin and metformin on physical, cardiovascular and cognitive performance in the elderly

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Background and Aims: Sitagliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor, is a newly developed oral hypoglycemic agent. The aim of this study was to analyze (I) physical performance, (II) cardiovascular and cognitive function (III) in elder patients during treatment with sitagliptin or metformin.
Materials and Methods: Sitagliptin was investigated in diabetic patients (mean age of 64,5 years) over a period of 36 months using a double-blind randomized study with two parallel groups: metformin 500 mg/thrice a day (MET) and sitagliptin 100 mg/day (SIT). Tinetti Balance and Gait Scale, Short Physical Performance Battery (SPPB), Borg Scale, Ejection fraction (EF), Cardiac Double product (DP) and Mini Mental State Examination (MMSE) were measured at the beginning and at the end of the treatment.
Results: A total of 45 patients (20 males and 25 females) were randomly assigned to the two groups. Sitagliptin and metformin were safe and well tolerated in the daily subministrations. Tinetti Scale, SPPB, Borg Scale, EF and DP showed a very significant difference between the two groups ($p < 0,05$). SIT group also showed a better diastolic function than the MET group. MMSE presented a mild, but not significant improvement ($p > 0,05$).
Conclusions: Sitagliptin appears to improve significantly physical performance and delays the onset of heart failure in the elderly. These findings will be useful in patient selection in future clinical trials with sitagliptin in long term studies.
This contribution has been awarded as Best Communication.

Prediction of empirical antibiotic therapy in respiratory infections using an artificial neural network: preliminary results

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Introduction: Lower respiratory tract infections are among the most common diseases in developed countries and include Chronic Obstructive Pulmonary Disease (COPD), one of the most frequent conditions. We know bacterial infections in the elderly show a higher morbidity and mortality. The need for a quick initiation of antibiotic therapy often requires to chose on empirical grounds. To date there are no official guidelines for empirical antibiotic therapy of COPD exacerbations. The aim of our study is to identify a tool to guide the choice of the most effective empirical antibiotic therapy when symptoms are acute and bacteriological tests can't be performed.
Materials and Methods: We used an artificial neural network (ANN) to study 117 patients aged between 55 and 97 years (mean 81.5, SD : +/- 8.7), admitted with a diagnosis of pneumonia, COPD exacerbation or pneumonia with respiratory failure. We registered symptoms at onset and some individual variables such as age, sex, risk factors, comorbidity, current drug therapies. Then the ANN was applied to choose antibiotic therapy in 20 patients versus 20 subjects whose therapy was chosen by the physicians, comparing these groups for therapy's efficacy, mean durations of therapy and hospitalization.
Results: In the learning phase the ANN could predict the resolution index 99.05% of the time (i.e. 104 times) with a standard deviation (SD) of 0.23. After the training, during the test phase, the network predicted the resolution index 91.67% of the time (i.e. 11 times) with a SD of 0.54, thus proving the validity of the relations identified during the learning phase. Application of our tool in 20 patients shows the ANN allowed to greatly reduce the duration of the antibiotic therapy and subsequently of the hospitalization: the test group in comparison to the control group, shows a reduction of the durations of the antibiotic therapy by 44% and by 41% of the hospitalization. (Fig. 1)
Conclusions: The use of ANN can make a valuable contribution in the choice of empirical antibiotic therapy in the course of acute lung diseases in elderly.

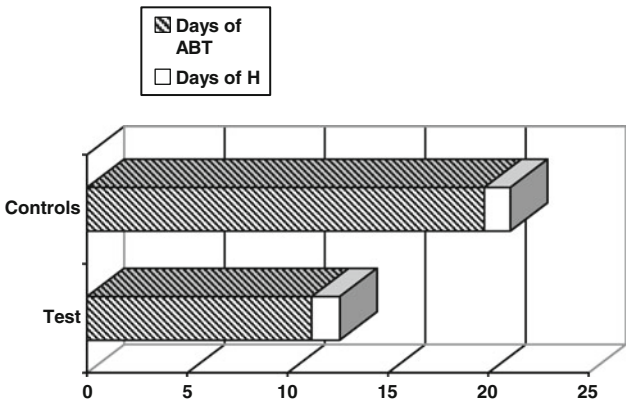

Fig. 1 Preliminary results.
(ABT: antibiotic therapy; H: Hospitalization)

Adenosine receptors A2A are associated with neurodegeneration and its clinical evolution

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Aims: Adenosine is a metabolite produced in response to metabolic stress and injury, such as ischemia, hypoxia, inflammation and trauma. The physiological effects of adenosine are transduced through four different subtypes of G-protein coupled specific receptors (A_1, A_{2A}, A_{2B} and A_3). A_{2A} receptors ($A_{2A}R$) are found ubiquitously in the body and a grooving body of evidence supports the notion that they represent the major immuno-regulatory arm of the Ado-receptor system and serve to down-regulate inflammation and immunity. Innate immune reactions are though to be implicated in the pathogenesis of neurodegenerative diseases and, in particular, in Alzheimer disease (AD).
Our previous study showed a significantly increased expression of $A_{2A}R$ in peripheral blood mononuclear cells (PBMC) of amnestic mild cognitive impairment (a-MCI) subjects compared to AD patients and controls; while in multiple impaired cognitive domains-MCI (mcd-MCI) the expression was lowest [1].
The aim of this work is to follow-up MCI subjects for at least 4 years in order to establish a possible correlation between clinical progression and $A_{2A}R$ expression.

Methods: We analysed $A_{2A}R$ mRNA levels by real-time-PCR and protein concentrations by western-blot analysis in PBMC of a-MCI, mcd-MCI, AD and CT at recruitment (T0) as previously described and after a 4-year follow-up (T1).
Results: At this time, among the patients that completed the follow-up, 4 a-MCI progressed to AD (a-MCI→AD) and 5 mcd-MCI were diagnosed with other types of dementia (e.g. vascular dementia or Lewy body disease).
At T1 a-MCI→AD showed decreased levels of mRNA (1,19 ± 1,05) and protein (0,33 ± 0,10) compared to the levels at T0 (mRNA: 3,76 ± 2.15; protein: 0,90 ± 0,85).
On the contrary, mcd-MCI patients that remain stable, after the follow-up, showed similar mRNA and protein levels at T1 and T0 (mRNA: T0: 1,05 ± 0,74 and T1: 1,47 ± 0,61; protein: T0: 0,54 ± 0,24 and T1: 0,38 ± 0,15).
Conclusion: Our data demonstrate that $A_{2A}R$ are up-regulated in peripheral cells of subjects in the pre-clinical stage of the disease and decrease when the a-MCI converted to AD. In conclusion, despite the limited number of patients that completed the follow-up period, our data seem to support the hypothesis of the involvement of $A_{2A}R$ in the early stage of neurodegenerative processes.

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Metabolism, Diabetes and Clinical Nutrition II

Epicardial fat thickness is associated with the severity of obstructive sleep apnea in obese patients

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Context: Physical activity and dietary patterns have received much attention in attempts to explain the rise in obesity of past decades; however, many other factors have been identified that may have contributed to the obesity epidemic that we observe today. One contributor receiving much interest recently is sleep quality. Obstructive sleep apnea (OSA) is a sleep disorder characterized by repeated partial or complete collapse of the upper airways, which leads to oxygen desaturation, fragmentation of sleep, and daytime sleepiness, with associated features including cardiovascular and metabolic consequences. The correlation between obesity and severity of OSA is controversial. Although fat excess is a predisposing factor for the development of OSA, it has not been determined whether body fat distribution rather than obesity per se is associated with OSA severity. Epicardial fat is the visceral fat depot of the heart and reflects visceral adiposity rather than general obesity. It is considered a metabolically active tissue, being a local source of pro-inflammatory factors associated with the development of metabolic syndrome and cardiovascular diseases.
Objective: To investigate the relation between body fat distribution, including epicardial fat thickness (EFT), and cardiometabolic risk factors with the severity of OSA in obese patients.
Patients and Methods: 115 obese patients (56 males, 59 females) with polysomnographic evidence of OSA (≥ 5 apnea/hypopnea events per hour) of various degree, were evaluated. The following parameters were measured: body mass index (BMI), waist circumference (WC), body composition by dual energy x-ray absorptiometry, EFT and common carotid intima-media thickness (cIMT) by ultrasound measurement, arterial blood pressure, fasting glucose, HDL-cholesterol, triglycerides.
Results: EFT, trunk/legs fat mass ratio and cIMT showed a positive correlation with OSA severity in univariate analysis ($r = 0.536$, $P < 0.001$; $r = 0.330$, $P < 0.001$; $r = 0.345$, $P < 0.001$). In sex-stratified analyses, the likelihood of detection of higher EFT values was higher in men ($r = 0.571$; $P < 0.001$) than in women ($r = 0.359$; $P < 0.005$). The significance of linear regression between AHI and trunk/legs FM ratio was conserved in men ($r = 0.298$; $P < 0.026$) but not in women ($r = 0.192$; $P = 0.145$). In multivariate linear regression analysis, EFT was the most significant independent correlate of the severity of OSA ($P < 0.0001$) beyond any other classical index of obesity in general and of visceral obesity in particular.
Conclusion: The present study identifies EFT as a possible predictor of the severity of OSA independent of other covariates, and associates EFT with OSA independently of obesity as defined by BMI. These findings suggest that epicardial fat may be a fat depot whose evaluation allows identifying obese patients at higher risk for severe OSA. Our data reinforce the view that sleep disorders may be contributing factors for an altered fat distribution that may increase the risk of cardiometabolic diseases in obese patients.

DNT-proBNP and 5 years survival in a large cohort of type 2 diabetic patients: the casale monferrato study

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Objective: Diabetes confers per se a high cardiovascular risk; although diabetes and other known risk factors are responsible for most part of this risk, other unknown variables are involved. Thus, researchers are interested in new "biomarkers" able to improve the performance of models for risk prediction. As few studies have highlighted a possible association between NT-proBNP and mortality in clinical cohorts of diabetic people, we studied this relation in a wide population-based cohort of type 2 diabetic subjects.
Research Design and Methods: The study base was a casual sample of 666/2381 (23%) type 2 diabetic patients belonging to the prospective population-based cohort of the Casale Monferrato Study. More than 80% of type 2 diabetic persons living in Casale Monferrato in 2001 were identified from two administrative databases and completeness of ascertainment assessed by mean of capture-recapture methods. Participants underwent a medical visit and anamnestic interview, blood and urine samples were drawn as with anthropomorphic measures. All measurements were centrally done. The role of NT-proBNP on mortality was assessed by univariate analysis and multivariate Cox models on a 5 years follow-up.
Results: We observed 156/666 deaths of whom 72 (46.2%) due to cardiovascular diseases. Patients included in the highest quartiles of NT-proBNP were older, exhibited a higher prevalence of hypertension, previous history of cardiac disease, diabetic nephropathy, were on insulin injective therapy and had higher levels of both fibrinogen and CRP. In the univariate analysis, the incidence rate ratio for the highest quartile of NT-proBNP was 7.1 (95% CI 4.0-12.6) and 15.8 (95% CI 4.9-51.1) respectively for all-cause and cardiovascular mortality (first quartile as reference). In the multivariate Cox analysis, after adjustments for age, sex, disease duration and other risk factors (hypertension, apoB/apoA1 ratio, CVD, HbA1c, AER, eGFR, anti-diabetic therapy, uricemia, BMI and fibrinogen), HRs for all-causes mortality (first quartile as reference) were 3.62 (95% CI, 0.94-13.9,

second quartile), 3.88 (95% CI, 1.01-14.86, third quartile) and 8.96 (95% CI, 2.35-34.17, upper quartile). A significant trend was observed ($p = 0.011$). Similarly, HRs for cardiovascular mortality were 2.11 (95% CI, 0.18-24.87), 6.88 (95% CI, 0.74-63.64) and 8.07 (95% CI, 0.88-73.48) with a highly significant trend ($p < 0.001$).

Conclusions: In people with type 2 diabetes NT-proBNP levels are strongly associated with both all-causes and cardiovascular mortality, independently of the other known cardiovascular risk factors. Further studies should evaluate its ability in improving discrimination and calibration of currently used models for individual risk prediction.

	All cause mortality		Cardiovascular mortality	
	HRs Model 1	HRs Model 2	HRs Model 1	HRs Model 2
NT-proBNP (quartiles, pg/ml)				
0 – 48	1.00	1.00	1.00	1.00
49 – 102	1.41	3.62	2.50	2.11
	(0.72 – 2.75)	(0.94 – 13.9)	(0.67 – 9.33)	(0.18 – 24.87)
103 – 232	2.14	3.88	5.92	6.88
	(1.13 – 4.06)	(1.01 – 14.86)	(1.72 – 20.38)	(0.74 – 63.64)
> 233	4.42	8.96	10.03	8.07
	(2.41 – 8.08)	(2.35 – 34.17)	(2.97 – 33.85)	(0.88 – 73.48)
p for trend	<0.0001	0.011	<0.0001	<0.001

Model 1: corrected for age, sex and diabetes duration

Model 2: corrected also for apob/apoA1, HbA1c, smoking, previous cardiovascular events, hypoglycemic therapy, AER (albumin excretion rate), C-reactive protein, BMI, fibrinogen, uric acid, eGFR (estimated glomerular filtration rate).

This contribution has been awarded as Best Communication.

High prevalence of advanced nafld in type 2 diabetic patients with normal liver enzymes and effect of liraglutide on nafld: meta-analysis of the lead program

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Non-alcoholic fatty liver disease (NAFLD) is the commonest cause of liver disease in patients with type 2 diabetes. Predicting patients at risk of progressing to advanced NAFLD (liver inflammation and fibrosis) is a clinical challenge as the majority of patients are asymptomatic and have normal liver enzymes. The NAFLD Fibrosis Score (NFS) is a well-validated non-invasive scoring system used to predict the severity of NAFLD fibrosis in the absence of a liver biopsy. The aims of this study are: (i) to estimate the severity of NAFLD using the NFS and (ii) to evaluate the efficacy of 1.8 mg liraglutide (VictozaR), a once-daily human GLP-1 analog, on NAFLD in a cohort of poorly controlled diabetic subjects. Meta-analysis was performed on individual data from patients enrolled in the Liraglutide Effect and Action in Diabetes (LEAD) program. ANCOVA analysis was performed on the intent-to-treat population to estimate change from baseline after 26 weeks.

Data from 3967 adults (sex, 53% male; ALT 24:33 [F:M] IU/L; mean [SD], age 56 years [10], HbA1c 8.3% [1.0], BMI 31.5 kg/m^2 [5.60]) were analyzed. 54% of subjects had an abnormal baseline ALT, but their mean ALT (39 IU/L) was only marginally raised. In contrast, the NFS predicted that 6.4% had advanced liver fibrosis (score > + 0.676) and 61.0% had an indeterminate score (–1.455 to 0.676) requiring further evaluation with a liver biopsy. Liraglutide reduced ALT *versus* placebo (LS mean 3.48 *vs.* 1.36; $p < 0.001$), although the proportions of patients normalizing their ALT were similar (25.2% *vs.* 22.7%). Liraglutide significantly improved NFS in comparison to placebo (LS mean –0.40 *vs.* –0.13; $p < 0.0001$), with significant changes in BMI (LS mean –0.66; $p < 0.0001$) and platelet count (LS mean +15.90; $p < 0.0001$). The increase in platelets may indicate that liraglutide has an effect independent of weight loss in NAFLD.

Advanced NAFLD is found in a significant proportion of patients in the LEAD program despite the presence of relatively normal serum transaminases. 26 weeks' treatment with liraglutide reduces ALT and markedly reduces the NFS score, thus reducing risk of further NAFLD progression.

Comparison of A1C, fasting and 2-H post-load plasma glucose criteria to diagnose diabetes in italian caucasians

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Background: Recently, the American Diabetes Association (ADA) has revised criteria for the diagnosis of type 2 diabetes on the basis of a comprehensive analysis carried out by an International Expert Committee. The ADA introduced A1C test to diagnose diabetes with a cut-point of ≥6.5% in addition to the criteria based on plasma glucose (PG), i.e. either fasting PG (FPG) ≥126 mg/dl or 2-h 75-g oral glucose tolerance test (OGTT) PG (2-hPG) value of >200 mg/dl. As there is a moderate concordance between the FPG and 2-hPG tests to diagnose diabetes, it is likely that there is not perfect concordance between A1C and either glucose-based test.

Aims: The aim of the study was to compare A1C, fasting and 2-h post-challenge glucose for the diagnosis of type 2 diabetes in adults at risk for diabetes. Additionally, we evaluated the cardio-metabolic risk profile for subjects diagnosed with diabetes by each of these tests.

Methods: This study included 1019 Caucasians without known diabetes, but having one or more cardio-metabolic risk factors including elevated blood pressure, dyslipidemia, overweight/obesity, and family history for diabetes. On the first day, after 12-h fasting, subjects underwent anthropometrical evaluation, including body mass index (BMI), and waist circumference, and a venous blood sample was drawn for laboratory determinations. On the second day, after a 12-h fasting, a 75 g OGTT was performed with sampling for plasma glucose and insulin. A1C was measured with high performance liquid chromatography. Insulin sensitivity was evaluated by an OGTT-derived index.

Results: Subjects were classified into four groups: 1) nondiabetic subjects by the three ADA criteria (FPG < 126 mg/dl, 2-hPG < 200 mg/dl and A1C < 6.5%); 2) subjects who had A1C < 6.5% but FPG ≥ 126 mg/dl and/or 2-hPG ≥ 200 mg/dl; 3) subjects who had A1C ≥ 6.5% but FPG < 126 mg/dl and 2-hPG < 200 mg/dl; and, 4) subjects who had A1C ≥ 6.5%, FPG ≥ 126 mg/dl and/or 2-hPG > 200 mg/dl.

Moderate agreement existed for A1C and OGTT criteria for diagnosis of diabetes (κ coefficient = 0.446), with 80.1% of individuals classified as not having diabetes by both A1C and OGTT criteria, and 7.0% classified as having diabetes by both A1C and OGTT criteria. Discordant classifications occurred for 4.3% of individuals who had an A1C $\geq$ 6.5% and FPG < 126 mg/dl and 2-hPG < 200 mg/dl and for 8.6% who had an A1C < 6.5% and FPG $\geq$ 126 mg/dl and/or 2-hPG $\geq$ 200 mg/dl. When diagnosis of type 2 diabetes based on the A1C criterion was compared with diagnosis based only on FPG $\geq$ 126 mg/dl, the agreement was higher (κ coefficient = 0.522), while the agreement of the A1C criterion with diagnosis based only on 2-h PG $\geq$ 200 mg/dl was lower (κ coefficient = 0.427). Compared with subjects who had A1C $\geq$ 6.5% but fasting plasma glucose < 126 mg/dl and 2-h post-challenge glucose < 200 mg/dl, those who had only OGTT-based diagnosis exhibited lower obesity associated with lower insulin sensitivity, and higher risk for having metabolic syndrome and hypertension.

Conclusions: In this cohort of adult Caucasians at risk for type 2 diabetes, the concordance between type 2 diabetes diagnosis made by A1C and OGTT criteria is relatively low. In our study, 38.2% of individuals with A1C $\geq$ 6.5% were not classified as diabetic by OGTT-based criteria and 55.3% of the individuals with diabetes by OGTT-based criteria would be classified as nondiabetic by A1C criterion.

We conclude that significant discordance exists between A1C and FPG or 2-hPG based diagnoses of type 2 diabetes. The use of A1C could have some advantages compared with FPG or 2-hPG, including greater convenience, since fasting or OGTT is not required, greater pre-analytical stability, lower between and within-subject variations and less day-to-day perturbations during periods of stress and illness. However, these advantages must be balanced by greater cost, limited availability of A1C assay in certain regions of the developing world, incomplete correlation between A1C and average glucose values in certain subjects, age-specific and race-specific differences.

Nephrology

A model to calculate fat mass in hemodialysis patients accounting for expansion of extracellular fluid: a comparison between BIS and DXA

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Introduction: Fat mass (FM) can be measured noninvasively with dual-energy X-ray absorptiometry (DXA), but is expensive and not portable. Multifrequency bioimpedance spectroscopy (BIS) measures total body water (TBW) and intracellular and extracellular water (ICW and ECW). Adiposity is calculated by subtracting Fat Free Mass (FFM) from weight assuming fractional hydration of FFM of 0.73. Hemodialysis patients (HD), however, have non physiologic expiation of ECW.

We developed an estimate of total-body FM in adult healthy subjects with BIS using a formula that calculates hydration of FFM that allows ECW and ICW to vary (TBW/FFM = (1 +ECW/ICW)/(1.569 + 1.16*(ECW/ICW)) (Wang Z. et. al. AJP Endocrinol Metab 276:995-1003,1999). We then derived a value for hydration of FFM correcting for the effects of non physiologic expansion of ECW to be applied to HD.

Materials and Methods: FM was measured by DXA in 11 healthy subjects (6 male and 5 female; C) median age 32.5 y (23-53 y), weight 76.5 kg (47.9-102.8 kg), body mass index (BMI; kg/m^2) 26.5 (17.5-29.8), and in 8 male HD with median (range) age 44.5 y (28-55 y), weight 102.5 kg (73-119.3 kg), BMI of 31.15 (24.7-36.9). We measured TBW, ECW and ICW with BIS and calculated FM using either Weight - TBW/.73 or with the formula to account for ECW/ICW to estimate FM.

Results: ECW/ICW was significantly greater in HD than in C (0.860.02 vs 0.780.01; p = 0.01) and hydration of FFM significantly greater in HD than in C (0.724 $\pm$ 0.004 vs 0.7195 $\pm$ 0.003; p < 0.01). FM (kg) estimated by DXA, BIS and the model were not different in C (20.0 $\pm$ 2.7, 20.3 $\pm$ 2.9, 19.4 $\pm$ 3.0, p = n.s. and correlated r^2 p < 0.001) or in HD (27.8 $\pm$ 3.2, 28.4 $\pm$ 2.9, 27.6 $\pm$ 2.9, p = n.s and correlated r^2 p < 0.001.). Neither Bland–Altman plot regressed (r^2 = 0.00). BIS reported FM 0.6 kg greater than DXA in HD, while the model underestimated FM by only 0.2 compared to DXA (P < 0.001).

Conclusion: This algorithm provides an estimate of FM using both ICW and ECW measured by BIS that can be used to evaluate adiposity when ECW is changed independently of physiologic constraints in HD patients that may have less error.

This contribution has been awarded as Best Communication.

Oxidative stress is associated with aortic stiffness in hypertensive subjects with chronic kidney disease

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Background: It is well know that arterial stiffness and oxidative stress are features of chronic kidney disease (CKD). Several studies have consistently demonstrated that arterial stiffness becomes progressively worse as CKD progresses and a negative correlation of oxidative stress with renal function has been described. There is also sound experimental evidence indicating that oxidative stress is involved in atherogenesis. The contribution of oxidative stress to aortic stiffness is less clear.

Objective: The aim of our study was to analyse the relationship between plasma levels of 8-ISO-prostaglandin F2alpha (8-ISO-PGF2alpha), an index of lipid peroxidation, considered a reliable biomarker of oxidative stress, and aortic stiffness in a group of hypertensive patients with chronic kidney disease.

Methods: We enrolled 90 pharmacologically treated hypertensive patients (mean age 59 $\pm$ 12 years, males 53%) with chronic kidney disease [estimated glomerular filtration rate (eGFR) < 60 ml/min/1.73 m^2]. In all the subjects routine biochemical parameters and 8-ISO-PGF2alpha plasma values, measured by a solid-phase specific sandwich enzyme-linked immunosorbent assay (Assay Design Inc), were obtained. Moreover, ambulatory blood pressure monitoring and measurement of c-f PWV, by a computerized automatic method (Arteriograph), were performed. The GFR was estimated by the four-variable MDRD study equation.

Results: The study population comprised 90 hypertensive subjects with eGFR ranging from 60 to 15 ml/min/1.73 m^2 (mean value: 37 ml/min/1.73 m^2). Thirty-one patients (34%) had type 2 diabetes. The patients (n = 41) with elevated values of c-f PWV (> 12 m/sec) showed significantly higher 8-ISO-PGF2alpha plasma levels than those of subjects with PWV < 12 m/sec (423.3 $\pm$ 117.8 vs 359.8 $\pm$ 105.9 pg/ml; p = 0.009 and p = 0.02, before and after adjustment for age, gender and mean arterial pressure). A statistical

significant correlation was found between 8-ISO-PGF2alpha and c-f PWV in the whole study population ($r = 0.33$; $p = 0.001$). This association held even after adjustment for age, gender, mean arterial pressure, smoking habit, presence of diabetes (or glycaemia, as continuous variable), total cholesterol, calcium x phosphate product and eGFR (beta = 0.23; $p = 0.006$) in a stepwise multiple regression model.

Conclusions: Our results seem to suggest that in hypertensive subjects with CKD there is an independent relationship between oxidative stress and aortic stiffness and that the unfavourable influence of a reduced renal function on large artery elastic properties may be partly mediated by an increased oxidative stress.

Cardio-renal syndrome should be recognized in patients admitted to internal medicine wards

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Introduction: Because of the increasing incidence of cardiac failure and chronic renal failure due to the progressive aging of the population, the extensive application of cardiac interventional techniques, the rising rates of obesity and diabetes mellitus, coexistence of heart failure and renal failure in the same patient are frequent. More than half of subjects with heart failure had renal impairment, and mortality worsened incrementally across the range of renal dysfunctions (1). Cardiorenal syndrome (CRS) defines a new clinical entity identifying an association between renal insufficiency and cardiac dysfunction (2-4). The general definition has been expanded into five subtypes reflecting the primacy of organ dysfunction and the time-frame of the syndrome (3): CRS type 1 = acute worsening of heart function leading to kidney injury and/or dysfunction; CRS type 2 = chronic abnormalities in heart function leading to kidney injury or dysfunction; CRS type 3 = acute worsening of kidney function leading to heart injury and/or dysfunction; CRS type 4 = chronic kidney disease leading to heart injury, disease and/or dysfunction, and CRS type 5 = systemic conditions leading to simultaneous injury and/or dysfunction of heart and kidney.The aim of this study was to define the clinical characteristics of CRS in a cohort of consecutive patients admitted to an Internal Medicine ward.

Methods: From July 2007 to December 2009, 438 patients with CRS were observed, and data relating to anthropometric, anamnestic, clinical, biochemical and therapeutic characteristics were obtained. Glomerular filtration (eGFR) was calculated by means of the MDRD equation, using two different coefficients ($eGFR_{MDRD186}$, $eGFR_{MDRD175}$), the MAYO equation ($eGFR_{MAYO}$), and the new CKD-EPI equation ($eGFR_{CKD\text{-}EPI}$).

Results: The mean age of the population (222 males, 50.6%) was 80 ± 8 years, 321 (73.2%) were smokers, 229 (52.2%) were diabetic, 207 (47.2%) had a clinical history of acute myocardial infarction, 167 (38.1%) suffered from angina, 135 (30.8%) were affected by cerebrovascular disease, 339 (77.3%) suffered with peripheral vascular disease. Diuretics were the most prescribed medication (78.7%), but only 5 patients were under hemodialytic treatment. CRS type I was found in 211 cases (48.2%), type II in 96 (21.9%), type III in 88 (20.1%), type IV in 29 (6.6%), and type V in 14 (3.2%). In CRS type I, glomerular filtrate was between 30.7 and 35.7 ml/min/1.73 m^2, between 37 and 44.5 in type II, between 24.2 and 27 in type III, between 27.4 and 33.4 in type IV, and between 25 and 28.2 in type V. Mean hospitalization length of stay was 9.8 ± 6.3 days.

Conclusions: CRS is a common illness found most frequently in elderly patients. CRS type I was the prevalent subset observed in the Internal Medicine ward, and patients showed a stage 3-4 renal insufficiency. Results obtained from the different GFR equations are similar, although the MAYO equation tends to overestimate the value. Since the management of cardiorenal patients requires a tailored therapy that prioritizes the preservation of the equilibrium of each individual patient, in an Internal Medicine ward (ed especially in elderly subjects), the identification of patients and the pathophysiological mechanisms underlying each syndrome subtype will help to provide adequate therapeutic strategies.

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High dose intravenous furosemide plus small volumes of hypertonic saline solutions (HSS) in treatment of acute prerenal failure. a pilot study

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Background: Our group recently reported that the combination of high-dose i.v furosemide and small-volume hypertonic saline solution (HSS) intravenous infusion is effective both in the treatment of refractory congestive heart failure and ascites We conducted a pilot study to compare the efficacy of intravenous high-dose furosemide + hypertonic saline solutions (HSS) and rehydratation in patients with pre-renal acute renal failure in comparison with rehydratation alone.

Materials: 40 subjects patients with acute pre-renal failure were randomized into 2 groups: *group A* received an IV 30-minute infusion of furosemide (500-1000 mg) plus HSS (150 mL of 1.4%-4.6% NaCl) twice a day in parallel with NaCl 0.9% (1500-2000 ml/day) for 6-8 days; *group B* received an IV infusion of NaCI (0.9%) (1500-2000 ml/day), without HSS for 6-8 days.

At the end of treatment period as primary efficacy endpoint were evaluated the difference (Δ) between admission value and discharge value of some renal variables (*Δ-Diuresis, Δ-serum creatinin, Δ- serum sodium, Δ-urinary Na, Δ-urinary K, Δ- GFR, Δ-BUN*).

Results: Subjects of group A treated with high dose furosemide + small volume hypertonic saline solutions (HSS) in comparison with subjects of group B treated with hydratation showed at discharge an higher Δ-diuresis, an higher Δ-serum creatinin, an higher Δ- serum sodium, an higher Δ-urinary Na, an higher Δ-urinary K, an higher Δ- GFR and a higher Δ-BUN.

Conclusions: Further studies are needed to confirm our findings and to evaluate hemodynamic and neurohormonal changes after this type of treatment.

Gerontology and Geriatric Medicine III

Is the hematopoietic effect of testosterone mediated by erythropoietin? the results of a clinical trial in older men

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The prevalence of anaemia is higher in older individuals where it has been recognized as an independent predictor of disability and mortality (1-2). Anaemia is often multifactorial (1). One of the causes of anemia is the decline of testosterone levels in both sexes (1). In older subjects low testosterone levels are predictors of anemia (3), which is consistent with the known stimulatory effect of testosterone on erythropoiesis (4). However, there are several mechanisms underlying the erythropoietic effect of testosterone (4). One of the most common has been considered the stimulating effect on erythropoietin levels (5). However this "dogma" has not been confirmed in more recent studies using injectable testosterone (6).

Aim of the Study: To test the effects of transdermal testosterone on haemoglobin and erythropoietin levels in older men with low-normal levels of testosterone.

Methods: 108 men $>$ 65 years old were selected according to serum testosterone concentration $\geq$ 1 SD below the mean for normal young men ($<$ 475 ng/dL) and randomized to receive a testosterone patch or placebo in a double blind fashion for 36 months. Ninety-six subjects completed 36 months of treatment. The present analysis was performed on 70 men, 42 in the testosterone treatment group and 28 in placebo group who had sufficient sera remaining for assays of testosterone, haemoglobin, erythropoietin, and creatinine. Total testosterone was assessed by electrochemiluminescence immunoassay with minimum detective concentration (MDC) of 2 ng/dl and interassay coefficients of variation (CV) less than 10%. Erythropoietin was assessed by ELISA with MDC of 2.5 mIU/ml and interassay CV less than 10%. Changes within groups from baseline to treatment end were evaluated with paired *t* tests. Finally the association between treatment and change of haemoglobin and erythropoietin over time was examined with random coefficient analyses. Treatment status and time point (T0-T1) were entered as fixed factors; subjects were treated as a random factor and a random intercept was estimated. To test whether the changes in hemiglobin and erythropoietin levels were different according to testosterone levels at treatment-by-T1 an interaction term was entered in the same models.

Results: The mean age $\pm$ SD of the 70 subjects population at baseline was 71.8 $\pm$ 4.9 years. The testosterone and placebo groups did not differ at baseline in terms of age, haemoglobin, and erythropoietin. The testosterone-treated group had lower baseline testosterone and BMI than the placebo group but neither was quite statistically significant ($p = 0.06$ and $p = 0.16$, respectively). As expected, 36 months testosterone treatment was associated with an increase in testosterone levels (from 488.20 $\pm$ 230.53 to 689.41 $\pm$ 307.26) that was statistically significant ($p = 0.0003$). The delta change in testosterone levels was 201.21 and 22.73 in testosterone and placebo group, respectively. Testosterone therapy for 36 months induced an increase in haemoglobin levels of about 1 g/dL (from 14.72 $\pm$ 1.02 to 15,53 $\pm$ 1.36).The delta change in haemoglobin levels was 0.81 and -0.06 in testosterone and placebo group, respectively. During the treatment period, participants on testosterone therapy, as compared to those on placebo, had a steeper increase of haemoglobin levels (Treatment-by-T1: $\beta \pm$ SE 0.86 ± 0.31, $p = 0.01$). Testosterone therapy for 36 months did not induce any significant increase in EPO levels (from 15.24 $\pm$ 19.32 to 15.40 $\pm$ 17.47). No significant change was also found in placebo group. The change in EPO levels during treatment did not differ between subjects on testosterone and those on placebo. The difference between 2 groups was not statistically significant (Treatment-by-T1: beta $\pm$ SE = -0.24 $\pm$ 2.16, $p = 0.91$).

Conclusion: Transdermal testosterone treatment for 36 months in older men significantly increased haemoglobin but not erythropoietin levels. Other mechanisms should be explored to explain the hematopoietic effect of testosterone.

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This contribution has been awarded as Best Communication.

Neuroserpin expression in peripheral blood mononuclear cells of patients with alzheimer's disease

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Aim: Amyloid-beta (Aβ plaques are pathological hallmarck of Alzheimer's disease.

One possible mechanism that describes the accumulation of Aβ is inefficient clearance of the peptide. Several proteases are know to cleave/remove Aβ including plasmin, the product of tissue plasminogen activator (tPA) cleavage of the pro-enzyme plasminogen.

Proteases are inhibited by members of the serine proteinase inhibitor (serpin) superfamily that include neuroserpin (NS), that is mainly expressed in the brain.

NS is synthesised in neurons of central and peripheral nervous system, where it is secreted from the growth cones of these cells. Previous data have demonstrated that NS is a potent inhibitor of tPA, urokinase plasminogen activator and plasmin modulating many processes as neurite outgrowth, synaptic plasticity and neuroprotection.

It was show that NS levels are significantly elevated in brains of AD patients, where is co-localized with Aβ laque forming a binary

complex. This in turn prevents fibril formation and renders the Aβ eptide less toxic to neuronal cells.
Moreover higher levels of NS were previously described in CSF of AD patients compared to controls (CT).
By considering the expression of NS in peripheral blood mononuclear cells (PBMC) of patients with established AD and CT, we sought to identify a potential bio-marker for neurodegeneration in easily accessible cells.
Methods: We analysed NS mRNA levels by real-time PCR in PBMC of 33 subject with AD, and 35 sex and age-matched CT.
At the moment, we are performing home-made ELISA test to analyse plasma and CSF levels of NS in the same patients.
Results: mRNA levels (expressed as mean ± standard error) were significantly different in the two groups (1,09 ± 0,20 and 1,99 ± 0,39 respectively in AD and CT; $p < 0,05$).
Conclusions: Our data show that NS gene expression is down-regulated in PBMC of AD compared to CT. If we, also, confirm an altered NS expression (gene and protein expression) in mild cognitive impairment subjects (the preclinical stage of AD), these findings will support the theory that NS could affect neurodegeneration mechanisms.

Predictors of destination at discharge in a population of hip fractured elderly patients

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Background and aim: in the last years, orthogeriatric care has become a recommended approach to manage hip fracture in frail elderly subjects. The aim of our study is to identify possible predictors of the outcomes at discharge in a population of older patients admitted to the Orthogeriatric Unit (OGU) at the San Gerardo University Hospital – Monza with a proximal femur fracture (PFF).
Materials and methods: 255 patients consecutively discharged after PFF surgery from the OGU during the period March 2007 - December 2010 have been considered. Baseline characteristics, time of management, occurrence of complications, and the destination at discharge, i.e. home, rehabilitation facilities, permanent institutionalization were evaluated. Statistical analysis has been performed with PASW Statistics 18.
Results: patients have been firstly divided in three groups according to the outcome at discharge: those transferred to rehabilitation facilities (group 1, $n = 228$), those returned home because not eligible for rehabilitation or institutionalized (group 2, $n = 20$), and those died (group 3, $n = 7$). Patients in the groups 2 and 3 were significantly older, more frequently dependent in functional status and demented before PFF, had more postoperative complications and a longer length of stay than their counterparts. Because of their small size, groups 2 and 3 were collapsed in a unique group. In a logistic regression analysis, with "eligibility to rehabilitation" rather than "ineligibility to rehabilitation or death" as the dependent variable, and demographics, clinical, cognitive and functional variables as independent variables or confounders, the full dependence in functional status before PFF and the persistence of delirium in the pre- and post-operative phases were the only two variables significantly and independently associated with the poor outcome (ineligibility to rehabilitation or death).
Conclusion: the functional level pre-existing PFF (i.e. full dependence in ADL) and the persistence of delirium in the pre- and post-surgical phases are independent predictors of eligibility to rehabilitation in a population of elderly patients discharged from the OGU. Especially delirium, which is by definition a modifiable condition, needs to be taken into the account in the assessment of elderly patients after PFF.

Table Univariate and multivariate models assessing the association of clinical, cognitive and functional variables with eligibility to rehabilitation at discharge from OGU in 255 elderly patients after PPF surgical correction

	Univariate models			Multivariate model		
Characteristics	OR	95% CI	P	OR	95% CI	P
Functional level (ADL)	1.1	0.4 - 2.5	.909	–	–	–
Partial dependence	5.2	2.3 - 12.0	<.001	4.2	1.4 - 13.0	.004
Full dependence						
Pre- and post-operative delirium (present/absent)	10.6	4.3 - 26.4	<.001	8.1	2.8 - 23.8	<.001
Dementia pre-existing PFF (present/absent)	2.2	0.9 - 5.3	.072	–	–	–
Time from HF to surgery (≥ 96 h)	2.9	1.2 - 6.9	.019	–	–	–
Time to mobilization after surgery (> 4 days)	2.1	0.9 - 5.0	.075	–	–	–
Intercurrent adverse clinical events (≥ 2)	4.1	1.4 - 11.5	.009	–	–	–

OR denotes Odds Ratio; 95% CI denotes 95% confidence intervals; p denotes significance at logistic regression analysis; ADL denote Activities of Daily Living; PFF denotes proximal femur fracture

A rare opportunistic infectious disease

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Introduction: Mucormycosis is a rare infectious disease caused by mold belonging to the order of *Mucorales* (Absidia, Rhizopus e Mucor), these mold contaminate the ground and derived from the decomposition of organic materials, moreover are present in foiled foods. *Rhizopus Orizae*, is the most common species, which is transmitted by inhalation of the spores, from the airway the mold pass inside the blood and metastasizes to the tissues.
Case Report: A 67-old-year man was admitted to our Department because of loss of consciousness associated with tonic clonic seizures shock. In his past medical history there was arterial hypertension, cardiovascular disease and diabetes mellitus with end stage renal failure on dialysis. Physical examination showed a dramatic peripheral deficit of the right facial nerve. At chest examination hypophonia and crackles were appreciable in the left lower lobe. Laboratory tests showed normochromic normocytic anemia (Hb 8.4 g/dl), a slight neutrophilia with normal white blood cell count (WBC 4.400; N82%), increased C-RP(10.28 mg/dl) and ESR (73 mm/h) values and impaired renal function (creatinine 8.4 mg/dl, BUN 75 mg/dl, K 5.5 mg/dl). Brain CT scan documented no evidence of acute injury in place, but, on a side, note the complete resorption of the nasal bones and cartilage, with subtotal disappearance of the nasal septum and turbinate bone, part of the ethmoid cells, and the medial wall of both maxillary sinus and obliteration of hypodense material and sphenoid

sinuses. On the basis of such result a brain and facial RMN was performed which showed character likely to chronic inflammatory tissue in relation to the presence of hyperostotic aspect of the bony walls… against the nasal septum, nasal turbinate bilaterally, ethmoid cells, of the hard palate and maxillary sinus to the right. In order also to have an histological test, a surgical cleaning of the cable nose, ethmoid and skull base was therefore performed which showed fragment of bone in the context of which are fungal spores and hyphae to support the clinical suspicion of fungal infection. Fragments of Sino-nasal mucosa characterized by chronic inflammation marked lympho-histiocytic, giant cell formation of some non-necrotizing granulomas charged to the chorion, associated with marked vascular ectasia. Thus the final diagnosis was mucormycosis and the patient underwent to amphotericin B i.v. therapy. A facial RMN was repeated showing reduction of potentiation loading of the lining of the residual facial bone components previously documented in a report likely to surgical outcomes known. Appears reduced the percentage of mucosal thickening involving the maxillary sinuses bilaterally, following surgery.

Conclusions: Mucormycosis normally has a low pathogenicity, but in patients severely debilitated (transplants, leukaemia, burns) may acquire a significant clinical value, sometimes it represents a life-threatening disease. Diagnosis is based on the clinical suspicion and on the evidence of large and no septate hyphae at histology. Facial RMN represent the main diagnostic tool to initiate the appropriate diagnostic and therapeutic iter.

Oncology

Short treatment with sorafenib is associated to tumor progression and accelerated time to progression in patients with hepatocellular carcinoma

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Introduction: Sorafenib is one of the most diffused treatments for advanced hepatocellular carcinoma (HCC). Dose reduction or withdrawal after a short time of treatment is sometimes necessary, due to adverse events or to scarce response in aggressive tumors. The effects of an incomplete cycle of treatment on tumor progression are still unknown.

Aims & Methods: We report our experience with sorafenib in the treatment of HCC, reporting the main factors associated to tumor progression (TP), time to progression (TTP) and survival. 28 HCC patients were included in the analysis (Table 1); they were all treated with sorafenib 400 mg/bid; withdrawal was due to adverse events (15 pts) or TP (13 pts).

Results: The mean daily administered sorafenib dose was of 695.9 mg (295-800 ± 137.7) and the mean treatment duration of 150.5 days (5-481 ± 136.2). Therefore, 19/27 (70.4%) patients underwent a reduced scheduled of treatment for dose or duration. 25 patients (92.6%) experienced TP. The mean TTP was of 232.2 days (12-1113 ± 251.6) after the beginning of sorafenib. Among all the investigated factors, treatment duration shorter or equal to 2 months seems to be associated with TP ($p = 0.039$) and to a faster TTP ($p = 0.021$). The reported mean overall survival was of 412.6 days (35- 1113 ± 295.4). The presence of metastasis and an advanced Child Pugh score before treatment seem the most important factors affecting patients' survival ($p = 0.05$ and $p = 0.023$, respectively).

Conclusion: In HCC patients receiving sorafenib a short treatment schedule should be avoided, due to a high risk of TP and a fast TTP. Patients' survival is almost related to an advanced stage of liver disease and extra-hepatic tumor spreading.

Table 1

CHARACTERISTIC	MIN	MAX	MEAN	SD	FREQUENCY
AGE	49,00	79,00	64,1429	8,33873	-
PRE-TREATMENT					
Nodules (> 3/diff)	-	-	-	-	15/28 (53.5%)
Dimension (> 5 cm/diff)	-	-	-	-	12/28 (42.9%)
Vascular invasion	-	-	-	-	13/28 (46.4%)
Metastasis	-	-	-	-	11/28 (39.3%)
Alphafetoprotein	2	7619	839	1997,2	-
BCLC	-	-	-	-	1A (3.6%)/5B (17.9%)/22C (78.6%)
CHILD	-	-	-	-	26A (92.9%)/ 2B (7.1%)
POST-TREATMENT					
N nodules (> 3 or diffuse)	-	-	-	-	16/26 (38.5%)
Dimension (> 5 or diffuse)	-	-	-	-	15/26 (53.6%)
Vascular invasion	-	-	-	-	15/26 (57.7%)
Metastasis	-	-	-	-	13/26 (50%)
Alphafetoprotein	3	7700	1078,4	1924,4	-
BCLC	-	-	-	-	3B (11.1%)/ 20C (74.1%)/ 4D (14.8%)
CHILD	-	-	-	-	15A (53.6%)/ 8B (28.6%)/ 5C (17.9%)

CD133 Stem cell marker regulates genes associated with invasiveness in colon cancer

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Introduction: Several in vitro assays have been used to identify cancer stem cells (CSC), including the presence of surface cell markers and by Hoechst dye efflux properties, the so-called Side-Population (SP). The CD133 antigen has been proposed as a marker for CSCs in colon cancer (CC), but the conflicting results reported in the literature indicate the need of a better characterization of CSC.

Aims & Methods: Aims of this study were to confirm that CD133 expression is a valid method for isolating CSC in CC and identify new antigens in order to increase the specificity of this marker.

CD133 and CD133-cells were isolated from four human CC lines (Caco-2, HT29, Lovo, HCT-116) by FACS sorter and the tumor-initiating potential of CD133 + cells was assessed in vitro and in

vivo. Furthermore, the gene expression profile of CD133 + versus CD133- CaCo-2 cells was compared by the means of microarray analysis and the most relevant transcripts found to be overexpressed in CD133 + CaCo-2 cells were evaluated, by real-time PCR, in the CD133 + fractions isolated from other CC cell lines. We have also compared, by real-time PCR, the gene expression profile of CD133 + cells and SP fractions, isolated from the same CC cell lines. Finally, we deplete CD133 expression in the CaCo-2 cell line by the means of siRNA and verified by Western Blot analysis whether there was a functional correlation between CD133 and the target genes. Finally, we tested by in vitro assay the effect of CD133-siRNA on tumor-initiating properties of CC cells.
Results: We confirmed that only CD133 + cells have a tumor-initiating potential in vitro and in vivo. Microarray analysis of CD133 + versus CD133- CaCo-2 cells revealed a significant overexpression of various transcripts involved in cell proliferation, invasion and stemness in CD133 + cell fraction. Comparison of the transcripts revealed that the genes of Endothelin-1 (END-1) and NR4A2 are highly expressed in both CD133 + cells and in SP fractions. Finally, when we deplete CD133 expression in Caco-2cells, we observed a significant attenuation of END-1 and NR4A2 expression, thus demonstrating that CD133 is involved in the transcriptional regulation of these genes. Finally, in vitro assay has also shown that down-regulation of CD133 expression correlated with loss invasiveness and clonogenicity of CC cells.
Conclusion: This study demonstrates for the first time that CD133 is involved in transcriptional regulation of genes associated with clonogenicity and invasiveness in the CC. This discovery could shed light on the role of CD133 antigen in defining the phenotype of the CSC and resolve disputes relating to its role as a marker of CSC in the CC.
This contribution has been awarded as Best Communication.

A novel metabolic action for the multikinase inhibitor sorafenib:targeting mitochondria in liver tumors

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Introduction/Objectives: One of the basis of modern oncology resides on the metabolic alteration of cancer cells, that explicates through an increase of glycolytic pathway, the so called "Warburg Effect".
Glycolysis can be seen as a cancer antioxidant response to allow tumor dissemination protecting cells from ROS-induced senescence and apoptosis. In the present study a novel metabolic effect of the multikinase inhibitor Sorafenib (SFB) was investigated, alone and in combination with the glycolytic inhibitor 2-deoxyglucose (2dg), in a liver tumor model, the LCSC -2 (Liver Cancer Stem Cells) cell line. In particular we investigated the mitochondrial action of SFB.
Aims & Methods: Our intent is to elucidate the metabolic effects of SFB and investigate the possibility of a mitochondrial action of the compound. Drug toxicity was evaluated through Propidium Iodide (PI) assay. Gene expression was investigated by microarray analysis. Protein expression was evaluated by western blotting. Intracellular energetic balance was assessed by ATP measurement. Mitochondrial activity was assessed by Oxygraph Respirometric Analysis. Mitochondrial potential was measured with the JC-1 probe.
Results: FACS analysis on LCSC revealed an increase of intracellular ROS content after SFB exposure. Since mitochondria are the main sources of ROS in normal and tumor cells, we reasoned that SFB may target these organelles. Actually, oxymetryc analysis of cellular respiration showed that SFB impaired both basal respiration and mitochondrial Complex I activity. Moreover, SFB depolarizes mitochondrial potential ($\Delta\psi$), and intracellular ATP was also significantly decreased. In agreement with these data, our gene expression profile showed an increase in glycolytic pathway induced by SFB, strengthening a metabolic action of the compound. Since SFB increases glycolysis we hypothesized that its effects might be enhanced by the glycolytic inhibitor 2DG; indeed, SFB plus 2DG results in a synergistic action on cell toxicity assays.
Conclusions: Our preliminary results outline a metabolic effect of SFB, in particular at the mitochondrial level; moreover, the synergistic action of SFB plus 2DG outline a novel combined metabolic therapy to eradicate liver tumors.

Cardiovascular Diseases III

Systolic dysfunction in diastolic heart failure with preserved ejection fraction: the case of cardiac amyloidosis

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Introduction and Aim: Cardiac amyloidosis represents an archetypal form of restrictive heart disease, that is characterized by profound diastolic dysfunction. Ejection fraction, an index of global systolic pump function, is preserved until the late stage of the disease. Therefore, the majority of patients with cardiac AL amyloidosis do fulfil the definition of diastolic heart failure, i.e. heart failure with preserved ejection fraction. However, systolic regional dysfunction is well evident when performing echocardiographic or nuclear magnetic resonance studies. Aim of the present study was to analyse different indexes of diastolic and systolic function in a large cohort of cardiac AL amyloidosis patients with preserved ejection fraction.
Methods: We enrolled 221 consecutive never-treated subjects, in whom a first diagnosis of cardiac AL amyloidosis was concluded between 2006 and 2009, according to the International Society of Amyloidosis criteria. Patients in whom cardiac involvement was excluded served as controls ($n = 121$). All patients underwent a complete echocardiographic evaluation. Systolic function was evaluated as: left ventricular (LV) ejection fraction (EF), longitudinal mitral annulus septum excursion (MAPSE), isovolumic (IVVm) and systolic (Sm) tissue Doppler peak velocity. Diastolic function was characterized in terms of: transmitral Doppler early (E) and atrial (A) velocities, E deceleration time, pulmonary venous flow velocity, early diastolic tissue Doppler peak velocity (E') and E/E' ratio. Patients with significant valve disease, previous myocardial infarction, atrial fibrillation, or chronic obstructive lung disease were excluded. All patients with ejection fraction below 50% ($n = 28$) were also excluded.
Results: When compared with AL patients without myocardial involvement, cardiac AL was characterized by increased wall thickness ($p < 0.001$) and reduced end-diastolic LV volumes ($p < 0.001$). As expected, diastolic dysfunction was evident in all cardiac AL patients, as evident by increased E/E' ratio ($p < 0.001$). By definition, global systolic function, as assessed by LV ejection fraction, was preserved (i.e. $EF > 50\%$ in the whole cohort). In contrast, both M-Mode and TDI-derived indices of systolic regional function were markedly depressed. When compared with patients without cardiac involvement, in patients with cardiac AL septal MAPSE was 7.3 ± 2.3 vs. 12.4 ± 2.1 mm ($p < 0.01$), lateral MAPSE 9.3 ± 1.9 vs 14.9 ± 2.4 mm ($p < 0.01$), whereas tissue Doppler IVVm was

10.9 ± 2.6 vs. 7.0 ± 2.6 mm/sec ($p < 0.01$), and Sm 11.5 ± 2.9 vs 8.2 ± 2.5 mm/sec ($p < 0.01$). All these parameters of systolic regional function were correlated with the extent of cardiac damage, as expressed by NT-proBNP serum levels.
Conclusions: In cardiac AL amyloidosis, diastolic heart failure is characterised by marked regional systolic dysfunction despite preserved ejection fraction.
This contribution has been awarded as Best Communication.

Functional correlates of NT-PROBNP and free light chain response to chemotherapy in cardiac al amyloidosis

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Introduction and Aim: Primary (AL) amyloidosis is a systemic disease caused by the extracellular deposition of insoluble fibrils derived from amyloidogenic immunogloblin light chains (LC), that are synthesized by clonal plasma cell proliferation in the bone marrow. Although clinical presentation is extremely variable, depending on the organ(s) that are involved by amyloid deposition, cardiac involvement represents the most common cause of death. It has been shown that in patients with cardiac AL amyloidosis a decrease in circulating free light chains (FLCs) higher than 50% is associated with a reduction in N-terminal pro brain natriuretic peptide (NT-proBNP) serum concentration, improvement of symptoms of heart failure and prolonged survival. However, little is known on the cardiac functional correlates of these changes.
Methods: In 32 patients with cardiac AL amyloidosis who responded to chemotherapy echocardiographic indices of systolic and diastolic regional function were compared at diagnosis and after response achievement. Diastolic function was characterized in terms of: transmitral Doppler early (E) and atrial (A) velocities, E deceleration time, pulmonary venous flow velocity, early Tissue Doppler (TDI) peak velocity (E') and E/E' ratio. Systolic function was evaluated as: left ventricular (LV) ejection fraction (EF), longitudinal excursion of the mitral annulus at the septum (MAPSE) and lateral wall (LAPSE). FLCs and NT-proBNP were concomitantly measured. Patients with significant valve disease, previous myocardial infarction, atrial fibrillation, or chronic obstructive lung disease were excluded.
Results: No significant change in left ventricular wall thickness, end-diastolic and end-systolic chamber dimension, indices of diastolic dysfunction or ejection fraction was observed after chemotherapy. In contrast, longitudinal excursion of both the interventricular septum and the lateral wall was increased (from 5.2 ± 1.4 to 6.8 ± 1.5 mm, and from 6.2 ± 1.3 to 7.7 ± 1.4 mm, respectively; $p < 0.05$ for both). Changes in systolic longitudinal wall motion were significantly correlated with the extent of both FLCs ($p = 0.05$) and NT-proBNP ($p = 0.05$) reductions.
Conclusions: In cardiac AL amyloidosis, hematological response to chemotherapy and reduction of cardiac biomarkers are associated with improved indices of regional systolic function.

Cerebellar damage in an experimental model of obstructive sleep apnea syndrome

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Background: The cerebellum is among the brain areas most vulnerable to hypoxia and several clinical studies have demonstrated that, in subjects affected by obstructive sleep apnea syndrome (OSAS), transient, repeated episodes of intermittent hypoxia (IH) induce alterations of grey matter, fiber integrity, and functional response in the cerebellum. OSAS is a breathing disease that affects millions of individuals and represents a challenge for our health system. However, surprisingly few experimental studies have investigated the effects of IH on the cerebellum. The aim of this study was to characterize the damage induced by IH on cerebellar tissue, both at the cellular and electrophysiological level.
Methods and Results: Using an established experimental in vivo model of OSAS, we have generated novel data demonstrating that IH induces significant morphological and electrophysiological changes in Purkinje cells (PCs), which are the major output neurons in the cerebellum and are crucial to the integration of sensory-motor coordination. Indeed, upon exposure to IH, PCs display a significant decrease in membrane potential and a significant increase in dendritic branching and spine density. We have also performed preliminary experiments to study the molecular effects of OSAS in the cerebellum and found significant changes in the local expression levels of genes involved in cell apoptosis, PC proliferation and differentiation, neuron regeneration, control of cell cycle, and response to oxidative stress. Most importantly, we have found that the developmental Sonic hedgehog (Shh) signaling pathway is strongly upregulated in the adult cerebellum in response to IH.
Conclusions: This study provides the first comprehensive characterization of the molecular, morphological, and electrophysiological damages induced by OSAS in the adult cerebellum. It also demonstrates for the first time that hypoxia upregulates Shh expression in the adult cerebellum. Based on the fact IH is the central key-point in the pathogenesis of OSAS-induced organ damage and that Shh, in addition to be important during cerebellar development, is also a survival factor for neurons in the adult brain and a regulator of neural stem cell maintenance and proliferation in response to ischemic/hypoxic injury, our study indicates that the Shh pathway is functionally important in the adult hypoxic cerebellum. These data may have important biological, clinical, and therapeutic implications not only for OSAS, but also for all those diseases in which reduced oxygenation of the cerebellum plays a pathogenic role.

Prognostic value of fragmented QRS in cardiac al amyloidosis: a further advantage of 12-lead ECG analysis

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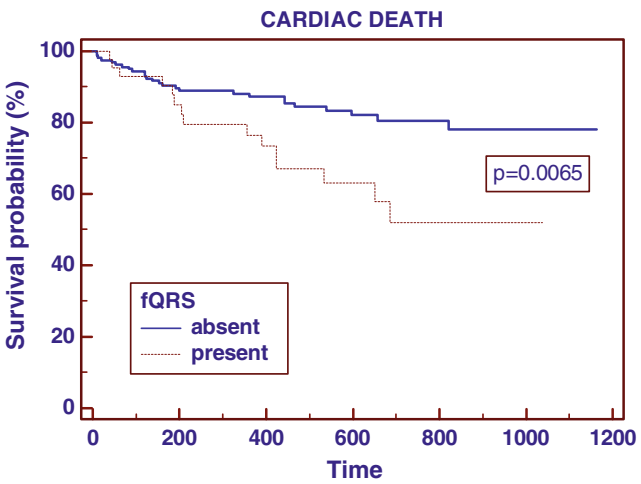
Introduction and Aim: The amyloidoses constitute a large group of diseases in which aggregates of insoluble toxic protein are deposited in forms of fibrils in several tissues. AL amyloidosis, in which fibrils are composed mainly by the N-terminus of a monoclonal immunoglobulin light-chain, has an incidence of approximately 1 case per 100000 person–years in western countries. Cardiac involvement is not only frequent, but it is also the most common cause of death. In cardiac AL, the 12-leads electrocardiogram (EKG) reflects the generalized infiltrative nature of this disease with low voltages in the limb leads, pseudoinfarction patterns in the anterior precordial and/or in the inferior limb leads, and conduction abnormalities such as fascicular

block or varying degree atrioventricular block. Moreover, it is not unusual to see "aspecific" abnormalities of the QRS complexes, such as notches and RsR' pattern in the absence of QRS prolongation. In the setting of ischemic heart disease and dilated cardiomyopathy, these alterations in QRS morphology, leading to a terminal conduction delay or a fragmentation of QRS complexes (fQRS), have been associated with regional myocardial scars. Since cardiomyocyte damage and interstitial fibrosis are associated with cardiac amyloid deposition, aim of the present study was to analyze the prevalence of fQRS in patients with cardiac amyloidosis, and to assess whether this finding has a prognostic value in predicting advanced heart failure and sudden cardiac death.

Methods: We enrolled all consecutive never-treated subjects undergoing extensive multiteam evaluation at the Amyloidosis Research and Treatment Centers of Pavia and Florence, in whom the diagnosis of primary AL amyloidosis was concluded between 2008 and 2010. Diagnosis was made according to the International Society of Amyloidosis criteria. The study population included 456 consecutive patients (Pavia: n = 386; Florence = 70), in whom 12-leads EKGs and cardiac echo-color Doppler data were collected at diagnosis. To avoid any possible interference of ischemic heart disease on the presence of fQRS, patients with a positive history of coronary disease were excluded from the analysis. The cohort was divided into two groups according to the presence (n = 307) or absence (n = 149) of heart involvement, as defined by echo-derived left ventricular wall thickness > 12 mm, and increased NT-proBNP levels. Prognosis was evaluated during a median follow up of 477 days.

Results: As expected, when compared with patients without cardiac amyloidosis the presence of cardiac involvement was associated with a different electrocardiographic pattern, with a 63.9% proportion of low voltages, a 38.5% of left ventricle strain pattern, and an 52.2% proportion of pseudonecrosis. Moreover, cardiac involvement was associated with prolonged PQ, QRS and QT intervals. fQRS were observed in 23.5% of patients, 28.5% with and 11.7% without cardiac involvement. The prevalence of fQRS was significantly higher in patients with cardiac AL amyloidosis ($p = 0.0008$). After a median follow-up of 477 days, Kaplan–Meier survival analysis revealed a significantly higher mortality in the fQRS group when compared with the "normal" QRS group, both in the general AL population ($p = 0.0065$) and in the group with cardiac involvement ($p = 0.0189$). No association was found between the presence of fQRS and either the duration of PQ, QRS, and QTc intervals, or the presence of peripheral low voltages, pseudonecrosis or a strain pattern. Moreover, no association was found between the presence of fQRS and NT-proBNP or cardiac wall thickness.



Conclusion: Analysis of a simple 12-lead ECG may unveil several aspects that are altered in cardiac amyloidosis. Among them, the presence of fQRS has an independent prognostic values both in the general population of AL patients and in cardiac amyloidosis. Integration of such a simple and cheap analysis in the diagnostic work-up may improve diagnosis and prognostic stratification not only in patients with AL amyloidosis, but also in the population at risk of developing amyloidosis, such as monoclonal gammapathy and multiple myeloma patients.

Thrombosis and Hemostasis III

Evaluation of the prevalence of severe hyperhomocysteinemia in adult patients with thrombosis who underwent screening for thrombophilia

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Background: The association of hyperhomocysteinemia with thrombosis is well established. The risk of thrombosis in patients with severe hyperhomocysteinemia due to rare congenital abnormalities of homocysteine (Hcy) metabolism (homocystinuria), is dramatically decreased by B-vitamins (± betaine). In contrast, there is no demonstration that B-vitamins reduce the risk of thrombosis in patients with mild hyperhomocysteinemia: for this reason the clinical utility of measuring plasma total Hcy (tHcy) in patients with thrombosis is uncertain. However, since homocystinuria may not be easily recognized, measurement of tHcy in adult patients with thrombosis may unravel the presence of undiagnosed homocystinuria. *Aim.* The primary objective of this study was to gather information on the prevalence of severe, previously undiagnosed, hyperhomocysteinemia among patients with thrombosis who underwent thrombophilia screening. Secondary objective was to profile these patients on the basis of their clinical characteristics. *Methods.* Six Italian Thrombosis Centers completed an online questionnaire, reporting the plasma tHcy levels measured in patients with thrombosis who underwent thrombophilia screening. A second, more detailed questionnaire reporting the characteristics of patients with severe hyperhomocysteinemia (tHcy > 100 μM) was also completed. *Results.* Among 19,574 patients who underwent thrombophilia screening for previous arterial or venous thrombosis in the last 12.5 years (median value; range 6-17 in different Centers), 34 had severe hyperhomocysteinemia (0.17%). In the same cohort of patients, the frequency of antithrombin deficiency, a severe thrombophilic defect that is commonly included in thrombophilia screening, was 0.6%. Among patients with severe hyperhomocysteinemia, 1) the median age at diagnosis was 49.5 years (range 19-83); 2) the median plasma level of tHcy was 130 μM (range 101-262); 3) venous thromboembolism (62%) was more frequent than arterial thromboembolism (35%); 4) concomitant thrombophilic defects were found in 17%; 5) recurrent thrombotic events were observed in 29%, 6) mild to severe renal insufficiency was present in 21%. *Conclusions.* Measurement of plasma tHcy in adult patients with thrombosis may unravel the presence of severe hyperhomocysteinemia that had not previously been diagnosed. Since treatment of patients with severe hyperhomocysteinemia may decrease the risk of thrombosis, measurement of tHcy in adult patients with thrombosis may prove clinically useful.

Platelet glycoprotein receptor polymorphisms in infective endocarditis: prevalence and pathophysiological significance

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Background: The pathogenesis of infective endocarditis (IE) comprises a two-step process: the initial formation of a non-bacterial clot is followed by bacterial seeding on the clot during bacteremia. Despite high rates of cardiac abnormalities as well as spontaneous bacteremia in the general population, only a limited number of patients develop IE during their lifetime. Inherited polymorphisms in platelet receptors affecting their function are common causes of thrombosis and may be over-represented in heart disease patients. However, no study has evaluated the potential role of inherited platelet receptor polymorphisms in the pathogenesis of IE.
Objective: To assess the possible role of human platelet alloantigens (HPA) associated with platelet glycoprotein (GP) receptor polymorphisms in patients with infective endocarditis (IE).
Methods: We studied 110 Caucasian patients admitted to our centre because of definite IE. As controls, we studied 125 Caucasian blood donors from the same geographical area. All patients underwent blood cultures and echocardiography (> 90% transesophageal). Genomic DNA was extracted with a spin-column method and subjected to a multiplex allele-specific PCR analysis to seek for HPA-1, -2, -3, -4 and -5 polymorphisms in platelet GPIIIa, GPIb-α, GPIIb and GPIa.
Results: Patient median age was 61 years (17-82), 76% were males. 77 (70%) had left- and 33 (30%) right-sided IE. 98% of patients showed intracardiac vegetations with a median length of 14 mm. Blood cultures grew gram-positive cocci in most cases. The allelic frequencies of platelet GP polymorphisms in IE patients and controls were as follows: HPA-1b 21% vs 13% ($p = 0.143$); HPA-2b 25% vs 10% ($p = 0.004$); HPA-3b 8.4% vs 34% ($p = 0.0001$); HPA-4b 12% vs 0.25% ($p < 0.0001$); HPA-5b 11% vs 8% ($p = 0.574$). IE patients showed a significantly higher prevalence of HPA2b of GPIbα, that is associated with atherothrombotic events, and a much lower prevalence of HPA-3b of GPIIb. A striking increase in GPIIIa HPA-4b allele prevalence was also observed.
Conclusions: Platelets actively bind IE causative microorganisms within the endocardial vegetation. This may contribute to both initiation and growth of IE vegetations. Our preliminary data suggest that HPA-3 and HPA-2/4 genetic polymorphisms could confer protection or susceptibility, respectively, towards the development of IE.

Rosuvastatin reduces platelet recruitment by inhibiting NADPH oxidase activation

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Background: The pleiotropic effects of 3-hydroxy-3-methylglutaryl–coenzyme A (HMG-CoA) reductase inhibitors include reduction of reactive oxygen species (ROS), and improvement of NO bioavailability. Although rosuvastatin increased vascular endothelial NO production and attenuated myocardial necrosis and platelet activation after ischemia–reperfusion in mice, the influence of rosuvastatin on the activation of human platelets and the underlying mechanism is still unclear. Platelet activation, and thrombus propagation is dependent upon NADPH oxidase activation. Thus, NADPH oxidase is involved in the production of pro-aggregating molecules like ROS and isoprostanes. Moreover NADPH oxidase reduces NO bioactivity and is associated with arterial thrombosis in animal models. We investigated whether the HMG-CoA reductase inhibitor rosuvastatin would beneficially modulate platelet NADPH oxidase activity and in turn platelet activation in an experimental in vitro model of thrombus formation.
Methods and Results: Platelets were obtained from 8 healthy donors (males 4, females 4, age 30-40 years) who had provided their informed consent. Samples were immediately mixed with sodium citrate (ratio 9:1) and processed within 2 h of sampling. To obtain platelet-rich plasma samples were centrifuged at 160 g for 15 min. Samples were incubated with scalar doses of rosuvastatin (1-10 μM) (30 min at 37°C under stirring condition) before activation and platelet recruitment (PR), that mimics the propagation of platelet aggregation and is dependent upon isoprostane formation, was investigated. PR was inhibited by rosuvastatin in dose-dependent manner coincidentally with down-regulation of platelet activation of NOX2, the catalytic sub-unit of NADPH oxidase. This effect was also associated with lower production of platelet ROS, isoprostane and activation of the glycoprotein IIb/IIIa; conversely rosuvastatin dose-dependently increased platelet NO generation. Finally, platelet incubation with rosuvastatin resulted in a dose-dependent inhibition of PKC, an enzyme which has a pivotal role in NADPH oxidase activation.
Conclusions: This study shows that rosuvastatin impairs platelet recruitment via inhibition of PKC-dependent NADPH oxidase activation. This effect, that was dependent on both reduced isoprostane formation and enhanced NO generation, may represent a novel mechanism accounting for the anti-atherosclerotic property of rosuvastatin.
This contribution has been awarded as Best Communication.

Atorvastatin acutely inhibits platelet activation by lowering both isoprostane and thromboxane A2 formation

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Background: Statins have been shown to reduce platelet activation as early as after 48-72 h from administration. It is still unclear if this antiplatelet action can be detected even earlier and if it is independent from the inhibition of cholesterol biosynthesis.
Methods: Patients with hypercholesterolemia were randomly allocated to a Mediterranean diet with low cholesterol intake(< 300 mg/day; $n = 15$) or atorvastatin (40 mg/day; $n = 15$). Platelet activation, as assessed by platelet recruitment (PR), platelet formation of reactive oxidant species (ROS), isoprostanes and thromboxane (Tx) A2, and platelet activation of NOX2, the catalytic sub-units of NADPH oxidase, as assessed by soluble NOX2-derived peptide(sNOX2-dp), were determined at baseline and after 2 h,24 h and 7 days from statin administration. In vitro study was also performed to see if atorvastatin affected platelet activation.

Results: Compared to patients following the Mediterranean diet, those on atorvastatin showed a significant reduction of PR (−17%, $p < 0.001$), platelet isoprostanes (−22%, $p < 0.001$) and TxA2 (−9%, $p = 0.03$), platelet ROS(−26%, $p < 0.001$) and platelet sNOX2-dp(−20%, $p < 0.001$) 2 h after atorvastatin administration. Such reductions were even higher 24 h after atorvastatin administration with no further decrease after 7 days with the exception of platelet TxA2 that continued to decline. Serum cholesterol did not change 2 and 24 h (-1%, and -2% respectively, p = NS) after atorvastatin administration but was significantly reduced after 7 days ($p < 0.001$). Multivariate analysis showed that the reduction of the above reported variables were independent from the changes of LDL cholesterol at any time. In vitro experiments demonstrated that atorvastatin dose-dependently reduced PR, platelet isoprostanes and TxA2, platelet ROS and sNOX2-dp. Also, atorvastatin and an inhibitor of NOX2 significantly reduced platelet PLA2 activation.
Conclusions: The study provides the first evidence that atorvastatin acutely decreases platelet activation by inhibiting platelet ROS generated via NOX2 activation. Low generation of ROS results in down-regulation of platelet isoprostanes and reduced activation of PLA2 with ensuing lowered production of TxA2. Such effects are independent from LDL-cholesterol lowering; however, the progressive decrease of platelet TxA2 suggests that LDL-cholesterol lowering is also implicated in the inhibition of arachidonic acid metabolism by atorvastatin.

Gastroenterology and Hepatology III

Abnormal expression of thymic stromal lymphopoietin in active coeliac mucosa

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Background & Aims: Intestinal immune homeostasis is regulated by the crosstalk between dendritic cells and epithelial cells. The latter constitutively express thymic stromal lymphopoietin (TSLP), which is able to condition dendritic cells towards a tolerogenic phenotype. Defective TSLP production secondary to chronic inflammation is responsible for depletion of tolerogenic dendritic cells in Crohn's disease mucosa. Though mucosal dendritic cells have been shown to be elevated in active coeliac mucosa and display an activated and mature phenotype, no information is available on TSLP production in this condition. Therefore, aim of our study was to measure TSLP expression in coeliac mucosa and to verify in ex vivo organ culture experiments the effect of gluten challenge on its production.
Patients & Methods: Perendoscopic duodenal biopsies were collected from 8 untreated coeliac patients, 8 coeliac patients on a gluten-free diet for at least 12 months, and 8 biopsied controls. Some biopsies were snap-frozen, other were put in organ culture and incubated in the absence or presence of the peptic-tryptic digest of gliadin (PT-gliadin) for 24 h. In order to exclude the presumable effect of lipopolysaccharide contained in the PT-gliadin solution, each experiment was also performed using boiled PT-gliadin. TSLP was detected on tissue omogenates by immunoprecipitation. Briefly, 5 μg antibody/mg total proteins were incubated overnight with protein G bound to Sepharose beads. After several washes with lysis buffer, this complex was added to the tissue omogenates, and samples were incubated overnight at 4°C with gentle rotation. After several washes, sample buffer was added and samples were boiled for 5 min. Then, immunoprecipitated proteins were separated by SDS-PAGE and transferred onto nitrocellulose filters. For immunoblots, filters were incubated for 2 h at 25°C in TBS containing 5% of milk. Filters were incubated for 1 h at 25°C with 0.3 μg/ml anti-TSLP antibody. After three washes with TBS Tween, filters were incubated with secondary antibody for 30 min at 25°C. TSLP was then immunodetected by enhanced chemiluminescence. Concentrations of IFN-γ in organ culture supernatants were measured using the Human IFN-γ ELISA (R&D Systems), according to the manufacturer's instructions.
Results: We found that in vivo mucosal TSLP expression is significantly ($p < 0.05$) reduced in the duodenum of untreated coeliac patients in comparison to treated coeliac patients and controls, without any difference between treated coeliac patients and controls. After 24 h-incubation with PT-gliadin, duodenal biopsies from treated coeliac patients produced significantly ($p < 0.01$) higher amounts of TSLP in comparison to those cultured with medium only. No difference was found between boiled and not-boiled PT-gliadin. PT-gliadin challenge was effective in inducing a significant ($p < 0.001$) up-regulation of IFN-γ production (from mean 32.8 ± 9.7 to 121.2 ± 20.4 pg/ml), thus supporting the functionality of our ex vivo system.
Conclusions: Our results point on defective mucosal TSLP expression in active coeliac disease as an additional factor contributing to the pathogenesis of this condition. The defect of TSLP production could be secondary to the enterocyte damage underlying villous atrophy. The positive ex vivo effect of gliadin in inducing TSLP production in treated coeliac mucosa might be interpreted as an attempt of the innate immune system to tolerate gluten by polarizing dendritic cells. A better clarification of the innate immune mechanisms modulating the mucosal response to oral antigens might be useful in identifying possible approaches alternative to gluten-free diet in coeliac patients.

Interleukin-17A but not interleukin-17E is profibrotic in the gut of patients with Crohn's disease

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Background and Aims: While interleukin (IL)-17A is pro-inflammatory, IL-17E (also known as IL-25) has been shown to exert an anti-inflammatory action in Crohn's disease (CD). There are no studies investigating the role of IL-17E in modulating extracellular matrix in the gut, while it is known that IL-17A increases matrix metalloproteinase (MMP) production by intestinal myofibroblasts. Here we have studied the role of both IL-17A and IL-17E in CD fibrogenesis.
Materials and Methods: Myofibroblasts were isolated from colonic surgical specimens collected from strictured and non-strictured areas of 10 patients with fibrostenotic CD (mean age 44, range 21-63), and normal areas of 10 subjects undergoing colectomy for colon cancer (mean age 52, range 37-70). The expression of IL-17R, which is common to both IL-17A and IL-17E, was determined on myofibroblast lysates by immunoblotting. Myofibroblasts were cultured for 24 h with recombinant human IL-17A and IL-17E. Then supernatants were used for detection of soluble collagen by Sircoll Collagen Assay and tissue inhibitor of MMPs (TIMP-1) by immunoblotting. Myofibroblast migration was assessed using the in vitro wound-healing scratch assay.

Results: IL-17R was expressed on CD strictured, CD non-strictured and control fibroblasts, with no significant differences between the three cell populations. IL-17A but not IL-17E significantly increased collagen production and TIMP-1 expression, mainly in the supernatants of CD strictured myofibroblasts. Migration of CD strictured, CD non-strictured and control myofibroblasts was significantly inhibited by IL-17A but not IL-17E, with no significant differences between the three cell populations.
Conclusions: Our results suggest that IL-17A but not IL-17E is profibrotic in CD. Further studies are needed to clarify the molecular mechanisms underlying this profibrotic action, and the possible role of anti-IL-17A blocking antibodies in counteracting the fibrogenic process in Crohn's disease, in order to prevent the development of intestinal strictures.
This contribution has been awarded as Best Communication.

Molecular and pathological characterization of an experimental model of non-aganglionic congenital megacolon

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Congenital megacolon (CM) is a colonic dysfunction characterized by severe constipation usually associated with an underlying aganglionosis of the enteric nervous system. CM unrelated to aganglionosis may also occur and its pathogenesis is still unknown. Experimental models of non-aganglionic CM, which may improve our understanding of such condition in humans, are rare and usually developed in rodents.
Aim: To characterize a new, non-rodent natural model of non-aganglionic CM. The CM phenotype is related to an incomplete dominant allele at the *English spotting* locus (*En*) and appears only in homozygous *En/En* animals.
Methods: An F1 population of 80 animals was created crossing *En/en* rabbits. *En/En* rabbits (almost completely with a white fur due to the absence of melanocytes in the skin) and littermate controls (*en/en*) (normally coloured) have been monitored since birth up to severe deterioration of *En/En* animals with the CM phenotype. Ascending colon of controls ($n = 4$) and CM ($n = 6$) were processed for quantitative double label immunohistochemistry (using antibodies for structural and neurochemical markers of the ENS: Hu, substance P [SP] and neural nitric oxide synthase [nNOS]) and electron microscopy analysis. DNA was extracted from blood samples collected from all F1 animals and used for candidate gene analysis.
Results: Compared to controls (*en/en*), *En/En* rabbits were subvital showing feeding abnormalities, reduced body weight and a massive colonic distension predominant in the cecum and ascending colon. Genetic tests confirmed the effects and segregation of the *En* alleles in the F1 population. Sequencing and genotyping of identified single nucleotide polymorphisms (SNP) in a few candidate genes showed complete co-segregation of a SNP in the *KIT* gene with the coat colour effects of the *English spotting* locus (LOD = 37.93; $\theta = 0.00$). Quantitative gene expression in colon and cecum specimens showed that the level of *KIT* gene in *En/En* rabbits was only 5-10% vs that of *en/en* rabbits. Morphometric data on whole mounts of the ascending colon showed a decreased number of Hu- and SP-immunoreactive (IR) neurons in *En/En* vs. *en/en* rabbits (950 ± 110 vs 1440 ± 120 and 76 ± 14 vs 160 ± 24, respectively; $P < 0.05$). Although not statistically significant, nNOS-IR neurons were less abundant in *En/En* vs *en/en*. Compared to *en/en*, electron microscopy analysis of *En/En* tissues showed neuronal (rough endoplasmic reticulum with dilated cisternae, a chaotically arranged cytoskeleton and nerve endings with empty synaptic vesicles) and interstitial cells of Cajal (ICC) (few cells with immature or altered features) abnormalities, particularly in the ascending colon.
Conclusions: Combined neuronal and ICC network alterations contribute to this non-aganglionic CM phenotype. *KIT* mutations may account for ICC abnormalities. The present findings can help understanding neuro-muscular changes occurring in human non-aganglionic CM.

High prevalence of atherosclerosis among HCV infected patients: HCV and steatosis play a pathogenic role

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Background/Aims: Hepatitis C virus (HCV) infection is endemic worldwide, with an estimated global prevalence of 3%, resulting in more than 170 million HCV-infected persons. Chronic HCV infection is often associated with severe hepatic and extra-hepatic diseases. Recently, HCV antibodies have been reported to be associated with atherosclerosis in studies on general population. These data suggest a possible role of HCV in the development of atherosclerosis aggravating the health problem associated with HCV infection. However, the prevalence of atherosclerosis in HCV infection as well as associated host and viral factors are not known. We hypothesised that HCV per se and HCV-related steatosis that is associated with pro-atherogenic factors play a role in development of atherosclerosis. Thus, in a cohort of chronic hepatitis C we assess: a) the prevalence of atherosclerosis b) the role of HCV, liver histological damage and, cardio-metabolic risk factors and, c) the occurrence of clinical cardiovascular events.
Patients/Methods: Overall, 758 subjects were enrolled, of which 326 patients with liver biopsy-proven chronic hepatitis C (CHC) and 432 matched subjects as control group. Subjects included in the control group were matched with HCV infected patients with respect of age, sex and associated conditions and including: health, 246; type 2 diabetes, 31; hypertension, 53 and 102 with a well characterized group of NAFLD patients. Among HCV patients 20.4% had cirrhosis, 53.5% had steatosis. Carotid atherosclerosis (CA) was assessed by high-resolution B-mode ultrasonography evaluating intima-media thickness (IMT: > 1 mm) and plaque (≥ 1.5 mm). Patients were evaluated for: serum HCV RNA levels, HCV genotype, BMI, visceral obesity, smoking, glucose and lipid metabolisms, Homa-IR, metabolic syndrome, homocysteinemia, hypertension, inflammatory markers. All cardio-vascular events were recorded. Data were analysed by univariate and multivariate analysis utilizing a model of logistic regression analysis.

Results: Overall, HCV patients showed a prevalence of CA of 53.7%, IMT, 25.8% and plaques 27.9%. The prevalence of atherosclerosis in HCV patients was significant higher than control (34.3%). Younger CHC group (< 50 yrs) had higher prevalence of CA than control (34% vs 16.0%), plaques were present in 25% of younger HCV patients and in 3% of control subjects. CHC patients without steatosis had higher prevalence of CA than control (26,0% vs 14.8%), similarly those with steatosis had higher CA (77.7%) than NAFLD (57.8%). HCV RNA levels were associated with serum CRP and fibrinogen levels. Atherosclerosis, at univariate analysis was associated with Age, BMI, HCV RNA, steatosis, smoking, hypertension, serum CRP and fibrinogen. At multivariate analysis, independent factors associated with atherosclerosis in HCV patients were: Steatosis, HCV RNA levels, smoking, hypertension, CRP and fibrinogen. Patients with diabetes and metabolic syndrome were at risk of advanced CA (plaques). Cardio-vascular events occurred in 11% of patients with higher prevalence (77%) in those with CA, such events were associated with HCV RNA levels and steatosis. The ROC curve showed that atherosclerosis is predictable in about 80% of steatosis cases, with a positive specificity of 81.7% and sensitivity of 74.2%.
Conclusions: Chronic HCV infection is associated with an earlier and higher prevalence of CA than that observed in the general population. HCV infection plays a pathogenic role likely modulating inflammation. HCV-related steatosis plays a role in the development of atherosclerosis by modulating a milieu of cytokines and metabolic pro-atherogenic factors. Steatosis is a marker to identify HCV patients at high risk of atherosclerosis. Metabolic alterations favour the development of advanced atherosclerosis. Effective treatment for HCV infection, steatosis and metabolic factors (metabolic syndrome, diabetes) could improve the liver disease and to have an impact on the development and progression of atherosclerosis.

Thrombosis and Hemostasis IV

Variability in the recovery rate of platelet cyclooxygenase activity during chronic therapy with low-dose aspirin in type 2 diabetes

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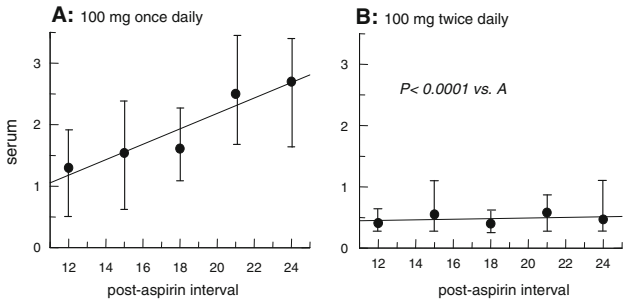
Aspirin is currently recommended for cardiovascular prevention in type 2 diabetes (T2DM). However, two primary prevention trials failed to substantiate its efficacy in T2DM and incomplete platelet inhibition has been reported. We hypothesized that faster recovery of platelet cyclooxygenase (COX)-1 activity may explain incomplete thromboxane (TX) A_2 inhibition by low-dose aspirin during the 24-hour dosing interval. One-hundred T2DM patients on chronic therapy with aspirin 100 mg daily were studied. Serum TXB_2, an index of platelet COX-1 activity, was measured every 3 h, between 12 and 24 h after a witnessed aspirin administration, to characterize the kinetics of platelet COX-1 recovery (phase 1). Thirty-three patients with the steepest COX-1 recovery slopes were randomized to receive aspirin 100 mg once daily, 200 mg once daily or 100 mg twice daily for 28 days (phase 2). On day 29, COX-1 recovery was reassessed over the 12 to 24 h dosing interval. COX-1 activity displayed linear kinetics with large interindividual variability in recovery slope, ranging from 0.001 to 0.46 ng/ml/hr. Multiple linear regression analysis showed that mean platelet volume (Beta = 0.42, SEM = 0.005, $p < 0.0001$), higher body-mass index quartiles ($\beta = 0.26$, SEM = 0.022, $p = 0.007$), and age (Beta = –0.18, SEM = 0.002, $p = 0.049$) predicted serum TXB_2 recovery slope, independently of other variables. A twice daily aspirin regimen, but not a doubling of the dose, completely corrected the abnormal recovery slope of platelet COX-1 activity: absolute values were 0.11 (0.1–0.14), 0.06 (0.05–0.08), 0.01 (0.002–0.02) ng/ml/hr (medians, IQR), for 100 mg once daily, 200 mg once daily and 100 mg twice daily, respectively; the median (IQR) changes versus phase 1 slopes were –33.3 (0–50.5, $p = 0.08$), –55.5 (40–60.9, $p = 0.02$) and –87.5 (69.1–99.7, $p < 0.0001$) %, respectively. We conclude that interindividual variability in the recovery rate of platelet COX-1 activity during the aspirin dosing interval most likely reflects abnormal megakaryopoiesis associated with T2DM; inadequate thromboxane inhibition can be overcome by a twice daily regimen; the clinical efficacy and safety of a tailored regimen of aspirin therapy remain to be tested.



Thyroid disease, antithyroid or thyreomimetic agents, and the risk of pulmonary embolism

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Background: Thyroid hormone is known to affect the coagulation and fibrinolytic systems, but the effect of both hyperthyroidism and hypothyroidism on the occurrence of venous thromboembolism remains to be elucidated. We therefore aimed to evaluate the risk of pulmonary embolism (PE) associated with thyroid disease, while at the same time analyzing the influence of treatment.
Methods: A nested case–control study was conducted using the PHARMO Record Linkage System, a Dutch population-based registry that links medication histories to hospital admission records. Cases were patients hospitalized for PE and the date of hospitalization was set as index date. Controls were sex- and age-matched subjects without a history of PE prior to this index date. New use of antithyroid or thyreomimetic agents, and thyroid-related hospitalizations, were used as indicators for diagnosis of thyroid disease.
Results: The study population consisted of 3479 cases and 11830 controls. Diagnosis of hypothyroidism prior to the index date, i.e. treated hypothyroidism, was significantly associated with PE (OR 2.05, 95% CI 1.11-3.78), especially within the first three months after diagnosis (OR 4.98; 95% CI 1.39-17.82). No association was found for diagnosis of hypothyroidism after the index date, i.e. untreated hypothyroidism. Also, no clear association was found for hyperthyroidism, but odds ratios were highest for diagnosis within three months after the index date (OR 3.46; 95% CI 0.57-21.10).

Conclusions: Our findings suggest that patients with hypothyroidism are at increased risk of pulmonary embolism. This most likely relates to treatment with thyreomimetic agents rather than hypothyroidism itself.

Congenital and acquired risk factors for cerebral venous thrombosis

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Background: The role of thrombophilia in determining venous thromboembolism is well established, even in cerebral vein thrombosis (CVT). CVT is more common in women (3:1 ratio), oral contraceptive and pregnancy/puerperium being important risk factors. Patients: We screened for G20210A prothrombin gene and G1691A Factor V (FV-Leiden) polymorphisms; antiphospholipids, hyperomocysteinemia, protein S, C, and/or antithrombin deficiency, 50 consecutive patients (13 men and 37 women; mean age 34.9 ± 10.88 years) with early-onset-CVT, compared to 159 age-and-sex-matched controls (mean age 35.03 ± 11.48 years), from the same ethnic background.
Results: 11/49 (22.9%) CVT patients showed G20210A FII polymorphism, compared to 3.9% of controls (OR 7.284, 95% CI 2.53-20.98; $p < 0.0001$). The FV Leiden frequency was 4.1% in patients and 12.7% in controls ($p = NS$). No risk of recurrence is related to genetic polymorphisms. None of patients was affected by natural anticoagulants deficiency. The 19.5% of cases and 18.7% of controls was affected by hyperomocysteinemia ($p = NS$). As lupus anticoagulant and antiphospholipids antibodies, not significant difference among cases and controls were observed. Common cardiovascular risk factors, such as cigarette smoking, obesity (BMI $\geq$ 30), hypercholesterolemia and arterial hypertension were not significantly associated to CVT. Seven cases out of 48 (14.6%) had cancer. Seven cases out of 49 (14.3%) had infectious diseases. Interestingly, 20/37 female developed CVT while assuming oral contraceptives (54.1%) versus 17/121 (14%) in controls ($p < 0.0001$).
Four out of 36 cases developed the event during pregnancy/puerperium (11.1%).
Conclusion: Despite the limitations of the sample size, these data confirm the role of the FII G20210A mutation and underline the importance of acquired factors like oral contraceptives. Screening for selected inherited and acquired thrombophilic risk factors might be justified to identify highest risk for early onset CVT.

Endogenous secretory rage in obese women: association with oxidative stress and platelet activation

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Background: The ligand-RAGE axis has been implicated in obesity-related metabolic disease and accelerated atherothrombosis. The splice isoform endogenous secretory (es)RAGE has been shown to act as a protective decoy receptor. In the present study, we tested the hypothesis that low esRAGE levels are associated with increased platelet activation in obese women. Furthermore, we examined the association of esRAGE with adiponectin plasma levels, and lipid peroxidation, in order to test in vivo the association between adipose tissue dysfunction, oxidative stress and RAGE hyperactivation.
Materials and Methods: Eighty otherwise healthy obese women (mean age 43 ± 11, body mass index [BMI] 37 ± 6 kg/m^2) were recruited as outpatients of the Obesity Center of University of Chieti. As control group, 20 non-obese women (mean age 43 ± 12 years, BMI 23 ± 3 kg/m^2) were also studied. A cross-sectional comparison of urinary 8-iso-prostaglandin (PG)$F_{2\alpha}$ and 11-dehydro-TXB_2, as in vivo indexes of lipid peroxidation and thromboxane (TX) biosynthesis, respectively, was performed between obese women and control subjects. Plasma esRAGE and adiponectin levels were also evaluated, as potential contributors to TX biosynthesis in this setting.
Results: Obese women had significantly higher urinary 11-dehydro-TXB_2 [795 (567-1089) vs 233 (158-327) pg/mg creatinine, $P < 0.0001$] and 8-iso-$PGF_{2\alpha}$ [544 (401-699) vs 172 (91-292) pg/mg creatinine, $P < 0.0001$] as compared to non-obese women. Furthermore, both plasma adiponectin [4.9 (3.2-6.6) vs 10.0 (6.8-12.6) μg/mL, $P < 0.001$] and esRAGE [0.18 (0.13-0.26) vs 0.38 (0.20-0.49) ng/mL, $P = 0.001$] were lower in obese than in controls. In obese women, a direct correlation was found between urinary 8-iso-$PGF_{2\alpha}$ and 11-dehydro-TXB_2 (Rho = 0.36; $P = 0.001$) and between plasma adiponectin and esRAGE (Rho = 0.44; $P < 0.0001$). Moreover, plasma esRAGE and urinary 11-dehdro-TXB_2 (Rho = -0.29; $P = 0.008$) were inversely related. On multiple linear regression analysis, urinary 8-iso-$PGF_{2\alpha}$ and plasma esRAGE were independent predictors of 11-dehydro-TXB_2 excretion rate (adjusted $R^2 = 0.37$, $P < 0.0001$).
Discussion: In otherwise healthy obese women, low plasma esRAGE levels are associated with adipose tissue dysfunction and enhanced TX biosynthesis that is mediated, at least in part, by increased lipid peroxidation. Thus, our data support the hypothesis that excess adiposity may be implicated in RAGE hyperactivation and TX-dependent platelet activation, thus contributing to obesity-related metabolic and vascular disease.
This contribution has been awarded as Best Communication.

Gastroenterology and Hepatology IV

Nonalcoholic fatty liver disease is associated with low circulating levels of insulin-like growth factor-1

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Background: Nonalcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease. NAFLD is associated with obesity, insulin resistance, metabolic syndrome, and is an independent predictor of incident type 2 diabetes and cardiovascular disease. Among factors that may account for increased cardio-metabolic risk observed in NAFLD, IGF-1 is a plausible candidate because liver is the main site of its production, and IGF-1 levels are decreased in patients with cirrhosis.
Aims: In this study, we determined the relationship of NAFLD with IGF-1 levels, and tested the hypothesis that free fatty acids (FFA)-induced insulin resistance might impair insulin-induced increase in GH receptors expression in human hepatoma cell line.
Methods: The study group consisted of 503 Caucasian subjects participating to the CATAnzaro MEtabolic RIsk factors (CATAMERI) Study, a cross-sectional study assessing cardio-metabolic risk factors in individuals carrying, at least, one risk factor including elevated blood pressure, dyslipidemia, dysglycemia, overweight/obesity, and family history for diabetes or cardiovascular disease. After 12-h

fasting, subjects underwent complete anthropometrical evaluation and a venous blood sample was drawn for laboratory determinations. Liver ultrasound scanning was performed in all participants by the same trained operator who was blind to clinical characteristics of participants. The sonographic features of NAFLD included the presence of a bright hepatic echotexture (compared with the kidneys), deep attenuation, and vascular blurring either singly or in combination to diagnose hepatic steatosis. In "in vitro" study, HuH7 hepatoma cells were exposed for 24 h to palmitate and insulin-induced expression of GHRs was determined by western blotting and real-time PCR.

Results: Of the 503 subjects examined (252 men and 251 women), 308 had NAFLD as assessed by ultrasonography. Significant differences between the two groups were observed with respect to gender with higher prevalence of men among individuals with NAFLD After adjustment for age and gender, individuals with NAFLD had significantly higher BMI, waist circumference, fasting insulin, triglycerides, HOMA index, liver enzymes, and lower HDL cholesterol compared with control subjects. IGF-1 levels were significantly lower in individuals with NAFLD ($P = 0.001$). A stepwise multivariate regression analysis revealed that the variables independently associated with IGF-1 levels were age, BMI, NAFLD, and HOMA accounting for 22.8% of its variation. Exposure of HuH7 human hepatoma cells to palmitate caused a dose-dependent reduction in insulin-induced increase in GH receptor expression.

Conclusions: Our data show that IGF-1 levels are reduced in subjects with NAFLD and that IGF-1 levels are independently related to both NAFLD and hepatic insulin resistance estimated by HOMA. The molecular mechanisms by which NAFLD and hepatic insulin resistance could contribute to modulate circulating IGF-1 levels are undefined. Our in vitro data indicate that hepatic insulin resistance due to FFA overload may contribute to the down-regulation of circulating IGF-1 levels by impairing insulin-induced increase in GH hepatic receptor expression at both protein and mRNA level, resulting in reduced GH-stimulated synthesis of hepatic IGF-1.

Treatment of chronic hepatitis c by pegylated interferon plus ribavirin combination therapy in aged patients: why not?

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Background & Aims: Pegylated interferon (PEG-IFN) plus ribavirin combination therapy has significantly improved the successful rate in virus eradication in patients affected by chronic hepatitis C. However, only few data are available with respect to antiviral effect and safety in aged patients. This study aimed at investigating the efficacy and tolerability of pegylated interferon (Peg-IFN) plus ribavirin therapy in aged patients with chronic hepatitis C (CH-C).

Methods: A total of 473 patients [319 (67.4%) naive, 195 (41,2% female) with CH-C (genotype 1, n = 266; genotype 2, n = 112, genotype 3 = 72, genotype 4 = 23), of whom 68 (14.4%) over 65 years old (y.o.) (mean age 69 ± 2 years), were treated with Peg-IFN (alfa-2a or alfa-2b) plus ribavirin according to international guidelines. These patients were assessed for sustained viral response (SVR) rate and for all known main predictors of SVR in CH-C.

Results: The overall SVR rate resulted similar in both age groups (270/405 (66.6%) in subjects < 65 y.o vs. 41/68 (60.3%) in subjects ≥ 65 y.o, respectively, p = 0.334). No significant difference in therapy discontinuance rate was observed between patients over and under 65 y.o. (4.4% vs. 4.9%, respectively), the most common reason being anemia in both groups. The table resumes the distribution of main known SVR predictors in the two considered groups

	<65 years (n = 405)	≥65 years (n = 68)	p
Genotype (1-4/2-3)	252/153	37/31	0.229
High viral load (cut off 500.000 UI/ml) (yes/no)	154/251	16/52	0.028
PegIFN alfa 2a/PegIFN alfa 2b use	244/161	59/9	<.001
Rapid Viral Response (RVR) (yes/no)*	106/107	28/28	1.000
Early Viral Response (EVR) (yes/no)	308/97	50/18	0.76
Naive (yes/no)	275/130	44/24	0.675
Sex (male/female)	248/157	30/38	0.011
Grading (Ishak score)	4.89 ± 2.13	5.77 ± 1.88	0.022
Staging (Ishak score)	2.08 ± 1.49	2.73 ± 1.51	0.029
Liver cirrhosis (yes/no)	42/363	13/55	0.043
Therapy reduction (yes/no)	123/282	20/48	0.888
Ribavirin reduction (yes/no)	87/318	18/50	0.430
Use of erythropoietic fatctors (yes/no)	43/362	26/42	<.001

* data not available for all patients

For patients over 65 y.o., at multivariate analysis, genotype 2/3 (OR, 2.56,95% CI 1.89-5.65 p = 0.026) and EVR (OR, 45.5,95% CI 26.2-125.3 p < 0.001) were significant predictors of SVR. Factors related to EVR at multivariate analysis were naive status (OR 2.58, 95%CI 1.26-3.69, p = .001), therapy with PEGIFNalfa 2a (OR 3.56, 95% CI 1.68-5.65, p = .014) and ribavirin reduction (OR 0.789, 95% CI 0.568-0.895, p = .015).

Conclusions: Aged patients can be candidates for Peg-IFN plus ribavirin therapy. The appropriate use of erythropoietic factors in these patients may be useful to achieve a significant reduction in the rate of therapy discontinuation due to hematological side-effects. The response-guided therapy may be applied in predicting therapy efficacy in these patients.

This contribution has been awarded as Best Communication.

Rapid postprandial gallbladder refilling and increased turnover of bile prevent cholesterol crystallization in small heterodimer partner (SHP) knockout mice

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The gallbladder is a dynamic organ with complex motor functions under a precise neurohormonal control during fasting and postprandially. Gallbladder emptying and refilling allows the flow of bile into the intestine for micellization and absorption of dietary fat, which are actively regulated by two endocrine pathways CCK and fibroblast growth factor 15 (FGF15). However, it is unclear what the role of

gallbladder refilling in cholelithogenesis is. Our **aim** was to explore whether rapid postprandial gallbladder refilling and increased turnover of bile prevent cholesterol crystallization and gallstones.
Methods: Biliary lipid secretion and gallstone studies were performed in male SHP (−/−) and (+/+) mice (n = 8 per group) before and during feeding a lithogenic diet (1% cholesterol, 0.5% cholic acid and 15% butterfat) for 56 days. Dynamic gallbladder emptying and refilling functions were measured by physical methods. Intestinal bile salt (BS) concentrations were measured biochemically. Gene expression levels were determined by real-time PCR.
Results: Deletion of the SHP gene resulted in significantly increased hepatic BS synthesis, and increased biliary BS outputs by 2 fold in SHP (−/−) mice than (+/+) mice. Intestinal bile acid pool size was significantly greater in SHP (−/−) mice than (+/+) mice. The increased bile acids are most efficacious ligands of FXR and stimulate expression of intestinal FGF15 through the FXR signaling pathways, which are consistent with expanded bile acid pool size in SHP (−/−) mice. At 14 days on the lithogenic diet, fasting gallbladder volume was significantly larger in SHP (+/+) mice than (−/−) mice. Feeding corn oil (i.e., a high-fat meal) induced gallbladder emptying and residual gallbladder volume was significantly larger in SHP (+/+) than that in (−/−) mice. At 30 min after the high-fat meal, gallbladder volume began to increase, indicating that postprandial gallbladder refilling started. This increase began to accelerate after 60 min and cumulative gallbladder refilling volume reached 43 microL at 120 min in SHP (−/−) mice. In contrast, there was minimal postprandial gallbladder refilling volume in SHP (+/+) mice, suggesting sluggish gallbladder kinetics. The mean turnover index was significantly greater in SHP (−/−) mice than (+/+) mice. Whereas gallstones were found in 60% (day 28) and 100% (day 56) of SHP (+/+) mice, no cholesterol crystals or gallstones were ever detected in SHP (−/−) mice.
Conclusions: Rapid fluctuations in gallbladder refilling with increased turnover of bile within the gallbladder provide a strong washout effect that is more crucial in eliminating solid cholesterol crystals than gallbladder emptying alone. Our findings suggest that these two parameters are novel, important factors that need to be assessed when studying gallbladder motor function in humans and its role in the pathogenesis of gallstones.
This contribution has been awarded as Best Communication.

Cardiovascular Disease IV

The electrocardiographic/echocardiographic mass ratio in the diagnosis of cardiac AL amyloidosis

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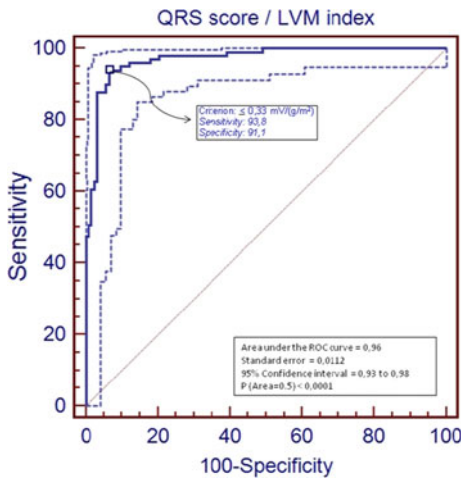
Introduction and Aim: In cardiac AL amyloidosis the increase in wall thickness caused by extracellular amyloid deposition leads to marked increases in left ventricular (LV) mass. At variance with other forms of cardiac hypertrophy, this is often associated with abnormally low electrocardiographic (EKG) voltages, due to the negative effects of amyloid infiltration on intramyocardial electrical conduction. Although almost 1/3 of cardiac AL patients do not strictly fulfil the definition of EKG low voltages (i.e.: < 5 mV in all peripheral leads), such a discrepancy (low EKG "electrical" LV mass/high echo-derived LV mass) might be a powerful clue suggesting the diagnosis of cardiac amyloidosis in the setting of unexplained LV hypertrophy.
Methods: To evaluate the possible relevance of this finding, we compared an index of the EKG/echo mass estimate in patients with cardiac AL amyloidosis (n = 218), non-cardiac AL amyloidosis (n = 69), hypertension with different degrees of LV hypertrophy (n = 125), and in normotensive patients with normal LV mass (n = 99). Echo-derived LV mass was estimated via the Devereux's formula and indexed to body surface area (g/m^2), whereas the peripheral lead QRS score (i.e. the sum of QRS voltages in the conventional peripheral leads, mV) was used as an index of "electrical" LV mass. EKG/Echo ratio was expressed as [$mV/(g/m^2)$]. Care was taken to exclude patients with other possible causes of low EKG voltages, such as large pericardial effusions, obesity, chronic obstructive lung disease, and severe peripheral oedema.
Results: As expected, indexed LV mass was higher in patients with cardiac AL amyloidosis when compared with all the other groups ($p < 0.01$ for all comparisons). The peripheral lead QRS score was lower in cardiac AL patients than in AL patients without cardiac involvement, hypertensive, and normotensive patients ($p < 0.01$ for all comparisons). No difference was observed in the EKG/echo mass ratio when comparing patients with non-cardiac AL with either normotensive or hypertensive subjects, indicating a "normal" relationship between LV mass and EKG voltages [0.51 ± 0.12, 0.54 ± 0.13, and 0.49 ± 0.15 $mV/(g/m^2)$, respectively]. In contrast, the EKG/echo mass ratio was markedly depressed in patients with cardiac AL amyloidosis [0.19 ± 0.10 $mV/(g/m^2)$, $p < 0.001$]. The area under the ROC curve for the detection of cardiac AL involvement was high: 0.96 (95% CI, 0.93 to 0.98). The best cutoff for the diagnosis of heart involvement was 0.33 $mV/(g/m^2)$, giving a 93.8% sensitivity and a 91.1% specificity.
Conclusions: In patients with unexplained LV hypertrophy, an abnormally low EKG/echo mass ratio might be a powerful clue suggesting the diagnosis of cardiac amyloidosis. The recognition of such a discrepancy, based on the side-to-side comparison between easily obtainable EKG and echo data, may be very important for patients with AL amyloidosis. Indeed, a high degree of clinical suspicion may lead to an early diagnosis, allowing potentially lifesaving treatment.
This contribution has been awarded as Best Communication.



Genetic influence in liver steatosis prevalence and pro-atherotrombotic/pro-inflammatory profile in patients affected by familial combined hyperlipoproteinemia

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Non-alcoholic fatty liver disease (NAFLD) has been associated with increased cardiovascular risk and is a common feature in patients affected by several risk factors including the features of the metabolic syndrome. Familial-combined hyperlipoproteinemia (FCHL) has been described as the most common pro-atherogenic dyslipidaemia in various populations and NAFLD is a common feature in such patients.

The aim of the present study was to determine whether the occurrence of NAFLD in FCHL patients was associated with pro-inflammatory and/or pro-atherothrombotic state and a putative genetic influence.

We studied 122 clinically diagnosed FCHL patients (70 male and 52 female, mean age 51.4 ± 3.07 yrs, 62 with NAFLD diagnosed on ultrasonography (NAFLD +) and 60 matched control subjects for comparison of complete lipid profile (enzymatic-colorimetric methods), transaminases (nephelometry), HOMA-index, soluble CD40 ligand (sCD40L) (ELISA), Tumor-necrosis factor-α, Interleukin 6 and 10, adiponectin, leptin, and C-reactive protein (hs-CRP) (all in ELISA). Each patient was genotyped for polymorphisms of some inflammation and oxidative stress mediators using specific primers and polymerase chain reaction (PCR) technique [oxidized-LDL receptor-1 (LOX-1), nitric oxide synthase (NOS) and adiponectin.

NAFLD + subjects had significantly increased sCD40L ($p < 0.001$) with respect to NAFLD- FCHL patients and it was associated with reduced levels of adiponectin and IL-10 ($r = -0.39$ and -0.31 $p < 0.01$, respectively) and increased ox-LDL ($r = 0.32$, $p < 0.05$). No association was found with waist circumference, BMI and HOMA-index while the 45TT and 276GT/TT in the adiponectin gene were confirmed to be associated with NAFLD as previously described in the multivariate analysis. Furthermore the K167 N polymorphism of LOX-1 seems to exert a protective role in the studied population also in the presence of adiponectin polymorphisms (O.R. 2.11 95% C.I. 1.49-3.02 $p < 0.01$ and 1.93 95% C.I. 1.12-2.99 $p < 0.01$, respectively).

Our data indicate that in patients with FCHL the occurrence of NAFLD is related with a pro-atherogenic profile of inflammatory mediators independently of other metabolic features such as insulin resistance. Furthermore polymorphisms of genes involved in protective response to oxidative stress seems to influence the occurrence of NAFLD and an aggressive pro-atherogenic biochemical pattern. Present evidences suggest that NAFLD could be related in FCHL patients to other mechanisms with respect to insuline resistance as frequently occurs in subjects affected by the metabolic syndrome. The presence of different pro-atherothrombotic profile in patients with the same degree of lipid changes in the context of FCHL according to the presence of NAFLD suggest that this condition could be a relevant tool in the definition of subjective pro-atherombotic risk.

This contribution has been awarded as Best Communication.

Functional capacity determinants in patients with non obstructive hypertrophic cardiomyopathy: role of chronotropic response to exercise

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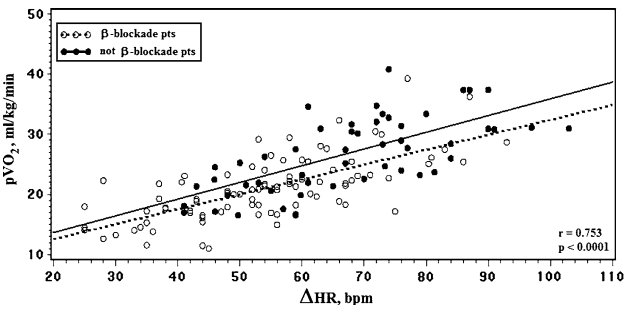
Aims: Patients with hypertrophic cardiomyopathy (HCM) usually complain of fatigue and exertional dyspnea. Previous studies which have attempted to evaluate the underlying mechanisms of exercise impairment were not conclusive, and they usually involved inhomogeneous HCM cohorts. We assessed exercise capacity by means of cardiopulmonary exercise testing in a large cohort of non-obstructive HCM patients.

Methods and Results: Starting from a study sample of 186 HCM patients, a total of 137 patients with non-obstructive HCM were actually enrolled in the current study. Eighty-six (63%) were on treatment with β-blockers. Forty healthy subjects acted as control group. Each subject underwent a maximal cardiopulmonary exercise test. HCM patients showed a peak oxygen consumption (pVO_2) significantly lower than controls, (23.1 ± 6.4 Vs 34.2 ± 10.2 ml/kg/min, $p < 0.0001$) and, within the HCM population, pVO_2 was lower in patients on β-blockers than in those not treated (20.6 ± 5.4 Vs 27.2 ± 6.3 ml/kg/min, $p < 0.0001$). Exercise capacity was not different between the groups treated with high or low β-blocker dose according to an arbitrary cut-off of 50 mg for atenolol or atenolol dose equivalent (20.9 ± 5.4 Vs 20.5 ± 5.3 ml/kg/min, p = Ns). At multivariate analysis with covariance model, the heart rate increase from rest to peak exercise values (ΔHR) was the variable more strongly and independently associated with pVO_2 (t value: 7.51; standard error: 0.024; $p < 0.0001$), whereas β-blocker therapy and dose were not related to exercise capacity.

The figure below shows the relationship, at Pearson univariate analysis, between exercise-induced increase in heart rate (ΔHR) and peak oxygen consumption (pVO2) in the HCM population without (filled circles) and with β-blocker (empty circles).



Multivariate analysis with covariance model confirms that ΔHR was the variable more strongly and independently associated with pVO_2 (t value: 7.51; standard error: 0.024; $p < 0.0001$), whereas β-blocker therapy and dose was not related to exercise capacity.

Conclusions: The chronotropic response is the strongest determinant of exercise capacity in non-obstructive HCM, whereas β-blocker therapy seems to have no influence on pVO_2. Whether chronotropic response to exercise could carry prognostic significance needs, however, further prospective studies.

Inferior vena CAVA ultrasnography and body hydration in ambulatory chronic heart failure

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Background: Inferior vena cava (IVC) diameter is an accepted method to evaluate hydration status in patients on haemodialysis and has been correlated with right atrial pressures in heart failure (HF). Bioelectrical impedance vector analysis (BIVA) is a less laborious method to assess hydration variables and more suitable for routine patient care. We investigated whether BIVA can replace IVC measurements in ambulatory HF patients.
Methods: In 95 ambulatory patients with diagnosis of chronic HF we performed 1) the ultrasonography of IVC to measure diameters and collapsibility (CI), and 2) BIVA to assess hydration status by detecting resistance (R), reactance (Xc) and phase angle (PA). We then sub-grouped study's population in overloaded and euvolemic patients.
Results: showed that many HF patients are hyper-hydrated at BIVA in comparison to euvolemic patients and those controls without HF. Some patients with normal IVC indexes and without clinical symptoms of decompensation may result hyper-hydrated at BIVA. We found a significant correlation ($p < .05$) between Xc and IVC indexes (diameters and CI), in particular at fluid distribution variation (increasing extracellular water), CI decrease (Figure). We also found a significant correlation ($p < .05$) between R and hemoglobin (Hb), in particular at increasing total body water content, Hb decrease.
Conclusion: We conclude that: 1. Hyper-hydration is a frequent condition in ambulatory HF and represents a therapeutic target to explore and compensate in these subjects. 2. BIVA may help to configure a subset of patients with a subclinical fluid overload, candidate to additional medical interventions or strict surveillance 3. Body hydration and IVC indexes are strictly correlated in HF, in particular BIVA and IVC are interchangeable tools to evaluate fluid overload in this setting. 4. Combining both methods may lead to a better estimation of hydration status because both techniques provide complementary information. 5. Hb decline in ambulatory HF may be a transitory condition due to hemodilution. It can return in a near-normal value after resolution of fluid overload.