EDITORIAL

WIDESPREAD MEMBRANE POTENTIAL CHANGES AND CARDIORESPIRATORY SYNCHRONIZATION INVOLVED IN ANXIETY AND SLEEP-WAKE TRANSITIONS

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Located within the ascending reticular activating system are nuclei which release neurotransmitters such as acetylcholine, serotonin, dopamine, and norepinephrine. These nuclei have widespread projections that extend into the limbic system and throughout cortex. Activation of these neurotransmitters during awake states leads to arousal, while inhibition leads to the loss of consciousness experienced during slow-wave sleep. Previously, we proposed a mechanism in which cardiorespiratory synchronization may underlie the widespread hyperpolarization that occurs throughout the brain during slow-wave sleep. We further propose that a similar homeostatic mechanism may be involved in sleep-wake transitions and maintaining various arousal states including rapid eye movement sleep, waking, and anxiety. Widespread depolarization associated with more rapid, shallow breathing and desynchronized cardiorespiratory oscillatory activity may underlie waking, anxiety, and rapid eye movement sleep states. The exact voltage values of these widespread membrane potential changes remain unknown and possibly highly variable between different neural areas and cell types. Here, we place these consciousness states on a spectrum of approximated widespread membrane potential values with anxiety states being the most depolarized, followed by waking states, and rapid eye movement sleep states. We propose that although these widespread membrane potential changes are minor, they may underlie transitions between and maintenance of varying levels of arousal. Further research on these mechanisms could provide insights into how the brain functions. This homeodynamic arousal mechanism involves the established feed-forward and feedback signaling between the ascending reticular activating system and the hypothalamus, as well as the modulation by cardiorespiratory oscillatory feedback from the body. Understanding the basic mechanisms responsible for the states of sleep, waking, and anxiety could lead to better treatment options in health and disease.
EDITORIAL

MAST CELL AND CANCER WITH SPECIAL EMPHASIS ON IL-37 AN ANTI-INFLAMMATORY AND INHIBITOR OF INNATE IMMUNITY: NEW FRONTIERS

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Mast cells (MCs) are mediators of allergy and inflammation and participate in the growth of cancer cells. MCs can promote both neoangiogenesis and tumor growth. They increase in the stroma of certain tumors where they can be recruited by tumor-derived chemoattractants, such as monocyte chemotactic protein-1 (MCP-1), RANTES and stem cell factor (SCF) to selectively secrete inflammatory molecules including chemical mediators and cytokines (TNF, IL-6 and IL-1). However, MC differentiation pathways and heterogeneity in cancer are still poorly understood. Human interleukin 1 (IL-1) plays a pivotal role in the pathogenesis of many diseases and functions, including host response to microbial invasion, injury inflammatory processes, immunologic challenges and cancer. Inflammation around the tumor includes the infiltration of mast cells and facilitates cancer growth. MCs are activated by IL-1 which can be produced by certain cancer cells and stimulate the stromal cells to selectively release IL-6, contributing to the development of Th-17 cells and increasing inflammation. IL-37, mainly generated by macrophage cell line, is an IL-1 family member which binds IL-18 receptor α (IL-18Rα) chain, and acts as a natural inhibitor of immune responses. IL-37 down-regulates cJun induced by IL-1, pro-inflammatory signals and reduces the expression of the mitogen-activated protein kinase (MAPK) p38α, STAT transcription factors and p53, affecting cellular differentiation and proliferation. In the present study we report the relationship between inflammatory mast cells, cancer and the beneficial effect of IL-37.
DIFFERENTIAL EXPRESSION AND DISTRIBUTION OF CYTOKERATINS AND VIMENTIN IN BUCCAL POUCH MUCOSAL CELLS DURING REAL-TIME CELL PROLIFERATION: RESEARCH BASED ON A PORCINE MODEL

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In recent years, buccal pouch oral mucosa cells were used as a source of potential biological grafting material in advanced tissue engineering. However, there are several limitations in the process of graft fabrication: donor and recipient patient availability as well as an incomplete knowledge of in vitro procedures related to tissue surgical recovery, in vitro cell culture (IVC) and/or tissue processing in “human somatic cell therapy.” Therefore, the animal model for oral mucosa grafting is still recognized as a source for xenografts and a useful model for biomedical research. In this study, the porcine buccal pouch oral mucosa cells were used in analysis of the stromalization/epithelialization process during short-term, in vitro real-time cell proliferation. We evaluated cytokeratin 18 (CK18), cytokeratin 8 + 18 + 19 (panCK), and vimentin (Vim) expression as epithelial and stromal cell markers, respectively. The porcine buccal pouch oral mucosa cells were cultured in vitro for 168 h, and the protein expression/distribution was analyzed every 24 h during real-time cell proliferation. In our analysis of protein expression using fluorescence intensity (FI), followed by confocal microscopic observations, we found the highest expression of CK18 occurred after 24 h of IVC, panCK after 72 h, and Vim after 48 h of IVC, as compared to other cultivation periods. We also found a substantial increase in Vim expression (3-4 fold) as compared to CK18 and panCK, and all of the investigated proteins were distributed in the cellular cytoplasm. The lag phase of cell proliferation occurred during the first 24 h of IVC, whereas the log phase was observed between 24 h-120 h of IVC. Throughout 7 days of IVC, statistically significant differences were found in Cell Index (CI) of the analyzed cells. Increased Vim expression in buccal pouch oral mucosa cells, as compared to CK18 and panCK, suggested that the stromal cells substantially predominated during in vitro cell cultivation. This may be a result of significant specificity of porcine oral mucosa cells isolated from the buccal pouch.
REMISSION EFFECT OF VITAMIN C ON ISOFLURANE-INDUCED APOPTOSIS AND ITS MECHANISM

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This study aims to discuss the remission effect of vitamin C on isoflurane-induced apoptosis of rats and its possible mechanism of action, to provide a theoretical basis for postoperative cognitive impairment. Reactive oxygen species (ROS) detection, adenosine triphosphate (ATP) test, MTT method and Morris water maze were applied for detection tests. For data statistics, double factor analysis of variance (ANOVA) and post hoc Bonferroni test were adopted. It was found that vitamin C could slow down the isoflurane-induced accumulation of ROS in H4-APP cells; moreover, it could relieve the activation of caspase-3 and increase cell survival rate to inhibit the occurrence of apoptosis, indicating that ROS was the source of cell toxicity. On the other hand, vitamin C could protect the cells with its antioxidant effect. It was proved that vitamin C could remit isoflurane-induced apoptosis and relieve the decline in learning and memory ability of rats.
ASSOCIATION BETWEEN EXPRESSION OF CUMULUS EXPANSION MARKERS AND REAL-TIME PROLIFERATION OF PORCINE FOLLICULAR GRANULOSA CELLS IN A PRIMARY CELL CULTURE MODEL

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Folliculogenesis is a compound process that involves both ovarian follicle growth and oocyte development, which is tightly attached to the follicular wall. During this process, cells that form the follicle structure undergo substantial morphological and molecular modifications that finally lead to differentiation and specialization of ovarian follicular cells. The differentiation of ovarian cells encompasses formation of follicle, which is composed of theca (TCs), mural granulosa (GCs), and cumulus cells (CCs). It was previously hypothesized that GCs and CCs represent undifferentiated and highly specialized follicular cells, respectively, which may have similar primordial cell origins. In this study, we investigated the expression pattern of cumulus expansion markers such as COX2, HAS2, PTX3, and TSG6 in porcine GCs during short-term, in vitro culture. We hypothesized that these genes may display an important function in GCs in relation to cellular real-time proliferation. The expression pattern of COX2, HAS2, PTX3, and TSG6 was evaluated after using RT-qPCR in relation to confocal microscopy observations of protein expression and distribution during real-time proliferation of porcine follicular GCs. The COX2 and HAS2 mRNAs were highly expressed after 120 h of in vitro culture (IVC), whereas PTX3 and TSG6 mRNAs were increased during the first 24-48 h of IVC (P<0.001, P<0.01). Conversely, all of the encoded proteins were highly expressed after 144-168 h of IVC as compared to other culture periods (P<0.001, P<0.01). When analyzing the real-time proliferation of GCs in vitro, we observed a logarithmic increase of cell proliferation between 0 h and 120 h of IVC. However, after 120-168 h of IVC, the cells reached the lag phase of proliferation. Since it is well accepted that porcine GCs undergo luteinization shortly after 24-48 h of IVC, the expression pattern of investigated genes indicated that Cox2 and Has2 are independent from the LH surge, but their increased levels may be upregulated by cell proliferation in vitro. Moreover, higher expression of PTX3 and TSG6 during first 24 h and/or 48 h of IVC suggested that their levels are accompanied by porcine GCs luteinization process.
A NATURAL FORMULA CONTAINING LACTOFERRIN, *EQUISETUM ARVENSIS*, SOY ISOFLAVONES AND VITAMIN D3 MODULATES BONE REMODELING AND INFLAMMATORY MARKERS IN YOUNG AND AGED RATS

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A pivotal role in osteoporosis development is played by radical oxygen species (ROS), the increased production of which is related to inhibited osteoblastic activity and bone formation. A new field of research could involve medicinal plants with antioxidant and protective effects in osteoporosis. Furthermore, considering the multifactorial metabolic aspects of osteoporosis, the pharmacological association of multiple medicinal plants could improve patient response. The aim of the present study is to evaluate *in vitro* and *in vivo* the protective effects of a natural formula containing lactoferrin 12%, *Equisetum arvensis* ES 54%, soy isoflavones 34% and vitamin D3 0.002%, in PBMC and C2C12 cells and in the bone matrix of young (3-month-old) and aged (12-month-old) female Sprague-Dawley rats, following chronic (21 days) administration. In this context, we assayed the activities of several inflammation and bone homeostasis mediators, such as IL-6, TNFα, PGE₂, osteoprotegerin, RANK, RANKL and NFkB. *In vitro* studies showed that natural formula (5-1000µg/ml) was able to significantly inhibit ROS and PGE₂ production. In the same concentration range, the natural formula inhibited both TNFα and IL-6 gene expression. In the *in vivo* studies, we administered to young and aged female rats the natural formula at 5mg/rat for 21 days, finding a significant reduction in inflammatory PGE₂ and NFkB activity. Nevertheless, we observed a significant increase in osteoprotegerin/RANKL ratio only in aged rats, compared to the respective control group. In conclusion, our findings corroborate the rational use of natural formula in the prevention and management of osteoporotic disease.
EFFECT OF LOW ENERGY LIGHT IRRADIATION
BY LIGHT EMITTING DIODE ON U937 CELLS

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Photobiomodulation (PBM) can induce a set of different biological modulators either in vitro or in vivo. Experimental evidence has highlighted the role of light effects on the mechanisms related to inflammation, apoptosis and autophagy. The goal of this project was the evaluation of PBM on U937, an established cell line of histiocytic lymphoma origin. Several aspects of modulation of proinflammatory pathways were analyzed and autophagic and proapoptotic mechanisms related to low laser light exposure of cells were studied. As a source of low energy light emission, we used an NIR-LED device, characterized by an 880 nm-wavelength as light source. Flow cytometry analysis was performed on supernatants of controls and treated U937 cells to detect inflammatory cytokine levels. In order to evaluate NF-kB and caspase3 expressions, Western blot analysis was performed according to standard procedures. In this report, we show the effect of PBM on a monocyte/macrophage established tumor cell line (U-937). We demonstrate that LED exposure, in the presence or absence of lipopolysaccharide (LPS), activates cell degranulation, increased expression of Interleukin-8 (IL-8) and modulation of beta galactosidase activity. Evidence shows that the well-known pro-inflammatory nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) and the apoptotic marker (caspase3/cleaved-caspase3 ratio) are up-regulated in response to a proinflammatory biochemical pathway.
miR-2861 IS INVOLVED IN OSTEOGENIC COMMITMENT OF HUMAN PERIODONTAL LIGAMENT STEM CELLS GROWN ONTO 3D SCAFFOLD

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miR-2861 endorsing osteoblast differentiation through the overexpression of Runt-related transcription factor 2 (RUNX2) protein has been recently described. In this study we evaluated: the performance of living construct, composed by human Periodontal Ligament Stem Cells (hPDLSCs) and 3D scaffold (EXg), and the behaviour of miR-2861/RUNX2 expression pathway on the osteogenic commitment. Human PDLSCs were seeded with and without EXg scaffold and cultured under basal and osteogenic conditions. Morphological features, adhesiveness and differentiation abilities were analysed using scanning electron and confocal laser scanning microscopy. Time-course of RUNX2, ALP, OPN and miR-2861 were evaluated through RT-PCR analysis. Our results highlighted that the osteogenic differentiation was mostly obvious in the hPDLSCs, grown onto 3D scaffold in presence of osteoinductive medium. Moreover, the overexpression of miR-2861 and RUNX2 in hPDLSCs cultured in presence of EXg under osteogenic and standard conditions was demonstrated. In synthesis, the increased expression of miR-2861/RUNX2 provides new insights regarding miRNA signaling network in the presence of scaffold providing an additional method to evaluate the performance of biomaterial in bone regeneration.
LETTER TO THE EDITOR

REAL-TIME SHEAR WAVE ELASTOGRAPHY AND APRI INDEX FOR EVALUATING AUTOIMMUNE HEPATITIS FIBROSIS

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The objective of this study was to investigate the significance of real-time supersonic shear imaging (SSI) and serum biochemical index in evaluating the degree of autoimmune hepatitis fibrosis. Retrospective analysis was carried out to study 291 cases of patients with autoimmune hepatitis, and discuss the value of SSI application, APRI index and bilirubin on autoimmune hepatitis. The area under the receiver operating characteristic curve (AUROC) was taken for statistical analysis to determine its diagnostic accuracy. In the high degree of hepatic fibrosis, the hepatic SSI measured value of autoimmune hepatitis positively correlated to ALT, AST, APRI ratio, AST and AST/ALT. The SSI measured value significantly and positively correlated to the degree of hepatic fibrosis ($r=0.598$, $p<0.01$). In chronic hepatic fibrosis, the elasticity values of AIH, PBC, AIH-PBC overlap syndrome (OS) were in a rising trend, and the difference in the elasticity value of each fibrosis stage was statistically significant ($P<0.01$). Hepatic SSI measured value was employed to respectively detect the AUROC of S3 stage and S4 stage for AIH, PBC, and AIH-PBC OS groups, which resulted as being higher than the APRI index detected in S3 stage and S4 stage. SSI measured index had better diagnostic significance than APRI index on hepatic fibrosis for AIH, PBC and AIH-PBC OS groups.
LETTER TO THE EDITOR

CLINICAL EFFECTS OF THERMOTHERAPY IN COMBINATION WITH INTRACAVITARY INFUSION OF TRADITIONAL CHINESE MEDICINE IN THE TREATMENT OF MALIGNANT PLEURAL EFFUSION

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Malignant fluid, a commonly seen tumor-associated complication, mainly includes peritoneal effusion, malignant pleural effusion and pericardial effusion. It can produce huge negative influence on the quality of life of patients and even lead to death. Treatment of malignant effusion is one of the effective measures for improving life expectancy of patients. To evaluate the effect of thermotherapy in combination with intracavitary infusion of Kangai injection in treating malignant pleural effusion, 195 patients who received treatment from April 2010 to October 2014 in the First Affiliated Hospital of Zhengzhou University were selected and divided into an observation group and two control groups (group A and B). The observation group was treated by thermotherapy in combination with intracavitary infusion of kangai injection. Control group A was treated by intracavitary infusion of kangai injection and control group B was treated by hyperthermal perfusion in combination with intracavity chemotherapy. Clinical effects, quality of life, treatment safety and untoward reactions were compared between the groups. It was found that differences of WBC, RBC and PLT levels before and after treatment had no statistical significance comparisons within group and comparisons between groups (P>0.05); hepatic and renal functions of the groups had no remarkable difference before or after treatment (P>0.05). The clinical effect of the observation group was superior to that of control groups A and B (P<0.05); the Karnofsky performance status (KPS) score of the observation group was much higher than that of control groups A and B (79.34±10.58 vs 71.11±9.64), but the difference of the ZPS score between groups had no statistical significance (P>0.05). It can be concluded that thermotherapy in combination with intracavitary infusion of traditional Chinese medicine can be safely applied as it has positive effects and remarkably improves quality of life, therefore it is clinically worth promoting.
LETTER TO THE EDITOR

EFFECTS OF CARBOMER EYE DROPS IN COMBINATION WITH ORTHOKERATOLOGY LENS IN TREATING ADOLESCENT MYOPIA

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To investigate the effects of carbomer eye drops (CED) during long-time wearing of overnight orthokeratology lens of adolescents with myopia, 260 teenagers with myopia treated in the Henan Provincial People’s Hospital from June 2012 to August 2014 and followed-up for more than 2 years were enrolled. All the patients underwent regular fitting of orthokeratology lens. They were divided into a CED (Vidisic) group (130 cases, 260 eyes treated with CED) and rewetting drops (RD) (Baushe and Lomb) group (130 cases, 260 eyes treated with RD). The effects in the two groups were observed. The incidence of corneal epithelial defects one day, one week and one month after treatment of the CED group was lower than that of the RD group, and the difference was statistically significant ($P<0.05$); the tear break up time (TBUT) of the CED group was higher than that of the RD group at different time points, and the difference had statistical significance ($P<0.05$); the difference of the value of Schirmer I test between the two groups had no statistical significance ($P>0.05$). It is concluded that carbomer eye drops can stabilize tear film and protect and repair corneal epithelium during the wearing of orthokeratology lens.
LETTER TO THE EDITOR

ACUPUNCTURE IN ENDOCRINE DISORDERS: A CRITICAL APPRAISAL

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Acupuncture is an integral part of ancient Chinese medical practice. The technique has been used extensively in pain relief and is being tried for many other chronic conditions. Industrial development and affluence lead to the increase in the prevalence of many endocrine disorders such as diabetes, obesity, and polycystic ovarian disease. The rising prevalence of the endocrine morbidity is observed in both the developing and developed nations. The management of these disorders involves major lifestyle modification coupled with a long-term drug intake. In such situations, patients often look at alternative therapeutic options existing in complementary and alternative medicine. The globalization of the world medical practice has led to the spread of acupuncture beyond China to other parts of the world. Acupuncture has been tried extensively in the management of various endocrine disorders with inconsistent results. In this review, we highlight the principles of acupuncture and its role in the management of various endocrine disorders.
LETTER TO THE EDITOR

MECHANISM OF SERUM miR-21 IN THE PATHOGENESIS OF FAMILIAL AND TRIPLE NEGATIVE BREAST CANCER

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The aim of this study was to clarify the mechanism of miR-21 in familial and triple-negative breast cancer (TNBC) by exploring the expression of serum miR-21. The sera were collected from healthy women at high risk of breast cancer. miR-39 was employed as the external reference, and real-time fluorescence quantitative PCR was used to detect the expression of serum miR-21 in 77 subjects. The miR-21 expression of the familial breast cancer group, TNBC group, and breast cancer high risk group were significantly higher than those in the normal control group and other breast cancer groups (P < 0.01). A high serum miR-21 expression level was associated with lymph node metastasis and Ki67 expression (P < 0.01). Serum miR-21 was closely associated with TNBC and familial breast cancer, and its expression was associated with genetic expression, degree of malignancy, and prognosis.
Study has shown that stem cell–based therapies are promising strategies in the treatment of several chronic diseases, but their overall benefit in the treatment of diabetic nephropathy (DN) remains controversial. The purpose of this study is to summarize the evidence of the effect of cell-based therapy in the treatment of DN to guide future clinical trials. We searched PubMed, EmBase, and the Cochrane Library for studies from the inception of cell-based therapies up to July 2015. We included animal trials that reported the effects of cell-based therapy on kidney function, cardiovascular risk factors, and body factors. A random-effects model was used to process the data, and the standard mean difference (SMD) was used to evaluate the efficacy of cell-based therapy. We included eight studies that reported data on 159 mice. Overall, we noted that cell-based therapies were associated with significantly reduced plasma creatinine level \( (P = 0.003) \), glomerular filtration rate \( (P < 0.001) \), plasma glucose level \( (P = 0.004) \), serum cholesterol level \( (P = 0.010) \), serum triglyceride level \( (P = 0.032) \), plasma urea level \( (P < 0.001) \), proteinuria \( (P = 0.008) \), and Cl\(^-\) fractional excretion \( (P = 0.023) \). Furthermore, cell-based therapies were associated with lower kidney weight \( (P = 0.003) \), and kidney/body weight \( (P = 0.004) \). A sensitivity analysis suggested that cell-based therapy might play an important role in increased body weight. In conclusion, cell-based therapies significantly improve kidney function, cardiovascular risk factors, and body factors in the treatment of DN.
Asthma is a type of chronic airway inflammation. Corticosteroids are inadequate for asthma therapy. However, it remains unclear whether oxidative stress is a distinct clinical and pathologic feature in asthma. We reviewed the articles on asthma-associated oxidative stress. The exposures to airborne allergens, such as house dust mite (HDM) and birch pollen, may not only trigger innate and adaptive immune responses but also cause oxidative stress damage in the airways. Allergen-induced reactive oxygen species (ROS) is involved in p38 MAPK, phosphoinositide-3-kinase (PI3K)/Akt and nuclear factor erythroid 2-related factor (Nrf2) kinase pathway signaling. Airborne particulate matter (PM) is an important environmental contaminant and is related to asthma development through increasing oxidative stress in the airways. Whether oxidative stress status is associated with the degree of asthma is needed to be further studied. Oxidative stress-induced corticosteroid insensitivity was associated with p38 MAPK, PI3K/Akt and Nrf2 signaling, and inhibited histone deacetylase 2 (HDAC2) activity and corticosteroid receptor (GR) function. Antioxidant treatments may be useful for oxidative stress in asthma.

OXIDATIVE STRESS IN ASTHMA: A DISTINCT CLINICAL AND PATHOLOGIC FEATURE?

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LETTER TO THE EDITOR

CHANGES OF EXPRESSION OF ESTROGEN AND PROGESTRONE RECEPTORS, HUMAN EPITHELIAL GROWTH FACTOR RECEPTOR 2 AND KI-67 AFTER NEOADJUVANT CHEMOTHERAPY IN THE TREATMENT OF BREAST CANCER

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Recent studies suggest that the development and prognosis of breast cancer is in close correlation to molecular subtype of breast cancer. Neoadjuvant chemotherapy has been extensively applied in the treatment of local breast cancer in advanced stage. In order to verify the correlation between expression changes of estrogen receptor, progesterone receptor, human epithelial growth factor receptor 2 and Ki-67 after neoadjuvant chemotherapy and neoadjuvant chemotherapy, we studied 120 patients with stage IIA-IIIC breast cancer who underwent neoadjuvant chemotherapy in Binzhou Medical University Hospital, Shandong, China from February 2011 to February 2015. Clinical characteristics were retrospectively analyzed. The expression of estrogen receptor, progesterone receptor, human epithelial growth factor receptor 2 and Ki-67 of patients were detected using the immunohistochemical method before and after neoadjuvant chemotherapy. The results suggest that the overall remission rate of neoadjuvant chemotherapy was 76.7% (92/120) of which 16.7% (20/120) of cases had complete remission, 60% (72/120) had partial remission and 23.3% (28/120) were stable. There were no cases of progressive disease. The property of estrogen receptor and the expression of Ki-67 of primary tumor were correlated to the remission rate of neoadjuvant chemotherapy (P<0.05). The expression of Ki-67 had a significant decline after neoadjuvant chemotherapy, and the difference had statistical significance (P<0.05). The difference in expression of estrogen receptor, progesterone receptor and human epithelial growth factor receptor 2 before and after neoadjuvant chemotherapy had statistical significance (P>0.05). Hence, it can be concluded that breast cancer patients with negative estrogen receptor expression and high Ki-67 expression before neoadjuvant chemotherapy can achieve better curative effects. Neoadjuvant chemotherapy cannot change the expression states of estrogen receptor, progesterone receptor and human epithelial growth factor receptor 2, but it can lower the expression level of Ki-67. Ki-67 can also be used for predicting the curative effect of neoadjuvant chemotherapy.
LETTER TO THE EDITOR

THE IMPACT OF ELECTROMAGNETIC FIELD AT A FREQUENCY OF 50 HZ AND A MAGNETIC INDUCTION OF 2.5 mT ON VIABILITY OF PINEAL CELLS IN VITRO

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The impact of electromagnetic fields (EMF) on the pineal gland has been described in numerous studies, but many questions still remain unanswered. The aim of the experiment described in this study was to evaluate the effect of EMF on the viability of the pineal gland cells of pig in vitro. Primary culture of the pineal gland cells has been exposed to the influence of an EMF at a frequency of 50 Hz with 1, 2 or 3 hours and for 3 hours every 2 or 3 days. After the experiment, viability of cells was assessed by MTT assay and compared to a control culture not exposed to electromagnetic fields. We noticed that in respect to the control, exposure of the cells to the EMF induced a significant increase in viability of cells at 2 and 3 hours of exposure. After three days of 3-hour exposure to EMF, we observed a significant decrease in cell viability in relation to the control. The results of these studies suggest that EMF can have a significant biological effect on the cells of the pineal gland in a time-dependent exposure to its action.
LETTER TO THE EDITOR

DECREASED IMMUNOREACTIVITY OF VISFATIN IN THE PANCREAS AND LIVER OF RATS WITH RENOVASCULAR HYPERTENSION

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Hypertension is one of the major endocrine and metabolic disorders, in which visfatin plays a significant role. The objective of this study was to evaluate the immunoreactivity of visfatin in pancreas and liver of “two kidney, one clip” (2K1C) renovascular hypertension model in rats. The studies were carried out on the pancreas and liver of rats. After a 6-week period of the renal artery clipping procedure, 2K1C rats developed a stable hypertension. Paraffin sections were stained with hematoxylin and eosin (for general histological examination) and processed for immunolocalization of visfatin. The intensity of immunohistochemical reaction was measured using Nikon NIS-Elements Advanced Research software. The hypertension significantly weakened the immunohistochemical reaction exhibiting visfatin in the pancreas and liver of hypertensive rats, compared to control animals. The changes induced by hypertension in the visfatin-containing cells in the pancreas and liver of the rats are discussed and needs further study.
LETTER TO THE EDITOR

EFFECTS OF SEVOFLURANE ON CARDIOPULMONARY FUNCTION IN PATIENTS UNDERGOING CORONARY ARTERY BYPASS

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The objective of the current study was to investigate effects of sevoflurane on cardiopulmonary function in patients undergoing coronary artery bypass grafting (CABG). In this study, 60 cases of patients with coronary heart disease (CHD) were selected and randomly divided into the sevoflurane group (group S) and the control group C (group C) with 30 cases in each group. The two groups received intravenous anesthesia. The patients of group C were only given oxygen mask and physiological saline to keep vein open; while the patients of group S were administered with 1% sevoflurane immediately after the beginning of cardiopulmonary bypass (CPB) until the end of the treatment. The cardiopulmonary functions at 30 min before operation (T₀), postoperative 2 h (T₁), 6 h (T₂), 24 h (T₃) and 48 (T₄) were observed. The mean arterial pressure (MAP) of the group S at T₁, T₂, T₃ was lower than that of the group C, as were the heart rate (HR) and left ventricular ejection fraction (LVEF). The creatine kinase isoenzyme (CK-MB) during T₁ to T₄ in the group S was less than that of the group C, and there were significant differences between the two groups (P < 0.05). The tidal volume (Vt), vital capacity (Vc) and oxygenation index (PaO₂/FiO₂) of the two groups during T₁ and T₂ were decreased, while respiratory frequency (RR) and alveolar-arterial blood oxygen partial pressure (Pa-aO₂) were increased and they began to decrease during T₃ and T₄. Vt and Vc of the group S were higher during T₁ and T₂ periods than those of the group C, while RR was lower than that of the group C; PaO₂ / FiO₂ during T₁ to T₄ period of group S was higher than that of group C, while PA-aO₂ was significantly lower than that of the control group (P < 0.05). In conclusion, although LVEF was not improved in the sevoflurane group, sevoflurane may contribute to stabilizing the cardiopulmonary function and preventing from myocardial injury.
LETTER TO THE EDITOR

USE OF LACTATED RINGER’S SOLUTION DOES NOT ELIMINATE THE RISK OF STRONG ION DIFFERENCE-RELATED METABOLIC ACIDOSIS FOLLOWING ON-PUMP CARDIAC SURGERY

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There is a growing interest in the effects of plasma sodium levels on postoperative outcomes. A trend of using balanced crystalloid solutions is based on the extensive data on chloride and the strong ion difference-related acidosis. However, effects of sodium are often overlooked in this context. The aim of the study was to establish the effects of the routine use of Lactated Ringer’s Solution (RL) on postoperative changes in sodium concentrations and whether these changes result in metabolic acidosis. We performed database analysis of 358 consecutive elective on-pump cardiac surgery cases in a tertiary referral university hospital. Approval from the institutional ethics committee was obtained for this study. Intraoperative fluid balance was 2726±1073 ml and the total volume of intravenous infusions in the first 24 hours was 5865 (±1073) ml, 95% of which was RL; 58% of the patients had metabolic acidosis with a base excess below (–)2 mmol L⁻¹ on arrival at the intensive care unit. There was a significant correlation between a strong ion difference and base excess (p < 0.01). A significant improvement in metabolic acidosis was noted within the first 24 hours, from a base excess of (–)2.49±2.8 to 0.32±2.6 mmol L⁻¹ (p < 0.001). All of the improvement in the base excess is explained by a change in the strong ion difference from the mean value of 31±4.3 to 34.2±3.6 mmol L⁻¹ (p < 0.001). Changes in the strong ion difference were primarily driven by changes in the serum sodium concentration, which were three-fold higher compared to those of chloride [–2.36 (±2.6) mmol L⁻¹ (p < 0.001) and 0.84 (±3.2) mmol L⁻¹, respectively (p = 0.01)]. In conclusion, our data confirm that there is a direct correlation between a strong ion difference and base excess following on-pump cardiac surgery. The use of RL prevented significant hyperchloraemia, but did not eliminate the risk of strong ion difference-related metabolic acidosis. The change in the strong ion difference was primarily linked to perioperative changes in the serum sodium concentration.
LETTER TO THE EDITOR

ROLE OF VITACAMPHORE IN IMPROVING CENTRAL PRO-INFLAMMATORY CYTOKINES FOLLOWING TRANSIENT GLOBAL ISCHEMIA

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Pro-inflammatory cytokines (PICs) including interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) are responsive to ischemic stress. This process thereby modulates the responsiveness of many cell types under diseased conditions. The current study was to examine the role played by vitacamphore (VCP) in regulating the levels of PICs and protein expression of PIC receptors in the cerebral cortex and hippocampus of rats after cardiac arrest (CA)-induced transient global ischemia. CA was induced by asphyxia followed by cardiopulmonary resuscitation (CPR) in rats. ELISA and Western blot analyses were employed to determine PICs and their receptors in the cortex and hippocampus. Our results show that IL-1β, IL-6 and TNF-α were significantly elevated in the cortex and hippocampus after CA. This was accompanied with increasing of PIC receptors, namely IL-1R, IL-6R and TNFR1. Systemic injection of VCP attenuated amplification of PIC signal pathway in these brain regions. VCP also improved Neurological Severity Score and brain tissue edema in CA rats. Notably, VCP resulted in a significant increase in survival of CA rats as compared with controls. In conclusion, VCP is likely to play a beneficial role in modulating transient global ischemia induced by CA via PIC signal mechanisms.
LETTER TO THE EDITOR

EFFECTS OF TWO DIFFERENT ANESTHETIC METHODS ON CELLULAR IMMUNITY OF PATIENTS AFTER LIVER CANCER RESECTION

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This study aims to investigate the effects of epidural block in combination with general anesthesia and general anesthesia alone on the immune function of patients undergoing primary liver cancer resection. Twenty-four patients with liver cancer who received treatment in the Third Hospital of Harbin Medical University, Heilongjiang, China, were enrolled and randomly allocated into group A and group B, with 12 in each group. The data on the T lymphocyte subpopulation, pro-inflammatory cytokines and anti-inflammatory cytokines were recorded before, immediately after and 24 h after liver cancer resection to compare differences and changes. It was found that CD4⁺ of patients who underwent combined anesthesia decreased after surgery and CD8⁺ of those patients was lower than that of the general anesthesia group. The content of interleukin (IL)-10 of patients who underwent general anesthesia combined with epidural block showed a decreasing tendency immediately after surgery, but increased 24 h after surgery, and the increase was greater than that of the general anesthesia group; furthermore, those patients had lower levels of IL-1β and interferon (IFN)-γ. In addition, patients who underwent epidural block and general anesthesia had a higher ratio of IFN-γ to IL-4 (Th1/Th2). These findings suggest that general anesthesia combined with epidural block has little passive influence on the cellular immunity of the body and can be selected as an anesthetic approach for patients with liver cancer.
LETTER TO THE EDITOR

INTERLEUKIN-6 AND INTERLEUKIN-8 IN DIAGNOSING NEONATAL SEPTICEMIA

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Neonatal septicemia (NS) is a common cause of death of newborn infants, hence early diagnosis and treatment are of the utmost importance. However, lack of specific clinical symptoms and late detection delay a correct diagnosis. It is therefore of great importance to establish auxiliary indexes for the early diagnosis of NS. To evaluate the value of interleukin (IL-6 and IL-8) in the diagnosis of NS, a prospective study was carried out. Seventy-five newborns who developed septicemia and received treatment in our hospital from January 2013 to December 2014 were selected as research subjects; also, 50 healthy newborns were set as a control group. The levels of serum IL-6 and IL-8 were compared between the two groups. Results demonstrated that levels of C-reactive protein (CRP), IL-6 and IL-8 of the septicemia group were higher than those of the control group on admission, although the difference had no statistical significance (P<0.05); the septicemia group had higher sequential organ failure assessment (SOFA) scores but lower pediatric critical illness scores (PCIS) compared to the control group (P<0.05); levels of CRP, IL-6 and IL-8 were in positive correlation to the SOFA scores and in negative correlation to PCIS. Analysis of receiver operating characteristics (ROC) curve demonstrated that the sensitivity, specificity and accuracy were 85.7%, 80.2% and 81.8%, respectively, when IL-6 level was set as 32 pg/mL, 78.1%, 64.2% and 66.9%, respectively when IL-8 level was set as 54 pg/mL, and 71.4%, 86.3% and 82.7% respectively, when detection of IL-6 and IL-8 were combined together. Hence it can be concluded that: IL-6 and IL-8 are involved in inflammatory reactions; levels of IL-6 and IL-8 were correlated to the severity of the infection; the value of IL-6 is higher than that of IL-8 in the diagnosis of neonatal septicemia and the combined detection of IL-6 and IL-8 can improve the accuracy of the diagnosis of neonatal septicemia.
A PLACEBO-CONTROLLED TRIAL OF A PROPRIETARY LIPID-LOWERING NUTRACEUTICAL SUPPLEMENT IN THE MANAGEMENT OF DYSLIPIDEMIA

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There is an ever growing emergence in the popularity of patient-driven care. As this health and wellness model grows, inquiries into diet, lifestyle, and supplemental approaches will continue to become a focal point for the healthcare consumer. Because of this, the aim of this study is to determine the tolerability, and overall effectiveness of a proprietary multi-ingredient lipid-lowering supplement in subjects with dyslipidemia. Forty participants were recruited for a single-center, double-blind randomized, placebo-controlled trial. Study participants were recruited between December 2014 and March 2015. Initial screening included a physical examination, renal and hepatic function, serum lipid, serum electrolytes, complete blood counts, and urine analysis. The 40 participants were randomly assigned to receive either the proprietary multi-ingredient lipid-lowering supplement (PMILLS) n= 20 or placebo n= 20. The trial consisted of a screening visit, a two-week run-in, and a four-month treatment period. Samples were taken at baseline, one month and four months of treatment. Results from the trial showed that the PMILLS significantly reduced total cholesterol (TC), low density lipoprotein (LDL-C), very low density lipoprotein (VLDL-C), oxidized LDL (oxLDL), Apo-lipoprotein B, triglycerides (TG), LDL particle number (LDL-P), heart rate, and diastolic blood pressure compared to placebo at one month and four months. The PMILLS significantly increased high density lipoprotein (HDL) particle number (HDL-P), and low density lipoprotein (LDL) particle size from dense type III and IV to larger type I and II LDL particle, compared to placebo at one month and four months. In addition, the PMILLS significantly reduced high sensitivity C-reactive protein (hs-CRP), tumor necrosis alpha (TNF-α), and interleukin 6 (IL-6) within the treatment group from baseline. There were no adverse effects noted in the treatment group after four months of supplementation. The present study demonstrates this PMILLS improves all relevant lipid parameters, such as particle numbers and particles sizes, as well as showing a significant reduction in inflammatory markers linked to cardiovascular health. With such combined changes in lipids, lipid sub-fractions, and inflammation, which are considered among the most effective means of reducing coronary heart disease (CHD), this PMILLS represents a new addition to safe and effective lipid-modifying strategies.
LETTER TO THE EDITOR

NASAL IRRIGATION WITH NASIR® IN CHILDREN: A PRELIMINARY EXPERIENCE ON NASAL CYTOLOGY

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Allergic rhinitis (AR) and upper airway respiratory infections are frequent in children, and both have a relevant impact on some social aspects, including school attendance and performance, sleep, quality of life (also of the parents), and costs. Saline nasal irrigation is widely employed to reduce nasal congestion and mucopurulent secretion, to stimulate cleansing of the nasal and paranasal cavities, and to induce restoration of mucociliary clearance. The present study evaluated the effects of nasal irrigation on nasal cytology, using the new device Nasir® in 66 children (40 males, 26 females, mean age 7.31±1.7 years, age range 4-17 years) with allergic rhinitis. The patients were treated with nasal irrigation with warm (36°C) Nasir® (250 mL sacs of premixed solution): one sac twice daily for 12 days. Nasal irrigation significantly reduced the neutrophilic infiltrate (baseline median value 2.8±0.7; post treatment value 2±0.5; p<0.05). In addition, there was a reduction of eosinophil infiltrate (T0= 3.2±1.1; T1= 2.6±1.2; p= <0.05). There was no significant change with regard to bacteria (T0= 2.7±0.9; T1= 2.3±1.02; p= 0.17). In conclusion, this pilot study reports that nasal irrigation with Nasir® might be useful to attenuate upper airway inflammation.
LETTER TO THE EDITOR

HUMAN HERPESVIRUSES-6 AND -7 ENCEPHALITIS IN IMMUNOCOMPETENT INFANTS: ARE THEY REALLY SO UNCOMMON?

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Human herpesviruses-6 and -7 (HHV-6 and 7) are considered uncommon causes of central nervous system infection and may occasionally cause encephalitis in young infants, however, the clinical syndrome and incidence are not well defined. In immunosuppressed hosts, reactivation is associated with a worse outcome such as encephalitis, hepatitis, or graft rejection. In immunocompetent hosts, this persistent infection is generally of no consequence. We report 4 cases of immunocompetent critically ill children, affected by HHV-6 and -7 encephalitis, admitted to our Pediatric Intensive Care Unit. In three patients, herpesvirus polymerase chain reaction in blood and cerebrospinal fluid was positive for HHV-6, while one patient was positive for HHV-7. In our cases, a typical clinical picture of viral infection was not present but neurological symptoms were predominant. In all 4 children, neurological involvement rapidly regressed after acyclovir therapy. In this report, we offer evidence that HHV-6 and -7 primary infections can cause several clinical manifestations, such as encephalitis, also in immunocompetent hosts. In our experience, children with neurological symptoms suggestive of viral encephalitis should be fully investigated for these two viruses.
LETTER TO THE EDITOR

PARASYMPATHETIC NERVOUS SYSTEM INVOLVEMENT IN FOOD ALLERGY:
DESCRIPTION OF A PAEDIATRIC CASE

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The latest research data emphasize the interaction between the nervous and the immune systems. It has been demonstrated that the central nervous system (CNS) can be involved secondarily due to blood brain barrier (BBB) disruption via pro-inflammatory cytokines released in allergy. More recently it was demonstrated that the parasympathetic nervous system (PNS) could also be equally involved in models of peripheral inflammation such as food allergy; although this last clinical presentation has rarely been described. Herein, the authors report the case of a five-year-old Caucasian female who was admitted to our Pediatric Acute and Emergency Operative Unit for cyclic vomiting. Her vomiting, which was preceded by objective torque vertigo, headache and weakness, had been recurring with constant frequency every two months since she was 3 years old. After a complex diagnostic flow-chart, it was found that this spectrum of neurologic symptoms was due to a food allergy syndrome, which postulates some etiopathogenic hypotheses to explain the relationship between the two mentioned diseases.
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LETTER TO THE EDITOR

FAVOURABLY EFFECTIVE FORMULATION OF SODIUM IODIDE AND SALICYLIC ACID
PLUS PROFESSIONAL HYGIENE IN PATIENTS AFFECTED BY DESQUAMATIVE GINGIVITIS

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The aim of this prospective pilot study was to evaluate the efficiency of an oral hygiene protocol, in combination with a solution of sodium iodide associated to salicylic acid (SISA), in patients affected by desquamative gingivitis (DG). Twenty patients not totally responding to conventional topical therapies, were selected. They received oral hygiene instructions with non-surgical periodontal therapy in a 21-day cohort study (during 3 weekly appointments). The SISA was used at the end of each session, with an impregnated gauze (with 5 ml of the solution) applied for 15 minutes for the upper jaw, and for a further 15 minutes with a new gauze for the lower. Evaluated clinical outcome variables included the full mouth plaque (FMPS) and bleeding (FMBS) scores, probing depth, patient related outcome and clinical gingival signs. Two months after concluding the planned protocol, a statistically significant reduction was observed for FMPS (P=0.032), FMBS (P=0.038), reported pain (P=0.000) and gingival clinical improvement (P=0.005). Topical application of SISA and professional oral hygiene procedures are connected with improvement of gum status, and decrease of related pain in subjects affected by severe DG.
PLASMA ENDOCANNABINOID BEHAVIOUR IN TOTAL KNEE AND HIP ARTHROPLASTY

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Endocannabinoids are a class of lipid mediators involved in a wide range of physiological pathways including pain perception, and immunological defences. In particular, the involvement of endocannabinoids in bone metabolism and bone resorption has recently been studied. Moreover, one study on total knee arthroplasty describes the probable role of endocannabinoids in pain perception after surgery. The aim of the present study was to evaluate variations of endocannabinoid concentrations in patients undergoing total hip or total knee arthroplasty before and after surgery. Sera from 23 patients were collected at three different times: before surgery and at two different times during rehabilitation, and endocannabinoids were quantified by HPLC-MS/MS analysis. Mean values of endocannabinoids in presurgical serum samples were: 6.11±0.5 ng/ml for N-palmitoylethanolamide, 1.39±0.08 ng/ml for N-stearoylethanolamide, 4.84±0.04 ng/ml for N-oleoylethanolamide, 0.44±0.03 ng/ml for N-arachidonoylethanolamide, 0.84±0.05 ng/ml for N-linoleoylethanolamide, 0.17±0.01 ng/ml for N-α-linolenoylethanolamide. Statistical analysis showed a significant decrease of all the endocannabinoids after surgery, while there were no remarkable differences between total hip and total knee arthroplasties or between genders. Moreover, the results show no significant correlation between endocannabinoid concentrations and C-reactive protein and Erythrocyte sedimentation rate. The present study shows for the first time a specific and univocal behaviour of six endocannabinoids and N-acylethanolamides in orthopaedic surgery, suggesting the endocannabinoid system as a possible pharmacological target for presurgical therapeutics.
LETTER TO THE EDITOR

MINOXIDIL DOSE RESPONSE STUDY IN FEMALE PATTERN HAIR LOSS PATIENTS DETERMINED TO BE NON-RESPONDERS TO 5% TOPICAL MINOXIDIL

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Topical minoxidil is the only US FDA approved drug for the treatment of female pattern hair loss (FPHL). 5% minoxidil foam is only effective at re-growing hair in a minority of women (approximately 40%). Thus, the majority of FPHL patients remain untreated. Previously, we demonstrated that non-responders to 5% minoxidil have low metabolism of minoxidil in hair follicles. As such, we hypothesized that increasing the dosage of topical minoxidil to low metabolizers would increase the number of responders without increasing the incidence of adverse events. In this study, we recruited FPHL subjects that were identified as non-responders to 5% topical minoxidil utilizing the previously validated assay for minoxidil response. Subjects were treated for 12 weeks with a novel 15% topical minoxidil solution. At 12 weeks, 60% of subjects achieved a clinically significant response based on target area hair counts (>13.7% from baseline), as well as significant improvement in global photographic assessment. None of the subjects experienced significant hemodynamic changes or any other adverse events. To the best of our knowledge, this is the first study to demonstrate the potentially beneficial effect of a higher dosage of minoxidil in FPHL subjects who fail to respond to 5% minoxidil.
LETTER TO THE EDITOR

HIGH LEVEL LASER THERAPY FOR THE TREATMENT OF LOWER BACK PAIN: CLINICAL EFFICACY AND COMPARISON OF DIFFERENT WAVELENGTHS

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High energy laser therapy (HELT) could be a new alternative treatment for lower back pain (LBP), which is a significant public health problem. Nevertheless, differences between the various light waves of HELT have not yet been fully researched. Sixty-six patients with lower back pain were treated using a high energy laser therapy. They were randomized into three different protocols which differed according to wave length (650 nm, 810 nm and TRIAX, which is simultaneous emission of 810 nm, 980 nm e 1064 nm). The other parameters remained constant (5 W and 50 J/cm² for ten daily sessions). The visual analogue scale (VAS), the Roland Scale, and the Oswestry Score were measured before treatment (T0), and at end of the treatment session (T1) and 1 month (T1), 2 months (T2) and 4 months of follow-up (T4). In each group we verified a statistically significant improvement over time and that there was a relationship between the time and treatment (p<0.01). At T1 for all wavelengths we found a statistically significant improvement of three scores (p<0.01), which was maintained up to T4. The group treated with 810 nm HELT, showed a better remission of pain on the VAS scale, and disability on the Oswestry Scale at T4 (p=0.01). Comparing T0-T1 the variation in the Roland Score was significant in the patients treated with 810 nm (p<0.01). All the wavelengths analyzed proved to be efficacious for LBP. The greater efficacy of 810 nm in promoting nerve regeneration and in modulating the nociception transmission could explain the better outcomes.
The “Risk Of Malignancy Algorithm” (ROMA) combines the diagnostic power of the CA125 and HE4 markers with menopausal status to predict the risk for developing epithelial ovarian cancer (EOC). The aim of this study was to evaluate the association between 25-OH vitamin D levels and ROMA score in obese women. One hundred and eighteen patients with a Body Mass Index (BMI) > 30 kg/m² (Group 1) and 80 women with a BMI <25 kg/m² (Group 2) were studied. The 25-OH vitamin D was quantified with LUMIPULSE® G 1200. As a threshold value, identified by ROC curve analysis, 20.2 ng/mL (sensitivity 73.3%, specificity 84%) was chosen corresponding to the limit between sufficient and insufficient 25-OH vitamin D according to the World Health Organization (WHO). Low 25-OH vitamin D levels were observed in 64% of obese women and in 11% of normal-weight women (p<0.001). ROMA score above 13% was detected only in obese women (19%). An association between low levels of 25-OH vitamin D and ROMA score was observed. Indeed, 64% of obese women with ROMA score >13% had concomitant insufficient levels of 25-OH vitamin D, while only 36% of obese women with ROMA score >13% had sufficient 25-OH vitamin D levels (p< 0.0001). This study suggests that the deficiency of 25-OH vitamin D in obese women has a possible correlation with high ROMA score.
LETTER TO THE EDITOR

ANTI-PLAQUE AND ANTIMICROBIAL EFFICIENCY OF DIFFERENT ORAL RINSES
IN A 3-DAY PLAQUE ACCUMULATION MODEL

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The idea of incorporating a mouthrinse with normal tooth brushing could be a useful adjunct to oral hygiene. Despite the principle nature of the toothpaste vehicle, most alcohol-based chemical plaque-control agents have been evaluated and later formulated in the mouthrinse vehicle. The current study was aimed to investigate the persistence of antimicrobial action and plaque inhibitory properties of a new alcohol-free mouthrinse when compared with positive control, chlorhexidine 0.12%, and placebo control, physiologic saline solution mouthrinses. The evaluation of the antimicrobial activity was performed by saliva samples collected during the 3 days of usage. The results of this study indicate that this new oral rinse has an equivalent plaque inhibitory action to chlorhexidine, and the plaque inhibitory action of the rinse appears to be derived from a persistence of antimicrobial action in the mouth. Furthermore, no side effects were reported during the study, and the additional benefit of no alcohol presence in the rinse solution.
During the period January 2013-December 2015, 175 cases of human salmonellosis were reported in the Apulia Region of Italy. The aim of this study was to characterize salmonella strains from the standpoints of serovars prevalence, antimicrobial resistance and clonal origin. The serological typing was performed by agglutination against antisera followed by a multiplex polymerase chain reaction (m-PCR). The obtained results were analyzed following the Kauffmann-White scheme. Susceptibility to antimicrobial agents was tested using the disk diffusion method on Muller-Hinton agar plates. All strains were tested by pulsed-field gel electrophoresis (PFGE) according to the PulseNet protocol, and cluster analysis was performed using BioNumerics software. It was found that the most prevalent isolated serovars were in order: i) S. Enteritidis, ii) S. Typhimurium and iii) S. 4,[5],12:i:–. The most common resistances were: i) Ampicillin (A) (38%), ii) Amoxicillin/Clavulanic Acid (AmC) (11%), iii) Streptomycin (S) (19%), iv) Sulphonamides (Su) (19%), v) Tetracycline (T) (30%), and vi) Piperacillin (Pip) (25 %). Ten multidrug-resistant (MDR) patterns were identified among the isolates, and the two most diffused ones were ASSuT and ASSuTPip, respectively. MDR patterns were predominantly expressed by Salmonella Typhimurium and Salmonella 4,[5],12:i:–. Molecular typing by PFGE yielded 60 different macrorestriction profiles among 33 serotypes.
LETTER TO THE EDITOR

FINE NEEDLE ASPIRATION CYTOLOGY OF 650 THYROID NODULES OPERATED FOR MULTINODULAR GOITER: A CYTO-HISTOLOGICAL CORRELATION BASED ON THE NEW ITALIAN CYTOLOGICAL CLASSIFICATION (SIAPEC 2014)

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The new Italian cytological classification (SIAPEC 2014) of thyroid nodules, in line with those of Bethesda and BTA-RCPath, replaces the previous TIR3 class with two new classes (TIR3A and TIR3B), which correspond to different risks of malignancy and clinical actions required. The present study was conducted to evaluate the diagnostic accuracy of the new SIAPEC classification as opposed to its previous version (SIAPEC 2007). Preoperative cytology was compared with the final histology obtained from 650 consecutive patients who underwent total thyroidectomy for multinodular goiter. Of this total, 434 patients (group A) had their cytological diagnosis based on the old SIAPEC 2007 classification and 216 patients (group B) had their cytological diagnosis based on the SIAPEC 2014 classification. In group A 111 patients (25.6%) had a TIR3 diagnosis, while in group B 52 patients (24.1%) received a TIR3 diagnosis, of whom 30 had TIR3A and 22 had TIR3B. In group A, 46 (41.4%) out of the 111 patients with TIR3 diagnosis had, based on histology, a thyroid carcinoma. In group B, only 2 (6.7%) out of 30 patients with TIR3A diagnosis had a thyroid carcinoma. This rate of malignancy was significantly lower (p<0.001) than that observed in patients with TIR3B diagnosis, in which 12 (54.5%) out of 22 patients had a carcinoma. The observations here reported show that, in respect to the previous version, the new Italian cytological classification provides greater diagnostic accuracy for detecting thyroid nodule malignancy.
Extracorporeal shock wave therapy (ESWT) is widely used for calcific tendonitis of the shoulder. The initial rationale for this therapy was to break the calcification, but this effect does not always occur. To date, we do not know how calcifications evolve or why they may be less responsive to the action of the shock waves. One hundred and seventy-four shoulders with calcific tendinitis were prospectively evaluated before and after ESWT, using the radiographic classifications according to Gartner and Heyer, to Bosworth and to Molè. Three months after ESWT therapy, we observed the disappearance of calcification in 36.8% of the shoulders, a reduction in size in 21.8% and no change in 41.4%. The calcifications that disappeared were large according to Bosworth (p=0.004). The probability of disappearance of calcification increased with increasing age (p=0.011), for medium calcifications according to Bosworth (p=0.001), and calcifications of type A according to Molè (p=0.043). The results of our study suggest that the radiographic aspects of calcific tendonitis of the rotator cuff could influence the disruptive effects after ESWT. With this knowledge we could define the timing of treatment and therapeutic choice for each patient.
Despite the clinical importance of metastasis to the skeleton, the diagnostic tools for early detection and monitoring of bone metastasis lack sensitivity and specificity. We evaluated a promising new serum biomarker, the soluble form of the Receptor of Advanced Glycosylated End-products (sRAGE). sRAGE is involved in the Wnt-signaling pathway, and has been reported to reduce the risk of cancer. We investigated the diagnostic potential of sRAGE to improve the detection and monitoring of bone metastasis.

We measured sRAGE in the serum of control healthy subjects, patients with primary tumors and patients with bone metastasis. sRAGE was also correlated with the Wnt inhibitors DKK-1 and sclerostin, the bone resorption markers MMP-2, MMP-9 and TRAP5, and the metastatic marker survivin. sRAGE was significantly lower in primary tumor and metastatic patients than in healthy subjects. sRAGE also showed a strong negative correlation with DKK-1, sclerostin, MMP-2, MMP-9, TRAP5b and survivin. These results indicated that sRAGE might play a protective role in bone metastasis progression, and it may diagnostic significance for detecting and monitoring osteolytic metastases.

CIRCULATING sRAGE IN THE DIAGNOSIS OF OSTEOLYTIC BONE METASTASIS

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LETTER TO THE EDITOR

COMPARATIVE MOLECULAR ANALYSIS OF BACTERIAL SPECIES ASSOCIATED WITH PERIODONTAL DISEASE

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Periodontal disease is an inflammatory disorder affecting the supporting teeth structures, including gingiva, periodontal ligament and alveolar bone, causing loss of connective tissue, reabsorption of alveolar bone and formation of periodontal pockets. The aim of this study is to find a correlation between bacterial growth and periodontal disease. Fifty-seven patients aged between 21 and 65 years, median age 46 years, were enrolled. According to gingival pocket depth, ranging from 3 to 7 mm, patients were divided into two groups: the first (30 patients, 53%) with deep pockets ≥ 5 mm and the second (27 patients, 47%) < 5 mm. The samples taken were processed for microbiological analysis by absolute quantitative real-time Taq-Man technique. Patients affected by periodontal disease were 32 (56%) and patients with gingival bleeding were 35 (61%). This data showed that the presence, the type and the bacterial load in gingival pockets were strongly correlated with gingival depth, periodontal disease and gingival bleeding. Quantitative microbiological analysis is a key point to improve patient compliance, allowing to choose the specific antibiotic treatment, avoiding antibiotic resistance and ensuring the successful outcome of therapy for periodontal disease.
CONGENITAL MUSCULAR DYSTROPHY AND EPILEPSY:
A PROSPECTIVE CASE SERIES OF PEDIATRIC PATIENTS

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Congenital Muscular Dystrophies (CMDs) can be considered as a heterogeneous group of diseases
characterized by marked weakness, generalized hypotonia and joint contractures. They are divided into
pure and classical forms, without ocular and cerebral involvement, and complex forms, which are
associated with cerebral abnormalities. Seizures have rarely been described in the pure forms while they
seem to occur more frequently in complex forms. The aim of our study was to evaluate the incidence of
seizure in CMD. Herein, the authors describe 16 cases of congenital muscular dystrophy (CMD) associated
with different kinds of epileptic events, in order to study the pathogenic connection between the two
clinical manifestations. In all described patients we reviewed the clinical, neurophysiologic, and neuroim-
maging data to determine any associations with epilepsy. The patients were divided into two groups: 14
cases with merosin positive CMD in one group and 2 patients with Walker Warburg syndrome (WWS)
in the second group. In our study we found that in the first group, one benign myoclonic epilepsy (BME),
one benign febrile convulsions had occurred. Also in one patient, the EEG revealed a moderately high
voltage slow background with diffuse sharp waves reaching 300mV in amplitude with no clinical signs.
In the merosin positive CMD patients, the presence of two different epileptic diseases, benign myoclonic
epilepsy (BME) in one and febrile convolution with tonic clinic seizures, may represent a new expression
of merosine-positive congenital muscular disease (PCMD) in which the deficiency of an undiscovered
muscular protein with a cerebral isoform may be the cause of epileptic events in this group of patients.
LETTER TO THE EDITOR

VITAMIN A DECREASES AFTER A MAXIMAL INCREMENTAL STRESS TEST IN NON-PROFESSIONAL MALE RUNNERS WITH LOW AEROBIC PERFORMANCE

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The data on the effects of aerobic training on plasma antioxidant vitamins are conflicting. Additionally, most studies focus on the oxidative profiles of professional athletes, but limited information is available for amateur athlete populations. The aim of this study was to evaluate the effects of high-intensity exercise on antioxidant vitamins in non-professional runners with varying levels of aerobic power. Eighty-one male runners underwent an incremental test to exhaustion. The study population was then divided into the following tertiles according to VO₂max: Group L (Low VO₂max, <44.2 mLkg⁻¹min⁻¹), Group M (Medium VO₂max, 44.2-49.7 mLkg⁻¹min⁻¹) and Group H (High VO₂max, >49.7). Comparative analyses were performed between Groups L and H. The total antioxidant capacity (TAC), Vitamin (Vit) E, Vitamin A, β-carotene, lycopene and thiobarbituric acid-reactive substances (TBARS) were determined before and 60 min after exercise testing. After the stress test, Vit A decreased and TBARS increased in Group L, whereas no changes in the vitamin concentrations, TAC induction and TBARS reduction were observed in group H. In individuals with low VO₂max, an incremental test determined lipid-peroxidation and Vitamin A consumption, whereas H Group increases TAC that buffer TBARS production.
LETTER TO THE EDITOR

ZENKER DIVERTICULUM IN THE RIGHT SIDE OF THE NECK RESEMBLING A THYROID MASS AT ULTRASOUND

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Zenker’s diverticulum represents the most common form of pharyngo-oesophageal diverticula usually occurring on the left side of the neck. Due to its anatomical proximity to the thyroid, it can mimic a thyroid mass. Here we describe the case of an asymptomatic 49-year-old man referred to the Thyroid Clinic of the Policlinico Umberto I Hospital-“Sapienza” University of Rome for thyroid sonography due to a family history of autoimmune thyroid disease. The patient’s thyroid blood tests did not reveal any abnormalities. The sonographic examination showed a dishomogeneous and hypoechoic thyroid gland. In addition, in the third middle of the right lobe, a mass (with a diameter greater than 26 mm), with heterogeneous internal echogenicity, hypoechoic margins and internal hyperechoic spots was recorded, with no appreciable flow at the Doppler evaluation. The TI-RADS score was 4c. Hence, the patient underwent ultrasound-guided fine-needle aspiration cytology that revealed the presence of squamous cells without cytological atypia, erythrocytes, muscular and vegetable fibres, colonies of bacteria in the absence of inflammatory infiltrate. This was consistent with the diagnostic hypothesis of oesophagus diverticulum, which was confirmed by means of a barium-swallow oesophagography. This case report underlines the possibility that a suspicious thyroid mass may result from a Zenker’s diverticulum, even if located on the right side, especially if the lesion has a heterogeneous echo-texture, a hypoechoic rim and internal hyperechoic spots.
Orthodontic tooth movement results from the response of the periodontal tissue to orthodontic force, which leads to modeling and remodeling of the surrounding alveolar bone. The response is considered to occur through the activation of specific signaling pathways, many of which are known, all acting to ultimately result in tooth movement. Much is known about the actions of these two cells, and the signaling pathways that affect them, both in bone and orthodontic literature, however, to date, little work has been carried out to examine the effect of the insulin-like growth factor binding proteins (IGFBP) in orthodontics. Therefore, we investigated the presence of IGFBP-5 in the gingival crevicular fluid (GCF) of 6 healthy subjects, and assessed the effects of orthodontic treatment on the levels and molecular state of this protein.