University of Modena and Reggio Emilia

PhD COURSE OF CLINICAL AND EXPERIMENTAL MEDICINE

PhD DAY 2018

Abstracts

May 8

2:30 p.m., Lecture Room H1.1

Department of Biomedical, Metabolic and Neural Sciences

(287 Campi street, Modena)

New horizons in Urology

The 22nd of March 2018 in Modena was hosted one of the most influential exponents of European Urology: Professor Estevao Lima. Professor Lima is 49 years old and he is the Director of the Urology Department at the hospital of Braga, Portugal, as well as associate professor of Urology at the School of Health and Sciences at the University of Minho.

He started an early career as a "Fellow of the European Board of Urology" (FEBU). Subsequently, from 2002 to 2010 he was Assistant of Urology at the "Hospital Geral de Santo António" and responsible for the endourological and laparoscopical departments. He obtained his PhD in 2008 with the thesis "Development of Transvesical Port for Scarless surgery". His intense scientific activity is well documented. In the "Scopus" has 64 documents referenced with 1272 citations in 836 documents, 12 book chapters, 7 of them with international Edition and h index of 19. He has received 16 research awards, many of them international. He is a member of several national and international scientific societies and reviewer of several international journals. He has 5 patents. He has distinguished himself for his constant need of innovation that drove all his work, so much that we can define him an urological pioneer. Professor Lima come to Modena as Member Committee on the awarding of a PhD scholarship in Experimental Medicine Department, directed by Professor Giuseppe Biagini.

In this occasion the residents had the pleasure to discuss the most difficult clinical cases with Prof. Lima and, in the afternoon, he gave a magisterial lecture on "Predicting new developments in Endourology - Looking beyond the horizon". The lecture touched themes as Scarless Surgery in Laparoscopy (NOTES technique); confocal microscopy in tumor diagnostics; pioneered techniques in percutaneous kidney accesses, like magnetic guideed puncture of the renal calyx; and biodegradable ureteral stenting witch he has invented and produced through "HydrUStent Company" (Spin-Off from University of Minho).

We are hoping to continue a prolific partnership with our Portuguese's colleague for an international scientific and professional growth.

Figure 3. From left to right. Prof. E. Lima, Prof. G. Bianchi, Prof. B. Rocco and Prof. S. Micali

Figure 2. Prof. E. Lima with Pavarotti statue

The International Doctorate School in Clinical and Experimental Medicine (CEM) is organized by the Department of Biomedical, Metabolic and Neural Sciences in collaboration with other Departments of the University of Modena and Reggio Emilia and is under the direction of Prof. Giuseppe Biagini.

The educational program and research opportunities are directed towards the acquisition of skills required for basic and clinical research at Universities, public or private Research Institutes, and Hospitals. A Faculty of internationally recognized professors is responsible for the educational activities and takes part into the organization of the doctoral program.

From 2018 (XXXIV cycle) the PhD Course of "Clinical and Experimental Medicine" is organized in 3 curricula:

Nanomedicine, Medicinal and Pharmaceutical Sciences
Translational Medicine
Health Sciences

From XXIX to XXXIII cycle the PhD Course of "Clinical and Experimental Medicine" was organized in 3 curricula:

Medicinal and Pharmaceutical Sciences
Translational Medicine
Health Sciences

From XXV to XXVIII cycle the Doctorate School of "Clinical and Experimental Medicine" was organized in 5 curricula/thematic areas:

Oncology
Public Health
Cellular and Molecular Pathophysiology
Clinical, Genetic and Molecular Medicine
Surgery

XXXI cycle

Dr. Gianluca Rompianesi

CEM Curriculum: Translational Medicine
Tutor: Prof. Giorgio E. Gerunda

INCIDENCE AND OUTCOME OF COLORECTAL CANCER IN LIVER TRANSPLANT RECIPIENTS: A NATIONAL, MULTICENTRE ANALYSIS ON 8115 PATIENTS

Background

Liver transplant (LT) recipients have a higher risk of developing malignancies, particularly skin cancers and lymphoproliferative tumours. The risk of developing solid tumours is less well documented. Retrospective studies have demonstrated conflicting results regarding the risk of colorectal cancer (CRC) development and post-LT survival. According to the European Liver Transplant Register, primary sclerosing cholangitis (PSC) is the primary indication for LT in approximately 5% of all adult recipients and is associated with inflammatory bowel disease (IBD) in up to 70% of patients. In comparison to the general population, patients with PSC-IBD have a 10-fold increased risk of CRC, while patients with PSC alone have a 5-fold increased risk of CRC. Moreover, CRC in this group of patients appears to develop at a younger age and is diagnosed at a more advanced stage compared with the general population, is predominantly localised in the ascending colon and represents one of the leading causes of death.

Objectives

In the present study, we used national data to assess the risk and outcome of CRC after LT. We therefore analysed: i) the incidence of post LT CRC and ii) whether cancer survival outcomes are comparable to the general population in LT patients with or without ulcerative colitis and with or without PSC by performing a retrospective analysis of prospectively collected national data in the UK.

Methods

This is a national multicentre retrospective cohort study. Data from all adult patients undergoing a LT in 6 out of the 7 Liver Transplant Units of the United Kingdom between January 1st 1990 and December 31st 2010 were analysed. National ethical approval, National Health Service Blood and Transplant (NHSBT) approval and National Information Governance Board approval were obtained to perform this study. Prospectively collected liver transplant data was obtained from NHSBT. Retrospective data was obtained from electronic patient records, patient notes and hospital databases. The Yorkshire Cancer Registry, which is the lead UK registry for colorectal cancer, cross matched the datasets against the cancer registry database and provided a list of liver transplant patients with a diagnosis of CRC as well as the survival data on all colorectal cancer patients within the specified dates. Patients were followed from the first recorded liver transplant until death or 31st December 2012. Those with evidence of colectomy prior or during first transplant were excluded from the analysis.

Results

8178 patients underwent a liver transplant between 1990 and 2010 in one of 6 UK transplant centres. 63 patients (0.8%) with a colectomy prior to or at the time of transplant were excluded as they were not considered to be at risk of developing CRC, leaving 8115 for the analysis. Median survival (95% CI) following first transplant was 15.6 (15.0-16.4) years and 5 and 10-year post-LT patient survival probabilities were 72% and 62% respectively. 52 (0.6%) patients were diagnosed with CRC at a median of 5.6 years after LT. 677 (8.3%) patients had either UC or PSC. Amongst individuals with CRC, 27 (51.9%) had either PSC or UC. The tumour localisation was equally distributed in the proximal and distal colon. The tumours were moderately differentiated (grade 2) in 76.9% of cases and were predominantly T3 tumours (50% of cases in the whole LT population, 47.6% in the PSC/UC group and 66.7% in patients without PSC/UC). There were a total of 63,609 person years at risk with a median (IQR) follow up time of 6.7 (2.9-12.2) years. This gave a crude incidence rate (95% CI) of 8.2 (6.0-10.4) cases of CRC per 10,000 person years of follow up. The SIR of CRC in the liver transplant population compared to the general UK population was 0.92 (95% CI 0.69-1.20). The probability of developing a CRC after LT increases linearly over time after transplantation. Among 677 patients with either UC or PSC, there were 27 (4.0%) diagnosed cases of CRC over a total of 5094 person-years of follow-up, giving an incidence rate (95% CI) of 53.0 (33.0-73.0) per 10,000 years. The rate of CRC among those with UC or PSC was therefore 12.3 (95% CI= 7.20-21.4) times the rate in those without UC or PSC ($p<0.001$). When compared with the UK population, the rate of CRC was 7 times higher in those with UC or PSC (SIR, (95% CI)= 7.00, (4.71-10.04)). Thirty-one (59.6%) patients with CRC died. Median (95% CI) survival from cancer diagnosis was 4.7 (2.1-8.0) years. One, five and ten-year survival rates from CRC diagnosis were 71%, 48% and 31% respectively. The probability of death due to CRC at 1, 5 and 10 years was 17.4%, 17.4% and 23.8%. There were 27 patients diagnosed with CRC who also had either UC or PSC. Of these, 13 (48.2%) died during follow up. Median survival time was 8.0 years. One, five and ten-year survival rates from CRC diagnosis were 81%, 59% and 47% respectively.

Conclusions

Our study has demonstrated significantly increased risk of CRC in the PSC/UC group of transplanted patients (SIR 7.0) but not in the whole LT group (SIR 0.92), when compared with the general population. It has further shown that tumours are frequently diagnosed at an advanced stage, despite increased awareness and vigilance. In this scenario, prophylactic colectomy could play a role, since it has been demonstrated that PSC patients who had a pre-LT colectomy or don't suffer from UC carry a significantly lower risk of recurrent PSC when compared to patients with UC and no colectomy, which is strongly associated to an increased rate of graft failure and patient death. The uni- and multivariate analysis of risk factors for CRC development is still ongoing.

Dr. Francesca Faillaci

CEM Curriculum: Translational Medicine
Tutor: Prof. Erica Villa

EXPLORATION OF THE ROLE OF INFLAMMATION, NEO-ANGIOGENESIS AND BIOLOGICAL AGGRESSIVENESS IN HEPATOCELLULAR CARCINOMA (HCC): CLINICAL STUDY AND EXPERIMENTAL APPROACH IN VITRO E IN VIVO

Background

In hepatocellular carcinoma (HCC)(4th leading malignancy worldwide), tumor microenvironment affects tumor progression and determines its high heterogeneity. Liver tumor microenvironment is a complex ensemble of tumor cells within extracellular matrix (ECM), stromal cells and secreted proteins. All together contribute to carcinogenesis. Patients with aggressive HCC can be identified by means of neo-angiogenetic transcriptomic signature (ANGPT2, DLL4, NETO2, ESM1, NR4A1) which also strongly related with rapid progression of tumor, risk of recurrence after therapy, and extremely median survival (E. Villa et al., 2015). Although all five genes contribute to the diagnostic power of the signature, the leader gene is Angiopoietin-2 (ANGPT2), which has, on its own, a ROC AUC of 0.943, 95 % CI 0.875 to 1000; $p<0.0001$. ANGPT2 is an extremely interesting gene: its product is secreted by endothelial cells at sites of active vascular remodelling. High ANGPT2 mRNA and protein levels are found in highly vascular and poorly differentiated HCCs. The different biochemical composition of tumor microenvironment derives from underlying chronic liver disease, correlated as well to hepatitis C virus (HCV) infections. Tumor-surrounding non-tumor tissue has an important role in dynamic molecules cross-talk, explaining the relevance of the peritumoral tissue also as potential therapeutic target. Some recent evidences show that the tissue microenvironment may engrave on tumor formation. New treatments are recently developed for HCV chronic patients, these new antivirals are called Direct-Acting Antiviral Drugs (DAAs). Recent studies reported a putative association between increased risk of recurrence, or of de novo development of HCC, in patients treated with DAAs.

Objectives

Aim of the project was to identify in HCCs, with a particularly severe course, the circulating and tissue components of the microenvironment affecting the aggressiveness and the ensuing clinical outcome. And in particular, in a subgroup of patients, treated with DAAs, our goal was evaluate the risk factors for HCC recurrence/de novo occurrence in relation with molecular characteristics of the tumor microenvironment, as angiogenesis, circulating and cellular components.

Furthermore by means in vitro e in vivo (using zebrafish model) approach, we aim to investigate behavior of cells or organs, respectively, under inflammatory stimulus or under stress condition, as increase of blood flow to simulate shear-stress effects.

Methods

To investigate the impact of tissue microenvironment components on HCC clinical outcome, we performed an extensive study in the prospective cohort of 132 HCC-patients.

We analyzed the baseline paired HCC and the surrounding tissue biopsies at the first diagnosis of HCC for immunolocalization of PD-1/PD-L1, FoxP3, E-cadherin, CLEC2 and for a panel of 82 microRNA associated with regulation of angiogenesis, cell proliferation, cell signaling, immune control and autophagy. Serum samples were analyzed for a panel of 19 cytokines. Data were associated with biochemical data, histopathology and survival.

We mainly focused on neo-angiogenesis, and in the subgroups of chronic HCV patients (pts) treated with DAAs, with advanced fibrosis and with cirrhosis of different etiologies, evaluating neo-angiogenic transcriptomic signature and the hepatic expression of Angiopoietin-2, as potentially favoring HCC onset and recurrence. Circulating Angiopoietin-2, vascular-endothelial growth factor (VEGF), and C-reactive protein were also measured.

We are now studying the effect of blood flow modification (as direction and turbulence) on liver microenvironment and on cell behavior through a test of microfluidic chip-system, set up in the laboratory of our collaborator Prof. Ute Schepers (KIT), and kindly provided to us. In these liver-on-a-chip system, the flow can be modulate and different types of shear-stress can be applied, in order to mimic human condition.

Results

Patients with a more aggressive disease and shorter survival, who we named fast-growing accordingly to the tumor doubling time, at presentation had significantly higher AFP levels, TGF-β 1 and Cyphra 21-1 levels. Transcriptomic analysis evidenced a significant downregulation of CLEC2 and upregulation of several metallo-proteinases. A marked local upregulation of both PD-1 and PD-L1, a concomitant FoxP3-positive lymphocytic infiltrate, a loss of E-cadherin, gain of epithelial-mesenchymal transition (EMT) phenotype and extreme poor differentiation at histology were also present. Upregulated microRNA in fast-growing HCCs are associated with TGF-β signaling, angiogenesis and inflammation. Our data show that fast HCCs are characterized not only by redundant neo-angiogenesis but also by unique features of distinctively immunosuppressed microenvironment, prominent EMT, and clear-cut activation of TGFβ 1 signaling in a general background of long-standing and permanent inflammatory state (Critelli et al., 2017).

The results of HCV DAAs-treated patients study, described above, were very recently published. Recurrent and de novo HCCs had significantly higher liver fibrosis scores, portal pressure, and systemic inflammation than non-recurrent HCC or patients never developing HCC. In recurrent/de novo HCC patients, tumor and non-tumor Angiopoietin-2 showed an inverse relationship with portal vein velocity and a positive relationship with liver stiffness. Baseline circulating VEGF and cirrhotic liver Angiopoietin-2 levels were significantly related. Angiopoietin-2 expression in the primary tumor or in cirrhotic tissue before DAAs was independently related with the risk of HCC recurrence or occurrence (Faillaci et al., 2018).

Conclusions

In conclusion, we have characterized the circulating and tissue components of the microenvironment of fast HCCs. Apart from redundant neo-angiogenesis, fast HCCs bear very unique features of distinctively immunosuppressed microenvironment, prominent EMT, and noticeable activation of TGFβ1 signaling in a general background of long-standing and permanent inflammatory state. This suggests that every effort should be made throughout the long natural history of patients with liver disease to switch off the inflammatory process, whatever the cause, as early as possible.

Secondly, our study findings suggest that DAAs are not per se able to determine the occurrence or recurrence of HCC, but that the DAA-mediated increase in VEGF acts as a trigger in predisposed patients, i.e., those with severe fibrosis and splanchnic collateralization, who already show high activation of neo-angiogenic pathways in cirrhotic tissue. The combination of the clinical and biologic risk factors that we have identified gives the unique possibility of selecting the patients at real risk of developing HCC after DAAs, without the need of holding back from treatment patients with cirrhosis at low risk of HCC.

Dr. Simone Pecorini

CEM Curriculum: Translational Medicine
Tutor: Prof. Andrea Cossarizza

INTRACELLULAR HIV-DNA LEVELS IN DIFFERENT CD4+ T CELL SUBSETS INDEPENDENTLY INCREASE IN VIROLOGICALLY-SUPPRESSED HIV+ PATIENTS WITH LOWER CD4:CD8 RATIO AND SHORTER TIME UNDER THERAPY

Background

CD4+ T cells represent the main reservoirs that support the HIV latent infection. Understanding the factors that influence the cell reservoirs in HIV is crucial, particularly, in patients with undetectable viremia, to develop new therapeutic strategies able to target these sanctuaries. In fact, HIV-DNA persists in cells of these patients, even after years of therapy. It is to note that CD4+ T cell subsets are different in terms of maturation, metabolism and, as consequence, homeostatic maintenance: signal joint TCR excision circle (sjTREC) and the length of telomere represent two validated markers that allow the evaluation of the cellular homeostatic proliferation. The combined approach of cell sorting and digital PCR permits to measure the content of HIV-DNA and sjTREC in naïve (TN), central memory (TCM) and effector memory (TEM) cells.

Objectives

Focusing on virologically-suppressed HIV+ patients under combined-antiretroviral therapy (cART), we aimed to clarify the role of key factors involved in homeostatic proliferation that could influence the intracellular viral reservoir in different CD4+ T cell subsets.

Methods

Thirty-two HIV+ patients were enrolled (mean age 49.0±7.2 years, 11 females), successfully treated for >2 years, with a CD4+ T cell count >500 cells/µL and undetectable plasma viremia from at least one year. After staining with fluorochrome-labeled monoclonal antibodies, TN, CM and EM CD4+ T cells were sorted with an S3e sorter (Bio-Rad, CA, USA) equipped with a specifically-designed biosafety containment hood (Biobubble, UK). Cell purity was always >95%. After DNA extraction, proviral HIV-DNA and sjTREC levels were quantified in each lymphocyte subset with QX200 droplet digital PCR (Bio-Rad); telomere length was quantified by using CFX9600 Real-time PCR (Bio-Rad) and plasma IL-7 and IL-15 levels were measured by ELISA. Viro-immunological parameters, such as CD4+ and CD8+ T cell counts and viral load, were collected for each withdrawal together with the clinical characteristics of patients.

Results

HIV proviral DNA, measured as LTR copies/1,000 cells, was significantly lower in TN cells (mean±SEM: 0.77±0.23) compared to CM (2.42±0.38) or EM (2.34±0.33), with $p<0.0001$ in both cases. Conversely, TN cells contained a higher number of sjTREC copies/1,000 cells (11.62±1.54) compared to CM (0.99±0.23) or EM (1.26±0.44); $p<0.0001$ in both comparisons. No significant

changes were observed in telomere length among CD4+ T cell subsets. After stratification of patients by different parameters, HIV-DNA content was higher in TN and TCM cells of patients with shorter time of treatment, or with lower CD4:CD8 ratio, in an independent manner. A similar but not significant trend was also observed in TEM cells. HIV reservoir was not influenced by other parameters, such as CD4+ T cell pre-therapy count and CD4+ T cell nadir.

Conclusions

We demonstrated that the length of treatment or the normalization of CD4:CD8 ratio (>1) independently correlate with the reduction of viral reservoir in both TN and TCM. These findings highlighted the importance of starting cART as soon as possible. Hence, to link cell sorting and digital PCR in an innovative method could be important for better comprehension of the features of HIV reservoir. Moreover, it could also be useful for bench translation to new therapeutic strategies.

Dr. Laura Anselmi

CEM Curriculum: Translational Medicine
Tutor: Prof. Sandra Marmiroli

RELATIONSHIP BETWEEN METABOLIC AND PI3K/Akt/mTOR SIGNALING FEATURES IN T-ACUTE LYMPHOBLASTIC LEUKEMIA (T-ALL) CELLS

Background

T-Acute Lymphoblastic Leukemia (T-ALL) is a heterogeneous malignant hematological disease, characterized by the abnormal accumulation of T-cell progenitors. Despite many efforts in designing novel treatment protocols, aggressive high-dose multiagent chemotherapy is currently the first therapeutic choice, and the outcome of primary chemoresistant and relapsed cases is still poor. The knowledge of T-ALL genetic alterations has significantly contributed to identify oncogenic drivers of its pathophysiology, which has opened the possibility of targeting cell signaling to prevent and/or treat relapse. In particular, T-ALL patients often display Notch1 mutations, as well as c-Myc overexpression and ectopic PI3K/Akt/mTOR signaling overactivation. These alterations frequently lead to reprogramming of metabolism, whereby cancer cells swap fuel sources to cope with boosted energy demand, enabling cancer cells rapid proliferation. However, possibly because of its heterogeneity, T-ALL remains poorly characterized from a bioenergetical point of view.

Objectives

Based on our previous experience and on the abovementioned recent findings, the broad aim of our study is to perform a comprehensive evaluation of the energy metabolism, as well as of the phosphoproteomic profiles, in order to find novel therapeutic protocols for T-ALL patients, designed according to their molecular hallmarks.

Specific aims: 1) To describe the signaling and metabolic profile of both primary cells from T-ALL patients and T-ALL cell lines, with particular attention to the role of PTEN and its phosphorylation on S380 of the C-terminal domain, frequently higher in cells carrying wild-type PTEN.

2) According to individual profiles from point (1), to examine whether combining signaling inhibitors (PI3K/mTOR or CK2 inhibitors) with glycolysis inhibitors modulates the profiles, obtained from the above analysis, and may represent an alternative therapeutic approach.

Methods

This objective is being pursued in preclinical models represented by fully characterized cell lines, primary cells from wt or mutant Notch1 pediatric T-ALL patient-derived xenografts (PDX) (in collaboration with the Paul O' Gorman Leukaemia Research Centre, Glasgow, UK), and primary cells from T-ALL patients. We analyzed the abovementioned cell models by reverse phase protein array (RPPA), using antibodies to key molecules of PI3K/Akt/mTOR and MAPK/ERK cascades. By means of specific inhibitors (the glucose analog/hexokinase inhibitor 2-deoxy-D-glucose, the dual

PI3K/mTOR inhibitor PF-4691502 and the CKII inhibitor CX-4945) we examined the responses to both individual and combined treatments, through viability assays. Specific inhibition and interesting checkpoints (as cleaved Notch1 and phospho-PTEN S380) were verified by Western Blot. We measured L-lactate secretion in the growth medium, indirect index of glycolytic rate, by a colorimetric assay. We further analyzed their metabolic profile using a Seahorse XFe96 Analyzer and two different types of assays (Mito Stress Test, Glycolysis Stress Test). A Gene Expression Profiling analysis, with particular focus on genes implicated in metabolism regulation downstream of main signaling pathways, was performed. Eventually, according to the stratification criteria derived from the above approaches, the best putative combinations of signaling and metabolic inhibition will be proposed.

Results

Overall, we confirmed the mutational analysis of PTEN and Notch1 status in the cell lines we used, which pointed out that PTEN wild-type cells display a high degree of PTEN phosphorylation at S380, indicating that, even if present, PTEN activity is highly inhibited. In a first qPCR analysis no significant differences are present in the expression of Notch1-2 receptors and Hes1. Our results, though preliminary, indicate that cells carrying both Notch1 and PTEN mutations display a higher signaling and a more glycolytic phenotype, compared to those with wild type and/or a single mutation. Besides, in these cells 2-DG and PF-4691502 show a higher cytotoxic effect. CX-4945 has a relevant impact on viability, in particular in the context of cells with high PTEN phosphorylation. Further insights into metabolic analysis in these cells, made possible by using a Seahorse XFe96 Analyzer, revealed that this inhibitor is able to let OXPHOS prevail over glycolysis. Overall, basal levels of extracellular acidification rate (ECAR) are comparable between distinct cell lines we analyzed, even though their response to the inhibitors is different, with cells harboring at least one mutation more prone to glycolysis inhibition. On the other hand, oxygen consumption rate (OCR) is remarkably different at basal levels, PTEN-mutated cells display high level of OXPHOS, and the effects of all the inhibitors, with particular attention to PF-4691502, are dramatic.

Conclusions

This project takes an important step towards precision medicine, aiming to provide new risk stratification strategies by evaluating mutational, metabolic and phosphorylomic background of primary cells from T-ALL patients, and to correlate these features with responsiveness of individual samples to treatment ex-vivo with specific modulators of signaling and metabolism, that are already tested in clinical trials for hematological or solid tumors.

Dr. Amelia Spinella

CEM Curriculum: Translational Medicine
Tutor: Prof. Clodoveo Ferri

CARDIO-PULMONARY DISEASE MANAGEMENT IN PATIENTS WITH SYSTEMIC SCLEROSIS: CARDIO-RHEUMATOLOGY CLINIC AND PATIENT CARE STANDARDIZATION PROPOSAL

Background

Systemic sclerosis (SSc) is a chronic connective tissue disease characterized by endothelial dysfunction, dysregulation of fibroblasts with excessive fibrosis of the skin and internal organs, as well as autoimmune abnormalities. Cardiopulmonary involvement is common in SSc: pulmonary fibrosis, pulmonary arterial hypertension (PAH), and electrical disorders are the most serious complications and frequent causes of death. In spite of the increasing recognition of these severe manifestations and new therapeutic strategies, the diagnosis is often delayed with consequent unfavorable outcome and poor overall prognosis. The detection of these complications in the early stage of the disease as well as their careful monitoring and follow-up are mandatory in order to counteract their impact on the disease outcome. Despite the need and importance of establishing a proper methodology, literature provides few reports about this issue.

Objectives

The aim of the present study was to analyse the activity of our Cardio-Rheumatology Clinic in order to optimize diagnostic management of cardio-pulmonary disease in SSc patients.

Methods

We retrospectively analyzed data from 350 consecutive SSc patients referred to our University-based Rheumatology Centre (F/M 308/42; lcSSc/dcSSc 45/305; mean age 50.8 ± 14.7 years; mean disease duration 10.9±7.0 years). The diagnosis of SSc was done by an expert rheumatologist based on a panel of clinical, serological and capillaroscopic data. Moreover, all subjects satisfied the new ACR/EULAR classification criteria for SSc. All patients underwent general (demographic and clinic-serological features) and cardio-pulmonary assessment, in particular they were evaluated in the Cardio-Rheumatology Clinic. The following parameters were considered: physical examination; blood pressure; past and current drugs; blood tests, including Erythrocyte sedimentation rate-ESR, C-reactive protein-CRP, blood cell count, hemoglobin, serum creatinine, transaminases, CPK enzymes, troponin, NT-pro-BNP, D-dimer, serum autoantibodies, 25-OH-vitamin D; pulmonary function tests; high resolution scan of the lungs (HRCT); standard electrocardiogram (ECG) and 24-Hour Holter ECG monitoring; Doppler echocardiography; cardiac stress test; coronary angiography and right heart catheterization (RHC); cardiac MRI and CT; vascular ultrasound, with main attention to carotid intima-media thickness, carotid-femoral pulse-wave velocity (cf-PWV) and brachial-ankle PWV (ba-PWV). The clinicians decided to perform these examinations according to clinical picture and current methodologies. Examination of time between the request and the assessment was measured in detail as indicator of process-of-care quality.

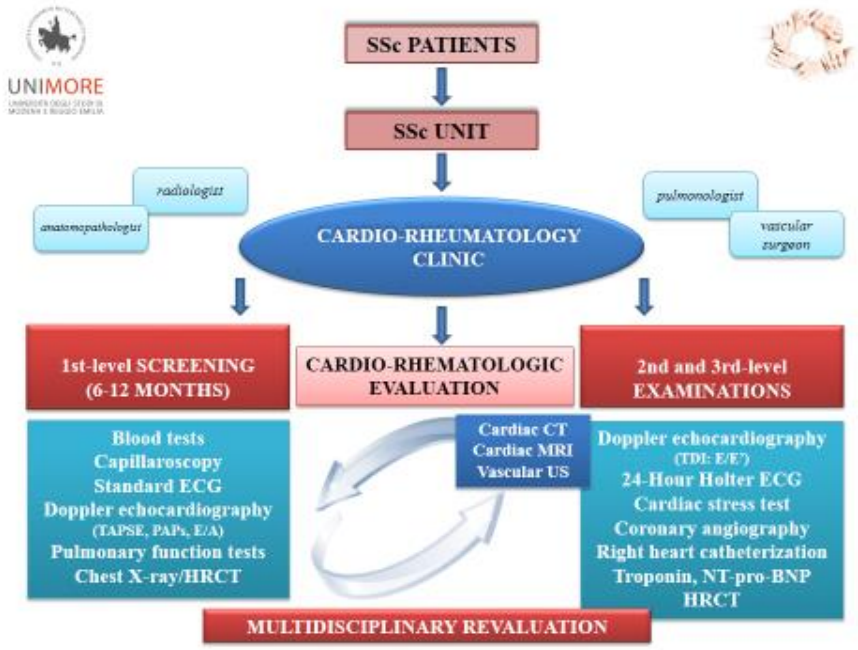
Results

In the last 12 months we assessed 300 patients with 1st-level screening (cardiologic and rheumatologic evaluation, standard ECG, Doppler echocardiography, pulmonary function tests, thoracic imaging). Among 2nd-level, 30 procedures of 24-Hour Holter ECG and 15 right heart catheterization tests were performed. Cardiac MRI, coronary CT angiography and vascular ultrasound were assessed, when requested, as 3rd-level examinations (30 procedures). After one year we reported that the overall mean time interval between request and evaluation was 10±5 days, 20±12 days for 1st-level screening, 25±15 days to execute the 2nd-level examinations, and direct access to the 3rd-level procedures. Figure 1 shows Cardio-Rheumatology clinical algorithm for the management of cardiovascular and cardio-pulmonary disease in SSc.

Conclusions

The activity of Cardio-Rheumatology Clinic tries to optimize the cardio-pulmonary assessment, determining an early detection of these harmful complications with reduced waiting times which are critical issues. Screening algorithms and non-invasive diagnostic tests are useful to stratify the risk and to establish the most appropriate diagnostic-therapeutic protocols, improving outcome and quality of life of scleroderma patients. The development of a cardio-pulmonary risk score and the standardization of a patient care approach, according to international quality indicators, could represent further tools to improve management and care for SSc patients.

Figure 1



Dr. Isabella Campanini

CEM Curriculum: Public Health
Tutor: Prof. Annalisa Bargellini

RISK OF FALL EVALUATION IN REHABILITATION SETTINGS AND IN ELDERLY INPATIENTS

Background - Risk of fall in rehabilitation settings

Falls are the first cause of adverse events in patients during hospitalization. Commonly known consequences that typically influence, and therefore determine higher costs, are reduced autonomy, fractures and even death of the patients. Fall risk assessment has been made mandatory in the guidelines issued by Regione Emilia Romagna. The first PhD project focused on the fall risk assessment in rehabilitative inpatients. In literature there are many risk assessment tools but none have been tested when taking into consideration the rehabilitation settings. Out of all these diagnostic tools, we tested the Hendrich Fall Risk Model II (HIIFRM). This showed satisfactory feasibility and predictive performances in the rehabilitation wards. Following PhD project step was to investigate both the fall rate and the predictability in patients living in community-dwellings after being discharged from the rehabilitation wards.

Objectives

The aim of this study, when evaluating predicted falls, is to determine the predictive performance of the HIIFRM administrated at discharge from the rehabilitation wards.

Methods

This prospective observational study was conducted during a 6-month period at the AUSL-IRCCS of Reggio Emilia. All patients discharged from the rehabilitation settings (Orthopedic, Pulmonary, Neurological Rehabilitation Wards) were enrolled. The patients' risk of falling was evaluated by means of HIIRFM. This was administrated during their last day of hospitalization. Six months after discharge, each patient was contacted by a standardized telephone call interview in order to evaluate the occurrence and the causes of actual falls after their return home.

Data analysis: predictive power of the HIIFRM was obtained as the area under the ROC curve. Sensitivity and specificity of the scale were computed along with their 95% confidence intervals (95%CI). According to HIIFRM threshold subjects were classified at risk of falling when their score was ≥ 5.

Results

A total of 145 patients was evaluated at discharge. Of these, 89 (35M, 54 F; age range 16-92 years) responded to the telephone interview providing comprehensive information to be included in the study. The remaining 56 subjects were respectively: admitted to other unknown healthcare facilities (23%), we were unable to get in touch with them (19%), they were subjected to bed rest conditions and were unable to answer (9%), and the remainder simply refused to answer the questions (6%). Eighteen of the 89 interviewed patients (rate=20%) fell at least once during the 6-

month period. Approximately, one third of these patients (28%) suffered fractures subsequent to the fall.

Predictive performance of HIIFRM was computed based on 18 falls and 89 subjects. The area under the curve was AUC=0,686 (IC 95% 0,556-0,817), thus indicating a moderate predictive power of the scale. The results obtained using the HIIFRM standard cutoff score were a sensitivity of 67% (IC95% 42-86), a specificity of 65% (IC95% 52-76), a positive predictive value of 32% (IC95% 18-50) and a negative predictive value of 88% (IC95% 76-95).

Conclusions

Patients discharged from rehabilitation wards are considered at high risk of fall (20%). The fracture rate in our sample (28%) was higher than the rate reported in literature for the elderly living in community-dwellings, which typically ranges between 7 and 15%. These rates suggested that risk fall assessment is an appropriate tool for rehabilitative patients at discharge, along with other prevention strategies. In literature, rehabilitative patients that are not considered “elderly” per se are typically more active and therefore also tend to engage in riskier everyday activities. HIIFRM scale can be a useful tool for fall risk assessment in rehabilitative patients at discharge. Further studies are needed to increase the sample size.

Risk of fall in elderly inpatients. The final part of the PhD project regarding risk assessment in elderly inpatients is still underway. A comprehensive checklist (CL) of evidence-based risk factors was produced. This included the items of HIIFRM and the items listed by the Regione Emilia Romagna guidelines on fall prevention. These guidelines require the evaluation of all inpatients over 65 (nearly 75% of all inpatients at our AUSL facilities) by means of a CL of all known fall risk factors and this is followed by interventions on each and every positive factor. This entails a substantial commitment of public resources, subtracting time and manpower for the everyday clinical routine. The identification of patients at high fall risk would allow professionals to focus on a limited number of patients, avoiding unnecessary interventions and would improve fall prevention. The aim of the study is to develop and validate a case-mix tailored procedure based on CL items to classify the elderly patient’s fall-risk status at admission. By the beginning of June 2018 all over 65 inpatients, admitted to our selected AUSL-IRCCS of Reggio Emilia wards with high fall risk rate, will be assessed by means of the CL. A predictive capability study will be carried out in six months’ time. Within this time frame, more than 5000 patients with > 250 falls are to be expected. A logistic regression will be computed on ¾ of available data to create the classifier. This will be tested on remaining ¼ of data.

Dr. Eleonora VANDINI

CEM Curriculum: Translational Medicine
Tutor: Dr. Alessandra Ottani
CoTutor: Prof. Daniela Giuliani

STUDY ON PROTECTIVE ACTION OF HYDROGEN SULFIDE IN A MOUSE MODEL OF ALZHEIMER'S DISEASE

Background

Alzheimer's disease (AD) is a chronic disorder characterized by progressive neurodegeneration associated with cognitive decline and several behavioral deficits.

In some regions of AD brains, such as cortex and hippocampus, presenilin-1 (PS1) and presenilin-2 (PS2), members of the γ-secretase complex, and β-secretases process the amyloid precursor protein (APP) to generate extra-cellular β-amyloid (Aβ) fibrillar deposits (Aβ plaques); moreover, intraneuronal tau neurofibrillary tangles composed of hyperphosphorylated tau protein develop. Both these hallmark lesions trigger pathophysiological pathways that lead to synaptic dysfunction, neurodegeneration and marked neuronal loss with consequent impairment in cognitive functions. Furthermore, free radicals, nitric oxide, glutamate, several cytokines, mitogen-activated protein kinases, Bcl-2 family members and caspases play an important role in the above mentioned pathophysiological pathways. A combination of impaired cholinergic transmission and high glutamate activity underlies the main symptomatology of AD, which is characterized by memory loss and severe cognitive decline [1].

Hydrogen sulfide (H2S) is a colorless, flammable, water-soluble gas and Tabiano's spa-waters are particularly rich in H2S (strong sulfydrometric degree, that is, more than 100 mg/l) [2]. H2S is increasingly being considered as an important signaling molecule in various body systems, and accumulating evidence demonstrates that H2S donor compounds exert significant beneficial effects in several animal models of inflammation and ischemia/reperfusion injury [3]. H2S is endogenously produced also in the brain, probably exerting a neuromodulatory role. It has been previously reported in literature that brain H2S synthesis is severely decreased in AD patients, and plasma H2S levels are negatively correlated with the severity of AD [4]. Recent data showed that the H2S donor sodium hydrosulfide reduces Aβ generation in cultured cells, and Aβ-induced cognitive impairment in rats, as detected in a short-term study [5]. Further, inhaled H2S has resulted to be able to prevent neurodegeneration in a mouse model of Parkinson's disease [6].

Objectives

The aim of my research project has been to evaluate the possible neuroprotective effects of a long-term treatment with sodium hydrosulfide -an H2S donor- and Tabiano's spa-water, rich in H2S, to counteract the progression of early AD.

Methods

An AD mouse model, harboring human transgenes APPswe, PS1M146V, tauP301L (3xTg-AD mice) at the age of 3 months at the start of the study, was used. Animals were treated for 3 months with

sodium hydrosulfide (NaHS) and Tabiano's spa-water, intraperitoneally, at doses of 0.5 mg/Kg and 12 ml/Kg respectively. In this study we investigated learning and memory, amyloid/ tau cascade, excitotoxic, inflammatory and apoptotic responses.

Results

Animals were significantly protected against impairment in learning and memory by treatment with H2S and spa-water. This improvement in behavioral performance was associated with the hippocampus size of β-amyloid (Aβ) plaques and the preservation of brain morphology. Furthermore, lowered concentration/phosphorylation levels of amyloid precursor protein (APP), presenilin-1 (PS1), Aβ1-42 and tau phosphorylated at Thr181, Ser396 and Ser202 were detected in 3xTg-AD mice treated with spa-water. These proteins, in fact, are considered the central events in AD pathophysiology. Likewise, in 3xTg-AD mice treated with spa-water, the levels of malondialdehyde and nitrites were decreased, showing that oxidative and nitrosative stress were counteracted. In the hippocampus of these treated mice, we found a reduction of activity of c-jun N-terminal kinases, extracellular signal-regulated kinases and p38, which have an important role not only in phosphorylation of tau protein but also in inflammation and apoptosis. Consequently, levels of tumor necrosis factor-α (TNF-α) were decreased, while Bcl-2 was up-regulated and BAX and caspase-3 were down-regulated in the hippocampus of 3xTg-AD mice treated with Tabiano's spa-water, suggesting the ability to modulate inflammation and apoptosis.

Conclusions

These favorable results would suggest that appropriate treatments with H2S donors or spa waters rich in H2S might represent an innovative approach to slow down AD progression in humans.

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Dr. Francesco Fontana

CEM Curriculum: Translational Medicine
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PRECLINICAL VALIDATION OF A WEARABLE PERITONEAL DIALYSIS DEVICE: IN VITRO EFFICACY AND BIOCOMPATIBILITY

Background

End Stage Renal Disease (ESRD) epidemic is steadily expanding, with maintenance dialysis prevalence rates peaking at almost 2000 individuals per million population in industrialized countries. Peritoneal dialysis (PD) represents today the main choice for home renal replacement treatment for patients with ESRD. Weakid (WK) is a wearable/portable PD device developed by Nanodialysis (Oirschot, The Netherlands) which redefines treatment by using a continuous recirculation of dialysate in the peritoneum in a tidal mode (instead of a static filling) and by employing a combination of new sorbent technology for toxins removal based on nanomaterials and electro-catalytic urea oxidation (EO). A small volume of dialysate is continuously recycled and refreshed; no large amount of sorbents is needed since they can be easily regenerated. The continuous mode of dialysis provides a stable internal environment and reduces the number of exchanges (time-consuming and at risk for peritoneal infection).

Objectives

Eurostars project Minikid, founded by "Fondazione Cassa di Risparmio dell'Emilia Romagna", had the objective to assess in vitro efficacy and biocompatibility of the WK device.

Methods

Two batches of spent peritoneal dialysate from a single patient were treated by WK in continuous flow for 8 hours with EO-on (four parallel units, 6.5A, 3.5V) and with EO-off. Sorbents composition was as follows: active carbon ≈ 1000g, potassium (K+) resin 180g, phosphate (PO43-) resin 100g. Treated peritoneal dialysate batches were sampled at different time points; samples were frozen after endotoxins exclusion. For efficacy testing, the removal of Urea, Creatinine, PO43-, K+, □2-microglobulin (□2m) from dialysate were assessed. In order to evaluate cytotoxicity, peripheral blood mononuclear cells (PBMCs) and human mesothelial cells (Met-5A) were incubated with a solution containing 50% of treated dialysate, both with EO-on and off; controls consisted in fresh dialysate, 50% PBS/50% RPMI medium, 100% RPMI medium. Flow cytometry assessments of cell viability and death were performed using Annexin V and TO-PRO-3 Iodide for PBMC, Annexin V and Propidium iodide for Met-5A. In addition, a scratch wound healing assay was used to assess cell migration in Met-5A cultures. After 24h of culture in complete medium, a "scratch'' was created in the cellular monolayer; cells were incubated with previously described solutions, and were photographed at different time points up to 72 hours. Scratch area was calculated with cellB software (Olympus).

Results

The PD effluent after 8 hours of treatment with WK device was endotoxin free. WK removed 140.43 mmol of urea with EO-on and 167.39 mmol of urea with EO-off, corresponding to a Urea Reduction Rate (URR) of 53.37% and 65.66%, respectively. The latter value meets the target for a single hemodialysis session (no such target is available in PD); considering the continuous mode of treatment of WK, results on URR are extremely promising. WK also showed very good removal of small molecules: creatinine (14.85 mmol with EO-on and 14.68 mmol with EO-off, for a creatinine reduction rate (RR) of 98,31% and 97,29% respectively), PO43- (12.44 mmol with EO-on and 10,64 mmol with EO-off, for a PO43- RR of 95.01% and 94.34% respectively), K+ (36.34 mEq with EO-on and 31.77 mEq with EO-off, for a K+ RR of 69,52% and 59,96% respectively). Middle molecules were also nicely removed, as evidenced by 2m dosing (2m RR 95.81% both with EO-on and off). Data were in line with previous experiments performed elsewhere. Dialysate treated with EO-on for 8 hours affected strongly cell viability at four days cultures (only 4% PBMCs and 1% Met-5A cell viability); there was an incremental effect on cell toxicity with increase in dialysate treatment time (starting from 4 hours). Dialysate treated with EO-off for 8 hours had no effects on cell viability (results were comparable to controls). Met-5A cells incubated with dialysate treated with EO-on showed a reduced wound closure capacity; dialysate treated with EO-off apparently had no effects on cells wound closure capacity.

Conclusions

WK showed good small and middle molecules removal from spent peritoneal dialysate both with EO-on and off. EO appeared to affect cell viability (on both PBMCs and Met-5A) and cell wound closure capacity (on Met-5A); on the contrary, peritoneal dialysate treated with EO-off had no effects on cell viability and wound closure capacity. Although promising in terms of urea removal, biocompatibility of EO should be questioned. Further studies are needed in order to elucidate mechanisms of EO cytotoxicity.

Dr. Anna Maria Costa

CEM Curriculum: Translational Medicine
Tutor: Prof. Giuseppe Biagini

A HYDROXYPYRONE-BASED INHIBITOR OF METALLOPROTEINASE-12 DISPLAYS NEUROPROTECTIVE PROPERTIES IN *STATUS EPILEPTICUS* MODEL

Background

Matrix metalloproteinases (MMPs) are endopeptidases whose expression is mainly triggered in pathological conditions involving inflammation. Matrix metalloproteinase-12 (MMP-12), also known as macrophage metalloelastase or macrophage elastase, is able to degrade a wide variety of extracellular matrix components, such as laminin, type IV collagen, fibronectin, chondroitin sulfate and vitronectin. By degrading basal membrane components, MMP-12 permits the entry of macrophages and other immune cells into injured tissues during inflammation. MMP-12 has a long-established role in the pathogenesis of respiratory inflammatory diseases, but only recent studies highlighted the detrimental role of this metalloproteinase in cerebrovascular diseases. Recently, we showed that MMP-12 is highly expressed in microglia and myeloid infiltrates, which are presumably involved in blood-brain barrier (BBB) leakage and subsequent neuronal cell death that follows status epilepticus (SE).

Objectives

In the current study, we used an hydroxypyrone-based MMP-12 inhibitor to test whether specific MMP-12 inhibition may represent a putative drug against neuronal cell death occurring after SE.

Methods

We assessed the effects of a hydroxypyrone-based inhibitor specific for MMP-12 in the pilocarpine-induced SE rat model to determine hippocampal cell survival.

Results

We showed that intra-hippocampal injections of the MMP-12 inhibitor protect CA3 and hilus of dentate gyrus (DH) neurons against cell death and reduce the extent of the ischemic-like lesion that typically develops in the stratum lacunosum-moleculare of the hippocampus. Moreover, we demonstrated that MMP-12 inhibition suppresses IgG and serum albumin extravasation into brain parenchyma.

Conclusions

Overall, these results support the hypothesis that MMP-12 inhibition can directly counteract neuronal cell death and that the specific hydroxypyrone-based inhibitor used in this study could be a potential therapeutic agent against neurological diseases/disorders characterized by an important inflammatory response and/or neuronal cell loss.

Dr. Giulia Lancellotti

CEM Curriculum: Translational Medicine
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FRACTURES IN A COMMUNITY-DWELLING ELDERLY POPULATION: THE ROLE OF DYNAPENIA

Background

Dynapenia, the loss of muscle strength as assessed by the hand grip test, has been associated with negative outcomes, including disability and falls, in the elderly population. The incidence of falls increases with aging and they can have serious and disabling consequences such as fractures. Among the community-dwelling elderly population prevention of falls is mandatory because of the extremely relevant individual, social and economic impact of fractures.

Objectives

Aims of the study are: 1) to detect the prevalence of dynapenia in a community-dwelling elderly population; 2) to determine the incidence of falls and fractures at 1-year follow-up; 3) to identify the factors associated with dynapenia, falls and fractures.

Methods

We carried out a prospective study enrolling 268 community-dwelling subjects aged over 65 years. All of them could walk alone for 10 meters and were not known to have cognitive impairment. Patients underwent a multidimensional geriatric assessment and data about autonomy, comorbidity, drugs, nutritional status, depressive symptoms and cognitive performance, were registered. The following tests/scores were applied: IADL (Instrumental Activities of Daily Living), CIRS (Cumulative Illness Rating Scale), BMI (Body Mass Index), MNA (Mini Nutritional Assessment), GDS (Geriatric Depression Scale), ACE-R (Addenbrooke's Cognitive Examination), orthostatic hypotension test and hand grip strength test. The hand grip test was performed using a validated dynamometer and according to the American Society of Hand Therapists protocol. The criteria considered for the diagnosis of dynapenia were <30 kilograms (kg) for males and <20 kg for females, as stated by the European Working Group on Sarcopenia in Older People. The occurrence of falls and their consequences, such as fractures, were registered quarterly till one year of follow-up.

Results

The study population mean age was 76±6 years (63% females) and the subjects were largely autonomous (IADL 5±2/8) and cognitively preserved (ACE-R 84±11/100). CIRS severity index was 1,7±0,3. The prevalence of diabetes, history of stroke, Parkinson disease were respectively 14%, 18% and 22%. The number of regularly used drugs was 6±3 and 67% of the patients regularly used ≥1 fall-risk-increasing drugs (diuretics, antidepressants, hypnotics, neuroleptics, antiepileptics, opioids). Nutritional status resulted in normal ranges: MNA 26±2, BMI 27±4. Depression

prevalence was 45%. Orthostatic hypotension was registered in 17% of the sample. Dynapenia was found in 34% of the community-dwelling elderly population.

The 1-year follow-up showed a falls incidence of 53%. Post-traumatic fractures were observed in 11% of the sample.

Dynapenia resulted significantly and positively associated with age, CIRS, diabetes, number of drugs, depression, falls and fractures. Whereas dynapenia was significantly and negatively associated with cognitive performance, functional and nutritional status. The multivariate analysis identified age, IADL, diabetes and fractures as independent predictors of dynapenia.

Falls resulted significantly and positively associated with the number of drugs, the use of ≥1 fall-risk-increasing drugs, CIRS, dynapenia, diabetes, Parkinson disease; whereas a negative correlation was found with cognitive performance and history of stroke. The multivariate analysis identified CIRS, Parkinson disease, history of stroke and cognitive performance as predictors of falls during the 1-year follow-up.

Fractures were significantly associated with dynapenia, cognitive performance and the use of ≥1 fall-risk-increasing drugs. Only dynapenia was an independent predictor of fractures at multivariate analysis.

Dynapenia resulted inversely associated with both fall-free and fracture-free survival at 1 year (Log Rank: p=0.029 and p=0.004 respectively). At the Cox Regression after adjustment for other factors associated to these outcomes, dynapenia was an independent predictor of fractures (p=0,035), but not of falls.

Conclusions

The incidence of falls and fractures at 1 year follow-up in our community-dwelling elderly population is 53% and 11% respectively, and the prevalence of dynapenia is 34%. Parkinson is the disease associated with the highest risk of falls. Dynapenia is an independent predictor of fractures, and diabetes is the condition associated with the highest risk of dynapenia.

The oldest old patients, those with disabilities, or subjects affected by diabetes or Parkinson disease, have a higher risk of dynapenia and falls and therefore also of negative outcomes such as fractures. For this reason they must be considered the target population who should promptly receive multidisciplinary interventions such as physical therapy, nutritional program, and polypharmacy reduction, with the aim of preventing dinapenia, falls and fractures, in order to avoid disability.

Dynapenia is a well-known predictor of negative outcomes such as disability, mortality and falls. This study shows that dynapenia is also an independent predictor of fractures and this is extremely relevant in clinical practice. In fact, dynapenia is a preventable and easily detectable condition and as a consequence it could be considered for fracture risk screening. Future research should introduce Hand Grip strength test in fracture risk evaluation.

Dr. Cinzia Puzzolante

CEM curriculum: Translational Medicine
Tutor: Prof. Cristina Mussini

PET-CT SCAN ROLE IN EARLY EVALUATION OF CLINICAL SUCCESS IN NATIVE VERTEBRAL OSTEOMYELITIS

Background and Aims

Native vertebral osteomyelitis (NVO) is a rare, potentially serious disease with long-term sequelae. In this setting the role of Positron-Emission Tomography/Computed Tomography (PET-CT) scan in predicting the outcome of patients with NVO is still unclear. The aim of my research project was to determine if PET-CT Standardized Uptake Value (SUV) change evaluated at baseline (SUVt0) and at week 2 (SUVt15) could predict the length of antimicrobial therapy (ABTx) in patients with NVO.

Materials and methods

We performed a retrospective and prospective since 2016, observational study enrolling patients with diagnosis of bacterial NVO referring to ID Clinic in Modena. For this study were collected: demographic, clinical and microbiological data, SUVt0 and SUVt15, C-reactive protein (CRP) values at baseline and at week 2. PET improvement was defined as SUVt15 decrease ≥ 25% from baseline. "Response" was defined as clinical cure within 6- (from 2015 IDSA guideline) or 12-weeks of ABTx and "Delayed response" as the prolongation of ABTx >6 (from 2015) or >12 weeks of ABTx. We performed a first analysis collecting all cases of NVO with PET performed from January 2010 to June 2016 regardless of aetiology (bacterial, fungal, tubercular). To obtain more homogeneous data we performed a second sub-analysis including only bacterial NVO collected from January 2010 to March 2018; in this sub-group CRP values at day 0, 15, 30 were also recorded.

Results

In the first analysis 46 cases of NVO with PET performed were recorded between 2010-2016; 32/46 (69.5%) had a PET-CT scan performed at week 2 and were included into the first analysis (1 fungal NVO, 1 TB NVO, 16 Gram + and 6 Gram – NVO, 7/8 cases of unknown aetiology treated as bacterial and 1/8 as TB NVO). Mean SUVt0 value was 7 (SD=±3.6) with no difference between TB and non-TB NVO (p=0.948). No significant correlation between SUVt15 decline and clinical "Response" was found both in patients with microbiological diagnosis (p=0.388) and in patients without aetiological diagnosis (p=1.000).

In the sub-analysis including only bacterial NVO, 35 NVO cases were collected: 22 due to Gram +, 6 due to Gram -, 7 of unknown aetiology treated as pyogenic NVO. Median [IQR] SUVt0 value for known and unknown NVO was 6 [4.7-8.5] and 5 [4.2-6.7] respectively. SUVt15 decline occurred statistically more often in the "Response" group (p=0.007) compared to the "Delayed response"

group (p=0.445). CRP decline at week 2 was significant in “Response” group (p=0.040) with a similar trend was in the “Delayed response” group (p=0.059); at day 30 no difference in CRP decline was noted within and between the “response” and “delayed response” group. Regardless of clinical response, SUVt15 decline in Gram-positive NVOs was more rapid compared to Gram-negative NVOs.

Conclusion

In the setting of NVO the role of CRP and nuclear medicine in diagnosis and follow-up is still unclear. In our first analysis, SUVt15 did not correlated with clinical cure in the whole population of NVOs. On the contrary, in a more homogeneous population of bacterial NVOs a decrease of SUVt15 of at least 25% from baseline correlated with a shorter course of ABTx also when considering a 6-weeks course of ABTx as suggested in a recent randomized trial. In our population CPR decline was significant at week 2 in the “response” group; a similar trend was present also in the “delayed response” group. At day 30 no difference in CRP decline was observed within and between the two groups confirming CRP’s poor predictive value in NVO setting. Finally we observed a more rapid decrease in SUV uptake after 2 weeks of appropriate ABTx in patients with Gram + NVO compared to Gram – NVO that might suggest a different 18F-FDG uptake.

In conclusion, in our population PET-CT improvement defined as SUVt15 decline ≥ 25% from baseline may be considered a valid tool for early assessment of clinical response in patients with pyogenic NVO.

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Dr. Stefania Guida

CEM Curriculum: Translational Medicine
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NEW INSIGHTS INTO PATHOPHYSIOLOGICAL MECHANISMS OF SKIN AGING

Background

Skin physiology includes the regulation of several activities. These activities comprise pigmentation, proliferation and immunity. A disruption of regulatory mechanisms can occur with passing years, leading to the development of different types of skin changes.

Objectives

The main purpose of the research is to explore the effect of some gene polymorphisms on skin variations related to the aging process, in order to provide possible correlations between genetic background and morphological variations of both epidermis and dermis.

Methods

A total of 100 women were enrolled in this study, after achieving informed consent. Gene polymorphisms analysis were performed. Reflectance confocal microscopy (RCM) and optical coherence tomography (OCT) images were collected and analysed for all subjects. A statistical evaluation was carried out in order to estimate the correlation between genetic status and morphologic variations.

Results

A significant correlation between the genetic background and morphologic variations, as revealed by means of non-invasive skin imaging techniques, was found. These results enable the identification of two main patterns of skin aging.

Conclusions

This is the first research exploring the potential correlation of genetic polymorphisms and specific skin features related to skin aging, as revealed by non-invasive skin imaging. These correlations enable the identification of two main different models of aging in subjects with different skin phototypes.

Dr. Stefania Paduano

CEM Curriculum: Health sciences
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THERMAL WATER AND MUD: A PILOT STUDY ON THE MICROBIOME CHARACTERIZATION FROM SPRING TO POINTS OF USE

Background

The use of thermal water for therapeutic or recreational purposes is a tradition dating back to Roman times[1,2,3]. Their specific therapeutic properties depend on chemical and physical characteristics but also on the microbial diversity of these water[3]. Thermal muds (peloids) are produced by mixing clayey materials with thermo-mineral waters. They have peculiar healing properties depending on the kind of clay minerals, the physico-chemistry of the thermal water and the growth and colonization of microorganisms during the maturation process1.

The diffusion of Next Generation Sequencing (NGS) and bioinformatics tools offers the opportunity for a more extensive approach for examining the microbial diversity of these matrices[4,5].

In literature, the microbial diversity of hot spring water has been extensively investigated[4,6], whereas studies focusing on thermal water distribution network and mud of spa centres are lacking.

Objectives

The aim of our study is to characterize by Next Generation Sequencing (NGS) technologies the microbial community of sulphurous-bromine-iodine thermal water and mud inside an Italian spa complex. This is a pilot study concerning the microbiome characterization along water network from spring to points of use.

Methods

The study is conducted on a sulphurous water with high concentrations of sodium chloride, bromine and iodine, with various therapeutic properties. Hot spring water gushes out at temperature of 69°C. Thermal water from the three drilling wells and from the spring is blended into a mixing plant and then distributed to hotels and spas by an aqueduct. In this thermal facility, muds are prepared in situ by maturation of clayey virgin materials mixed with sulphurous water.

We collected 12 water samples from spring, wells, tanks and points of use. Samples were analysed by NGS technologies in collaboration with University "Foro Italico" of Rome. Briefly, DNA was extracted, amplified using primers specific for 16S rDNA and subsequently sequenced through Illumina MiSeq platform. Bioinformatic analysis was performed with 16S Metagenomics app, while microbial biodiversity was computed through EstimateS software. Mud samples were collected from outdoor pools at 3 different stages of maturation (young, intermediate and mature) and analysed by NGS technologies as described above.

Results

Our data highlight the presence of a microbial community in line with physicochemical characteristics of this thermal water, mostly constituted by sulphur-cycling bacteria belonging to Desulfomonile, Thermodesulfovibrio, Geothermobacterium, Thermus, Thiofaba and Syntrophomonas genera. A progressive transformation of microbial community from spring to distal points is observed.

The characterization and evolution of bacterial community of muds during maturation process have been studied. The most important finding is that the microbiome of mature mud is dominated by bacteria, such as Pelobacter spp, able to participate to biosynthesis of lipids, already known as responsible for anti-rheumatic properties of thermal mud.

Conclusions

Our investigation approach based on the use of NGS technologies for microbiome characterization in both thermal water and muds can significantly improve our knowledge on the relationship between microbial diversity of a thermal spring and its peculiar therapeutic and cosmetic properties. Moreover, in this preliminary study no genera enclosing opportunistic pathogens were detected. However, sequencing-based microbial community analysis can provide new information on the unexpected presence of waterborne opportunistic and pathogenic bacteria useful to select control measures aimed to guarantee the best water quality and safety for persons attending the thermal facilities.

Full paper In press Paduano S, Valeriani F, Romano-Spica V, Bargellini A, Borella P, Marchesi I. Microbial biodiversity of thermal water and mud in an Italian spa by metagenomics: a pilot study. Available Online 25 October 2017, ws2017209; Water Science & Technology Water Supply doi 10.2166/ws.2017.209.

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Dr. Angelo Territo

CEM Curriculum: Translational Medicine
Tutor: Prof. Giampaolo Bianchi
CoTutor: Prof. Salvatore Micali and Dr. Alberto Breda

THE EUROPEAN EXPERIENCE ON ROBOT-ASSISTED KIDNEY TRANSPLANTATION: MINIMUM OF ONE-YEAR FOLLOW-UP

Background

Open kidney transplantation (KT) is the gold standard treatment for patients with end-stage renal disease. In order to reduce the morbidity of the open KT, a robot-assisted approach has been recently introduced. Nowadays, several European Centers perform robot-assisted kidney transplantation (RAKT). Therefore, an European Robotic Urology Section (ERUS) group was created in March 2016 with the aim to collect data on RAKT. No study, however, has previously evaluated outcomes in RAKT patients after one year of follow-up or compared kidney function in the immediate postoperative period and after one year; the present study was designed to address these issues.

Objectives

The aim of this study to evaluate functional results, graft survival, and late complications in patients who underwent RAKT, with a minimum follow-up of one year. Furthermore, the correlation between surgical data and functional results at a minimum 1-year follow-up and the correlation between renal function in the immediate postoperative period and after one year were analyzed.

Methods

A common prospective recruitment database of RAKT was created by the ERUS RAKT working group, including eight different European centers. In each center, RAKTs were performed from living donation. Data on demographic variables, surgical results, graft survival, functional outcomes [creatinine and glomerular filtration rate (eGFR)] at postoperative days (POD) 7 and 30 and at 1 year, and late complications were extracted from the common database.

Results

A total of 147 RAKTs were performed by the ERUS RAKT working group. Of the 147 patients, 83 had a minimum one-year follow-up (mean 21 months; range 13–27). The patients' demographic characteristics were: 30 female and 53 male patients, median age 43 years (range 30–75), median BMI 25.3 kg/m2 (range 20–40), median pre-transplantation serum creatinine and eGFR 517 μmol/L (range 198–1414) and 10 ml/min per 1.73 m2 (range 3–29). Of the 83 cases, 46 were pre-emptive. Overall ischemia time was 116 min (range 53–377). The average rewarming time was 60 min (range 35–110). At one-year follow-up, the median serum creatinine was 131 μmol/L (range 66–244) with a median eGFR of 57.4 ml/min per 1.73 m2 (range 28–97) (figures 1 and 2). There

was no statistically significant difference between functional data at POD 30 and at 1 year for creatinine (p=0.78) or eGFR (p = 0.91). Regarding the correlation between the surgical data and the functional outcomes, the data showed that overall operative time and rewarming time did not affect the graft function at one year. Regarding graft survival, three cases of graft loss occurred due to massive arterial thrombosis within the first postoperative week. Late complications comprised one case of ureteral stenosis and one case of graft pyelonephritis. No late vascular complications or cases of incisional hernia were recorded.

Conclusions

Findings at 1-year follow-up indicate RAKT from a living donor to be a safe procedure in a properly selected group of recipients. It seems to provide a low complication rate with maintenance of excellent graft survival and function. This is the first and largest report with a minimum follow-up of one year on functional results after RAKT from a living donor.

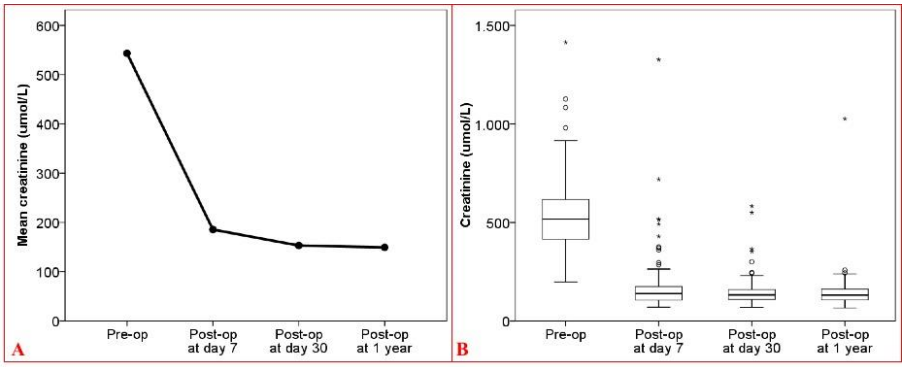

Figure 1. A: Trend with respect to serum creatinine over time. Values are shown at four time points: preoperative, POD 7, POD 30, and 1 year. B: In each box plot, the central horizontal line indicates the median value, and the lower and upper box horizontal lines indicate the 25th and 75th percentiles. Whiskers above and below the box indicate the 90th and 10th percentiles. Circles and asterisks indicate outliers and extremes values, respectively.

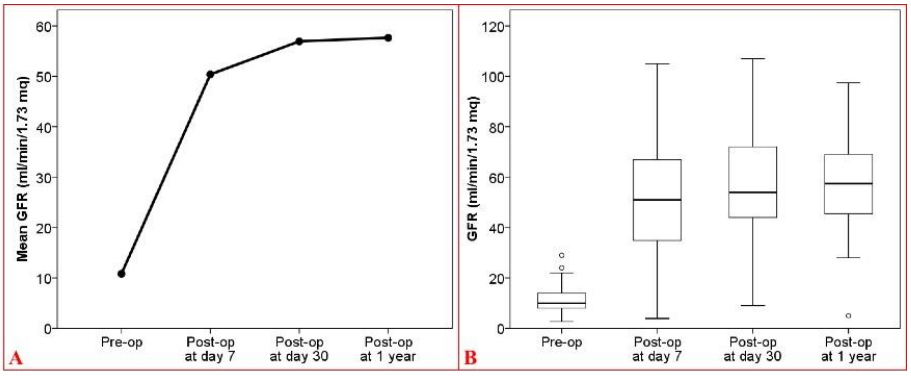

Figure 2. A: Trend with respect to eGFR over time. Values are shown at four time points: preoperative, POD 7, POD 30, and 1 year. B: In each box plot, the central horizontal line indicates the median value, and the lower and upper box horizontal lines indicate the 25th and 75th percentiles. Whiskers above and below the box indicate the 90th and 10th percentiles. Circles indicate outliers.

Dr. Eleonora Truzzi

CEM Curriculum: Medicinal and Pharmaceutical Sciences
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SMALL-ANGLE NEUTRON SCATTERING CHARACTERIZATION OF LIPOSOMES FOR ANTI-TUBERCULOSIS INHALED THERAPY

Background & Aims

The present investigation studied the effects of two first-line anti-tuberculosis drugs, rifampicin (RIF) and isoniazid (INH), on the structure of multilamellar liposomes. Liposomes are biocompatible vehicles for drug delivery that in recent years have been shown to be a promising system for inhaled therapy. [1] An in-depth characterization of drug delivery systems is necessary to highlight liposome-drug interactions and morphological changes in liposomal structure. Among all the available methods, small-angle neutron scattering (SANS) technique is a useful tool in gaining a detailed understanding of the structure of macromolecular systems, although it is poorly applied in nanomedicine. In fact, SANS provides valuable and unique data about steric bilayer thickness, particle dispersion, number of lamellae and drug localization under physiological conditions. [2]

Methods

Unloaded, single drug-loaded and co-loaded liposomes were prepared using different amounts of drugs by reverse phase evaporation method. Liposomal suspensions were prepared using D2O, in order to emphasize the contrast between the aqueous and the lipid/drug phases. The samples were characterized by dynamic light scattering, atomic force microscopy and finally by SANS technique (Rutherford Appleton Laboratory, U.K.). Neutron scattering curves were analyzed using a multi-shell spherical model of the fitting routine SASView 2.2.0.

Results

Liposomes have been shown to be physico-chemically stable until the end of the experiments, efficiently drug-loaded, and able to control drug release. In keeping with morphological studies, preliminary dimensional analysis demonstrated that particle sizes are in the range of SANS dimensional detection. SANS curves exhibited Bragg peaks for all samples, confirming the multilamellar liposome structure. By fitting the data with the multi-shell spherical model, significant differences among the samples have been highlighted. RIF-loaded liposomes were less ordered than unloaded liposomes. In addition, a reduction of the lamellae number was observed and the periodicity of the lipid bilayers slightly increased with the increment of the drug loading. In INH-loaded liposomes, the drug payloads did not change vesicle structure, because INH is a hydrophilic drug. However, INH induced a change in the inter-bilayer periodical spacing, which could be compatible with the formation of drug-liposome complexes at the water-lipid interface. Finally, the RIF-INH co-loaded liposomes exhibited the same characteristics of unloaded liposomes. In fact, no destabilization and no changing in inter-bilayer periodical spacing were observed.

Conclusion

In conclusion, SANS analysis provides fundamental information about drug-liposome interactions to better understand the relation between system structure behaviour and its biological activity. Moreover, data suggest that the co-encapsulation of the two anti-tuberculosis drugs may have a synergic effect on liposome stability.

The same samples were also prepared using deuterated drugs and phospholipids and analyzed at Helmholtz Zentrum Berlin, in order to obtain a better contrast in neutron scattering. The fit of neutron scattering curves is in progress in collaboration with Sapienza University of Rome.

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DESIGN, SYNTHESIS AND BIOLOGICAL EVALUATION OF NEW INHIBITORS REDUCING THYMIDYLATE SYNTHASE PROTEIN LEVEL IN CANCER CELLS

Background

Colorectal cancer (CRC) is one of the major causes of mortality throughout the world and it is the third most common form of cancer with 610.000 deaths per year. The treatment is mainly based on chemotherapy, employing 5-FU, Capecitabine and Raltitrexed as first line drugs. These antimetabolites target Thymidylate synthase (hTS), a key homodimeric enzyme involved in the synthesis of DNA. While the catalytic activity is specific of the homodimeric structure of the enzyme, the monomers of hTS can bind to its mRNA controlling the levels of enzyme expression by repressing its translational efficiency [1]. The presence of excess substrate or TS inhibitors stabilize the homodimeric conformation leading to decrease its binding to mRNA, resulting in increased translational efficiency and ultimately increased levels of TS protein. hTS overexpression due to increased gene transcription and mRNA translation can mediate toxicity and drug resistance. Moreover, decreased cellular uptake and polyglutamylation of TS-targeting drugs (raltitrexed), increased drug efflux, altered metabolism of cytotoxic drugs (5-FU) and other intracellular events can decrease the effectiveness of TS-targeting drugs. New inhibitors showing a different mechanism of action from classical inhibitors (5FU and antifolates) is needed to impair the TSmRNA regulation mechanism with respect to the catalytic activity [2, 3]. A previous work identified new TS inhibitors that interfere with dimeric protein assembly inducing dimer-monomer equilibrium, thus increasing the monomer concentration. The identified compounds were characterized with respect to the mechanism of action, that has been in part disclosed [unpublished data]. The final lead compound discovered showed in vivo antitumor activity.

Objectives

The aim of my PhD project is to develop new dissociative inhibitors able to induce a perturbation in the dimer-monomer equilibrium in favour of the monomeric form, thus inhibiting the catalytic function and preserving the regulatory activity. With this purpose, my work is specifically focused on the design, synthesis, characterization and evaluation of the enzymatic and cellular inhibitory activity of new compounds, potentially endowed by dissociative mechanism.

Methods

Compound design: A pharmacophore model has been obtained in collaboration with Prof. A. Cavalli (University of Bologna) using different our AIRC compounds that have been synthesized.

Compounds synthesis: Novel compounds have been synthesized by means of specific reaction steps whose number strictly depended on their retrosynthetic analysis, using conventional or microwave-assisted synthesis. The single intermediates and the final compounds were purified by crystallization or chromatographic techniques. The chromatography process was carried out either

with ISOLERA (Biotage® automatic purification system) or using flash column chromatography. The compounds were fully characterized through Nuclear Magnetic Resonance (NMR) spectroscopy, mass spectral techniques (Ion Trap LC-MS) and elemental analysis. NMR spectra (1D-NMR: 1H and13C-NMR; 2D-NMR: COSY, HSQC, and HMQC) were recorded on a Bruker Advance 400 MHz WB spectrometer operating at 400 MHz for 1H and 100 MHz for 13C.

Biological evaluation: Enzymatic inhibition assays towards the target (hTS) and MTT assays for anticancer activities towards HT29 and A2780/CP were performed at Drug Discovery and Biotechnology Lab - University of Modena and Reggio Emilia (Italy).

Results

Based on a mixed ligand-based and structure-based approach, I focused on the design and synthesis of 43 new derivatives, analogues of LEAD compounds (AIC-1, AIC-2) obtained from tethering approach in previous studies, with the intent to improve the dissociative-inhibitor activity and the biological features. Moreover, AIC-1 was re-synthetized in a scale-up approach in order to study the in vivo test tumour growth in orthotopic cancer mice model. This compound shows an efficacy 2 times higher than 5-Fluorouracil (5-FU). The decrease in TS levels seems to be ascribable to a reduced enzyme stability and an increased degradation by proteasome. So, it is evident that our novel inhibitors act differently from classical TS inhibitors at a cellular level.

Aiming to explore the benzothiazole moiety present in AIC-1, different new benzothiazole derivatives have been synthesized in order to obtain new derivatives with a potential improved activity

Moreover, a chiral semipreparative HPLC method has been developed to perform the enantiomeric separation.

In details, among the 43 new compounds that have been synthesized, 15 of these present an IC50 below 30 µM but the correlation with FRET data is impossible to do due to difference in the concentrations used in the assays. The 43 compounds have been tested against the 2 cell lines. Half of them induce cellular death around 20-40% at 40 µM.

An important goal was obtained with compound AIC-C16 (EC50 4.66 µM HT29; 3.71 µM A2780), which resulted to be more active respect to lead compound AIC-1. Moreover, compound AIC-C37 was designed for a further future derivatization, since a functional group was introduced on it with the aim to improve the dissociative-inhibitor activity, its pharmacokinetic profile and increase the solubility.

Conclusions

On the basis of the obtained data about the hTS enzyme, specifically related to FRET and cell growth in MTT assays, we have selected the most promising compounds in order to expand structure activity relationships (SAR) studies to find a new lead candidate. According to literature, the compound survey studies indicate that PPI inhibitors tend to be more hydrophobic, more rigid, and contain multiple aromatic rings. However, these characteristics directly translate in a reduced solubility, and this represent a problem for the assessment of the enzymatic in vitro inhibition

assays. For this reason, the forthcoming compounds will include a polar group to balance the LogP value and PPI activity of compounds. The results demonstrate that it was possible to identify new promising compounds, representing novel therapeutic agents acting as dissociative inhibitors of hTS, with the peculiarity to avoid target protein overexpression.

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Acknowledgement

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Dr. Simone Vitiello

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MOLECULAR ASPECTS IN THE ACTIVE / INACTIVE EQUILIBRIUM OF HUMAN THYMIDILATE SYNTHASE

Background

Human thymidylate synthase (hTS) relevance as an antitumoral drug target has been thoroughly documented in scientific literature. The folate dependent enzyme catalyzes the methylation of 2'-deoxyuridine – 5' – monophosphate (dUMP) to 2' – deoxythymidine – 5' – monophosphate (dTMP), using a methyl group given by a cofactor, the N5N10methylentethrahydrofolate (mTHF).

Structure has been resolved via X-ray diffraction, highlighting two monomers linked together to form the unit enzyme. In an aqueous solution, the monomeric and dimeric forms of the enzyme are at equilibrium. Structurally different from all analogous enzymes, the human thymidylate synthase sports a rather unique feature: the ability to switch between an active state and an inactive one. The active and inactive conformers have been resolved via X-ray diffraction: structure comparison among the two has produced important elements to understand such behaviour. Current literature agrees in considering two main differences, which identify each conformer. First, the orientation of loop 181 – 197, containing the Cys193 residue, responsible for the catalytic activity; second the loop 107-128, regularly defined in the active conformer, while disordered in the inactive one.

While it has been established how some molecules may affect such equilibrium, acting as "effectors" favoring one of the two conformers, current literature shows however little to none information regarding how the transition process occurs, its molecular properties, mechanisms and conditions. It is widely accepted for example, that dUMP acts as an effector towards the active conformer, while phosphate ions shift the balance towards the inactive one. Drawbacks of the current pharmacological treatment – among the most important, protein over expression leading to pharmacoresistance – call for new molecular targets to be identified and inhibited by novel molecules. Being able to obtain information regarding the structure of a certain target as well as the molecular details of its interactions with an inhibitor may constitute a huge input in the drug development process.

Objectives

The long-term object of the current thesis work delves into the characterization of the molecular aspects underlining the hTS active – inactive equilibrium and the switch from one conformer to the other. Such characterization will be carried out by taking into account different observables such as energy and structure interaction. Forthcoming data will be then analyzed in the search for any relevant information in both energies and molecular interactions. Further developments may take into account the interactions with larger macromolecules, such as mRNA, whose interaction

with the enzyme has been reported in literature as a pathway towards its inactivation via a negative feedback mechanism.

The main objective of this PhD year will be to analyze the interaction of TS with milder effectors than dUMP, while still capable of pushing the equilibrium towards one of the two conformers – such as phosphate and ammonium salts. Interactions with molecular candidates that either stabilize the inactive form of the protein [2] or destabilize its dimeric assembly, thus acting as dissociative inhibitors [1] will also be taken into account, to establish directions for the improvement of current molecular candidates. TS mutants' deployment in the project is expected later on this year to assess, if any, differences in thermodynamic prints.

Methods

hTS wild type (hTSwt) was expressed in competent BL21 cells following an established protocol. Enzyme purification has been achieved by means of a MPLC system, AKTA Prime, in a 20mM sodium phosphate and 30mM NaCl buffer. Functionality of the purified protein was assessed by means of a spectrophotometric kinetic assay (Varian Cary 100 UV – Vis Spectrophotometer). Together with the hTSwt, one mutant was also expressed and purified, F59A. Both wild type and mutant enzyme have been conjugated with fluorescein and tetramethylrhodamine maleimide, attempting to obtain 1:2:1 (F-TS-T) molar ratio. Fluorescence spectroscopy has been employed to follow enzyme folding and unfolding in the absence and presence of selected dissociative inhibitors. FRET - Förster Resonance Energy Transfer – based experiments were performed to quantitatively define the equilibrium between the monomeric and the dimeric assemblies of hTS and F59A in the presence of candidate dissociative inhibitors. Due to possible issues with the phosphate contained in the elution buffer of election during the purification process for successive experiments, hTSwt has been dialyzed in an opportune buffer (30mM TRIS + 150mM NaCl buffer). This step was deemed necessary in order to ensure that all evaluation of data started from a situation of equilibrium between the two-conformer types. ITC was performed to initiate thermodynamic assessments of the interactions between the hTSwt and dUMP in the new buffer, while future assessments will regard single and multiple interactions with other effectors on both wild type and mutant.

Results

Expression and purification of hTSwt and mutant has been carried out as per protocol: throughput was on par with the expected results. Changing buffer did not cause relevant stability issues with the wild type enzyme, although further analysis is still ongoing to fully assess, if existing, any problem that should arise from such change. Protein dialysis was successful, although some sample dilution was noted and is being currently addressed. Enzyme conjugation was successful, although not without challenges, especially in achieving 1:2:1 molar ratio (F-TS-T). Fluorescence spectroscopy preliminary data is still under review; fluorescence anisotropy data obtained from the analysis of the interaction between the wild type enzyme and a dissociative inhibitor shows a decrease in the observable that still needs to be fully evaluated. ITC data obtained from the evaluation of the interaction between hTSwt and dUMP within the new buffer shows that the data is coherent and comparable with results previously obtained within the same concentrations of enzyme and dUMP in the old buffer.

Conclusions

In conclusion, the thesis project aims at defining molecular aspects of the active – inactive switch of the human thymidylate synthase, especially by analyzing both single and multiple interactions from an energetic and a structural standpoint. The evaluated interactions between wild type and mutant enzymes will be then confronted to obtain relevant information regarding the switching process from one conformer to the other. Current drawbacks and challenges, mainly in the realm of conjugation of both wild type and mutant and dialysis are being addressed, with special care towards the full understanding of the limits of the phosphate free buffer, which will be used as canon for all new experiments. Further endeavors will be aimed at both resolving current issues and drawbacks and to the set-up of new experiments. On the latter, ITC will be employed to confront the thermodynamic footprints of the titration of both wild type and mutant enzyme with phosphate first, then with a mild (ammonium salt for instance) and a strong effector (dUMP) towards the active conformer.

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Acknowledgements

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CEM Curriculum: Medical and Pharmaceutical Sciences
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Cotutor: Prof. Barbara Ruozi

INNOVATION IN BBB-TARGETED AND ROS-RESPONSIVE POLYMER DDSs FOR THE TREATMENT OF BRAIN DISEASES

Background

Nowadays, there is a lack of efficient therapeutics to treat brain diseases such as Alzheimer's (AD), Parkinson's (PD) and brain cancer. This is mainly due to the inability of therapeutics to cross the Blood Brain Barrier (BBB). The development of drug delivery systems (DDSs) transporting therapeutics across the BBB into the CNS is mandatory to resolve this issue. Our research group has previously demonstrated the utility of DDSs by surface engineering PLGA NPs to target the BBB and deliver therapeutics to the brain1–3.

Owing to its increased oxygen consumption, the brain is highly susceptible to oxidative stress damage, which is caused by an overload of Reactive oxygen Species (ROS). This ROS overload is a key determinant of the onset and pathogenesis of many Central Nervous System diseases (CNS)4,5.

Currently, DDSs that deliver therapeutics in response to specific bio-signals, such as pH, GSH and ROS are under investigation6. To the best of our knowledge, studies that take advantage of the increased ROS levels in CNS diseases for the design of ROS responsive DDSs, are lacking. Hence, the design of ROS responsive DDSs constitutes a novel topic for innovative therapeutics. Linkers containing the ROS responsive moiety Thioketal (TK), due to their easy synthesis and biocompatibility, represent good candidates as linking groups for the design of ROS-responsive DDSs7.

Objectives

The overall goal of this PhD is to apply TK-technology for the design of ROS-responsive and BBB-targeted polymeric DDSs for the delivery of drugs and genes aiming to treat brain diseases. The first objective in this second year of PhD is to fully characterize the TK-diacid linker and TK-conjugated polymers, namely methoxypolyethylene glycol (mPEG) and poly-lactide-co-glycolide (PLGA) that were synthesized during the first year. The second objective is to confirm the ability of TK based polymers to release drugs under ROS conditions by studying the triggered release of Cyanine5 (Cy5) from the mPEG-TK-Cy5 model prodrug in proof-of-concept reactions. The third objective is to synthesize and evaluate in ROS-simulated conditions, the specific ROS response of mPEG-TK-Melphalan (MPH) prodrug designed for glioblastoma treatment. The test in tumor cell lines as well as in in vivo models are planned to be exploited in the third year of PhD project, to be performed in Limerick and Anger Universities as consequence of international mobilities. As last objective, regarding AD application, since it is known that A peptide aggregation (which create AD plaques) takes place at extracellular level, we plan PLGA NPs loaded with drugs active against

AD plaque formation and functionalized in their surface with TK conjugated with those kinds of drugs. At this stage of the study, we therefore completed PLGA-TK conjugation, characterization and NPs formation.

Methods

Synthesis, characterization and ROS responsive studies of mPEG-TK-Cy5: after TK-diacid linker fully characterization by 1H NMR and ESI-MS, ROS responsive polymer (mPEG-TK-COOH) was synthesized starting from mPEG-NH2 (5 KDa MW) and TK diacid linker. The ROS responsive mPEG-TK-Cy5 prodrug was prepared by EDC/NHS crosslinking of mPEG-TK-COOH with Cy5-NH2. Similarly, a control polymer without the insertion of TK, mPEG-Cy5, was prepared. A RP-HPLC method to control prodrug purity and indirectly quantify percent of Cy5 derivatization to mPEG-TK-COOH, was developed. This chromatographic technique was afterward employed to study the ROS responsive Cy5 release from the prodrug. The procedure to perform this study was as following: mPEG-TK-Cy5 was incubated with a mixture containing 400 mM H_2O_2 + $CuCl_2$ for 48 h at 37°C and at determined fixed times, samples for RP-HPLC and fluorometric analysis were taken. The same ROS assay procedure was employed to evaluate Cy5 release from mPEG-Cy5 (ROS unresponsive prodrug) as control.

Synthesis, characterization and ROS responsive studies of mPEG-TK-MPH: with the same technology, the preparation of the active prodrug, mPEG-TK-MPH, was also carried out by EDC/NHS crosslinking of reactants: mPEG-TK-COOH and MPH. The control of purity, quantification of MPH derivatization and ROS responsive analysis of mPEG-TK-MPH were also performed by RP-HPLC. As control, mPEG-MPH was also synthetized. The procedure followed for the study of ROS responsive release of MPH from these prodrugs, was the same as the used for mPEG-TK-Cy5 and mPEG-Cy5 prodrugs.

Synthesis and characterization of PLGA-TK-COOH and NP formulation: with a similar and adapted protocol to the one used for mPEG-TK-COOH synthesis, PLGA-TK-COOH polymer was prepared by conjugating TK diacid linker to PLGA-NH2 (15.9 KDa MW). After proper characterization by NMR, we attempted to prepare PLGA-TK NPs by simple emulsion and nanoprecipitation, employing 10 and 20% of PLGA-TK-COOH in the formulation. The NP size and zeta potential were measured by Zetasizer. As control, 100% PLGA NPs were prepared with the same formulation protocols.

Results

The ROS responsive mPEG-TK-COOH polymer and their derivative prodrugs: mPEG-TK-Cy5 and mPEG-TK-MPH, were obtained and characterized with derivatization yields of 83 %, 31.5 % and 13 %, respectively. In order to improve the derivatization yield of mPEG-TK-MPH prodrug, we are currently optimizing its synthesis conditions. When mPEG-TK-Cy5 was exposed to a simulated ROS-environment, RP-HPLC analyzed samples showed a time-dependent release of Cy5 confirmed by a reduction of the prodrug peak, with the concomitant increase of a peak corresponding to Cy5 excised form, based on UV and fluorescent detection. In the case of control samples (mPEG-Cy5), a second peak was not observed, indicating that Cy5 was not released from the prodrug. Conversely, the fluorescence intensity from mPEG-Cy5 samples at 48 h that were fluorometer measured, was reduced 2.17 times with respect to initial, while the fluorescence intensity from mPEG-TK-Cy5 samples was not changed. This evidence clearly indicated that in presence of ROS-enriched

environment, Cy5 fluorescence from mPEG-Cy5 resulted to be quenched by ROS. This event (namely the quenching of Cy5 by ROS) did not occur with Cy5 fluorescence from mPEG-TK-Cy5, possibly due to the preferential ROS reactivity over TK bonds, highlighting the role of TK in protecting Cy5 from ROS and therefore the success in ROS-protecting aim.

Otherwise, mPEG-TK-MPH prodrug response to ROS is at present under study. As a preliminary result, we found a time dependent increase of MPH peak. This result is in agreement with the one obtained from mPEG-TK-Cy5 prodrug. We are planning to perform RP-HPLC coupled to MS analysis to unequivocally establish whether the aforementioned peak corresponds to excised MPH. Regarding the application of TK-technology for the formulation of TK functionalized PLGA NPs, PLGA-TK NPs were obtained through simple emulsion and nanoprecipitation techniques. Their particle size and zeta potential did not differ considerably from non-functionalized PLGA NPs (around 150 nm, surface charge close to -20 mV). In order to accomplish the last objective, surface analysis of the NPs validating that TK moiety is surface accessible for drug attachment, are currently ongoing.

Conclusions

We were able to synthesize mPEG-TK-COOH with a high derivatization yield and reproducibility as well as PLGA-TK-COOH for PLGA-TK NP formulation. We also demonstrated that, in a simulated ROS-environment, both Cy5- and MPH- mPEG-TK-COOH derivatives were shown to be ROS responsive. In addition, from simulated ROS-environment studies with mPEG-TK-Cy5, we found that TK display ROS buffer capabilities that prevented Cy5 from being quenched. Altogether, these results validate that TK-technology can be applied for the design of CNS targeted DDSs.

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DECIPHERING IMMUNE RESPONSE TO CHECKPOINT INHIBITORS AND FINDING NOVEL BIOMARKERS IN METASTATIC RENAL-CELL CARCINOMA

Background

Nivolumab represents the new second-line treatment for metastatic renal cell carcinoma (mRCC). This drug is a fully human IgG4 against PD-1 and his role is to inhibits programmed death-1 (PD-1)/PD-1 ligand 1 (PD-L1) immune checkpoint. In the majority of patients, this drug is able to restore the patient's tumour-specific T-cell-mediated response thus improving both overall survival and objective response rate. However, a lack of clinical response occurs in a number of patients, which varies according to the drug itself, the underlying disease, and other factors, hence raising questions about how to predict and increase the number of patients who receive long-term clinical benefit from immune checkpoint therapy. Unlike traditional cancer therapies, checkpoint inhibitors act primarily on cells of the immune system. The requirement for the immune system as a mediator of the drug's activity suggests that the balance of positive and negative regulators of the immune response at the time of therapy may be critical for therapy efficacy. Among these regulators, cytokines, chemokines, and other soluble factors regulate the survival, activity, and location of immune cells and thus represent potential players in determining drug efficacy. Of particular interest are soluble factors involved in the recruitment and regulation of effector T cells, the frequency of different subsets of regulatory T cells and the ratio between effector T cells and regulatory T cells.

Objectives

The main aim of this project is to identify immune and serum biomarkers that are modulated in patients with metastatic renal cell carcinoma during and treated with immune checkpoint inhibitors and that can discriminate patients who most likely benefit from such therapy.

Methods

This is a prospective, longitudinal, interventional study on patients with mRCC who will receive Nivolumab in standard clinical practice. The project investigates changes in main immune parameters in patients with mRCC treated with nivolumab by analysing blood samples at baseline and after 1, 2, 3, 6 and eventually 12 months. Thirty mL of blood were collected and peripheral blood mononuclear cells (PBMC) were isolated according to standard procedures. PBMC were stored in liquid nitrogen. Then, PBMC were thawed according to standard procedures and stained with a viability probe and the following antibodies recognizing: CD3, CD4, CD8, CD25, CD127, FoxP3, ICOS, CXCXR6, CXCR3, CD95, CD45RA, CCR7, CD95, HLA-DR, CD38, CD28, CD27, CD71, CD87, CD39, TIM3, TIGIT, CCR4, Glycoforin, PD-1/IgG4, CD57, KI-67. This 28-color multicolour flow cytometry panel was set up in collaboration with Dr. Lugli (Humanitas, Milan). Samples were acquired by using a BD Symphony flow cytometer. Compensation was set using single stained

controls and gating strategy was checked by using FMO. Data analysis was performed using FlowJo 9.6 under Mac OSX.

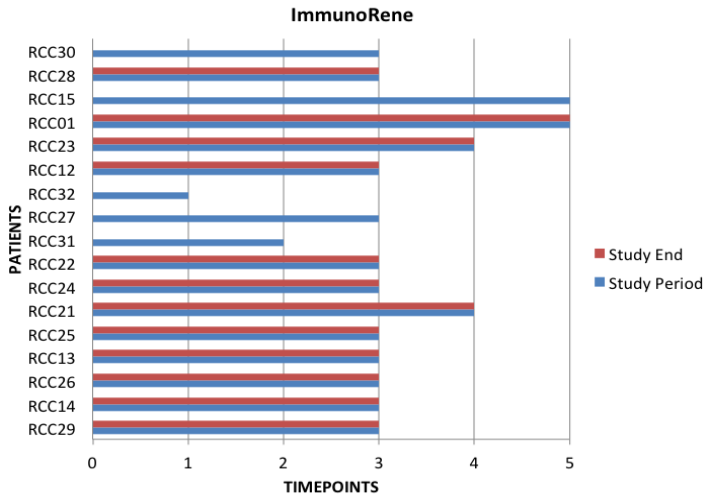
Results

From January 2016 until March 2018 we enrolled 17 patients. The median age was 68 years (52-79). The majority of patients had clear cell histology (94%). Nivolumab was given as second-line therapy in 53% of patients, as third line therapy in 29% of cases. According with International Metastatic Renal Cell Carcinoma Database Consortium Score (IMDC score) 70% of patients were in the intermediate prognostic risk group and 12% in poor risk. With a median follow-up of 13 months (min: 1 max: 26), 6-months survival rate was 70% (95%CI 38-87). Median progression-free survival (PFS) was 3 months (95%CI 2.8 - 5). Disease control was achieved in 4 of 15 evaluable patients (27%). Treatment discontinuation rate was 71%. Figure 1 shows enrollment and patient acquisition time-points. Preliminary data on PBMC show that Ki-67, a marker of cell proliferation, is increased after 15 days of therapy in some patients. Accordingly, the expression of HLA-DR and CD38 are increased.

Conclusions

Reactivation of the immune system is one of the main goal of nivolumab. We expect to identify easily measurable immune biomarkers that predict the responsiveness to nivolumab. Finding novel biomarkers that predict the response to therapy with nivolumab and monitor its efficacy can be of great benefit for the success of treatment not only to increase the number of patients who assume this therapy but also to identify those who have to change treatment without losing time so allowing an optimal allocation of economic resources. Longer follow up is required to assess preliminary immunological data.

Fig 1. Enrolment and patients acquisition time-points



Dr. Nathalie de Carvalho

CEM Curriculum: Translational Medicine
Tutor: Prof. Giovanni Pellacani

ASSOCIATION BETWEEN MELANOMA CYTO-ARCHITECTURAL FEATURES AND THE INTRATUMORAL MICRO-VASCULARIZATION AS DETECTED IN VIVO BY MEANS OF REFLECTANCE CONFOCAL MICROSCOPY AND SPECKLED-VARIANCE OPTICAL COHERENCE TOMOGRAPHY FOR THE PREDICTION OF AGGRESSIVENESS AND RISK OF PROGRESSION OF MELANOMAS

Background

Histology is the gold standard examination for melanoma diagnosis and provides important data for the staging of the tumor, as tumor thickness and presence of ulceration and mitosis rate. Some other exams, such as sentinel lymph node biopsy and Radiology imaging, may be necessary for the final definitive staging. However, these 3 complements modalities of investigation are expensive and may delay the beginning of the therapy. Reflectance confocal microscopy (RCM) is a noninvasive imaging technique, and which has already proved to be able to diagnose and correlate the morphology of the atypical cells with melanoma behavior. Speckle-variance optical coherence tomography (SV-OCT) is a novel in vivo imaging technique that generates images of the skin micro-angiography in transversal and enface/horizontal view. Changes in the skin micro vascularization has been seen with this technique when comparing normal from skin tumors, different skin tumors among them and when comparing melanomas with different Breslow thickness, the last presenting a vascular implement in accordance to the thickness of the melanoma during.

Objectives

Association between RCM features and SV-OCT micro-vascularization analysis for the evaluation of progression and aggressiveness of melanomas. The benefit of this association may represent a future advantage for in vivo staging of this skin cancer, which may lead to a reduction on late diagnosis and implement in patient's targeted approaches, with a final benefit in prognosis and quality of life.

Methods

Eighty lesions suspicious or consistent for melanoma diagnosis upon dermoscopy were evaluated by RCM and SV-OCT, prior to excision. In order to quantify the tumor rate of growth, during the evaluations patients informed since how long the lesions have been there. Once RCM confirmed the in vivo diagnosis of melanoma, a morphologic classification of the predominant atypical cell-types has been done as well as the description of RCM features previously proposed by Pellacani et al. Upon SV-OCT, an evaluation of the tumor micro-vascularization was done in the enface/horizontal view in 2 different depths (150 and 300 μm) The morphology of the vessels (dotts, blobs, coils, lines, curves and serpiginous) and the presence of branching (and its classification as arborizing or bulging) were evaluated. To consider a certain vascular morphology as present, it had to be seen at least 3 times independently in each depth.

According to histologic descriptors and clinical indication, immunohistochemical markers related to tumor aggressiveness (Ki67, CD271, HIF, -1aCD31) and research of BRAF, NRAS and c-Kit mutations will be performed in a subset of cases. Lymph node biopsy and radiologic imaging were performed for the staging of the tumor. Moreover, sentinel lymph node biopsy and radiologic imaging were performed when indicated.

RCM and SV-OCT evaluations were then correlated with melanoma stages, tumor growth pattern, the progression of the disease (in cases when it was possible to make a follow-up of the patients) and the biomolecular profile.

Results

In the first part of the study there was obtained a correlation between the vascular morphologies seen upon SV-OCT and the Breslow thickness of melanomas. Dotted vessels were frequently seen in both depths and independently from tumor thickness, but the irregular distribution of those vessels was an indicator for lesions thicker than 1.0mm.

At 150 µm the irregular distribution of dots and the presence of curved vessels were predominant on Breslow > 1,0mm, whereas coiled and serpiginous vessels were almost only present on Breslow > 2,0mm. At that depth, bulging branches were only present in melanomas thicker than 2,0mm.

At 300 µm, the irregular distribution of dots and the presence of serpiginous vessels and bulging branches were predominant on melanomas with Breslow thickness > 2,0mm.

The correlation of this first part of the results with the RCM findings and immunohistochemical markers are still in progress.

RCM evaluations are being performed.

Conclusions

Still to be done.

Publications about SV-OCT and vascular evaluations that have been already published:

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Themstrup L, De Carvalho N, Nielsen SM, Olsen J, Ciardo S, Schuh S, Nørnberg BM, Welzel J, Ulrich M, Pellacani G, Jemec GBE. In vivo differentiation of common basal cell carcinoma subtypes by microvascular and structural imaging using dynamic optical coherence tomography. Exp Dermatol. 2018;27:156-165.

Dr. Claudia Omarini

CEM Curriculum: Translational Medicine
Tutor: Dr. Federico Piacentini

ANAEMIA AS NEGATIVE PREDICTIVE FACTOR FOR TUMOR RESPONSE TO NEOADJUVANT CHEMOTHERAPY IN EARLY BREAST CANCER PATIENTS

Background

Neoadjuvant systemic therapy (NST) is a treatment option in patients with early-stage breast cancer (BC). Tumor response to NST well correlates with survival. In particular, pathological complete response (pCR) significantly predicts long-term outcomes. Anaemia is one of the most common side effects of cytotoxic drugs. Biologically, anaemia produces low intra-tumoral oxygen levels that seem to induce many adaptive responses in cancer cells such as overexpression of hypoxia inducible factor-1 (HIF-1), epidermal growth factor receptor (EGFR) and vascular epidermal growth factor receptors (VEGFR). These biological modifications could be responsible for increase in chemo-resistance. In literature, data on the predictive role of anaemia and hypoxia induced by chemotherapy during NST in BC patients are lacking. We have hypothesized that the grade and the duration of anaemia could represent a negative predictive factor for tumor response.

Objectives

The aim of the study is to evaluate the influence of Hb level throughout treatment course on tumour shrinkage induced by NST. Moreover, we want to investigate the relationship between anaemia and the expression of hypoxia-related biomarkers and genes in anaemic women with residual BC disease after NST.

Methods

317 patients diagnosed with stage I-III BC treated with primary chemotherapy were evaluated. Patient and tumor characteristics and treatment information were collected. Standard biological parameters (Ki67, nuclear grade, hormone receptors and HER2 status) were correlated to pCR. We focus on Hb level (at baseline, at the end of NST, drop in Hb throughout treatment, duration of anaemia) and its correlation with the pCR rate. Anaemia was defined as a drop of Hb under the local limit of normal in women (12 mg/dl). Moreover, we analysis the expression of HIF-1, PHD2, VEGFR and EGFR expression and their correlation with pCR and survival outcomes in patients with residual disease after NST and anaemia. The expression of these biomarkers will be compare with the expression of them in a control group of patients with no-pCR and no anaemia.

Results

Globally, pCR was achieved in 83 patients (26%), mainly HER2 positive disease (Hormonal receptors positive/HER2 negative = 6%, HER2 positive = 41%, triple negative = 37%; p<0,0001).

Median baseline Hb was 13.3 g/dl while median Hb level at the end of NST was 10 g/dl. pCR rate was not influenced by baseline Hb level. Anaemia due to chemotherapy was reported in 60% of patients. No difference in Hb levels was observed stratifying patients according to nuclear grade, tumour stage and cancer subtypes. Anaemia at the end of NST was an independent negative predictive factor for pCR in univariate and multivariate analysis (p=0.009). In the subgroup of anaemic patients the decrease in Hb ≥ 2 g/dl from baseline was associated with a significantly lower rate of pCR (15% vs 28%, p=0.009). Moreover, in anaemic patients with duration of anaemia longer than two months and decrease in Hb ≥ 2 g/dl the rate of pCR was the lowest (10%, p=0.01). The evaluation of expression of HIF-1, PHD2, VEGFR and EGRF are ongoing.

Conclusions

Preliminary results show an independent negative predictive role of anaemia in women treated with NST for BC. This evidence suggest that anaemia should be corrected in order to obtain the best response to primary treatments.

Dr. Ilaria Giovannacci

CEM Curriculum: Translational Medicine
Tutor: Prof. Cristina Magnoni
Co-Tutor: Prof. Marco Meleti

USEFULNESS OF AUTOFLUORESCENCE AS DIAGNOSTIC AID IN DYSPLASTIC LESIONS OF THE ORAL MUCOSA

Background

A great interest for non-invasive tools possibly improving diagnostic accuracy has been noticed in several fields of surgical oncology. Among these, the use of auto-fluorescence (AF) of the tissues is particularly being investigated.

It seems that malignant or potentially malignant changes in some tissues are reflected in variations of the tissue AF spectra, that can be visualized through a real-time non-invasive method.

Despite a large use of AF in clinical practice there is still a gap of knowledge about the specific molecules responsible for such a phenomenon and the mechanisms responsible of their reduced or increased capacity to emit fluorescence in case of malignant or pre-malignant lesions.

During the first year of the present PhD research a systematic review focused on identification of fluorophores of the oral mucosa and of the skin, as well as their excitation and emission wavelengths and the devices used for inducing fluorescence has been performed.

The best known endogenous fluorophores of oral mucosa and skin stimulated by an ultraviolet or blu excitation wavelength seem to be some proteins such as collagen, elastin, keratin, tryptophan. Most probably, during dysplastic processes their structure and concentration within tissues are modified and this is reflected in variations of the normal AF.

Other fluorophores are molecules involved in tissue metabolism such as NADH and FAD. Metabolic activity of the relative amounts of reduced NADH and FAD and the microenvironment of these metabolic electron carriers can be used to non-invasively monitor changes in metabolism, which is one of the hallmarks of carcinogenesis

Such molecules are stimulated by wavelengths between blue and violet / ultraviolet light.

The analysis of tissue AF for improving sensitivity and specificity in cancer diagnosis has been proposed for different organs, such as colon, lung, cervix, esophagus. Particularly, there are several evidences supporting the effectiveness of AF in head and neck cancer diagnosis.

Objectives

To investigate the correlation between degree of AF and histopathological features of oral mucosa, in order to assess the usefulness of AF in the diagnosis of malignant and potentially malignant lesions.

To propose a digital scoring system that objectively quantifies the degree of AF.

Methods

A system emitting 400-460 nm light (VELscopeVx - LED Medical Diagnostics Inc., Barnaby, Canada) was used to assess AF.
On the basis of clinical appearance, lesions were classified as red (erosive, ulcerated or atrophic areas), white (hyperkeratosis) or combined (lichenoid or leuco-erythroplastic lesions).

With regard to the histological diagnosis, lesions were graded as having: no dysplasia; dysplasia (mild or moderate) and carcinoma (in situ, micro-invasive, verrucous or invasive).

Both visual and a numerical assessment of AF has been considered and it was graded as hypo-fluorescence, normo-fluorescence and hyper-fluorescence.

White light and fluorescence images were acquired and a digital elaboration was performed with the software ImageJ (National Institute of Health - NIH, USA). Color image was converted to grayscale and LUT spectrum image to indirectly measure the AF intensity.

Statistical analysis was performed through the IBM-SPSS statistical package v.20. Inferences about the groups have been performed using t test for independent data and the equivalent nonparametric Kruskal-Wallis test. Inferences about categorical data have performed using chi-squared test and Fisher exact test, when appropriate.

Results

Red lesions were related to hypo-fluorescence, whereas white lesions were mostly related to hyper-fluorescence most probably because of keratin increase. There was a statistically significant association between histological alteration (Oral Epithelial Dyspasia - OED or carcinoma) and AF alteration considering both hypo- and hyper-fluorescence ($p<0,05$ for visual AF, $p<0,001$ for numerical AF). OED and carcinoma are particularly associated with hypo-fluorescence, excluding verrucous carcinomas, which appear hyper-fluorescent because of intense keratinization.

It was also found a statistically significant difference in the AF numerical value distribution related to diagnosis, with decreasing values progressing from no dysplasia to dysplasia and then carcinoma.

Numerical ranges corresponding to hypofluorescence, hyperfluorescence and normofluorescence were identified (K, concordance measure Landis and Koch = 0.748, meaning substantial correspondence).

Conclusions

AF alteration is statistically related to histological alteration. The present evaluation demonstrated that not only hypo-fluorescence, but also hyper-fluorescence should be investigated, especially if it is associated with anamnestic and clinical suspicious features.

Since the correlation is stronger with numerical AF then visual AF, it could be useful adding an AF simultaneous quantification method to the mere observation.

AF analysis could be a valid adjunctive technique, when associated to the clinician experience and knowledge. It may be used to spot lesions at risk, to identify suitable sites for incisional biopsies and to define excision margins of the lesions.

XXXIII cycle

Dr. Aida Meto

CEM Curriculum: Public Health
Tutor: Prof. Elisabetta Blasi
Co-tutor: Dr. Eva Pericolini

EFFECTS OF CUPRAL AGAINST PATHOGENS OF THE ORAL CAVITY: STUDIES ON SPECIFIC TRAITS OF VIRULENCE

Background

Problems concerning quality of endodontic and mucosal treatments have remained unsolved so far. Pulpitis and periodontitis represent common caries complications, accounting for at least 50% of dental diseases in patients of different ages. Studies on different forms of periodontitis, treated with traditional methods, show that the quality of canal filling materials in problematic roots is another important factor that greatly influences the success of treatment.

Furthermore, it is known that phenols, linked with para-chlorophenol aldehyde, allow for optimal bactericidal action against most microorganisms. On the other hand, 1% sodium hypochlorite, used as an irrigant in endodontic treatment, reaches per se an antimicrobial effectiveness of about 70%.

Nowadays, transcanalar inductive currents, in combination with different medications, are widely employed in order to enhance the performance of the treatment. In particular, it has been shown that the effect of electrophoresis with Cupral, coupled to transcanalar current, guarantees antibacterial and long-term action when applied to dental roots affected by problematic curves and periapical destructive damage.

Objectives

Our primary objective is to evaluate in vitro the antimicrobial activity of Cupral against microorganisms commonly harboured as commensals in the oral cavity, often becoming associated with soft and hard oral tissues diseases. Special emphasis will be laid on the impact of Cupral on the microbial ability to produce biofilm, a well-known trait strictly related to virulence.

Methods

Both bacterial and fungal cells will be tested. Initially, collection references strains will be used; then, clinical isolates will be selected and assessed. The possibility of using drug-resistant isolates will be taken into account. Different protocols will be employed in order to investigate in vitro the effects of Cupral on biofilm formation and persistence. In order to mimic oral conditions as closely as possible, mono-species and multi-species biofilms will also be assessed for susceptibility to Cupral.

Expected results

This project will provide novel information on Cupral anti-microbial efficacy, ultimately imparting wider knowledge of such a tool for a more efficient treatment of oral infections.

Data on the potential efficacy of Cupral against conventional drug-resistant strains will clear the way for innovative therapeutic protocols.

Dr. Silvia Tanzi

CEM Curriculum: Translational Medicine
Tutor: Prof. Stefano Luminari

EARLY PALLIATIVE CARE IN HEMATOLOGIC ADVANCED PATIENTS

Background

Integration of palliative care (PC) into standard oncology care has been established by the literature to be essential and it serves as a guideline all over the world. Oncological patients do not differ from hematological patients in the deterioration of several Quality of life (QoL)' dimensions. Despite this acknowledgement, hematological patients do not access palliative care services, or access only occurs in last days of their life.

Objectives

The aim of my PhD project is to pilot and evaluate a new integration model between Palliative care and standard hematological care in an Italian hospital.

Methods

My project can be interpreted as a Phase 0-II, according to the MRC Framework for the assessment of the complex interventions. Within this framework, the study can be described as follows:

a systematic literature review on hematological patients and palliative care is ongoing. This review will provide the theoretical background and the historical comparison of the intervention (phase 0, so called the theory)

the intervention: a feasibility mix method study will be adopted, in which a group of hematological patients received integrated hematological care and palliative care services throughout the course of the predictive last active treatment. The intervention will be compared with the standard care for hematological patients (phase I-II. Modelling and exploratory trial). Quantitative and qualitative data will be collected.

Expected results

Expected results will be the description of new feasible integration between palliative and hematological care for advanced patients. Important preliminary results on the efficacy in QoL's dimensions will be collected, such as qualitative data on patients, caregivers and professionals involved.

Dr. Michele De Maria

CEM Curriculum: Translational Medicine
Tutor: Dr. Luigi Fontana
CoTutor: Dr. Domenico Merlo

ANALYSIS OF INTRAOCULAR INFLAMMATION AFTER EYE SURGERY: NON-STEROIDAL ANTI-INFLAMMATORY DRUGS VERSUS CORTICOSTEROIDS

Background

The past decades have witnessed a surge in the scenario of eye surgery because of the continuous development of new surgical techniques, sophisticate instrumentations and innovative eye drugs. However, managing intraocular inflammation and preventing its complications still remain a topic of concern among the ophthalmologists worldwide. There is much evidence in literature that uncontrolled inflammation after eye surgery increase the risk of post-operative complications both for routine procedures such as cataract surgery, and more complex ones such as corneal and vitreo-retinal surgery. Several previous studies have shown the positive effect of corticosteroids and non-steroidal anti-inflammatory drugs (NSAID) eye drops, administered alone or in combination, for the treatment and prevention of intraocular inflammation, especially after cataract surgery. The REPEX study (Bromfenac to Reduce Inflammation in Patients with Pseudoexfoliation Syndrome After Cataract Surgery) showed that a combined therapy with NSAID and corticosteroids after cataract surgery in pseudoexofliation syndrome provides a better control of intraocular inflammation if compared to the corticosteroids alone.

However, despite the large number of published papers on the management of post-operative intraocular inflammation, no widely accepted guidelines have been published. Still, scientific evidences are much needed to clearly establish the better therapeutic regimen to increase the post-operative outcomes and reduce complications.

Objectives

The main objective of this project is to extend the understanding on topical NSAIDs (alone or in combination with topical corticosteroids) versus topical corticosteroids alone in controlling intraocular inflammation after uncomplicated eye surgery especially for cataract surgery, corneal surgery for endothelial decompensation and vitreoretinal surgery for pucker and macular holes.

Methods

This project will be a phase IV, single centre, randomized, active-control, parallel design, open-label trial comparing different topical NSAIDs and topical corticosteroids in patients who will undergo uncomplicated cataract surgery, vitreoretinal surgery for macular pucker and macular hole and corneal surgery for endothelial dystrophy or pseudophakic endothelial decompensation.

To be eligible to randomization the following inclusion criteria will need to be met: age > 60 years old, eligibility for surgery under evaluation, uneventful surgery, no history of ocular comorbidities,

no history of systemic comorbidities. In order to quantitatively establish the intraocular inflammation and to assess the efficacy of different topical anti-inflammatory treatments, the anterior chamber flare of the operated eye will be measured using the laser flare photometry (LFP), in which a diode laser beam measures the light scattered by small molecules or cells in the anterior chamber. Results are expressed by photon counts per milliseconds ph/ms. The laser flare photometry will be performed before surgery and at scheduled follow-up visits after surgery. In addition, the assessment of visual acuity, the anterior biomicroscopy and fundus examination and the optical coherence tomography (OCT) will be performed in order to provide e detailed analysis of post-operative outcomes and incidence rate of complications.

Expected results

This study project is expected to define a standardized post-operative therapeutic protocol after different types of eye surgery. In the light of the results from the REPEX study, the "Bromfenac vs Dexamethasone Study" (BVD study) has already started and patients' enrollment is about to be completed. The aim of this study is to compare bromfenac 0.09% to dexamethasone 0.1% for reducing ocular inflammation measured by laser flare after uncomplicated cataract surgery. To date, 64 patients have been enrolled. An interim analysis shows that both drugs are effective in reducing anterior chamber flare as it was expected. However, the end of the follow-up will provide statistically valid results. In detail, The BVD study will provide an estimate of the time since the intervention needed to revert the postoperative flare to the preoperative or lower level. It is expected that the anterior chamber flare will increase the day after surgery and then progressively decrease towards baseline in all patients.

In line with the preliminary results from the BVD study, the next phases of this project will investigate the effect of different anti-inflammatory therapy protocol after more complex procedure. It is awaited that a more invasive surgery requires a combined therapy, with both NSAIDs and corticosteroids.

Dr. Francesco Venturelli

CEM Curriculum: Public Health
Tutor: Dr. Paolo Giorgi Rossi
CoTutor: Prof. Annalisa Bargellini

NEW TOOLS FOR HPV-BASED CERVICAL CANCER SCREENING

Background

In high-income countries cervical cancer is a well-controlled disease, thanks to the diffusion of Pap-test and organized screening programmes. Indeed, this is one of the few oncologic diseases that can be prevented through the identification and treatment of pre-cancerous lesions. Since persistent carcinogenic human papillomaviruses (HPV) infection has been identified as the necessary, but not sufficient, cause of cervical cancer, vaccination and HPV-DNA test-based screening have become the main prevention strategies.

The new challenge in cervical cancer prevention is the reduction of over-diagnosis and over-treatment. These phenomena may increase with the introduction of the HPV test. Indeed, since the HPV-DNA test is less specific than Pap-test, we need evidence-based guidelines defining the best management of HPV positive women and how to use available biomarkers. We need triage tests to reduce colposcopy referral of HPV-DNA positive women, and we need appropriate follow up strategies for women who had a colposcopy and received a treatment for a high-grade cervical intraepithelial neoplasia (CIN2 or CIN3).

Objectives

To assess the accuracy of biomarkers (HPV E6/E7 mRNA and p16/Ki67) as test of triage in HPV-DNA based screening protocols.

To assess the prognostic value of biomarkers (HPV E6/E7 mRNA and p16/Ki67) for the identification of regressive lesions.

To update guidelines for the follow up of women treated for CIN2 and CIN3 with evidence-based recommendations.

Methods

Reggio Emilia is coordinating the follow up and the analyses of the New Technologies in Cervical Cancer 2 (NTCC2) study. NTCC2 is a double testing accuracy study with a nested randomized study, funded by the Italian Ministry of Health, that involved 10 centres in 7 Italian regions. NTCC2 aims to measure the accuracy of mRNA and p16/Ki67 and their negative predictive value for CIN2 or more severe lesions (CIN2+). The project recruited women who were invited for a new screening round based on HPV-DNA test within the screening programs (i.e. aged 25-59). HPV-DNA positive women were tested for cytology, E6/E7 mRNA and p16/Ki67. Women with ASC-US or more severe (ASC-US+) cytology were referred to colposcopy whereas women with negative cytology were randomized to immediate colposcopy or to 1-year follow up by HPV-DNA test. All women referred

to 1-year control were tested for HPV-DNA, mRNA and p16. Women negative to all tests were referred to 4-year follow up. Regardless of mRNA and p16 results, HPV-DNA positive women were referred to colposcopy. The final endpoint of the study is the confirmed CIN2+. The follow up of the study was fixed at 5.5 years from recruitment of each woman to include the subsequent screening round for the majority of the women. All the lesions found during the 5.5-year follow up will be included in the endpoint. To minimize the effect of loss to follow up, cancer registries and pathology unit registries will be searched at the end of the follow up for the identification of CIN2+ which occurred in the recruited cohort. The prospective sensitivity of biomarkers for CIN2+, the proportion of CIN2+ HPV-DNA positive and mRNA or p16 negative that could regress in one year and the relative sensitivity of a HPV-DNA screening followed by mRNA or p16 triage will be calculated. In addition, the study design enables the evaluation of other biomarkers, more specific than HPV-DNA (i.e. genotyping and methylation).

Regarding the third objective, as part of the Italian Group for Cervical Cancer Screening (GISCi), we started an evidence-based revision process of protocols for the follow up of women treated for CIN2 and CIN3. A multidisciplinary team comprising all the professions contributing to cervical cancer screening was built and the GRADE methodology was used for the first time in this specific field.

Expected results

To date, the recruitment phase of NTCC2 has been completed and the 12-month follow up is ending. More than 40 thousand women were recruited. We are now collecting data on the 12-month follow up. Complete data on baseline and 12-month follow up will be available by the end of 2018. Data on the 5-year follow up will be available by the end of 2021. Baseline results will allow three main analyses: 1) cross-sectional accuracy of triage biomarkers; 2) determinants and predictors of CIN2+ and infection persistence; 3) reproducibility of morphological biomarkers (p16/ki67).

The scoping phase of the revision of follow up protocols for treated women was conducted from January to March 2018. Ten specific research questions were defined by the team members using the PICO framework (Population, Intervention, Comparator, Outcomes). Question specific outcomes have been scored for their relevance. Systematic review of the literature for each PICO are now starting and their protocols will be published in the Prospero database. Analysis of literature search will be synthetized in Evidence to Decision tables and discussed with working group members in plenary sessions to formulate recommendations. Updated GISCi guidelines are expected to be published by the end of 2019.

Dr. Mariarosa Maiorana

CEM Curriculum: Translational Medicine
Tutor: Dr. Domenico Merlo

EFFECTIVENESS ASSESSMENT OF AN ONCONEPHROLOGICAL BUNDLE TO REDUCE THE RISK OF ACUTE KIDNEY INJURY AND THE PROGRESSION OF CHRONIC KIDNEY DISEASE IN HIGH-RISK PATIENTS ADMITTED TO THE ONCOLOGY DEPARTMENT OF SANTA MARIA NUOVA HOSPITAL, REGGIO EMILIA

Background

Onco-nephrology is a new and evolving subspecialized area in Nephrology that deals with kidney diseases in cancer patients.

Chronic kidney disease (CKD), acute kidney injury (AKI) and cancer are connected in several ways.

Not only does can cancer lead to the development of CKD, but the presence of CKD can be associated with cancer.

Although the overall incidence and prevalence of CKD among cancer patients are still uncertain, there is growing evidence to suggest that the risk is high and still increasing.

The improvement in survival rates of neoplastic patients due to new chemotherapeutic agents, including biological drugs, has increased the number of patients who develop renal disease due to neoplasia.

However, there are no definitive and solid data on the frequency of AKI and CKD in neoplastic patients in relation to the stage of renal disease and the characteristics of progression.

Objectives

The aim of the project is to set up an onco-nephrological bundle, for both research and a wide array of diagnostic-therapeutic applications such as the following: identification of cancer patients with CKD; evaluation of the incidence rate and risk factors for the development of AKI; evaluation of risk factors for the progression of renal damage; the effects of AKI on clinical outcomes (recovery of renal function or evolution towards CKD, prolonged hospitalization, development of further clinical complications, etc.); a specialized nephrological counseling intervention for high-risk patients.

Methods

We undertook a retrospective study collecting data from patients admitted to the Oncology Department of Santa Maria Nuova Hospital, Reggio Emilia, from January 2012 to December 2017. Primary outcomes were AKI, defined according to the KDIGO model and progression of a pre-

existing CKD. Clinical data were used to define the incidence of CKD in oncological patients based on stage of kidney disease and type of cancer, risk of progression of CKD, prognostic features of proteinuria and microhematuria or AKI in patients with previous normal renal function.

Expected results

The application of measures to reduce the risk of AKI, especially in high-risk patients, would reduce its incidence, the risk of progression of CKD and while at the same time improving the outcomes of hospitalized patients with cancer.

The risk factors, if confirmed by prospective studies, could be used to create a predictive score to identify high-risk patients for developing AKI.

Furthermore, data obtained from the study will be evaluated for epidemiological purposes.

Dr. Giulia Besutti

CEM Curriculum: Translational Medicine
Tutor: Dr. Paolo Giorgi Rossi
CoTutor: Prof. Guido Ligabue

EFFICIENCY AND APPLICABILITY OF INTERNATIONAL GUIDELINES FOR NON-ALCOHOLIC FATTY LIVER DISEASE ASSESSMENT IN HIGH RISK PATIENTS AND ACCURACY OF IMAGING TESTS IN THE DIAGNOSIS OF NON-ALCOHOLIC STEATOHEPATITIS AND FIBROSIS

Background

People with type 2 diabetes mellitus (T2DM) or Metabolic syndrome (MeTS) have a high prevalence of Non-Alcoholic Fatty Liver Disease (NAFLD). Moreover, they are at high risk for Non-Alcoholic Steatohepatitis (NASH), which can progress to fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). Recently, the European Associations for the Study of the Liver, Diabetes and Obesity, as well as the American Association for the Study of Liver Diseases proposed recommendations for the diagnosis, treatment and follow-up of NAFLD. However, these guidelines have not been validated in high risk patients yet.

Liver biopsy is considered the gold standard for NAFLD assessment. Given the upcoming introduction of new therapeutic agents, the lack of a non-invasive tool to identify patients who need a therapeutic intervention is a central issue. Newer ultrasound (US) and magnetic resonance (MR) techniques have shown promising results in the diagnosis of NASH and staging of fibrosis. Should liver biopsy be avoided, or at least reserved to a limited number of patients with undefined risk level, the benefit-risk balance of NASH/fibrosis screening and therapies would undergo a major change.

Objectives

AIM 1: Preliminarily, we are conducting a quality assessment of the most influential and recent guidelines on NAFLD diagnosis and management according to the AGREE2 criteria. The primary objective is to evaluate the efficiency of international recommendations in the diagnosis of NASH and fibrosis among high risk patients, by calculating the positive predictive value of specialist referral (PPV1) and liver biopsy (PPV2). Secondary objectives are:

to evaluate PPV1 and PPV2 in subgroups of patients with different clinical criteria for referral;

to evaluate the applicability of the recommendations in terms of burden of clinical examinations and respective resources absorption generated by the application of the recommendations on the overall number of eligible patients in the population;

to evaluate the number of subjects excluded for other liver disease/other causes of steatosis;

to evaluate patients' adherence to hepatologic evaluation and liver biopsy referrals.

AIM 2: Preliminarily, we are conducting a systematic review of the literature on the accuracy of imaging tests in NASH diagnosis (the protocol has been published in the Prospero database). The

primary objective is to evaluate the sensitivity and specificity of non-invasive imaging methods (US and MR) in the diagnosis of NASH/fibrosis in high-risk NAFLD patients, using liver biopsy as the reference standard. Secondary objectives are to evaluate the association of imaging biomarkers of steatosis, fibrosis and NASH with:

- histologic biomarkers used for NAFLD assessment (steatosis, activity and fibrosis);

- volume and MR characteristics of visceral adipose tissue (VAT);

- demographic, clinical, and anthropometric characteristics of the included patients.

Methods

AIM 1: This is a pilot observational study including T2DM and MeTS patients who will be stratified in different risk categories based on their fatty liver index (FLI), liver enzymes, and NAFLD fibrosis score (NFS). As proposed by the recommendations, patients at higher risk for fibrosis or NASH will be referred for hepatologic evaluation and, after US confirmation of liver hyperechogenicity and exclusion of other liver disease or secondary causes of steatosis, for liver biopsy. An expert pathologist will evaluate steatosis grade, hepatocellular ballooning, lobular inflammation, and fibrosis stage. According to these findings patients will be classified in 1) Not NAFLD; 2) NAFL, Not NASH; 3) NASH. The score SAF (steatosis, activity and Fibrosis) will be calculated. Positive Predictive Values of hepatologic evaluation referral (PPV1) and liver biopsy referral (PPV2) for NASH/fibrosis diagnosis will be calculated as a measure of efficiency of the recommended algorithms. The burden of clinical examinations and respective resources absorption generated by the application of the recommendations will also be estimated.

AIM 2: High risk NAFLD patients who are referred for liver biopsy will undergo: 1) US examination with evaluation of US-fatty liver index (a potential surrogate for NASH) and calculation of liver stiffness by means of Shear Wave Elastography (SWE), 2) Multiparametric MR examination without contrast administration, with different techniques (including Proton Density Fat Fraction, T2* and T1 mapping, MR Spectroscopy, virtual MR Elastography), obtaining multiple biomarkers of steatosis, inflammation and fibrosis, and VAT measurement. Sensitivity and specificity of different imaging tests, alone and in combination, for NASH and fibrosis diagnosis will be calculated, using liver histology as the reference standard.

Expected results

The application of international recommendations to a selected cohort of high-risk patients is expected to generate a high burden of hepatologic evaluations and liver biopsies, leading to increased resources absorption and biopsy-related risks. Our results could help in assess their feasibility and will allow the evaluation of different scenarios according to changes in the referral criteria. Furthermore, we expect to identify which combination of imaging tests is accurate enough to potentially replace histologic evaluation.

Dr. Jovana Milic

Curriculum: Clinical and Experimental Medicine
Tutor: Prof. Giovanni Guaraldi

THYMUS IS THE BAROMETER OF AGING IN HIV PATIENTS, ASSOCIATED WITH METABOLIC SYNDROME AND FRAILTY

Background

People living with HIV (PLWH) may experience accentuating aging in relation to immuno-activation. Thymus (THY) size and function decline across time: they are more conspicuous at the onset of puberty, afterwards, they decrease by approximately 3% per year until middle age, and subsequently by 1% per year. In the general population, THY function failure has been described as an independent predictor of all-cause mortality in uninfected elderly humans. Little is known regarding the role of THY decline in immuno-activation, which characterizes accentuating aging processes in people living with HIV (PLWH). At a clinical level, this immuno-metabolic phenomenon is often described as “inflammaging”, and it is pathogenically linked with metabolic syndrome (MetS), non-infectious co-morbidities, functional decline and geriatric syndromes including frailty.

Objectives

In PLWH, we sought to investigate the relationship between THY imaging detection and size with clinically relevant ageing outcomes such as metabolic syndrome (MetS), multi-morbidity (MM) and frailty.

Methods

This was a cross sectional observational study including 665 HIV patients (81% males, median age 53 years) attending Modena HIV Metabolic Clinic from 2014 to 2017. These were evaluated for immuno-metabolic abnormalities, comorbidities, multi-morbidity and frailty. Immuno-metabolic disorder was defined using MetS ATPIII classification. Co-morbidities were defined according to EACS guidelines. Multi-morbidity (MM) was defined as ≥3 comorbidities in the same individual. Frailty was measured with both the frailty phenotype (FP) and a 37-item frailty index (FI) previously validated at MHMC and constructed from health variables collected at the same study visit. In addition, all participants underwent thoracic CT scan as part of the routine follow-up, in which THY detection and size were reported using a semi-quantitative score. To avoid collinearity between age and HIV duration the latter was derived from univariate linear regression as a residual time effect depicting HIV duration after correction for age.

Results

THY was detected in 27% of subjects. Of these, 71.1% showed THY size of grade 1-2 and 28.9% exhibited grade $\geq$3. Negative predictors for THY detection were older age, male gender, higher body mass index (BMI) and longer residual HIV duration. Independent predictors for MetS were older age, longer residual HIV duration, higher BMI and THY grade 1-2. Independent predictors for

MM were older age, longer residual HIV duration and lower CD4 nadir. Independent predictors for frailty index were older age, longer residual HIV duration, lower CD4 nadir, higher BMI and THY detection.

Conclusions

Our study defines THY as an indicator of biological ageing in HIV patients, predicting MetS and frailty.

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IMPLEMENTATION AND VALIDATION OF A MODEL OF POST-STATUS EPILEPTICUS TEMPORAL LOBE EPILEPSY

Background

Epilepsy is a heterogeneous group of diseases that affects 65 million people around the world. The burden of epilepsy is mostly due to uncontrolled seizures, which affect at least a third of people with epilepsy receiving standard treatment. This still remains a largely unsolved problem in spite of advances in pharmacotherapy and in the understanding of pharmacoresistance mechanisms in the last two decades. Therefore, an urgent need for well-characterized models with better validity has been claimed in recent years.

Temporal lobe epilepsy (TLE) accounts for 75% of epilepsies in adulthood. Such syndrome is characterized by focal limbic seizures, usually with impaired consciousness that may progress to bilateral convulsive seizure (so-called generalized tonic-clonic seizures, GTCS). Many people of TLE present poorly controlled focal seizures, even though generalized ones are effectively prevented by antiseizure drugs (ASD) in the appropriate posology.

Amongst the epilepsy models, that of post-status epilepticus (SE) induced in rodents by kainic acid (KA) reproduces many characteristics of TLE in humans. KA is an orthosteric agonist of KA-type ionotropic glutamate receptors. The original "single high dose" systemic KA protocol presents a high mortality rate. Many researchers modified the former protocol in order to mitigate this important shortcoming. Some of those modifications are represented by intracerebral administration routes, the "repeated low dose" protocol and the block of SE by benzodiazepines, even though SE resumes some hours later. Therefore, modified KA protocols have been characterized and used for several purposes over the last twenty years.

Previous findings from our laboratory pointed to a very low (<10%) mortality rate in Sprague-Dawley (SD) rats undergoing "single high dose" systemic KA protocol. After an extensive analysis of electrophysiology, pathology, neurochemistry and pharmacology data about this specific protocol, we became aware of the relative scarcity of such data.

Objectives

In line with this, we intend to carry out a characterization of single high dose systemic KA protocol in SD rats.

Methods

For this purpose, we aim to evaluate lesions provoked by KA in several brain areas by means of Cresyl violet (Nissl) and Fluorojade B stainings, immunohistochemistry and immunofluorescence procedures for NeuN, GFAP, Iba-1, GAD-65, parvalbumin, cholecystokynin, and somatostatin

markers. Besides, we will evaluate behavioral (by Racine scale staging) and electrographic seizure parameters by means of video-electrocorticography (V-EcoG) in 4-6 monopolar electrodes in referential arrangement. V-EcoG are intended to be recorded 24 h per day over 6 to 8 weeks. A later phase of the study may include groups to undergo chronic treatment for the assessment of pharmacological response profile.

Expected results

This work is expected to provide a robust model of TLE with clear predictive and construct validity. In particular, the model is expected to be able to strongly support the development of drug response biomarkers, ASD prototypes with improved efficacy on focal pharmacoresistant seizures, and possibly antiepileptogenic therapies, as well as further dissection of pharmacoresistance/-responsiveness mechanisms.

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DEEP CHARACTERIZATION OF BIO-MOLECULAR PATTERNS IN METASTATIC COLORECTAL CANCER: AN EXPERIMENTAL ANALYSIS ON A SURGICAL COHORT

Background

Metastases from CRC are the leading cause of cancer-related mortality and are present in approximately 25% of patients at diagnosis. The liver is the most common site of metastases from CRC and about 50% of patients will develop hepatic metastases during the course of disease. The lung is the second most frequently involved site in metastatic disease and affects 10% - 15% of patients with CRC at diagnosis.

Rewarding survival rates have been reported after resective surgery of isolated hepatic or pulmonary metastases (25% - 58% and 32% - 68% at 5 years, respectively). Currently, there are few data on long-term results in colorectal cancer patients who underwent surgery for both hepatic and pulmonary metastases after colorectal resection. In such patients, the standard of treatment is still under debate. However, one of the main issue regards of the lack of bio-molecular factor to be considered when stratifying the long-term prognosis and personalizing the treatment of such patients.

Therefore, surgical resection of both hepatic and pulmonary metastases is nowadays recommended based only on clinical aspects, while genetic/bio-molecular aspects involved in colorectal tumor carcinogenesis are poorly investigated.

Objectives

AIM 1: We will analyze the long-term results (overall survival and progression-free survival) of a large surgical series of patients undergone surgery for both hepatic and pulmonary metastases after colorectal cancer resection in order to identify every clinical, pathological or surgical variable able to influence long-term outcomes.

AIM 2: We will perform a lab analysis on the surgical specimens consisting of a deep genetic sequencing with the double aim of exploring the bio-molecular alterations in both metastatic sites and comparing them with bio-molecular profile of primary colorectal cancer.

Methods

AIM 1: A retrospective analysis of clinical, pathological, surgical and follow-up information will be performed using a dedicate anonymous database. All consecutive patients who underwent surgery for hepatic or pulmonary colorectal metastases in 2 Institutions (IRCCS - Santa Maria Nuova Hospital of Reggio Emilia and Catholic University of the Sacred Heart of Rome) from January 2000 to June 2015 will be enrolled in the present study. Patient, treatment, and outcomes variables were analyzed using Log-rank, Cox regression, and Kaplan-Meier methods.

AIM 2: We will collected specimens of primary site (colorectal cancer) and metastatic site (liver and lung) at the lab of Promoting Center (IRCCS - Santa Maria Nuova Hospital of Reggio Emilia).

In cooperation with biologists of the Lab, tumor tissue will be microdissected from formalin-fixed paraffin-embedded (FFPE) sections under microscope guidance and DNA will be extracted using the FFPE Plus LEV DNA Purification Kit (Promega) and the Maxwell 16 instrument (Promega). Then, samples will be processed with the TruSight Tumor 26 genes panel (Illumina), a 174-amplicon multiplexed targeted resequencing assay. The process will include the DNA Quality Control test, followed by the generation, quantification and normalization of the libraries, which will be sequenced with the MiSeq Desktop Sequencer instrument (Illumina). Finally, data will be analyzed with the AmpliconDS protocol and with VariantStudio (Illumina) and dedicated softwares. Somatic variant will be identified by selecting mutations with no reported minor allele frequency in germline mutations repositories, according to VariantStudio software.

Expected results

AIM 1: We will add new information on long-term results of patients underwent surgical resection of both metastatic sites (lung and liver) from colorectal cancer. New clinical prognostic factor could be identified, allowing physicians to better manage this selected subset of patients.

AIM 2: We will explore the complexity of genetic profile of metastatic colorectal cancer by investigating bio-molecular alterations of 26 genes directly involved in cancerogenesis. Moreover, by comparing the genetic profiles of metastatic sites and primary tumor, we will investigate the association between genetic asset and cancerogenetic process. These speculative results could be the proof of principle for future pre-clinical or experimental analyses on this issue.

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