

EDITORIAL

**DYSMICROBISM, INFLAMMATORY BOWEL DISEASE AND THYROIDITIS:
ANALYSIS OF THE LITERATURE**

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The human body is colonized by a large number of microbes that are collectively referred to as the microbiota. They interact with the hosting organism and some do contribute to the physiological maintenance of the general good health thru regulation of some metabolic processes while some others are essential for the synthesis of vitamins and short-chain fatty acids. The abnormal variation, in the quality and/or quantity of individual bacterial species residing in the gastro-intestinal tract, is called “dysmicrobism”. The immune system of the host will respond to these changes at the intestinal mucosa level which could lead to Inflammatory Bowel Diseases (IBD). This inflammatory immune response could subsequently extend to other organs and systems outside the digestive tract such as the thyroid, culminating in thyroiditis. The goal of the present study is to review and analyze data reported in the literature about thyroiditis associated with inflammatory bowel diseases such as Ulcerative Colitis (UC) and Crohn’s Disease (CD). It was reported that similarities of some molecular bacterial components with molecular components of the host are considered among the factors causing IBD through an autoimmune reaction which could involve other non-immune cell types. The axis dysmicrobism-IBD-autoimmune reaction will be investigated as a possible etiopathogenic mechanism to Autoimmune Thyroiditis. If such is the case, then the employment of specific probiotic strains may represent a useful approach to moderate the immune system.

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

PERIODONTAL DISEASE AND BONE PATHOGENESIS: THE CROSSTALK BETWEEN CYTOKINES AND *PORPHYROMONAS GINGIVALIS*

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Periodontal disease is the most frequent cause of tooth loss among adults. It is defined as a plaque-induced inflammation of the periodontal tissues that results in a loss of support of the affected teeth. This process is characterized by destruction of the periodontal attachment apparatus, increased bone resorption with loss of crestal alveolar bone, apical migration of the epithelial attachment, and formation of periodontal pockets. Although the presence of periodontal pathogens such as *Porphyromonas gingivalis* is a prerequisite, the progression of periodontal disease is dependent on the host response to pathogenic bacteria that colonize the tooth surface. Nowadays, a growing body of literature has accumulated to investigate the association between bone diseases, periodontal pathogens and periodontal diseases. The integration of pathogen-associated molecular patterns from microorganisms with their surface receptors in the immune cells, induces the production of several cytokines and chemokines that present either a pro- and/or anti-inflammatory role and the activation of mechanisms of controlling this and the related disease, such as osteoporosis and rheumatoid arthritis. This review focuses on the evidence and significance of bone host cell invasion by *Porphyromonas gingivalis* in the pathogenesis of bone disorders, as well as the different lines of evidence supporting the role of cytokines in bone diseases.

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EDITORIAL

CROSSTALK BETWEEN VITAMIN B AND IMMUNITY

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Vitamin B1 (thiamin) is considered to be the oldest vitamin and in 1936 R.R. Williams and colleagues determined its chemical structure and were able to synthesize this vitamin. Vitamin B1 influences pro-apoptotic proteins, mitochondrial membrane potential, cytochrome C release, protein kinases, p38-MAPK, suppresses oxidative stress-induced NF-kappaB and has anti-inflammatory properties. Deficiency of vitamin B1 may cause beriberi, dysfunction of the nervous system, neuroinflammation, T cell infiltration, chemokine CCL2 activation, over expression of proinflammatory cytokines, such as IL-1, TNF, IL-6, and arachidonic acid products, and induces expression of CD40 by the microglia and CD40L by astrocytes which provoke the death of neurons. Here we report the relationship between vitamin B complex and immunity.

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

THE INFLUENCE OF CARBON MONOXIDE ON THE SECRETION OF MELATONIN BY PINEALOCYTES MEASURED *IN VITRO*

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Photoperiod is considered the most important factor entraining the circannual physiological rhythms through changing circadian patterns of melatonin (MEL) secretion from the pineal gland. The pineal gland of mammals does not respond directly to light but is controlled by light via neuronal phototransduction originating in the retina. In accordance with humoral phototransduction hypothesis, the aim of this study was to determine whether an increased concentration of CO, as a carrier of a light signal in pineal cell culture, affects the synthesis of melatonin. This study demonstrates that a commonly used carbon monoxide donor (CORM-2) markedly stimulated melatonin release from pineal cells incubated *in vitro* in a time-dependent manner, but the mechanism whereby CO modulates MEL release needs to be further explored.

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INHIBITORY EFFECT OF TETRAMETHYLPYRAZINE ON HEPATOCELLULAR CARCINOMA: POSSIBLE ROLE OF APOPTOSIS AND CELL CYCLE ARREST

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The first three authors contributed equally to the paper

Hepatocellular carcinoma (HCC) is the fifth most common cancer. An important approach to control HCC is chemoprevention. This study aims at investigating the antitumor effect of Tetramethylpyrazine (TMP). Rats were injected with N-Nitrosodiethylamine (DEN) to establish HCC. Tumor development was observed. Liver function was evaluated. Apoptosis and cell cycle arrest-related makers and signaling cascades were determined by Western blot, RT-PCR and flow cytometric analysis. The administration of TMP could significantly inhibit tumor development in DEN-induced HCC rats, shown by reduced incidence of tumor, decreased number of tumor nodules and reduced maximal size of tumor. DEN-induced increase of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase and alkaline phosphatase activities were significantly inhibited by TMP. TMP exhibited inhibitory effect on HCC through induction of apoptosis and cell cycle arrest in rats. TMP induced apoptosis through increasing Bax, decreasing Bcl-2, increasing the release of cytochrome c, and activating caspase, which consisted of the mitochondrial apoptotic pathway. TMP induced G2/M cell cycle arrest through down-regulation of cyclin B1/cdc2. In addition, inhibition of Akt and ERK signaling and the antioxidant activities of TMP may also contribute to its antitumor effect. These data provide new insight into the mechanisms underlying the antitumor effect of TMP.

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KNOCKDOWN OF CASEIN KINASE 1 ϵ INHIBITS CELL PROLIFERATION AND INVASION OF COLORECTAL CANCER CELLS VIA INHIBITION OF THE Wnt/ β -CATENIN SIGNALING

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Deregulation of casein kinase 1 epsilon (CK1 ϵ) is involved in the development of multiple pathological disorders such as cancer, however the function and molecular mechanism of CK1 ϵ in cancer are still unclear. In the present study, we aimed to investigate the role of CK1 ϵ in human colorectal cancer (CRC). The expression of CK1 ϵ was examined by immunohistochemical assay using a tissue microarray procedure. A loss-of-function experiment was performed to observe the effects of lentivirus-mediated CK1 ϵ shRNA (Lv-shCK1 ϵ) on cell proliferation and invasive potential by MTT and Transwell assays in CRC cell line (SW480). As a result, we found that the expression of CK1 ϵ protein was significantly increased in CRC tissues compared with that in adjacent non-cancerous tissues (ANCT) (68.9% vs 42.2%, $P=0.017$), and was correlated with the Duke's staging and depth of invasion in CRC patients ($P=0.012$; $P=0.015$). Knockdown of CK1 ϵ reduced cell proliferation and invasion of CRC cells followed by the downregulation of wnt3 α , β -catenin, PCNA and MMP-9. In conclusion, our findings show that high expression of CK1 ϵ is positively associated with the Duke's staging and depth of invasion in CRC patients, and knockdown of CK1 ϵ suppresses the growth and invasion of CRC cells through inhibition of the wnt/ β -catenin signaling, suggesting that CK1 ϵ may serve as a promising therapeutic target for the treatment of CRC.

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FLOW CYTOMETRIC DETECTION OF SUBHAPLOID NUCLEI IN HUMAN SPERM AS A MEASURE OF DNA FRAGMENTATION AND APOPTOSIS

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The use of assisted reproductive technologies (ARTs) is increasing worldwide. In order to predict the rate of pregnancy after ART the DNA fragmentation index (DFI) of ejaculated spermatocytes may be a better marker than conventional semen quality parameters. Spermatocytes with fragmented DNA are associated with apoptotic stages and are characterized by a low DNA content. The subhaploid nuclei of DNA-damaged spermatocytes can be easily detected by flow cytometry. We here analyzed the percentage of subhaploid nuclei of semen samples from 163 patients aged 26 to 74 years who consulted one of the ten centres for reproductive medicine which routinely send sperm samples to our laboratory in order to determine special sperm parameters. The percentage of subhaploid nuclei indicating the DFI of spermatocytes did not correlate with age and sperm volume, but inversely correlated with sperm concentration and the percentage of motile spermatocytes. This is in concordance with previous studies which demonstrated that DNA damage of spermatozoa correlates with conventional semen quality parameters. Since DNA-damaged spermatocytes are associated with an impaired outcome of assisted conception technologies, this method could help to monitor sperm quality of subfertile men after measures to increase sperm quality and to improve selection criteria of cryopreserved sperm samples in assisted reproduction medicine.

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MELATONIN ALLEVIATES BLEOMYCIN-INDUCED PULMONARY FIBROSIS IN MICE

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Pulmonary fibrosis occurs as a common end-stage sequela of a number of acute and chronic lung diseases. Eicosanoids exert crucial roles in inflammatory processes pertinent to fibrogenesis induction, however, the role of cyclooxygenase 2 (COX-2) is not fully elucidated in most pulmonary fibrosis related-disorders. Recently, melatonin (MLN) has been introduced as an effective immuno-modulator and anti-oxidant agent. The present study aimed to investigate the effect of MLN on COX-2 expression in idiopathic pulmonary fibrosis (IPF). Animals were divided into five groups, including: 1) saline control, 2) 1% ethanol control, 3) MLN control, 4) bleomycin (BLM), in which mice were injected with BLM (15 mg/kg, i.p.) two times per week for four weeks, and 5) BLM+MLN, in which MLN was given to mice (10 mg/kg, i.p.) 30 minutes prior to BLM injections for four weeks. MLN administration significantly reduced body weight loss ($P<0.05$), the rate of mortality, edema formation, lung injury, COX-2 expression ($P>0.05$), interstitial tissue percentage volume ($P<0.05$), and also increased the alveolar space percentage volume. MLN attenuated the BLM-induced lung injury responses such as collagen accumulation and airway dysfunction in mice. Finally, histological evidence supported the ability of MLN to inhibit COX-2 expression. Thus, it may serve as a novel potential therapeutic agent for IPF.

THE EFFECT OF DIHYDROARTEMISININ ON THE PROLIFERATION, METASTASIS AND APOPTOSIS OF HUMAN OSTEOSARCOMA CELLS AND ITS MECHANISM

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This study aims to research the effect of dihydroartemisinin on the proliferation, metastasis and apoptosis in human osteosarcoma cells 143B and the underlying mechanism. This study designed five groups for experiment and control, using dimethylsulfoxide (DMSO), and docosahexaenoic acid (DHA) at concentrations of 15, 25, 35 $\mu\text{mol.L}^{-1}$ respectively. Experiments including methyl thiazolyl tetrazolium (MTT) assay, clone formation assay, Hoechst 33258 staining assay, luciferase reporter plasmid assay, Western blot and scratch test were carried out. In addition, SPSS 18.0 software from IBM was used for statistical analysis and all the data obtained from the experiments were expressed as mean \pm SD, and variance was used to compare the difference between the groups. DHA is proved to be able to inhibit the proliferation and metastasis of osteosarcoma cells, as well as leaving a positive effect on apoptosis in the cytomorphosis. It achieves regulation over the human osteosarcoma cells by keeping the expression of related protein under control.

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4(α -L-RHAMNOSYLOXY)-BENZYL ISOTHIOCYANATE, A BIOACTIVE PHYTOCHEMICAL THAT DEFENDS CEREBRAL TISSUE AND PREVENTS SEVERE DAMAGE INDUCED BY FOCAL ISCHEMIA/REPERFUSION

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Natural compounds are a promising source to treat several pathologies. The present study shows the *in vivo* pharmacological beneficial effect of 4(α -L-rhamnosyloxy)-benzyl isothiocyanate (glucomoringin isothiocyanate; GMG-ITC) obtained from glucomoringin (GMG; 4(α -L-rhamnosyloxy)-benzyl glucosinolate), purified from *Moringa oleifera* seeds and hydrolyzed by myrosinase enzyme (β -thioglucoside glucohydrolase; E.C. 3.2.1.147). Cerebral ischemia/reperfusion (CIR) was induced in rats according to a classic model of carotid artery occlusion for a time period of 1 h and the reperfusion time was prolonged for seven days. GMG-ITC (3.5 mg GMG/ml plus 30 μ l enzyme/rat; one ml i.p./rat) was administered 15 min after the beginning of ischemia and daily. The results clearly show that GMG-ITC possesses the capability to counteract the CIR-induced damage reducing TNF-alpha release, I κ B-alpha cytosolic degradation/NF κ Bp65 nuclear translocation, as well as several other direct or indirect markers of inflammation (phospho-ERK p42/44, p-selectin) and oxidative stress (inducible Nitric Oxide Synthase (iNOS), MMP-9). GMG-ITC was shown to exert neuroprotective properties in preventing CIR-induced damage and the related cascade of inflammatory and oxidative mediators that exacerbate the progression of this disease in an experimental rat model. Our results clearly show that the tested phytochemical GMG-ITC possesses the capability to counteract CIR-induced damage.

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DISCLOSURE: ALL AUTHORS REPORT NO CONFLICTS OF INTEREST RELEVANT TO THIS ARTICLE.

ANTIPSYCHOTICS REVERSE P-GLYCOPROTEIN-MEDIATED DOXORUBICIN RESISTANCE IN HUMAN UTERINE SARCOMA MES-SA/Dx5 CELLS: A NOVEL APPROACH TO CANCER CHEMOTHERAPY

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Multidrug resistance (MDR) mediated by P-glycoprotein (Pgp) remains one of the major obstacles to effective cancer chemotherapy. Several chemosensitizers have been used *in vivo* and *in vitro* to reverse MDR but have exhibited several unwanted side effects. Antipsychotics are often administered to treat psychiatric disorders such as delirium, anxiety and sleep disorders in cancer patients during chemotherapy. The present *in vitro* study, examined the effects of two common antipsychotic compounds, haloperidol and risperidone, and a natural compound such as theobromine on reversing MDR Pgp-mediated, to evaluate their potential use as chemosensitizing agents. The human doxorubicin (doxo) resistant uterine sarcoma cells (MES-SA/Dx5) that overexpress Pgp (100-fold), were treated with the antipsychotic alone (1, 10 and 20 μ M) or in combination with different concentrations of doxo (2, 4 and 8 μ M). The accumulation and cytotoxicity of doxo (MTT assay) and cellular GSH content (GSH assay) in comparison with verapamil, a well-known Pgp inhibitor, used as reference molecule were examined. It was found that the three compounds significantly enhanced the intracellular accumulation of doxo in resistant cancer cells, when compared with cells receiving doxo alone ($p < 0.05$). Furthermore, compounds showed strong potency to increase doxo cytotoxicity toward resistant MES-SA/Dx5 cells, when compared with untreated control cells. The antipsychotic compounds also significantly increased GSH content at all concentrations ($> 30\%$) in resistant cells, when compared to untreated control cells ($p < 0.05$). These findings suggest that the antipsychotics or their derivatives might represent a novel class of reversal agents for overcoming MDR in cancer therapy, in particular theobromine showed to be an effective Pgp inhibitor with the lowest toxicity.

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LETTER TO THE EDITOR

**INFLUENCE OF miR-373 ON THE INVASION AND MIGRATION
OF BREAST CANCER AND THE EXPRESSION LEVEL
OF TARGET GENES TXNIP**

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An increasing number of people die from breast cancer every year. Consequently, more research has been concentrated on the study of this type of tumour, and miR-373 resulted as an important gene for treating breast cancer. To explore the influence of miR-373 on the invasion and migration of breast cancer and the expression level of target gene TXNIP, a set of therapeutic methods were designed based on miR-373. The transfection was performed using miR-373 inhibitor; the concentration of miR-373 was controlled by inhibitor, and it was transfected into MCF-7 cell by lipofectin. Fluorescent quantitative polymerase chain reaction was used to detect the expression level of miR-373 in cells after transfection as well as that of Caspase-3 and Caspase-8. MTT assay was used to detect the influence of miR-373 inhibitor on MCF-7 cells. The expression quantity of miR-373 in cell and tissue of breast cancer with high-low invasion and migration ability was detected by qRT-PCR (quantitative real-time polymerase chain reaction), thus the influence of the expression quantity of miR-373 on the invasion and migration of cell was determined. The expression of miR-373, EMT and TXNIP was determined by Western blot. Through the identification of proteomics and bioinformatics, it was finally found that TXNIP was regulated by miR-373. The protein expression level of TXNIP was negatively correlated with the level of miR-373. Thus it was concluded that miR-373 could promote the invasion and migration of breast cancer. In addition, in the tissue and cell of breast cancer with different invasion and migration abilities, the expression level of TXNIP was negatively correlated with the level of miR-373

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LETTER TO THE EDITOR

HOW EXPRESSIONS OF CLAUDIN-1 AND MMP-2 IN RETINOBLASTOMA CORRELATE WITH HISTOLOGICAL DIFFERENTIATION AND OPTIC NERVE INVASION

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Retinoblastoma is a commonly seen and dangerous intraocular malignant tumor in infants. Studies have found that Claudin-1 and MMP-2, whose expressions may be connected, play roles in tissues of retinoblastoma. In this study we analyze and discuss changes of Claudin-1 and MMP-2 expressions, and the correlation between the expressions and retinoblastoma histological differentiation and optic nerve invasion. MaxVision™ was applied to detect expressions of Claudin-1 and MMP-2 in 45 samples of retinoblastoma and 15 paraffin-embedded samples of normal retina. The correlation between Claudin-1 expression and MMP-2 expression was analyzed based on *chi*-squared test and Spearman's correlation test. Positive expressions of Claudin-1 in retinoblastoma were fewer than those in retina; higher positive expressions were found in differentiated tissues than in undifferentiated tissues; while compared to expressions in invasive optic nerves, Claudin-1 expressed more positively in optic nerves without invasion. As for MMP-2, its expressions were higher in retinoblastoma than in normal retina; undifferentiated tissues had higher positive expressions than differentiated tissues, which were not statistically significant; higher positive expressions were detected in invasive optic nerves. Thus, it could be concluded that the correlation between Claudin-1 expression and MMP-2 expression in retinoblastoma was negative. Expressions of Claudin-1 were positively related to histological differentiation and optic nerve invasion of retinoblastoma; while MMP-2 expression had negative correlation with histological differentiation and optic nerve invasion of retinoblastoma. Claudin-1 and MMP-2 played a negative role in the optic nerve invasion and tumor development of retinoblastoma.

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LETTER TO THE EDITOR

SIDE EFFECTS OF TUBERCULOSIS TREATMENT WITH FIXED-DOSE COMBINATIONS

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This paper aimed to explore the therapeutic effect and safety of Fixed-dose Combinations (FDCs) on tuberculosis. A computer search was carried out to review the literature related to clinical randomized controlled trials (RCTs) and clinical controlled trails (CCTs) on the curative effect and safety of treating pulmonary tuberculosis with FDCs. The results demonstrated that, in the 22 studies examined, comparison of sputum negative conservation rate of treating smear-positive pulmonary tuberculosis with FDCs and single drug, the relative risk (RR) value and 95% confidence interval (CI) were 1.02 (1.01, 1.03) and 1.01 (1.00, 1.02), respectively, at the end of the 2nd month and 6th month ($P < 0.05$), while comparison of the relapse rate within six months showed that RR value and 95% CI was 1.72 (0.98, 3.02) ($P > 0.05$). No statistically significant differences were found between the two groups in total occurrence of the rates of side effects pertaining to skin reaction, gastrointestinal tract side reaction, occurrence rate of liver and gall side reaction or occurrence rate of drug withdrawal because of side effects ($P > 0.05$). After sensitivity analysis, it was found that occurrence rate of gastrointestinal tract side effects and occurrence rate of liver and gall side effects were unstable. All the findings suggest that the curative effect of treating tuberculosis with FDCs is better than that of a single drug. More reliable evidence is required since the safety evaluation results are not stable.

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LETTER TO THE EDITOR

THE INFLUENCE OF POSTERIOR APPROACH CERVICAL INTRASPINAL TUMOR RESECTION ON THE STABILITY OF CERVICAL VERTEBRAXP. GENG¹, X. WANG², T. MENG¹, JJ. LI¹, XC. LI¹ and GY. FU¹

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The first two authors contributed equally to this manuscript

This paper discusses the influence of posterior approach cervical intraspinal tumor resection on the stability of cervical vertebra. A total of 32 patients with cervical intraspinal tumor were included and divided into a group undergoing posterior approach bilateral vertebral lamina resection (group A) (n=16) and a group undergoing posterior approach semi-laminectomy (group B) (n=16). It was found, through follow-up visits, that the incidence rate of cervical instability of the patients was 25% and the incidence rate of cervical curvature deterioration of the patients was 37.5% in group A, whereas the two incidence rates of group B were 6.25% and 12.5% respectively; the incidence rates of cervical curvature deterioration and instability were significantly increased compared to group B (P< 0.05). It is concluded that, both regular posterior approach vertebral lamina resection and semi-laminectomy influence the biomechanical change of cervical vertebra, but the influence of the latter is less. Also, it is found that, applying titanium connectors and titanium nails for rigid internal fixation maintains the completeness and stability of the structure of the cervical vertebra.

LETTER TO THE EDITOR

**BERBERIS VULGARIS FRUIT CRUDE EXTRACT AS A NOVEL
ANTI-LEUKAEMIC AGENT**

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Tumor protein p53 encoded by the *TP53* gene in humans is known as a cancer biomarker in patients diagnosed with cancer, and it plays an essential role in apoptosis, genomic stability, and inhibition of angiogenesis. Cancer therapies with common chemotherapy methods are effective, as known, but have some side effects. *Berberis vulgaris* is traditionally administrated as a cancer drug. The current research aims to evaluate p53 as a biomarker in WEHI-3 cell line and to demonstrate the *Berberis vulgaris* fruit crude extract (BVFCE) as a new anticancer drug. For this purpose, we evaluated the effect of BVFCE in different concentrations against WEHI-3 cell line *in vitro* and determined the quantitative level of p53 gene in the treated WEHI-3 cells. The results demonstrated that even at only 1 mg/ml concentration of *Berberis vulgaris* crude extract, there was a low level of p53 biomarker expression on WEHI-3 cells in comparison with doxorubicin. Therefore, the current study suggests BVFCE as a reliable anti-leukaemic drug and candidate for anticancer therapy. However, further investigation need be carried out to confirm its efficiency *in vivo*.

LETTER TO THE EDITOR

**EFFECT OF PREGNANE XENOBIOTIC RECEPTOR ACTIVATION ON
INFLAMMATORY BOWEL DISEASE TREATED WITH RIFAXIMIN**Y-C. WAN¹, T. LI², Y-D. HAN², H-Y. ZHANG³, H. LIN⁴ and B. ZHANG⁵

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The causes and pathogenesis of Inflammatory Bowel Disease (IBD) are still not clearly understood. This study aims to prove the important role of rifaximin played in inflammatory reaction caused by abnormality of the intestinal mucosal immune system. Intestinal microflora can greatly promote and maintain the inflammatory reaction of IBD, therefore, antibiotics can be used to treat IBD. Rifaximin is a medicine usually used for local intestinal infection. Many clinical and basic studies have shown that both a single application of rifaximin and the joint application with other medicines could achieve a good efficacy. This paper studied the activation of Pregnane Xenobiotic Receptor (PXR) in treating IBD with rifaximin and analyzed its efficacy in IBD when PXR was involved in the transport of medicine and metabolism. The results prove that rifaximin can not only serve as an anti-microbial drug, but can activate PXR and actually weaken the reaction of IBD. Thus it is safe to say that rifaximin has great potential in treating IBD.

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LETTER TO THE EDITOR

CLINICAL DIAGNOSIS OF CAROTID ATHEROSCLEROSTIC PLAQUE IN HYPERTENSIVE PATIENTS WITH HIGH RESOLUTION MAGNETIC RESONANCE ANGIOGRAPHY

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As the incidence of ischemic cerebrovascular disease increases continuously over the years, carotid atherosclerosis as an important dangerous factor has drawn a lot of attention from many experts and scholars. To explore the clinical significance of high resolution magnetic resonance angiography (MRA) in carotid atherosclerosis and ischemic cerebrovascular disease, a group contrasting method was adopted. One hundred patients with ischemic cerebrovascular disease and 100 patients without ischemic cerebrovascular disease were taken as observation group and control group, respectively. High resolution MRA was used for examining and observing the development of carotid atherosclerotic plaque in patients in the two groups. We found that the proportion of carotid atherosclerotic plaque in the experimental group and control group had statistical difference ($P<0.05$); and the proportion of carotid atherosclerotic plaque in patients over 60 years of age was higher than in patients under 60 years, and the difference was statistically significant ($P<0.05$). The proportion of carotid atherosclerotic plaque in patients with high blood pressure was also higher than in patients without high blood pressure, and the difference was statistically significant ($P<0.05$). Moreover, the proportion of carotid atherosclerotic plaque in patients with hyperlipidemia was higher than in patients without hyperlipidemia, and the differences were statistically significant ($P<0.05$). We can therefore draw a conclusion that the development of carotid atherosclerotic plaque affects the occurrence of ischemic cerebrovascular disease, which is related to patient's age, level of blood pressure and blood lipids. In addition, high resolution MRA is helpful to early discovery of the formation of carotid atherosclerotic plaque.

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LETTER TO THE EDITOR

**A CASE OF MULTIPLE BILATERAL TESTICULAR CAPSULE
MAST CELL TUMOURS IN A DOG**

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A 5-year-old intact male German Shepherd dog was referred with a diagnosis of leishmaniasis. Several testicular masses were palpated during the physical examination, while the diagnostic screening yielded no remarkable findings. Fine needle aspiration cytology of the masses revealed the presence of intermediately differentiated mast cell tumours. Scrotal ablation and orchietomy were performed as a definitive treatment option. The pathological examination of the surgical specimens confirmed the diagnosis of grade II mast cell tumours and showed that they were all confined to the testicular capsule. At 7 months post-admission, the dog exhibited neither postsurgical complications nor metastatic foci and was, therefore, given a favourable prognosis. Despite their exceptionally rare occurrence, mast cell tumours should be considered for the differential diagnosis of testicular tumours.

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LETTER TO THE EDITOR

EXPRESSION MECHANISM AND CLINICAL SIGNIFICANCE OF NOB1 IN GASTRIC CANCER TISSUE AND ADJACENT NORMAL TISSUEW-P. ZHOU¹, X. LIU², Y. YANG³ and Y-F. LIU⁴

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This paper studies the effect and relationship of NOB1 in the development of gastric cancer, based on an analysis of NOB1 expression in gastric cancer tissue and adjacent tissue. Thirty gastric cancer tissue samples taken during surgery with complete pathological data and their related adjacent normal tissue were examined in this study. NOB1 protein expression in gastric cancer tissue and adjacent normal tissue was detected by immunohistochemistry (IHC). Real-time PCR was used to detect NOB1 mRNA expression, which provided a basis on which to explore the clinical pathological characteristics for patients with gastric cancer. Results show that NOB1 protein in gastric cancer tissue and adjacent normal tissue were diffusely expressed both in the cytoplasm and nucleus. The positive expression rate in gastric cancer tissue was 73%, higher than that in adjacent normal tissue (47%). Both the reference GAPDH and NOB1 amplification are reflected in the amplification curve in standard S-shape and the unimodal solubility curve which was not altered by non-specific amplification and primer dimer. NOB1 mRNA relative expression in cancer tissue was 4.899 ± 1.412 . NOB1 expression had no direct relationship with the patients' age, gender, tumor differentiation or infiltration degree, lymphatic metastasis, distant metastasis nor pTNM periodization, but was directly related to the size of the tumor. All the findings in this paper suggest that NOB1 can be one of the focuses for diagnosing and treating gastric cancer and that its protein expression is likely to increase with the growth of tumor, thus playing a great role in the incidence and development of gastric cancer.

*LETTER TO THE EDITOR***EXPRESSION AND CLINICAL SIGNIFICANCE OF APOPTOSIS-ASSOCIATED PROTEINS SURVIVIN AND LIVIN IN CONDYLOMA ACUMINATUM**

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The objective of the present study was to explore the expression and significance of survivin and Livin in lesions of Condyloma acuminatum (CA). Streptavidin-peroxidase (SP) immunohistochemistry method was used to measure the expression of survivin, Livin and Ki-67 in 48 cases of CA and 25 cases of normal foreskin tissues. The positive expression rates of survivin, Livin and Ki-67 were 72.91% (35/48), 77.08% (37/48) and 85.42% (41/48) in CA tissues, and 4% (1/25), 4% (5/25) and 60% (15/25) in the control group, respectively. The expression intensity of survivin, Livin and Ki-67 in CA tissues (++ ~ +++) was significantly higher than that in the normal control group (- ~ ++). There were significant differences ($P < 0.05$) both in the positive rates and the expression intensity of survivin, Livin and Ki-67 between the two groups. There was positive correlation between the expression of survivin and Livin in CA group ($P < 0.01$); the expressions of survivin and Ki-67 were positively correlated with each other ($P < 0.01$); Livin and Ki-67 expressions were positively correlated with each other ($P < 0.01$). There were over-expressions and excessive proliferations of survivin and Livin in CA tissues, and apoptosis suppressors survivin and Livin were correlated with CA.

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LETTER TO THE EDITOR

EFFECT OF RELATIVE GENE EXPRESSION ON PLAQUE VULNERABILITY IN PATIENTS WITH STABLE ANGINA AND PATIENTS WITH ACUTE CORONARY SYNDROMEN. CHEN¹, J.Y. ZHANG¹, S.Z. YANG² and Y.D. LI²*¹Department of Cardiology, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, China; ²Department of Cardiology, Nanyang Central Hospital, Nanyang, China**Received February 13, 2015 – Accepted April 29, 2015*

This study was conducted to investigate the effect of relative gene expression on plaque vulnerability in patients with either stable angina or acute coronary syndrome (ACS). A total of 30 patients with ACS, 28 patients with stable angina and 17 healthy volunteers were selected. High resolution ultrasound was used to detect carotid arterial intima-media thickness (IMT) and plaque score, Sandwich enzyme linked immunoassay to determine the change of matrix metalloproteinase (MMP)-9 and tissue inhibitor of matrix metalloproteinase (TIMP)-1. The three groups had no statistically significant difference in age, gender, total cholesterol, triglyceride, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. MMP-9, TIMP-1, MMP-9/TIMP-1 and IMT, total plaque score, soft plaque score and hard plaque score of patients' acute coronary syndrome were obviously higher than those with stable angina and normal people. It was also found that MMP-9 was in a positive correlation with IMT, total and soft plaques score, TIMP-1 was positively correlated with IMT as was MMP-9/TIMP-1. Regardless of age, IMT was in a positive correlation with MMP-9, TIMP-1 and MMP-9/TIMP-1 in partial correlation analysis. All these findings suggest that ACS patients have remarkably higher MMP-9, TIMP-1, MMP-9/TIMP-1, IMT, total plaque score, soft plaque score and hard plaque score compared to patients with stable angina pectoris and healthy subjects ($P<0.05$) and there are positive correlations between MMP-9, TIMP-1, MMP-9/TIMP-1, total plaque and soft plaque score.

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LETTER TO THE EDITOR

EVALUATION OF ANTIVIRAL THERAPY TREATMENT FOR LIVER CIRRHOSIS CAUSED BY CHRONIC HEPATITIS C AND HEPATITIS C BY ^{31}P -MRS, BASED ON METABOLITE DETECTION

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This study discusses the application of magnetic resonance spectrum (MRS) to evaluate the efficacy of antiviral therapy in the treatment of liver cirrhosis caused by chronic hepatitis C and hepatitis C, based on metabolite detection. A total of 54 patients with liver cirrhosis caused by chronic hepatitis C and hepatitis C were selected and divided into treatment group and control group. ^{31}P -MRS imaging was carried out on patients in the two groups both before receiving antiviral treatment and 6 months after treatment to compare the change of metabolite ratio $(\text{PE}+\text{PC})/(\text{GPE}+\text{GPC})$. It was revealed that no statistically significant difference was found in the comparison of $(\text{PC}+\text{PE})/(\text{GPC}+\text{GPE})$ ratio in the two groups before treatment, but the difference was found 6 months after treatment; ratio of $(\text{PC}+\text{PE})/(\text{GPC}+\text{GPE})$ in the treatment group distinctly decreased 6 months after treatment compared to before treatment, with a statistically significant difference, while the control group had no remarkable change or statistical significance. Moreover, 32 patients were found with sustained virus response to antiviral therapy. Of these, 25 patients possessed a decreased ratio of $(\text{PC}+\text{PE})/(\text{GPC}+\text{GPE})$, 4 remained without change and 3 had a slightly increased ratio after antiviral treatment. Of 12 patients with no response, 1 had a decreased ratio of $(\text{PC}+\text{PE})/(\text{GPC}+\text{GPE})$, 2 remained without change and 9 had a slightly increased ratio. The differences were all statistically significant in comparison of the two groups. ^{31}P -MRS is thought to be effective for evaluating the efficacy of antiviral therapy through non-invasive detection of liver energy metabolism.

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LETTER TO THE EDITOR

**META-ANALYSIS OF HIGH RISK FACTORS OF RESIDUE OR RELAPSE OF
CERVICAL INTRAEPITHELIAL NEOPLASIA AFTER CONIZATION**J. JIN¹, L. LI² and F. ZHANG³

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This study assesses the high risk factors of residue or relapse after conization of cervical intraepithelial neoplasia. Literature on high risk factors of residue or relapse after conization of cervical intraepithelial neoplasia from January 2006 to June 2011 were selected from the Pubmed Database, Elsevier Database, Chinese Biomedicine Database and Chinese Journal Full-text Database of China National Knowledge Internet (CNKI). Software RevMan 4. 2 provided by Cochrane collaboration network was used in the statistical analysis of the data. According to the inclusion criteria, 10 essays were retrieved, including 348 cases in case groups and 1,608 cases in control groups. Information about residue or relapse after conization, incisal edge, HIV infection after six months of surgery, age, menopause status was obtained through the above method. Meta-analysis showed that positive surgical margin groups had a higher residual or recurrence rate than negative surgical margin groups after conization; groups where glands were involved had a higher residual or recurrence rate than non-involved glands groups after conization; positive HR-HPV infection after six months of conization groups had higher residual or recurrence rates than negative HR-HPV infection groups; 50 years or older groups had higher residual or recurrence rate than under 50 year-old groups after conization; postmenopausal groups had higher residual or recurrence rate than premenopausal groups. Menopause, 50 years old or older, gland involvement, positive surgical margin and HR-HPV infection after six months are high risk factors of residue or relapse after α - β conization of CIN.

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LETTER TO THE EDITOR

**PROTEIN EXPRESSION OF METADHERIN AND SENSIBILITY OF BREAST CANCER
MDA-MB-231 CELLS TO TREATMENT BEFORE AND AFTER TRANSFECTION**H. ZHAO^{1,2}, QT. WANG², SQ. GENG³, J. XU³, YZ. YU² and ZG. YU¹

¹Department of Breast Disease, the Second Hospital of Shandong University, Jinan, China; ²Department of Breast Disease, the Second Affiliated Hospital of Qingdao University Medical College, Qingdao, Chin; ³Department of Pathology, the Second Affiliated Hospital of Qingdao University Medical College, Qingdao, Shandong, China

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Breast cancer tends to have an increasing mortality, severely threatening the health of females. The invasion and metastasis of breast cancer are the leading causes of death. It has been reported that breast cancer is caused by the activation of a series of proto-oncogenes and inactivation of anti-oncogenes. In the present study, Real-time PCR and Western blot were used to detect the protein expression level of metadherin before and after transfecting MDA-MB-231 cells to identify the effect, while the sensitivity of MDA-MB-231 cells to 1 mg/L doxorubicin and 8mg/L taxol was measured by methylthiazolyldiphenyl-tetrazolium bromide (MTT). The results demonstrated that mRNA and protein expression level of metadherin both improved after transfection. The inhibition effect of 1 mg/L doxorubicin and 8 mg/L taxol on breast cancer cells decreased after transfection. Detected by flow cytometry, the apoptosis rate of breast cancer cells was $39.68\pm 0.42\%$, $20.64\pm 0.55\%$, respectively, under the effect of 1 mg/L doxorubicin; while under the effect of 8 mg/L taxol, the rate was $24.89\pm 0.41\%$ and $13.8\pm 0.63\%$, respectively. Thus the inhibition effects of 1 mg/L doxorubicin and 8mg/L taxol to breast cancer cells and their effects on apoptosis were different, and the differences were statistically significant ($P<0.05$). Based on the statistics on the expression level of metadherin after transfecting breast cancer cells MDA-MB-231 and the exploration of the sensitivity of the cells to treatment, the effect of metadherin on breast cancer MDA-MB-232 cells was proved.

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*LETTER TO THE EDITOR***EFFICACY OF DIFFERENT RESECTIONS ON NON-MUSCLE-INVASIVE BLADDER CANCER AND ANALYSIS OF THE OPTIMAL SURGICAL METHOD**

GF. CHEN, TP. SHI, BJ. WANG, XY. WANG and Q. ZHANG

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This study aimed to analyze the clinical efficacy of different resections in treating non-muscle-invasive bladder cancer (NMIBC), including partial cystectomy, transurethral resection of bladder tumor (TURBT) and holmium laser resection of bladder tumor. Two hundred and sixteen patients were recruited with NMIBC who were available for follow-up visits in hospital, including 62 cases treated with partial cystectomy, 90 cases treated with TURBT and 64 cases with holmium laser resection. Analysis was made on the cases with tumor relapse in the two years, on operation time, blood loss, time for indwelling urinary catheter, hospital stay and complications after operation. Results were compared to the clinical efficacy of these operation patterns. It was found that the two-year relapse rate for TURBT group, partial cystectomy group and Holmium laser resection group was 41%, 31%, and 33% respectively, and the difference had no statistical significance ($p>0.05$). Both the TURBT group and holmium laser resection group had shorter operation time, hospital stay and time for indwelling urinary catheter as well as much less blood loss when compared with the partial cystectomy group; the difference had statistical significance ($p<0.001$). In terms of complications, the TURBT group was likely to induce obturator nerve reflex and bladder perforation while the partial cystectomy group was likely to induce bladder spasm. Therefore, this study presumes that holmium laser resection and TURBT are much safer and quicker for recovery and obviously superior to the partial cystectomy.

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CORRELATION BETWEEN C-erbB-2 WITH GASTRIC MUCOSAL ATYPICAL HYPERPLASIA AND GASTRIC CARCINOMA

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C-erbB-2 is a cancer gene originating from cells. The high-expression and amplification of C-erbB-2 and its protein products (P185) are found in a wide variety of tumors. The abnormal expression of C-erbB-2 has great influence on the occurrence and development of gastric carcinoma. This paper aimed to analyze the expression of C-erbB-2 in the tissues of gastric carcinoma, gastric mucosal atypical hyperplasia and gastritis, and discuss its role in the occurrence and development of gastric carcinoma. The morphological differences and connections among simple intestinal metaplasia (SIM), atypical intestinal metaplasia (AIM) and dysplasia in intestinal metaplasia through hematoxylin and eosin (HE) staining were studied. Three groups were set to detect the expression condition of C-erbB-2 by immunohistochemical method (IHC). The result showed that C-erbB-2 had no significant difference in AIM and gastric carcinoma, that is, AIM was closely related to gastric carcinoma. The positive expression was demonstrated of C-erbB-2 products (P185) in medium and gastric mucosa dysplasia tissues and was 29.41% and 66.67%, respectively, while it was 25%, 50% and 77.78% in high, medium and low differentiation of gastric carcinoma. It can be seen that there was a significant difference between them ($P < 0.05$), and the expression degree was significantly enhanced ($P < 0.05$); the expression degree in high differentiation gastric cancer tissue was significantly higher than the middle and low differentiation gastric cancer tissue. It was concluded that C-erbB-2 played an important role in the pathogenic mechanism of gastric carcinoma, and it might act on the later period of the gastric carcinoma, which provides objective reference index for the diagnosis and prognosis of gastric carcinoma and meanwhile provides instructional theoretical reference for the application of targeted drugs in the clinical treatment of gastric carcinoma.

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LETTER TO THE EDITOR

**THIN-LAYER CHROMATOGRAPHY IDENTIFICATION FOR RHUBARB AND
PHELLDENDRI AMURENSIS CORTEX IN SHUANG-BAI CATAPLASM AND STUDY
OF SKIN IRRITATION ASSAY**

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Received January 29, 2015 – Accepted April 21, 2015

This paper aimed to raise a thin-layer chromatography (TLC) identification method for rhubarb and Phellodendri Amurensis Cortex and inspected skin irritation induced by them. It applied the TLC identification for Rhubarb and Phellodendri Amurensis Cortex in Shuang-bai cataplasm prescription. In this study six rabbits were divided into two groups to observe the skin irritation from Shuang-bai cataplasm on intact and defected skin. Another 36 were randomly divided into 6 groups to observe the acute toxicity from Shuang-bai cataplasm on intact and defected skin. Also 30 guinea pigs were divided into 3 groups to observe skin allergy to Shuang-bai cataplasm. The results showed that the average weight of the group of intact-skin rabbits was 2.026 ± 0.10 kg and 2.427 ± 0.023 kg after medication; the average weight of the group of defected-skin rabbits was 2.170 ± 0.05 kg and 2.540 ± 0.15 kg after medication; Shuang-bai cataplasm produced no irritation on intact or defected rabbit skin, no acute toxicity in rabbits and no allergy on the skin of guinea pigs. The skin allergy rate on guinea pigs of the medication group was 0 at each time quantum. Therefore, it can be concluded that this preparation produces no extreme skin irritation for rabbits, guinea pigs or human beings, and it can be safely put into practice.

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LETTER TO THE EDITOR

**THE INFLUENCE AND REGULATORY MECHANISM OF Y-BOX BINDING PROTEIN 1
IN OSTEOSARCOMA AND ITS SIGNIFICANCE**W-P. ZHOU¹, X. LIU², Y. YANG³ and Y-F. LIU⁴

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This study quantified the expression of Y-box binding protein 1 (YB-1) by the immunohistochemical method based on pathological paraffin block specimens of aspiration biopsy from patients with osteosarcoma to explore the influence and regulatory mechanism of YB-1 in osteosarcoma and its significance. Patients were divided into two groups with high and low expressed YB-1, and results showed that 7 cases (13.7%) and 18 cases (26.1%) were in level III, and 44 cases (86.3%) and 51 cases (76.9%) were in level IV respectively, and patients with high YB-1 expression quantity had higher malignant tumor degree ($p=0.03$). Moreover, the tumor necrosis rate induced by chemotherapy in the two groups were 21 cases (41.2%) and 38 cases (51.8%), respectively. By survival analysis, it was found that a 5-year overall survival rate of patients with high YB-1 expression and low YB-1 expression were 61.2% and 76.6%, respectively ($p = 0.054$), and 5-year event free survival rates were 52.5% and 72.4%, respectively ($p = 0.033$). Furthermore, metastasis rate of high YB-1 expression and low YB-1 expression were 41.8% and 22.7%, respectively ($p = 0.036$), indicating that patients with high YB-1 expression had higher pulmonary metastasis rate. Through further study, we discovered that possibly miR-382 plays a regulatory role in YB-1 gene in osteosarcoma.

LETTER TO THE EDITOR

GLYOXALASE I A111E, PARAOXONASE 1 Q192R AND L55M POLYMORPHISMS IN ITALIAN PATIENTS WITH SPORADIC CEREBRAL CAVERNOUS MALFORMATIONS: A PILOT STUDY

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It is already known that the conditions of increased oxidative stress are associated to a greater susceptibility to vascular malformations including cerebral cavernous malformations (CCMs). These are vascular lesions of the CNS characterized by abnormally enlarged capillary cavities that can occur sporadically or as a familial autosomal dominant condition with incomplete penetrance and variable clinical expression attributable to mutations in three different genes: *CCM1* (*Krit1*), *CCM2* (*MGC4607*) and *CCM3* (*PDCD10*). Polymorphisms in the genes encoding for enzymes involved in the antioxidant systems such as glyoxalase I (GLO I) and paraoxonase I (PON I) could influence individual susceptibility to the vascular malformations. A single nucleotide polymorphism was identified in the exon 4 of GLO 1 gene that causes an amino acid substitution of Ala for Glu (Ala111Glu). Two common polymorphisms have been described in the coding region of PON1, which lead to glutamine → arginine substitution at 192 (Q192R) and a leucine → methionine substitution at 55 (L55M). The polymorphisms were characterized in 59 patients without mutations in the CCM genes versus 213 healthy controls by PCR/RFLP methods using DNA from lymphocytes. We found that the frequency of patients carrying the GLO1 A/E genotype among the case group (56%) was four-fold higher than among the controls (14.1%). In the cohort of CCM patients, an increase in the frequency of PON192 Q/R genotype was observed (39% in the CCM group versus 3.7% in the healthy controls). Similarly, an increase was observed in the proportion of individuals with the genotype R/R in the disease group (5%) in respect to the normal healthy cohort (0.5%). Finally, the frequency of the PON55 heterozygotes L/M genotype was 29% in patients with CCMs and 4% in the healthy controls. The same trend was observed in PON55 homozygous M/M genotype frequency (CCMs 20% vs controls 10%). The present study aimed to investigate the possible association of GLO1 A111E, PON1 Q192R and L55M polymorphisms with the risk of CCMs. We found that individuals with the GLO1 A/E genotype, PON192/QR-RR genotypes and PON55/LM-MM genotypes had a significantly higher risk of CCMs compared with the other genotypes. However, because CCM is a heterogeneous disease, other additional factors might be involved in the initiation and progression of CCM disease.

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LETTER TO THE EDITOR

**QUALITY OF LIFE FOLLOWING SURGICAL TREATMENT OF LOWER LIMB
METASTASES IN LONG BONE**

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Pathological fractures have a high incidence in musculo-skeletal oncology, and localization in long bone causes severe pain, disability and poor quality of life. The aim of this retrospective case series is to evaluate the clinical results, in particular regarding the quality of life, in patients affected by lower long bone pathological fractures surgically treated. We analyzed 93 patients with pathological fractures of tibia and femur surgically treated in our Orthopaedic Department and followed up for at least 3 years or until their death. Intramedullary nailing or endoprosthetic reconstruction for pathologic fractures located in the metadiaphyseal and diaphyseal or proximal regions in advanced-stage cancer patients are suitable methods for a stable fixation or reconstruction. These approaches guarantee a good mechanical stability, a faster mobilization, a better control of pain with an overall improvement in quality of life in all patients, confirmed also by the trend of the ECOG performance status and QOL-ACD.

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LETTER TO THE EDITOR

POTENTIAL USE OF MELATONIN IN PROCEDURAL ANXIETY AND PAIN IN CHILDREN UNDERGOING BLOOD WITHDRAWAL

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The recognition of the value of pain, especially in the pediatric population, has increased over the last decade. It is known that pain-related anxiety can increase perceived pain intensity. There are several different approaches to the treatment of pre-procedural anxiety and procedural pain in children. Melatonin, a neurohormone with the profile of a novel hypnotic-anaesthetic agent, plays an important role in anxiolysis and analgesia. This study investigated the effects of oral melatonin premedication to reduce anxiety and pain in children having blood samples taken. The investigations were carried out on 60 children, aged 1-14 years, divided into 2 equal groups. Using a computer-generated randomization schedule, patients were given either melatonin orally (0.5 mg/kg BW, max 5 mg) or placebo 30 min before blood draw. Pre-procedural anxiety was assessed using the scale from the Children's Anxiety and Pain Scales, while procedural pain used the Face, Legs, Activity, Cry and Consolability assessment tool for children under the age of 3 years, Faces Pain Scale-Revised for children aged 3-8 years and Numeric Rating Scale for children over the age of 8 years. Oral administration of melatonin before the blood withdrawal procedure significantly reduced both anxiety ($p < 0.0005$) and pain levels than placebo ($p < 0.0002$ for children under 3 years and $p < 0.0039$ for children over 3 years). These data support the use of melatonin for taking blood samples due to its anxiolytic and analgesic properties. Further studies are needed to support the routine use of melatonin to alleviate anxiety and pain in pediatric patients having blood samples taken.

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LETTER TO THE EDITOR

**MIXED DENTITION SPACE ANALYSIS OF A SOUTHERN ITALIAN POPULATION:
NEW REGRESSION EQUATIONS FOR UNERUPTED TEETH**

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Mixed dentition analysis forms a critical aspect of early orthodontic treatment. In fact an accurate space analysis is one of the important criteria in determining whether the treatment plan may involve serial extraction, guidance of eruption, space maintenance, space regaining or just periodic observation of the patients. The aim of the present study was to calculate linear regression equations in mixed dentition space analysis, measuring 230 dental casts mesiodistal tooth widths, obtained from southern Italian patients (118 females, 112 males, mean age 15±3 years). Student's *t*-test or Wilcoxon test for independent and paired samples were used to determine right/left side and male/female differences. On the basis of the sum of the mesiodistal diameters of the 4 mandibular incisors as predictors for the sum of the widths of the canines and premolars in the mandibular mixed dentition, a new linear regression equation was found: $y = 0.613x + 7.294$ ($r = 0.701$) for both genders in a southern Italian population. To better estimate the size of leeway space, a new regression equation was found to calculate the mesiodistal size of the second premolar using the sum of the four mandibular incisors, canine and first premolar as a predictor. The equation is $y = 0.241x + 1.224$ ($r = 0.732$). In conclusion, new regression equations were derived for a southern Italian population.

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