Atherosclerosis is an inflammatory disease due to a diet high in saturated fat, hypercholesterolemia, obesity, hypoglycemia, etc. mainly mediated by the infiltration of macrophage and T cells into the vascular wall. Once the endothelial is damaged monocytes penetrate the tissue and are transformed in scavenger cells. Upon stimulation of Th1 cells, a group of cytokines is released and contributes to the inflammatory response of atherosclerotic tissue. When macrophages proliferate they amplify inflammatory response through the secretion of growth factors and cytokines such as TNF and IL-1. In addition, chemokines such as RANTES and other C-C chemokines are generated, and matrix metalloprotease 9 (MMP-9) are produced by activated monocytes. However, the immune system in atherosclerosis still remains unclear. Here, in this study we revisited the inter-relationship between atherosclerosis and inflammation.
EDITORIAL

BRIDGING THE GAP BETWEEN THE CLINICIAN AND THE PATIENT WITH CRYOPYRIN-ASSOCIATED PERIODIC SYNDROMES

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Cryopyrin-associated periodic syndromes are categorized as a spectrum of three autoinflammatory diseases, namely familial cold auto-inflammatory syndrome, Muckle-Wells syndrome and chronic infantile neurological cutaneous articular syndrome. All are caused by mutations in the NLRP3 gene coding for cryopyrin and result in active interleukin-1 release: their rarity and shared clinical indicators involving skin, joints, central nervous system and eyes often mean that correct diagnosis is delayed. Onset occurs early in childhood, and life-long therapy with interleukin-1 blocking agents usually leads to tangible clinical remission and inflammatory marker normalization in a large number of patients, justifying the need to facilitate early diagnosis and thus avoid irreversible negative consequences for tissues and organs.
EFFECTS OF SUBCHRONIC INHALATION EXPOSURE TO ETHYL TERTIARY BUTYL ETHER ON SPLENOCYTES IN MICE

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Ethyl tertiary-butyl ether (ETBE) is a motor fuel oxygenate used in reformulated gasoline. The current use of ETBE in gasoline or petrol is modest but increasing. To investigate the effects of ETBE on splenocytes, mice were exposed to 0 (control), 500 ppm, 1750 ppm, or 5000 ppm of ETBE by inhalation for 6 h/day for 5 days/wk over a 6- or 13-week period. Splenocytes were harvested from the control and exposed mice, and the following cell phenotypes were quantified by flow cytometry: (1) B cells (PerCP-Cy5.5-CD45R/B220), (2) T cells (PerCP-Cy5-CD3e), (3) T cell subsets (FITC-CD4 and PE-CD8a), (4) natural killer (NK) cells (PE-NK1.1), and (5) macrophages (FITC-CD11b). Body weight and the weight of the spleen were also examined. ETBE-exposure did not affect the weight of the spleen or body weight, while it transiently increased the number of RBC and the Hb concentration. The numbers of splenic CD3+, CD4+, and CD8+ T cells, the percentage of CD4+ T cells and the CD4+/CD8+ T cell ratio in the ETBE-exposed groups were significantly decreased in a dose-dependent manner. However, ETBE exposure did not affect the numbers of splenic NK cells, B cells, or macrophages or the total number of splenocytes. The above findings indicate that ETBE selectively affects the number of splenic T cells in mice.
IDENTIFICATION OF DIFFERENTIALLY-EXPRESSED PROTEINS BETWEEN EARLY SUBMUCOSAL NON-INVASIVE AND INVASIVE COLORECTAL CANCER USING 2D-DIGE AND MASS SPECTROMETRY

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Early detection and diagnosis of colorectal cancer (CRC) are closely related to a better therapeutic outcome, and the five-year survival rate of early CRC is over 90%. Though endoscopic minimally invasive treatment has become a quick and effective therapy for early CRC, endoscopic biopsies are usually not deep enough to obtain tissues from the submucosal layer and it is difficult to determine whether early CRC has infiltrated into the submucosa. Therefore, in the present study, we constructed tumor models of early submucosal non-invasive CRC (SNICRC) and submucosal invasive CRC (SICRC) in Fischer-344 rats induced by N-methyl-N-nitrosourea (MNU). The differentially-expressed proteins were analyzed and identified in SNICRC, SICRC and normal control (NC) tissues using highly sensitive two-dimensional differential gel electrophoresis (2D-DIGE) coupled with mass spectrometry (MS). Proteomic data revealed 132 protein spots between SNICRC and SICRC, 162 protein spots between SICRC and NC and 154 protein spots between SNICRC and NC which were found differentially expressed. These differential spots were picked, in-gel digested and peptide mass fingerprints were obtained by MALDI-TOF-MS/MS. Finally, five differentially-expressed proteins in SNICRC, SICRC and NC were identified, and increases in Transgelin, peptidylprolyl isomerase A (PPIA) and tropomyosin alpha isoform d were observed, while decreases in carbonic anhydrase 2 (CAII) and an unnamed protein were detected in SICRC compared with SNICRC and NC. Furthermore, Fluorescence-based quantitative polymerase chain reaction (FQ-PCR), Western blotting and immunohistochemistry assays also revealed significant upregulation of Transgelin expression and down-regulation of CAII expression in SICRC tissues. In conclusion, 2D-DIGE is confirmed to be an efficient strategy that enables us to identify differentially-expressed proteins between early SNICRC and SICRC. The potential biomarkers such as Transgelin and CAII may be used for the detection of early SICRC.
EFFECTS OF PARAQUAT AND CAPSAICIN ON THE EXPRESSION OF GENES RELATED TO INFLAMMATORY, IMMUNE RESPONSES AND CELL DEATH IN IMMORTALIZED HUMAN HaCat KERATINOCYTES

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The present experiments were aimed to characterize in immortalized human HaCat keratinocytes the gene expression induced by paraquat and capsaicin, two agents known to induce cell death or to affect inflammatory and pain pathways, respectively. In particular, the following set of genes were analysed by qRealtime PCR: CXCL10, CXCL11, IL-10 (inflammatory and immune responses), TP73, BCL2, (apoptotic and anti-apoptotic genes), MMP9 (proteolysis), SOD-1, BAK-1 and CAT (peroxysomal and microsomal oxidation pathways). In this way, we were able to differentiate the two toxins since they had a different profile of gene expression. In fact, paraquat was found to activate set of genes involved in inflammatory (CXCL10, CXCL11 and IL-10), and cell death (BCL2, BAK-1, MMP9) pathways. Another specific site of action of paraquat was represented by an activation of the gene involved in SOD-1 transcription. On the contrary, capsaicin was found to produce only an up-regulation of BCL2, an anti-apoptotic gene and MMP9, whereas no significant changes were reported in genes involved in inflammatory and immune responses. Finally, in comparison to previous experiments carried out with TNF-α and IL-1β, we have shown that paraquat produced a similar pattern of activation of set of genes involved both in inflammation and apoptosis.
Immune parameters show rhythmic changes with a 24-h periodicity driven by an internal circadian timing system that relies on clock genes (CGs). CGs form interlocked transcription-translation feedback loops to generate and maintain 24-h mRNA and protein oscillations. In this study we evaluate and compare the profiles and the dynamics of variation of CG expression in peripheral blood, and two lymphoid tissues of mice. Expression levels of seven recognized key CGs (mBmal1, mClock, mPer1, mPer2, mCry1, mCry2, and Rev-erbα) were evaluated by quantitative RT-PCR in spleen, thymus and peripheral blood of C57BL/6 male mice housed on a 12-h light (L)-dark (D) cycle and sacrificed every 4 h for 24 h (3-4 mice/time point). We found a statistically significant time-effect in spleen (S), thymus (T) and blood (B) for the original values of expression level of mBmal1 (S), mClock (T, B), mPer1 (S, B), mPer2 (S), mCry1 (S), mCry2 (B) and mRev-Erbα (S, T, B) and for the fractional variation calculated between single time-point expression value of mBmal1 (B), mPer2 (T), mCry2 (B) and mRev-Erbα (S). A significant 24-h rhythm was validated for five CGs in blood (mClock, mPer1, mPer2, mCry2, mRev-Erbα), for four CGs in the spleen (mBmal1, mPer1, mPer2, mRev-Erbα), and for three CGs in the thymus (mClock, mPer2, mRev-Erbα). The original values of acrophases for mBmal1, mClock, mPer1, mPer2, mCry1 and mCry2 were very similar for spleen and thymus and advanced by several hours for peripheral blood compared to the lymphoid tissues, whereas the phases of mRev-Erbα were coincident for all three tissues. In conclusion, central and peripheral lymphoid tissues in the mouse show different sequences of activation of clock gene expression compared to peripheral blood. These differences may underlie the compartmental pattern of web functioning in the immune system.
**LACTOBACILLUS PARACASEI** LP6 FAVORS IMMUNE MODULATION INDUCED BY ALLERGOID TREATMENT IN RAGWEED SENSITIZED MICE

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It has been hypothesized that lactic acid bacteria (LAB) could be used as adjuvant for specific immunotherapy (SIT), as various studies conducted on humans and animals converge to define LAB as anti-Th2 modulators and Treg inducers. In the present study we evaluated the effects of LAB, in particular *Lactobacillus paracasei* Lp6 (Lp6), in a mouse model of ragweed (RW) allergy. Groups of Balb/c mice, experimentally sensitized towards ragweed, were treated by viable Lp6 or by RW-allergoid with or without co-administration of Lp6. A control group was sham-sensitized with PBS and sham-treated with water and a group was sensitized with RW and treated with water. Serum IgE, RW-induced release of IFN-γ, IL-4 and IL-10 from splenocytes and the frequency of CD4CD25 regulatory T cells (Tregs) expressing Foxp3 or IL-10 were evaluated in various groups. RW-allergoid treatment induced a reduction of serum IgE, with a decrease in RW-induced release of IL-4, and an increase in IL-10 and IFN-γ, along with a significant change in the frequency of Tregs, both CD25+ and -. The joint RW-allergoid+Lp6 treatment induced the highest degree of suppression of allergen-driven IL-4, the greatest reduction of IL-4/IFN-γ and IL-4/IL-10 ratios and the most significant increase of Foxp3 and IL-10 expressing Tregs. The study shows that Lp6 strengthens the immune modulation induced by allergoid-SIT in RW-sensitized mice, essentially characterized by a differential induction of Tregs associated to a reduction of IL-4; data converge to define a role of SIT adjuvant for Lp6.
STIMULATION OF TH1 RESPONSE BY HELICOBACTER PYLORI NEUTROPHIL ACTIVATING PROTEIN DECREASES THE PROTECTIVE ROLE OF IgE AND EOSINOPHILS IN EXPERIMENTAL TRICHINELLOSIS

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Th2 responses seem to play an important role in defence against Trichinella spiralis (Ts). The Neutrophil Activating Protein of Helicobacter pylori (HP-NAP), that induces IL-12, and IL-23 expression and shifts to Th1 allergen-specific Th2 cells in vitro was used as an anti-Th2 agent in BALB/c mice infected with T. spiralis. The muscle larvae (ML) burden was lower (p < 0.02) in untreated infected animals than those infected treated with HP-NAP. In both groups there was an inverse relationship between ML burden of each animal and total IgE level (controls: r -0.617, p = 0.0013 and HP-NAP-treated: r -0.678, p = 0.0001) or eosinophil count, evaluated in the same mouse on day 42 (r -0.390, p = 0.0592 and r -0.803, p = 0.0001, respectively). Inflammatory response around the nurse cell-parasite complex was significantly higher in HP-NAP-treated infected animals than in those untreated infected, on the contrary the number of eosinophils, counted around each complex was significantly lower in the first animal group. This study provides evidence of a powerful anti-Th2 activity in vivo by HP-NAP and for the partial protective effect of Th2 responses in T. spiralis infection.
GALECTIN-3 PLASMA LEVELS AND CORONARY ARTERY DISEASE:
A NEW POSSIBLE BIOMARKER OF ACUTE CORONARY SYNDROME

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Inflammation plays a key role in atherosclerosis. Galectin-3 is a macrophage- and endothelium-derived mediator actively involved in the regulation of many aspects of inflammatory cell behaviour. The aim of this study is to quantify plasma Galectin-3 in patients with coronary artery disease (CAD) and different clinical manifestation at the moment of observation in order to verify whether Galectin-3 could be a useful biomarker of atherosclerotic state. We enrolled 125 patients affected by CAD, angiographically documented (70 stable, 55 unstable). They underwent accurate examinations and anamnestic data was collected. The most important traditional risk factors, such as age, hypertension, and body mass index, were reported. Plasma Galectin-3 was quantified using an ELISA kit. Unstable patients (n = 55) had a higher plasma Galectin-3 levels in respect to the stable subjects (27.75 ng/mL (19.27-39.09) vs 6.48 ng/ml (4.88-8.83), p<0.001. A trend in correlation between plasma Galectin-3 levels and number of vessels compromised seems to be present: CAD patients with three-vessel disease had higher levels of Galectin-3 than patients with one-or two-vessel disease (17.39 ng/ml (10.75-29.82) vs 9.18 ng/ml (5.56-23.22), p= 0.058. The significantly higher plasma Galectin-3 levels in patients with unstable angina in respect to the stable angina confirm the involvement of Galectin-3 in promoting macrophage activation and monocyte attraction. Despite the distribution of CAD in patients with acute and chronic coronary disease being similar, we may hypothesize that Galectin-3 could be a useful biomarker of atherosclerotic plaque and in particular of its destabilization.
CIRCULATING REGULATORY T CELLS IN “CLINICAL” MONOCLONAL B-CELL LYMPHOCYTOSIS

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Regulatory T-cells (Tregs) constitute a small subset of cells involved in antitumour immunity and are generally increased in patients with chronic lymphocytic leukemia (CLL). No data is available on Tregs in monoclonal B-cell lymphocytosis (MBL), a disease entity characterized by less than 5000/μL circulating clonal B-cells in absence of other features of lymphoproliferative disorders. We used multicolour flow cytometry to evaluate the number of circulating Tregs in 56 patients with “clinical” MBL, 74 patients with previously untreated CLL and 40 healthy subjects. MBL patients showed a lower absolute number of Tregs, compared to CLL patients, but slightly higher than controls. Moreover, the absolute cell number of Tregs directly correlated both with more advanced Rai/Binet clinical stages and peripheral blood B-cell lymphocytosis. Of note, the absolute number of Tregs was found lower in MBL patients than in CLL patients staged as 0/A Rai/Binet. The study showed that Tregs increase gradually from normal subjects to “clinical” MBL patients and are significantly higher in CLL patients as compared to MBL patients. Moreover, a significant direct relationship was found between higher Treg values and a higher tumor burden expressed by B-lymphocytosis or more advanced clinical stages. In light of this data, MBL seems to be a preliminary phase preceding CLL. The progressive increase of Treg numbers might contribute both to the clinical evolution of MBL to overt CLL and to CLL progression.
KMUP-1 INHIBITS H441 LUNG EPITHELIAL CELL GROWTH, MIGRATION AND PROINFLAMMATION VIA INCREASED NO/CGMP AND INHIBITED RHO KINASE/VEGF SIGNALING PATHWAYS

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This study investigates whether KMUP-1 protects soluble guanylate cyclase (sGC) and inhibits vascular endothelial growth factor (VEGF) expression in lung epithelial cells in hypoxia, therapeutically targeting epithelial proinflammation. H441 cells were used as a representative epithelial cell line to examine the role of sGC and VEGF in hypoxia and the anti-proinflammatory activity of KMUP-1 in normoxia. Human H441 cells were grown in hypoxia for 24-72 h. KMUP-1 (1, 10, 100 μM) arrested cells at the G0/G1 phase of the cell cycle, reduced cell survival and migration, increased p21/p27, restored eNOS, increased soluble guanylate cyclase (sGC) and PKG and inhibited Rho kinase II (ROCK-II). KMUP-1 (0.001-0.1 μM) concentration dependently increased eNOS in normoxia and did not inhibit phosphodiesterase-5A (PDE-5A) in hypoxic cells. Hypoxia-induced factor-1α (HIF-1α) and VEGF were suppressed by KMUP-1 but not by L-NAME (100 μM). The PKG inhibitor Rp-8-CPT-cGMPS (10 μM) blunted the inhibition of ROCK-II by KMUP-1. KMUP-1 inhibited thromboxane A2-mimetic agonist U46619-induced PDE-5A, TNF-α (100 ng/ml)-induced iNOS, and ROCK-II and associated phospho-p38 MAPK, suggesting multiple anti-proinflammatory activities. In addition, increased p21/p27 by KMUP-1 at higher concentrations might contribute to an increased Bax/Bcl-2 and active caspase-3/procaspase-3 ratio, concomitantly causing apoptosis. KMUP-1 inhibited ROCK-II/VEGF in hypoxia, indicating its anti-neoplastic and anti-inflammatory properties. KMUP-1 inhibited TNF-α-induced iNOS and U46619-induced PDE-5A and phospho-p38 MAPK in normoxia, confirming its anti-proinflammatory action. KMUP-1 could be used as an anti-proinflammatory agent to reduce epithelial inflammation.
ACTIVATION OF HUMAN MONOCYTE-DERIVED DENDRITIC CELLS IN VITRO BY THE BIOLOGICAL RESPONSE MODIFIER ARABINOXYLAN RICE BRAN (MGN-3/BIOBRAN)

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Arabinoxylan rice bran (MGN-3/Biobran) is a potent biological response modifier (BRM) that activates natural killer (NK) cells, T cells and monocytes. Currently, little is known regarding the effects of MGN-3 on dendritic cells (DCs), the cell type that bridges innate and adaptive immunity. Therefore, we examined the stimulatory effects of MGN-3 on DCs. Human monocyte-derived DCs were treated with MGN-3 at different concentrations (5-20 \( \mu \text{g/ml} \)) for 24 hours \textit{in vitro}. Activation of DCs was determined by assessing the expression of co-stimulatory and maturation markers (CD40, CD80, CD83, CD86 and HLA-DR) by flow cytometry, and production of cytokines by ELISA. DC function was determined by assessing their ability to activate naïve T cells. Activation of T cells was assessed by measuring cell proliferation and cytokine production. MGN-3 treatment, in a dose-dependent manner, resulted in: 1) up-regulation of the surface expression of CD83 and CD86, on DCs; 2) an increase in the production of pro-inflammatory and immuno-regulatory cytokines (IL-1\( \beta \), IL-6, IL-10, TNF-\( \alpha \), IL-12p40 and low levels of IL-12p70 and IL-2) by DCs; and 3) MGN-3 stimulated DC induced CD4\( ^{+} \)T cell proliferation and their production of cytokines, IFN-\( \gamma \), IL-10, IL-17. Results suggest that MGN-3 functions as a natural adjuvant for DC activation and thus may be used in DC-based vaccine strategies against infections and cancer.
2B4 (CD244) IS INVOLVED IN EOSINOPHIL ADHESION AND CHEMOTAXIS, AND ITS SURFACE EXPRESSION IS INCREASED IN ALLERGIC RHINITIS AFTER CHALLENGE

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A role for the subtypes of CD2 Ig superfamily receptors has been recently demonstrated in eosinophilic inflammation in experimental asthma and atopic asthmatics. We investigated the functions of 2B4 (CD244) molecules in eosinophil adhesion and chemotaxis, and correlated the results to the pathophysiology of allergic rhinitis (AR). Herein, we show that agonistic stimulation of 2B4 by C1.7, the anti-human 2B4 functional grade purified antibody, resulted in significant increase of eosinophils and eosinophil cell line (Eol-1 cells) adhesion to collagen type IV, and random migration. These functions were associated with tyrosine kinase phosphorylation of several protein residues of low molecular weight. Flow cytometry (FACS) experiments demonstrated that Eol-1 cells, normal peripheral blood eosinophils and eosinophils from AR patients, express surface 2B4 molecules. In vitro AR model demonstrated that the CC-chemokine receptor CCR3 stimulation by eotaxin induced significant increase in the expression of surface 2B4 in eosinophils and Eol-1 cells. Immunofluorescence confocal microscopy images showed that eotaxin induces also redistribution of 2B4 molecules towards the pseudopods in eosinophils and Eol-1 cells, changing their shape. Blocking of 2B4 molecules by the corresponding neutralizing antibody inhibited eotaxin induced Eol-1-adhesion, chemotaxis and the cytoskeleton changes. Pretreatment of Eol-1 cells with 1 μM genistein blocked eotaxin-induced Eol-1 adhesion, chemotaxis and 2B4 up-regulated expression. In vivo correlation demonstrated the expression of 2B4 molecules in eosinophils from AR patients to be significantly increased, after nasal provocation challenge. These results identify a novel role for 2B4 molecules in eosinophil functional migratory response and may point to a novel tyrosine kinase-mediated ligation between CCR3 receptor and 2B4 co-receptor in eosinophil chemotaxis. If so, then 2B4 molecules would be a novel target for therapeutic modalities in diseases characterized by eosinophilia such as AR.
EXPRESSION OF IL-23, VEGF AND TLR2/TLR4 ON MONONUCLEAR CELLS AFTER EXPOSURE TO PSEUDOMONAS AERUGINOSA

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Pseudomonas aeruginosa is a Gram-negative, aerobic bacillus causing infections of the respiratory and other organ systems in susceptible hosts. Although it does not cause pulmonary infections in immunocompetent individuals, P. aeruginosa causes chronic lung infection in individuals with cystic fibrosis and nosocomial pneumonia resulting in significant morbidity and mortality. Exogenous administration of an important P. aeruginosa virulence factor, lipase, present in P. aeruginosa culture supernatant, induces potent mononuclear cell activation leading to the production of numerous proinflammatory cytokines. In particular, P. aeruginosa culture supernatant stimulated increased proliferation of THP-1 cells and monocytes (MN). The addition of culture supernatant to THP-1 cells and MN also induced Interleukin (IL)-23 and vascular endothelial growth factor (VEGF) release in a time-dependent manner. To investigate whether any compounds present in the supernatant lipase contributed to releasing IL-23 and VEGF, the culture supernatant from P. aeruginosa containing lipase was treated with hexadecylsulfonfluoride (AMSF). The AMSF-treated culture supernatant (CS) did not show any induction on the IL-23 and VEGF release compared to the cells treated with CS without AMSF. We also showed that Toll-like receptors (TLR)2/TLR4 are expressed in THP-1 cells and MN treated with P. aeruginosa CS in a time-dependent fashion. Flow cytometry analysis revealed a higher TLR4 and a lower TLR2 expression at 48 and 72 h of treatment. The treatment of cells with TLR4 neutralizing antibody, and to a lesser extent with TLR2 neutralizing antibody, resulted in a decrease in P. aeruginosa CS-induced IL-23 and VEGF production.
Magnesium is one of the most important cations for an organism. The aim of our study is to evaluate whether the use of a magnesium formulation as a diet supplement or medical treatment is necessary. The 24-hour recall method was used to obtain information regarding the daily magnesium consumption of 949 people. The results were compared with the Estimated Average Requirement (EAR) and Recommended Daily Allowance (RDA) values. The average daily requirement for magnesium was exceeded by 292 (183 women and 109 men) of the 949 respondents. This research confirmed excessive magnesium intake by both men and women that exceeded both the EAR and the RDA. Uncontrolled, excessive dietary supplementation or medical treatment with magnesium by this group may constitute a health threat.
HYPER-IgM, NEUTROPENIA, MILD INFECTIONS AND LOW RESPONSE TO POLYCLONAL STIMULATION: HYPER-IgM SYNDROME OR COMMON VARIABLE IMMUNODEFICIENCY?

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A young woman presenting respiratory infections, polyarthritis, severe neutropenia, and increased serum IgM was treated with Intravenous Immunoglobulin (IVIG) with good clinical and laboratory outcome followed by a loss of efficacy. The increased serum IgM associated to recurrent infections and autoimmune manifestations suggested the diagnosis of a hyper–IgM syndrome (HIGMs). The frequency of peripheral T cells, the expression of CD40 on the patients’ B cells and CD40L on T cells and the Activation-Induced cytidine Deaminase (AID) and Uracyl-DNA glycosylase (UNG) at mRNA level was comparable to controls. In contrast, the frequency of B cells was one half of the healthy control and all cells showed an atypical phenotype. Although AID and UNG were normal, class-switch recombination was not very efficient because circulating switched memory were reduced and, once stimulated with CpG, generated less antibody-secreting cells than controls. An increase in serum B Lymphocytes stimulator (BLyS) was also found. The patient presented a peculiar clinical and immunological phenotype fitting for many aspects of both HIGM4 and Common Variable Immunodeficiency (CVID). These findings underline the need to better explore the complex link between these two diseases.
MICRO OPIOID RECEPTOR A118G POLYMORPHISM AND POST-OPERATIVE PAIN: OPIOIDS' EFFECTS ON HETEROZYGOUS PATIENTS

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The Single-Nucleotide-Polymorphism (SNP) 118A>G in the μ-1 Opioid Receptor gene (OPRM1) is associated with a decrease in the analgesic effects of opioids. The aim of this study is to assess whether 118A>G polymorphism could influence the analgesic response to opioid-based postoperative pain (POP) therapy. The study consisted of two parts: section α, observational, included 199 subjects undergoing scheduled surgical procedures with pain management standardized on surgery invasiveness and on expected level of postoperative pain; section β, randomized, included 41 women undergoing scheduled caesarean delivery with continuous intra-operative epidural anesthesia and post-operative analgesia (CEA). In both sections, POP was measured over 48 h (T6h-T24h-T48h) by the visual analogue scale (VAS). In section β we also tested the responsiveness of hypothalamic-pituitary-adrenal axis (HPA) expressed by cortisol levels. In section α, with cluster analysis, subjects were analyzed according to their genotype: a group (#1) of 34 patients reporting VAS score >3 at every time lapse was identified and included only A118G carriers, while wild-type (A118A - absence of 118A>G polymorphism) patients were unevenly distributed between those with cluster #2 (VAS score <3 at every study steps) and those with cluster #3 (VAS score progressively reducing from T6h). In section β, A118G carriers receiving epidural sufentanil had the lowest VAS scores at T24h; also in these patients, cortisol levels remained more stable, with a mild decrease at T6h. This study shows that the OPRM1 118A>G polymorphism affects postoperative pain response in heterozygous patients: they have a different postoperative pain response than patients with wild-type genes, which may affect the efficacy of the analgesic therapy.
NEO-ADJUVANT CHEMO/IMMUNOTHERAPY IN THE TREATMENT OF STAGE III (N2) NON-SMALL CELL LUNG CANCER: A PHASE I/II PILOT STUDY

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In a previous randomized study, we showed that adjuvant immunotherapy with tumor-infiltrating lymphocytes and recombinant interleukin-2 (rIL-2) significantly improved survival in resected N2-Non Small Cell Lung Cancer (NSCLC) patients. The present study assesses feasibility, safety and potential efficacy of combined neo-adjuvant chemotherapy and immunotherapy with peripheral blood mononuclear cells (PBMC) and rIL-2 in resectable N2-NSCLC patients. Eighty-two consecutive N2-NSCLC patients underwent neo-adjuvant chemotherapy with cisplatin and gemcitabine. Out of the 82 patients, 23 were also subjected to leukapheresis prior to neo-adjuvant chemotherapy while the remaining 59 did not. Collected PBMC were analyzed for viability and phenotype and then stored frozen in liquid nitrogen. Thawed PBMC were infused intravenously, 5 days before surgery. After the infusion, rIL-2 was administered subcutaneously until surgery. Only patients with a partial or complete response to neo-adjuvant chemotherapy underwent surgery: 13 patients in the experimental immunotherapy group (A) and 32 in the reference group (B). The two groups were homogeneous for all major prognostic factors. Median leukapheresis yield was 10 billion PBMC, (range 3-24 billions). Two to six billion PBMC were infused. The phenotypic analysis showed that similar proportions of CD4 and CD8 cells were present in leukapheresis products, and thawed PBMC, as well as in T lymphocytes isolated from the removed tumours. No severe adverse effects were observed following immunotherapy. No significant differences in overall survival (OS) and event-free survival (EFS) were seen between the two groups. However, the 5-year OS in group A was almost twice as much compared to group B (59% vs 32%). After adjustment for major prognostic factors, a statistically significant 66% reduction in the hazard of death was seen in patients receiving immunotherapy. The OS benefit was more evident in patients with adenocarcinoma than in those with squamous cell carcinoma. This study supports the favorable toxicity profile and potential efficacy of combining neo-adjuvant chemotherapy and immunotherapy with PBMC and rIL-2 in the treatment of N2-NSCLC patients.
Patients with Chronic Fatigue Syndrome (CFS) often report a comorbid depressive disorder. Comorbid depression may negatively influence the long-term outcome of CFS therefore it must be correctly diagnosed and treated. The aim of the present study is to provide a clinical and psychometric assessment of CFS patients with and without depressive features. A comparative analysis between 57 CFS subjects (CDC, 1994), 17 of whom with a comorbid depression, and 55 matched healthy volunteers was assessed to evaluate the presence of any psychophysical distress and alexithymic traits, by means of Symptom Checklist-90-R (SCL-90R) and Toronto Alexithymia Scale (TAS-20). The severity of fatigue was also assessed in all CFS patients using the Fatigue Impact Scale (FIS). With regard to psychiatric comorbidity, the SCL-90R scores showed higher levels of somatic complaints in CFS patients than in healthy subjects, whereas augmented depressive and obsessive-compulsive symptoms were observed only in the depressed CFS subgroup. When comparing the TAS-20 scores, we observed a selective impairment in the capacity to identify feelings and emotions, as measured by the Difficulty in Identifying Feelings subscale (DIF), non-depressed CFS patients showing an intermediate score between depressed CFS and healthy controls. Finally, in terms of FIS scores, a statistical trend versus a higher fatigue severity in depressed CFS patients, with respect to non-depressed ones, was observed. In conclusion, comorbid depression in CFS significantly increased the level of psychophysical distress and the severity of alexithymic traits. These findings suggest an urgent need to address and treat depressive disorders in the clinical care of CFS cases, to improve social functioning and quality of life in such patients.
MODULATION OF BIOFILM OF STRAINS ISOLATED FROM PATIENTS WITH CHRONIC OBSTRUCTIVE PULMONARY DISEASE BY LEVOFLOXACIN, MOXIFLOXACIN, CIPROFLOXACIN, AMOXICILLIN/CLAVALANIC ACID AND CEFTRIAXONE

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The ability of levofloxacin, moxifloxacin, ciprofloxacin, amoxicillin/clavulanic acid and ceftriaxone to interfere on biofilm produced by Pseudomonas aeruginosa, Haemophilus influenzae and Streptococcus pneumoniae isolated from patients with chronic obstructive pulmonary disease was evaluated. The effects of antibiotics were evaluated on formation of biofilm (at 1/2, 1/4 and 1/8 X MIC) and on pre-formed biofilm (at epithelial lining fluid peak concentrations) by means of a spectrophotometric method. Levofloxacin was the most active compound followed by ciprofloxacin, moxifloxacin and amoxicillin/clavulanic acid and ceftriaxone. Levofloxacin may contribute to clear the reservoir of pathogens involved in chronic obstructive pulmonary disease, thus leading to decreased occurrence of acute exacerbations.
EFFECTS OF *LACTOBACILLUS SALIVARIUS* LS01 (DSM 22775) TREATMENT ON ADULT ATOPIC DERMATITIS: A RANDOMIZED PLACEBO-CONTROLLED STUDY

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Atopic dermatitis (AD) is a common inflammatory skin disease characterized by xerosis, pruritus and eczema. The role of probiotics in the prevention and the treatment of AD have been extensively studied in children with controversial results while there are few studies on an adult population. The aim of this randomized, double-blind, placebo-controlled study is to evaluate the clinical efficacy of the intake of a probiotic strain (*Lactobacillus salivarius* LS01) in the treatment of adult patients with AD. A group of 38 patients was treated with probiotics or placebo (maltodextrin) for 16 weeks. The study was performed from January (T0) to May, 2009 (T16). The assessment of efficacy was based on change in SCORAD (SCORing Atopic Dermatitis) index, dermatology life quality index (DLQI) improvement, cytokine production by PBMCs and ability to modify faecal microbial flora. No significant adverse events were recorded during the study. Patients treated with probiotics showed a statistically improvement of both clinical parameters (SCORAD p< 0.0001 and DLQI p= 0.021) at the end of treatment (T16) compared with the placebo group. Furthermore, after four months of treatment there was a significant reduction of Th1 cytokines (IL-12+IFNγ) (p= 0.03) and Th1/Th2 ratio (IL-12+IFNγ/IL-4+IL-5) (p= 0.019) only in placebo-treated patients. A statistically relevant decrease of staphylococci in faeces of the probiotic-treated group was also observed at the end of treatment. In our study, the administration of *L. salivarius* LS01 was well tolerated and was associated with a significant improvement of clinical manifestation and QoL. This probiotic strain could have an important role in modulating Th1/Th2 cytokine profiles and could be considered as an important adjunctive therapy in the treatment of adult AD.
A COMPARISON BETWEEN IgE AND IgG4 AS MARKERS OF ALLERGY IN CHILDREN: AN EXPERIMENTAL TRIAL IN A MODEL OF NATURAL ANTIGEN AVOIDANCE

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IgG4 have been hypothesized to act as blocking antibodies capable of preventing IgE-mediated effector cell triggering. This study aims to evaluate the changes in IgG4 in children during a period of natural antigen avoidance. Serum IgE and IgG4 were evaluated in a group of asthmatic children, aged between 7 and 17 years, admitted to the residential house Istituto Pio XII (Misurina, BL, Italy), located at 1,756 m, in a natural model of antigen avoidance. All the patients were skin prick test positive to at least two of the following allergens: *Dermatophagoides pteronissynus*, *Dermatophagoides farinae*, cat epithelium, timothy grass pollen and Parietaria pollen. During the 180 days of hospitalization, serum specific IgE and IgG4 were measured six times. A significant decrease (p≤0.05) in serum specific IgE to house dust mite and pollen allergens was observed; by contrast, no significant variations were shown by IgG4 and IgG4/IgE ratio. No significant relationship was found between serum specific IgE, IgG4 and IgG4/IgE ratio variations and the re-exposure to house dust mite allergens during the Christmas holidays. A positive correlation between specific IgE and specific IgG4 was observed at each considered time (T0: r=0.57, p=0.08; T1: r=0.85, p=0.001; T3: r=0.76, p=0.01). The positive correlation between specific IgE and specific IgG4, enduring throughout the entire time of study, suggests a relationship between these classes of immunoglobulins.
LACTOFERRIN DECREASES INFLAMMATORY RESPONSE BY CYSTIC FIBROSIS BRONCHIAL CELLS INVADED WITH *BURKHOLDERIA CENOCEPACIA* IRON-MODULATED BIOFILM

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In cystic fibrosis (CF) high iron concentration in airway secretion plays a pivotal role in bacterial multiplication and biofilm formation as well as in inflammatory response. *Burkholderia cenocepacia*, an opportunistic facultative pathogen responsible for chronic lung infections and cepacia syndrome, recurrently infects CF patients. Lactoferrin (Lf), an iron binding multifunctional glycoprotein synthesized by exocrine glands and neutrophils, has been found at higher concentration in the airway secretions of infected CF patients than in healthy subjects. Here the influence of milk derivative bovine lactoferrin (bLf), an emerging important regulator of iron and inflammatory homeostasis, on invasiveness of *B. cenocepacia* iron-modulated biofilm, as well as on inflammatory response by infected CF bronchial (IB3-1) cells, is reported. bLf did not significantly affect invasion efficacy by biofilm-forming *B. cenocepacia* clinical strains. Conversely, the addition of bLf to cell monolayers during infection significantly decreased the pro-inflammatory Interleukin (IL)-1β and increased the anti-inflammatory IL-11 expression compared to that observed in cells infected in the absence of bLf. The bLf ability to modulate genes expressed following *B. cenocepacia* infection seems related to its localization to the nucleus of infected IB3-1 cells. These results provide evidence for a role of bLf in the protection of infected CF cells from inflammation-related damage, thus extending the therapeutic potential of this multifunctional natural protein.
LETTER TO THE EDITOR

EVALUATION OF BRONCHIAL HYPERREACTIVITY WITH MANNITOL DRY POWDER CHALLENGE TEST IN A PAEDIATRIC POPULATION WITH INTERMITTENT ALLERGIC ASTHMA OR ALLERGIC RHINITIS

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We evaluated the bronchial hyperreactivity (BHR) with a new bronchial challenge test, mannitol dry powder, in a paediatric population with intermittent allergic asthma or allergic rhinitis who did not respond to an exercise challenge test. We selected 50 children, aged 9-16 years, with intermittent allergic asthma (Group 1) or allergic rhinitis without clinical manifestation of asthma for at least 12 months (Group 2). All patients performed the following tests in three different days (≥ 48 hours apart): Day 1: exhaled nitric oxide (FeNO) determination followed by baseline spirometry and reversibility to inhaled β₂-agonists; Day 2: exercise challenge test followed by FeNO determination; Day 3: mannitol challenge test followed by FeNO determination. Forty children completed the study. Eighteen subjects of Group 1 (90%) and 5 subjects of Group 2 (25%) resulted positive to the mannitol test. Positive mannitol challenge subjects showed no statistically significant differences compared to negative subjects as regard baseline spirometry, reversibility to salbutamol and response to the exercise challenge test, but they had significantly higher FeNO values. In conclusion, the mannitol challenge test can be a diagnostic tool more useful than the exercise challenge test to identify BHR in a paediatric population with intermittent allergic asthma or allergic rhinitis because it is better reproducible, quick and easy to perform and well tolerated.
LETTER TO THE EDITOR

ANETODERMA: EVIDENCE OF THE RELATIONSHIP WITH AUTOIMMUNE DISEASE AND A POSSIBLE ROLE OF MACROPHAGES IN THE ETIOPATHOGENESIS

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Anetoderma is a benign condition characterized by round or oval macular lesions with focal loss of dermal elastic tissue resulting in localized areas of flaccid or herniated saclike skin. Often, the anetoderma is associated with immuno-mediated pathogenetic mechanism. In this article, we describe the association between anetoderma and autoimmune diseases, by underlining the role and the action of macrophages as a possible etiopathogenesis.
LETTER TO THE EDITOR

CHURG-STRAUSS AND MONTELUKAST

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Churg-Strauss syndrome (CSS) is a systemic small vessel vasculitis involving lungs, skin, heart, gastrointestinal tract and peripheral nerves. We report the case of a 36-year-old woman with a necrotic lesion on the left foot of two months duration, associated with hypereosinophilia, patchy lung infiltrates, cardiac damage and a mononeuritis. The personal history was remarkable only for an asthma, treated with Montelukast, a leukotriene receptor antagonist (LRA). Clinical symptoms, laboratory exams and instrumental examinations led us to the diagnosis of CSS. In recent years several studies have reported the possible relationship between use of leukotriene receptor antagonist (LRA) and CSS expression. We report this case to underline the possible relationship between LRA and CSS and its etiopathogenetic mechanism.
LETTER TO THE EDITOR

AGGRESSIVE LARGE B-CELL LYMPHOMA IN A SYSTEMIC LUPUS ERYTHEMATOSUS PATIENT WITH CHRONIC ACTIVE EPSTEIN-BARR VIRUS INFECTION: A CASE REPORT

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A link between Epstein-Barr Virus (EBV) infection, Systemic Lupus Erythematosus (SLE) and Non-Hodgkin’s Lymphoma (NHL) has been recently reported in literature. Here we report a case of diffuse Large B-Cell Lymphoma (DLBCL) with a particularly aggressive clinical course in an SLE patient with EBV infection. A 49-year-old woman with a long history of SLE was admitted to the Department of Experimental and Clinical Medicine and dramatically died a few hours later. The autopsy described no evidence of active lymphoproliferative disorder. Instead, histological examination demonstrated an atypical lymphocytic proliferation in lymph node, kidneys, pericardium and uterus. Immunoistochemically, the lymphomatous cells were positive with CD19, CD20, CD22 and CD79a, which was consistent with a DLBCL. The cells were also reactive to EBV markers, indicating the possible role of previous EBV infection in DLBCL pathogenesis.
LETTER TO THE EDITOR

VIDEOCAPILLAROSCOPIC PATTERN OF ALOPECIA AREATA BEFORE AND AFTER DIPHENYLICLOPROPENONE TREATMENT

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Alopecia areata (AA) is an inflammatory skin disease the most effective therapy for which is diphenylcyclopropenone (DPCP). Videodermatoscopy and intra-vital capillaroscopy (IVCP) are two non-invasive techniques that help in the differential diagnosis of alopecias. It is known that, after DPCP therapy, there is a histologically proven significant increase of VEGF in hair follicle keratinocytes and a consequent increase in capillary vessels in the dermis of the same follicles. The aim of our study is to emphasize any clinical and videodermatoscopic-videocapillaroscopic changes after DPCP treatment in 20 patients affected by alopecia areata. Videodermatoscopic images and an intravital videocapillaroscopic analysis were performed at T0, T12 and T24 to emphasize clinical modifications and microscopic changes in vascular pattern before and after DPCP treatment. At T0, videodermatoscopy showed the presence of “exclamation point” hairs, hair follicles filled with hyperkeratotic plugs (“yellow dots”), hair follicles containing cadaverized hairs (“black dots”) and broken hairs. IVCP highlighted a pale scalp, and vessels were not visible. At 24 weeks (T24), videodermatoscopy revealed the disappearance or a statistically significant reduction of AA hallmarks and an increase of number of vellus hairs. Videocapillaroscopy showed a statistically significant increase of new vessels and, where neoangiogenesis were more marked, a major hair regrowth was evident. Our study emphasizes that, after DPCP therapy, neoangiogenesis is detectable by videocapillaroscopy and these new capillaries could be considered an initial positive attempt to compensate capillary loss of T0 alopecia areata images.
LETTER TO THE EDITOR

SEVERE NECROTIZING PNEUMONIA COMPLICATING INFLUENZA A (H1N1): THE ROLE OF IMMUNOLOGIC INTERACTION

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This report describes the successful management of a documented necrotizing pneumonia due to *Streptococcus pneumoniae* in a child with pandemic influenza A (H1N1). The importance of early recognition of bacterial superinfection in patients with influenza and the immunologic interactive mechanisms between viruses and bacteria in determining respiratory diseases are highlighted. The role of modern molecular techniques in improving diagnostic microbiology sensitivity and informing consequent clinical care is emphasized.
LETTER TO THE EDITOR

RHEUMATOID ARTHRITIS: A COMPLICATION OF AROMATASE INHIBITOR THERAPY?

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We report the case of a 56-year-old woman treated with aromatase inhibitors for a breast cancer. Following one year of such therapy, the patient presented with widespread osteoarthralgia. The clinical picture worsened 3 years later when the pain became more severe with swelling and stiffness involving several joints in a symmetric fashion. Biochemical analysis showed an increase of ESR, CRP and rheumatoid factor, as well as of anti-CCP antibodies. The x-ray was compatible with a diagnosis of rheumatoid arthritis (RA). Therapy with methotrexate, prednisolone, bisphosphonates and vitamin D was started, achieving a quick clinical remission. Aromatase inhibitors have been shown to alter the distribution of Th1/Th2 lymphocytes and increase the level of RANKL. A possible role of aromatase inhibitors in RA development has been further addressed.
LETTER TO THE EDITOR

EFFECTIVENESS OF ISchia THERMAL WATER NASAL AEROSOL IN CHILDREN WITH SEASONAL ALLERGIC RHINITIS: A RANDOMIZED AND CONTROLLED STUDY

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Allergic rhinitis is characterized by local inflammation. Nasal lavage may be a useful treatment, however, there are few studies on this topic. This study aims to evaluate the effects of Ischia thermal water nasal irrigation on allergic rhinitis symptoms and airway inflammation during the period of natural exposure to Parietaria pollen in children with allergic rhinitis and intermittent asthma. Forty allergic children were randomly divided into two groups: the first group (Group 1) practiced crenotherapy with thermal water aerosol for 15 days per month, for three consecutive months, the control group (Group 2) was treated with 0.9% NaCl (isotonic) solution. In addition, all children were treated with cetirizine (0.5 gtt./kg/day once daily). Nasal symptom assessment, including Total Symptom Score (TSS), spirometry, and exhaled nitric oxide (FeNO) were considered before the treatment (T0), at the end of the treatment (T1) and again 2 weeks after the end of the treatment (T2). The study was registered in the Clinical Trials.gov (NCT01326247). Thermal water significantly reduced both TSS and FeNO levels and there was a significant relationship between reduction of nasal symptoms and FeNO values at the end of treatment with thermal water. In conclusion, this study shows that nasal crenotherapy with the hyper-mineral chloride-sodium water of Ischia was effective in children with seasonal allergic rhinitis based on the sensitivity to Parietaria. These results demonstrate that this natural treatment may be effective in a common and debilitating disease such as the allergic rhinitis.
LETTER TO THE EDITOR

CONTACT ALLERGIC DERMATITIS TO GOLD IN A TATTOO: A CASE REPORT

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The art of tattooing has increasing in recent decades. Allergic sensitivity to one of the pigments is the most frequent cause of dermatological reactions at the site of the tattoo. Gold is a new pigment used in tattooing, because of its bright yellow color and luster. Allergy to this metal is uncommon. To our knowledge, this is the first reported case of allergic contact dermatitis to gold in a tattoo.
LETTER TO THE EDITOR

ACQUIRED ANGIOEDEMA WITH C1 INHIBITOR DEFICIENCY ASSOCIATED WITH ANTICARDIOLIPIN ANTIBODIES

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Acquired angioedema (AAE) with C1 inhibitor deficiency is often associated to B cell lymphoproliferative disorders or autoimmune diseases. We report a case of AAE associated with IgM anti-cardiolipin antibodies, with frequent edematous attacks, that disappeared completely after a slight immunosuppression and danazol therapy.
LETTER TO THE EDITOR

EXHALED NITRIC OXIDE AS A MARKER OF LUNG INVOLVEMENT IN CROHN’S DISEASE

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Crohn’s Disease is an inflammatory bowel disease associated with a variety of systemic manifestations, including large and small airway involvement. The latter is most often a subclinical one, and requires expensive and invasive diagnostic approaches. Nitric Oxide (NO) can be detected non-invasively in the exhaled air (eNO) and be considered as a surrogate marker of airway inflammation. eNO tested at multiple expiratory flows can be used to distinguish the alveolar concentration of NO (C_{alv}NO) from the total amount of fractional eNO (FeNO). The aim of our study is to compare FeNO and concentration of alveolar nitric oxide (C_{alv}NO) levels and to assess their relationship with pulmonary involvement in Crohn’s patients differing in clinical stage and therapeutic regimens versus a group of healthy subjects. Thirty Crohn’s patients not showing clinical evidence of pulmonary diseases and 21 non-smoking, non-atopic healthy controls were enrolled. FeNO (14.9±10.2 ppb vs 10.1±6.3 ppb, p=0.049) and C_{alv}NO (4.4±2.2 ppb vs 2.6±1.9; p=0.006) values were found to be significantly higher in Crohn’s patients than in healthy controls. Both FeNO and C_{alv}NO correlated positively with the Crohn’s Disease Activity Index. In conclusion, our results for FeNO and C_{alv}NO confirm the presence of subclinical pulmonary involvement in Crohn’s disease. eNO measurement may be of clinical value in the follow-up of Crohn’s patients.
LETTER TO THE EDITOR

LUPUS MASTITIS IN SYSTEMIC LUPUS ERYTHEMATOSUS: A RARE CONDITION REQUIRING A MINIMALLY INVASIVE DIAGNOSTIC APPROACH

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Breast involvement is a rare event in SLE patients. The most frequent presentation is lupus panniculitis with skin erythema, tenderness, and parenchymal nodules. However, when breast masses are detected in SLE patients without significant superficial inflammation, it is mandatory to rule out breast carcinoma. Here, we report the case of a 47-year-old woman with an 18-year-long history of SLE, who presented with a suspicious breast mass. Since surgical trauma has been reported to be able to exacerbate breast inflammation in lupus mastitis, an ultrasound-guided minimally invasive Mammotome biopsy was performed to obtain tissue samples for histological and immunohistochemical examinations. Histology was consistent with lupus mastitis. The patient was already on mycophenolate mofetil and hydroxychloroquine. At the latest follow-up visit 6 years later, no progression of the breast lesion was observed.