FREE PAPERS - MODERN PATHOLOGY

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Giant axonal neuropathy: Case report and two novel mutations in the gigaxonic gene

Hereditary neuropathy is clinically and genetically heterogeneous group of disease. Giant axonal neuropathy (GAN) corresponds to a generalized disorganization of the cytoskeletal intermediate filaments. Nerve fibers are distorted by giant axonal swellings, filled with densely packed bundles of neurofilaments. Myelin sheaths were thin or absent around swellings. Size of nerve fibers average 20 μm up to 50 μm. GAN appears to be an autosomal recessive disorder. GAN gene, located in chromosome 16q24, encodes a novel, ubiquitously expressed protein - gigaxonin. Gigaxonin plays an important role in the cross talk between the IF and microtubule (MT) networks. Mutations in the gigaxonin gene have been identified as the underlying genetic defect. Our patient with GAN has two novel missense mutations, Tyr89Cys in exon 2 and Gly368Arg in exon 7 (Nelis et al. in press).

KEYWORDS: Heredodegenerative Disorders, Nervous System; Nerve Fibers; Axons; Cytoskeletal Proteins; Mutation, Missense

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Study of p53 in patients with CML by immunohistochemistry and DNA analysis

Chronic myelogenous leukemia (CML) is a clonal myeloproliferative disorder of the hematopoietic stem cell. In the initial chronic phase, myeloid progenitors and mature cells accumulate in the blood and extramedullary tissue. After 3-4 years, the disease transforms and terminates in a blast crisis characterized by a maturation arrest in the myeloid or lymphoid lineage. The molecular mechanisms responsible for progression of diseases have not been well defined but blast crisis may be partially related to inactivation of tumor suppressor gene such as p53. Mutation and/or deletion of the p53 gene have been reported to be associated with disease progression in a wide variety of human cancer, including adult-type CML. Molecular alterations of p53 gene were investigated in 25 patients with CML (19 patients in chronic phase and six at blast crisis). We analyzed mut p53 expression using the monoclonal antibodies by immuno-alkaline phosphatase (APAAP) techniques. DNA structures of exon 5-8 of the p53 gene were scanned by PCR-SSCP (single strand conformation polymorphism analysis of polymerase chain reaction products). We did not found structurally altered genes in either the chronic phase or blast crisis. Our study suggested that p53 protein alterations have not an important role in pathogenesis of CML.

KEYWORDS: Leukemia, Myeloid, Chronic; Genes, p53; Gene Expression; Immunohistochemistry

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p53 gene mutations and human papillomavirus in oral squamous cell carcinomas: Correlation with clinical and histopathological findings

Squamous cell carcinoma of the head and neck is the sixth most common human malignancy, although it only accounts for 2% of all cancers in Western population. The incidence of head and neck cancer in particular tumors of the oral cavity and larynx, are increasing in developed countries, with the increase of risk being seen in younger people. The incidence of oral cancer is variable from region to region and the highest rates are seen in India, Sri Lanka, Hong Kong and Taiwan. Oral squamous carcinogenesis is a multistep process in which multiple genetic events occur that alter the normal functions of oncogenes and tumor suppressor genes. The most frequent genetic change in human cancer is ocurring on the short arm of chromosome 17 (17p) in the region that contains the TP53 gene. The TP53 is a tumor suppressor gene, which arrests the cell cycle at the late G1-phase in the cells with DNA damage, on that way allowing DNA repair or apoptosis. Mutations in p53 tumor suppressor gene have been suggested to play a significant role in the development of various epithelial carcinomas and their response to therapy. High-risk human papillomavirus (HPV) is known to be tumorigenic in human epithelial tissues. Over 120 types of HPV have been identified. HPV types are often described as high-risk (oncogenic) or low-risk (nononcogenic). Two viral oncoproteins of high-risk HPVs, E6 and E7, promote tumor progression by inactivating the TP53 and retinoblastoma tumor suppressor gene products, respectively. Genetic changes are studied to determine the biologic of oral squamous cell carcinomas OSCC. The tumor is characterized by poor prognosis despite great efforts made in the field of prevention, early diagnosis and treatment, as well as in the field identification of new markers. The aim of the study was to investigate the incidence of p53 mutations and infections of HPV in patients with OSCC of the tongue and floor of the mouth and their correlation with clinical and histopathological findings. Thirty-two primary tumors (64%) involved the tongue, 17 (34%) the tongue with floor of the mouth, and 1 (2%) the floor of the mouth. Median age was 55.4 years (range 43-80 years). The clinical T- and N-categories (UICC 2002) were: T1 (5), T2 (38), T3 (7), N0 (6) and N1 (44). The tumors were histologically graded (histological grade G1-G3 and nuclear grade NG1-NG3). We analyzed the presence of HPV infection (n=50), as well as p53 gene mutations (n=42) by PCR-SSCP method in the specimens of OSCC tumors. TP53 mutations (exons 4-8) were identified in 11/42 (26%) patients' tumors. HPV infection was detected in 32/50 (64%) - 10 patients were infected with HPV16 (31.2%), 6 with HPV 18 and HPV 31 (18.7%) and none with HPV 33. Distribution of p53 status and HPV infection within host tumor characteristics showed that p53 mutation was detected in 9/16 (56%) tumors of histological grade I (G1). This incidence was significantly higher then those found for tumors with grade II (G2) and III (G3). Tumors with the highest nuclear grade had significantly highest incidence of p53 mutations. Our results showed significantly higher incidence of p53 mutations in the tumors with histological grade I. Concerning nuclear grade, on the contrary the highest incidence of p53 mutations was found in the grade III tumors. So, it is questionable if nuclear grade better describes tumor proliferative ability than the histological one. The finding of the scattering of p53 mutations within histological grade I (9/11 cases) can indicate that p53 mutations are early event in the OSCC carcinogenesis.

KEYWORDS: Gene, p53; Mutation; Papillomavirus, Human; Carcinoma, Squamous Cell; Mouth Neoplasms

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Early detection of K-*ras* mutations in lung cancer

Lung cancer is the leading cause of cancer related deaths in all western countries, and newly diagnosed lung cancer patients in our population are being increased during last decade. Since only 25-40% of all lung tumors are considered resectable at the time of initial assessment, problem of early diagnosis is one of the biggest. The goal of our study was to determine whether molecular genetic analysis of K- and H-*ras* gene mutations in bronchoalveolar aspirate could help in the early diagnosis, and also to asses the relation between the gene alterations and cytogenetic findings. Alterations in codons 12 and 13 of K- and H-*ras* genes in bronchoaloveolar aspirate of 53 patients were examined by PCR-SSCP method. The same samples were examined by conventional cytological analysis. 40/53 pts were in advanced stage of non-small cell lung cancer (NSCLC, IIIA, IIIB and IV stage), 11/53 with no-malignant disease and 2/53 with small cell carcinoma. In our study, mutations of K-*ras* oncogenes were detected in 16/42 patients with malignant disease (38.1%) and in 2/11 patients with benign non-specific inflammation. High percentage of K-ras mutations was found in squamocellular carcinoma (68%). Also, 92% of detected mutations were in active smokers (>35 cigarettes per day). In 3/40 (7.5%) patients mutations in H-*ras* oncogenes were detected. Correlation between cytological and PCR-SSCP results were found in 34 of 40 analyzed patients. Mutation in exon I of K- and H-*ras* oncogenes could be an additional criteria for early diagnosis of NSCLC. High incidence of ras gene mutations in NSCLC has already been reported in western populations, but there are no relevant studies for significance of ras oncogene mutations in early diagnosis and prognosis for our population, indicating that further studies in our population should be continued.

KEYWORDS: Lung Neoplasms; Genes, ras; Mutation; Bronchoalveolar Lavage; Polymerase Chain Reaction; Polymorphism; Single-Stranded Conformational

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Vascular endothelial growth factor and K-*ras* mutation in colorectal carcinoma

Neovascularization is one of the main features that enable neoplastic progression of colorectal carcinoma (CC) and the subsequent occurrence of metastases. For instance, microvessel count (MVC) has proven to be a strong predictive marker for the risk of metastatic disease in colorectal cancer. In this study we investigated the prognostic significance of vascular endothelial growth factor (VEGF) expression and microvessel count (MVC) at the deepest invasive site in colorectal carcinoma tissue as well as their relationship with K-ras mutation in order to asses their clinical and therapeutic implications. Formalin fixed paraffin-embedded tumor tissue of 81 surgically removed colorectal carcinomas (21- Duke's stage A, 24- stage B and 36- stage C+D) were studied by immunohistochemical staining methods for detection of VEGF and CD31 positive microvessels, as wall as by polymerase chain reaction (PCR) in order to analyze mutations in codons 12 and 13 of the K-ras gene. The type of mutation was identified by autoradiography. MVC was expressed as mean of the microvessels counts per field obtained in 10 fields at magnification x200. VEGF assessment and semiquantitative analysis was performed according to a method described by Mulcahy et al. Tumor sections with grade 3 or 4 were judged as positive. Median follow up was 31.4 month (range, 2-66 months). VEGF expression at the deepest invasive site of the tumor was found in 66.7% of CC. It was significantly more frequent in Duke's stage C and tumors with high histological grade (80.5% and 82.2% respectively) than in Dukes without lymphonodal metastasis and low-grade tumors (55.5% for stages A and B and 47.2% for low grade tumors). High MVC were significantly more frequent in VEGF positive tumors (64.8%) comparing with VEGF negative tumors (25.9%). The overall frequency of K-*ras* gene mutations was 43.2%, including two patients with double K-*ras* gene mutations. The frequency of mutations was significantly higher in Dukes stage C+D comparing with Dukes stages A and B (45.7% in stage C vs. 20% in stage A and 34.3% in stage B). The K-*ras* mutation was present in 55.6% VEGF positive tumors and only in 11.1% VEGF negative tumors ($p<0.001$). Survival analysis showed that VEGF expression and the presence of K-*ras* mutation as well as hypervascularity of colorectal carcinomas correlated with poor survival ($p\leq0.01$). Our findings suggest the presence of K-*ras*-VEGF pathway regulating tumor angiogenesis in human CC. VEGF analysis may be used in a clinical setting to identify patients at high risk for relapse who may benefit from adjuvant treatment including new therapeutic strategies such as monoclonal antibody neutralizing VEGF.

KEYWORDS: Colorectal Neoplasms; Endothelial Growth Factors; Genes, ras; Angiogenesis Factor; Neovascularization, Pathologic

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Gene alterations in patients with lymphoproliferative disorders

Our aim was to study gene alterations in patients with lymphoproliferative disorders and to assess diagnostic significance of these findings. Formalin-fixed and paraffin-embedded tissue from 34 patients with large cell B-NHL, 6 with chronic lymphadenitis (LC), 17 with cutaneous T-cell lymphoma (CTCL), and peripheral blood mononuclear cells from 6 healthy donors were analyzed for mutations in codons 12, 13 of K- and H-*ras* genes; c-*myc* amplification (differential PCR); *bcl-2* translocation (mbr); B-cell clonality based on immunoglobulin (IgH-CDR3 and CDR2) gene rearrangements and T-cell clonality based on TCR-γ gene rearrangements (multiplex PCR). For identification of amplified products 10% vertical polyacrylamide gels were used. Presence of mutations was examined by single-strand conformation polymorphism analysis of polymerase chain reaction products. The results of this study are presented in Table 1. Monoclonality of B lymphocytes, as evidenced by DNA fragment length homogeneity, was detected in 100% of B-NHL when two primer pairs were used, but never in LC, CTLC or in normal PBL. Also, in 14/17 of CTCL dominant clone of T lymphocytes was detected by multiplex PCR. In the case of LC and normal PBL these gene alterations were not detected.

Table 1. Distribution of gene alterations among different non-Hodgkin's lymphomas

Diagnosis	Gene alteration						
	FRIII/JH	VH4a/JHa	TCRγ	H-ras	K-ras	bcl-2	c-myc
B-NHL	29/34	15/34	0/32	3/32	2/32	7/31	11/31
CTCL	0/8	0/8	14/17	∅	0/17	∅	2/17
LC	0/6	0/6	0/6	0/6	0/6	0/6	0/6

PCR analysis of clonal IgH and TCR-γ rearrangements is given priority when diagnostic assistance is required. This technique has also great potential in tracking minimal residual disease in lymphomas and leukemias and for monitoring clonal evolution in acute and chronic lymphoblastic leukemias and lymphomas. A presence of other genetic alterations, that we detected, could serve as an additional factor in assessment of tumor biology in patients with NHL.

KEYWORDS: Lymphoproliferative Disorders; Mutation; Mutagenesis; Gene Rearrangement, T-Lymphocyte; Gene Rearrangement, B-Lymphocyte; Polymerase Chain Reaction

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Detection of *Chlamydia trachomatis* in clinical specimens of patients with Reiter's syndrome by PCR

Chlamydia trachomatis is the most common sexually transmitted bacterial pathogen in humans. Some forms of reactive arthritis have been linked with chlamydial infection. The rapid identification of chlamydial infection is essential for the proper treatment of infected patients. Also, it is very important for the prevention of transmission to susceptible individuals. The aim of this study was to detect bacterial DNA in sensorial tissue, sensorial fluid and peripheral blood of patients with Reiter's syndrome. Synovial tissue, synovial fluid and peripheral blood mononuclear cells were used as the source of DNA for PCR amplification of bacterial 16S RNA gene. Samples were obtained from 20 patients with Reiter's syndrome. DNA was isolated by phenol/chloroform extraction and after PCR amplification bacterial DNA was detected by 10% PAGE. Of 20 clinical specimens, 6 were found to be positive for *Chlamydia trachomatis* DNA in joint by the polymerase chain reaction. No one sample from peripheral blood was positive. Our results implicated that in 30% of patients with urogenital form of the Reiter's syndrome *Chlamydia trachomatis* could be etiopathogenetic trigger for disease.

KEYWORDS: Chlamydia trachomatis; Reiter Disease; Synovial Membrane; Genes, Bacterial; Polymerase Chain Reaction

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Significance of B lymphocyte clonality, c-myc amplification and BCL-2 translocation in the salivary gland tissue of Sjögren's syndrome patients

Sjogren's syndrome is a systematic chronic autoimmune inflammatory disorder of exocrine glands, primarily manifested through reduced or absent secretion of saliva and tears. Altered function of exocrine glands is caused by continuous activity of infiltrated lymphocytes and plasmocytes. Although B lymphocytes represent about 20% of mononuclear infiltrate, they have dominant role in pathogenesis characterized by local auto antibody production and by regulation of autoimmune response mediated by T lymphocytes. Additionally, these patients demonstrate about 40 times higher risk of developing lymphoma originating from malignantly transformed B lymphocytes that infiltrate exocrine glands. Consequently, determination of dominant B lymphocyte clones together with molecular activity markers such as *c-myc* and *bcl-2* in salivary gland tissue could have great predictive value in monitoring of patients with Sjogren's syndrome. The aim of our study was to demonstrate type of B lymphocyte clonality in the salivary glands tissue of Sjögren's syndrome patients, together with level of *c-myc* amplification and *bcl-2* translocation. DNA samples were isolated from minor labial gland biopsies from 32 Sjogren's syndrome patients hospitalized in Clinic for rheumatology, MMA. After PCR amplification of CDR3 immunoglobulin heavy chain gene region, level of *c-myc* amplification (compared to D2R gene) and amplification of translocated bcl-2 region were identified on polyacrilamide gel electrophoresis. Both amplification of *c-myc* protooncogene and translocated *bcl-2* were found in significant percent of patients who had monoclonal B lymphocyte infiltration (27%), against group of patients with polyclonal (8%) or oligoclonal (0%) type of infiltration. Amplification of *bcl-2* alone was almost equally frequent in groups with monoclonal (27%) and oligoclonal (29%) type of infiltration. Amplification of *c-myc* without *bcl-2* amplification was most frequently detected in patients whose salivary gland was infiltrated with one B lymphocyte clone (27%), than who had oligoclonal type of infiltration (14%) and was the less numerous in group with polyclonal infiltration. Finally, absence of both genetic alterations was significantly associated with polyclonal type of B lymphocyte infiltration (76% versus 57% with oligoclonal and 19% with monoclonal type). Monoclonal type of B lymphocytes infiltration in the salivary glands of Sjögren's syndroma patients was significantly associated with *c-myc* amplification and *bcl-2* translocation and can be used to further assess course of disease, potentially selecting those with high risk for lymphoma development.

KEYWORDS: Sjorgen Syndrome; Salivary Glands; B-Lymphocytes; Polymerase Chain Reaction

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Nuclear accumulation of beta-catenin protein in Wilms' tumor

The Wnt-signaling pathway plays an important role during both normal kidney development and Wilms' tumorigenesis. Activation of this pathway involves stabilization, intracellular accumulation, and nuclear translocation of the beta-catenin and may be caused by specific mutations in the beta-catenin gene itself. Such mutations have been found in about 15% of Wilms' tumors. Beta-catenin expression was investigated using streptavidin-biotin technique, applying antibody to beta-catenin. Correlation of semi quantitatively scored beta-catenin nuclear expression with histological type, tumor stage and prognostic group was performed on 30 cases of Wilms' tumors (25 classical and 5 anaplastic) and four metastases. Nuclear immunoreactivity for beta-catenin was detected in 6 cases of Wilms' tumor. Nuclear positivity, in each case, was found to be very strong, but was usually present only in a fraction of cells ranging from 5% to 10%. Among the different histological subcompartments, blastemic and mesenchymal cells nuclei preferentially stained positive, whereas cells of epithelial differentiation displayed nuclear localization of beta-catenin only in anaplastic type. Furthermore, nuclear positive cells were found more often in Wilms' tumors of lower stage (I/II) than in higher stage (III/IV). Also, nuclear positive cells were found in tumors of both favorable and unfavorable histology. These data support the idea that activation of the Wnt-signaling pathway is a key oncogenic step in Wilms' tumorigenesis.

KEYWORDS: Nephroblastoma; Immunohistochemistry; Cytoskeleton Proteins; Cell Nucleus; Mutation

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Apoptosis and expression of $BclX_{S/L}$ in Wilms' tumor

Wilms' tumor usually has a good outcome, although a poor prognosis is often related to more advanced stages and anaplastic features. Apoptosis occurs with variable frequency in malignant tumors, and may have a role in reducing their growth rate. Different proteins-regulators of apoptosis, such as Bcl-2, BclX, and Bax have influence on rate of apoptosis in various tumors. Twenty-eight cases of Wilms' tumor (2 cases with metastasis) and 2 samples of normal kidney tissue were studied using streptavidin-biotin-complex technique. $BclX_{S/L}$ expression levels were semi quantitatively scored. The expression of $BclX_{S/L}$ was observed in the majority of cases (60.7%), more often in blastemic than in epithelial component of Wilms' tumor: 60.7% and 28.6%, respectively ($p=0.02$). There was statistically significant inverse relationship between $BclX_{S/L}$ expression and tumor stage ($p=0.015$). $BclX_{S/L}$ was found less frequently in high-risk tumors then in tumors with good prognosis ($p=0.02$). Treated Wilms' tumors more often showed $BclX_{S/L}$ expression comparing to non-treated tumors, but relationship was not statistically significant ($p=0.08$). Expression of $BclX_{S/L}$ was detected in various histologic types of Wilms' tumor, but there was no statistically significant association ($p=0.82$) except in cases with diffuse anaplasia ($p=0.012$), which was always negative. $BclX_{S/L}$ immunostaining was not observed neither in two cases of metastasis nor in one case of bilateral Wilms' tumor. Our results suggest that the expression of $BclX_{S/L}$ protein is associated with prognostic group, tumor stage and presence of anaplasia.

KEYWORDS: Nephroblastoma; Proto-Oncogene Proteins c-bcl-2; Immunohistochemistry; Apoptosis

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Immunolocalization of BRCA1 protein in women with sporadic breast invasive ductal carcinomas: A correlation with other biological parameters

Mutation of the BRCA1 tumor suppressor gene has been demonstrated in 80% of familial breast cancer (BC). However, there is much controversy with regard to the importance of BRCA1 protein expression. Immunohistochemical studies have demonstrated a loss or reduction of protein expression not only in BRCA1 associated BCs but also in non-BRCA1 associated familial and sporadic BCs. The expression of BRCA1 protein in large series of different histological types of BCs with correlation with other biological parameters has not been clarified. Fifty-one cases of sporadic invasive ductal carcinomas (IDC), not otherwise specified, were included in this study. All the patients were women, ranging in age from 38 to 77 (mean 58.8). Immunohistochemistry (IHH) carried out using a standard avidin-biotin immunoperoxidase method on paraffin wax embedded primary breast tumor tissues, and the commercially available anti-BRCA1 antibody MS110 (Ab-1). In addition, the tumor specimens were IHH stained for estrogen receptor (ER), progesterone receptor (PR), p53, HER-2/*neu*, and bcl-2. All tumors were histologically graded according to the modified Scarff, Bloom, and Richardson (SBR) grading system. Axillary lymph node status and vascular invasion were determined by histological examination. BRCA1 protein was exclusively localized in nuclei of both ductal and lobular epithelia of normal tissue adjacent to the tumor, and demonstrated in 70% of epithelia. In 51 of sporadic IDC, 30 (60%) had BRCA1 expression exclusively in the nucleus, 5 (9%) cases had expression exclusively in the cytoplasm, 3 cases (6%) had both nuclear and cytoplasmic expression, and 13 (25%) had absent BRCA1 expression. Complete loss of BRCA1 nuclear expression found in 34% of IDC. In this study we demonstrated that complete loss of nuclear BRCA1 expression correlated well with high histological grade and bcl-2 negativity. Our results regarded relationship of loss of BRCA1 nuclear expression to other biological markers and clinicopathological factors in IDC, indicated that loss of BRCA1 nuclear expression was also significantly more frequent in ER-negative tumors. There was no significant correlation between complete loss of BRCA1 nuclear staining and expression of p53, HER-2/*neu*, axillary lymph node status, tumor size and patient's age. Although the BRCA1 gene is not mutated in sporadic BCs, the loss of BRCA1 protein expression (as detected by immunohistochemistry) indicates that BRCA1 gene probably features in the genesis of sporadic breast cancer through mechanisms other than mutation.

KEYWORDS: Breast Neoplasms; Carcinoma, Infiltrating Duct; BRCA1 Protein; Immunohistochemistry; Protein p53; Receptor, erbB-2

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Detection of brc-abl gene expression in the Philadelphia chromosome negative patients with chronic myeloid leukemia

Chronic myeloid leukemia (CML) is the hematopoietic stem cell disease, characterized by the presence of the Philadelphia (Ph1) chromosome in the more than 95% of the patients (pts.). Philadelphia chromosome is the product of reciprocal translocation t(9;22) (q34;q11). Molecular consequence of this translocation is the formation of the chimeric bcr-abl gene. In dependence of brake point in bcr gene two forms of bcr-abl gene could be found in CML: b3a2 and b2a2. In about 5% of the CML patients Ph1 chromosome is not detectable by the cytogenetic analysis because of the submicroscopic micro insertions. Manifestations of the chronic myeloproliferative disorders often overlap and in the cases of Ph1 negative patients only molecular techniques can solve this diagnostic problem. We present the 5 patients with manifestations of chronic myeloproliferative disease, most likely CML. Preparation of the bone marrow chromosomes was done by the direct method and after 24h cell culture. 20 metaphases were analyzed after GTG banding technique. RNA was isolated from the peripheral blood leukocytes. RT-PCR was done with primers for bcr-abl sequence and the products were visualized on 8% polyacrilamide gel after electrophoresis. In all 5 patients only normal karyotype was detected. RT-PCR analysis detected expression of the b3a2 form of bcr-abl gene in all cases. Those results mean solution of diagnostic dilemmas and adequate therapeutic approach for this group of patients.

KEYWORDS: Leukemia, Myeloid, Chronic; Philadelphia Chromosome; Genes, abl; Gene Expression

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Frequency of estrogen and progesterone receptor positivity by immunohistochemical analysis in women with breast invasive ductal carcinomas

Estrogen receptor (ER) and progesterone receptor (PR) are important regulators of growth and differentiation in the mammary gland. Both are also involved in the development of malignant tumors and have been reported to be prognostic as well as predictive parameters in primary breast cancer patients. This study was carried out with a group of 65 tumor tissues from IDC patients. Patients' age ranged from 38 to 77 years; the median age was 59 years. ER and PR were determined by sensitive IHH method (labeled streptavidin-biotin complex). For the quantitification of both ER and PR, a "simple semi quantitative scoring system", with a maximum score of 8, by Leake and co-workers (2000) was used. Fresh tissue specimens served to measured tumor size. All tumors were histologically graded according to the modified Scarff, Bloom, and Richardson (SBR) grading system. Nodal status and vascular invasion were determined by histological examination. The χ_2 test, U test, Kruskal-Wallis analysis of variance and Student's test were used for statistical analysis. ER positivity (+) was determined in 75%, while PR+ was determined in 49, 5% of patients with IDC. Moreover, 67% of ER+ tumors were considered strong positive (with scores 7 or 8). The highest proportion of tumors have combined hormonal receptor status ER+/PR+ and ER+/PR-(45% and 31%, respectively). A strong positive correlations were found between each frequency of ER+ tumors, strong ER+, and expression of combined phenotype ER+/PR+, and patient's ages (>50 years) ($p<0.01$). Also, a strong negative correlation was found between strong ER+, and frequency of ER+/PR+ expression, and SBR grade ($p<0.01$). The highest proportion of tumors negative for both receptors (ER-/PR-), or ER+/PR+, or ER+/PR- occurred in patients with tumor size <5cm ($p<0.01$). Both frequency of ER+ or PR+ tumors and IHH-intensity of receptor expression were independent of axillary lymph node involvement. Also, both frequency of ER+ and intensity of expression were significantly correlated to lymphatic invasion. ER and PR were frequently expressed in IDC. This expression correlated with patient age, tumor size, histological grade and lymphatic invasion.

KEYWORDS: Receptors, Estrogen; Receptors, Progesterone; Breast Neoplasms; Carcinoma Infiltrating Duct; Immunohistochemistry

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Immunohistochemical assessment of steroid hormone receptor status in invasive breast carcinoma

The assessment of steroid hormone receptors in resected breast cancer tissues is essential to decide whether endocrine therapy is indicated and to select the best treatment for each patient on the basis of receptor status. The aim of this study was the assessment of estrogen (ER) and progesterone (PR) receptor status in invasive breast carcinomas of various histological types and grades. Immunohistochemistry was performed on paraffin sections of 80 invasive breast carcinomas (38 ductal, 18 lobular, 18 ducto-lobular, 2 medullar, 2 mucinous, 1 tubular and 1 papillary). The same scoring system used for immunohistochemicaly stained ER and PR receptors: a score was given to the proportion of cells staining positive (0-5) and an intensity score also was given (0-3). A tumor with a total score 0-2 was classified as negative, score of 3-4 as low positive and score 5-8 as positive. The results were compared with the histological grade of the breast carcinomas and the results analyzed by the chi-squared test. ER and PR receptors positivity (low positive and positive cases were considered positive) were seen in 71.25% and 60% cases respectively. In grade I tumors both ER and PR receptors positivity were 100%, while in grade II tumors ER and PR receptors positivity were 76.36% and 61.62% respectively. The corresponding figures for grade III tumors were 41.18% and 35.29% respectively. Significant association between different histological grades of breast carcinomas and ER and PR receptor status (negative, low positive and positive) were found ($p<0.05$). This study showed that ER and PR receptors positivity declined with increase in tumor grade.

KEYWORDS: Breast Neoplasms; Receptors, Estrogen; Receptors, Progesterone; Immunohistochemistry

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Chemodectoma: Case report

Tumors of the paraganglia of head and neck region are known as chemodectomas. Paraganglia of head and neck region are closely aligned with parasympathetic nervous system and often have a close spatial relationship with neural or vascular structures. Carotid body and aorticopulmonary paraganglia have been shown to have chemoreceptor role and modulate respiratory and cardiovascular function. Since the largest collection of chemoreceptor cells is in the carotid body located in adventitia on the posterior aspect of the common carotid artery at bifurcation, it is the most common site of these tumors. The majority of reported cases have been in women, usually in the middle age group. Grossly, the tumors are firm red-brown, extremely vascular, usually well circumscribed and at least partially encapsulated by compressed connective tissue. The tumor can extend into the adventitia or even the media of the carotid arteries so that its removal necessitates vessel resection. Needle aspiration is safe procedure for correct diagnosis. Improved surgical procedures improve prognosis, with only 10% of cases with local recurrence. Malignant behavior, as evidenced by lymph node, bony, visceral or soft tissue metastases, is observed in about 6 to 10% of tumors. We present a case of chemodectoma in middle-aged woman with the history of previous therapy for endometrial adenocarcinoma. Patient had painless, slow growing mass on the right side of the neck and was forced to see the surgeon for the suspicion of cervical lymph node metastasis of endometrial carcinoma. Irregularly ovoid mass of firm, dark brown, well encapsulated tissue, and 4cm in largest diameter was extracted from the bifurcation of right carotid artery. Morphological examination reveals typical chemodectoma: solid nests (Zellballen) separated by fibrous trabeculae containing prominent capillary vessels and occasionally bundles of nerve fibers. Stoma changes include: focal hemorrhage and prominent perivascular sclerosis. Tumor cells vary in shape from round to polygonal. Their cytoplasm is rather abundant. Some cells had oncocytic appearance. Nuclei were ovoid and vesicular. The tumor cells were strongly positive to antichromogranin A (DAK - A3). There was no familiar history of this type of tumor, but it is positive for endometrial carcinoma. It is of interest to underline the differential diagnosis of incidentally observed non-tender anterolateral neck mass which include: metastatic lymph nodes, carotid body aneurisms, salivary gland tumors, branchial cleft cyst and thyroid and neurogenic tumors.

KEYWORDS: Head and Neck Neoplasms; Carotid Body Tumor; Paraganglioma; Diagnosis, Differential

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Merkel cell carcinoma: Clinical and histopathological findings - a case report

Merkel call carcinoma (MCC) is rare and aggressive dermal malignant tumor, which derives from the neuroendocrine cell system with features of epithelial differentiation. The most frequently affects elderly patients. Eighty-five percent of all MCC appear on sun-exposed areas, with 50% to 55% ocurring on the head and neck. MCC is characterized with local or locoregional extension and distant spread by hematogenious or lymphogenic way. Our aim wad to describe the clinocopathological and immunohistochemical features of rare neuroendocrine MCC of the skin. A 90-year-old woman has been operated of the dermal tumor at the Clinic of Maxillofacial Surgery of the Military Medical Academy in Belgrade. The primary tumor location was naso-ethoido-orbital region in the first stage of the disease. The patient was undergone surgical excision and than locoregional radiotherapy. Histologically the tumor was composed of solid islands of mostly basophilic densely packed cell with a scant cytoplasm, which was suggestive of a neuroendocrine origin. Results of immunohistochemical studies using antibodies against neurone-specific enolase, chromagranin and cytokeratin allowed classification of the lesion as a Merkel cell tumor. In a 2-year follow-up the patient had a two regional recurrences. Ten months after the operation, an regional recurrence appeared in the bilateral parotidomasseterical region, because of which we performed total bilateral parotidectomy and radical neck dissection on the right side. Twenty-one months later the regional recurrence appeared in the maxillary sinus, because of which we had to perform the total maxilletomy on the right side. We haven't found local recurrence at the site of the primary tumors, neither by clinical or radiological investigation, and the patient is still alive. Recent studies revealed that the MCC is a rare neuroendocrine neoplasm of the skin, with highly aggressive spread with a predisposition for local recurrence and local regional and distant metastases5. Helmbold P et al 2002 also showed that the a high frequency of local recurrence (25% -77%), and lymph node metastases (50%) are characteristic features of MCC. During of establishing the diagnosis about half of the patients has positive lymph nodes with a 3-year-survival rate of 60%, and 5-year-survival rate of 30%-74%. Surgical excision of tumor and regional lymphadenectomy is a first step of treatment MCC. MCC is a highly radiosensitive tumor and the surgical treatment is completed with locoregional radiotherapy and in some case with chemotherapy. Surgical treatment and radiotherapy should be included into the treatment concept in every stage disease MCC. Elective lymph node dissection should decrease the rate of regional recurrences. Extensive surgical resection with free margins of the tumor should decrease the appearance of local recurrences.

KEYWORDS: Carcinoma, Merkel Cell

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Cystic dystrophy of pancreatic heterotopia of the duodenal wall: Its relation to myoepithelial hamartoma

Pancreatic heterotopia is defined as aberrant pancreatic tissue without contact with the normal pancreas and possesses its own duct system and vascular supply. If pancreaticobiliary-type ducts dominate, they are often surrounded by thick bundles of smooth muscle and have been designated as myoepithelial (adenomyomatous, myoglandular) hamartomas or as adenomyomas. It is not uncommon but mostly found incidentally unless complicated with inflammation, obstruction, cystic dystrophy and malignant alteration. Since cystic dystrophy of pancreatic heterotopia in the duodenal wall has been recognized as a separate entity a decade ago, its etiopathogenesis and relation to similar lesions in gut wall remain unclear. We analyzed twelve cases of cystic lesions in the duodenal wall consistent with cystic dystrophy of pancreatic heterotopia from the Department of Histopathology Registry during last 10 years (1994-2003) in 9 male (average age 44.67 years) and 3 female patients (average age 30 years) operated in Clinical center of Serbia. Detailed histopathological analysis was performed searching for elements of myoepithelial hamartoma and pancreatic parenchyma using standard H&E multiple serial sections of samples, histochemical stains for periodic acid-Schiff and Masson's trichrome, immunohistochemical studies for cytokeratin 7, α_1-antitrypsin, α_1-antichymotrypsin, chromogranin A, neurone-specific enolase, desmin, smooth muscle actin and CD34. All cases revealed admixture of fibromuscular bundles and diffuse or ill-defined inflammatory infiltrate surrounding irregular cysts. After careful examination elements of pancreatic tissue were found only in 8 cases, primarily of ducts and simple mucin-producing glands and in 5 cases only ducts or islets are present. According to Heinrich who classified pancreatic heterotopia into 3 types, we found the following: type I, ducts plus acini plus endocrine islets - in 5 cases; type II, ducts plus acini - in 1 case; and type III, ducts with few acini or dilated ducts only (adenomyoma) - in 2 cases. It was impossible to ascertain the pancreatic origin of this lesion in the absence of acini and/or islets of Langerhans in 4 out of 12 cases. Furthermore, ductal structures may have a local origin by means of metaplastic phenomena or following prolonged mucosal injury (duodenitis cystica profunda). It seems that myoepithelial hamartoma or so-called adenomyoma could be frequent association of heterotopic pancreas rather than the end of the same spectrum. Therefore, we propose these cases of duodenal wall cysts should not be classified as heterotopic pancreas.

KEYWORDS: Choristoma; Pancreas; Duodenum; Cysts

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Cytochemical and immunocytochemical characteristics of Meckel's diverticulum with heterotopic rests of pancreatic tissue: A case report

Meckel's diverticulum, a persistent omphalomesenteric of vitelline duct, is the most frequent congenital anomaly of the digestive tract, occurring in approximately 2 percent of autopsied adults. The diverticulum is wide-mouthed, about 5 cm long, and arises from the antimesenteric border of the ileum, usually within 100 cm of the ileocecal valve. Clinical symptoms and heterotophic rests of the pancreatic tissue are the reasons for this report. The patient, a 20 years old girl, complained of the 20-hour pain in the lower quadrant, suprapublically. Except normochromic anemia, other laboratory findings were non-contributing. The patient was operated on the first day of admission. During the appendectomy, Meckel's diverticulum located in the terminal ileum, within 80 cm of the ileocecal valve, was discovered incidentally and resected. Appendiceal and Meckel's sections were fixed in 10% formalin, embedded in paraffin and stained with: HE, PAS, HID-AB, pH: 2.5, Massons for detection of EC endocrine cells and immunocytochemical LSAB2, using epithelial marker Pan Cytokeratin, for detection the hypoplastic pancreatic aberrant tissue. Results are as follows: a) Meckel's diverticulum: a blind segment having a lumen-like ileum and a length of 4.5 cm; the mucosal lining was of that of normal small intestinal, only with nodular polypoid hyperplasia of glands of Lieberkh¸n, enriched with both Paneth's and endocrine EC cells but without peptic ulceration. Histochemically, brush border of the lining epithelium was PAS positive and goblet cells AB positive. Nodular hyperplasia of glands of Lieberkh¸n contained the associated hyperplasia and hypergranulation of Paneth's and of EC cells. The pancreatic tissue was formed only of exocrine pancreatic tissue (duct and small acini). Imunocytochemical reaction showed widespread and large quantity the CK filaments arranged in a mesh of loose bundles. Localization of the ectopic pancreatic tissue was immediately beneath the serosa and inside muscularis propria of the wall. b) Appendix: scant neutrophilic exudate was found through the mucosa, submucosa and mucularis propria, showing characteristics of early acute appendicitis. The most plausible theories of the origin of Meckel's diverticulum with pancreatic tissue, as well as the possible reasons for presenting symptoms, will be discussed.

KEYWORDS: Meckel's Diverticulum; Choristoma; Pancreas; Cytodiagnosis; Immunohistochemistry

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Structure and function of interstitial cells of Cajal

Morphologists have long been interested in interstitial cells of Cajal (ICC) since Cajal first mentioned them as cells intercalated between nerve terminalis and their effector smooth muscle cells in the gastrointestinal (GI) tract. The function of ICC had remained unknown. Recently it was realized that ICC express proto-oncogene c-kit, resulting in great studies of the morphology and function of ICC. They are located from the esophagus to the anal channel, wherever smooth muscle cells exist, and show a distinct distribution and local differences within the GI tract. ICC has long cell process and show bi-polar or multi-polar configurations. At the level of the myenteric plexus, ICC has their own interconnecting network processes. They also situate closely to the nerve elements such as ganglion and nerve strands. Electron microscopy analysis showed the characteristic features of ICC: electron dense cytoplasm, prominent mitochondria, smooth ER and many of intermediate filaments composed of vimentin. ICCs can also be identified by their membrane-bound tyrosine kinase receptor, Kit (CD117), the ligand of which is stem cell factor (SCF). Within the GI tract C-Kit expression is limited to mast cells and ICCs. ICCs can be distinguished from mast cells histochemically by use alcian blue pH 0.5. By use of Kit immunohistochemistry, ICCs have been shown to form a network of cells in the myenteric plexus of the intestines, and at the submucosal border of the circular and longitudinal muscle layers. ICCs are now recognized to form an integral part of the physiology of gut motor functions as pacemaker cells, controlling peristalsis and muscle contraction, and possibly as mediators of neurotransmission. They have been described as having similarities to fibroblasts as well as to smooth muscle cell.

KEYWORDS: Proto-Oncogene Protein c-kit; Gastrointestinal System; Immunohistochemistry; Enteric Nervous System

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Prognostic significance of PCNA and Ki-67 labeling indices in gastrointestinal stromal tumors

Gastrointestinal stromal tumors (GISTs) present a distinctive group of mesenchymal tumors with very difficult prediction of biological behavior. The advent of new adjuvant therapeutic agent, STI-571 (imatinib Gleevec; Novartis, Basel, Switzerland) in treatment of metastatic GISTs put attention to the proper selection of patients for this therapy and even the possibility to use it in nonmetastatic GISTs with malignant potential. Therefore, there is a need for additional and more reliable prognostic markers. We examined 32 cases of GISTs, which were immunohistochemically positive for c-kit and/or CD34 with clinical follow-up of up to 4-year recurrence-free survival. The malignancy of GISTs was estimated as probably benign, probably malignant and uncertain (potentially malignant or intermediate) with respect to tumor size ($\leq$2 cm, $\leq$5 cm, $\leq$10 cm, >10 cm), mitotic rate ($\leq$5 mitoses per 50 HPFs or >5 mitoses per 50 HPFs) and tumor site (gastric or intestinal) according to modified traditional criteria by Mietinnen et al. Immunohistochemical expression of proliferative markers was evaluated by percentage of PCNA and Ki-67 labeled cells per 1000 tumor cells in most proliferative area and correlated with estimated malignancy. In examined series there were 18 gastric GISTs ranging from 25 to 145 mm in size and 14 intestinal GISTs ranging from 4 to 160 mm in greatest diameter. According to morphological criteria, we found 4 gastric and 1 intestinal probably benign GISTs, 8 gastric and 7 intestinal tumors with uncertain malignancy and 6 gastric and 7 intestinal probably malignant GISTs. Proliferation indices (PI) were semiquantitave scored and subdivided into 4 groups: $\leq$1%, $\leq$5%, $\leq$15%, >15% immunopositive cells (for Ki-67) and $\leq$10%, $\leq$25%, $\leq$50%, >50% immunopositive cells (for PCNA). The Kruskal-Wallis statistical analysis showed significant difference between benign and uncertain GISTs categories for PCNA PI ($p=0.0302$) and uncertain and malignant categories for Ki-67 PI ($p=0.0060$). Furthermore, the mean Ki-67 PI of benign tumors was significantly different from that of uncertain GISTs category ($p=0.0401$) and the range of Ki-67 PI values did not varied considerably, i.e. they were more consistent ($\leq$5%). Proliferation index of Ki-67, but not PCNA could be very helpful in discrimination probably malignant GISTs from other categories of GISTs. Determination of GISTs with uncertain malignancy should respect both Ki-67 and PCNA PI values in addition to traditionally well-established importance of mitotic rate, tumor size and site.

KEYWORDS: Gastrointestinal Neoplasms; Stromal Cells; Proto-Oncogene protein c-kit; Ki-67 Antogen; Proliferation Cell Nuclear Antigen; Prognosis

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Serrated adenoma of the colorectum: A morphological, histochemical and immunohistochemical study

Serrated adenoma is a relatively newly defined entity of colorectal neoplasms characterized by a saw-toothed structure of hyperplastic polyp and cytologic atypia of tubular adenoma. The optimum model for the histological identification of the serrated adenoma includes the presence of a serrated architecture in >/= 20% of crypts in association with surface epithelial dysplasia. Its histogenesis and natural history still remain unclear. They have a significant malignant potential; in one series 11% contained foci of early carcinoma. The concept of serrated adenoma proposes an independent pathway for the pathogenesis colorectal cancer, namely hyperplasia-dysplasia/adenoma-adenocarcinoma sequence. The aim of this study was to provide a histological, histochemical and immunohistochemical evaluation of the serrated adenomas and compare results to those of hyperplastic polyps and conventional tubular adenoma, and to evaluate the prognostic significance of these lesions. Five-micrometer sections of the formalin-fixed and paraffin-embedded tissue blocks from 10 cases of serrated adenomas, 10 hyperplastic polyps, and 10 traditional tubular adenomas were used in this study. The cases were selected from the files of the Department of Pathology, Medical School of Nis. Hematoxylin-eosin, HID-Alcian blue (pH-2.5) and immunohistochemistry for Ki-67 (Dako, Denmark) were used. Immunohistochemical staining was visualized using LSAB (labeled streptavidin-biotin) method. Histologically, serrated adenomas showed the classic architecture of hyperplastic polyps with a serrated appearance of the elongated crypts (although they showed an increased content of immature goblet cells). However, serrated adenomas were composed of less mature eosinophilic cells with elongated, hyperchromatic nuclei resembling those found in typical adenomas. Two lesions contained a focus of severe atypia with numerous mitotic figures in the surface epithelium. This comprised closely spaced serrated glands with pseudocribriform appearance and round vesicular nuclei with prominent nucleoli. Histochemically, serrated adenomas contained a mucin-depleted cells exhibiting nuclear pseudostratification and occasional loss of the polarity line the glands. Immunohistochemically, Ki-67-positive cells in hyperplastic polyps were localized mainly in the bottom of crypts and those in tubular adenomas were diffusely distributed, while Ki-67 positive cells in serrated adenomas were mainly aggregated in the upper half of the crypts. Serrated adenomas had significantly higher number of Ki-67-immunopositive cells compared with hyperplastic polyps, but the number of Ki-67- positive cells was significantly higher in the tubular adenomas compared with serrated adenomas. These results suggest that serrated adenomas may be committed to independent growth. The role of the serrated adenoma in the histogenesis of colorectal cancer should be examined.

KEYWORDS: Colorectal Neoplasms; Adenoma; Colonic Polyps; Hyperplasia; Immunohistochemistry; Ki-67 Antigen

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Micromorphologic and immunophenotypic features of Lennert's lymphoma subtype

Lymphoepitheloid lymphoma (Lennert's lymphoma subtype) shows diffuse or more rarely interfollicular infiltrates. It is among the most aggressive of the non-Hodgkin lymphomas. Patients often respond poorly to therapy, relapses are frequent and the overall and failure-free survival rates at 5 years. So far the only factors consistently associated with the prognosis are stage and the international prognostic index. Because of the diagnostic challenge for clinical and pathologists we have decided presenting this case. One case of T-cell lymphoma involving liver was presented. A 49-year-old woman with rapidly progressive Lennert's lymphoma terminating in fulminant hepatic failure was presented. Staging radiological studies revealed that she had cervical and mediastinal lymph node swellings and multiple nodular lesions in the liver. Lymph node biopsy specimens showed diffuse infiltrates consisting of large blastic lymphocytes interspersed with the proliferation of small clusters of epitheloid cells. Reed-Sternberg-like cells, eosinophils and plasma cells were also present. These lymphocytes were $CD3^+$, CD45RO (UCHL-1)$^+$, $CD8^+$ and $CD19^-$. Under the diagnosis of Lennert's lymphoma, she was treated with standard chemotherapy. After two courses of the therapy, despite the decreased size of cervical lymph nodes, high-grade fever appeared. The liver function deteriorated rapidly. This case is an example demonstrating that at least some of the Lennert's lymphomas phenotypically correspond with cytotoxic T-cell lymphomas. It should be also emphasized that Lennert's lymphomas containing cytotoxic proteins may have a fulminant clinical course, which cannot be rescued by the conventional chemotherapy.

KEYWORDS: Lymphoma; T-Cell; Liver Neoplasms; Biopsy; Immunohistochemistry

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Endometrial adenosarcoma

Several hundred cases of endometrial adenosarcoma have been reported since 1974, when it was first described. Endometrial adenosarcoma is defined as mixed epithelial and mesenchymal tumor with malignant mesenchymal component, either homologous or heterologous by cellular origin. We report three cases of endometrial adenosarcoma diagnosed in women (mean age 47 years) in Clinical Center of Serbia, Belgrade in last year, one of them as heterologous type. All cases underwent surgical treatment because of severe perimenopausal bleeding. Tumors were examined after standard histological H&E staining on 4 microns sections from formalin-fixed and paraffin-embedded samples. Immunohistochemical analysis was performed in order to determine cell lineage differentiation and evaluate malignant potential of stromal components. All sections were examined using LSAB immunostaining method with AEC visualization for vimentin, smooth muscle actin, desmin, S-100, CD34, HHF35, GFAP, NSE, EMA, pancytokeratin (AE1/AE3), p53 protein, as well as proliferative activity factors, namely Ki-67 protein and PCNA. Morphologically readily seen sarcomatous component after immunohistochemical examination showed presence of strong cytoplasmic positivity for desmin in all cases as well as strong positivity for smooth muscle actin and vimentin. Additionally, myogenic differentiation was proved for immunoreactivity to desmin and HHF 35 in one case and in two cases we found slight and focal positivity for EMA, cytokeratin, S-100 protein and NSE. There was no significant reactivity to GFAP, thus excluding endometrial stromal sarcoma. Ki 67 protein labeling index was 10-25% (mean value 20%), contrary to prominent immunopositive portion of examined tumor cell population for p53 and PCNA (about 50%). In two cases of endometrial adenosarcoma, the stroma was of homologous type and showed smooth muscle differentiation. The third case of endometrial adenosarcoma was heterologous type with rhabdomyosarcoma overgrowth. The presence of immunohistochemically demonstrated heterologous elements; high grade sarcoma and high mitotic index of the stromal component have been reported as unfavorable prognostic factors but also can be very helpful in differential diagnosis of these tumors.

KEYWORDS: Endometrial Neoplasms; Adenosarcoma; Immunohistochemistry

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Ovarian immature teratoma

Ovarian immature teratomas comprise only 1% of all teratomas but still represent the third most common germ cell tumors of the ovary in young women. Conservation of fertility is essentially important in these women, so unilateral surgical treatment is often appropriate in stage FIGO IA, but in higher stages radicalization is mandatory. Thus, correct diagnosis, grading and staging is very important in overall prognosis and treatment. Four cases of unilateral ovarian immature teratoma were diagnosed in last decade in women ranging in age from18 to 30 years in Clinical Center of Serbia, Belgrade. All cases underwent surgical treatment after incidental ultrasound finding of ovarian masses. Tumors were examined after standard histological H&E staining on 4-microns sections from formalin-fixed and paraffin-embedded samples. Immunohistochemical analysis were performed in order to determine cell lineage differentiation and evaluate its malignant potential, using LSAB+ immunostaining method with AEC visualization for vimentin, S-100, GFAP, PGP9.5, NSE, neurofilaments, CD34, desmin, smooth muscle actin, alpha-feto-protein, alpha-1-antitrypsin, EMA, pancytokeratin (AE1/AE3), CEA, PLAP, p53 protein, as well as proliferative activity factors, namely Ki-67 protein and PCNA. All four tumors were semicystic well-defined ovarian masses with maximal diameters from 90 to 140 mm. Besides histological evident derivates of all three germ layers, on histological examination we regularly find domination of neuroectodermal elements; in three cases with focal and in one diffuse histologically overt immaturity. Immunohistochemical analyses showed various cell phenotypes with preponderance of NSE and PGP9.5 immunopositivity and focally positive immunostaining with neurofilaments and synaptophysin in all cases. Other antibodies showed inconsistent and variable positivity in examined immature tissue. GFAP was negative in immature neuroepithelial elements, but positive in glia in all cases. PCNA labeling index showed range from 10% to 25% but only 1% to 5% of Ki 67 protein was found; p53 protein did not show any significant reactivity. Detailed morphological examination should be supported by immunohistochemistry in order to confirm histogenetic cell types and resolve some differential diagnostic dilemmas. We could not find any correlation of proliferative indices with histological grade or stage.

KEYWORDS: Ovarian Neoplasms; Teratoma; Immunohistochemistry; Cytodiagnosis

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Osteoprotegerin and related molecules suppress bone resorption in multiple myeloma and breast carcinoma patients

Multiple myeloma and breast carcinoma have a strong propensity to produce lytic skeletal metastases. The establishment of bone metastases in carcinoma patients is not possible without previous resorption of the bone tissue. The only cells capable of resorbing bones are osteoclasts. Osteoclasts are specialized cells, derived from the monocyte/macrophage hematopoietic lineage, which secrete acid and lytic enzymes that degrade mineralized bone matrix. A dominant role in the differentiation and activation of osteoclasts plays the interaction between receptor activator of nuclear factor kappa B (NFkappaB) ligand (RANKL) and its specific receptor, receptor activator of NFkappaB (RANK). RANKL is expressed by stromal cells and osteoblasts in the local bone marrow microenvironment, but it can be released from the cell surface as a soluble molecule after proteolytic cleavage by the metalloprotease disintegrin TNF-(convertase (TACE) (23). Both soluble and membrane bound RANKL can function as ligands for receptor RANK expressed on the surface of osteoclast precursors and mature osteoclasts. The binding of RANKL to RANK plays a key role in the promotion of osteoclast formation, activation and bone resorption. Laboratory researches have demonstrated that the RANKL-RANK axis for osteoclastogenesis contributes to pathologic osteoclast formation in several disease states, including osteolysis caused by cancer metastases. Binding of the RANK ligand to its receptor can be prevented by osteoprotegerin (OPG), a neutralizing soluble decoy receptor produced by osteoblasts and marrow stromal cells. Osteoprotegerin negatively regulates RANKL signaling. Administration of OPG in animal tumor models prevents establishment and progression of osteolytic metastasis, development of hypocalcaemia of malignancy and skeletal pain. OPG is currently in first phase of clinical trials. Preliminary data suggest that OPG may represent a future therapeutic option for treating conditions in which bone loss occurs, such as postmenopausal osteoporosis and bone metastases in multiple myeloma and breast carcinoma patients. Beside the natural decoy receptor osteoprotegerin, in the therapy of excessive bone resorption diseases can be used a recombinant OPG fusion protein (OPG-Fc) or inhibitory RANK antibodies (RANK-Fc).

KEYWORDS: Osteoclasts; Bone Resorption; Breast Neoplasms; Multiple Myeloma; NF-kappa B

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Metastatic bone disease: Clinicopathological correlation

Bone is the third most common site of metastatic disease. The axial skeleton is seeded more than the appendicular skeleton. Pain, pathological fractures and hypercalcemia are the major sources of morbidity with bone metastasis. Patients may have no other manifestation of cancer other than their painful bone lesion. In 55% of metastaic bone disease patient has a metastasis of unknown origin. Diagnosis of bone metastases is accomplished by a bone scan, CT, MR, biopsy and pathological analysis of bioptic material using standard, histochemical (AB, PAS, Grimelius) and immunohistochemical stains by using the following antibodies: common CK, CK 7, CK 20, VIM, Synaptophisin, Chromogranin A, LCA, NSE, heppat, alpha-fetoprotein, to appointed both histological type and prime location of cancer. In the course of last year 68 cases of metastatic carcinoma in bones were diagnosed at the Institute of pathology, School of Medicine, Belgrade. The youngest patient was 27 years old woman; the oldest was a man 83 years old (the mean age 46. 8). Second deposition was predominately distributed in axial skeleton (40% from all localization). Even 38 patients (57%) with diagnosed bone metastatic carcinoma have a metastasis of unknown origin. In spite of clinical diagnostic procedures which are based on pathological results, about 25-30% of all metastatic bone carcinomas remain as metastasis of unknown origin.

KEYWORDS: Neoplasm Metastasis; Bone and Bones; Bone Neoplasms; Neoplasm, Unknown Primary

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Immunophenotypization of round cell bone tumors: Answer to clinicomorphological dilemmas

The descriptive term "round cell bone tumors" means heterogenic tumors group very similar in cells morphology, but strong different in histogenesis. It includes: Ewing's sarcoma, PNET, mesenchymal chondrosarcoma, round cell osteosarcoma, lymphoma of bone, embryonal rhabdomyosarcoma and metastatic oath-cell bronchiolar carcinoma. In the last 5 years 72 cases of "round cell bone tumors" were registered on the Institute of pathology, School of Medicine, Belgrade. Clinical and pathological diagnostic procedure is very complex and clearly definite. It started with native radiography of bone, passing CT, MR scan, biopsy, and finished with pathologic analysis. For the correct diagnostic procedure it is necessary to use special histochemical and immunohistochemical stains. We stained tumors tissue with PAS and AB for the first orientation, but definitive typisation required specific antibody application. The main antibody panel includes: vimentin, common citokeratin, desmin, LCA, CD 99, S-100 protein, synaptophisin, kappa, lambda, CD68, CD79a, CD3, and CD20. Finally, in last 5 years we diagnosed: 18 Ewing's sarcomas, 8 PNETs, 6 mesenchymal chondrosarcomas, 1 round cell osteosarcoma, 26 lymphomas of bone, 2 embryonal rhabdomyosarcomas, and 1 metastatic oath-cell bronchiolar carcinoma. Using this immunohistochemical stains we could make the final diagnosis and gave answer to clinicomorphological dilemmas.

KEYWORDS: Bone Neoplasms; Cytodiagnosis; Immunohistochemistry

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Dedifferentiated leiomyosarcoma with osteosarcomatous differentiation: A case report

Abrupt change in differentiation of soft tissue sarcomas to high-grade morphology is known as "dedifferentiation". It is mostly seen in recurrent tumors. A heterologous sarcomatous line of differentiation may be additionally found within the dedifferentiated portions of the tumor. There have been very few publications concerning the dedifferentiated leiomyosarcoma. To the best of our knowledge the dedifferentiated leiomyosarcoma with heterologous osteosarcomatous differentiation has never been reported. We describe a case of a perineal leiomyosarcoma with dedifferentiation in two recurrent tumors in a 57-year-old woman. The primary tumor was well and moderately differentiated. The leiomyomatous nature of the tumor was confirmed by desmin and SMA immunopositivity. Both recurrent tumors had the same histologic appearance. It revealed the dedifferentiated sarcomatous pattern with prominent areas of osteosarcomatous differentiation. The dedifferentiated portions were very cellular and composed of atypical polygonal, ovoid and small cells with mitoses up to 50/10 HPF. The myogenic phenotype of the dedifferentiated tissue was confirmed by the positive smooth muscle markers immunoreactivity in some dedifferentiated cells (<10%). The osteosarcomatous differentiation consisted of large areas of narrow anastomosing irregular trabeculae of osteoid, which was focally mineralized. The osteoid was rimmed by pleomorphic round and ovoid cells, resembling malignant osteoblasts. This case demonstrates that a leiomyosarcoma may undergo dedifferentiation, containing heterologous sarcomatous component(s) analogous to the dedifferentiated liposarcoma and chondrosarcoma.

KEYWORDS: Leiomyosarcoma; Osteosarcoma; Cell Differentiation; Immunohistochemisty

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Correlation of cytologic and histopathologic findings of bone tumors

The objective of this study is to determine the role of imprint cytology in the diagnosis of bone tumors. From 56 surgical biopsies of bone lesions imprints were made, air-dried and stained with May-Grünwald Giemsa method. The findings were compared with histopathologic diagnoses. In two cases there was no material for cytologic analysis. In 19 patients with malignant bone tumors (chondrosarcoma 2, malignant giant cell tumor of bone 1, Ewing's sarcoma 2, ameloblastoma 2, malignant fibrous histiocytoma 1, malignant schwannoma 1, leomyosarcoma 1, synoviosarcoma 2) cytologic and histopathologic findings matched in 18 cases. There was one false negative case. In 14 patients with metastases (squamous cell carcinoma 11, anaplastic carcinoma 1, melanoma 2) cytologic and histopathologic diagnoses correlated in all cases. In 21 patients with benign lesions (enchondroma 5, osteoma 2, aneurismal bone cyst 1, fibroma desmoplasticum 1, osteonecrosis 3, osteomyelitis 3, exsostosis cartilaginea 1, fibrous dysplasia 3, connective tissue 1, bone tissue 1) cytologic diagnosis was consistent with benignancy in19 patients. In one case with fibrous dysplasia cytologic finding was suspicious of fibrosarcoma. In one patient with histopathologic diagnosis of bone necrosis, the groups of cells with cytologic criteria of malignancy were found. Cytologic analysis of imprints of bone lesions is valuable for early orientation of the clinicians because the procedure of decalcination needs time but the cytologist must be experienced and provided with detailed clinical and radiological data.

KEYWORDS: Bone Neoplasms; Cytodiagnosis; Histological Techniques

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Histiocytic sarcoma: Clinicopathological and immunohistochemical analysis of two cases

Histiocytic sarcoma (HS)/ true histiocytic lymphoma is an extremely rare malignant histiocytic tumor, with incidence of 0.004% of non-Hodgkin's lymphoma. About one-third of HS are present in lymph nodes. It is usually an aggressive neoplasm with poor response to therapy. We report two cases of HS, both with presentation in lymph node: the first patient, 62-year-old man, with 5cm large lump in the right axilla; and the second patient, 62-year-old woman, with well circumscribed 3cm large mass in the right inguinal region. Staging procedures showed no evidence of disease elsewhere. Excision biopsies of a tumor were performed. The diagnosis was obtained on review of the hematoxylin and eosin-stained slides and immunophenotype data. Both lesions showed similar histological features. The normal architecture was effaced by a diffuse non-cohesive proliferation of pleomorphic neoplastic cells with areas of necrosis, numerous neutrophils and phagocytosis by some tumor cells. The individual neoplastic cells were usually large with abundant eosinophilic cytoplasm, occasionally finely vacuolated, and large vesicular eccentrically placed nuclei. Bizarre multinucleated forms were commonly seen. Mitoses were numerous. Immunohistochemically, the tumor cells stained positively with vimentin and CD68 (histiocytic marker), whereas S-100 was weak and focal positive. They showed no reactivity for CK, EMA, HMB-45, CD45 (LCA), HLA-DR, CD20, CD3, CD15 and CD30. Despite multiagent chemotherapy, the first patient suffered from the relapse at the same place with fatal rapid outcome. The second patient was recently diagnosed and she will be carefully followed up. The cases reported here highlights the diagnostic difficulties encountered clinically and morphologically in patients with HS. Immunophenotyping was essential in discriminating HS from other neoplasm with similar morphological features, such as: large cell lymphoma, undifferentiated carcinoma, Hodgkin's disease, malignant melanoma, malignant fibrous histiocytoma etc.

KEYWORDS: Histiocytic Disorders, Malignant; Sarcoma; Lymph Nodes; Immunohistochemistry; Antigens, CD

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Unusual form of dyserythropoesis in immunocompromized patients

Anemia, thrombocytopenia, lymphocytopenia, monocytopenia, neutropenia and permutations of these abnormalities are found in most immunocompromized patients including the patients with AIDS. Although immune mechanism is a common cause of thrombocytopenia, the majority of other types of cytopenia usually reflect bone marrow dysfunction. We report two cases of unusual form of dyserythropoesis in immunocompromized patients. Both patients presented with profound anemia. Both were females, one HIV+ and the other with previous long history of autoimmune anemia and thrombocytopenia that was treated with immunosuppressive therapy. Bone marrow examination revealed a profound hypoplasia of erythroid cells with dysplastic features. In both biopsies we observed scattered large cells with large round-to-oval nuclei with one or more basophilic nucleoli. Some of these nucleoli resemble intranuclear viral inclusions. There was a moderate amount of basophilic cytoplasm. Those cells resembled atypical lymphoid cells but they were negative for lymphoid markers and positive for glycophorin A. In both cases the strong expression of Epstein-Barr virus LMP antigen was found in those cells. Epstein-Barr virus infection has a high prevalence in immunocompromized patients and may contribute to this unusual morphological abnormality of erythroid cells.

KEYWORDS: Erythroid Progenitor Cells; Red-Cell Aplasia, Pure; Immunocompromised Host; Bone Marrow Cells; Biopsy; Epstain-Barr Virus Infection

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Metastatic tumors of unknown origin: Clinicohistopathological presentation

Unknown primary tumors are unique in that patient has a microscopically confirmed metastatic malignancy but no primary site can be identified on clinical and other investigations. The incidence of cancer of unknown origin is between 0.5%-15%. Metastatic carcinoma from an unknown primary site does not have a characteristic presentation and the frequency of predominant presenting site(s) varies from series to series. Histopathological presentation unknown primary malignant lesions metastatic to lymph nodes and skeletal sites was the initial manifestation. Between August 2000 and November 2003, 15 patients with lymph nodes and skeletal metastasis of unknown origin were evaluated at the Military Hospital of Niš. There were seven men and two women. The youngest patient was 45 years and the oldest 78 years old. The median age was 64 years. Only nine of these patients were referred prior to biopsy with a diagnosis of suspected metastatic carcinoma. Criteria for inclusion in this series were: (1) no previous history of malignancy, (2) biopsy-proven evidence of malignant neoplasm, and (3) no clinical evidence of primary site. Excisional biopsy was performed in all cases. Tumor biopsy specimens were routinely fixed and processed. Deparaffinized sections were stained by H&E. There were 2 inguinal lymph node metastases, 2 axillary lymph nodes, 2 neck lymph nodes, and 3 skeletal metastases. Pathologic subtypes of primary tumors: 1 rectal carcinoma; 2 melanomas; 1 prostate carcinoma; 2 hypernephromas; 1 thyroid carcinoma; 1 urothelial carcinoma; and 1 lung carcinoma. On the basis of this study diagnostic strategy for patients with histopathologically confirmed metastases of unknown origin it is necessary: precise medical history, physical examination, routine laboratory studies, chest radiograph, CT examination of the abdomen and pelvis.

KEYWORDS: Neoplasm Metastasis; Cytodiagnosis; neoplasms, Unknown Primary; Lymphatic Metastasis; Bone Neoplasms

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Radical prostatectomy in the treatment of local prostatic cancer - histopathological evaluation

The aim of the study is histopathological evaluation of radical prostatectomy in the treatment of local prostate cancer. We analyzed 49 radical prostatectomies due to prostate cancer in Clinic of Urology in Clinical Center of Serbia in the period 1996-2000. We analyzed average age of the patients, Gleason score and stage of the tumors and premalignant lesions. We used H2 statistical analysis. In our study average age of the patients was 65,6 years (range 44-76), Gleason score 3 was in 6.1%, 4 in 12.2%, 5 in 8.1%, 6 in 16.3%, 7 in 24.5%, 8 in 26.6% and 9 in 6.1%. Premalignant lesions we found: PIN in 28 cases (57.1%), LG PIN in 10 (20.4%) and HG PIN in 18 cases (36.7%). Stage T2 of the tumors was in 34 cases (68%), $p<0.5$, T3 in 13 (26.5%) and T4 in 2 cases. We suggest that radical prostatectomy is adequate method in the treatment of local prostate cancer.

KEYWORDS: Prostatectomy; Prostatic Neoplasms; Prostatic Intraepithelial Neoplasia; Neoplasm Staging

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Total serum PSA level and prostatic biopsy findings concerning Gleason grade

A level of total PSA (prostatic specific antigen) in serum represents a good clinical and biochemical indicator of possibly existing prostatic adenocarcinoma and has used routinely for years in diagnostic purposes, as well as for postoperative follow up. Ordinarily, a level tPSA up to 4 ng/ml should be considered as normal, with slight difference which follows male age <49 years 0.0 to 2.5 ng/ml, 50 to 59 years 0.0 to 3.5 ng/ml, 60 to 69 years 0.0 to 4.5 ng/ml, 70 to 79 years of age 0.0 to 6.5 ng/ml. Levels between 5 and 10 ng/ml. should be consider suspect for carcinoma and perform additional screening for free PSA level. Level between 10 and 20ng/ml indicate tumor extension beyond capsule, and level above 40 ng/ml indicates existence of bone metastases. As a segment of one ample investigation, we had to determine usefulness of tPSA serum level in prostatic FNA biopsy material, concerning Gleason grade, and age of patient, which is the aim of this study. We examined 371 prostatic biopsies, and selected patients with prostatic carcinoma (152, 41%). All histological types were included. In 59.9% (91) of cases we had the information about tPSA level. Average age of patients with biopsy-diagnosed carcinoma was 69 years; the youngest patient was 39 and the oldest 92 years old. In 92.6% percent of cases a level of serum tPSA was 10ng/ml or more, in 5.3% of cases between 4ng/ml and 9ng/ml, and only in 2.1% of cases was 3ng/ml and lower. The lowest tPSA level detected was 0.45 ng/ml, and the highest 1369 ng/ml. Relative risk (13.297) indicates excellent correlation between tPSA level (10ng/ml or more) and the existence of prostatic carcinoma, with high statistical significance (Mantel-Haenszel test 36.680 $p<0.001$). There is excellent correlation between tPSA serum test sensitivity and specificity (ROC curve for serum tPSA level and existing of prostatic carcinoma). In 13.7% of cases, prostatic carcinoma is graded as Gleason grades I, in 66.7% of cases Gleason grades II and in 19.6% Gleason grades III but we did not detect any statistical significance between serum tPSA in estimated groups on one hand and Gleason grades on the other (Kruskal Wallis Chi-Square=0.51 $p=0.822$). Total serum PSA level determination is useful indicator in prostatic cancer screening but it cannot indicate Gleason grade.

KEYWORDS: Prostatic Neoplasms; Adenocarcinoma; Prostatic Specific Antigen; Biopsy; Neoplasms Staging

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Decoy cells in the urine in the renal transplant recipient: A case report

Polyoma BK virus is nonenveloped, double-stranded DNA virus. Although it was first isolated in 1971 from the urine of renal transplant recipient, it was only recently recognized it's association with renal allograft nephropathy. Renal allograft recipients are at risk of reactivation of polyoma BK virus because of permanent immunosuppression, especially if tacrolimus or mycophenolate mofetil are included. The diagnosis of polyomavirus nephropathy can only be made histologically, and urine cytology is the most sensitive screening method for polyomavirus infection. Here we presented a case of polyoma BK virus infection in a renal transplant recipient, diagnosed by the urine cytology and confirmed by finding BK virus DNA in plasma, detected by polymerase chain reaction (PCR). A 31-year old man with terminal renal insufficiency due to renovascular hypertension, had received a kidney form his mother. Initial and maintenance immunosuppressive treatment was performed using antithymocyte globulin, mycophenolate mofetil, cyclosporine and metilprednisolone/prednisone, with ganciclovir as prophylaxis against cytomegalovirus infection, because both, recipient and donor, had positive antibodies IgG class against cytomegalo virus (CMV). In the first 55 days after the transplantation, the patient had three episodes of cyclosporine nephrotoxicity manifested by clinical and biochemical findings and proved by increased serum levels of cyclosporine. During this period urine cytology also pointed out to renal parenchyma injury because of the finding of vacuolised cytoplasm in renal tubular cells, eosinofils and casts. Clinical signs disappeared on lowered dose of cyclosporine but level of serum creatinine continued to increase because of CMV reactivation, serologically proved. On the day 69 after renal transplantation, the cells with large hyperchromatic nuclei and intranuclear inclusions in some of them were found in the urine sediment, stained by May Grunwald Giemsa method. The infection with polyoma BK virus was suspected which was proved by finding decoy cells with ground-glass type intranuclear inclusions in urine sediment stained by the Papanicolau. In spite of ganciclovir therapy and higher dose of corticosteroids, serum creatinine continued to increase. Because there were no signs of acute rejection of renal allograft, an overimmnunosupression and BK virus infection or nephropathy was suspected, and doses of mycophenolate mofetil and cyclosporine were lowered. Quantitative PCR assay of the plasma detected high values of copies of the viral genome (788 000), one hundred time higher amount of recommended "cut off" level for infection. Biopsy of renal allograft was performed but histopathological examination showed only mild cyclosporine nephrotoxicity, without signs of rejection or BK virus nephropathy. According to these findings, cyclosporine was excluded and immnunosuppression continued with appropriate doses of corticosteroids and mycophenolate mofetil. After that the values of creatinine level were satisfactory and decoy cells disappeared from urine. A month and a half later, in three plasma specimens viruses were not detected, and it was concluded that BK virus infection was successfully treated. Routine screening of urine cytology in renal allograft recipients is very important. It may point to acute rejection, cyclosporine nephrotoxicity and, if decoy cells are present, to polyomavirus replication in the renourinary tract. Finding decoy cells in our patient's urine enabled to discover BK virus infection and to treat the patient in an appropriate way.

KEYWORDS: Kidney Transplantation; Polyomavirus hominis 1; Urine; Cytodiagnosis; Kidney Diseases; Polyomavirus Infections

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Prostatic volume as a corrective factor improving cancer detection rate in patients with PSA below 20 ng/ml

The detection rate of cancer in prostatic biopsies averages about 25%. Patients in whom cancer was ruled out at first biopsy are subjects of growing interest, since it is not yet defined if and when the repeated biopsy should be performed. The improvement of detection rate is important in order to avoid unnecessary first or repeated biopsies. The aim of our work was to find a single or multiple factors, which most strongly influence the ability to detect a cancer in prostatic biopsy. During the 6-year period, we performed 155 TRUS guided sextant biopsies in patients with PSA under 20. The mean PSA was 9.88, ranging from 0.41 to 19. The mean PSA density was 0.30, ranging from 0.03 to 1.06. F/T PSA ratio was performed in 99 patients, the mean index being 0.13, ranging from 0.02 to 0.74. The cancer was detected in biopsy specimens in 39 patients (25%). This group of patients was further stratified in three groups according to the PSA value (under 4, from 4 to 10 and from 10 to 20ng/ml). In subgroups, cancer was diagnosed in 3 (0.13%), 14 (0.22%), and 22 (0.30%) patients, respectively. PSA was significantly different ($p<0.05$) in cancer (mean PSA > 9.36) and noncancerous patients (mean PSA 11.68). PSA did not significantly differ in any subgroup according whether cancer was diagnosed or not ($p>0.05$). There was no difference in F/T PSA ratio in cancer and noncancerous patients. The most significant difference between cancer and non-cancer patients was in PSA density ($p<0.001$). A significant negative correlation was detected between F/T PSA and PSA density ($p<0.001$) in all patients. Introducing prostatic volume through PSA density should be an important corrective factor improving the cancer detection rate. According to our results, if PSA is under 20, prostate volume should be considered before performing a prostatic biopsy.

KEYWORDS: Prostatic Neoplasms; Prostatic-Specific Antigen; Biopsy; Diagnosis

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Prostatic *corpora amylacea*

The localized amyloidosis is a heterogeneous group of disorders with regard to the chemical nature of the amyloid protein, morphology and clinical manifestation, as well as the course and prognosis. This group includes most of the senile forms of amyloidosis, polypeptide hormone-related (AE) amyloidosis associated with endocrine tumors, amyloidosis of the pancreatic islets in diabetes mellitus type II, localized AL amyloidosis, presenting often as amyloidoma, and many other forms. *Corpora amylacea* (CA) in certain organs are also encountered as localized amyloid. Previous studies have shown that prostatic corpora amylacea differ morphologically from those in other organs, including the lung. Therefore, the purpose of this study was to determine the morphology and frequency of prostatic *corpora amylacea*. The specimens of the prostate were selected from 15 autopsies of individuals aged 70 years and older. The paraffin sections were stained with HE, PAS, von Kossa and Congo red methods. The corpora amylacea of the prostate were detected in 9 cases, and localized only in hyperplastic areas. Most of these had a polymorphic shape and irregular inner structure. However, some were of round or ovoid shape and laminated, so that, morphologically, they resembled the pulmonary CA as observed in one patient. The prostatic concretions found in five patients were predominantly localized in the atrophic areas. The mixture of corpus amylaceum and prostatic concretion were also observed. Staining of the prostatic concretions with von Kossa was variable with some concretions being positive and other negative. CA stained positive for PAS reaction. They were Congo red positive and revealed apple-green birefringence under polarized light; no CA stained with von Kossa. The pulmonary CA, as well as some prostatic showed a Maltese cross-like shape birefringence. The obtained results indicate that the prostatic CA are morphologically different, while some have laminated structure, showing a Maltese cross-like shape birefringence as those seen in the lung, which suggests the possibility that not all prostatic CA have the same constitution.

KEYWORDS: Amyloidosis; Prostate; Cytodiagnosis

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Localized amyloidosis of the urinary bladder

Amyloidosis encompasses a heterogeneous group of diseases characterized by the tissue deposition of proteinaceous material with distinctive tinctorial, ultrastructural and conformational features. In the amyloid light-chain (AL) amyloidosis, fibrillar material is composed of the immunoglobulin light-chain fragments. AL amyloidosis usually involves multiple organs and tissues. Localized AL amyloidosis is uncommon. Its etiology and pathogenesis remains obscure. Localized amyloid deposition in the urinary bladder is a rare entity, having a typical presentation, with hematuria and findings mimicking neoplasm. A case of a localized amyloidosis of the urinary bladder in a 45-year-old man is reported. This patient presented with intermittent macrohematuria. Cystoscopic evaluation showed two tumors in the right bladder wall. Pathological examination of the obtained "tumor" tissues revealed large, nodular amorphous eosinophilic material, which was especially prominent in the lamina propria. Characterization of the amyloid protein was performed using PAS, alkaline Congo red, crystal violet, thioflavine T, electron microscopy and immunohistochemical methods. Congo red staining was positive and revealed apple-green birefringence under polarized light. Yellow-green fluorescence with thioflavine T-stained sections was observed in ultraviolet light. Amyloid fibrils were also observed by an electron microscope. Immunohistochemical study showed anti- λ chain staining within the amorphous material. The patient's history, physical examination and laboratory evaluation excluded the involvement of other organs, confirming a diagnosis of localized AL amyloidosis of the urinary bladder.

KEYWORDS: Amyloidosis; Bladder Diseases; Bladder Neoplasms; Diagnosis Differential

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Staging of infiltrative bladder tumors: Comparison between preoperative evaluation and postoperative surgery obtained specimens

We examined the reliability of standard diagnostic procedures (IVP, ultrasound, CT, cystoscopy, TURBT, and bimanual examination) in preoperative tumor staging. From 1997 to 2002, radical cystoprostatectomy was performed in 54 patients (48 men and 6 women; mean age: 62.2 years) with infiltrative bladder cancer (T-2 to T-4a). Pretreatment evaluation of T-stage (TNM 1997) in all cases, using the examined diagnostic procedures separately, was done and compared with postoperative specimens. We assessed diagnostic procedures by establishing the level of diagnostic error. We also established the level of misdiagnosed patients, which could lead to a wrong therapeutic decision (clinically significant error) for every examined diagnostic method. Correlation with final pathologic stage was measured by Pearson's non-parametric test for $p<0.05$.

Table 1. Imaging diagnostic methods

Dg. method	Staging error No (%)	Clinically signific. error No (%)	Pearson's test p<0.05
IVP	30 (55%)	11 (20%)	-0.09
Ultrasound	18 (33%)	6 (11%)	0.48
CT	33 (41%)	2 (4%)	0.35

Table 2. Endoscopic diagnostic methods

Dg. method	Staging error No (%)	Clinically signific. error No (%)	Pearson's test p<0.05
Cystoscopy	29 (54%)	5 (9%)	0.10
TURBT	27 (50%)	0 (0%)	0.45
BE	18 (33%)	3 (6%)	0.76

Using all examined diagnostic methods simultaneously we did not have mistreated patients. As seen in table 1 and 2 CT achieved satisfactory staging accuracy. Other examined imaging methods correlated in majority number of patients with postoperative staging (ultrasound 67%), but with significant number of misdiagnosed patients who could be over or under treated (IVP 37%). We used these methods for upper urinary tract evaluation. Bladder tumor must be confirmed by endoscopic methods (cystoscopy and TURBT). Preoperative TURBT of urinary bladder carcinoma seems to be the most accurate diagnostic tool in preoperative infiltrative bladder tumor staging. None of the evaluated procedures is supreme for staging bladder tumors. Using all examined diagnostic methods we could provide appropriate therapeutic strategy for every patient.

KEYWORDS: Bladder Neoplasms; Neoplasms Staging; Diagnosis; Diagnostic Errors

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Malignant mesothelioma of the tunica vaginalis: A case report

Tunica vaginalis of testis is invagination of peritoneum. The tumors of testicular tunica vaginalis are rare, mostly malignant and with aggressive progression. They are clinically presented as painful or painless enlargements of scrotum that were caused by hydrocoella or solid tumor masses. Patients with these diagnoses are 20 to 75 years old. The tumor can be 0.6 cm to 6 cm. Malignant mesothelioma cells can show histologically epithelial, mesothelial or mixed cell morphology. The patient, 74 years old, was hospitalized due to painless enlargement of right scrotum, which had lasted for several months. Preoperative diagnosis was hydrocele and the surgical treatment was suggested. The material was macroscopically examined and there was found unilocular cystic formation of smooth, tense surface, which consisted of eccentrically located tissue of testis, funiculus spermaticus, soft dark red material of 12 x 9 x 8 cm. The inner surface of the cystic formation had soft, not smooth proliferation of diameter 2 cm. Microscopic examination showed papillary formation with fibrovascular axis covered with one or more layers of atypical cubical or cylindrical cells, oval shaped and light colored, pleomorphic nucleuses and moderate eosinophilic cytoplasm. Mitotic index was 3/10 HPF. Tumor cells were found in fibrous tissue of funiculus spermaticus. The results of imunohistochemical analysis were: mesothelial antigen (-), cytokeratin 8(+), cytokeratin 5/6(+), EMA (+), vimentin (+), CEA (-). Diagnosis was established after histopathological analysis: mesothelioma malignum tunicae vaginalis testis infiltrans funiculi spermatici. Malignant mesothelioma of tunica vaginalis is a rare tumor, mostly diagnosed after histopathological examination of the surgical material. It is necessary to exclude reactive papillary proliferation of mesothelial cells of tunica vaginalis among long lasting scrotal hernias, then carcinoma of rete testis and epydidimis, and also metastatic adenocarcinomas. Anamnesis, clinical examination, routine histopathology, and imunohistochemical analysis enable a diagnosis of malignant mesothelioma of tunica vaginalis testis.

KEYWORDS: Mesothelioma; Testicular Neoplasms; Immunohistochemistry; Microscopy; Hydrocele; Diagnosis

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The type, localization, and total number of rat cardiac mast cells in acute T-2 mycotoxicosis

In previous experiments, we showed that T-2 toxin induced a massive degranulation of mast cells (MCs). The goal of the present experiment was to evaluate the type, localization and number of degranulated MCs. Adult female Wistar rats were poisoned with a single injection of one LD-50 of T-2 toxin (0.18 mg/kg sc). Rats were divided into two groups: (1) control group and (2) T-2 toxin group. T-2 toxin was produced in laboratory conditions from Fusarium sporotrichoides fungi. Animals were killed at the end of the day 1, 3, 5, and 7 of the study. Cardiac MCs were counted in whole visual fields, magnified by 40-fold on paraffin section stained by Giemsa method. In the control group of rats the majority of MCs were tiny and hypogranular. MCs, with discreet granules of the so-called hypergranular MCs, were discovered only in the subepicardium. Only a few of these cells showed degranulation. In the heart of rats treated with T-2 toxin, blood vessels were congested, with thickened walls, and a large number of mononuclear cell infiltrations were present nearby. Total number of the hypergranular MCs was similar to the control group 24 hours after administration of T-2 toxin. However, more than 300 percent of MCs showed degranulation in comparison with the control group. During the one-week period, total number of the hypergranular MCs was similar to the poisoned group of animals sacrificed after 24 hours. After the end of the day 3, the total number of degranulated MCs was decreased by 33 percent in comparison with the ones counted in the first poisoned group. T-2 toxin assured the strongest degranulation of MCs on day 5. Their number was significantly increased (up to 20%) in comparison with the second poisoned group and it was similar to the values on the day 1. At the end of the experiment, hypergranular and MCs that secreted a large amount of granules were diffuse situated in myocardium and endocardium. Minority of these cells was accumulated on the internal wall of the epycardium. T-2 toxin increased by 372 percent the number of these cells in all parts of heart tissue in comparison with the control group of rats. Our results showed that T-2 toxin caused perivascular localization and diffuse tissue accumulation of MCs. Later massive degranulation of MCs probably plays an important role during the acute inflammatory reaction in the heart of rats poisoned by T-2 toxin.

KEYWORDS: T-2 Toxin; Mast Cells; Heart; Rats; Cell Degranulation

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Ultrastructural changes of renal epithelial cells during postmortal autolysis

Determination of schedule and certain predictable regularities of ultrastructural changes of proximal tubular epithelium of kidney during post mortal interval would be very useful in forensic medicine, when it is needed to determine exact time of death. In this research 52 Wistar rats were used. They were killed by choking. Four animals were selected to be a control group right immediately after death, and rest of 48 rats were divided into three equal groups. Rats were than kept on different temperatures: 8˚C-10˚C, 18˚C-20˚C, 28˚C-30˚C, respectively. In each and every group, rats were divided into four subgroups based on time interval after death: 1, 2, 4 and 6 hours. There were four rats in each one of those four subgroups. Preparations have been analyzed and photographed using transmission electronic microscope. It was found that pace of ultrastructural changes of proximal tubular epithelial cells of kidney cortex is directly dependable on duration of autolysis and temperatures that body have been stored at. First changes on nucleus, which are separation of external and internal membranes, occurred during fourth hour of autolysis. Decomposition of external membrane occurred also during fourth hour on temperatures of 8-10˚C and 18-20˚C. When body was stored on 30˚C lysed decomposition of both membranes of nucleus and loss of natural nucleus shape is noticeable even during first hour of autolysis. During sixth hour, nucleus membranes were almost lysed around perimeter and that led to leaking of chromatin in sarkoplasm. Mitochondria kept normal shape six hours after death when body was stored on 8-10˚C and 18-20˚C, and lysis and fragmentation of cristae are noticeable from the first hour of autolysis. Mitochondria lost natural shape and inner composition during first hour when body was stored on 30˚C. Therefore, after fourth hour only balloon like and lightened remaining of mitochondria and fragmented peaces of their cristae were noticeable. All of the predictable changes found in morphological changes on subcells level of kidney tissue can be useful to determine very precisely the time of death. They can be also used to determine vital value of tissue and organs.

KEYWORDS: Kidney; Epithelise cells; Kidney Tubules, Proximal; Autolysis; Death; Time; Rats; Forensic Medicine

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Langerhans cell histiocytosis of the yaw: Clinical and histopathological analyses - a case report

Langerhans cell histiocytosis (LCH) is a rare disease with variable clinical appearance. So far, the etiology of LCH remains unclear. Historical terms for LCH include "histiocytosis X", "eosinophilic granuloma", "Letterer-Siwe disease", "Hand-Schüller-Cristian disease", and others. The key issues are whether the disease is unifocal or multifocal in a single-organ system with or without other "nonrisk" organs involved. The "risk" organs are liver, spleen, bone marrow, and lungs. The mandibulae was more frequently involved in the maxillofacial region. The clinical examination should be confirmed radiologically with magnetic resonance imaging (MRI). Histological analyses should include light and electronic microscopy. Birber's bodies in Langerhans cell confirmed the diagnosis of LCH. The gene codinig for this infolded surface protein has been identified and the protein called "langerin". Now, the diagnosis of the LCH can be confirmed by staining biopsy tissue specimens with anti-langerin, as well as the standard marker anti-CD1a. In the present study we analyzed a 48-year-old woman with recurrence LCH on the left and right side of the yaw and systemic manifestation of LCH in the third left rib. After radiographic (ortopantomograph and MRI) examination showed inflammatory changes with bone erosion on the left and right side of the yaw. The patient has been operated to in the Clinic of Maxillofacial Surgery, Military Medical Academy, Belgrade. During surgical exploration (curettage) fragile, slightly yellowish tissue with perforated cortex areas of the yaw was found. Biberk's bodies were found in Langerhans cells. After primary surgical treatment the patient was treated with postoperative chemotherapy. In conclusion, the patients with systemic manifestation or recurrent LCH, after surgical treatment with curettage for solitary bone lesions should be included into clinical trials initiated by the Histiocytosis Society with postoperative adjuvant chemo or radiation therapy.

KEYWORDS: Histiocytosis, Langerhans-Cell

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Effects of bromadiolone on rodent spleen: A histopathological analysis

Bromadiolone, 3-(3-(4'-bromo (1,1'-biphenyl)-4-yl)-3-hydroxy-1- phenylpropyl)-4-hydroxy-2H-1-Benzopyran-2-one, is one of the most commonly used second-generation anticoagulant rodenticides. It was patented in 1967 for the control of commensal rats and mice, including those resistant to warfarin and first-generation anticoagulants. As well known, 4-hydroxycoumarin derivatives are antagonists of vitamin K. Its use as rodenticide is based on the inhibition of the vitamin K-dependent step in the synthesis of a number of blood coagulation factors, such as prothrombin, proconvertin, Christmas factor and Stuart-Prower factor. The aim of this study was to thoroughly investigate histopathological changes in the spleen after multiple oral application of bromadiolone in rodents, since up to date data are scarce. Adult Wistar rats, weighing 200-250 g, and Swiss mice, (20-24g), of both sex, were used in these experiments. One day before the experiment animals were fasting. The first three consecutive days animals were fed with a ready-to-use baits of low bromadiolone concentration (0.005%). During the rest of the experiment they were fed with standard laboratory food *ad libitum*. They were allowed access to fresh tap water *ad libitum*. Rats were randomly allocated to eighth groups, each of them consisting of 10 animals. Survival rates, general health status and spleen histopathological changes were noticed everyday during the one-week period, starting with 24h after the beginning of the experiment. Spleen paraffin sections were stained by hematoxylin and eosin (HE) as well as by periodic acid-Schiff's (PAS) method. The histological changes detected ranged from degeneration to necrosis of lymphatic tissue accompanied by massive circulatory changes. Fat degeneration, diffuse edema, hyperemia and multifocal hemorrhages were predominant in the spleen of animals sacrificed during the first three days of experiment. Atrophy of lymphoid follicles with a presence of a large number of round or ovoid foam cells in spleen were seen. Thickening of the blood vessels with necrosis of sinusoidal endothelial cells were particularly prominent from the fourth to seventh day of experiment. The most interesting finding is the presence of massive hemorrhages with focal macro-megacariocyte cells infiltrations. The majority of lymphocytes were small, irregular with atrophy of cytoplasm. Large multifocal necrotic fields were noticed in cortical lymphoid follicles of some mice spleen on the day 7 of the study. Our results suggest that multiple oral ingestion of bromadiolone induced irreversible histopathological alterations, such as diffuse hemorrhages as well as atrophy and necrosis of lymphoreticular tissue in rodent spleen, especially mice.

KEYWORDS: Anticoagulants; Rodenticides; Rats; Mice; Spleen; Histological Techniques; 4-Hydroxycoumarins

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Pre-leukemic granulocytic sarcoma of the female genital tract: Report of two cases

Granulocytic sarcoma (GS) with no demonstrable abnormalities in the peripheral blood or bone marrow is a rare but recognized initial manifestation of acute myeloid leukemia (AML). The diagnosis is often difficult, the most common problem being distinction from malignant lymphoma and undifferentiated sarcoma. We report two cases of GS presenting as tumors of female genital tract. The diagnosis of GS in both cases was established by light microscopic and immunohistochemical investigations. An isolated GS of the uterus occurred in the absence of AML in a 67-year-old woman. The myometrium was heavily infiltrated by immature myeloid cells; scattered megakaryocytes were also seen. The tumor exhibited a prominent cellular stroma with fibroblasts, T lymphocytes and follicle-like collections of B-lymphocytes. Peripheral blood and bone marrow were normal. Cytogenetic study of bone marrow revealed no abnormality. Eleven months after operation, the patient developed the AML, M2 type, in bone marrow and blood and died during induction chemotherapy. 41 years old female, was treated with surgery for tumor of the right ovary. The ovary, abdominal lymph nodes and peritoneal tissue were infiltrated by undifferentiated myeloid cells. Two months after the operation she developed AML-M4 in peripheral blood and bone marrow. Cytogenetic study of bone marrow revealed inversion of chromosome 16(p13,q22). She was treated with aggressive chemotherapy and complete remission was achieved.

KEYWORDS: Genital Neoplasms, Female; Leukemia, Myeloid; Sarcoma; Granulocytes; Leukemia, Myelocytic, Acute; Leukemia, Myelomonocytic, Acute

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Pericardial extramedullary hematopoiesis

Extramedullary hematopoiesis (EMH) after fetal development is uncommon and is usually seen in the context of a few well-known clinical conditions. Most often it is associated with hematologic disorders, both neoplastic (myelofibrosis, the spent phase of polycythemia vera, chronic myelomonocytic leukemia, chronic myeloid leukemia) and non-neoplastic (as in thalassemias). It has also been described in association with some tumors: including cerebellar hemangioblastoma, hemangiomas, hepatoblastomas, leiomyomas, pilomatricomas, hepatic angiosarcoma, endometrial carcinoma, mesoblastic nephroma, liposarcoma, miofibroblastic tumors and renal cell carcinoma, and in variety of non-neoplastic environments such as allograft livers , acute tubular necrosis, breast biopsy cavities and cardiac congenital or acquired disease. We present a case of 76-year-old woman with history of hypertension for 20 years and atrial fibrillation during several years, who developed recurrent pericardial effusion. X-ray examinations showed the heart increased due to left ventricle of myopathic configuration, with significant stasis in lung. Echocardiography demonstrated circular pericardial effusion around the whole heart, 2.5 cm in diameter. Subxiphoid pericardiocentesis was sucsessfully performed and 1300 ml of clear, serous fluid was obtained. Pericardial sample was sent on histopathologic analysis. Pericardial fluid was routinely sent for chemical, microbiological and cytological analysis. Biochemical assays and cultures were negative. Cytological examination of the pericardial effusion fluid has shown EMH with clusters of erythroblasts, promyelocytes, myelocytes, megacaryocytes, granulocytes and macrophages. Pathological evaluation did not demonstrated foci of EMH in pericardium, only fibrosis without inflammation. The results of laboratory analysis of peripheral blood were within normal ranges. Bone marrow examination, cytologically and pathologically, has shown hypercellularity, normoblastic erythroid elements, maturation of myeloid elements with increased early forms and regular megakaryocytic cell line. Ultrasound examination of liver, spleen and kidneys has shown no pathological changes. According to all investigations there was no evidence of underlying hematologic disorder. Extramedullary hematopoiesis, the presence of blood-forming cells in the heart, has been only rarely described. Myocardial EMH was observed in patients with myocardial infarcts, dilated cardiomyopathy, congenital heart disease and viral myocarditis. First, the report of Luban et al. suggests that myocardial EMH may be a cause of significant pericardial effusions with hematopoiesis. Extension of myocardial EMH into the pericardium was seen in six cases associated with myocardial infarcts. We made no definitive conclusions about the cause of the presence of hematopoetic stem cells in the pericardial fluid. Can we consider it as are extension of myocardial EMH into the pericardium, based on cardiac disease, or is this a first report of pericardial EMH in adult hearts with acquired disease? Also, we can consider it as the first sign of approaching hematologic disease. We present the case of exramedullary hematopoiesis found in pericardial fluid. The patient had underlying acquired heart disease. We made no definitive conclusion about the cause of the presence of hematopoietic stem cells in the pericardium.

KEYWORDS: Haemopoiesis, Extramedullary; Pericardial Effusion; Heart Diseases

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Autoimmune diseases and non-Hodgkin's lymphoma

Autoimmune diseases (AID) and lymphoid malignancies are related and this association is bi-directional. Non-Hodgkin's lymphomas (NHL) occur more frequently in the course of AID and autoimmune manifestations occur in the course of lymphoid malignancies. An increased incidence of NHL is present in patients with rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome, autoimmune thyroid disease, and coeliac disease. We report 20 cases of NHL (14 of B-cell type, 6 of T-cell type) occurring subsequently to AID. The patients were 17 females and 3 males aged 24 to 72 (median 49). Systemic lupus erythematosus was most frequent preceding autoimmune disease (6 patients). Four patients had Sjogren's syndrome, four had rheumatoid arthritis and coeliac disease was present in four. In seven patients NHL was the first manifestation of underlining AID. The intervals from the onset of AID to that of NHL were 1 to 13 years (median 8). All these patients were treated with immunosuppressive agents and one had been given methotrexate. The possible causal role of the immunosuppressive drugs used in those patients before the development of NHL cannot be excluded from its pathogenesis.

KEYWORDS: Autiimmune Diseases; Lymphoma, Non-Hodkin