EDITORIAL

PSYCHOLOGICAL STRESS AND CANCER

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All the concepts reported in this editorial are based on recent literature data obtained through a PubMed search, using both Medline and manual searches, with particular reference to articles, which could be relevant to clinical practice. This paper contributes to the existing literature on depression and stress and provides important information for the development of effective strategies to manage these conditions among patients with cancer.
DECREASED mRNA EXPRESSION OF TWO FOXP3 ISOFORMS IN PERIPHERAL BLOOD MONONUCLEAR CELLS FROM PATIENTS WITH RHEUMATOID ARTHRITIS AND SYSTEMIC LUPUS ERYTHEMATOSUS

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Both the number and functional capacity of T-regulatory (Treg) cells are known to be decreased in various autoimmune diseases. FOXP3, an essential transcription factor for Treg cells, has three isoforms in humans, wild, and exon 2- and exon 2-exon 7-lacking, although their role in autoimmunity is not clearly understood. Here, we investigated the messenger RNA (mRNA) expression of the major wild and exon-2 isoforms in peripheral mononuclear cells by quantitative PCR methods in 56 subjects, consisting of 23 rheumatoid arthritis (RA) and 25 systemic lupus erythematosus (SLE) patients, and 8 healthy controls (HCs). Although mRNA expression of the two isoforms did not directly correlate with clinical disease activity, relative expression of both was significantly lower in SLE and RA patients than in HCs. Furthermore, we found a significant statistical correlation between the two isoforms, suggesting that they are similarly regulated. Decreased expression of these isoforms in RA and SLE may reflect Treg cell abnormalities in these autoimmune diseases.
CORRELATIONS OF SERUM INTERLEUKIN-16, TOTAL IgE, EOSINOPHIL CATIONIC PROTEIN AND TOTAL EOSINOPHIL COUNTS WITH DISEASE ACTIVITY IN CHILDREN WITH ATOPIC DERMATITIS

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Several laboratory parameters have been investigated for assessing disease activity in children with atopic dermatitis (AD). Analyses of the correlation between these parameters and clinical severity can help to choose a convincing tool. This study compared the significance of serum interleukin-16 (IL-16), serum total immunoglobulin E (IgE), serum eosinophil cationic protein (ECP), and total eosinophil count (TEC) in reflecting AD severity in order to identify the most relevant objective tool for assessing AD activity and to assess the correlation between these laboratory parameters. The Severity Scoring of Atopic Dermatitis (SCORAD index) was used for the assessment of disease activity in 48 pediatric patients in the acute exacerbation phase and in the maintenance phase after improvement of clinical findings with conventional treatment for 8 weeks. Serum levels of total IgE, ECP, and IL-16 as well as TEC were measured on the same time points and compared with healthy non-atopic controls. The correlation between SCORAD and each laboratory parameter was tested for significance and compared. Serum levels of total IgE, IL-16, ECP, and TEC in the acute exacerbation phase and with SCORAD for serum IgE (r=-0.317, p=0.028), TEC(r=-0.434, p=0.002), IL-16 (r = 0.321, p=0.026) in the acute exacerbation phase, and with SCORAD for serum IgE (r=-0.510, p<0.001), TEC(r=-0.489, p<0.001), serum ECP (r=-0.468, p=0.001) in the maintenance phase. Serum levels of total IgE, IL-16, ECP, and TEC correlated with the SCORAD index in pediatric patients with atopic dermatitis. Thus, they can serve as serum markers for monitoring disease activity in childhood atopic dermatitis.
ISOENZYMES OF ADENOSINE DEAMINASE AND METALLOPROTEINASES AS BIOMARKERS IN IN VITRO FERTILIZATION AND EMBRYO TRANSFER

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It is estimated that approximately 1% of babies born per year result from in vitro fertilization and embryo transfer, and other assisted reproductive technologies. In humans, the exact mechanisms that lead to embryonic attachment to the endometrial epithelium and invasion into the endometrial stroma have not been fully characterized. The aim of the study is to estimate serum total adenosine deaminase and isoenzymes ADA1, ADA2, as well as MMP-2, MMP-3, MMP-13 and MIP-1a as parameters for pregnancy following IVF-ET. The study group comprised seventeen women who conceived (Group A) and nineteen women aged 21-42 years who did not conceive (Group B) after IVF-ET. Blood samples were collected between 09.00 and 10.00 a.m. during IVF-ET treatment at two different periods. The first blood sample was collected before ET and the second sample 14 days after ET. All serum samples were assayed for the MMP-2, MMP-3 MMP-13 and MIP-1a concentrations with ELISA assay. Serum tADA activity was measured by a spectrophotometer using adenosine as the substrate (Method by Giusti). According to our results it was demonstrated that women who successfully conceived after IVF-ET showed significantly lower serum concentrations of ADA1, MMP-2, MMP-3 and higher serum concentration of MMP-13 at 14 days following ET. In conclusion, ADA1 may play a protective role at the hemochorial interface. Thus, our results suggest that ADA1 may have a modulatory role in the implantation and duration of the pregnancy. In women with successful or unsuccessful pregnancy compared with normal women the levels of ADA and MMPs may be affected by the exogenous hormone therapy according to the protocol of ovarian stimulation during IVF-ET.
EFFECT OF INHIBITION OF THE UBIQUITIN-PROTEASOME-SYSTEM AND IκB KINASE ON AIRWAY INFLAMMATION AND HYPERRESPONSIVENESS IN A MURINE MODEL OF ASTHMA

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The current treatment of asthma is far from optimal and there is a need for novel therapeutic approaches. NFκB has recently been highlighted as an important pro-inflammatory transcriptional factor and its blockade is believed to represent a new therapeutic approach for asthma. The purpose of this study is to investigate the effects of blocking the actions of NFκB, through inhibition of the ubiquitin-proteasome system (UPS) or IκB kinase (IKK), in a murine model of asthma. Treatment with the UPS inhibitor, MG-132 (0.03 and 0.1 mg/kg), did not significantly affect the ovalbumin-induced increase in total and differential cell numbers, histological changes such as perivascular and peribronchial inflammatory cell infiltration, perivascular and peribronchial fibrosis or the increased Penh to methacholine. In contrast, treatment of mice with the IKK inhibitor, BAY 11-7085, (3 and 10 mg/kg) dose-dependently inhibited the ovalbumin-induced increase in airway leukocyte influx and decreased the percentage of airway lymphocytes, neutrophils and eosinophils. Also, BAY 11-7085-treated (10 mg/kg) mice showed a significant decrease in the histologically assessed inflammatory indices as well as a significant reduction in the ovalbumin-induced increase in Penh to inhaled methacholine. Furthermore, BAY 11-7085 significantly inhibited the ovalbumin-induced increase in the level of phosphorylation of IκBα and extracellular regulated kinases (ERK) 1/2, whilst MG-132 significantly increased the phosphorylation of (ERK) 1/2. These findings confirm the critical role that NFκB plays in airway inflammation, highlight the importance of IKK in regulating the pro-inflammatory activity of NFκB and also suggest that UPS may not be a useful drug target for asthma treatment.
TOLL-LIKE RECEPTOR 4-DEPENDENT ADJUVANT ACTIVITY OF KAKKON-TO EXTRACT EXISTS IN THE HIGH MOLECULAR WEIGHT POLYSACCHARIDE FRACTION

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Kakkon-to, a traditional herbal medicine (Kampo formula), has been used historically in China and Japan for the treatment of infectious diseases such as influenza and the common cold. However, the biological mechanism of its therapeutic action has not yet been elucidated. In this study, we investigated the immunological function of Kakkon-to and found that the high molecular weight fraction of the extract activated macrophages in vitro. This fraction was found to be composed primarily of saccharides and in vitro intensively stimulated mouse peritoneal macrophages that produce Th1 inflammatory cytokines such as tumor necrosis factor α (TNFα), interleukin-1β (IL-1β), interferon-γ (IFN-γ), and interleukin-6 (IL-6). The fraction did not activate macrophages from C3H/HeJ lacking Toll-like receptor 4 (TLR4) or MyD88-deficient mice, indicating that macrophage activation by the fraction was mediated by TLR4. The route of administration of the fraction into mice regulated the kinetics of TNFα production in immune organs. Intravenous administration induced TNFα production in the four target organs of spleen, liver, lung, and Peyer’s patch; however, the most abundant production occurred in the liver and peaked at 30-60 min post administration. Peritoneal administration induced similar kinetics but the most abundant production occurred in the spleen. In contrast, oral administration induced TNFα production in the liver, lung, and Peyer’s patch, but not in the spleen. Although liver and lung are TNFα-abundant organs, production peaks in these organs occurred later than in Peyer’s patch. We also found that the fraction induced antibody production as an adjuvant against a specific antigen [ovalbumin (OVA)] when administered simultaneously and subcutaneously in a dose-dependent manner. Interestingly, the fraction induced IgG-class antibody in response to low doses of the antigen, which induced only IgM-class antibody when administered alone, suggesting that the fraction induces a class switch of immunoglobulin as an adjuvant in vivo. The high molecular weight fraction of Kakkon-to extract could be applicable as a potent immunostimulating drug and adjuvant.
THE SPHINGOSINE KINASE ACTIVATOR K6PC-5 STIMULATES C2C12 MYOBLAST DIFFERENTIATION

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Previously, K6PC-5, a synthetic derivative of ceramide, was demonstrated to activate sphingosine kinase (SK)-1 in keratinocytes. In this study its potential biological effect in mouse myoblasts was examined. The obtained results show that K6PC-5 promotes myogenic differentiation by enhancing myogenic marker expression, differentiation index and fusion index. Interestingly, its biological action was prevented by pharmacological inhibition of SK or S1P$_2$ receptor, in full agreement with their recognized role in myoblast differentiation. This is the first evidence that pharmacological activation of SK accelerates myogenesis and suggests that this new therapeutic strategy could be possibly employed in skeletal muscle disorders where muscle regeneration is deficient.
INHIBITION OF THE COMPLEMENT SYSTEM BY GLUTATHIONE: MOLECULAR MECHANISMS AND POTENTIAL THERAPEUTIC IMPLICATIONS

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Glutathione (GSH), a component of the antioxidant defence system, plays a role in autoimmunity and the complement system is often responsible for tissue damage in autoimmune diseases. The aim of this study is to evaluate the effects of GSH on the complement system. The complement system was examined in the normal human sera (NHS) of 30 healthy subjects. Increasing quantities of GSH (1, 2, 10, 20 mg) were incubated in 1 ml of each NHS. The mixtures were evaluated for complement activities (THC, CPA and APA) and for the presence of cleavage fragments of activation of C3 and B. GSH was also incubated with human complement in the presence of classical and alternative pathway activators. The results showed an inhibitory effect of GSH on the complement system starting from a dosage of GSH≥1 mg/ml. Indeed, when NHS was incubated with GSH at such dosage, a significant reduction of the complement activities THC, CPA, and APA was observed (P<0.0001, P<0.005, P=NS, respectively), and no cleavage fragments of C3 or B were found. Further analysis demonstrated that the inhibition was exerted on C3-9 and to a lower extent on classical and alternative pathway C3-convertases. Our results indicate that GSH is capable of inhibiting the complement system. These findings are relevant for the design of interventions aimed at modulation of GSH metabolism to inhibit complement-mediated damage in autoimmune diseases.
INHIBITING PROLIFERATION IN KB CANCER CELLS BY RNA INTERFERENCE-MEDIATED KNOCKDOWN OF NICOTINAMIDE N-METHYLTRANSFERASE EXPRESSION

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The enzyme Nicotinamide N-methyltransferase (NNMT) catalyzes the methylation of nicotinamide and other pyridines, playing a pivotal role in the biotransformation and detoxification of many drugs and xenobiotic compounds. Several tumours have been associated with abnormal NNMT expression, however its role in tumour development remains largely unknown. In this study we investigated expression levels of Nicotinamide N-methyltransferase in a cancer cell line and we evaluated the effect of shRNA-mediated silencing of NNMT on cell proliferation. Cancer cells were examined for NNMT expression by semiquantitative RT-PCR and Western blot analysis. A HPLC-based catalytic assay was performed to assess enzyme activity. Cells were transfected with four shRNA plasmids against NNMT and control cells were treated with transfection reagent only (mock). The efficiency of gene silencing was detected by Real-Time PCR and Western blot analysis. MTT cell proliferation assay and the soft agar colony formation assay were then applied to investigate the functional changes in cancerous cell. NNMT mRNA was detected in cancer cells, showing a very high expression level. In keeping with the results of RT-PCR analysis, the protein level and NNMT enzyme activity were particularly high in KB cells. ShRNA vectors targeted against NNMT efficiently suppressed gene expression, showing inhibition observed at both the mRNA and protein levels. Down-regulation of NNMT significantly inhibited cell proliferation and decreased colony formation ability on soft agar. The present data support the hypothesis that the enzyme plays a role in tumour expansion and its inhibition could represent a possible molecular approach to the treatment of cancer.
AURORA B EXPRESSION AS A PROGNOSTIC INDICATOR AND POSSIBLE THERAPEUTIC TARGET IN ORAL SQUAMOUS CELL CARCINOMA

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The aim of this study is to investigate the expression of the chromosomal passenger protein Aurora B and its activated (phosphorylated) form in a large series of human oral squamous cell cancers (OSCC) and to evaluate its clinical and prognostic significance. Western blotting analysis revealed overexpression of both Aurora B and Thr-232 Phospho-Aurora B in OSCC lines as compared to normal keratinocytes and bladder cancer cells. Furthermore, protein expression was analysed by immunohistochemistry in 101 OSCC of different site, stage and histological grade and in normal peritumoural areas. The intracellular localization of Aurora B in tumour cells was mainly nuclear, especially in proliferative areas, and significant overexpression was found in tumours in comparison to normal peritumoural areas (P=0.012). Staining results were correlated with clinicopathological parameters and long-term follow-up, and a significant association was found between protein expression and tumour stage (stage II, III and IV vs stage I, P=0.030) and size (<2cm vs >2cm, P=0.010). Cox regression analysis confirmed a poorer disease-free survival in cases with high expression of Aurora B protein. Kaplan-Meier curves showed shorter time to progression in patients with high levels of Aurora B expression (p<0.05). Moreover, the tumoral group with nuclear Aurora B immunolocalization had the worst prognosis (P=0.0364 in disease free survival). Our results suggest that assessing Aurora B expression might help in patients’ risk stratification and serve as a novel therapeutic target in advanced OSCCs.
THE COMBINATION OF IMMUNOSUPPRESSIVE DRUGS WITH 8-METHOXYPSORALEN AND ULTRAVIOLET A LIGHT MODULATES THE MYELOID-DERIVED DENDRITIC CELL FUNCTION

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The functional properties of myeloid dendritic cells (DCs) differ, depending on microenvironmental factors as well as on their stage of maturation. The main approaches for the selective enhancement of the tolerogenic properties of DCs include the induction of a pharmacological arrest of the DCs maturation and the genetical engineering of DCs expressing immunosuppressive molecules. Several immunosuppressive/anti-inflammatory agents have been discovered that potentially inhibit DC maturation and immunogenicity. Photopheresis (ECP) is an immunomodulatory therapy in which leucocytes are exposed to 8-methoxypsoralen (8-MOP) and ultraviolet (UV) A radiation (PUVA). The combination of ECP with immunosuppressive agents has demonstrated efficacy in the management of transplanted patients by reducing either the incidence of organ rejection or the pharmacological toxicity. In particular, we have observed in hepatitis C virus (HCV)-positive patients that the same combination has reduced the immunosuppressive burden and improved sustainability and efficacy of pre-emptive antiviral therapy after liver transplantation. Therefore, in our work we investigated the in vitro effects of PUVA, combined with immunosuppressive drugs (IDs), on both in vitro human DC generation and maturation, in order to contribute to understanding the immunological mechanisms underlying this pharmacological combination. Monocyte PUVA-treatment was performed by using an in vitro experimental protocol that we previously described. PUVA-treated or -untreated highly purified CD14+ cells were incubated with the association of the immunosuppressive drugs, used in the management of liver transplantation, at two different concentrations, in the presence of IL-4 and GM-CSF. The treatment with IDs at the highest concentration (corresponding to that used in clinical practice), alone or in association with PUVA, induced an immunosuppressive effect, by impairing both DC generation and maturation. Neither immunosuppressive drugs at the lowest concentration nor their
CHARACTERIZATION OF THE IMMUNE RESPONSE OF HUMAN CORD-BLOOD DERIVED γδ T CELLS TO STIMULATION WITH AMINOBISPHOSPHONATE COMPOUNDS

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Vγ9Vδ2 T lymphocytes have been shown to respond to a variety of non-peptide antigens including alkylamines and phosphoantigens. Recently, aminobisphosphonates have also been shown to stimulate this subset of γδ+ T cells. In this study we analyzed the proliferative responses of freshly isolated γδ T lymphocytes obtained from human cord blood when challenged with pyrophosphomonoesters or aminobisphosphonates. Nitrogen-containing aminobisphosphonates, in contrast to phoshoantigens, readily stimulated expansion of Vδ2Vγ9 cells in human cord blood. Expanded cells displayed an activated mature phenotype, and were capable of producing TNFα and IFNγ but not perforin following secondary stimulation, consistent with the development of a regulatory, as opposed to cytotoxic, phenotype. This approach may provide a useful strategy for a new approach to the treatment of neonatal pathologies.
CONSERVATIVE MANAGEMENT OF CANINE TRACHEAL COLLAPSE WITH STANOZOLOL: A DOUBLE BLINDED, PLACEBO CONTROL CLINICAL TRIAL

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The objective of this study is to determine the efficacy of stanozolol in the treatment of tracheal collapse (TC) in dogs, which is the analogous disease to tracheomalacia (TM) in humans. Twenty-two dogs with endoscopically confirmed and graded TC were enrolled into five groups. Groups S1 (n=5), S2 (n=5) and S3 (n=4) with grade 1, 2 and 3 TC, respectively, received stanozolol orally for 75 days, while groups P1 (n=4) and P2 (n=4) with grade 1 and 2 TC, respectively, received placebo. The clinical score was evaluated every 15 days, whereas TC grade was reassessed at the end of the experiment. Clinical improvement was detected from the 30th day in S2 and S3 group dogs and from the 45th day in S1 group dogs and continued until the end of the experiment. Also, statistically significant differences were seen between S2 and P2 dogs from the 30th day, and between S1 and P1 dogs from the 60th day, and continued until the end of the study. Amelioration of the TC grade was seen in 13 of 14 (92.9%) dogs, which received stanozolol. Of the 14 dogs, 57.1% were cured and 35.8% demonstrated a less severe TC grade, while only one dog (7.1%) did not improve at all. Stanozolol seems to be an effective drug in the management of canine TC and it may have potential for use in humans with TM.
DIRECT HITS TO THE HEAD DURING AMATEUR BOXING IS ASSOCIATED WITH A RISE IN SERUM BIOMARKERS FOR BRAIN INJURY

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Boxing exposes participants to the physiological response to high intensity exercise and also to direct body and brain trauma. Amateur boxing is increasing and females have also been included in the Olympics. The aim of this study is to assess the stress response and possible brain injury incurred during a match by measuring serum biomarkers associated with stress and cellular brain injury before and after combat. Sixteen male amateur boxers were studied retrospectively. The study population was divided into two groups: (a) a group that received predominantly punches to the head (PTH) and (b) a group that received predominantly punches to the body (PTB). Blood samples were taken before and five minutes after each contest. They were analysed for S-100B, neuron-specific enolase (NSE), creatine kinase (CK) and cortisol. The PTH group received direct contacts to the head (not blocked, parried or avoided) and to the body (n=8, age: 17.6 ± 5.3, years; height: 1.68 ± 0.13, meters; mass: 65.4 ± 20.3, kg). The PTB group received punches to the body including blocked and parried punches, but received no direct punches to the head, (n=8, mean ± SD, age: 19.1 ± 3.2 years; height: 1.70 ± 0.75, meters; mass: 68.5 ± 15 kg). Significant increases (P<0.05) were observed between pre- and post-combat serum concentrations in serum concentrations in PTH of S-100B (0.35 ± 0.61 vs. 0.54 ± 0.73, μg.L⁻¹) NSE (19.7 ± 14 vs.31.1 ± 26.6, ng.ml⁻¹) and cortisol (373 ± 202 vs. 756 ± 93, nmol.L⁻¹). Significant increases (P<0.05) of creatine kinase were recorded in both groups. This study demonstrates significant elevations in neurochemical biomarkers in boxers who received direct blows to the head. However, further work is required to quantify this volumetric brain damage and long term clinical sequelae.
HIGH RISK HUMAN PAPILLOMAVIRUS GENOTYPING IN CLINICAL SAMPLES: EVALUATION OF DIFFERENT COMMERCIAL TESTS

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The aim of the present study is to compare the performance of several commercial human papillomavirus (HPV) tests in a cohort of 281 women. The hybrid capture II, the PreTect-HPV-Proofer, the linear array, and DR.HPV™ IVD were utilized to detect and type HPV in parallel with in-house PCR tests followed by direct automated sequencing or by sub-cloning and sequencing. The concordance levels along with other tests were evaluated with a Cohen’s K value varying between 0.60 to 0.88, indicating good correlation with nearly perfect agreement between hybrid capture II, (HCII) and the linear array test. High sensitivity was recorded by the linear array and HCII with 100% (95% CI, 0.8021 to 1.0000) detection of cervical intraepithelial neoplasia (CIN) III by both methods. Conversely, the PreTect-HPV-Proofer showed high specificity with 12% (95% CI, 0.7966 to 0.9163) positivity on normal samples. The genotyping analysis showed that agreement among tests was only low to moderate with great differences between different HPV types. Multiple infections were detected with poor concordance and sub-cloning assays revealed the presence of a lower number of HPV in comparison to the other methods. In summary, the use of different HPV tests applied to the same group of cervical smears may possibly lead to incongruent results, suggesting the need to standardize type-specific sensitivity of genotyping methods and the need to evaluate their accuracy in detecting multiple HPV infections. This would be a prerequisite for the use of genotyping assays in cervical cancer screening programs.
ZOLEDRONIC ACID ENHANCES V<sub>δ</sub>2 T-LYMPHOCYTE ANTITUMOR RESPONSE TO HUMAN GLIOMA CELL LINES

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Glioblastoma multiforme (GBM), the most frequent and aggressive primary brain tumor in humans, responds modestly to treatment: most patients survive less than one year after diagnosis, despite both classical and innovative treatment approaches. A recent paper focused on γδ T-cell response in GBM patients, suggesting the application of an immunomodulating strategy based on γδ T-cells which is already in clinical trials for other tumors. Human V<sub>δ</sub>2 T-cells recognize changes in the mevalonate metabolic pathway of transformed cells by activating cytotoxic response, and by cytokine and chemokine release. Interestingly, this activation may also be induced in vivo by drugs, such as zoledronic acid, that induce the accumulation of V<sub>δ</sub>2 T-cell ligand Isopentenyl-pyrophosphate by blocking the farnesyl pyrophosphate synthase enzyme. The aim of our work is to confirm whether bisphosphonate treatment would make glioma cell lines more susceptible to lysis by in vitro expanded γδ T-cells, improving their antitumor activity. We expanded in vitro human V<sub>δ</sub>2 T-cells by phosphoantigen stimulation and tested their activity against glioma cell lines. Co-culture with glioma cells induced V<sub>δ</sub>2 T-cell differentiation in effector/memory cells, killing glioma cells by the release of perforin. Interestingly, glioma cells were directly affected by zoledronic acid; moreover, treatment increased their activating ability on V<sub>δ</sub>2 T-cells, inducing an effective antitumor cytotoxic response. Taken together, our results show that aminobisphosphonate drugs may play a dual role against GBM, by directly affecting tumor cells, and by enhancing the antitumor response of V<sub>δ</sub>2 T-cells. Our results confirm the practicability of this approach as a new immunotherapeutic strategy for GBM treatment.
DO DNA-METHYLATION AND HISTONE ACETYLATION PLAY A ROLE IN CLEAR CELL RENAL CARCINOMA? ANALYSIS OF RADICAL NEPHRECTOMY SPECIMENS IN A LONG-TERM FOLLOW-UP

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We investigated global methylation and histone acetylation in 50 conventional clear cell renal carcinomas (RCC), treated with radical nephrectomy, to assess their possible role as diagnostic biomarkers. The features considered in this study were patient age, tumor size and grade, percentage and intensity of 5-methylcytosine (5mc) and Acetyl-Histone (Lys 9) expression in tumor tissue. All considered parameters were correlated with patient specific survival. The mean percentage of global cellular methylation in tumoral tissue was significantly higher compared to normal peritumoral tissue (p<0.0001), while the intensity of cellular methylation was significantly higher in normal tissue than in tumoral tissue (p=0.001). The mean percentage of histone cellular acetylation in tumoral tissue was significantly lower compared to normal peritumoral tissue (p=0.0005), while the intensity of mean acetylation in neoplastic tissue was similar to the normal tissue. The percentage of global DNA methylation was significantly higher in grades 3 and 4 tumors (p=0.033). Global DNA methylation and histone acetylation in tumoral tissue did not correlate with survival. Fuhrman grade was statistically significant for prognosis (p=0.031). In conclusion, global hypermethylation and histone hypoacetylation play an important role in RCC carcinogenesis; Fuhrman grade is still considered the most important factor for patient survival; 5mc can have a role as markers of aggressiveness.
ROLE OF THE QUANTIFERON-TB TEST IN RULING OUT PLEURAL TUBERCULOSIS: A MULTI-CENTRE STUDY

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Diagnosing pleural tuberculosis (pITB) might be difficult due to limited sensitivity of conventional microbiology tools. As M. tuberculosis (MTB)-specific T cells are recruited into pleural space in pITB, their detection may provide useful clinical information. To this aim, in addition to standard diagnostic tests, we used the QuantiFERON-TB Gold In-Tube (QFT-IT) test in blood and pleural effusion (PE) samples from 48 patients with clinical suspicion of pITB, 18 (37.5%) of whom had confirmed pITB. Four of them (22.2%) tested positive with a nucleic acid amplification test for MTB. The tuberculin skin test was positive in most confirmed pITB cases (88.9%). Positive QFT-IT tests were significantly more frequent in patients with confirmed pITB, as compared to patients with an alternative diagnosis, both in blood (77.7 vs 36.6%, p=0.006) and in PE samples (83.3% vs 46.6%, p=0.02). In addition, both blood and PE MTB-stimulated IFN-γ levels were significantly higher in pITB patients (p=0.03 and p=0.0049 vs non-pITB, respectively). In blood samples, QFT-IT had 77.8% sensitivity and 63.3% specificity, resulting in 56.0% positive (PPV) and 82.6% negative (NPV) predictive values. On PE, QFT-IT sensitivity was 83.3% and specificity 53.3% (PPV 51.7% and NPV 84.2%). The optimal AUC-derived cut-off for MTB-stimulated pleural IFN-γ level was 3.01 IU/mL (77.8% sensitivity, 80% specificity, PPV 68.4% and NPV 82.8%). These data suggest that QFT-IT might have a role in ruling out pITB in clinical practice.
A NEW CHANCE TO MAINTAIN REMISSION INDUCED BY ANTI-TNF AGENTS IN RHEUMATOID ARTHRITIS PATIENTS: CynAR STUDY II OF A 12-MONTH FOLLOW-UP

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The advent of biological therapies represented the beginning of a new era in the therapy of Rheumatoid Arthritis (RA), as demonstrated in several studies, but still many questions about their safety, especially in long term use, and correct administration time remain unanswered. Once remission is achieved, the orientation of clinicians regarding the maintenance of biological therapy or the switch to another immunosuppressive therapy is still uncertain. In our previous study 21 patients affected by RA who reached remission by the use of a combined therapy of anti-TNF drugs and methotrexate (MTX) underwent CyA-MTX combination therapy for maintaining remission state and were evaluated during a 6-month follow-up. The present study aims to investigate these data by a longer follow-up (12 months) and on a larger population. Fifty-three RA patients, with a disease duration of less than 3 years and DAS28<3.2 that reached a level of low disease activity within 6-8 months from the beginning of anti-TNF and methotrexate therapy, were enrolled in the study. By the suspension of anti-TNF therapy, patients underwent A-Cyclosporine (2-3 mg/kg/day) and methotrexate (15mg/week) therapy. DAS28, Pain VAS, Erythrosedimentation rate (ESR), C Reactive Protein (CRP) were all tested at time 0 and every 2 months after the interruption of the anti-TNF therapy and the beginning of A-Cyclosporine and methotrexate therapy, as well as liver and kidney profiles. Side effects were also recorded. Of 53 patients, 50 completed the study with a 12-month follow-up. Twenty-one (42%) patients maintained clinical parameters within low disease activity values at 12 months, while 29 (58%) patients showed an increase in DAS28 and other parameters: 16 (32%) patients at the 6-month control, 13 (26%) patients at the 12-month control. Our data show that 42% of the patients undergoing A-Cyclosporin and Methotrexate therapy maintained low disease activity parameters of rheumatoid arthritis, obtained after 6-8 months of anti-TNF therapy. Further studies on larger populations are necessary in order to confirm such results and identify predictor factors for different responses.
CD1A and CD1E gene polymorphisms are associated with susceptibility to multiple sclerosis

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Multiple sclerosis (MS) is thought to be an autoimmune T-cell-mediated disease directed at myelin antigens of the central nervous system. Besides myelin proteins, lipid components of CNS are supposed to play a role as antigens for T cells in MS. CD1 is a family of MHC-like glycoproteins specialized in capturing and presenting a variety of microbial and self lipids and glycolipids to antigen-specific T cells. CD1-restricted T cells specific for gangliosides and sulfatide have been isolated from subjects with MS and in mice with experimental allergic encephalopathy. We genotyped exon 2 of CD1A and CD1E in 205 MS patients and 223 unrelated healthy controls and determined their association with the presence of anti-ganglioside and anti-sulfatide antibodies. CD1E 01-01 is associated with a reduced risk of MS (OR 0.54, p=0.001); CD1A 02-02 (OR 1.99, p=0.012) or CD1E 02-02 (OR 2.45, p=0.000) with an increased risk. The combination of the genotypes CD1A 02-02 and CD1E 02-02 is present in 90.7% of patients but in only 9.4% controls (OR 94.16, p=0.000). CD1A and CD1E polymorphisms contribute to the polygenic susceptibility to MS. The functional effects of CD1 polymorphisms are unknown, however changes in CD1 alleles may affect numerous immunological functions.
MODULATION OF TOLL-LIKE RECEPTORS IN PSORIATIC PATIENTS DURING THERAPY WITH ADALIMUMAB

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Toll-like receptors (TLRs) are a key part of the innate immune system that detect pathogen-associated molecular patterns (PAMPs) of microorganisms and their stimulation results in the activation of signaling pathways leading to the modulation of inflammatory and immune responses. Since psoriasis is a complex, inflammatory and immune skin disease, characterized by an abnormal immune response and increased proliferation of keratinocytes, with an increased production of proinflammatory cytokines, TLRs could play an important role in the pathogenesis of the disease. We propose to assess the modulation of TLR expression on psoriatic skin of patients treated with Adalimumab and systemic conventional therapies. We therefore recruited fifteen patients: ten were treated with adalimumab and five with systemic conventional therapies; their clinical conditions were analyzed by PASI index and skin biopsies were evaluated for TLR1 and TLR2 expression by immunohistochemistry assays. Our data suggest adalimumab is not only able to improve the clinical condition of psoriatic patients, but also to modulate TLR1 and TLR2 expression involved in psoriasis, as in healthy skin. Adalimumab is a most promising biological drug able to orchestrate immune and inflammatory responses in psoriatic lesions, recovering TLR expression on basal keratinocytes and improving clinical conditions of psoriatic patients, with no evident side effects.
AEROSOL THERAPY WITH THIAMPHENICOL GLYCINATE: A RETROSPECTIVE STUDY ON EFFICACY AND SAFETY IN A GROUP OF SIXTY-SIX ONCOLOGICAL PATIENTS

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The purpose of this paper is to present the effectiveness of aerosol administration of TG in a group of oncological patients. Thiamphenicol is an antimicrobial agent active in the treatment of infection of different etiology and localisation due to its broad spectrum of antimicrobial activity as well as its pharmacokinetic properties. The data of the retrospective study analysis of the activity of TG, administered to oncological patients affected by infections of the respiratory tract, showed that TG administered alone or in association with other antibiotics was globally effective in more than 95% of patients. These positive results were obtained in immunologically compromised patients. The therapeutic advantages of using TG are: ease of use - aerosol therapy permits good local action; tolerability - no adverse reaction or intolerance; the possibility of using it in an ideal association with other antibiotics to combat the infectious pathology.
CYCLOSPORIN-A EFFICACY IN CHRONIC IDIOPATHIC URTICARIA

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Common drugs in the therapy of chronic idiopathic urticaria (CIU) include antihistamines alone or combined with corticosteroids, but severe unresponsive patients require alternative treatments. This retrospective study aims to evaluate clinical response and safety of low-dose and long-term oral Cyclosporin-A (CyA) in unresponsive patients. One hundred and ten CIU patients, unresponsive to a previous treatment (antihistamines plus prednisone 0.2 mg/kg/day), received additional oral CyA 1–3 mg/kg/day for 6 months. The patients were subdivided into three groups (A, B, C) according to the different CyA doses. Parameters of clinical efficacy including pruritus, and size and number of wheals were evaluated at baseline, after three and six months. All adverse events were recorded. The mean total symptom severity score decreased by 63% in Group A, 76% in Group B, and 85% in Group C after 6 months. Total disappearance of the symptoms was recorded in 43 patients (39.1%): 7 (28%) of Group A; 12 (37.5%) of Group B and 24 (45%) of Group C. After a mean of 2 months from CyA suspension, 14 patients (11%) had recurrence of symptoms. Minor side effects were noted in 8 patients (7%). Our study indicates that low-dose, long-term CyA therapy is efficacious and safe in severe unresponsive CIU.
CHANGE OF γδTCR-EXPRESSING T CELLS IN HEALTHY AGING

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A mature T-cell lineage with the capacity to proliferate in response to receptor-mediated signals and to display non-major histocompatibility complex (MHC)-restricted cytolysis expresses a CD3-associated heterodimer made up of the protein encoded by the T-cell receptor (TCR) gamma-gene. We investigated the possible differences in lymphocyte subpopulations between healthy young-middle-aged and elderly subjects, focusing attention on γδ-TCR-expressing cells. The study was carried out on fifteen healthy young-middle-aged male subjects (age range 36-55 years) and fifteen healthy elderly male subjects (age range 67-79 years). Lymphocyte subpopulations were analyzed in blood samples collected every four hours for 24 hours. The presence of circadian rhythmicity on absolute counts was validated to evaluate the periodicity of variation, and the fractional variation between single time point values was calculated to evaluate the dynamics of variation. In the group of young and middle-aged subjects a clear circadian rhythm was validated for the time-qualified changes of all the lymphocyte subpopulations (CD3, CD4, CD4/CD8 ratio, CD20, CD25 and HLA-DR with acrophase at night, CD8, CD16 and TcRγδ with acrophase at noon). In the group of elderly subjects a clear circadian rhythm was validated for the nyctohemeral changes of CD3, CD8, CD4/CD8 ratio, CD16, CD25. There was a statistically significant difference for the Midline Estimating Statistic of Rhythm (MESOR) of CD3 (p=0.001), CD25 (p=0.003) and γδ-TCR-expressing cells (p=0.004), higher in the elderly, and for the MESOR of HLA-DR (p=0.002) and CD20 (p=0.002) higher in the young and middle-aged subjects. There was a statistically significant difference between the groups in the fractional variation of TcRγδ-expressing cells between 18:00h and 22:00h values (higher in elderly subjects, p=0.007). In conclusion, specific lymphocyte subsets present different levels and different profiles of nyctohemeral changes in healthy young-middle aged in respect to elderly subjects, since B cells are decreased, whereas CD25 and γδ-TCR-bearing cells are higher in the elderly, but the rhythm and the dynamics of variation of this lymphocyte subset is severely altered and this phenomenon might contribute to the onset of age-related variations of the immune responses.
PERCUTANEOUS STEROIDAL TREATMENT IN RELAPSES OF CHRONIC TENDINOPATHIES: A PILOT STUDY

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Relapses are frequently observed in subjects with chronic tendinopathies. Corticosteroid injections are usually performed with positive results, but are uncomfortable for the patient and not free from side effects. The aim of this pilot study is to evaluate the short-term efficacy and tolerability of an occlusive Betamethasone Valerate medicated Plaster (BMVP). Fifteen subjects with relapses of chronic tendinopathies (clinical and ultrasound diagnosis) were enrolled, and treated according to RICE (Rest – Ice – Compression – Elevation) protocol. An BMVP plaster was also applied on the affected tendon. Clinical examination, at baseline and after 7, 14, 21 and 28 days, included pain (VAS at rest and during activities) and functional evaluation. Local side effects on the area and drop-outs were also recorded. Pain, both at rest and during activities, significantly decreased at 28 days (from 3.7 ± 2.7 to 1.1 ± 1.7 [p < 0.01], and from 7.3 ± 1.7 to 3.3 ± 1.4 [p < 0.0000], respectively). Moreover, the patients reported a significant improvement in the functional limitation. Five subjects dropped out of the study. No side effects were reported. The release of the steroid in pharmacologically-active concentrations over 12–24 hours and the good penetration of the drug in subcutaneous tissues explain the positive results. BMVP application may be considered a reliable first therapeutic approach in relapses of chronic tendinopathies.
INTERFERON-INDUCED GENE EXPRESSION IN CERVICAL MUCOSA DURING HUMAN PAPILLOMAVIRUS INFECTION

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The aim of this study is to monitor type I interferon (IFN) activation in the cervical mucosa of Human Papillomavirus (HPV)-infected and uninfected women attending a routine gynaecologic clinic. The expression of three IFN-induced genes (MxA coding for human Mixovirus resistance protein A, ISG15 Interferon Stimulated Gene coding for a 15 kDa ubiquitin-like protein and UBP43 coding for the ISG15 isopeptidase) was determined as the mRNA copy number in cervical cells, normalized to the mRNA ones of the beta-glucuronidase gene. Type-specific HPV-DNA load was concurrently determined in the HPV-positive samples. Out of 127 samples tested, 54 were sufficient for both DNA and RNA extraction. The type-specific HPV-DNA copy numbers in the 34 HPV-positive samples varied widely. No significant association was found between copy numbers of MxA, ISG15, UBP43 and HPV status or viral load. However, despite a marked inter-individual variability, ISG15 expression was significantly higher when low-risk HPV infections were compared with HPV-negative samples, while high-risk HPV infections had very low ISG15 levels. The lack of ISG15 activation in high-risk HPV-infected cervical cells could be due to the lack of p53-mediated induction or to HPV-directed specific inhibition of type I IFN pathways. This study approach might be of value in clarifying the role of type I IFN activation in determining the clearance or persistence of HPV infections.
THE USE OF FIRST GENERATION VERSUS SECOND GENERATION ANTIPSYCHOTICS AS ADD-ON OR AS SWITCH TREATMENT AND ITS EFFECT ON QTc INTERVAL: THE ITALIAN EXPERIENCE IN A REAL-WORLD SETTING

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Some psychotropic drugs are connected with prolongation of the QT interval, torsade de pointes and sudden death. Recent data suggest that with regard to this adverse effect, the atypical antipsychotic drugs are no safer than the older drugs. The purpose of this study is to evaluate the different use of first generation versus second generation antipsychotics as add-on (Group I) or switch treatment (Group II) and its effect on QTc interval in a sample of schizophrenic and bipolar inpatients without medical illness. All patients had been evaluated twice by using ECG: on admission and after two weeks of hospitalization. Exclusions criteria were: abnormalities in levels of potassium, magnesium and calcium, cardiovascular and metabolic diseases, alcohol or drug abuse. We found a significant (p < 0.01) greater use of first generation antipsychotic in Group I (73.80%) than in the Group II (33.33%). Also Group I showed a significant increase (p < 0.0001) in total chlorpromazine equivalent (476.78 ± 448.80 mg/day vs 845.48 ± 491.64 mg/day) and in QTc interval (369.14 ± 33.75 ms vs 387.09 ± 31.97 ms), while we did not find any statistical difference in Group II during hospitalization. Our results, in spite of the small sample size, indicate that antipsychotic add-on can increase QTc interval more than switching to other antipsychotic in psychiatric patients without other risk factors.
Natural killer (NK) cells play a fundamental role in innate and early phases of adaptive immunity against viral infections, both in humans and in animal models. To date, NK cell deficiencies in patients with severe herpetic infections have been reported in single cases, and their role as predisposing factor is still controversial. Five children affected by herpetic encephalitis were consecutively admitted to the Anna Meyer Children’s Hospital in Florence (Italy) between 2003 and 2005. We therefore investigated the presence of NK cell deficiencies in a consecutive series of children with herpetic encephalitis. Five healthy children were included in the study as controls. Differential WBC counts, main Ig and IgE class serum analysis, cytofluorimetric analysis of circulating T, B and NK cells were performed on our study population. Sequencing of a selected region of CD16A gene transcript was carried out in two patients. All patients resulted to be affected by deficiencies related to NK cells in respect to controls. One patient was also affected by lymphopenia, while no other significant deficits of immunity were detected in the study population. To date, this is the first survey that demonstrates isolated NK cell deficiencies in a cohort of consecutive patients affected by severe herpes simplex infections. These findings suggest a role for NK cell deficiencies as a predisposing factor for increased susceptibility and severe course of disease in these patients.
LETTER TO THE EDITOR

MONILETHRIX TREATED WITH MINOXIDIL

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In literature many different therapies are proposed to treat Monilethrix, but a definitive therapy still does not exist. We decided to treat four patients affected by Monilethrix, with topical minoxidil 2%, 1 ml night and day for 1 year. Minoxidil led to an increase of normal hair shaft without any side effects in all the patients. Therefore topical minoxidil 2% could be considered a good therapy to treat Monilethrix.
LETTER TO THE EDITOR

SEARCH FOR GENOMIC SEQUENCES OF MICROBIAL AGENTS IN Atherosclerotic Plaques

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Atherosclerosis is a complex, multifactorial disease. Several studies have reported a possible association between infection with microbial agents and atherogenesis. Chlamydia pneumoniae (C. pneumoniae), Herpes Simplex Virus 1 (HSV1), Human Cytomegalovirus (HCMV), and Epstein Barr Virus (EBV) have been widely investigated for their possible role in atherosclerosis development, but the results obtained to date are contradictory. The aim of our study is to search DNA of the aforementioned infectious agents by means of Quantitative Real Time PCR in atherosclerotic plaques from carotid arteries obtained from 17 patients. Genomic sequences of C. pneumoniae, HSV1, HCMV were not found in any atherosclerotic lesion. Therefore, our results do not support the hypothesis of an association between these infectious agents and atherosclerosis. Conversely, three patients were found to be positive for EBV DNA, thus indicating that, at least in a limited number of patients, EBV could play a role in atherogenesis.
LETTER TO THE EDITOR

RECURRENT ATRIAL FIBRILLATION IN A PATIENT WITH ULCERATIVE COLITIS TREATED WITH AZATHIOPRINE: CASE REPORT AND REVIEW OF THE LITERATURE

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We present a clinical case report regarding recurrent atrial fibrillation in a patient with ulcerative colitis treated with azathioprine. Atrial fibrillation represents the most common sustained cardiac arrhythmia, occurring in 1-2% of the general population and characterized by seemingly disorganized atrial depolarizations without effective atrial contraction. Several mechanisms determine this arrhythmia; in particular remodelling (structural, mechanical and electrical alteration related to atrial fibrillation). The pro-arrhythmic effect of azathioprine may be evaluated during immunosuppressive therapy to be aware of this serious but reversible adverse effect.
LETTER TO THE EDITOR

NEUROENDOCRINE TUMORS DIAGNOSED AT THE “ANTONIO CARDARELLI” HOSPITAL (NAPLES, CAMPANIA, ITALY) BETWEEN 2006-2009: A SINGLE-INSTITUTION ANALYSIS

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Neuroendocrine tumors (NETs) are rare, with an incidence of about 5 per 100,000 inhabitants. As no study on NETs has ever been specifically conducted on the population of Campania, we performed a retrospective analysis of all newly diagnosed NETs at the “Antonio Cardarelli” hospital between 2006-2009. A search of the registry of the Pathology Department of the “Antonio Cardarelli” hospital was carried out to retrieve available data on all newly diagnosed NET cases. Two hundred and ninety-nine NET tumors were diagnosed at our Institution from January, 2006 to December, 2009. Globally, 121 patients (40% of the population) had a lung NET, while 92 patients (30% of the population) presented a GEP-NET. The most common primary tumor site varied by sex, with female patients being more likely to have a primary NET in the lung, breast or colon, and male patients being more likely to have a primary tumor in the lung. Also, twenty-three cases of breast NETs were identified, and clinical information regarding therapy and response was available for 22 patients. Our study represents a pioneering effort to provide the medical community in Campania with basic information on a large number of patients with different types of NETs. The “Antonio Cardarelli” hospital could greatly benefit from cooperation with other hospitals in order to become a highly specialized center for NETs in the region and Southern Italy.
LETTER TO THE EDITOR

AN 18-YEAR FOLLOW-UP OF A CASE OF D-PENICILLAMINE-INDUCED ELASTOSIS PERFORANS SERPIGINOSA

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Elastosis perforans serpiginosa (EPS) is a rare complication of chronic therapy with a high-dose of D-penicillamine (1 g daily for more than 5 years), characterized by the elimination of abnormal elastic fibers from the upper dermis through the epidermis. D-penicillamine (DPA) is a heavy metal chelator primarily used for disorders such as cystinuria and Wilson disease. This therapy can lead to induction of EPS through a still unknown mechanism. We report the follow-up of a D-penicillamine-induced EPS in patient with Wilson disease, which prompted us to switch the therapy with trientine (another metal chelator). After 14 years the cutaneous lesions are still visible; therefore, we conclude that the DPA-induced cutaneous damage is irreversible.
LETTER TO THE EDITOR

EVALUATION OF THE EFFECT OF BOSENTAN TREATMENT ON PROINFLAMMATORY CYTOKINE SERUM LEVELS IN PATIENTS AFFECTED BY SYSTEMIC SCLEROSIS

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Systemic sclerosis (SSc) is a connective tissue disease characterized by vascular and fibrotic changes in the skin and in internal organs. Endothelin-1 (ET-1) is a peptide that has a role in promoting both vascular injury and the fibrotic process in SSc; indeed, patients with systemic sclerosis have higher levels of ET-1 compared with healthy subjects. Moreover, ET-1 enhances expression of pro-inflammatory cytokines in animal model. Bosentan is a dual endothelin receptor antagonist approved for the treatment of pulmonary arterial hypertension and digital ulcers in scleroderma patients. In animal models and in vitro models, after treatment with Bosentan, a significant reduction of cytokine (TNFα, IFNγ, IL-8, IL-4) levels was observed. The aim of the study is to verify whether Bosentan treatment in SSc patients can reduce circulating cytokines levels. We enrolled 10 patients affected by SSc with digital ulcers and/or pulmonary hypertension, treated with Bosentan 125 mg twice daily. Patients were tested for cytokines and ET-1 level before treatment and after 12 months. The cytokines tested were IL-10, IL-2, IL-4, IL-5, IL-6, IL-8, GM-CSF, IFN-γ and TNF. Levels of ET-1, IL-10, IL-4, IL-5, GM-CSF and TNFα did not show consistent modification during treatment with Bosentan in respect to baseline, while IL-2, IL-6, IL-8 and IFN-γ were significantly decreased. Bosentan significantly reduced IL-2, IL-6, IL-8 and IFN-γ levels in SSc patients, probably slowing progression to fibrosis and vascular damage. This is the first report showing a decrease of profibrotic and proinflammatory cytokines levels in humans during treatment with Bosentan.
LETTER TO THE EDITOR

THERAPEUTIC EFFECTIVENESS OF MINIMAL DOSES OF RITUXIMAB IN A PATIENT WITH RHEUMATOID ARTHRITIS

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We report the case of a man suffering from rheumatoid arthritis, resistant to common DMARDs and anti-TNF-alpha, who received an excellent response, in terms of effectiveness and depletion of CD20 positive B-lymphocytes, to minimal doses of anti-CD20 monoclonal antibody (rituximab). The dose used was only 100 mg, repeated after 2 weeks. Already after the first infusion of rituximab, a profound depletion of CD20 B-lymphocytes and an improvement of clinical symptoms were evident. The patient, after 4 months from the first two infusions, maintained an accentuated lymphocyte depletion and obtained a low disease activity, passing from an initial DAS28 of 6.3 to a DAS28 of 2.8. The possible practical implications of this observation are taken into consideration.
LETTER TO THE EDITOR

EFFICACY AND SAFETY OF LEFLUNOMIDE OR METHOTREXATE PLUS SUBCUTANEOUS TUMOUR NECROSIS FACTOR-ALPHA BLOCKING AGENTS IN RHEUMATOID ARTHRITIS

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Several smaller retrospective case series have concluded that leflunomide (LEF) in combination with anti-TNF-α blocking agents appears to be effective and safe. Prospective case series and cohort studies have generally confirmed the efficacy of this combination. Overall, there is currently no evidence from controlled trials that an anti-TNF-α combination with LEF is as effective as an anti-TNF-α combination with methotrexate (MTX). We compared the effectiveness and safety of a therapeutic regimen associating subcutaneous anti-TNF-α agents, etanercept (ETN) and adalimumab (ADA), with leflunomide (LEF) or methotrexate (MTX), in a two year open-label study performed in clinical practice. We evaluated 96 patients with active rheumatoid arthritis undergoing therapy with ADA at the dose of 40 mg every other week, or with ETN at the dose of 50 mg/week for two years added to prednisolone (PDN) at the mean dose of 5.2±2.6 mg/day. Fifty-four of these patients were also undergoing therapy with MTX at the mean dose of 11.7±2.6 mg/week, while 42 patients were undergoing therapy with LEF at the daily dose of 20 mg. At 12 months, the analysis of variance showed an improvement of DAS28 in both groups (p<0.001), with a reduction in 33.3% of the patients in treatment with LEF and in 51.8% of the patients in treatment with MTX (p = 0.20). At 18 months, improvement was present in 33.3% of the patients in the LEF group and in 81.5% of the patients in the MTX group (p=0.001). This improvement seems to be independent of the anti-TNF-α agent, even if MTX produces the highest DAS28 reduction when used in association with ETN (p<0.078). We found no difference in drug discontinuation rates or in effectiveness measures between anti-TNFα+MTX and anti-TNFα+LEF. Our data showed a greater reduction of DAS28 in the MTX group and, in combination with ETN, better results after two years of therapy.