lowing anatomic sites: orbit in 20 (83.4%), and eyelid and conjunctiva in 2 (8.3%) patients each. Three patients had prior or concurrent systemic disease and 21 patients had primary lymphoma (83.3% stage IE, 4.2% stage IIE, and 12.5% stage IV). The main subtypes of non-Hodgkin lymphoma according to the WHO classification were extranodal marginal zone B-cell lymphoma (83.3%), diffuse large cell B-cell lymphoma (8.3%), mantle cell lymphoma (4.2%) and plasmacytoma (4.2%). Six lymphomas were CD43 positive and five of them were extranodal marginal B-cell lymphomas. Local relapse was found in three patients and distant recurrence in four patients. Distant recurrence was found in four patients with stage IE disease (two of them also had a local relapse). In the group of patients with B-EMZL the estimated five-year overall survival rate was 92.9±6.6%, and five-year failure-free survival rate was 80.1±10.3%. Age, gender, side of involvement, anatomic localization of the lesion, clinical stage of disease, and mode of therapy had no prognostic significance during the median follow-up period of 52 (range 9-131) months. The immunohistochemical marker CD43 was the only parameter of prognostic significance (p=0.035). Patients with B-EMZL had almost 14 times higher chance for an unfavorable outcome if the tumor cells expressed CD43 on their surface as compared with the CD43 negative cases. These findings indicate that most ocular adnexal lymphomas usually have a B cell immunophenotype, the morphologic and immunohistochemical features of extranodal marginal zone B-cell lymphoma, and a favorable prognosis. Our data suggest that CD43 could be useful to distinguish the group of patients with B-EMZL with unfavorable prognosis from those that have a good prognosis. CD43 positive ocular lymphomas are associated with a higher rate of subsequent distant recurrence and a risk of lymphoma-related death (p=0.035).

The aim of the study was to define the possible malignant potential of OLR lesions by determining the intensity of c-erbB-2 antigen expression. The study included 30 patients with clinically and histopathologically confirmed diagnosis of OLR. Results were compared with a control group of 15 patients diagnosed with oral leukoplakia, verified as leukoplakia simplex. The aim of the study was to assess the intensity of c-erbB-2 expression in the clinical forms of lichen planus (LP) and lichen planus erosus (LRE), and to compare the antigen expression according to inflammation and degree of hyperkeratosis. The c-erbB-2 antigen was detected by the APAAP and LSAB immunohistochemistry methods after treatment in a microwave oven. The reaction of the study antigen was expressed as mosaic, delicately positive in the spinous layer cells and negative in basal layer cells. The reaction was of a strong intensity in tonofibrils of the spinous layer cells. In the control group, the reaction was uniform and strong in all epithelial layers. There was no difference in the expression intensity between the two clinical forms of oral lichen. The intensity of this antigen expression was independent of the extent of inflammation, but positively correlated with the extent of intralesional hyperkeratosis. It is concluded that such a modified expression of c-erbB-2 antigen in OLR lesions points to an altered nature of these lesions with a potential to undergo malignant transformation.

Proliferative activity of epithelial cells in oral lichen ruber detected by PCNA and Ki-67 antigens

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Proliferative activity of epithelial cells in oral lichen ruber (OLR) lesions may be caused by underlying inflammation following an immune reaction. The aim of the study was to detect PCNA and Ki-67 tissue antigens in correlation with the severity of clinicalpathologic alteration of oral mucosa in OLR, to assess the expression intensity of these antigens in the clinical forms of lichen planus (LP) and lichen planus erosus (LRE), and to compare antigen expression according to inflammation and hyperkeratosis degree. Patients (N=30) with the clinical status

Expression of C-ERBB-2 tissue antigen in patients with oral lichen ruber in correlation with clinical status

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According to WHO criteria, oral lichen ruber (OLR) is a precancerous lesion of oral mucosa.
and histopathologic diagnosis of OLR were selected. The control group included patients with a verified diagnosis of oral leukoplakia. The PCNA and Ki-67 tissue antigens were detected by the APAAP and LSAB immunohistochemistry methods after treatment in a microwave oven. The reaction of study antigens was mosaic-like, intracellular and focal prominent in particular cell groups. PCNA antigen was detected in the basal and parabasal cell layers, and in inflammatory infiltrate of lamina propria. The Ki-67 antigen was detected in basal cells and in some inflammatory cells of lamina propria. The reaction was negative in other epithelial layers. High intensity of PCNA antigen expression was observed in OLR lesions, without any notable difference in the expression intensity between the two clinical forms of the disease. The intensity of PCNA antigen expression positively correlated with the extent of inflammation and intralesional hyperkeratosis. The expression of Ki-67 tissue antigen manifested with mild to moderate reaction. Reaction of greater intensity was observed in erosive lesions of oral lichen. The reaction positively correlated with the extent of inflammation and intralesional hyperkeratosis. Accordingly, the immunohistochemical reaction of PCNA and Ki-67 antigens was found to alter according to the clinical status of OLR patients, and could be related to the modified nature of OLR lesions.

FHIT PROTEIN EXPRESSION IN HUMAN DENTAL CYSTS

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Fragile Histidine Triad (FHIT) has been shown to span the fragile chromosomal site FRA 3b at band 3p14.2. Alterations and deletions within the FHIT gene and concomitant perturbations of FHIT protein expression are strongly linked to the genesis and establishment of human tumors of the lung, cervix, breast, stomach, pancreas, oral cavity, and other tissues. FHIT gene acts as a tumor suppressor, and therapeutic significance of the restoration of its expression has been proposed. The aim of this study was to estimate the expression of the FHIT protein in the epithelial lining of oral cysts by use of immunohistochemistry. An immunohistochemical study was conducted on 21 oral cysts collected for pathohistologic diagnosis after cystectomy and apicectomy. Nineteen of these were inflammatory radicular cysts obtained from 10 male and 9 female patients, age range 14-69 (mean age 40.9) years. Two were developmental odontogenic cysts: 1 keratocyst obtained from a male aged 49, and 1 follicular cyst from a female aged 29. The primary antibody used was rabbit anti-FHIT (Zymed Laboratorires Inc., San Francisco, CA, USA). Seven radicular inflammatory cysts (5 from male and 2 from female patients) showed positive reaction implying normal expression of FHIT. Three radicular inflammatory cysts showed weak positive reaction (all 3 from female patients). The reaction was negative in 9 radicular inflammatory cysts (5 from male and 4 from female patients). Neither of the 2 developmental cysts showed positive reaction. It is concluded that expression of FHIT protein is to a certain extent altered in inflammatory dental cysts. Due to the different origin and nature of developmental cysts, it is possible that the aberration of FHIT protein is even more frequent, however, additional studies in more specimens should be performed to make any firm conclusion.

References

EPSTEIN-BARR VIRUS EXPRESSION IN BREAST CANCER

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Epstein-Barr virus (EBV), a ubiquitous herpes virus, was the first virus shown to cause malignant alterations in humans. Recently, several studies have pointed to the important but controversial role of EBV in the development of breast cancer, reporting on different percentage of EBV positive tumor cells (21%, 32% and 51%). In the present study, we analyzed the frequency of EBV in 44 specimens of invasive breast cancer diagnosed at the Department of Pathology, Sarajevo University School of Medicine. EBV expression was correlated with histopatholog-
ic and clinical data as well with disease-free survival (DFS) and overall survival (OS). For immunohistochemical staining, the EBNA-1 monoclonal antibody (DAKO, Glostrup, Denmark) was used. EBNA-1 scoring system was as follows: 0 – no positive cells; 1 – rare cells positive or staining could be identified with certainty only by using magnification of at least ×200; 2 – staining can be identified by low-power examination, but it is weak; and 3 – staining can be identified by low-power examination, and it is intense. Nine (20.5%) tumors were negative and 35 (79.5%) positive for EBNA-1. Of the positive tumor cells, 8 (18.2%), 14 (31.8%) and 13 (29.5%) tumors showed intensity 1, 2 and 3, respectively. There was no correlation between EBNA-1 expression of EBV and estrogen, progesterone, Bcl-2 and cyclin D1 expression in breast cancer (Mann-Whitney test). There was no statistically significant correlation between age and EBV presence, however, 21 of 22 women (95%) aged <50 and 14 of 22 (63%) women aged >50 were positive for EBV. We recorded a considerably higher expression of EBNA-1 EBV in our biopsy samples compared with data from the available literature. The reason for this is not clear, and more sophisticated and molecular methods should be used to elucidate it.

**CYCLIN D1 IS A USEFUL PROGNOSTIC FACTOR IN BREAST CANCER**

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The aim of the study was to evaluate and compare basic histopathologic data (type, grade, tumor size, lymph node status, mitotic activity) and immunohistochemical markers (estrogen receptor (ER), progesterone receptors (PR), Bcl-2 and Cyclin D1) with relapse-free survival (RFS) and overall survival (OS). Medical records of 52 patients from Central Database of Department of Oncology, Sarajevo University Clinical Center, diagnosed in 1998, were analyzed. The mean follow-up was 58 (range 4-99) months. Routine histopathologic evaluation was performed on 52 formalin fixed and paraffin embedded tumor tissues. For immunohistochemistry ER, PR and Bcl-2 staining with DAKO monoclonal antibodies were used. For cyclin D1 NovoCastra monoclonal antibody was used. Kaplan-Meier test and Cox regression were used on statistical analysis. Patients with smaller tumor size had longer OS and RFS (p=0.003 and p=0.04, Kaplan-Meier test). Tumor grade showed inverse correlation with OS (p=0.006). Patients with four or more positive auxiliary lymph nodes had significantly shorter OS and RFS (p=0.001 and p=0.003). Higher mitotic activity correlated with shorter OS (p=0.003). Higher ER and PR density correlated with longer OS (p=0.04 and p=0.01, respectively). Stronger Bcl-2 expression was associated with longer OS and RFS (p=0.006 and p=0.005). Weaker cyclin D1 expression correlated with longer OS (p=0.02). Cox regression yielded cyclin D1 as the only independent prognostic factor (p=0.05). Although a number of factors are of prognostic significance for OS and RFS, only cyclin D1 was demonstrated to be an independent prognostic factor in this study.

**EVOLUTION OF THE NECK DISSECTION PHYLOSOPHY**


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We followed the evolution of planning and performing neck dissection since the first Crile ‘en block resections’. Many new, more selective methods, the choice of which depends on the tumor site and its metastases, have been developed since the first procedures. There has been strict acceptance of the neck dissection accomplishment according to contemporary protocols for removal of micrometastasis in the 90’s to differentiate it from the 80’s. Due to this, a new methodology of preoperative staging as well as postoperative follow-up and systematized nomenclature have been introduced; the number of elective and bilateral dissections has increased; and the number of elective radiotherapies and postoperative irradiation has decreased. The main purpose of the report is to show the impact of these changes on the number of larynx and neck dissection procedures, and on changes in their interaction during the last two decades. The number of dissections and the choice of the method of dissection depending on tumor site and type of laryngection are discussed. The results showed a significant improvement in the indications, better selectivity, and an increase of the overall number of dissections.
in the second decade compared with the first decade, thus confirming the previously mentioned significant change in the surgical approach to head and neck carcinoma as well as strict acceptance and introduction of the new procedures. Neck dissections performed at University Department of ENT, Sestre milosrdnice University Hospital, from 1982 till 2001, are elaborated, with retrospective analysis of larynx carcinoma.

STRATEGIES FOR TREATMENT OF KELOID AND HYPTERTROPHIC SCARS

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Keloids and hypertrophic scars are consequences of excessive collagen deposition during the wound healing process. The increased number of operations and injuries, widely accepted culture of piercing, and higher esthetic criteria have resulted in a higher interest in the methods of their treatment. Due to the lack of animal models, research of any kind can only be based on clinical experience. A great number of therapeutic options show that no ideal therapy has yet been found. The use of multiple modalities is often necessary to treat the lesions successfully. Molecular, biochemical and clinical features of keloids and hypertrophic scars as well as treatment modalities are discussed.

SECONDARY HYPERPARATHYROIDISM; PARATHYROIDOMATOSIS – CASE REPORT

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Secondary hyperparathyroidism (SHPT) is a disorder most frequently caused by chronic renal failure in its terminal stage. Sometimes it is caused by various disorders of the metabolism of vitamin D, phosphates and calcium. Chronic renal failure leads to phosphate retention, low levels of D3, and parathyroid hormone bone resistance. This leads to hypocalcemia and decrease in the number of calcium and D3 receptors on parathyroid cell surface. In the beginning of the disease parathyroid glands respond with an increased synthesis and excretion of parathyroid hormone. As the disease develops, there is hyperplasia of parathyroid glands, first diffuse, and then nodular in the final stage. Blood levels of calcium, phosphate, intact PTH and alkaline phosphatase are measured for the diagnosis of SHPT. Ultrasound examination of the neck is required to evaluate the stage of the disease and to determine an indication for surgery. In the early stages the treatment is focused on phosphate reduction, phosphate binders and oral vitamin D intake. In the advanced stage of the disease calcitriol pulse therapy, calcium mimetics and vitamin D analogs can be added. In the terminal stage percutaneous inactivation with ethanol or application of calcitriol directly into the parathyroid glands can be tried. As a final solution, parathyroidectomy, subtotal, total or total with autotransplantation can be performed. Parathyroidomatosis is described as multiple nodules of parathyroid tissue scattered through soft tissues of the neck and/or mediastinum. It is believed that parathyroidomatosis is caused by autoimplantation of parathyroid cells during surgery of parathyroid glands. A female patient with chronic renal failure is presented. In 1997, she underwent subtotal parathyroidectomy for advanced SHPT. After 4.5 years free of symptoms, the disease recurred with all findings characteristic of SHPT. On ultrasound examination 8 nodules were found, with extension from the postoperative scar on the neck through soft tissues deeply next to the left thyroid lobe (parathyroid cells proved on cytologic biopsy). Reoperation was performed, when 7 and half nodules were resected.

PERIPHERAL NERVE SHEATH TUMOR OF NASAL SEPTUM – CASE REPORT

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A 62-year-old female patient with an unusual tumor of the posterior nasal septum is presented. The patient complained of difficult and worsening nasal breathing during the last several years. She was a diabetic with appropriate
insulin control of glycemia. Routine nasal examination at the onset of symptoms showed no evidence of nasal pathology. She denied epistaxis, headaches or nasal discharge. As nasal obstruction progressed, another ENT examination showed bulging of the posterior septum that obstructed both nasal cavities but was covered with normal mucosa. CT scan revealed a round tumor occupying posterior septum, measuring 4 cm in diameter, located just in front of the sphenoid rostrum. Biopsy showed the tumor to be composed of myxomatous stroma and numerous vascular spaces. The stroma contained small fusiform to stellate cells without mitotic activity. Initial finding pointed to a ‘hemangiopericytoma-like’ tumor. The tumor was removed endoscopically. Negative margins were confirmed by intraoperative biopsy. Definitive histologic and immunohistochemical analysis (IHA) revealed the same histologic pattern as described earlier. IHA was positive for vimentin, CD31, CD34, GFAP, NSE, S100, CK-pan, and SMA. Differential diagnosis was a ‘hemangiopericytoma-like’ tumor again, however, strong reactivity to S100 suggested a definitive diagnosis of peripheral nerve sheath tumor. Clinical finding of very moderate bleeding during biopsy also supported this diagnosis instead of vascular tumor. Benign peripheral nerve sheath tumors are rare in the head and neck area, with only one case arising in the nasal septum, according to the literature cited in Medline.

LYMPH VESSELS OF HUMAN PARATHYROID

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There is a lack of studies on lymph vessels of the human fetal, postnatal and adult parathyroid gland. Few studies dealing with the above-mentioned topic have produced controversial results. Therefore, the aim of the current study was to investigate the presence of lymph vessels in the human parathyroid gland at various ages. Forty-four human parathyroid glands (patients aged 4-90 years) were divided into three age groups: 4-30 (1), 31-60 (2) and 61-90 (3) years. After standard histologic procedure (fixation dehydration, embedding) tissue samples were serially cut and stained with hematoxylin-eosin and PAS. The slides were analyzed by light microscopy and immersion. The study showed the structure of parathyroid glands of groups 1 and 2 to be quite similar. In both groups the parenchyma of the gland mainly consisted of chief cells with pale stained cytoplasm, whereas the morphology, location and content of lymph vessels were identical. Surprisingly, no lymph vessels were recorded in group 3. The parenchyma of the gland mainly consisted of chief cells with a dark stained cytoplasm. Study results indicated the morphology of human parathyroid changes that could be attributed to different functional status/activity of the gland at a particular age.

EXPRESSION OF INTRATUMORAL MICROVESSEL DENSITY IN DIFFERENTIATED CARCINOMAS OF THYROID GLAND WITH AND WITHOUT METASTASES

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Differentiated carcinoma of the thyroid is considered to be a biologically relatively indolent neoplasm characterized by a favorable outcome following appropriate surgical treatment. However, about 30% of the tumors take an unexpected course and behave in a highly malignant fashion, showing poor prognosis. Therefore, in order to investigate whether intratumoral microvessel density could be used to identify a subgroup of patients with more aggressive behavior of the tumor as potential candidates for radical surgical treatment, adjuvant radiotherapy, antiangiogenic therapy and more intensive clinical follow-up, intratumoral microvessel count (MVC) and intratumoral microvessel density (MVD) were analyzed in 50 localized papillary carcinomas (LPC), 50 papillary carcinomas of the thyroid gland with metastatic involvement of regional lymph nodes (PCMLN), 50 associated metastatic tumors (M), and normal thyroid gland tissue. Also, intratumoral MVD and tumor histologic grade were compared between LPC and PCMLN groups, and the relationship between intratumoral MVD and clinical parameters of age, sex and tumor size was analyzed. The study was carried out by immunohistochemistry on the paraffin embedded material. Formalin fixed, paraffin embedded tissue was cut at 5 mm, deparaffinized and stained with monoclonal antibody to human Von Willebrand Factor H0079 (Factor VIII related antigen, Dako, Denmark) following Microwave Streptavidin Immuno Peroxidase (MSIP) protocol on DAKO Tech-
Mate™ Horizon automated immunostainer. Invasive tumors were often heterogeneous with respect to the amount and distribution of microvessels, therefore sections were examined at low magnifications (x40, x100) to identify the most vascular area of the tumor (‘hot spot’). Within these ‘hot spots’ counting was done in 10 non-overlapping consecutive high power magnification fields (x400/0.144 mm²). The average MVC was calculated from 10 vascular ‘hot spots’, and was also expressed as MVD/mm² of the tumor area. Statistical analysis was performed by the SAS 6.12 and STATISTICA 6.0 statistical package. The level of significance was set at p<0.05 in all cases. A statistically significant difference in MVD was observed between LPC and PCMLN (p<0.001). Mean MVD in LPC was 118.00/mm² as compared with 201.29/mm² in PCMLN. A statistically significant difference was observed in MVD between G1 (low grade) and G2 (high grade) papillary carcinomas (p<0.001). Mean MVD in G1 carcinomas was 132.14/mm² as compared with 208.53/mm² in G2 carcinomas. Finally, our results suggest that intratumoral MVD expression in papillary carcinomas of the thyroid gland may help identify the group of high risk patients with a more aggressive biologic behavior of these tumors, who can benefit from new therapies such as angiogenesis-inhibiting drugs and adjuvant radiotherapy following more extensive surgical treatment, accompanied by careful follow-up. This selective approach may lead to the prevention of unnecessarily aggressive treatments for tumors that are likely to take a benign course and of inappropriate therapy for others with anticipated aggressive behavior display.

PERITUMORAL RETRACTION CLEFTING IN BASAL CELL CARCINOMA OF THE SKIN

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Peritumoral lacunas or peritumoral retraction clefting are frequently observed around buds of basal cell carcinomas (BCC) and appear as a clear halo on hematoxylin-eosin section. They were considered as technical artifacts related to fixation methods. However, they can be observed on frozen sections where there are no similar fixation artifacts. Peritumoral clefting of BCC may be considered as a good example of carcinoma-stroma interaction. The aim of the study was to analyze the presence of peritumoral clefting in different BCC types. The surgical pathology registry at Ljudevit Jurak University Department of Pathology was canvassed for the year 2003 to identify patients with skin BCC. Twenty-nine tumors were selected and further classified according to established histopathologic criteria as solid undifferentiated and adenoid differentiated. All relevant patient data including age, sex and histologic appearance were analyzed. There were 16 patients with adenoid and 13 patients with solid form of BCC. In the group with adenoid form there were eight males and females each, age range 48-89 (mean 67.9) years. In the group with solid form there were eight males and five females, age range 46-81 (mean 65.8) years. Tumors nests with more than 10 adenoid structures were analyzed. We also observed peritumoral clefting present in more or less than 50% of the adenoid structure circumference. All tumors were localized in the head and neck region. In the group of 13 solid BCCs there were three tumors with retraction clefting around complete circumference of the nests. Five tumors had less than 50% and five had more than 50% circumference included in retraction clefting. In the group of 16 adenoid BCC type there were two tumors in which there was clefting retraction around whole adenoid formation circumference. One tumor had less than 50% and thirteen had more than 50% circumference included in clefting retraction. We confirmed the existence of peritumoral retraction clefting in both solid and adenoid types of BCC. Clefting was found to be more pronounced in adenoid BCC type. Obviously, this phenomenon is due to the interaction between tumor cells and stroma, and should be additionally analyzed.

References
OCULOPHARYNGEAL MUSCULAR DYSTROPHY – CASE REPORT

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Oculopharyngeal muscular dystrophy (OPMD) is an adult-onset autosomal dominant disease with a worldwide distribution. It usually presents in the sixth decade with progressive dysphagia, ptosis, and proximal limb weakness. Unique nuclear filament inclusions in skeletal muscle fibers are its pathologic hallmark. It is caused by stable (GCG)8-13 expansions in exon 1 of poly(A)-binding protein 2 gene (PABP2). (GCG)6 represents normal repeat length, while (GCG)7 is a polymorphism that acts as a modifier of the disease severity or as a recessive mutation. More severe phenotypes were observed in compound heterozygotes for the (GCG)9 mutation and (GCG)7 allele that is found in 2% of the population. We report on the histologic study in a 56-year-old woman who had progressive eyelid dropping from the age of 48. She had mild dysphagia, hardly noticed ophthalmoplegia, and severe proximal limb girdle weakness that had started around the age of 56 when she had been hospitalized and found to have a myogenic pattern of EMG, high serum level of creatine kinase and stenocardia. Muscle biopsy was performed. The biopsy specimen was examined by light and electron microscopy. Tissue samples for light microscopy were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin. Electron microscopy samples were prepared according to standard procedure. Light microscopy showed normal arrangement and different thickness of muscle fibers. Examination of semi-thin sections showed the presence of a clear zone in some muscle fiber nuclei (intranuclear inclusions). On electron microscopy, these inclusions were made of tubular filaments arranged in tangles or palisades. The filaments were seen in muscle fibers but not in the cytoplasm or other cells found in the samples. The muscle fibers had otherwise normal ultrastructure. The intranuclear inclusions are specific for oculopharyngeal muscular dystrophy. Several years later DNA testing from peripheral blood has now replaced this approach. The test is reliable, can be done in Zagreb, and permits accurate genetic counseling.

References

HYALINIZING SPINDLE CELL TUMOR WITH GIANT ROSETTES OF THE UTERUS – CASE REPORT

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A case of a hyalinizing spindle cell tumor of the uterus with giant rosettes (HSTGR) is presented. To our knowledge, this is the first case of HSTGR described in the Croatian medical literature. A 38-year-old woman presented with painless uterus enlargement on routine gynecologic examination. Preoperative CT, ultrasound and radiographic examinations did not reveal any distant metastases. The patient underwent total hysterectomy and bilateral adnexectomy with selective pelvic and para-aortal lymphadenectomy. No complications occurred postoperatively. No adjuvant treatment was performed. The resected enlarged uterus measured 20x18x12 cm. Serial slicing of the uterus revealed a tumor mass of 15 cm in maximum length. The margins of the tumor merged with the uterine wall and the tumor infiltrated more than one half of the myometrium. Pathohistologic analysis confirmed the diagnosis of HSTGR. The tumor consisted of spindle stroma with predominating picture of large collagen nodules surrounded in rosette-like fashion by tumor cells in axial array, which in some areas coalesced into long serpiginous cords of dense hyalinization. Spindled stroma of varied cellularity consisted of fibroblastic cells that formed a storiform pattern in the hypocellular hyalinized or myxoid areas. Immunohistochemical staining confirmed the biphasic pattern of HSTGR; stromal reaction was positive for vimentin, NSE, SMA, desmin, but negative for S-100,
CD34, SA and cytokeratin, while the rosettes showed negative reaction with all performed staining. HSTGR was originally described in 1997 by Lane et al. At least morphologically it is closely related to low-grade fibromyxoid sarcoma. Although considered to be a tumor of low malignant potential, since the initial report a few cases with the development of pulmonary metastases have been described. At least one of them was associated with longterm survival. Fifteen months after operation, our patient is feeling well with no signs of recurrence. Extended follow-up will be necessary to rule out the development of local recurrence as well as metastatic disease.

References

PURE LIPOMA OF THE UTERUS – A VERY RARE ENTITY
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Introduction
Pure lipoma is a very rare benign tumor of the uterus which commonly occurs in postmenopausal women. In the literature, lipoleiomyomas are most often described, however, these were the ones that do not represent a particular entity but a combination of two different tumors having different histogenesis. Several cases of pure uterine lipoma in the presence of endometrial carcinoma have been reported. The histogenesis of pure uterine lipoma has not yet been fully clarified. Previously, these tumors also used to be considered as hamartomas or choristomas, however, this opinion has been abandoned. Recently, the origin of these tumors has been explained by chromosomal aberration at the molecular level. Cytogenetics of the uterine lipoma has pointed to chromosomal abnormality in the zones responsible for the control of cellular proliferation. Translocations of different genes, which become susceptible to aberration at several locations, were found in benign tumors of the mesenchymal origin, e.g., leiomyoma, pleomorphic adenoma, lipoma and chondrogenic pulmonary hamartoma. However, aberrant expression of HMGA2 protein should occur due to dysfunction of Tsc2 tumor suppressor gene in those cases in which structural abnormality is not present. Although rare, these tumors may pose a problem on the differential diagnosis versus other uterine mesenchymal tumors. Clinical symptoms and physical signs are similar to those in leiomyoma.

On gross examination, these tumors are yellowish in color, well confined, often presenting as a small node which is situated within the muscular layer. Histologically, the tumor is composed of lobules of mature lipid cells, which are divided by delicate soft tissue in which blood vessels of capillary type are often seen.

Case Report
An 85-year-old woman was admitted to the University Department of Gynecology and Obstetrics, Sestre milosrdnice University Hospital, for surgical removal of a left ovary tumor. A 2-cm large, round tumor with regular ultrasound flow on the left side of the uterus was clinically verified. Total hysterectomy and bilateral adnexectomy were performed, and a large cystic tumor mass of bluish color and smooth surface, which occupied the entire Douglas’ pouch, was found in the left ovary.

Histologically, a well-circumscribed, yellowish, soft, intramural node measuring 5 mm was incidentally found in the uterine corpus. The tumor was lobulated and composed of mature lipid cells, among them narrow stripes of loose connective tissue were seen. A diffuse adenomyosis was also found. Endometrium was fibrocystic and atrophic. An empty cystic formation filled with blood measuring 7x8 cm was found in the right ovary, which was microscopically verified as an endometrial hemorrhagic cyst. The right fallopian tube was 7 cm long and was attached to the uterus, with histologic signs of chronic inflammation. The left adnexa were normal. Tumor cells showed an intensive positive Sudan B reaction. Immunohistochemical expression of S-100 antigen was prominent in tumor cells.

Conclusion
Pure lipoma is an extremely rare benign tumor of the uterus, which is of a mesenchymal origin. Usually, it is an incidental finding in postmenopausal women. Pure lipo-
ma may present a problem on differential diagnosis of uterine tumors.

References


INFLAMMATORY PSEUDOTUMOR OF THE CERVIX – CASE REPORT

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Inflammatory myofibroblastic tumor, synonymously referred to as inflammatory pseudotumor (IPT), has become an almost ubiquitous non-neoplastic tumorous condition, most frequently reported in the lungs, however, no other anatomic site can be excluded. Recent literature emphasizes a frequent involvement of pediatric cases. Only seven cases of IPT have been reported in the uterus. To the best of our knowledge, we herein report on the histopathologic and immunohistochemical findings in the second case of cervical IPT in the literature. In December 2003, an 18-year-old woman presented to our hospital because of dysfunctional bleeding. Gynecologic examination revealed an incidental finding of a solitary leiomyoma-like mass measuring 4.2 and 3.8 cm in maximum diameter, originating from the cervix. Four months after surgical excision of the tumor the patient was feeling well with no signs of recurrence. A distinctive mesenchymal lesion composed of spindle cells, displaying morphological features of myofibroblasts admixed with considerable numbers of inflammatory cells, was found on histopathologic analysis of the tumor. Immunohistochemical staining was negative for SMA and positive for CD68, confirming the diagnosis of IPT. Although extremely rare at this location, IPT should be taken in consideration if differential diagnosis of mesenchymal malignant lesion or other non-neoplastic condition is questioned. No history of trauma or recent surgical procedure, absence of Michaelis Gutman bodies, and negative special stains for microorganisms, together with distinctive histologic appearance of the tumor, should ease the confirmation of IPT.

References


SECOND PRIMARY MALIGNANT TUMORS IN PATIENTS WITH PRIMARY COLORECTAL ADENOCARCINOMA

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The aim of the study was to determine the frequency and types of second primary malignancy in patients with primary colorectal adenocarcinoma. A total of 2035 (1200 male and 835 female) patients with colorectal carcinoma were analyzed. Data were obtained from the computer based colorectal cancer registry at Ljudevit Jurak University Department of Pathology for the 1995-2003 period. The database contains data on each patient including pathohistologic diagnosis. The following parameters were analyzed: sex and age distribution, and localization of second primary malignant tumors in patients with primary colorectal carcinoma. There were 107/2035 (5.3%) patients with second primary malignancy, 59 (3%) men and 48 (2.3%) women. Second primary malignancies in descending order of frequency were: stomach (16.5%), prostate (13.9%), skin (13%), urinary bladder (11.3%), and kidney cancer (6.9%). Ninety-eight (91.5%) patients had only one second malignancy, whereas nine (8.5%) patients had more than one second malignancy. It is concluded that histopathologic type and location of second primary malignancy can be of great importance for patients, their physicians and pathologist. In this series the frequency of secondary malignancies was much higher than expected, reaching up to 5% of patients. Had we excluded skin cancers from the analysis, the frequency of second primary malignancies...
would probably be lower, but data should be checked at the National Cancer Registry.

References


CHANGES IN UROPATHOLOGIC FINDINGS AT A 20-YEAR DISTANCE

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During the last 20 years, the occurrence of some diseases has changed due to a different way of living and new diagnostic and treatment possibilities. Diagnostic imaging (CT, MR and especially ultrasound) has increased the rate of identification of renal tumors, while there has not been any systematic screening for urologic diseases. Department of Urology is a referral center for prostate diseases since 1994. The procedure of needle core biopsy was introduced two years later and its utilization has increased significantly over the last few years. In the present study, the occurrence of some urologic diseases was compared at 20 years apart using the histopathologic database for two-year periods. Data on all patients who underwent biopsies at Department of Urology during the 1980-1981 and 2000-2001 periods were included in the study. In the 1980/81 period, tissue samples of 25,117 patients were analyzed at Department of Pathology, 1070 (4.3%) samples being obtained from urologic patients. In the 2000/01 period, there were 27,720 patients, 2,233 (8%) of them from Department of Urology. Urogenital tract tuberculosis was found in 13 (1.2%) patients during the 1980/81 period, and in only two (0.1%) patients in the 2000/01 period. In the 1980/81 and 2000/01 periods, there were 13 (12%) and 29 (45%) tumors of the testis in the 1980/01 and 2000/01 periods, respectively. Seminoma accounted for 30% (n=4) and 55% (n=16) of all germ cell tumors in the 1980/81 and 2000/01 periods, respectively. Of the total number of urology biopsies performed in 1980/81 and 2000/01, there were 416 (38.8%) and 963 (43%) prostate biopsies, respectively, 59 (14%) of them carcinomas in 1980/81 and 222 (23%) carcinomas in 2000/01. An increasing number of prostate biopsies was recorded in the 2000/01 period, mainly due to the large proportion of needle core biopsies (304 of 966 prostatic biopsies). Prostatic biopsies accounted for 40% of all urologic biopsies in 2000 and for 46% in 2001. On the basis of this study it is concluded that the total number of urologic biopsies increased 1.9-fold comparing the first (1980-1981) and second (2000-2001) period. There was a significant increase in the number of renal cell carcinoma (3.8-fold) and urothelial carcinoma of the pyelon (8.4-fold). The total number of testicular biopsies decreased, whereas the number of testicular tumors and seminomas increased. Tuberculosis of the urogenital system decreased 13-fold.

References


PERIACINAR RETRACTION CLEFTING AND P63 IMMUNOSTAINING IN PROSTATIC CARCINOMA

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The diagnosis of prostate carcinoma is based on three major histologic criteria: the infiltrative growth pattern, the absence of basal cell layer, and the presence of macronucleoli. Basal cells are invariably absent from the malignant
glands of prostate cancer. The ability of immunohistochemical staining to detect basal cells has proven to be diagnostically invaluable, especially in needle biopsy specimens. P63, a homolog of the tumor suppressor gene p53, has been shown in the basal cell component of epithelium from a variety of tissues, including prostate epithelium. One of the criteria favoring cancer is the presence of retraction clefting around neoplastic glands that is probably connected to the lack of basal cells. Therefore, the aim of this study was to correlate the presence and extent of retraction clefting and the expression of p63 in neoplastic glands in needle core biopsies. Fourteen cases with prostate carcinoma diagnosed on the basis of major and favoring criteria at Department of Pathology, Sestre milosrdnice University Hospital, were chosen for the study. The patients were aged 64-80 (mean 68.8) years. They underwent sextant biopsy after having an increased PSA serum value. In all cases retraction clefting was also described in biopsy findings. Immunohistochemical staining was performed following the Microwave Streptavidin ImmunoPeroxidase (MSIP) protocol on a DAKO TechMate Horizon automated immunostainer using antibodies to p63. Retraction clefting was observed in all 14 cases; in 8 (57.2%) it affected more, and in 6 (42.8%) less than 50% of the gland circumference. p63 immunostaining was negative in all carcinoma cases but positive in adjacent normal glands. Our results strongly suggested a connection between the lack of basal cells in neoplastic glands and the development of retraction artifacts. The results also showed invariably negative staining for p63 in all cases with periacinar retraction artifacts. We conclude that the clefts represent a reliable diagnostic criterion and that staining for p63 might be useful when the clefts affect less than 50% of the gland circumference or are not present at all.

References

NEGLECTED INFANT OR INFECTIVE DISEASE

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A rare case of infant death caused by an infective disease (probably congenital toxoplasmosis), which was discovered on forensic autopsy at Department of Pathology and Forensic Medicine, Osijek University Hospital (CHO) is presented. Autopsy was performed upon notification from the CHO Department of Pediatrics, characterizing it a case of criminal offence of child neglect and abuse. The offender was the child’s mother, currently in her fifth marriage with altogether 10 children. By court ruling she lost her parental right over 9 children for neglect and abuse, and was sentenced to one-year term of imprisonment. The tenth child was born from uncontrolled pregnancy on February 17, 2002. Thus, with social and medical supervision and with no reported signs of neglect, the child was admitted at the age of 70 days for hospital treatment for elevated temperature, dehydration, hypotrophy, convulsions, and progressing coma vigil. During hospital stay, doubts were raised of the possible child neglect and abuse, and judicial proceeding was started. Forensic autopsy, histopathologic findings and serology tests indicated that the cause of death was a congenital infectious disease, on the basis of which the mother was acquitted.

THE IMPORTANCE OF IMPLEMENTATION OF THE FIVE-TIER WHO CLASSIFICATION OF PITUITARY ADENOMAS

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Great changes have taken place in the basic knowledge of pituitary adenomas during the last decade, with a number of different classifications of pituitary adenomas proposed. In 2000, World Health Organization accepted the five-tier classification of pituitary adenomas proposed by Kovacs and Horvath. It is based on clinical and biochemical results, neuroradiologic imaging, operative findings, histology, immunocytochemistry and electron microscopy.
studies on more than 10000 surgically treated pituitary adenomas. Its importance is that it supplies the endocrinologist, neurosurgeon and oncologist with valuable information concerning the biologic behavior, growth potential, treatment response and prognosis of pituitary adenomas. Due to financial restraints, lack of facilities and unavailability of well trained personnel, this five-tier classification cannot be implemented in all institutions. Nevertheless, clinical, biochemical, neuroradiologic, operative, histologic and immunohistochemical data are generally available. Together with the novel biologic techniques that provide data on tumor growth rate, aggressiveness and invasiveness, they are necessary in establishing correct diagnosis which will direct the patient’s future treatment.

We strongly advocate this five-tier classification of pituitary neoplasms and hope it will find full implementation.

References


TISSUE TYPING OF HLA GENES, ANTIGENS, AND ANTI-HLA ANTIBODY SCREENING IN TISSUE AND ORGAN TRANSPLANTATION

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It is well established that transplantation is the method of choice in organ failure and to treat hematologic disorders and malignancies. In organ transplantation, organ donors can be relatives or cadavers. Bone marrow transplantation prefers HLA identical siblings, and now the use of unrelated bone marrow donors is in progress. However, the first condition for selecting a donor is ABO compatibility, which is followed by matching in major histocompatibility antigens. HLA antigens are detected by standard complement-dependent-cytotoxic assay. The same assay is used to determine anti-HLA antibodies in sera of patients waiting for transplantation. In combination with good matching, the pretransplant cross-match reaction between donor cells and recipient sera must be negative. Special condition is detection of autoanti-HLA antibodies. With all criteria fulfilled it is reasonable to expect a higher surviving rate and good graft function.

COMPARATIVE EVALUATION OF THE MYCOBACTERIUM GROWTH INDICATOR TUBE (MGIT) WITH SOLID MEDIUM FOR ISOLATION OF MYCOBACTERIUM

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Because mycobacteria are slow growing and require long incubation times, appropriate decontamination procedures, culture media, and conditions of incubation must be selected to facilitate optimal recovery from clinical specimens. The objective of the study was to evaluate isolation rates and time to detection of mycobacteria in clinical specimens using both BBL Mycobacterium Growth Indicator Tubes (MGIT) and Lowenstein-Jensen (LJ) medium as a reference method. Over a period of 2 years a total of 743 clinical samples were treated by N-acetylcysteine-NaOH method for decontamination and fluidification. Direct examinations were performed using Ziehl-Nielsen staining. For each sample, aliquots of 0.5 ml were inoculated onto Lowenstein-Jensen and to MGIT. Cultures were inoculated at 37 °C and daily observed for 2 months. It appears clearly that MGIT are a sensitive liquid medium containing a fluorescent sensor that allows for early detection of mycobacterial growth.

GIEMSA STAINING: METHOD OF CHOICE TO DETECT HELICOBACTER PYLORI IN CHRONIC GASTRITIS

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The bacterium Helicobacter(H.) pylori provokes gastritis and has also been associated with the possibility of cancer development. It is usually located on the mucous membrane of the stomach and in the area of gastric fave-
The dye is used on formalin-fixed, paraffin-embedded tissue sections. Before tissue section staining, it is extremely important that the tissue of the stomach mucous membrane is correctly oriented in paraffin and the slice thickness is 4-6 m. In this way, one layer of the gastric mucosa cells is obtained, with \textit{H. pylori} clearly visible if present.

There are several methods to detect \textit{H. pylori} in gastric mucosa of patients with chronic active gastritis and chronic gastritis: KWIK DIFF stain kit – Shandon kit; Warthin-Starry staining – silver method; Giemsa staining – Merck dye; and LSAB+/System HRP/DAB-IHC method for \textit{H. pylori} (Vissulation – LSAB+/HRP-DAB). Giemsa staining has a number of advantages: it is a quick, simple to perform, and differentiating method allowing for \textit{H. pylori} to be easily observed within glandular epithelium of the gastric mucosa biopsy specimen. And the last but not the least, because of its simple performance and favorable price it is widely applicable in laboratories with less sophisticated equipment. However, Giemsa staining also suffers from some drawbacks, as follows: the Giemsa solution has to be filtered each time before use. Otherwise, a sediment is formed, so stain artifacts may cover \textit{H. pylori}. The Giemsa working solution should be fresh. The acetate buffer should also be fresh, because the prepared tissue section has to be rinsed properly to ensure accurate diagnosis. Furthermore, the immersion time is also important for optimal staining of \textit{H. pylori}. If the immersion time is too long, it is not possible to differentiate \textit{H. pylori} due to vivid purple color of the whole tissue section. If the immersion time is too short, it is not possible to differentiate \textit{H. pylori} because it is pale whereas the whole tissue section is light blue. The following results are obtained: nuclei – blue; cytoplasm, connective tissue – pink; erythrocytes – salmon colored; bacteria – blue to violet. Accordingly, Giemsa staining can be recommended as a fast, reliable and inexpensive method in the routine diagnosis of \textit{H. pylori} in chronic active gastritis and chronic gastritis. According to our longlasting experience, a small series should be made (multiple cuts on the same glass). Furthermore, it is important to prepare an unstained cut for further studies, primarily IHC analysis of the mucus in the focal areas of a possible intestinal metaplasia. On gastric biopsy from different areas (antrum, pylorus, corpus), each specimen should be labeled in separate to make a precise diagnosis and to determine the location of \textit{H. pylori}.

**QUANTITY AND QUALITY OF IMMUNOHISTOCHEMICAL ANALYSIS BEFORE AND AFTER APPLICATION OF DAKO TECHMATE AUTOMATED IMMUNOSTAINER**

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Technical advances that have supervened in the recent years are responsible for immunohistochemistry becoming a staple of the histopathology laboratory. The aim of the study was to analyze the period before (1997-2000) and after (2001-2002) the introduction of automated immunostainer in daily routine to reveal the potential advantages/disadvantages in comparison with standard manual immunohistochemical procedures. The study was carried out on a DAKO TechMate™ Horizon automated immunostainer, which has a capacity of up to 40 slides that can be used with up to 20 different primary antibodies, providing 12 staining protocols and additional 20 custom made protocols. The number and quality of slides, duration of staining procedure, and possible reduction in reagent utilization were compared. There was a significant increase in the number of observed slides between the two periods of observation, from 1507 to 2687 per year. The number of applied antibodies increased from 30 to 81. The time required for the procedure was reduced and standardization more easily achieved. Owing to the mentioned advancements, there was more time and personnel left for other activities including educational and scientific projects using immunohistochemical analysis.

**DIAGNOSIS OF UROGENITAL TRICHOMONIASIS BY \textit{TRICHOMONAS VAGINALIS} CULTIVATION – OUR TEN-YEAR EXPERIENCE**

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The value of our own modification of axenic cultivation of \textit{Trichomonas (T.) vaginalis} on Diamond medium with the addition of nystatin was tested in a total of 15917 samples from patients with chronic disorders, including 12365 vaginal swabs, 2158 ejaculates collected over a 10-year period, and 1394 expressed prostate secretions collected.
over a 3-year period. Analysis of the results obtained by cultivation according to days of incubation for 24, 48 and 72 hours revealed 1036 (8.38%) vaginal swabs, 264 (12.23%) ejaculates and 90 (6.45%) expressed prostate secretions to showed the growth of *T. vaginalis*. Besides native slide microscopy, the fastest method used in laboratory diagnosis of trichomoniasis, a negative finding yet requires completion of the diagnostic procedure by cultivation for the result to be considered definitive. Comparison with clinical observations showed cultivation to be a more appropriate and efficient method. Analysis of the results recorded during the period of 10 years showed the identification by cultivation to be three times superior to the native slide microscopy identification. The increased incidence of positive cultures during spring and fall in patients of both sexes could be explained by biologic and socioethologic patterns, and calls for additional studies.

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**CEREBROSPINAL FLUID IN PATIENTS WITH AIDS**

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The aim of the paper is to present cytomorphological changes in the cerebrospinal fluid (CSF) of patients with the acquired immunodeficiency syndrome (AIDS). CSF cell count was done in a Fuchs-Rosenthal chamber and sedimentation in a Cytospin 3 (Shandan) at 700 rpm for 5 min, then the sediment was stained by the May-Grünwald-Giemsa (MGG) method. Cytomorphological analysis was done on an Olympus (1000 X) optical microscope. In general, pleocytosis in the CSF of patients with neuro-AIDS was low or even normal (5-150 ccm). According to our observation, the cytomorphological changes in CSF were not characteristic. Mononuclear cells, which predominated, were lymphocytes and monocytes with a small number of their reactive forms and different types of particular phagocytes. Very often, some erythrocytes could be found. Cryptococcal meningitis is a frequent secondary infection of the central nervous system in AIDS patients, and this yeast can be seen in the CFS sediment stained by MGG as well as in the Indian ink preparation. The number of yeast has to be counted in a FR chamber. Low mononuclear pleocytosis with frequent findings of cryptococci very reliably leads to the diagnosis of neuro-AIDS.