1° SESSIONE: SPECIALIZZAZIONI AREA SERVIZI CLINICI

DIAMOCI UN TAGLIO

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Riportiamo il caso di un uomo di 48 anni, con anamnesi positiva per storia di tossicodipendenza che, nel tentativo di asportare una neoformazione vascolare dal braccio destro, utilizzando un paio di forbici, è rimasto vittima di una emorragia venosa fatale. La morte causata da gravi lesioni di un vaso venoso periferico è scarsamente documentata nella letteratura scientifica: la maggior parte dei casi noti di lesioni venose riguarda vasi affetti da varici o altre patologie. Nella maggior parte dei casi la sede di riferimento delle lesioni è a livello dei polsi, di contro più rare sono quelle a livello femorale. Analizzando invece la modalità di produzione, i decessi avvenuti per emorragie sono per lo più riconducibili a cause accidentali o a finalità auto soppressive.

Il caso di specie non rientra in questa casistica perché l'emorragia è avvenuta a seguito di un atto su base volontaria ma senza finalità suicidarie: l'esame autoptico ha evidenziato una sezione in due punti della vena antibrachiale. Tale sezione è stata prodotta dal soggetto con lo scopo di asportare un aneurisma venoso, purtroppo però questa manovra maldestra è stata alla base del massivo sanguinamento ad esito fatale. Le successive analisi istologiche eseguite su campioni di organi e tessuti hanno confermato l'acuità della perdita ematica, evidenziando lo stato di anemizzazione poliviscerale: tale evidenza era compatibile con uno shock emorragico. La particolarità del caso descritto quindi è relativa alla sede (piega del gomito, vena periferica dell'avambraccio), alla modalità di produzione (taglio volontario, senza scopo suicidario) e alle caratteristiche della lesione (vaso venoso nell'ambito di una neoformazione cutanea con tessuto fibroso). Il soggetto, con la sua condotta volontaria, e non avendo alcuna nozione scientifica, si è autoprodotto una lesione le cui caratteristiche sono difficilmente riscontrabili in letteratura.

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CONTA E DIFFERENZIAZIONE CELLULARE SU CAMPIONI DI LIQUIDO CEFALORACHIDIANO (LCR) MEDIANTE SISTEMI DI MICROSCOPIA OTTICA AUTOMATIZZATI: POSSIBILE FUTURA APPLICAZIONE NELLA DIAGNOSTICA ROUTINARIA?

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L'esame del liquido cefalorachidiano (LCR) costituisce una vera emergenza per il Laboratorio di analisi chimico-cliniche, in quanto il tipo di campione è altamente instabile a causa della rapida degenerazione della componente cellulare. Nonostante l'esistenza di Linee Guida l'esame del LCR risente ancora di un approccio troppo spesso non standardizzato, che può ripercuotersi sull'esito clinico. L'esame citometrico del LCR, che consiste nella conta e nella differenziazione cellulare, ricopre fondamentale importanza per l'inquadramento diagnostico di patologie a carico del SNC, in quanto un'alterata cellularità è sempre indicativa di un processo patologico in atto. Il metodo in microscopia ottica (MO) con camera di conta è, ad oggi, il metodo di riferimento. I suoi limiti però sono l'elevata imprecisione, la variabilità intra- e inter-operatore, la necessità di personale altamente qualificato e TAT (time around time) elevato. Scopo di questo studio è stato quello di valutare le prestazioni diagnostiche di un sistema di microscopia ottica digitalizzato e automatizzato, al fine di definire nuovi modelli analitici da inserire nella diagnostica di routine.

È stata effettuata l'analisi citometrica di 41 campioni di LCR, pervenuti presso la UOC Laboratorio di Patologia Clinica della AOU Senese, in particolare: 1. è stata eseguita la conta cellulare mediante analizzatore ematologico automatizzato (Sysmex XN-9000, Dasit, Milano, Italia) utilizzando il canale per i fluidi biologici (XN-BF mode) validato per campioni a bassissima cellularità; 2. citocentrifugazione (Cytospin 4, Thermo Scientific, USA) e successiva produzione e colorazione automatizzata di vetrini secondo metodo May Grünwald-Giemsa (Sysmex SP-10, Dasit); 3. analisi microscopica mediante sistema di microscopia ottica digitalizzato e automatizzato (CellaVision DI-60 Body Fluid Software - Sysmex DI-60, Dasit). Su tutti i campioni è stata, inoltre, effettuata contestualmente la conta manuale tramite microscopio ottico (Nikon Eclipse Ci). Per verificare l'efficienza del microscopio automatizzato DI-60 sono stati confrontati i risultati percentuali di Mononucleati (MN) e di Polimorfonucleati (PMN) con quelli ottenuti mediante conta in MO. È stata inoltre valutata la possibile interferenza causata della presenza di globuli rossi (GR) sulla interpretazione dei preparati.

I dati ottenuti hanno mostrato che non esiste una differenza significativa tra i due metodi, sia per la conta dei MN ($t = -0,52$ con $p = 0,61$) che per i PMN ($t = 0,53$ con $p = 0,61$). La presenza di GR genera per entrambi i metodi analitici, uno sfondo rosa/viola che, se in quantità rilevante, comporta una falsata discriminazione cellulare, conseguenza di una visibilità notevolmente ridotta.

L'urgenza diagnostica del LCR pone come obiettivo l'ottimizzazione dei tempi e la standardizzazione dei metodi analitici. I risultati da noi ottenuti hanno dimostrato che l'utilizzo di analizzatori

automatizzati per la conta cellulare e la lettura dell'immagine permettono una riduzione del TAT ed una maggiore efficienza diagnostica, eliminando la soggettività dell'operatore e aumentando la standardizzazione dei dati, consentendo la memorizzazione digitale dell'immagine e l'ingresso automatico nel sistema informatico del laboratorio (LIS). Tali risultati pongono le basi per un possibile inserimento della microscopia digitale automatizzata su campioni di LCR nella pratica routinaria.

VALUTAZIONE DEGLI INTERVALLI DI RIFERIMENTO NELL'OTTICA DEL GENERE

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Dati epidemiologici, clinici e sperimentali mostrano come sia fondamentale un approccio di genere nella pratica clinica per promuovere l'appropriatezza e la personalizzazione delle cure. Nell'ambito della Medicina di laboratorio, questo concetto può essere applicato agli intervalli di riferimento, che sono specifici per i singoli laboratori per la diversità dei metodi e strumentazioni utilizzate. In questo studio abbiamo esaminato, dalla prospettiva della medicina di genere, lo stato dell'arte degli intervalli di riferimento utilizzati presso la UOC Laboratorio Patologia Clinica, Azienda Ospedaliera Universitaria Senese.

Dal Manuale degli analiti presente nel Sistema Qualità della UOC Laboratorio Patologia Clinica, certificata ISO9001:2015, sono stati estrapolati i 344 analiti erogati dalla struttura. Abbiamo selezionato gli analiti richiesti più comunemente al Laboratorio (39 analiti) che sono determinati sul sistema Roche/Hitachi Cobas, analizzatore dedicato ai test di chimica clinica e immunochimica eseguiti su siero, plasma e urine. In particolare, abbiamo analizzato gli intervalli di riferimento riportati da Roche sulle istruzioni dei singoli test e quelli indicati dalla letteratura; inoltre, abbiamo controllato se gli intervalli di riferimento caratterizzati per genere fossero presenti nel Manuale degli analiti del Sistema Qualità e nel referto.

Dei 39 analiti presi in esame, 10 (26%) hanno solo un intervallo di riferimento, mentre 29 (74%) presentano un range di riferimento per genere (es: creatinina sierica e urinaria, mioglobina, CK, colesterolo HDL, HE-4, che presenta range correlati allo stato di fertilità della donna.). Tra gli analiti caratterizzati per genere: ALT, GGT, ALP, Ferro e pro-BNP hanno range ulteriormente suddivisi per fasce di età; Beta-HCG sieriche e AFP sono anche suddivisi per periodo di gestazione; progesterone, estradiolo, FSH ed LH presentano anche valori differenti in relazione alle fasi del ciclo ovarico. Tra gli analiti caratterizzati per genere i range di riferimento differenziati tra maschio e femmina sono riportati nel Manuale degli analiti del Sistema Qualità in 22 casi (76%), mentre in 7 casi (24%) non viene riportata tale differenziazione.

Questo studio ha mostrato che nella UOC Laboratorio Patologia Clinica ci sono diversi analiti molto ben caratterizzati per sesso e genere. Al contrario, per alcuni analiti la caratterizzazione degli intervalli di riferimento per genere non è riportata né nel Manuale degli analiti del Sistema Qualità nè nel referto. La fase successiva dello studio sarà quindi quella di aggiornare gli intervalli di riferimento in modo da rispondere meglio alla medicina personalizzata.

NATURAL HISTORY OF KBG SYNDROME IN A LARGE EUROPEAN COHORT

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KBG syndrome (KBGS) is characterized by distinctive facial gestalt, short stature and variable clinical findings. With ageing, some features become more recognizable, allowing a differential diagnosis. We aimed to better characterize natural history of KBGS. In the context of a European collaborative study, we collected the largest cohort of KBGS patients (49). A combined array-based Comparative Genomic Hybridization and next generation sequencing (NGS) approach investigated both genomic Copy Number Variants and SNVs. Intellectual disability (ID) (82%) ranged from mild to moderate with severe ID identified in two patients. Epilepsy was present in 26.5%. Short stature was consistent over time, while occipitofrontal circumference (median value: -0.88 SD at birth) normalized over years.

Cerebral anomalies, were identified in 56% of patients and thus represented the second most relevant clinical feature reinforcing clinical suspicion in the paediatric age when shortstature and vertebral/dental anomalies are vague. Macrodontia, oligodontia and dental agenesis (53%) were almost as frequent as skeletal anomalies, such as brachydactyly, shortfifth finger, fifth finger clinodactyly, pectus excavatum/carinatum, delayed bone age. In 28.5% of individuals, prenatal ultrasound anomalies were reported. Except for three splicing variants, leading to a premature termination, variants were almost all frameshift.

Our results, broadening the spectrum of KBGS phenotype progression, provide useful toolsto facilitate differential diagnosis and improve clinical management. We suggest to consider a wider range of dental anomalies before excluding diagnosis and to perform a careful odontoiatric/ear-nose-throat (ENT) evaluation in order to look for even submucosalpalate cleft given the high percentage of palate abnormalities. NGS approaches, following evidence of antenatal ultrasound anomalies, should include ANKRD11.

GERMLINE DNA REPAIR PATHWAY DEFICIENCY AND CLONAL *TP53/KRAS ALTERATIONS* IN PANCREATICCANCER: PROSPECTIVE FOR TARGET THERAPY.

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Pancreatic Cancer (PC) continues to be a hard-to-treat cancer with a 5-year survival rate of approximately 11%. Germline variants in *BRCA1/2, PALB2, CDKN2A, CDK4, MLH1, MSH2, MSH6, PMS2 and PRSS1* are associated with a high-risk to develop PC. Somatic *KRAS*, *TP53, CDKN2A,* and *SMAD4* mutations are commonly found. Here, we investigated the mutation profilingin PC patients.

Exome sequencing and Next Generation Sequencing of Cell-free DNA were performed respectively on 33 patients with suspect familial PC and 19 advanced PC cases.

Fifteen patients carry at least one germline mutation in DNA repair genes (DRGs).
The homologous recombination (HR) genes (*PALB2, CHEK2, ATM, SLX4, RECQL4) were* found tobe the most frequently mutated (37%), followed by Fanconi anemia (FA) genes (*FAN1, ERCC4, NBN, FANCM) (21%)* and mismatch repair (MMR) genes (*MSH2, MSH3, MSH6) (21%).* Of note, Fanconi anemia genes were always mutated paired with a second germline mutation in other DRGs. Six patients processed for liquid biopsy presented both *TP53* and *KRAS* clonal mutations during tumor progression.

A hallmark of many cancers is an inactivation of DRGs. The disruption of one pathwayfrequently enhances cell dependency on compensatory pathways explaining why a combination therapy might be more efficient. An aggressive not specific chemotherapy intervention may expand clonal undruggable variants.
HR and FA deficiency is primarily targetable with PARP and histone deacetylase inhibitors whilst MMR deficiency tumors are sensitive to immunotherapy with checkpoint inhibitors. Recently, *KRAS*- mutated tumors became druggable with covalent and non-covalent inhibitors. Early-detection of germline susceptibility and expanding clonal variants in targetable genes could open a new personalized combined therapeutic era for PC patients.
A multidisciplinary approach concerning laboratory geneticists, clinical geneticists, oncologists, and other specialists involved in cancer patients care is mandatory to integrate the continuous biological,clinical, and technical advancements into the clinical management.

2° SESSIONE: SPECIALIZZAZIONI AREA CHIRURGICA

PERITONEAL TISSUE CHANGES AND SYSTEMIC CHANGES FOLLOWING INDUCTION OF PNEUMOPERITONEUM IN PAEDIATRIC LAPAROSCOPY.

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In recent years, laparoscopy has become the surgical approach of choice for many paediatric surgical procedures.
Despite the undoubted advantages of this technique, the induction of pneumoperitoneum through CO2 insufflation has been shown to alter the peritoneal environment.
The consequences of achieving pneumoperitoneum have only been studied on animals and adults so far, but given the developmental stages of paediatric patients, it is of paramount importance to replicate such studies also on the paediatric population to verify possible peritoneal morphological changes.

This multicentric cross-sectional study aims to investigate possible histological and serum pro-inflammatory changes after minimally invasive surgery in patients aged 0-18 years. In particular, the case series examined concerns paediatric patients enrolled at the Paediatric Surgery Department of the Azienda Ospedaliera Universitaria Senese.
The study protocol included four biopsies of the parietal peritoneum (two after entry into the abdominal cavity -T0 and two before the end of the operation -T1) and two blood samples (one before entry into the operating room and one pre-discharge, 12-24h after surgery).
The samples were analyzed at the Institute of Pathological Anatomy of the Veterinary Faculty of the University Federico II of Naples and the Morphology and Immunochemistry laboratories of the Department of Medical, Surgical and Neuroscience Sciences of the University of Siena.

No local peritoneal or systemic pro-inflammatory changes were evident from the analysis of the samples obtained:
-The peritoneal serosa was morphologically normal in both T0 and T1 samples.
-Quantitative analysis of the cytokines tested (MCP-1, IL-8, RANTES) showed no significant changes between the T0 and T1 blood samples.
These results can be attributed to certain factors peculiar to paediatric surgery, such as reduced insufflation pressure during pneumoperitoneum and a short duration of the laparoscopic procedures examined.

TECHNOLOGICAL INNOVATIONS IN ORTHOPEDIC SURGERY: THE USE OF COMPUTER NAVIGATION AND THE AID OF ARTIFICIAL INTELLIGENCE IN SHOULDER PROSTHESIS

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The shoulder prosthesis is the third most frequently used prosthetic implant after hip and knee replacement, used in the treatment of patients with severe cuff arthropathy, severe glenohumeral arthrosis, and complex fractures of the proximal humerus. The use of reverse prosthesis (RSA) has continued to grow in recent years, becoming the most widely used operation in shoulder prosthesis. The main cause of long-term failure of RSA is failure of the glenoid component, so appropriate positioning and fixation of the metaglena are the key point to ensure implant survival and prevents postoperative instability. For this reason, over the past few years, the use of intraoperative navigation technology has become widespread in prosthetic surgery. Initially developed and applied in knee and hip prosthesis, to date, it appears to be able to provide significant advantages and greater precision in shoulder prosthesis as well, in view of reduced gleno-osseous surface area and neurovascular structures (brachial plexus, subclavicular and axillary vessels) that might be at risk.

The computerized navigation technique involves an initial preoperative phase in which the patient's anatomy is studied with a CT scan with 3D reconstructions, and with dedicated software, personalized surgical planning can be performed for each individual patient, in view of the frequent anatomical variables of the glenoid due to bone loss in degenerative diseases. In addition, artificial intelligence, such as the Predict+ software, allows the surgeon to provide patients with predictions about the risks and benefits of surgery in terms of ROM and pain improvement by analyzing databases of thousands of patients undergoing shoulder prosthetic surgery. In the intraoperative phase, the surgeon is guided by sensors and a GPS station that project informations and images of the shoulder and prosthetic implant to a screen in real time, allowing the risk of complications to be reduced and greater precision in implant placement to be achieved. Thanks to innovative latest generation Verasense sensors, it is possible to obtain information on the range of motion (ROM) and joint tensions of the soft tissues involved, obtaining a more balanced and high-performing prosthetic implant. In our experience, intraoperative navigation based on 3D CT studies in patients undergoing RSA surgery can improve metaglene placement and fixation by allowing the use of fewer screws and longer lengths to achieve optimal primary stability, and simultaneously improve anatomical knowledge of the bone defect, allow more frequent use of baseplates with augment.

We conducted a retrospective study comparing a consecutive series of 30 patients who underwent RSA surgery using 3D CT study-based navigation and 20 patients who underwent the same surgery with the conventional technique. All surgeries were performed by the same operator, employing the same implant, on patients with eccentric arthrosis or cuff arthropathy. Patients with post-traumatic arthrosis, complex fractures of the proximal humerus, and prosthetic revision surgeries were excluded from the study.

We observed a longer surgical time in the navigated RSA group (average 11 min, range 7-16) probably attributable to the positioning of the camera and anatomic recognition systems. However, the intraoperative navigation system allowed the use of longer screws (36.3 mm vs 31.5 mm), a higher

rate of achieving optimal fixation with only two screws (90% vs 55%), and a higher rate of using baseplates with augment (30% vs 10%).

Our study highlights how preoperative 3D planning aids the orthopedic surgeon in identifying the optimal positioning of the metaglene, how intraoperative navigation allows continuous real-time feedback of medialization, version, and baseplate tilt, and allows for increased implant stability thanks to fewer and longer screws. In addition, with an eye toward the future, the humeral component of the prosthesis is also beginning to take on an important role; with the help of the Verasense sensor, it is possible to obtain dynamic feedback on the tensions and range of motion of the glenohumeral joint as a whole, directing the surgeon to custom surgery to the individual patient, and thanks to artificial intelligence , the possibility to provide the patient in advance with predictions about the possible outcomes and benefits of surgery.

PRECISION MEDICINE: THE RETINOBLASTOMA PARADIGM-A TEAM EFFORT

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Retinoblastoma is the most common intraocular tumor during childhood. Although it is an aggressive and life-threatening disease, retinoblastoma is curable.

The main challenge in managing the advanced tumor is eye globe salvage and preservation of visual acuity. The introduction of intra-arterial chemotherapy (IAC) and intra-vitreal chemotherapy (IViC) have greatly improved the prognosis of advanced retinoblastoma, but still 28-32% of patients have had a relapse that led to enucleation.

Liquid biopsy is a non-invasive procedure that allows us to identify and study tumor DNA from a simple blood sample. In fact, circulating tumor DNA fragments (ctDNA) are released from tumor cells in the systemic circulation. Thanks to DNA sequencing and findings of somatic mutations, it is possible to identify different tumor patterns. Circulating tumor DNA profiling can have a direct impact on patient care by modifying treatment decisions, enabling the monitoring of cancer response to therapy, revealing drug resistance mechanisms and detecting minimal residual disease or relapse.

In that way the liquid biopsy in retinoblastoma can improve a targeted treatment in order to have a better therapeutic response and increase the eye globe salvage and preservation of visual acuity.

SONOGRAPHIC STUDY OF UTERINE BIOMETRY FOR THE DIAGNOSIS OF DIFFUSE ADENOMYOSIS IN A TERTIARY OUTPATIENT CLINIC

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Transvaginal ultrasound (TVUS) can be considered the first-line imaging technique to diagnose adenomyosis. The aim of this prospective study is to systematically compare, in fertile-age women attending to a tertiary outpatient clinic, several uterine biometric parameters at TVUS between adenomyosis uteri and non-adenomyosis uteri and evaluate their potential role for the diagnosis of diffuse adenomyosis.

This prospective observational study was conducted between the 1st of February 2022 and the 30th April 2022. Exclusion criteria were age less than 18 years, virgo, postmenopausal status, ongoing or recent pregnancy (less than 6 months), gynaecological malignancy, uterine malformations, previous surgery for adenomyosis or fibroids, focal myometrial lesions larger than 1 cm. Patients were divided in two groups according to the appearance of the uterus - adenomyotic and non-adenomyotic - at TVUS performed by expert operators. The two study groups were compared in terms of baseline and sonographic variables. First, we compared sonographic uterine biometric parameters (longitudinal (LD), anteroposterior (APD) and transverse (TD) diameters, volume, simple and complex diameter ratios) between the two study groups; secondarily diagnostic performance of their optimal cut-off values in diagnosing diffuse adenomyosis was evaluated.

During study period, 56 eligible patients with a TVUS diagnosis of adenomyosis were included as adenomyosis uteri group. During the same period, a 1:1 ratio age and parity-matched group of eligible patients with non- adenomyosis uteri at TVUS was selected. Sonographic uterine diameters, volume simple and complex diameter ratios were all significantly different between the two groups, except for TD/(LD+APD). Optimal cut-off values of each biometric parameter demonstrated a low discriminative value for the diagnosis of adenomyosis. Of note, among all investigated parameters, the optimal cut-off values of APD and LD/APD showed the best sensitivity and specificity. In particular, APD diameter equal or superior to 39.5 mm (95% CI, 36.2-42.8) had sensitivity of 0.70 (95% CI, 0.57-0.80), specificity of 0.71 (95% CI, 0.59-0.82) and accuracy of 0.75 (95% CI, 0.66-0.84). LD/APD equal or inferior to 2.05 (95% CI, 1.96-2.13) showed sensitivity and specificity of 0.70 (95% CI, 0.57-0.80) each and accuracy of 0.72 (95% CI, 0.62-0.81).

Although several biometric uterine parameters at TVUS in fertile-aged women were statistically different between adenomyosis and non-adenomyosis uteri, their optimal cut-off values showed a low accuracy in diagnosing adenomyosis. Future larger studies are needed to confirm our data in different settings and conditions.

3° SESSIONE: SPECIALIZZAZIONI AREA CLINICA

RADIOFREQUENCY ECHOGRAPHIC MULTISPECTROMETRY (REMS): A NEW TOOL FOR BONE STATUS ASSESSMENT IN OSTEOGENESIS IMPERFECTA

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Osteogenesis imperfecta (OI) is a rare hereditary disorder of connective tissue, mainly characterized by qualitative and quantitative alterations of bone collagen responsible for bone fragility and increased risk of fractures. The most common clinical features in OI are frequent fractures that are often precipitated in absence of trauma or by minimal trauma, blue sclera, dentinogenesis imperfecta, joint hyperlaxity, short stature, progressive bone deformities, muscle weakness. BMD by Dual-energy Xray absorptiometry (DXA) is undoubtedly useful for assessing disease progression and response to bisphosphonate treatments in patients with OI. However, numerous studies have shown that in many patients with OI, BMD by DXA is only slightly reduced and sometimes even increased. In fact, in OI bone microarchitecture is markedly altered: trabecular thickness and volumetric bone mass are lower; cortical thickness is also reduced with higher intracortical porosity. All these alterations of bone structure alter bone biomechanics. A more recent and attractive tool for bone status is Radiofrequency Echographic Multi Spectrometry (REMS) technology. REMS is based on the analysis of native raw unfiltered radiofrequency (RF) ultrasound signals, obtained during an echographic scan of proximal femur and lumbar vertebrae. Our study aimed to evaluate the usefulness of REMS technique in the assessment of bone status in subjects with OI.

In a cohort of 35 subjects (39.5 ± 21.2 yrs) with OI and in 30 healthy controls we measured BMD at the lumbar spine (LSBMD), at femoral neck (FN-BMD) and total hip (TH-BMD) using a dual-energy X-ray absorptiometry device; TBS was calculated using TBS iNsight software. Moreover, REMS scans were also carried out at the same axial sites. According to the Sillence classification 27 (80%) OI subjects was Type I whereas 4 (11.4%) was Type III OI an 3 (8.6%) was Type IV. Moreover, in OI subjects the presence of prior fractures was reported.

A total of 85.7% of patients presented with a fracture history. The most common fracture sites were extremity long bones (femur, tibia-fibula and radius-ulna) (70.0%) and at vertebral site (54.3%). BMD evaluated by DXA and REMS technique at all measurement sites were all significantly ($p<0.01$) lower in subjects suffering from OI than in controls. BMD T-score by REMS were lower than those obtained by DXA technique at lumbar spine, on the contrary at femoral neck T-score by REMS were higher than those obtained by DXA technique, while at total femur the values are similar. Dividing OI patients on the bases of Sillence classification, no differences between the BMD-LS values carried out with the DXA technique between OI type I group and OI Type III and IV group. On the contrary, the OI Type III and IV group presented significantly lower values of both TBS and BMD-LS by REMS with respect to patients OI type I ($p<0.05$).

This preliminary study has shown that REMS appears to be an accurate non-ionizing technology able to assess the bone status in subjects with OI. An interesting result of this study is the significant correlation between REMS and TBS. The attractiveness of REMS for bone assessment in OI patients lies in:1. its lack of ionizing radiation so REMS can be used during fertile age and pregnancy; 2. its ease of use and repeatability, so it’s possible to follow up OI patients ; 3. its portability, in fact REMS device can be used directly on patient's bed, and this could represent an excellent method for assessing bone status even in OI subjects with a recent fracture.

HIPPOCAMPAL ATROPHY CAN PREDICT EVOLUTION TO DEMENTIA IN PATIENTS WITH VASCULAR MILD COGNITIVE IMPAIRMENT

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Vascular mild cognitive impairment (VMCI) is a transitional condition that may evolve to vascular dementia (VaD). A deeper understanding of the mechanisms of cognitive impairment in VMCI is crucial to develop markers of disease progression. Studies have suggested hippocampal atrophy as a putative MRI-based marker in early VaD. Our study aims to evaluate (i) differences in hippocampal volume among patients who converted to VaD vs those who did not convert and (ii) to what extent hippocampal involvement correlated with cognitive impairment.

In this longitudinal multicentre study, 110 subjects with VMCI (mean age [±SD]: 74,33 [± 6,63] years, 61 males/49 females) from the vMCI-Tuscany Study database underwent brain MRI and were scored on cognitive tests. For each subject, right and left hippocampi were semi-automatically segmented (following the EADC-ADNI protocol) and volumes calculated using FIRST (https://fsl.fmrib.ox.ac.uk/fsl/fslwiki). Information on whether patients evolved to VaD after 10 years was recorded. Linear regression models were used to assess differences in hippocampal volumes between groups and their correlations with cognitive tests, corrected for age, sex, center and education, as appropriate.

At baseline, VMCI subjects could be grouped into 4 groups according to their cognitive profile, respectively: 9 were amnesic single-domain (ASD), 74 amnesic multidomain (AMD), 10 non-amnesic single-domain (NASD), 15 non-amnesic multidomain (NAMD). In the whole cohort, lower hippocampal volume was associated with worse verbal memory assessment score, such as ray-auditory-verbal learning test delayed recall (beta coefficient: 101.19, p=0.004). At 10 years evaluation, 32/110 (29%) of VMCI converted to VaD. Lower

whole hippocampal volume was found in vMCI patients who converted to VaD (mean volume [±SD]: 4829 [±897] mm^3) with respect to those who did not convert (mean volume [±SD]: 5178 [±819] mm^3); ($p=0.03$).

Data reported here suggest that hippocampal atrophy can be detected in patients with VMCI, is closely related to subclinical memory impairment and can predict evolution to VaD.

EFFICACY AND TOLERABILITY OF INTRAVENOUS KETAMINE IN TREATMENT-RESISTANT DEPRESSION

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Many adult patients with a major depressive episode fail to achieve complete remission and global recovery of functioning despite treatment with antidepressants that act as modulators of monoaminergic neurotransmitter systems (McAllister- Williams et al, 2020). Clinical trials have shown that 30-40% of depressed patients do not respond to initial antidepressant treatment despite adequate adherence, dose and duration, 60-70% do not achieve complete remission, up to 20% are still acutely depressed after two years, and 10% remain depressed despite multiple interventions (Fagiolini and Kupfer, 2003). Therapeutic success rates are even lower when the patient presents with a complex clinical picture characterized by mixed symptoms such as anxiety, irritability, internal tension and suicidality (McIntyre et al., 2020). In addition, treatment failure hasbeen shown to increase the risk of relapse and suicidality (Rush et al., 2006).

The pharmacodynamic rationale for the use of ketamine in the treatment of depression is its effect on neurotropism and synaptogenesis, which is mediated by modulation of GABA/glutamatergic neurotransmission due to its high affinity for N-methyl-D-aspartate receptors (NMDAR) (Lumsden EW et al., 2019).

Our study investigated the efficacy, tolerability and safety of intravenous ketamine as an adjunct to standard psychopharmacological therapy (e.g. mood stabilisers in patients with bipolar depression) in people with a resistant major depressive episode. The treatment protocol was approved by the Department of Health and the Pharmaceutical Service of the University of Siena Medical Centre (AOUS) and by the Ethics Committee of the Area Vasta Toscana Sud-Est, Institutional ReviewBoard.

Statistical analyses of 31 patients (mean age, 55±9.1 years; 32.3% female) treated consecutively with ketamine showed a rapidand statistically significant reduction in depressive symptoms at each week of treatment ($p<0.05$), as assessed by the MADRS psychometric scale; this was maintained and improved by the final assessment at week 4 ($p<0.001$, compared with baseline). Significant improvements were also seen in the key MADRS symptoms of 'inner tension', 'insomnia' and 'suicidal thoughts' as early as the first week of treatment. In addition, analysis of the Y-MRS scale showed no worsening of mixed symptoms or the occurrence of manic episodes. Side effect data confirmed an acceptable tolerability profile.

Ketamine offers a favourable risk-benefit ratio in the treatment of resistant depression, thanks to a rapid clinical response, including important symptoms such as suicidal ideation, and good tolerability.

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THE IMPACT OF BENRALIZUMAB ON NK CELL PROLIFERATION AND MATURATION IN PATIENTS WITH SEVERE EOSINOPHILIC ASTHMA.

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Bronchial asthma is globally the second most frequent chronic respiratory disease after chronic obstructive pulmonary disease, affecting approximately 300 million people worldwide. Although most asthmatic patients are well controlled on inhaled therapy, approximately 3.7-10% has a severe disease, defined as clinically uncontrolled asthma despite use of high dose inhaled steroids doses associated with bronchodilators. Severe asthma is characterized by chronic and disabling respiratory symptoms and recurrent asthma attacks (defined as exacerbations) requiring additional treatments, such as oral corticosteroids (OCS), the chronic use of which is responsible for numerous side effects. Thanks to a better understanding of the immunological processes underlying airway inflammation in severe asthma, biological treatments have been developed that have completely redesigned the clinical management and quality of life of these patients. Among currently approved biologic drugs, benralizumab is a humanized monoclonal antibody that induces rapid depletion of eosinophils by binding IL-5 receptor α and activating antibody-dependent cell-mediated cytotoxicity (ADCC) through NK cells, macrophages and neutrophils. Although the clinical efficacy of the drug is undoubted, there are no data on the immunological impact of benralizumab on NK cells, which are directly implicated in the drug's pharmacodynamic activity.

The aim of the study is to investigate the effect of benralizumab on NK cells through flow cytometric analysis on blood. Cytometric assays were performed before and after 6 months of therapy and related with the clinical improvement induced by the drug.

We prospectively enrolled 10 patients with severe eosinophilic asthma and compared them with 15 healthy controls and 9 patients with mild-to-moderate asthma. At baseline (T0) and after 1 and 6 months of treatment (T1 and T6), the clinical control of asthma was assessed through validated questionnaires (ACQ-6 and ACT), respiratory functional tests and evaluating the use of oral and inhaled steroids (OCS and ICS). As well, NK cell analysis was performed on blood by multiparameter flow cytometry at T0, T1 and T6.

No differences emerged in the distribution by gender, mean age and smoking habits among the 3 groups examined. At baseline, patients with severe asthma showed higher percentages of immature NK cells than healthy controls ($p<0.05$). Benralizumab induced a maturation of the NK profile leading to an increase in cytotoxic phenotypes ($p<0.001$); this variation was further supported by a significant increase in the release of cytotoxic mediators responsible for ADCC such as IFN-γ ($p<0.001$). Accordingly, flow cytometric analysis of CD137 expressed on the surface of NK, CD4 and CD8 T cells showed a significant increase at T6 in respect with T0 ($p<0.001$ for all). NK cell activation status was shown to correlate with improvement in pulmonary function data and therapy-induced reduction of OCS ($p<0.01$ and $p<0.001$, respectively).

These data contribute to understanding the mechanisms of action by which benralizumab induces resolution of bronchial inflammation in patients with severe asthma. NK cell activation and proliferation, both stimulated by benralizumab, appear to be the primum movens of eosinophil depletion, correlating with clinical and functional improvement and steroid-sparing effect in treated

patients. We also demonstrate for the first time, through the analysis of CD137 expression, that benralizumab induces both active proliferation and increased activation of NK cells, which can therefore express their cytotoxic action against the eosinophil. The role of NK cells in severe asthma, as well as their pharmacodynamic involvement in benralizumab therapy, represent a still largely unexplored field which could represent, from the point of view of precision medicine, a valid clinical aid in the personalized choice of therapy biologic in this specific subset of patients.