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ABSTRACTS

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EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR (EGFR), CYTOKERATIN 7 (CK7) AND P53 IN BARRETT'S OESOPHAGUS AND ESOPHAGEAL ADENOCARCINOMAS

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The overexpression of receptors with kinase tyrosine activity and the markers of glandular differentiation are associated with the possibility of activation of autoregulation mechanisms in neoplastic transformation process. Using the immunoperoxidase test, we studied the expression of EGFR, CK7 and P53 protein in endoscopic biopsy specimens obtained from patients with Barrett's esophagus and suffering from esophageal adenocarcinomas. The preliminary results indicate that EGFR overexpression appeared in the late stages of Barrett's esophagus. The detection of EGFR staining, especially when accompanied by a strong CK7 immunoreactivity, may facilitate the selection of patient subgroup with an increased risk of esophageal adenocarcinoma development. The P53 protein in esophageal adenocarcinomas may be a clinically useful molecular marker for qualifying patients for future clinical studies.

IMMUNOHISTOCHEMICAL ANALYSIS OF KAI1 PROTEIN EXPRESSION IN PRIMARY AND METASTATIC OVARIAN CANCER

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The molecular changes involved in the metastatic process of ovarian cancer are still unclear. The expression of KAI1 metastatic-suppressor gene product was analyzed immunohistochemically in primary ovarian carcinomas and metastatic lesions, considering the histology of the tumors, grade of differentiation and clinical stage of disease. A significant heterogeneity of KAI1 immunostaining was observed, and no correlation with clinico--pathological parameters was found. However, KAI1 expression occurred more often in the early stage of tumors as compared to the advanced stage of disease. The expression of KAI1 was higher in primary ovarian carcinomas than in metastatic lesions. Our preliminary results showed that a decreased KAI1 expression in early stage of tumors indicates that it may be an early event in the progression of ovarian tumors. The increased percentage of KAI1-negative cells in primary advanced ovarian carcinomas and metastases indicates the role of KAI1 expression in progression and metastatic process of ovarian carcinoma.

EXPRESSION OF BAX PROTEIN AND CYTOKERATIN 18 (CK18) NEOEPITOPE (M30) AND CYTOTOXIC EFFECT IN OVARIAN CANCER CELL LINE OvBH–1 BEFORE AND AFTER PHOTODYNAMIC THERAPY

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Photodynamic therapy (PDT) is a well-known method of treatment of tumors and nonneoplastic diseases. In the present study we investigated: 1. the cytotoxic effect of PDT on the ovarian cancer cell line, OvBH–1, which demonstrates p53 protein accumulation in 95% of cells (employing the MTT assay); 2. the expression of BAX protein and cytokeratin 18 (CK18) neoepitope (M30) in OvBH–1 before and after PDT. In our study, OvBH–1 showed to be resistant to PDT during the treatment with hematoporphyrin derivative (HPD) and photofrin II (PhII) at the doses of 10 micrograms/ml of cell medium and the total light doses 21.6J/sq.cm at the fluence rate of 120mW/sq.cm and the established wavelength of 630 +/– 20nm. The rate of surviving cells was then from 90 to 99%. Higher photodynamic doses, i.e. light x sensitizer doses (20 micrograms of HPD or PhII and 43.2J/sq.cm) resulted in an increase in cell mortality, so that the rate of surviving cells decreased from 97 to 1%. This damage was time-dependent, in that after 6 hours following PDT and after 18 hours of incubation with PhII or HPD, the number of cells significantly decreased in comparison with time points 0 and 3 hours. The expression of BAX and M30 was observed in a larger number of cells (10–80% for BAX and 30–70% for M30) after the application of HPD in comparison with PhII and this was found to be irradiation time-dependent. Our preliminary results showed that the presence of BAX protein and CK18 neoepitope after PDT indicated that PDT induced apoptosis in a p53 protein overexpression-independent manner.

MENINGEAL HEMANGIOPERICYTOMA WITH DISTANT BONE METASTASES, A CASE PRESENTATION

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The authors report the case of a 53-year old female with an unusual presentation of meningeal hemangiopericytoma with bone metastases. The patient underwent two consecutive craniotomies with a tumor removal and the diagnosis of meningioma. Two years later, the patient had a local recurrence, another operation and again the diagnosis was meningioma. During a follow-up radiological examination the subsequent year, she was found to have a lesion in one of the ribs on the left side and another one in her right scapula. Histopathology was interpreted as synovial sarcoma and malignant hemangiopericytoma, respectively. Few months later, another lesion was found in the L1 vertebrae. The patient was referred to the Institute of Oncology in Warsaw for more precise diagnostic and therapeutic management. She was treated with both chemo- and radiotherapy.

ASSOCIATION OF E-CADHERIN GENE MUTATIONS WITH HISTOLOGICAL FEATURES OF GASTRIC CARCINOMA

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E-cadherin gene mutations are characteristic for hereditary, familial gastric cancers. In about one fourth of all hereditary, familial gastric cancers germline E-cadherin gene mutation can be found. These cancers are usually characterized by high histological grade (G3) and diffuse growth pattern. E-cadherin

gene mutations occur also in approximately 12% of sporadic gastric cancers. Sporadic gastric carcinomas with E-cadherin mutation usually express the E-cadherin protein, but the immunoreactivity is weaker than in non-neoplastic epithelial cells in the adjacent mucosa. Sporadic tumors with E-cadherin gene mutation show usually G3 histology, but in contrast to hereditary carcinomas this group encompasses diffuse, mixed and intestinal Lauren's types. There was no special region of E-cadherin gene where the sporadic mutations occur more frequently – the mutations were distributed along the entire gene.

LUNG SURFACTANT AND THE FEATURES OF RESPIRATORY SYSTEM MATURATION

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Important components of the peripheral part of the mature lung are the thin air-blood barriers, type II pneumocytes and the surfactant of proper molecular composition. In mature thin air-blood barriers, a capillary vessel is in a contact with the alveolar lumen on both sides of the alveolar septa. The epithelial cells form tight junctions. The epithelial surface should contain two types of pores: small (98.7%) and large (1.3%) ones, which are an important factor in maintaining the para-crystal water interphase. Such water form separates surfactant proteins from sugars bound with integral cell membrane proteins. It plays a significant role in the interaction between surfactant molecules and the cell membrane, which is a key element in lung drying and gas stabilization within the lung. The alveolar and saccular shape and lung compliance for the respiratory function, as well as the capillary resistance (which all have an impact on gas exchange) depend on extracellular matrix composition, e.g. the basement membranes and lung matrix. The features of maturation of the lung periphery include continuous and regular basement membranes, in which a continuous laminin and collagen type IV are observed. Moreover, fibronectin in larger amounts is found in the apical portion of the proliferating septa. Elastic fibers and collagen fibrils are formed in the saccular lung and then form and correlate a network around the alveoli and in higher amount are found in the apical portion of the developing septa. In the stroma of the alveolar septa, a fibrillar form of glycosaminoglycans (GAGs) is found regularly within the basement membranes on the abluminal portion in respect to the alveolar lumen. In the course of maturation, amorphic GAGs are organized as a granular form around collagen fibrils and laminin fibers. The proper lung function is achieved by the regular and systematic development of the aforementioned systems, which are synchronized and affect each other. The above mentioned statements are based on several studies.

MORPHOLOGICAL PICTURE OF THE VESTIBULAR SCHWANNOMA AND THE SIGNS OF FACIAL NERVE PARESIS

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No abstract available.

PROGNOSTIC SIGNIFICANCE OF HPV SEROTYPE 16/18 INFECTION FOR RADIOTHERAPY OF CERVICAL CANCER – *IN SITU* HYBRIDIZATION STUDY USING GentPoint SYSTEM (DAKO Cytomation)

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No abstract available.

IN VITRO STUDIES ON THE INFLUENCE OF QUERCETIN AND CISPLATIN ON THE CELLS OF SELECTED LUNG TUMORS

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No abstract available.

APPLICATION OF IMMUNOHISTOCHEMICAL TESTS FOR SMOOTH MUSCLE ACTIN AND FOR CD34 IN DIFFERENTIAL DIAGNOSIS OF BREAST CANCER

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Differential diagnosis of various forms of adenosis versus tubular carcinoma remains one of difficult problems in breast pathology. Identification of myoepithelial cells with the application of immunohistochemical detection of smooth muscle actin and of protein S–100 is considered to be useful in this respect. However, in practice these methods are sometimes unsatisfactory because in the stroma of tubular cancer multiple myofibroblasts are frequently present. Therefore, simultaneous detection of CD34 in order to evaluate stromal cells is recommended. In our diagnostic material we confirmed usefulness of simultaneous detection of smooth muscle actin and of CD34, especially in differential diagnosis of early breast cancer from so-called radial lesions. In our opinion the demonstration of protein S–100 is not useful in this context.

FLUORESCENCE *IN SITU* HYBRIDIZATION (FISH) IN DIAGNOSTICS OF ACUTE LYMPHOBLASTIC LEUKEMIA (ALL) IN A PATIENT WITH HYPERDIPLOID KARIOTYPE

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Numerical and structural chromosomal abnormalities occur in up to 90% of childhood acute lymphoblastic leukemias (ALL). Two thirds of these abnormalities are recurrent. The most common abnormalities are pseudodiploidy and t(1;19). Hyperdiploidy has the best prognosis, with 80–90% 5-year survival. We report a patient 4 years old, with common-ALL. Cytogenetic studies were performed on bone marrow. We observed abnormal, hyperdiploid, kariotype with structural aberration –60, XY, +X, +del(1)(p22), +3, +4, +6, +10, +14, +15, +16, +17, +18, +18, +21, +22 [28] / 46,XY [9]. Fluorescence *in situ* hybridization (FISH) and RT-PCR analysis revealed the fusion of the genes ***BCR*** and ***ABL*** in 7% of the interphase cells. High hyperdiploidy (51–65) is associated with a better prognosis than that of any other ploidy group. The presence of ***BCR/ABL*** fusion gene placed the patient into a high risk group ALL and treatment with ALLIC 2002 protocol for high risk group was applied. Clinical and hematological remission was achieved on the 33rd day. More sensitive complex cytogenetic analysis involving molecular tests (FISH and RT-PCR) allowed to detect diagnostically and prognostically relevant chromosomal aberrations and to classify the patient to proper treatment group.

COMPARATIVE ANALYSIS OF PATIENTS WITH AND WITHOUT CYTOGENETIC REMISSION, TREATED DUE TO ACUTE MYELOID LEUKEMIA

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Acute myeloid leukemia (AML) is a heterogeneous disease characterized by a wide variety of morphologic, cytogenetic, immunophenotypic and other parameters. A cytogenetic analysis performed at diagnosis is generally recognized as the single most valuable prognostic factor in AML. The characterization of adult patients with AML according to their presentation karyotype provides an important basis for the selection of therapy. Cytogenetic abnormalities were grouped according to the published criteria adopted by the Southwest Oncology Group (SWOG). Four cytogenetic categories were defined. The favorable risk category included patients with abnormalities (abn) of inv(16)/t(16;16)/del(16q) or t(15;17) with any additional abnormalities, or t(8;21) without either del(9q) or being part of a complex karyotype. The presence of del(9q) in patients with t(8;21) leukemia was reported as a poor risk indicator, requiring more aggressive treatment. The intermediate risk category included patients characterized by +8, –Y, +6, del(12p), or normal karyotype. The unfavorable risk category was defined by the presence of one or more of –5/del(5q), –7/del(7q), inv(3q), 11q, 20q, or 21q, del(9q), t(6;9), t(9;22), 17p, or complex karyotype defined as 3 or more abnormalities. The unknown risk category included all the other abnormalities. The examples of karyotypes of AML patients from particular risk categories illustrating the association between the type of aberration and the prognosis and course of treatment were presented.

MYOEPITHELIAL LESIONS OF THE BREAST – THE DIAGNOSTIC PROBLEMS

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Myoepithelial cells are an integral part of the normal histology of the mammary glands. Lesions with striking overgrowth of myoepithelial cells are rare. Histologically and cytologically they may disclose different patterns, and some additional features may result in diagnostic errors. We report two cases of adenomyoepithelioma (tubular variant and spindle cell variant) and one case of adenomyoepithelial adenosis. We analyzed their histologic, cytologic, immunohistochemical features and their differential diagnosis. The tumors were characterized by a bicellular pattern of gland-forming epithelial cells and proliferating myoepithelial cells. They showed foci of monotonous growth of myoepithelial cells devoid of glands with low mitotic rate (1–2 figures/10HPF) or moderate mitotic rate (3–6 figures/l0HPF). Immunohistochemically myoepithelial cells were reactive with SMA, Actin, Calponin, but negative for Desmin. The cytologic features of adenomyoepitheliomas characterized by hypercellularity and clusters of regular polygonal cells with abundant pale cytoplasm (tubular variant) or sheets of spindle cells (spindle cell variant). The nuclei were round to oval with dispersed chromatin and small nucleoli. A failure to recognize adenomyoepithelioma or adenomyoepithelial adenosis may lead to an inappropriate diagnosis such as fibroadenoma, sclerosing adenosis, tubular adenoma, and even invasive carcinoma. Immunohistochemical examination is needed to distinguish epithelial cell proliferation from myoepithelial cell proliferation.

SELECTED IMMUNOHISTOCHEMICAL REACTIONS IN PLEURAL MESOTHELIOMA DIAGNOSTICS

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Pleural mesothelioma belongs to tumors posing diagnostic difficulties in routine and selective staining methods, as in immunohistochemistry. The aim of the study was an assessment of selected immunohistochemical reactions in the group of selected cases of pleural mesothelioma. The material consisted of 16 cases of mesothelioma, diagnosed upon the routine staining. This group consisted of various morphological kinds of diffuse epithelial pleural mesothelioma. The immunohistochemical staining was performed using the primary antibodies: HBME-I, calretinin, TTF-l, CK 5/6. The tissue positive staining patterns were classified as: diffuse, mosaic, focal, and at cellular territory were identified as cytoplasmic, membranous, nuclear. Moreover, the intensity of the positive staining was assessed semiquantitatively. In slides stained with HBME-l the mosaic reaction was predominant (50%), the diffuse staining was present in 30%. In 75% of cases the immunostaining was classified as intensive (+++) or strong (++). In all cases the cellular pattern was membrano-cytoplasmic (mixed). The immunostaining with

calretinin varied greatly without the dominant pattern. The nuclear-cytoplasmic staining was analyzed for the intensity and the extent. TTF-l showed no positive reaction in all but one case. The reaction with CK5/6 showed predominantly mosaic staining in 56%, and was negative in 31%. In the selected group of antibodies, three of them showed high variety in intensity, extent, type of cellular staining. Our studies did not emphasize the selectivity of antibodies for CK5/6 for mesothelial cells.

INFLUENCE OF MUCOID FORMS OF *PSEUDOMONAS AERUGINOSA* ON HUMAN LUNG CELLS

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Gram-negative *Pseudomonas aeruginosa* bacteria are one of microorganisms causing chronic lung infections, particularly in patients with cystic fibrosis or artificially ventilated. Lung infections caused by these opportunistic microorganisms are difficult to eliminate due to drug resistance related not only to the presence of R plasmides, but also to an increase in the form of a biofilm surrounded with a layer of extracellular polysaccharide. The aim of our *in vitro* study was to determine levels of proinflammatory cytokines, essential in action against infections: INF- , IL–12, as well as anti-inflammatory: IL–4 and IL–10 produced by lung cells after stimulating with antigens of mucoid form of *P. aeruginosa* 219. Monolayer culture of lung epithelial cells of a human line A549 in concentration of 10^6/ml was treated with whole bacteria in concentration of 10^6/ml, with bacteria filtrate (100 l) or extract of extracellular polysaccharide (20 g/ml). Immunoenzymatic ELISA test was used to determine the level of IL–4, IL–10 and IL–12. The level of INF- was determined with a biological method. The obtained results showed that lung cells A549 incubated for 24 hours with the solution of raw mucoid extract synthesized only slightly higher amounts of tested cytokines as compared to the cells not treated with antigens. Bacterial filtrate stimulated A549 cells to produce a significant level of INF- (256U/ml) in comparison with the cells not treated with the tested antigens (2U/ml). The level of IL–12 was 10.3pg/ml (control 7.8pg/ml), IL–4 – 6pg/ml (control 4.5pg/ml) and IL–10 was 219pg/ml (control 82pg/ml). In supernatants from above lung cells treated with suspension of living *P. aeruginosa* 219 slightly larger amounts of INF- (284U/ml) than in supernatants from above cells treated with bacterial filtrate were found. The level of IL–12 was also higher and was 19.6pg/ml, but the amounts of IL–10 were the same for filtrate and living bacteria. *P. aeruginosa* 219 induced the production of higher levels of IL–4 but these were not significant differences. On the basis of the obtained results we can state that exopolysaccharide produced by *P. aeruginosa* 219 did not stimulate cell A549 to produce larger amounts of cytokines. The highest levels of proinflammatory factors were induced by living bacteria but numerous products of *P. aeruginosa* 219 (bacterial filtrate) stimulated cells to produce significantly lower amounts of these factors. Pathogenic microorganisms created a lot of ways allowing to avoid or to manipulate with the defensive reactions of the host. Balance disturbances of proinflammatory factors (engaged in quick elimination of infection) and anti-inflammatory factors leading to maintenance of a low level of proinflammatory cytokines can cause chronic infections. The knowledge on interaction mechanisms between the host's cells and microorganisms will allow for introducing of new methods to fight bacterial infections supporting antibiotic therapy.

DO NEW METHODS OF REMOVING OF NEOPLASTIC TISSUE INFLUENCE IMMUNOLOGICAL REACTION?

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The aim of the research was a comparison of synthesis of proinflammatory factors produced by normal lung tissue and lung neoplasms treated by cryodestruction. This method was used in inoperable lesions of the bronchial tree, in which radical treatment was impossible. Provisional improvement of general patient's condition and decrease of complaints were the clinical effects. Cryodestruction procedures of a tumor were performed endoscopically. The available surfaces of a tumor were frozen during 2min-cycles. Twenty-nine patients with non-small cell carcinoma aged 19–77 years (22 men and 7 women) were included in the study group. *In vitro* tests concerned determination of an activity of proinflammatory factors appearing in a frozen tumor in comparison with the level of these factors in frozen normal lung tissue. The level of cytokines in supernatant obtained from above frozen tissue was determined with biological methods: –TNF using the cytopathic effect on the cell line L929; – INF – by the method of EMC virus cytopathic effect inhibition in the cell line A549. Nitrogen oxides were revealed with colometric Ding's method. TNF level in supernatants from both normal and neoplastic lung tissue specimens was low (4–16U/ml). It was shown that in the same conditions INF production was slight, between 2 and 18U/ml; NO_2/NO_3 synthesis ranged from 8 to 36 M/ml. The results show the new method of removing of neoplastic lung tissue – cryodestruction do not result in increased synthesis of inflammatory mediators.

GASTRIC CARCINOMAS WITH LOW LEVEL OF MICROSATELLITE INSTABILITY

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Microsatellite instability (MSI) reflects the degree of damages in particular class of DNA genes – mismatch repair genes (MMR, such as hMSH2, hMLH1, hMSH6, hPMS1, hPMS2 etc.). This phenomenon originally discovered in colorectal carcinomas, subsequently was detected in other types of malignant neoplasms usually of epithelial origin, e.g. endometrioid, gastric, ovarian and pancreas cancers. As the pile of scientific data has been growing, it became evident that there is a "gray zone" of cancers with only small number of microsatellite unstable defects in DNA, which initially seem to fit in-between the traditional division of MSI-stable and MSI-highly unstable carcinomas. Surprisingly, such tumors called MSI-low cancers, in colon showed not "in-between" biology and prognosis. In the stomach, like in the colon, MSI-low carcinomas seem to be quite uncommon however they also form a group of "bad boys": compared with the other two groups of MSI-stable and MSI-high carcinomas, they usually are larger, with particularly high metastatic potential and exceptionally bad prognosis. Although the discovery of low level microsatellite instability in stomach tumors does not mean necessarily that they

form a distinct and homogenous group of gastric carcinomas, the diagnosis of tumors with "MSI-low" hallmark may have some influence on redefinition of surgical procedures and oncological treatment in the future.

FLOW CYTOMETRY RESULTS OF LYMPHOCYTE SUBSETS LEVELS IN NON-SMALL CELL LUNG CANCER PATIENTS TREATED WITH RADIO- AND CHEMOTHERAPY

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Blood samples were collected from 33 patients with III stage non-small cell lung cancer, treated at the Center in the years 2002–2003. In the first group of 22 patients conformal X-ray radiotherapy (20MV) was applied to the tumor area with appropriate margin and on the mediastinum area (lymph nodes). Two systems of fractional doses were used: conventional (5 days a week) and continuous (7 days a week). Medium dose was 70Gy and an average time of the treatment was 50 days. Blood samples were collected before treatment, then once a week and during the 5^{th} week after treatment. Eleven patients were treated with 3 cycles of chemotherapy in a 21-day schedule: 2 patients received Carboplatin (1^{st} day) and Navelbin (2^{nd} and 8^{th} day) injections, while 9 patients received Cisplatin (1^{st} day) and Navelbin (2^{nd} and 8^{th} day). Blood samples were collected before each cycle and on the 1^{st} and 8^{th} day of the treatment. Lymphocyte subsets: T, B, helper/inducer T, suppressor/cytotoxic T and natural killer cells were collected with COULTER EPICS XL flow cytometer using monoclonal antibodies from Becton Dickinson Simultest-IMK Lymphocyte Kit. Non-small cell lung cancer radiotherapy was associated with decreased number of all lymphocyte subsets during the first 2–6 weeks of the treatment (especially during the first phase). The level of leukocytes remained stable. An increased number of human T cells, especially suppressor/cytotoxic T cells at the end of radiotherapy pointed to their faster regeneration. Among the patients treated with chemotherapy a sharp increase of most lymphocyte subsets was noticed. During the breaks between cycles a return to prior values was observed. Both radio- and chemotherapy have a great influence on human lymphocyte subsets levels, depending on their sensitivity to cytotoxic factors, different times of regeneration and humoral factors eliciting subsets proliferation (probably cytokines mobilizing liberation of lymphocytes into patients' blood).

IMMUNOHISTOCHEMICAL ANALYSIS OF THE INTERSTITIAL MAST CELLS IN ACUTE REJECTION OF HUMAN RENAL ALLOGRAFTS

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Acute and chronic rejections are still the major causes of a renal allograft loss despite major advances in immunosuppression and transplant management. Acute rejection has been shown to be the strongest predictive factor of a subsequent chronic rejection. Recent evidence also suggests a role for mast cells in the pathogenesis of renal tubulointerstitial damage in these cases. Morphometric investigations were performed to evaluate whether mast cells could correlate with tubulointerstitial fibrosis in acute renal transplant rejection (ARTR) and to examine the relationship between the mast cells and interstitial -smooth muscle actin (-SMA) expression, as well as interstitial infiltrates. Twenty-four renal allograft biopsy specimens from patients with ARTR, for whom both light and electron microscopy as well as immunofluorescence microscopy and full clinical data were available, were examined quantitatively by means of a computer image analysis system. The specimens had similar histological Banff 97 (IA and IB) scores. As the controls, 11 allograft biopsy specimens from patients without any signs of rejection were used. The morphometric study revealed that the mean values of the interstitial tryptase-positive cells, expression of -SMA, interstitial volume, CD68+, CD43+ and CD20+ cells were in ARTR patients significantly increased in comparison with the control group. In ARTR group, there were significant positive correlations between interstitial tryptase-positive cells and interstitial expression of -SMA, interstitial volume, CD43+ and CD68+. Moreover, CD68-positive cells significantly correlated with interstitial volume The correlation between interstitial tryptase-positive cells and CD20+ cells was positive, but it did not reach a statistical significance. In conclusion, our findings demonstrate that mast cells are one of the constitutive cell types in the interstitium in ARTR. Although significant positive correlations between the interstitial mast cell count and relative interstitial volume, as well as the interstitial expression of -SMA suggest a role of these cells in the development of early interstitial fibrosis in renal allografts, these relationships need further investigations.

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PLACENTAL PATHOLOGY IN MULTIPLE PREGNANCIES

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The multiple pregnancy is a high risk pregnancy. Gross and microscopic evaluation of the placenta is useful to find out the nature of poor perinatal outcomes. From January 1999 to December 2003 in the Department of Reproductive Pathology examinations of 267 placentas from multiple gestations were performed. In placentas derived from twin pregnancies chorionicity was determined by gross and microscopic estimation of the intertwin membrane. Here passed discussion of relations among chorionicity and zygosity. Umbilical cord insertion and a number of umbilical vessels were examined. In monochorionic placentas the number and types of vascular anastomoses were examined using the injection technique. In light microscopy 15 features were evaluated (**for example:** infarcts, intervillous thrombi, fetal vascular thrombosis, fibrin deposits, chorioamnionitis, villous dysmaturity). The placental findings in twin pregnancies were compared with a control group (placentas from single pregnancies) – selected randomly (but matched according to gestational age) from Dept. of Reproductive Pathology database of 2400 placentas. In addition, this present paper describes such phenomena in multiple pregnancies as: *fetus papyraceus* (*vel* vanishing twin), twin reversed arterial perfusion (TRAP) with formationof a acardiac twin, conjoined twin, twin-to-twin transfusion syndrome (TTTS), and their pathological expressions in placenta. The paper describes also morphological changes in placentas from higher multifetal pregnancies (triplets, quadruplets).

CONGENITAL PULMONARY LYMPHANGIECTASIS COEXISTED WITH CONGENITAL HEART DISEASE — TWO CASES REPORT

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Congenital pulmonary lymphangiectasia (CPL) is a rare lesion with numerous separate etiologies. This disorder is characterized by marked dilatation of the subpleural and septal lymphatic channels. The disease is seen almost exclusively in neonatal period and infancy. The lymphangiectasia most frequently coexists with congenital heart disease with, and rarely without, pulmonary venous obstruction. We present the case of CPL with hypoplastic left heart syndrome (HLHS) — mitral stenosis and aortal valve stenosis. The male newborn, 30th week of pregnancy, birth weight 2080g, died 45 minutes after the birth. The autopsy revealed: non-immune fetal hydrops; heart – HLHS with premature closure of *foramen ovale* and endocardial fibroelastosis of the left ventricle and left atrium. The karyotype was normal, male – 46,XY. The second case of CPL was a female newborn, 32nd week of pregnancy, birth weight 1380g, who died 5 hours afterwards. The autopsy revealed the heart with transposition of great arteries and constricted (atrophied) arterial duct. In both cases microscopic examination of the lungs revealed a network of partly tubular, partly cystically enlarged lymphatic channels in the interlobular septa and bronchiovascular bundles. We discuss differential diagnosis and therapeutic implications of this rare pathological condition.

ACARDIAC-TWIN PREGNANCY — PLACENTAL PATHOLOGY, *ACARDIUS* AND NEUROPATHOLOGY OF THE SECOND TWIN. A CASE REPORT

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Acardiac twinning is a very rare complication of multiple pregnancy (1% of monochorionic twins, 1 in 35000 pregnancies). We present the case of a 24-year-old woman, primigravida with preterm labor at 26 weeks of gestation. The normal-looking female newborn (weight 900g) and the *acardius* were delivered by Cesarean section. The placenta weighing 740g was diamniotic-monochorionic. The umbilical cord (of normal twin) with two arteries and one vein was eccentrically inserted, hypertwisted and edematous. The second cord (of *acardius*) had vascular malformation (pseudo-false knot). Histology of the placenta showed dysmaturity of terminal villi, their swelling and presence of numerous Hofbauer's cells within the stroma of terminal villi. The acardiac twin, weighing 1540g, was grossly a round cystic mass with normal-looking skin, lower limbs but without the neck, head and upper extremities. The autopsy revealed the absence of thoracic organs, liver, spleen, pancreas and adrenals; and the presence of large kidneys, small and large intestines. There was omphalocoele with the stomach. A cytogenetic study was performed from skin fibroblasts and revealed normal female karyotype (46,XX). The normal-looking female twin died three days after the delivery. The autopsy revealed lung with hyaline membrane disease, obstructive hypertrophic cardiomyopathy and hepatomegaly. Neuropathological examination showed numerous hypoxic-ischemic lesions dispersed in the cortex and white matter. Foci of necrosis in the cerebral cortex, brainstem and cerebellum were seen. Periventricular germinal matrices were spongiosed bilaterally and accompanied by small foci of hemorrhages. Awareness of the diversity of neuropathological changes in the normal twin described in this report leads to the recommendation that autopsy and neuropathological examination should be carried out in cases of all fetuses and newborns from acardiac-twin pregnancies, including those which appear normal. The monitoring of neonates of such pregnancies for discreet changes in the central nervous system is also advisable.

THREE CASES OF CONGENITAL CYSTIC ADENOMATOID MALFORMATIONS OF THE LUNG

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Congenital adenomatoid cystic malformation (CCAM) is a rare lesion. CCAM is usually found in newborns or very young children. Antenatal diagnosis (by sonography) has been reported. The Stocker's classification (prepared in 1977, modified in 1994) is widely used. We present three cases of CCAM. First case – a baby was born at 27th week of gestation (birth weight was 1220g) in a poor general condition (Apgar score 2) and died 5 days after the birth. The morphological pattern of the left lung (a change occupied two lobes) corresponded to type II CCAM. The second and third cases were type III CCAM – diagnosed in 2 stillborn fetuses: 19th week of pregnancy, birth weight 350g and 24th week of pregnancy, birth weight 820g, respectively. The lesion occupied only one lobe of the lung. For light microscopy the sections were stained with HE, mucicarmine and Masson's trichrome. Immunohistochemical studies were also performed. The following antibodies were used: cytokeratins (cocktail), vimentin, desmin and smooth muscle actin. The differential diagnostics of cystic lung lesions observed in fetuses and newborns is discussed.

PLACENTAL MESENCHYMAL DYSPLASIA: A REPORT OF CASE WITH DIFFERENTIATION FROM PARTIAL HYDATIDIFORM MOLE

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Placental mesenchymal dysplasia (PMD) is a rare recently recognized placental vascular malformation. We report a case of a 23-year-old woman, gravida 1, para 0 with premature labor at 28 weeks gestation. She had first routine antenatal ultrasound scan at 22

weeks, which revealed thickened placenta with multicystic changes ("Swiss cheese" pattern). Amniocentesis showed a normal 46,XX female karyotype, -fetoprotein was 5.7mg/l. Premature labor was spontaneous and she delivered a 930g female infant vaginally. There were no obvious external dysmorphic features. After the following 8 months, the baby was found well with normal development and no features of Beckwith-Wiedemann syndrome were found. The placenta was partially fragmented and weighed 363g (over 90 percentile for 28 weeks of gestation) with the dimension of 160mm 140mm and up to 35mm in thickness. The cord was eccentrically inserted and contained three vessels. On the fetal surface aneurysmally dilated chorionic vessels were not observed macroscopically. On the maternal plate of the placenta there were grape-like, cystic vesicles measuring up to 20mm in diameter. The histology of the placenta showed marked hydropic swelling of the stem villi with cisterns formation. Some stem and terminal villi had focal marked chorioangiomatoid changes. The extramedullary hemopoiesis also was focally present in villi. PMD is most easily confused with a partial hydatidiform mole, clinically and pathologically. Postnatally, careful gross and microscopic examination of the placenta is required to distinguish these two conditions. The present case report highlights the importance to distinguish PMD from partial mole, as a management is entirely different. The need for detailed pathological examination of all abnormal placentas cannot be overemphasized.

USEFULNESS OF p16^{INK4a} ANTIBODY (CINtec^{TM} CYTOLOGY KIT, *DakoCytomation*) IN EVALUATION OF CERVICAL SMEARS WITH ABNORMALITIES OF SQUAMOUS CELLS

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Cytological screening of uterine cervix has been highly efficient to reduce the morbidity and mortality of cervical cancer. An evaluation of cervical smears is affected by a high rate of false-positive and false-negative results. More objective diagnostics parameters and tests are looked for. Cervical dysplasia is induced by persistent infections by high-risk types of human papillomaviruses (HPV). Increasing expression of the viral oncogenes E6 and E7 in dysplastic cervical cells might be reflected by abnormal expression of p16^{INK4a} – cyclin-dependent kinase (CDK) inhibitor, which plays a role in G1-S-phase transition of the cell cycle. We examined the potential role of p16^{INK4a} expression as a marker for cervical lesions by performing immunocytochemistry on cervical smears taken from routine screening (primary) or secondary follow-up after surgical intervention. Immmunocytochemical analysis of the expression of p16^{INK4a} antibody (CINtec^{TM} Cytology Kit, *DakoCytomation*) was performed on 20 normal cervical smears, 40 cervical smears from patients diagnosed with HSIL or CIN3 (histopathological diagnosis after conisation or amputation of the cervix) and 40 smears with "abnormalities of squamous cells" diagnosed only cytologically (10 ASC-US, 10 ASC-H, 20 LSIL). p16^{INK4a} immunoreactivity was absent in 95%(19/20) of normal cervical smears examined. Ninety-five per cent (38/40) of HSIL/CIN3 specimens demonstrated the presence of p16^{INK4a} overexpression. In ASC-US group the expression was various: 7/10 positive in ASC-H, but only 3/10 positive in ASC-US group. 13/20(65%) LSIL samples showed positive staining. Conclusions: these results demonstrate the potential use of p16^{INK4a} as a diagnostic marker for cervical squamous atypical (neoplastic) lesions. The technique can be used to identify individual dyskaryotic cells in cervical smears. Thus, p16^{INK4a} is a useful marker for cervical dyskaryosis.

PDEGF EXPRESSION AND MICROVESSEL DENSITY IN INVASIVE BREAST CANCER IN DIFFERENT AGE GROUPS

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Introduction: Breast cancer most commonly develops in women above 50 years however, 4% of patients are younger than 35 years. The prognosis in young patients (20–30 years) is much worse than in middle-aged women. The causes still remain uncertain. Previous studies showed that the microvessel density in the tumor tissue is an independent prognostic factor of the clinical course and the survival. Aim of the study: The evaluation of the microvessel density and the expression of PDEGF (platelet derived endothelial cell growth factor), a potent pro-angiogenic factor, in two different age groups. Material and methods: Cancer tissue samples obtained from patients below the age of 35 (50 cases) and above 60 years of age (20 cases). In all patients the diagnosis was invasive ductal carcinoma. Anti-CD34 monoclonal antibody was used in order to reveal microvessels. The vessels were counted in high power fields (HPF) under light microscopy within fields of the highest density (the so called "hot spots"). Moreover, slides stained with anti-PDEGF were evaluated in a semi-quantitative manner. The results were compared with the use of Kruskal Wallis test and ANOVA test. Results: The mean microvessel density in the first group was 20.02/HPF. In the second group, the structure and the distribution of capillaries were similar, however, their density ranged between 9 and 28/HPF. The mean microvessel density in ductal carcinoma among the younger age group was higher than among the older group. The intensive angiogenesis was accompanied by an increased PDEGF expression in the cytoplasm of cancer cells. The differences were statistically significant. Conclusions: The obtained results seem to confirm the thesis that the more aggressive clinical course in women under the age of 35 years may result from an increased angiogenesis.

ANALYSIS OF EXPRESSION OF PROTEIN PRODUCTS OF SOME CELL CYCLE REGULATORY GENES IN INVASIVE BREAST CARCINOMAS USING TISSUE MICROARRAY TECHNOLOGY

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The search for prognostic and predictive factors in breast cancer includes the assessment of protein products of cell cycle regulatory genes by immunohistochemistry. The purpose of this study is to assess whether cost and time consumed by such approach can be substantially reduced by tissue microarray technology. To this end the tissue microarray from 200 invasive ductal breast carcinomas has been made and used for immunohistochemistry with antibodies to p21/WAF1, p27/KIP1, p53 and with antibody MIB1. The results

indicate that: (1) preparation of an microarray is a time consuming procedure that requires involvement of experienced pathologist; (2) once a microarray has been made it allows for an easy and inexpensive testing of protein products of cell cycle regulatory genes simultaneously in hundreds of cases.

SNH – A NEW PROGNOSTIC CLINICO-PATHOLOGICAL CLASSIFICATION OF GASTRIC CARCINOMA

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A new prognostic clinico-pathological classification (SNH) is proposed for gastric carcinoma based on tumor site (site-S), status of local lymph nodes (nodes-N), and histopathological features (histopathology-H) of the tumor. The classification stratifies patients into three prognostic groups: Group 1–57% of 4-year survival, group 2–20% and group 3–8%.

SKELETAL STATUS ASSESSED BY QUANTITATIVE ULTRASOUND IN WOMEN WITH ASTHMA ON CORTICOSTEROID THERAPY

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The aim of the study was to assess the skeletal status in female bronchial asthma patients on corticosteroids (CS). Material: 82 female patients (25 with and 57 without fractures; mean age 58.0y.) were compared with 999 females (821 controls without fractures, mean age 58.6y., and 178 females with previous osteoporotic fractures – mean age 57.8y.). The therapy duration was 8.4y. and the daily mean dose of prednisone was 8.8mg. Methods: quantitative ultrasound (QUS) measurement at the heel (Achilles, Lunar). Results: all patient groups had significantly lower QUS values than the controls without fractures and their values did not differ significantly from those characteristic of women with fractures. There was no difference between patients with and without fractures. The ROC analysis was performed to assess the discriminatory capability of heel QUS by calculating the area under the ROC curve, which ranged from 0.68 to 0.70. Conclusion: in the studied females the skeletal status was affected, but did not differ from that in women with fractures.

PROLIFERATIVE INDEX AS A PROGNOSTIC FACTOR IN NEUROBLASTOMA

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Forty-one cases of stroma-poor neuroblastic tumors were evaluated immunohistochemically for Ki–67. The proliferative index (PI) was determined as % of Ki–67 positive tumor cells. The results in correlation with clinical characteristics (previous chemotherapy, age, stage, tumor primary site, bone involvement, LDH and ferritin levels) were assessed. Results: NB tumors showed varying average values of the proliferative index (1.5–79.6%). There was a statistically significant difference between PI in tumors before chemotherapy and after chemotherapy (35% and 23%). Further analyses revealed a lack of significant correlation between PI and age, stage, primary site and bone involvement. PI was, however, higher in children < 1 year old than in those > 1 year (34% vs. 22%). PI was higher in undifferentiated tumors vs. differentiating neuroblastoma. Lower PI values were characteristic of tumors from patients with stage III disease (23.9%) as compared to the other stages (II and IV) (32% vs. 40%) and of those located in the adrenal gland (26%) as compared to the retroperitoneal region (37%). Bone involvement was related to higher PI. Differences between PI values in relation to biochemical markers were slight. The analyses performed in 27/41 tumors from patients before chemotherapy confirmed similar relationships. Among 14 pretreated patients, 3 with high levels of PI > 40% manifested an aggressive course and poor outcome within a year. Conclusions: chemotherapy affects PI values in NB tumors. The proliferative activity should be assessed together with clinical characteristics of the disease. Further studies could evaluate the usefulness of PI as a marker of treatment response.

IMMUNOHISTOCHEMICAL STUDY OF EXPRESSION OF APOPTOTIC AND PROLIFERATIVE MARKERS AND MICROVESSEL DENSITY IN EPENDYMOMAS

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Ependymomas are relatively uncommon tumors of the central nervous system (2–5% of all CNS neoplasms). They have a great tendency to recurrence and spread through the cerebro-spinal fluid despite the fact that their microscopic picture does not demonstrate features of malignancy. Therefore, the prognosis in ependymomas based on classical histopathological features is uncertain. This study presents the results of the investigation of apoptotic and proliferative activity and microvessel density in 50 ependymomas of various histological types (including 5 cases of recurrences). In all tumor samples, immunohistochemical reactions with MIB–1 and PCNA antibodies (for estimation of proliferative index), Bcl–2 and Bax antibodies (for intensity of apoptosis) and CD31 and VIII factor antibodies (for microvessel density) were performed. The results of immunohistochemical studies were estimated quantitatively and analyzed statistically. The analysis revealed that as for the investigated markers, there were no statistically significant differences between various histological types of ependymomas of second grade of malignancy according to WHO classification. However, the proliferative index demonstrated in recurrent ependymomas was higher then in primary tumors.

THE INVESTIGATION OF EGFR AND HER2 EXPRESSION IN NON-SMALL CELL LUNG CARCINOMA

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The purpose of our study was to investigate the expression of epidermal growth factor receptor (EGFR) and HER2 in locally

TABLE 1
The levels of EGFR and HER2 expression in NSCL

Scale	Staining intensity number of patients		Percentage of positive cells number of patients	
	EGFR	HER2	EGFR	HER2
0	25	63	25	68
1	13	17	12	4
2	36	18	23	11
3	26	2	32	15
4	–	–	8	2

TABLE 2
The correlation between staining intensity and histological types

	0		1		2		3	
	EGFR	HER2	EGFR	HER2	EGFR	HER2	EGFR	HER2
Squamous cell carcinoma	11	39	9	13	25	10	17	0
Adeno-carcinoma	5	10	1	2	5	3	4	0
Unclassified carcinoma	9	14	3	2	5	4	3	0
Large cell carcinoma	0	0	0	0	1	1	2	2

TABLE 3
The correlation between percentage of positive cells and histological types

	0 (%)		1 (%)		2 (%)		3 (%)		4 (%)	
	EGFR	HER2	EGFR	HER2	EGFR	HER2	EGFR	HER2	EGFR	HER2
Squamous cell carcinoma	11	41	9	4	11	5	24	11	7	1
Adeno-carcinoma	5	10	1	0	5	1	3	3	1	1
Unclassifie d carcinoma	9	14	2	0	5	5	4	1	0	0
Large cell carcinoma	0	3	0	0	2	0	1	0	0	0

advanced inoperable non-small lung carcinoma (NSCLC) and the correlation between protein expression and histological type. The study was performed on 100 patients with histologically (97) or cytologically (3) diagnosed NSCLC; including squamous cell carcinoma (N=62), adenocarcinoma (N=15), large cell carcinoma (N=3) and unclassified carcinoma (N=20 patients). The percentage of positive cells and staining intensity were assessed by immunohistochemistry. The scale of staining intensity: 0 – negative, 1 – weak, 2 – moderate, 3 – intense. The scale of positive tumor cells: 0 – 0%, 1 – <10%, 2 – >50%, 3 – >75%, 4 – 100%. The results were summarized in Tables 1, 2 and 3.

Conclusion: we did not observe a statistically significant difference in correlation between EGFR and HER2 expression and histological types of NSCLC.

APOPTOTIC INDEX (AI) IN NON-SMALL CELL LUNG CANCER AND ITS CORRELATION WITH OTHER MARKERS

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The significance of apoptosis in cancers as a biological marker, especially as a prognostic factor, has not been clearly established. Therefore, the aim of this study was to assess the apoptotic index (AI), to evaluate the significance of AI as a prognostic factor in non-small cell lung cancer and to correlate AI with p53, mdm2, pRb and p21WAF1/CIP1 protein status in the same tumors. The study group included 50 patients, who underwent pulmonary resection between 1997 and 2000 (41 men and 9 women; aged from 44 to 78, median 62 years; 37 squamous cell carcinomas, 8 adenocarcinomas, 3 large cell cancers and 2 mixed type; 25 stage I, 9 stage II, 14 stage IIIA and 2 stage IIIB). AI was detected with the use of the TUNEL technique and defined as the number of apoptotic cells per 1000 tumor cells. The expression of p53, mdm2, pRb and p21WAF1/CIP1 was assessed immunohistochemically with the use of monoclonal antibodies. Samples showing any nuclear staining were considered positive for all proteins. The mean AI calculated for all 50 patients was 14 and the median AI was 9. According to the mean AI, the patients were divided into two groups: with higher AI (AI>14) and with lower AI (AI<14). Lower and higher AI was found in 35(70%) and 15(30%) cases, respectively. There was no relationship between AI and the clinical characteristics, including age, sex, stage of disease, tumor type and differentiation. There were also no differences between AI and p53, pRb and p21WAF1/CIP1 status; however, lower AI was correlated with the expression of mdm2 protein, whereas higher AI with the absence of mdm2 (Chi^2, p=0.02). The median survival for patients with lower and higher AI was 43 months and 22 months, respectively, and the 5-year survival probability – 60% and 25%, respectively (p=0.049). In multivariate analysis, the only characteristics associated with shortened survival was the apoptotic index (P=0.03, HR=2.6, 95% CI 1.72–3.50). These results suggest that AI correlates with mdm2 protein expression and influences survival in NSCLC patients. However, these results should be proven in a larger group of patients.

ROLE OF METALLOTHIONEIN EXPRESSION IN NON-SMALL CELL LUNG CARCINOMAS

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Metallothionein (MT) is a low molecular weight protein which participates in processes of differentiation and proliferation of normal and neoplastic cells. In certain malignant tumors (breast cancer, renal cancer, ovarian cancer) its augmented expression is thought to represent an unfavorable prognostic index. Non-small cell lung carcinoma (mainly squamous cell or adenocarcinoma) is characterized by difficult to predict prognosis, what poses problems in choosing effective modes of treatment following surgery. The present study aimed

at demonstrating MT expression in non-small cell lung carcinomas of lungs and at attempts to correlate intensity of the expression with grade (G) and intensity of expression of proliferation-associated antigen Ki–67. The studies were performed on archival paraffin blocks containing samples of 25 cases of non-small cell lung carcinoma (5 squamous cell cancers and 20 adenocarcinomas). In paraffin sections of the tumors immunohistochemical reactions were performed using mouse monoclonal antibodies to MT, Ki–67 (DAKO). The investigated antigens were visualized using LSAB2 kit and diaminobenzidine. MT expression was evaluated using the semiquantitative IRS scale of Remmele (0–12 points), which took into account intensity of the color reaction and the number of positive cells. The intensity of Ki–67 expression was expressed by proportion of cells manifesting positive nuclear reaction (1–4 points). The expression of MT and Ki–67 was noted in all examined tumors. The analysis of MT expression, Ki–67 expression and G grade demonstrated strongly positive relation between the two latter variables ($r=0.70$; $p<0.05$). Less pronounced correlations were noted between MT expression and grade ($r=0.44$; $p<0.05$) and between MT expression and expression of Ki–67 ($r=0.41$; $p<0.05$). In addition, survival analysis was performed on 15 cases of the study group, which demonstrated shorter survival of patients with high MT expression. The obtained results corroborated the relation between MT expression and the expression of Ki–67, which points to participation of the protein in proliferation of the cells. In turn, shorter survival of patients with tumors with high MT expression indicates prognostic significance of the protein in non-small cell lung carcinomas.

EXPRESSION OF METALLOTHIONEIN (MT) IN PLEURAL MESOTHELIOMAS

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Pleural mesothelioma represents a relatively rare malignant tumor. In its etiology, exposure to asbestos is of particular significance. The tumor poses difficulties in histopathological diagnosis and in prognostic evaluation. Metallothionein (MT) is a low-molecular weight protein which participates in processes of differentiation and proliferation of normal and neoplastic cells. In some malignant tumors (breast cancer, renal cancer, ovarian cancer) its augmented expression is thought to be an unfavorable prognostic factor. The present study aimed at demonstrating MT expression in mesothelioma cells and at correlating intensity of its expression with expression intensities of proliferation-associated antigens (Ki–67, PCNA). The studies were performed on archival paraffin blocks with samples of 23 cases of epithelioid type malignant pleural mesothelioma. On paraffin sections of the studied tumors immunohistochemical reactions were performed using mouse monoclonal antibodies directed against mesothelin, calretinin (in order to confirm the diagnosis), MT, PCNA, Ki–67. The antigens were visualized using LSAB2 kit and diaminobenzidine. MT expression was evaluated using the semi-quantitative IRS scale according to Remmele (0–12 points), which took into account intensity of the color reaction and the frequency of positive cells. The expression of Ki–67 and PCNA antigens was evaluated using a scale which took into account incidence of cells, which demonstrated color reaction in cell nucleus (1–4 points). The analysis of the obtained results demonstrated expression of mesothelin, calretinin, MT and of proliferation-associated antigens (Ki–67, PCNA) in all study tumors. The most pronounced positive correlation was disclosed between the expression of Ki–67 antigen and of MT ($r=0.82$; $p<0.05$). In the case of correlations between MT and PCNA expressions ($r=0.61$; $p<0.05$) and between Ki–67 and PCNA expressions ($r=0.58$; $p<0.05$) the relation was less pronounced but also positive and significant. The obtained results permit to conclude that in mesothelioma (epitheliod type) MT expression correlates positively with the expression of Ki–67 and PCNA and such correlation may occur for other proliferation markers.

IMMUNOHISTOCHEMICAL EVALUATION OF THE CELLS INFILTRATING THE SKIN IN MYCOSIS FUNGOIDES

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Mycosis fungoides (MF) is the most common primary T-cell lymphoma of the skin. Cell changes in the skin infiltrate during various stages of MF remain poorly understood despite the studies which have led to progress in this area. In this study we compare the immunophenotype of cells infiltrating the skin in various stages of MF and in other chronic skin diseases such as eczema and parapsoriasis. We examined skin biopsies obtained from: 20 patients with chronic non-allergic contact dermatitis, 11 patients with parapsoriasis en plaque and from 52 patients in various stages of MF. The immunohistochemical tests were performed using monoclonal antibodies and EnVision/HRP system (Dako Cytomation, Denmark). An expression of the following antigens was assessed: CD2, CD3, CD4, CD7, CD8, CD20, CD25, CD30, CD45, CD45RO, PCNA and Ki67. The results obtained were evaluated using the Kruskal-Wallis test. In all MF cases, irrespective of stage, high expression of CD2, CD45 and CD45RO was observed, while the expression of CD20 and CD7 was low. Along the progression of the disease the expression of some of the lymphocyte surface antigens, mainly CD3, CD4 and CD5 decreased. In some patients, especially in those who later died of MF, the expression of CD30 was noted. A statistically significant difference was observed between the expression of CD7 and Ki67 in MF and in inflammatory skin diseases. We conclude that the expression of CD7 and of Ki67 can facilitate differential diagnosis of MF versus chronic inflammatory skin diseases. In addition, the expression of CD30 may be indicative of a poor prognosis.

Bcl-xL EXPRESSION AS A PROGNOSTIC FACTOR IN NON-SMALL CELL LUNG CANCER

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The perturbations of programmed cell death caused by an improper genetic regulation are common during carcinogenesis. The Bcl-2 family proteins influence the important part of apoptosis signaling pathway that begins with the caspase

cascade activation. The alterations of genes belonging to the Bcl-2 family may be involved in the pathogenesis of non-small cell lung cancer (NSCLC). The aim of our study was to estimate the expression of the Bcl-xL gene, a member of the Bcl-2 family, in NSCLC patients. A total of 60 patients diagnosed with NSCLC that underwent chemotherapy prior to surgery were reviewed. Bcl-xL expression was assessed on paraffin sections by *in situ* hybridization (ISH) and immunohistochemistry (IMM). In IMM method, the value over 10% of positive cells was considered as overexpression, while in ISH, the expression was assessed as the presence or absence of Bcl-xL mRNA in the examined sample. We observed the presence of mRNA of the Bcl-xL gene and its protein product overexpression in most patients (60% and 81.7%, respectively). In the examined material, no significant correlation was observed between the pattern of Bcl-xL expression and the histological type of tumor, cell differentiation, stage according to TNM classification, performance status according to WHO, number of chemotherapy regimens administered and response to the therapy. The expression of Bcl-xL protein was low (less than 10% of positive cells) in 11 patients (median survival time 29.6 months) as compared to 46 patients with overexpression (median survival time 21.1 months). The difference was not of statistic significance. In the examined group, Bcl-xL mRNA was found in 36 patients, while it was absent in 24 cases. The median survival time was 14.8 months and 74.1 months, respectively (difference statistically significant, p=0.01). The Bcl-xL gene expression assessed on mRNA level correlated with survival time. Our results suggest that in patients with neither Bcl-xL mRNA nor protein expression longer survival time is observed.

MORPHOLOGICAL TRAIT OF FIBROSIS REGRESSION IN PATIENTS WITH HEPATITIS B AND C

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The aim of this study was to examine the degree of fibrosis regression in patients treated for chronic hepatitis, taking into account the frequency of morphological traits of the liver repair complex. We examined biopsies collected from 1236 chronic hepatitis patients treated at the Observation and Contagious Disease Ward of the Provincial Contagious Disease Hospital in Częstochowa, at the VII Department of Infectious and Contagious Diseases, Silesian Medical Academy in Sosnowiec and at the Observation and Contagious Disease Ward of the Provincial Contagious Disease Hospital in Kielce between 2001 and 2004. The population included 10 patients with viral hepatitis B, who were treated with Lamivudine, 12 patients with viral hepatitis C treated with Interferon and Rybavirine, and 1 patient with auto-immunological hepatitis, who was treated immunosuppressively. Morphological traits of the repair complex were found in 23 patients, 6 of which were women and 17 men, whose age ranged between 20 and 71 (44.4). The prevailing trait of the fibrosis regression process appeared to be the presence of vestigial fibrous septa, which was encountered in 18 patients (78%). Secondly, the findings revealed singular and thick bundles of collagen – 16 patients (70%), a radial arrangement of the fibers surrounding the portal spaces – 12 patients (52%), solitary portal veins – 9 patients (39%), and the residue after portal spaces – 3 patients (13%). Following the treatment, the average score of the regression fibrosis decreased by 1.7 points. In 4 patients we found regression of the traits of cirrhosis. Conclusions: in some chronic hepatitis patients we observed fibrosis regression as a result of the applied treatment. The most frequent traits of fibrosis regression were vestigial fibrous septa, singular and thick bundles of collagen, solitary portal veins and a radial arrangement of the fibers surrounding the portal spaces.

MORPHOLOGICAL AND IMMUNOHISTOCHEMICAL ALTERATIONS OF THE RECTAL MUCOSA AFTER HIGH-DOSE SHORT-TERM PREOPERATIVE RADIOTHERAPY

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The purpose of this study was the estimation of morphological and immunohistochemical alterations of the rectal mucosa immediately surrounding the tumor after high-dose short-term preoperative radiotherapy. This study included 39 patients with rectal cancer hospitalized in the Wielkopolska Oncology Center in 2000–2001. Twenty patients received a total dose of radiation equal to 2000–2500cGy during 4–5 days (the test group). In all the cases routine hematoxylin-eosin (HE) staining was done as well as mucicarmine and alcian blue/periodic acid-Schiff staining (AB/paS). Alcian blue (AB) staining was performed at pH 1.0 and pH 2.5. The slides were stained immunohistochemically with mouse anti-human antibodies against p53, Ki–67, CK7, CK20 and CK-MNF116. Relative to the control group, test patients were observed to display an increased degree of inflammation, surface epithelium destruction, atrophied and proliferative crypts after radiotherapy. Also, clusters of eosinophils and small mucus lakes within the atrophied crypts were observed. Atypically enlarged cell nuclei, apoptotic bodies and Paneth's cells were seen in the crypt epithelium. A decreased level of mucus secretion as well as *melanosis coli* was also seen. The results of this study show that the number of goblet cells decreased (p=0.006592) and the percentage of acidic and neutral mucin-secreting cells also decreased (p=0.002733). In the test group an increase in p53 (p=0.000656), Ki–67 (p=0.003757) and of cytokeratins (CK-MNF116) was observed while expression of CK20 decreased. In both groups of patients there was no correlation between p53 and Ki–67 expression (p=0.481104 and p=0.074672).

NON-SMALL CELL LUNG CANCER IN *IN VITRO* STUDIES

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Lung cancer represents one of the most frequent malignant tumors and the most frequent cause of mortality. About 80% of all lung tumors represent non-small cell lung carcinomas (NSCLC) of variable malignancy, reflecting defined histological type and a grade of tumor differentiation. No effective routine chemotherapy has been worked out for the patients since the biological nature of the neoplasm remains unclear. Cell cultures allow for recognition of biological and biochemical properties of the tumor and evaluation of its *in vitro* sensitivity. The latter does correlate well with observed *in vivo* responses to treatment.

Non-small cell lung carcinoma can successfully be cultured *in vitro*. In 115 out of 125 cases (92%) proliferating primary cultures of the solid tumor could be obtained and studies were performed. The studies were focused on various combinations of till now employed drugs, new drugs and on application of adjuvant drugs. At the beginning of the studies tumors sensitivity and resistance were tested to the following cytostatic drugs: cisplatin, epirubicin, vinblastin, vincristin, etoposide, methotrexate, bleomycin, fluorouracil. At the subsequent stage the studies included: cyclophosphamide, methotrexate, fluorouracil, gemcitabine, cisplatin, topotecan, etoposide, paclitaxel (tax), vinblastin, doxorubicin, novantron, mitomycin c. Multiple tests on small-cell lung carcinomas demonstrated low activity of cytostatic drugs. The noted resistance of the tumor to the used cytostatic drugs suggested a need of additional studies. For this reason, subsequent stages of the studies included polyphenols.

THE EXPRESSION OF HORMONAL RECEPTORS IN ORAL PERIPHERAL GIANT CELL GRANULOMAS

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Introduction: Peripheral giant-cell granulomas are benign lesions of the gingival, alveolar and buccal mucosa. They may occur at any age, and are more common in women. In their structure, they contain osteoclast-like giant cells. The etiology of granulomas remains unknown; however, sex hormones may play a role in their development. The aim of the study: the evaluation of hormonal receptors in peripheral giant cell granulomas. Material and methods: seventy-three peripheral giant cell granulomas diagnosed during the period of 1992–2001 (43 men, 30 women). The age of the patents ranged between 7 and 78 years. The peak incidence was in the group below 15y. and in the group aged 45–60 years (20 and 22 cases, respectively). Granulomas were usually located over the mandibular alveolar processes. The duration of symptoms ranged between 1 and 136 weeks. In 6 cases patients reported the history of trauma, in 4 cases tooth extraction, 2 patients suffered from type II diabetes mellitus, and 32 patients presented with poor oral hygiene. The clinical examination revealed parodontosis in 5 patients and oral inflammatory diseases in 15 patients. The expression of estrogen receptors, AB progesterone receptors, and androgen receptors was evaluated with the use of immunohistochemical methods in all the cases. The examinations were performed with Novocastra antibodies following the manufacturer's instruction. Results: a strong, positive nuclear reaction in numerous small mononuclear stromal cells was observed in 23 cases (31.5%), in 18 women and 5 men. A weak nuclear reaction in single small cells was present in 4 cases. A similar weakly positive reaction in some giant cells was obtained in 8 patients. AB progesterone receptors, and androgen receptors were negative in all 73 cases. Conclusions: the presence of estrogen receptors in granuloma cells suggests the possibility of estrogen influence upon these cells, and thus the effect of estrogen on the development of some granulomas. The examinations performed failed to show the presence of progesterone receptors and androgen receptors in peripheral giant cell granulomas.

ACUTE CHANGES IN THE RECTUM AND ANAL CANAL FOLLOWING RADIOTHERAPY FOR RECTAL CARCINOMA

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Background: adjuvant preoperative radiotherapy markedly reduces the risk of local recurrences, and in cases of inoperative tumors allows for a radical excision of the lesion and salvaging the sphincters. Changes observed in the gut have already been quite well described, but the descriptions of changes in the anus are still scarce. The aim of the study: evaluation of the acute post-irradiation changes in the anus and anal canal in patients with rectal carcinoma subjected to preoperative radiotherapy. Material and methods: 36 patients with rectal adenocarcinoma submitted to preoperative radiotherapy; according to the hyper- and hypofractionated protocol. The control group consisted of 8 patients treated by surgery alone. Changes in the epithelial and mesenchymal histological structures of the skin and anal canal were evaluated. Results: acute radiation-induced reactions were found mainly in patients submitted to hyperfractionated irradiation. The most common findings included degenerative changes in the epithelium (macronucleosis and vacuolization), inflammatory infiltrations, para- and dyskeratosis, as well as atrophy of skin adnexa. The internal and external sphincter was usually not affected; however, the mucosal and intramuscular plexus showed degenerative changes.

IMMUNOPHENOTYPING OF PERIPHERAL T-CELL LYMPHOPROLIFERATIVE DISORDERS BY FLOW CYTOMETRY

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IMPATH Inc. New York, USA

T-cell lymphomas comprise a heterogeneous group of lymphoid neoplasms, which are among the most challenging diagnoses in hematopathology. In many cases correlation between clinical data, cytology, histology, phenotyping, cytogenetic and molecular studies is required to establish correct diagnosis. We present flow cytometric phenotyping findings in 240 cases of peripheral (mature/post-thymic) T-cell lymphoproliferative disorders. Multicolor flow cytometric analysis was performed with fresh cell suspension using either 3- and 4-color panels. Immunophenotypic profiles of peripheral T-cell disorders, which included T-PLL (30 cases), T-LGL (41 cases), NK-LGL (16 cases), adult T-cell leukemia/lymphoma (8 cases), Sezary syndrome (9 cases), anaplastic large cell lymphoma (10 cases), gamma/delta T-cell lymphoma (5 cases), hepatosplenic T-cell lymphoma (10 cases), angioimmunoblastic T-cell lymphoma (15 cases), enteropathy T-cell lymphoma (2 cases), extranodal T/NK-cell lymphoma (5 cases) and peripheral T-cell lymphoma, NOS (89 cases) are presented with details. Additionally, immunophenotypic criteria that are most useful in differentiating T-cell lymphomas from atypical (reactive) T-cell populations are discussed.

FLOW CYTOMETRY IN THE DIAGNOSIS OF MEDIASTINAL TUMORS AND PLEURAL EFFUSIONS

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Flow cytometry has become the routine technique in the evaluation of hematopoietic neoplasms. Since the anterior mediastinum and pleura are the frequent sites of involvement by both primary and secondary lymphoma/leukemia, flow cytometry plays an important role in evaluation of mediastinal masses and pleural effusions. The present study reviews 110 flow cytometry cases from patients presenting with mediastinal lesions and 60 cases of effusion specimens. The advantages and disadvantages of flow cytometry in the diagnosis of different hematopoietic neoplasms are discussed. Special emphasis is placed on flow cytometric differentiation of precursor T-lymphoblastic lymphoma from thymic hyperplasia/thymoma. Flow methodology has the advantage of rapid turn-around time, as well as a high sensibility, enabling patients to begin treatment as soon as possible. No false positive results have been encountered. In the experienced hands, flow cytometry plays a valuable and complementary role to histology and immunohistochemistry in diagnosing mediastinal tumors and pleural effusions.

TRANSITIONAL CELL CARCINOMA OF THE RENAL PELVIS OCCURRING IN ASSOCIATION WITH AUTOSOMAL DOMINANT POLYCYSTIC KIDNEY DISEASE

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In this report, we describe transitional cell carcinoma of the renal pelvis in 62-year old man occurring in association with autosomal dominant polycystic kidney disease with a history of two-year hemodialysis. The association of transitional cell carcinoma of the renal pelvis with autosomal dominant polycystic kidney disease is infrequent. To our knowledge, only five such cases have been reported previously.

UNEXPECTED, RARELY DIAGNOSED, AND ATYPICAL MORPHOLOGICAL PICTURES OF PRIMARY LIVER TUMORS

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We presented here our experiences in histopathological diagnosis of primary liver tumors in adults. In the period from January 2002 till the end of 2003 in the Department of Pathology, Medical University of Warsaw we diagnosed 212 primary focal liver lesions and most of them belong to WHO Liver Tumors Classification. The most frequently diagnosed liver neoplasm was hepatocellular carcinoma – HCC (17%). Cholangiocellular carcinoma – CCC was described with nearly the same frequency (15%). Beside HCC with typical morphology we found variants: with macrovesicular steatosis, with pronounced bile secretion, and with many bizarre cells. In 3 cases of HCC the fibrolamellar variant was diagnosed. In one patient collision tumor (coexistence of two separated neoplasms – HCC and CCC) was found. Also in one patient combined tumor characterized by intimately mixed picture of HCC and CCC was diagnosed. In another patient the coexistence of HCC and poorly differentiated sarcoma (carcinosarcoma) was described. In our material we diagnosed 4 hepatocellular adenomas (1.9%). In one of them the differential diagnosis with well-differentiated HCC posed the real difficulties. In the picture of one hepatocellular adenoma the osseous tissue was found. Among the neoplastic proliferations deriving from bile ducts, beside the typical CCC and cholangiocellular adenoma with no clinical significance, more and more often, especially in the women, cystic neoplasms, such as bile duct cystadenoma and bile duct cystadenocarcinoma, are diagnosed. In our material they were diagnosed, respectively in 2.4% and 0.5%. Exceptionally rare papillomatous proliferations of bile duct epithelium develop (two cases). The unquestionable diagnosis of malignancy of these tumors poses very often the big problem for the pathologist. Among non-epithelial neoplasms cavernous haemangiomas were most frequently diagnosed (13.7%). Among other neoplasms deriving from vessels we diagnosed epithelioid heamangioendothelioma in three cases. In one of the patients liver transplantation was performed, the second one is on the waiting list. We described also one case of angiosarcoma and one angiomyolipoma. During analyzed period of time two liver lymphomas, and from 2000 year till now – 5 have been diagnosed. All the neoplasms were diffuse large B-cell lymphomas (DLBCL). One should remember that neoplasms typical for children can also develop in adults. In our material two cases of hepatoblastoma and one embryonal sarcoma and one mesenchymal hamartoma were found. Beside liver tumors described above, in our material the focal liver lesions not included in WHO classification were found. Among them, the first group consisted of the tumors with typical histological picture, but so far not described as the primary liver tumors: endocrine carcinoma (in 3 out of 10 an extrahepatic primary tumor was not found) and adenoid cystic carcinoma (one case). The second group contained described by us for the first time atypical liver lesions, neoplastic (tumor of bimodal differentiation) and non-neoplastic (myoid hamartoma). In two patients occurred huge liver tumors with the picture of macrovesicular steatosis in otherwise healthy liver, which were diagnosed as focal fatty changes (FFC). Focal nodular hyperplasia (FNH) is relatively frequent liver lesion, posing no problems in diagnosis. In our material the cases of it constituted 13.2%, and the indications for the surgical intervention were the following clinical signs: large tumor size or the suspicion of malignancy. The possibility of coexistence of FNH with other tumors is very interesting; we have found 6 such cases (with haemangiomas, adenomas, and one case with embryonal sarcoma). Among parasitic lesions the most often diagnosed was echinococcal cyst (usually diagnosed serologically). In our material we described 18 such cases (8.5%): *Echinococcus granulosus* and *Echinococcus multilocularis*. The last one was described 3 times, one patient had transplantation and the second one is on the waiting list. One should remember that among the typical non problematic focal liver lesions more and more often pathologists face rare and unexpected morphological pictures. Some of them one does not even find in actual classification of liver tumors and their clinical course is usually unpredictable. They demand the big experience and a special attention.

MORPHOLOGICAL FEATURES AND IMMUNOHISTOCHEMICAL EXAMINATION OF INFLAMMATORY PSEUDOTUMORS (IPTs) OF THE LUNG IN CHILDREN AND ADULTS

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Introduction: inflammatory pseudotumor (IPT) of the lung is a rare benign lesion, clinically and radiologically resembling a neo-

plasm. Its diagnosis and therapy can be difficult. The histogenesis and nature of IPT remain uncertain. The principal site of IPT is the lung, but it can also occur elsewhere in various other anatomic locations. The incidence of this lesion in children is significant, representing 20% of all primary lung tumors, in adults it is rare, accounting for about 1%. IPT is composed of spindle mesenchymal cells (fibroblasts, myofibroblasts) admixed with a prominent inflammatory infiltrate consisting of plasma cells, lymphocytes, histiocytes and macrophages. The mixture of these components is varied, and has allowed for dividing the lesions into three categories: fibrohistiocytic, lymphoplasmocytic and organizing pneumonia-like (named also BOOP-like). Individual cases may have microscopic features that overlap more than one group. This entity is generally regarded as a reactive process. However, some of clinical and pathological aspects have begun to suggest the possibility that these lesions are more similar to neoplasms than a post-inflammatory process. The aggressive features, such as vascular invasion, infiltrative local growth, recurrence, cytogenetic clonal aberrations and sometimes histological similarity to a spindle cell sarcoma, fibrous histiocytoma or fibrosarcoma, have been described. The aim of the study was the estimation of morphological features and immunophenotype of inflammatory pseudotumor of the lung. Material and methods: we carried out a histopathological analysis of 10 cases of IPTs. There were 6 patients, age range 22 to 71 years, and 4 children. All patients were admitted to hospital because of an abnormal shadow or mass found incidentally on chest X-ray, resembling a neoplasm of the lung. We evaluated the size of tumors, histopathological features, involvement of the pleura and invasion of pulmonary blood vessels, and determined the immunophenotype of ITPs using monoclonal antibodies against vimentin, SMA, desmin, CD68, light chains and light chains of immunoglobulin. Results: three patients underwent lobectomy, four – local excision, and 3 partial resection. The lesions ranged from 1.5 to 6cm in size. Two of the tumors involved the pleura and invaded the pulmonary blood vessels. Depending on the major histopathological features, the cases were divided into two groups: fibrohistiocytic type – 4 cases, and mixed type (fibrohistiocytic and lymphoplasmocytic) – 6 cases. The spindle cells displayed immunoreactivity for smooth muscle actin and vimentin in all cases, and focally for desmin in 3 cases. Immunohistochemical studies demonstrated a positive reaction for CD68 in all cases and polyclonal nature of the plasma cells. Conclusions**:** IPTs are rare entities, usually found incidentally in routine chest radiography. An accurate preoperative diagnosis is difficult. Vascular invasion and involvement of the pleura are seen, suggesting a more aggressive course of disease.

MORPHOLOGICAL CHANGES OF LYMPH NODES IN PATIENT TREATED WITH METHOTREXATE. A REPORT OF THREE CASES

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The recent WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues distinguishes a group of methotrexate-associated lymphoproliferative disorders as a group of changes in lymph nodes observed in patients treated with methotrexate. The authors describe the cases of 3 patients diagnosed because of lymph node enlargement and treated with methotrexate. The aim of this presentation is to show morphological pictures of lymph nodes and the results of immunophenotyping.

VILLOGLANDULAR ADENOCARCINOMA (VGA) OF THE UTERINE CERVIX AND ENDOMETRIUM: A REPORT OF TWO CASES WITH HISTOLOGICAL FINDINGS AND IMMUNOPROFILE

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Villoglandular papillary adenocarcinoma (VGA) of the uterine cervix was reported by Young and Scully in 1989 as a rare distinctive histological entity, which developed in relatively young women and had a favorable prognosis. While papillary endometrioid or villoglandular adenocarcinoma is a relatively common type of endometrial adenocarcinoma, studies describing its behavior have yielded conflicting results. We report two cases of papillary villoglandular tumors with their clinicopathologic features and immunohistochemical profiles. The first case is VGA of the uterine cervix detected in a 48-year-old woman who underwent a total hysterectomy and is alive and well with no evidence of recurrent disease after 2 years from diagnosis. The second one is VGA of the endometrium in a 56-year-old woman who underwent a total hysterectomy and pelvic lymphadenectomy. We present microscopic findings with a predominantly villoglandular papillary growth pattern and the immunohistochemical profile of both tumors with anti-PCNA, anti-Ki–67, anti-p53, anti-CD34, anti-c-erbB–2 and anti-bcl–2 antibodies. Our report and review of the literature suggest that villoglandular adenocarcinoma of the uterine cervix is often well differentiated, appears to have an indolent behavior with low metastatic capability and is usually associated with a favorable prognosis. The same type of cancer existing in the endometrium is often admixed with typical endometrioid adenocarcinoma (EA). In view of this frequent admixture and generally similar biological characteristics and prognosis, it is suggested that endometrial VGA should be considered a variant of EA.

PAPILLARY TRANSITIONAL CELL CARCINOMA OF THE UTERINE CERVIX (TCC): A CASE REPORT WITH EMPHASIS ON POTENTIAL DIAGNOSTIC PITFALLS

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Papillary transitional cell carcinoma (TCC) is rare in the female genital tract and it often resembles urothelial carcinomas of the urinary tract. Transitional cell carcinoma is most common in the ovary, less common in the fallopian tube, and only small series of cases in the cervix have been reported. Since its original description, it has been considered a distinct clinicopathologic entity with a morphologic spectrum formed by squamous cells at one end and transitional cells at the other. It remains unclear whether papillary carcinomas of the cervix represent two clinicopathologically distinct groups of tumors (squamous and transitional). We present the clinical, pathological and immunohistochemical features of transitional cell carcinoma of the uterine cervix in a 66-year-old woman. We describe the tumor with a distinctive surface papillary growth pattern, a similarity to

papillary transitional carcinoma of the urinary tract and a tendency to be deeply invasive. The immunohistochemical profile with antibodies against PCNA, Ki–67, cytokeratin 7 and cytokeratin 20 has been determined. We believe these transitional cell neoplasms further emphasize the potentialities of the cervical epithelium, thus, the recognition of subtle histological features and clinical correlations are essential in a correct diagnosis.

NEW MOLECULAR PATHOLOGICAL FINDINGS IN HEMATOPATHOLOGY

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No abstract available.

COINCIDENCE OF GASTROINTESTINAL STROMAL TUMORS (GIST) AND OTHER NEOPLASMS

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Aim of the study: clinical and morphological analysis of simultaneous gastrointestinal stromal tumors (GIST) and other benign and malignant tumors. Material and methods: patients with mesenchymal gastrointestinal neoplasms were selected from archives encompassing years 1989–2002. Tumors fulfilling the criteria of gastrointestinal stromal tumors (GIST), with typical histological pattern and positive reaction with the CD117a antibody were included into the study. In each case of GIST the department database was searched for the other tumors diagnosed in these same patients. Results: seventy four gastrointestinal stromal tumors were diagnosed during the period of 1989–2002. Sixty tumors fulfilled the criteria of stromal tumors (GIST). The mean age of the patients was 55.0 years (range: 10–89 years). The diameter of the tumors ranged from 0.7 to 35cm. In 4 cases GIST coexisted with gastric cancer, in 6 cases with colon carcinoma and in one with colon adenoma, in 3 cases with pancreatic tumor (2 carcinomas, and one cystadenoma). There were single adenomas in the colon and, in one patient – myelogenous leukemia. Additionally, in 2 cases the uterine leiomyomas and STH secreting hypophyseal adenoma were noted. Conclusions: a coincidence of stromal tumors with other neoplasms was found in 28.3% of cases. In most cases there were epithelial benign and malignant gastrointestinal neoplasms. These results confirm the suggestion of other authors about the possible simultaneous influence of carcinogens on the gastrointestinal mesenchymal tissue.

IMMUNOHISTOCHEMICAL PROFILING OF ADENOCARCINOMAS OF KNOWN AND UNKNOWN ORIGIN USING A TISSUE MICROARRAY

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The immunohistochemical profiles of tumors are often used in routine pathology departments to aid differential diagnosis, to evaluate the stage of the tumor and to help clinicians to form a prognosis for the patient. In some cases a tumor has no known origin – that is, it's immunohistochemical profile does not match or even approximate that of the surrounding tissue while at the same time it's morphology does not suggest any other organ system as being the original source. The immunohistochemical profile of such cases can be used to form a "best guess" diagnosis in order that patients' treatment may commence. In this study we test the Tissue Microarray (TMA) technology by comparison of the immunohistochemical profiles of 40 adenocarcinomas, including 20 cases of unknown origin. The TMA includes triplicate tissue cores from each case to ensure adequate tissue representation. The immunohistochemical profile under study includes antibodies to cytokeratins, tissue and tumor markers including: CAM 5.2, CK AE–1/AE3, CK7, CK20, EMA, Vimentin, CEA, FP, Mesothelial marker HBME–1, Hepatocyte marker OCH1E5, PSA, TTF–1 and GCDFP–15. The aims of this study are to assess the suitability of TMA technology for this type of study and to attempt to identify the origins of the unknown tumors.

A CASE OF INTESTINAL STROMAL TUMOR WITH DISSEMINATION TO THE LIVER

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The small intestine is a part of the gastrointestinal tract that is very rarely a site of primary neoplasms of both epithelial and non-epithelial origins. Among them, mesenchymal tumors, especially stromal tumors (GISTs), constitute a quite large group. According to Miettinen's report, GIST is a cKIT (CD117) positive neoplasm of unpredictable behavior, composed of spindle cells, epithelioid cells or both (mixed pattern), located anywhere in the GI tract or sometimes outside it, inside the abdominal cavity or retroperitoneally. The aim of the study was to present a case of a malignant stromal tumor of the small intestine with numerous metastases to the liver at the time of diagnosis. In a 46-year-old man, a tumor located in the wall of the small intestine was detected. The tumor measured 5cm in diameter and extended intraluminally, causing mucosal ulceration. Within the liver there were 7 metastatic foci measuring about 1cm in diameter. After intraoperative diagnosis, the tumor was resected and paraffin slides were prepared. The slides were stained with HE and immunohistochemical analysis was performed using antibodies against oncoprotein c-kit (CD117), CD34, desmin, SMA and S–100. Grossly, the primary tumor was solid, gray-white on the cut section with multiple hemorrhagic foci. Light microscopy revealed a mixed pattern tumor with the prevalence of spindle cells and slight cellular pleomorphism. Numerous mitotic figures; much more than 10/50HPF, were present. The metastatic tumors within the liver were solid and white. Microscopically, they were spindle cell type with a low mitotic activity. Immunohistochemistry revealed that neoplastic cells were strongly and diffusely positive for CD117. It allowed for confirming the diagnosis of GIST in both the primary tumor and in all metastatic foci. The reactions with CD34 and S–100 were also strong and diffuse. Only some neoplastic cells were positive for SMA, no cells were positive for desmin. The results of our study confirm the data from Miettinen's report that GISTs of the small intestine, especially these with the following features: size more than 5cm, mucosal invasion, ischemic

necrosis and high mitotic count (more than 5/50HPF), easily metastasize to organs situated within the abdominal cavity, among them to the liver. Small intestinal GISTs more frequently behave as malignant tumors than GISTs involving other sites in the GI tract, abdominal and retroperitoneal cavities.

VASCULAR PATTERNS IN NEUROBLASTOMA

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Vascularization in neuroblastic tumors is an infrequent subject of investigations, despite of the accepted classifications, where the quantity of tumor stroma is one of diagnostic criteria. Sixty-five neuroblastomas (NB) were examined, including 49 cases of stroma-poor type (9 undifferentiated UN, 16 poorly differentiated PD and 24 differentiating DF) and 16 cases of stroma-rich ganglioneuroblastoma (GNB; mixed type). A type of vascular pattern and vascular changes were evaluated in routinely stained slides (HE). Three types of vascularization were observed: reticular, trabecular and irregular. In the reticular type, the fibrovascular stroma forms a delicate meshwork surrounding small tumor nests; in the trabecular variant, a thick, band-like fibrous stroma separates tumor sheets. The reticular pattern was found in 15 NB cases (10 PD, 5 DF); trabecular – 13 (1 UN, 3 PD, 9 DF) and irregular – 37 (including all GNB). Basic vascular changes consisted of fibrosis, hyalinization, lumen widening and microvascular proliferation (MVP) – from slight to severe with glomeruloid tufts. MVP was observed in 22 cases (2 UN, 3 PD, 15 DF, 2 GNB) with glomeruloid form in 15 (including 10 DF). Fibrosis and hyalinization dominated in GNB and in post-chemotherapy cases. An evident collocalization of beginning/developing neuromatous stroma formation and vascular framework was encountered. Because of the pathological complexity of neuroblastoma, its vascular patterns are diversified. While observing tumors with consecutive maturation type from NB UN to GNB, the striking features are: 1) remodeling of the pattern: irregular stroma-poor > reticular trabecular > irregular stroma-rich, and 2) MVP in 30% of the examined group with the highest intensity in NB DF type.

LOH ON CHROMOSOME 10q AND CHOSEN CLINICO-PATHOLOGICAL FEATURES OF GLIOBLASTOMA

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The study was performed to establish the frequency of LOH on chromosome 10q in glioblastoma and to search for its relations to tumor morphology and microvascular density. Thirty cases of glioblastoma were examined. A predominant cell type and type of microvascular proliferation were established histologically. The microvessels immunostained for CD34 were calculated to assess vascular density. The representative neoplastic tissue and normal brain tissue were cut out with lancet from respective paraffin blocks. DNA was isolated with proteinase K with phenol/chloroform extraction and isopropanol precipitation. Polymorphic markers were localized close to suppressor genes known to be lost or mutated in glioblastoma – PTEN (D10S1765, D10S215), LGI1 (D10S1680), DMBT1 (D10S587). The fifth marker was D10S607 10q22.2. Fragments were PCR-amplified and analysed using automated sequencer. Signals from neoplastic and normal tissue were calculated and result below 0.6 was scored as LOH. In 14 cases at least one marker from chromosome 10q was lost (46.7%) and among them were 11 men with LOH. The mean patients' age with and without 10qLOH was similar. Five cases showed LOH in PTEN locus only. In 9 cases deletions were found in two or more loci, what probably points to the loss of longer part of chromosome 10. No relations to the tumor location nor to tumor cell type were found. Non-significant correlation was observed between LOH 10 and lower vascular density. LOH on 10q was found in about 50% of glioblastomas. In the study group LOH 10q was significantly more frequent in men (64% vs. 23%).

CENTRAL NEUROCYTOMA – CLINICAL, MORPHOLOGICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL ANALYSIS OF THREE CASES

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The authors present three cases of central neurocytoma – a rare, benign tumor of central nervous system and describe its clinical course, radiological data and histopathological, immunohistochemical and ultrastructural analysis. Two of three patients were males aged 45 and 41, and one patient was female 35 years old. Histologically the tumors were composed of isomorphous small round or ovoid oligodendroglioma-like cells alternating with irregularly shaped patches of fibrillar matrix similar to the neuropile. Multiple focal calcifications were present in one of the tumors. Immunohistochemical examination showed prominent synaptophysin positivity in all cases distinguishing investigated tumors from ependymomas and oligodendrogliomas. The immunoreactivity for neurofilaments and neuron-specific enolase was also marked in all cases and in two of three tumors glial fibrillar acidic protein positivity of single cells was visible. Reaction for chromogranin was always negative.

EVALUATION OF THE PERIPHERAL PART OF ADENOID CYSTIC CARCINOMA

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Adenoid cystic carcinoma (ACC) is a slowly growing tumor with a tendency to infiltrate soft tissues, nerves, and bones. It has propensity for local recurrence and for distal metastases. The lymph nodes metastases are rare. ACC occurs in large and small salivary glands (6% of all malignant tumors in this location). Histologically, three growth patterns are recognized: tubular, cribriform, and solid, but most commonly mixed patterns are observed. The earlier electron microscopic studies revealed the following cell types, which compose the tumor: cells forming intercalated ducts (found in all patterns), secretory cells (mainly found in the tubular pattern), cells resembling pluripotential cells (most numerous in the solid pattern) and myoepithelial cells (rare in the solid pattern). The aim of the present study was the evaluation of the peripheral part of ACC. The study group included 26 patients with ACC. All the studied tumors were evaluated using light and electron microscopy (TEM). Additionally, immunohistochemical (IHC) evaluation of fibronectin and laminin expression within the

tumor stroma was performed. In all cases ACC was of the mixed pattern, which was confirmed by TEM. In all patients the tumors were removed radically with margins of normal tissue. In the peripheral parts, there was lack of histoformative texture of the tumor. The tumor cells far to the periphery were isolated and situated at a long distance from vessels. They were accompanied by fibrillar stromal structures, such as collagen fibrils and fibronectin, which were surrounded by fibrillar forms of glycosaminoglycans. The IHC expression of laminin in the peripheral and central parts of the tumor was similar. A positive regular reaction was seen in the basement membranes of the capillaries. A linear and branching pattern of the IHC reaction was found in the areas of tumor cell proliferation, but the tumor cells were most commonly not encircled. The IHC reaction for fibronectin was more complicated. The strongest reaction was in the cribriform pattern. A positive reaction was observed as delicate or faint, ramified with a predilection to occur within the capillary wall; in the peripheral parts of the tumor it was seen as irregular but fairly positive. The detailed study of the tumor periphery leads to a conclusion that the tumor has a compact structure and only sporadically and focally distant infiltrating cells accompanied by fibronectin and collagen fibrils can be observed.

ASSESSMENT OF USEFULNESS OF THE MIB–1 ANTIBODY IN DIFFERENTIATION BETWEEN GRADE II AND III OR GRADE III AND IV GLIOMAS

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Tumors of the central nervous system (CNS) have a limited space to grow. Therefore, their proliferative activity directly affects the clinical course of the disease and the prognosis. The quantitative assessment of that process is very useful in the prediction of biological behavior of individual CNS tumors. The proliferative activity is especially important in cases of gliomas of the CNS, which have a tendency to progress. Information about cell kinetics is a useful adjunct to the histology-based tumor classification. Differentiation between GII and GIII is especially important because of different ways of treatment. The aim of the study was the assessment of the usefulness of the MIB–1 antibody in differentiation of gliomas with WHO grade II vs. III or III vs. IV in each lineage. The proliferative activity was assessed in 101 cases of glioma of the CNS representing 12 different subtypes. Tumor specimens were obtained from patients hospitalized in the Department of Neurosurgery and Pediatric Neurosurgery of the University Medical School in Lublin between January 1995 and May 2000. They had been routinely processed into paraffin blocks. Immunohistochemical staining, with the mouse monoclonal antibody MIB–1 against the Ki–67 antigen, was done by using streptavidin-biotin method. There were differences of MIB–1 LI values in the following pairs: 1) grade II vs. III among astrocytic gliomas (Kolmogorov-Smirnov's test, $p<0.01$); 2) grade III vs. IV among astrocytic gliomas ($p<0.05$); 3) grade II vs. III among oligodendrocytic gliomas ($p<0.01$); 4) grade II vs. III among oligoastrocytic gliomas ($p<0.05$). However, no differences in MIB–1 LI values were found between grade II and III ependymal gliomas. Conclusion: on the strength of the statistical analysis, the assessment of proliferative activity in individual subtypes of gliomas of the CNS allowed for demonstrating a high usefulness of the MIB–1 antibody in routine neurooncology.

GRANULAR CELL ASTROCYTOMA – A CASE REPORT

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Granular cell tumors are a well-known group of neoplasms. The tumors have a common pathological hallmark – the eosinophilic granular bodies. They are generally benign neoplasms in different locations, more frequent in the skin, oral cavity and also in the abdominal organs. They also arise in the nervous system, namely in the pituitary gland (their origin is probably the pituicyte) and in the cranial and peripheral nerves (which originate from the Schwannian cells), as well as in the cerebral hemispheres. The latter usually contain an astrocytic component (low- or high-grade). The grade of malignancy of the tumors depends on the non-granular astrocytic component. A 50-year-old woman was admitted to the hospital because of speech deterioration persisting for the period of two weeks. CT scan of the head showed a large tumor (4 3 2.5cm) involving the right and left frontal lobes, with heterogeneous contrast enhancement and mass effect. The tumor was well circumscribed. Craniotomy and tumor resection was performed. The condition of the patient worsened after operation and she died. The tumor tissue was fixed in 10% buffered formalin and embedded in paraffin. Sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff (paS), mucicarmine and Sudan IV. Paraffin sections were also immunostained with the following antibodies: GFAP, synaptophysin, S–100, CK, EMA, vimentin, HMB–45, CD68. The mitotic index was also established. The remaining paraffin-embedded tissue was recovered for electron microscopy. Histologically, the tumor consisted of neoplastic astrocytic cells (high-grade) and granular cells. Conclusion: multiple cells have been suggested as the origin of granular cell tumors (e.g. striated muscle fibers, fibroblasts, histiocytes, Schwann cells). The origin of cerebral hemispheric granular cell tumors is a matter of controversy. The occurrence of these tumors in the central nervous system lessens the likelihood of a Schwannian source in all instances, but an origin from Schwann cells of the perivascular nerves cannot be excluded. Microscopic and immunohistochemical examinations favor the glial origin of the tumor. It is also possible that granular cell tumors have more than one cell of origin. The prognosis of astrocytic granular cell tumors is poorer than in their pure astrocytoma counterparts.

THE CASES OF B-CELL LYMPHOCYTIC LEUKEMIA RECOGNIZED IN LYMPH NODES EXCISED DURING SURGICAL TREATMENT IN PATIENTS WITH OTHER NEOPLASMS

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The aim of the presentation is to draw attention to changes in lymph nodes, other than neoplastic metastases. Leukemic infiltration of lymph nodes resected because of breast carcinoma and melanoma were diagnosed in 7 patients. Two of them were treated because of B-CLL. The leukocyte count in peripheral blood in non-treated patients was lower than 10K/mL. The primary diagnosis of leukemia was established in the remaining 5 cases by histological examination and immunophenotyping of the lymph nodes and peripheral blood. The authors present morphological pictures and immunophenotyping results.

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) IN THE OBESE PATIENTS

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The prevalence of nonalcoholic steatohepatitis (NASH) may increase in parallel with the increasing epidemics of obesity and metabolic syndrome in affluent communities. The purpose of this work is to describe the clinical and hepatopathological findings in the group of patients who underwent surgery as obesity treatment. Seventy-eight patients with severe or morbid obesity were subjected to gastroplasty as obesity treatment and followed-up for 41 months. Their age, body mass index (BMI) and laboratory data correlated with pathological data. A remarkable improvement was noted in the biological markers of metabolic syndrome. Ninety six percent of the initial liver biopsies manifested fatty change, 18% patients developed hepatitis and mild perivenular fibrosis. There was a statistically significant association between BMI and grade III–IV steatosis, between BMI, elevated transaminases, NASH and fibrosis. A significant improvement of the degenerative and inflammatory hepatic lesions in repeated biopsies and hepatic function tests was noted within 8 months after the surgery. Obesity is a major and independent risk factor for NAFLD, NASH and fibrosis. Surgical treatment can improve hepatic lesions.

PRIMARY CUTANEOUS LYMPHOMA

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A 76-year-old female presented with a 6-month-long history of slowly developing plaques and tumors on her trunk and legs. The clinical history included fatigue and weight loss (15 kg in 6 months). She did not have fever and had no previous history of any other skin diseases (MF). On physical examination, the patient presented many lumps and tumors on the trunk and legs, measuring several cm in diameter. The overlying skin was reddish, without ulcers. There was no evidence of peripheral lymphadenopathy and organomegaly. Her chest X-ray and abdominal ultrasound scan were normal. The tumor biopsy was performed. The material was routinely processed. Paraffin sections were HE stained. The histologic features included diffuse infiltrates of large and medium sized pleomorphic cells in the upper and deep dermis and in the subcutaneous tissue. There was a focal epidermal invasion. Tumor sections were stained using the standard immunohistochemical technique with CD3, CD43, CD20 and CD30 antibodies (DAKO). The neoplastic cells were CD3, CD43-positive, and CD20, CD30-negative. The diagnosis was primary cutaneous large T-cell lymphoma CD30(–). Primary cutaneous lymphomas refer to the entities with primary presentation in the skin and with no evidence of extracutaneous disease at the time of diagnosis. They often manifest a different clinical behavior and prognosis compared with their nodal counterparts. The presented case belongs to a group of multifocal aggressive primary cutaneous lymphomas.

INITIAL EVALUATION OF TTF–1 USEFULLNESS IN DISCRIMINATING BETWEEN PRIMARY AND METASTATIC LUNG AND THYROID CANCERS

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It has been suggested that Thyroid Transcription Factor (TTF–1) is frequently expressed in lung and thyroid cancers. TTF–1 belongs to the family of transcription factors and is selectively expressed in some thyroid and lung normal and cancer cells. The aim of this study was the evaluation of the usefulness of TTF–1 in discriminating between lung and thyroid primary and metastatic cancers. Forty-three metastatic and primary tumors from 24 women and 19 men, 21–77 years old (median age, 49) were investigated. There were 29 metastatic and 11 primary tumors. Three tumors were not defined as either primary or metastatic. Tumor sections were stained using the standard immunohistochemical technique and the panel of commercial antibodies including TTF–1 (clone 8G7G3/DAKO). Only nuclear staining of TTF–1 was evaluated as positive. Eighteen (41.9%) out of 43 tumors were TTF–1 positive and they included 15 tumors suspected of being lung cancers, 2 thyroid cancers and 1 undetermined case. Among 11 primary tumors, 7(63.6%) were lung cancers and 2(18%) were thyroid ones. Among 25 TTF–1 negative tumors, in 9 cases was not possible to determine the primary site of disease. An immunohistochemical panel including TTF–1 is helpful in discriminating between primary and metastatic thyroid and lung cancer and suggesting the primary site of some tumors.

IDENTIFICATION OF LOSS OF HETEROZYGOSITY (LOH) ON CHROMOSOME 9, 10, 13, 17, EGFR AMPLIFICATION AND p53 MUTATIONS WITH RELATION TO IMMUNOHISTOCHEMICAL EXPRESSION OF PTEN, P16, p53 AND EGFR IN GLIOBLASTOMA MULTIFORME IN ADULTS

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Glioblastoma multiforme (GM) WHO GIV is a lethal primary astrocytic tumor with various molecular and epidemiological profiles. Genetic classification is mainly based on p53 mutations, loss of heterozygosity on chromosome 17p and EGFR amplification to distinguish between secondary glioblastoma evolving from pre-existing low-grade glioma with p53 mutations and LOH of chromosome 17p, and primary (*de novo*) GM that exhibits more frequent GFR and MDM2 amplification or overexpression, LOH 10p and 10q, p16 deletion, and PTEN mutation. The aim of our study was the identification of molecular profiling in GM with immunohistochemical expression of some proteins encoded by suppressor genes or oncogenes. The material consisted of 32 tumor tissue samples with GM and peripheral blood from patients. We examinated the loss of heterozygosity (LOH) on chromosome 9, 10, 13, 17, EGFR amplification, and p53 mutations in patients with EGFR amplification or/and LOH 10. LOH 10 was detected in 25%(8/32) of cases (in one case the mechanism of alteration was deletion on chromosome 10), LOH 9 in 15.5%(5/32); LOH 17 in 12.5%(4/32); LOH 13 in

9.3%(3/32); EGFR amplification was identified in 21.8%(7/32) of cases. In one case, the p53 mutation was associated with LOH 9, 10, 13. In another unusual case, the genetic alterations were more complex, consisting in the p53 mutation, LOH 10, LOH 17, and EGFR amplification. This case indicates an overlapping of two main genetic pathways of primary and secondary GM. The immunohistochemical study was performed using antibodies against PTEN, p16, p53, Rb, EGFR, p21 and p27.

Bak, Bax, Bcl-2 AND Bcl-xl PROTEIN EXPRESSION ESTIMATED IN CONJUNCTIVAL AND EYELID LESIONS

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Apoptosis is the process of programmed cell death or cell suicide, where the Bcl-2 protein family is involved in the response to apoptotic stimuli. Some of these proteins (such as Bcl-2 and Bcl-xl) are anti-apoptotic, while others (such as Bak or Bax) are pro-apoptotic. These proteins may form homo- or heterodimers and a balance between these proteins regulates the cell cycle and apoptosis. The aim of our study was the evaluation of programmed cell death markers such as Bak, Bax, Bcl-2 and Bcl-xl protein expression in conjunctival and eyelid tumors. In 45 cases of squamous cell papillomas (SCP) and 38 cases of squamous and basal cell cancers (SCC, BCC) Bcl-2, Bcl-xl, Bak and Bax protein expression was immunohistochemically detected using polyclonal antibodies (DAKO and Santa Cruz Biochemicals). To detect the antibody-antigen complexes LSAB technique and DAB were used. In SCP group Bcl-2 overexpression was observed in 24 cases (53.3%) Bak expression was seen in 28(62.2%), Bax in 31 cases (68.9%), and Bcl-xl protein expression was found in 45 cases (100%). In SCC, BCC groups Bcl-2 protein expression was observed in 18 cases (47.4%), Bak in 31 cases (81.6%), Bax expression was seen in 32 cases (84.3%), and Bcl-xl immunopositivity was observed in 34 cases (89.5%). In SCC, BCC group there was a statistically significant association between expression of Bax and Bak. In SCP group there was a significant correlation between expression of Bax and Bcl-2, Bax and Bak as well as between Bak and Bcl-xl. The higher percentage of Bax and Bak-positive cancers as well as lower percentage of tumors with overexpression of antiapoptotic proteins (Bcl-2, Bcl-xl) ($p<0.05$) might suggest a crucial role of apoptosis in conjunctiva and eyelid cancer development and progression.

GAP JUNCTIONAL INTERCELLULAR COMMUNICATION IN BREAST CANCER

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The most common way of communication between cells in multicellular organisms is gap junctional intercellular communication (GJIC), which plays a critical role in tissue development and differentiation and is important in maintenance of tissue homeostasis. Each gap junction channel is composed of two connexons, which are formed from members of a multigene family of transmembrane proteins called connexins (Cxs). Gap junctions are specialized cell membrane channels, which facilitate the transfer of small ($Mr<1000$) molecules and ions between adjacent cells. Presently, 20 different connexin isoforms have been established in humans. Perturbations of gap junctional intercellular communication (GJIC) between the precancerous cells and their normal counterparts and loss of communication via gap junctions appear to play a role in oncogenesis, and up-regulation of Cxs has been shown to restore normal phenotypes and retard tumor cell growth. In several studies, the lack of connexin expression and/or function of gap junction channels was demonstrated in tumors. The connexin genes have been classified as tumor suppressors. Three Cxs have been detected in normal rodent breast tissue: Cx26, Cx32 and Cx43. The normal human mammary ductal epithelial cells express Cx26. In addition, between myoepithelial cells, Cx43 has been found. Our study comprised 71 women, who underwent surgery for primary breast cancer and had not received any preoperative chemo- or hormone therapy. Tumor samples were examined by immunohistochemistry, using the avidin-biotin-peroxidase method, for the expression of Cx26, Cx32 and Cx43 proteins. In the present study, the expression of Cx26, Cx43, as well as Cx32 in breast cancer cell was observed. We concluded that neoplastic cells could produce connexins, also atypical to normal cells, what may indicate that neoplastic transformation can lead to activation of connexin genes and to production of new proteins. We observed cytoplasmic expression of Cx26, Cx32 and Cx43, as well as the lack of normal intercellular staining pattern. It suggests that in breast cancer cells there are alterations in connexins expression. Thus, our results suggest the lack of functional gap junctions between cancer cells in the breast.

CONNEXINS 26, 32 AND 43 IN COLORECTAL CANCER

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Intercellular communication plays a crucial role in tissue development, differentiation and in control of apoptosis. Gap junctional intercellular communication (GJIC) is a mechanism for direct cell-to-cell signaling and is mediated by gap junctions (GJs), which consist of transmembrane proteins called connexins (Cxs). Many physiological roles have been proposed for gap junctions, such as maintenance of tissue homeostasis, regulation of tissue development, electrical and metabolic coupling, as well as regulation of cellular growth, differentiation and apoptosis. Alterations of GJIC appear to play a role in carcinogenesis as a result of inhibition of signaling pathways, which control these processes. It has been demonstrated that in normal human epithelium of the colon, the expression of Cx32 and Cx43 is present. In our previous study we found the expression of Cx26 in normal colonic epithelium. The aim of the present study was to evaluate the expression and correlation between Cx26, Cx32, Cx43 and apoptotic proteins (Bak, Bcl-XL) in colorectal cancer, as well as to determine the relationship between these proteins and selected anatomoclinical features of the cancer. Tissue samples were obtained from 144 patients (mean 65.4 years) who underwent surgical resection because of colonic (78 cases) and rectal (66) carcinomas. Protein expression was examined by immunohistochemistry using the

antibodies for Cx26, Cx32, Cx43, Bak and Bcl-XL. Results and conclusion: immunohistochemical analysis of colorectal cancer sections revealed an altered expression and location of Cxs. We found a negative correlation between the expression of Cx26 and histological grade (G) ($p<0.03$). Moreover, a negative association between Cx26 expression and lymph node status was noted ($p<0.05$). We found a positive correlation between Cx26 and Bak expression ($p<0.00001$). Furthermore, in a group including 21 cases of mucinous carcinoma and adenocarcinoma with mucinous differentiation, in 13 cases no positive staining for Bcl-XL was seen. In the other cases of this group, positive Bcl-XL immunostaining was observed only in a few of the cells. In conclusion, these results showed that the expression of Cx26 in the colonic cancer correlates with good prognostic markers and the positive correlation between Cx26 and Bak expression suggests that Cx26 can play a role in signaling pathways of apoptotic regulation.

THE NUMBER OF NONINVASIVE AND DIAMETER OF INVASIVE BREAST CARCINOMAS OPERATED ON IN WEST-POMERANIAN REGION OF POLAND IN THE YEARS 1994–2002

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The number of noninvasive (intraductal carcinoma, lobular carcinoma in situ) and diameter of invasive breast carcinomas operated on in Regional Oncological Hospital in Szczecin, Poland have been analyzed. The number of noninvasive cancers increased from zero in 1994 to 9 in 1995 and 46 in 2002. The percentage of breast cancers 10mm and less in diameter rose from 13.87% in 1994 to 27.96% in 2002. The results document the increasing trend in detection of low clinical stage breast cancers with good prognosis, most likely due to better mammography screening programs and better health education of women.

EXPRESSION OF IL–2, IL–2R AND Ki–67 IN SELECTED LUNG CANCERS

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The receptor for interleukin 2 (IL–2R) undergoes expression in all classes of lymphocytes and on monocytes. Studies *in vitro* and *in vivo* prove that the receptor is produced also in tumor cells, both of hemopoietic and non-hemopoietic origin. In patients with advanced lung tumors, a significant increase as compared to the normal range is noted in serum concentration of the soluble receptor for IL–2 (sIL–2R), accompanied by lowered concentrations of IL–2. No correlation could be demonstrated between sIL–2R concentration and the so-called Tac-positive (i.e. carrying IL–2R) lymphocytes. The mechanisms responsible for sIL–2R release to the circulation and the expression of the receptors on cell membranes of the immune system cells and cellular expression of IL–2R (chain), IL–2 and Ki–67 in squamous cell carcinoma cells have not been fully recognized. The present studies aimed at evaluation of non-small cell lung carcinomas (non-SCLC), carcinoids (C) and small cell lung carcinomas (SCLC). The positive control involved reactive lymph nodes. The immunocytochemical techniques included the ABC technique and mouse monoclonal anti-IL–2, anti-IL–2R and anti-Ki–67 antibodies. The results were appraised semi-quantitatively using the scale of Remmele and Stegner (1987). A cytoplasmic (IL–2), membranous (IL–2R) or nuclear (Ki–67) pattern of the proteins was detected. The highest amounts of IL–2 were detected in lung carcinoid cells, as compared to the remaining groups of tumors. In the three groups of lung cancers, no significant differences could be found in the expression of IL–2R. The highest expression of Ki–67 was detected in the SCLC group, followed by, the non-SCLC and C groups. In the group of lung carcinoids, the expression of Ki–67 and of IL–2 was found to correlate with each other ($r=0.8839$; $p<0.05$).

ANALYSIS OF VEGF, VEGF-C AND flt–1, flt–4 RECEPTOR EXPRESSION IN GASTRIC ADENOCARCINOMA

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Markers of neoplastic malignancy include infiltration of adjacent tissues and formation of metastases. Both the growth of primary tumor and its metastasizing capability are dependent on neoangiogenesis. The family of the vascular endothelial growth factors (VEGF) is one of the factors influencing the intensity of this process. To investigate their impact on biological malignancy potential of gastric adenocarcinoma, tissue material from 52 patients after gastrectomy was analyzed. The immunohistochemical expression of VEGF, VEGF-C and their receptors (flt–1, flt–4) as well as microvessel density (MVD, using anti-CD34 antibody) was studied in samples from primary tumors. The expression was paired with clinico-pathological data. A variable intensity of cytoplasmic expression of both proangiogenic factors was observed in the majority of the studied tumors (of all histological types), while their receptors were also focally found on the surface of vascular endothelium. These findings were associated with high vessel counts and higher stages of the disease.

EXPRESSION OF IGF-IR IN BENIGN MAMMARY LESIONS AND IN PRIMARY AND METASTATIC BREAST CANCER

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The insulin-like growth factor I receptor (IGF-IR) is a transmembrane heterotetramer molecule with intrinsic tyrosine kinase activity. IGF-IR plays an important role in normal breast development. In addition, IGF-IR has been implicated in breast cancer. Numerous studies using animal and cellular models documented that tumorigenic activity of IGF-IR in mammary epithelial cells relates to its anti-apoptotic and mitogenic activities, and possibly to pro-metastatic potential. However, only limited studies addressed IGF-IR expression in human clinical material. Consequently, we assessed by immunohisto-

chemistry the expression of IGF-IR in 41 cases of benign mammary dysplasia, as well as in 50 primary tumors and 25 breast cancer metastases to lymph nodes, and correlated IGF-IR expression in tumors with selected clinicopathological features. We found that in 21 cases of benign dysplasia without intraductal proliferative lesions, IGF-IR was negative and only a few epithelial cells were IGF-IR-positive. Among 20 cases of intraductal proliferative lesions, mainly including the usual ductal hyperplasia, we found 8 cases with positive IGF-IR and 12 cases with negative immunostaining. IGF-IR was expressed in primary cancers, as well as in lymph node metastases, but the expression in primary tumors was more frequent. IGF-IR expression in primary tumors was associated with negative node status ($p<0.04$), but not with tumor size or grade. Both IGF-IR-positive and IGF-IR-negative primary tumors were found to produce IGF-IR-positive, as well as IGF-IR-negative metastases. The results suggest that IGF-IR expression is correlated with the cancer phenotype. Thus, IGF-IR could become a viable pharmaceutical target in breast cancer therapy, but such therapy should be based on IGF-IR assessment in primary tumor and metastasis in each potential patient.

EXPRESSION OF SELECTED APOPTOTIC MARKERS AND ASSOCIATIONS WITH THE INSULIN-LIKE GROWTH FACTOR-I RECEPTOR IN HUMAN COLORECTAL CANCER

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Dysregulation of apoptosis, as well as growth factors and their receptors play an important role in colorectal cancer development and progression. The main group of genes controlling apoptosis is the Bcl-2 family, which comprises both promoters (Bax, Bak, Bad, Bcl-XS) and inhibitors (Bcl-2, Bcl-XL, Mcl-1). These proteins may form homo- or heterodimers and the balance between these proteins regulates the cell cycle and apoptosis. But there are conflicting results with regard to expression of proteins involved in controlling apoptosis during colorectal carcinogenesis. Insulin-like growth factor (IGF) and its receptor (IGF-IR) are implicated in mitogenesis, transformation, apoptosis and aggressiveness of different kinds of cancers. Overexpressed IGF-IR in colorectal cancer is associated with an increase of cancer cell proliferation and migration, as well as inhibition of apoptosis. Recently, *in vitro* studies have shown that IGF-I and IGF-IR can also stimulate apoptosis. To study the relationships between Bax, Bak, Bcl-XL and IGF-IR, a total number of 144 cases of colorectal cancer were examined by immunohistochemistry, using the avidin-biotin-peroxidase method. The results were correlated with selected clinicopathological features of the cancer and with the expression of Bax, Bak, Bcl-XL and IGF-IR in normal colonic mucosa. Results and conclusion: a strong immunostaining for Bax, Bak, Bcl-XL and IGF-IR was observed in 55.5%, 49.3%, 72.4% and 50.8% of the tumors, respectively. In Bax, Bak or IGF-IR-positive cancers, the adjacent colorectal mucosa also revealed positive immunostaining for these proteins. In the majority of Bax, Bak or IGF-IR-negative tumors, we also did not observe staining for these proteins in the adjacent mucosa. We found a positive relationship between Bax and IGF-IR ($p<0.001$), between Bak and IGF-IR ($p<0.002$), as well as between Bax and Bak ($p<0.0001$). We observed negative associations between Bax, Bak and tumor grade ($p<0.01$, $p<0.003$, respectively), but no relationship was seen between Bax, Bak expression and tumor stage and between Bax, Bak and lymph node status. Moreover, no relationship was noted between IGF-IR expression and tumor grade, stage and lymph node status. The knowledge about relationships between apoptotic pathways and growth factor receptors could help in our understanding of colorectal cancer biology and the importance of the IGF-IR in cancer progression, as well as in effective treatment of patients with this disease.

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PROGNOSTIC VALUE OF FOUR COMMON HISTOPATHOLOGICAL CLASSIFICATIONS OF GASTRIC CARCINOMA

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The purpose of this study was to define an association of four histopathological classifications of gastric carcinoma (Lauren, Kubo, WHO and Goseki) with survival. Material: tissue samples from 184 patients with primary gastric carcinoma and an appropriate follow-up. Tumor size, location, depth of invasion, local lymph node involvement and histological subtype according to the above mentioned classifications were established in all cases. Results: in multivariate analysis none of the analyzed histological classifications had independent prognostic significance. Cancers of the cardia were associated with poorer prognosis as compared to carcinomas of the lower part of the stomach. Prognostic value of Goseki and Lauren classifications was different for carcinomas of upper and lower stomach.

EXPRESSION AND SUBCELLULAR LOCALIZATION OF MMP-9 mRNA IN RAT BRAIN AFTER STATUS EPILEPTICUS

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Temporal lobe epilepsy (TLE) is the most common form of epilepsy in adults. It is a severe, progressive, often intractable disorder that ultimately leads to brain damage and cognitive impairment. A key role in TLE progression is played by aberrant neuronal plastic responses, including abnormal synaptogenesis, which occur in the hippocampal dentate gyrus (DG). Recently, a family of matrix metalloproteinases (MMPs) has been implicated in some forms of neural plasticity. One of those enzymes, MMP-9, has been shown to be increased (at both protein and mRNA levels) within DG after kainic acid-induced status epilepticus, in a rat model of TLE induction. However, the precise cellular and subcellular distribution of MMP-9 and, in particular, its mRNA, after seizures has not been clarified. Here, we addressed the latter issue using a high-resolution fluorescent in situ hybridization. The studies were performed in six control, and six kainic acid-injected adult male Wistar rats. The

experimental group had severe status epilepticus lasting for several hours. The brains were perfusion-fixed with paraformaldehyde, cryoprotected and frozen. In situ hybridization reaction was performed in free-floating sections using fluorescein-labeled cRNA probes, and tyramide-based fluorescent signal enhancement. Some sections were double labeled for MMP-9 mRNA and MAP-2, for dendrite visualization. The specimens were examined under the confocal microscope. In the control hippocampi, the fluorescent in situ hybridization signal was present in the form of small (sub-micrometer), discrete, weakly-to-moderately positive foci, distributed sparsely over the neuronal cell-bodies, and, occasionally, within the neuropil. By double labeling with MAP-2, the neuropil foci were found to colocalize closely with both large- and fine dendritic processes. At 24 hours after kainic acid treatment, a dramatic increase in both the number and fluorescent intensity of MMP-9 mRNA-positive punctae was detected in the DG hippocampal subfield. Importantly, the strong in situ hybridization signal detected over the area of neuronal dendritic trees was almost invariably present within, or in close apposition to, dendrites. In such locations, mRNA foci formed minute protrusions, stemming out of the dendritic surface, closely resembling the dendritic spines. Virtually no signal was found in sections hybridized with the control sense probe. As an outcome, our studies show that after status epilepticus, MMP-9 mRNA is increased specifically within the neuronal dendritic compartment (most probably within the dendritic spines) and their synaptic domains. Since those are the structures that undergo aberrant plastic changes during TLE progression, the results suggest a role for MMP-9 in the pathogenesis of this disease.

IMMUNOHISTOCHEMICAL STUDY OF COMPLEMENT INHIBITORS CD55 AND CD59 IN PANCREATIC ADENOCARCINOMA

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The prognosis in advanced pancreatic adenocarcinoma is dismal, with less than 5% of patients surviving for 5 years after the diagnosis. In general, the cancer is resistant to conventional medical treatment, thus there is a need to find novel treatment options. A promising but underdeveloped approach appears to be immunotherapy with monoclonal antibodies, which is safe; however, its efficacy is limited by the expression of complement inhibitors in tumor cells. Since very little is known about the expression of the latter group of proteins in the cancer of the pancreas, we decided to immunolocate two such proteins: CD55 and CD59, in a range of pancreatic adenocarcinoma tissues. The sections of formalin-fixed paraffin-embedded tumor samples from 51 patients with pancreatic adenocarcinoma, grades G1 – G3, were immunostained for CD55 and CD59, as well as for Ki67 and p53 tumor markers using microwave antigen-retrieval and peroxidase-based- or fluorescent-confocal detection. Within the tumor tissues, CD55 and CD59 immunoreactivity was present almost exclusively in cancer cells. Several types of intracellular CD55 and CD59 distribution could be discerned: apical-linear, apical-cytoplasmic, diffuse-cytoplasmic, granular and basal. Among different grades, strong or moderate immunoreactivity of both proteins was present in G1 and G2 tumors, whereas in G3 they were virtually absent. Accordingly, they correlated inversely with the expression of Ki67 and p53. Moreover, similar inverse correlations were found within individual tumors when the areas of higher and low differentiation were compared. Our study indicates that the expression of CD55 and CD59 in pancreatic adenocarcinomas depends on the grade of their differentiation. Therefore, it is possible that a similar correlation occurs with respect to the resistance of cancer cells, derived from such tumors, to monoclonal antibody-directed, complement-mediated, cytotoxicity. Further studies are needed to explore this possibility and its putative clinical relevance.

FOLLICULAR LESION IN THYROID FNAB: AN ABSOLUTE INDICATION FOR OPERATION? AN ANALYSIS OF CASES FROM A SINGLE CENTER

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The main limitations in the application of thyroid fine-needle aspiration biopsy (FNAB) as a diagnostic tool are associated with follicular lesions and result from the lack of cytological criteria of differentiation between benign and malignant follicular changes. The term follicular lesion/neoplasm was coined for FNAB years ago, and represents an attempt to narrow the diagnostic spectrum to the follicular lesions. Such diagnosis, when stated in FNAB report, is an indication for surgical treatment. According to the majority of previous analyses, the risk that the lesion turns out to be a thyroid carcinoma (TC) has achieved the level of 20 – 30%. However, recent reports indicate that the risk might be much lower. The main goal of this work was to perform a histopathological and morphological-clinical analysis of cases diagnosed by FNAB as follicular lesions. From October 2001 to January 2004, 3033 cases of ultrasound-guided thyroid FNAB were diagnosed in our Department. Among them, 2867 (94%) were recognized as focal changes of the thyroid. The diagnostic material was obtained from 2762 FNAB cases (96.3%). Among them, 190 (6.9%) were diagnosed as follicular lesions classified into two categories: follicular neoplasm (FN; cell-rich, monomorphic smears, almost entirely consisting of follicular structures) and follicular proliferation (FP; smears, which besides follicular structures contained other cellular elements and colloid). FN was detected in 71 patients, while FP was found in 119 cases, which represented respectively 2.6% and 4.3% of the aspirates. The histopathological verification was possible in 76 patients diagnosed with follicular lesions (40%), in 58 patients with FN (81.7%) and in 18 patients with FP (15.1%). By postoperative histopathological examination, a neoplasm was diagnosed in 47 cases (61.8%), the majority being adenomas – 40 cases (52.6%). Seven (9.2%) cases were recognized as TCs – 4 follicular carcinomas (5.3%) and 3 papillary carcinomas (a follicular variant) (3.9%). TC was found in 6(10.3%) patients with FN, as well as in 1 patient (5.6%) with FP. Non-neoplastic nodules were diagnosed in 24 patients (31.6%). In 5 cases (6.6%), chronic thyroiditis was detected. In our study, among lesions diagnosed by FNAB as follicular ones, the overall prevalence of malignancy was 9.2%, amounting to 10.3% in FN and 5.6% in FP. Considering the relatively low risk of malignancy, the FNAB diagnosis of follicular lesions should be treated as a relative indication for surgical treatment, especially in case of follicular proliferation. In these patients, it seems advisable to consider conservative treatment with a subsequent FNAB control.

GASTRIC SCHWANNOMA – A VALID ENTITY IN DISTINGUISHING GIST FROM OTHER NONEPITHELIAL NEOPLASMS

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GIST is the most common nonepithelial neoplasm of the gastrointestinal tract, yet it is necessary to distinguish it from gastric schwannoma because of clinical and histological similarities. In this article we present two cases of gastric tumors that were originally considered to be GISTs, but histological examination led to the final diagnosis of a gastric schwannoma. Those cases prove that the diagnosis process should be very careful and precise and not all of gastrointestinal nonepithelial neoplasms should be considered GIST.

LIVER AND KIDNEY HISTOLOGICAL ASSAY IN METHANOL INTOXICATED RATS TREATED WITH ETHANOL OR 4-METHYLPYRAZOLE

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Clinical treatment of methanol intoxication focuses on prevention of metabolic acidosis or correction of acidosis, which has already occurred. It is now generally accepted that ethanol is administered to inhibit the first pass of methanol metabolism. However, ethanol is generally used in the treatment of methanol poisoning, but it shows a lot of side effects and the administration is difficult. In consequence, some new solutions concerning lower toxicity and higher treatment efficacy are still explored. In 1998, the Federal Food and Drug Administration gave approval for the use of 4-methylpyrazole (Atizole, Fomepizole) as an antidote for ethylene glycol poisoning and since 2000, 4-methylpyrazole has been enjoying the status of a pharmaceutical to use in methanol poisoning. The aim of this study was the comparative assays: methanol/ethanol and methanol/4-methylpyrazole interactions which could implicate liver and kidney dysfunction in rats. The N_2O rat model was used in our experiment to easily extrapolate data from animals to humans. The animals were kept in N_2O atmosphere and were methanol (3g/kg b.w.) intoxicated *per os*. After 4 hours, ethanol (0.5g/kg b.w.) or 4-methylpyrazole (50mg/ kg b.w.) was administered i.p. Twelve hours after methanol administration, the liver and kidney samples were removed and fixed in 10% buffered formaldehyde and paraffin embedded. The results will be presented during the Congress.

DOES THE MEAN AGE OF PATIENTS WITH ULCERATIVE COLITIS DECREASE? THE STATISTICAL ANALYSIS OF 1254 PATIENTS TREATED IN THE YEARS 1996–2003

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Ulcerative colitis is a disease of the large bowel characterized by chronic diarrhea and rectal bleeding with a pattern of exacerbations and remissions. The disease usually begins in an early adult life, with a peak incidence in the third decade of life. The purpose of this study was a retrospective analysis of patients with ulcerative colitis treated in the period 1996–2003. The patients were identified from computer records of our Department and divided into two groups: patients examined in the years 1996–1999 and 2000–2003. In each group, the following variables were studied: age, sex, the phase of the disease, and the presence or lack of dysplasia. We analyzed the data of 1254 patients (622 patients seen between 1996 and 1999 and 632 patients from the period of 2000–2003). Our study has shown an increasing incidence of ulcerative colitis in the group of patients under 18 years of age in the years 2000–2003 (59 patients, mean age 12.7) comparing with years 1996–1999 (20 patients, mean age 14.1) and a decrease of the mean age in this group of patients (the mean age of the patients examined in the years 1996–1999 was 45.8, while for the patients seen between 2000 and 2003 it was 44.4). These findings could be the result of a decrease in the mean age of patients with ulcerative colitis, but they also could result from early referrals and diagnoses associated with a more common employment of histopathological examinations in the last years in patients with chronic diarrhea and rectal bleeding.

ROLE OF ADENOSINE TRANSPORT INHIBITORS IN THE COURSE OF EXPERIMENTAL ACUTE PANCREATITIS

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The aim of this study was to assess the role of drugs that increase the endogenic adenosine level in the course of acute pancreatitis (AP) induced with sodium taurocholate at the dose 0.3ml/100g b.w. The experiment was performed on Wistar male rats divided into four groups: I – control healthy animals, II – control animals with AP, III – animals with AP, which were injected with an adenosine transport inhibitor – dipiridamole, at the dose of 3mg/kg b.w., IV – animals with AP, which were injected with an adenosine re-uptake inhibitor – dilazep at the dose of 2mg/kg b.w. Blood for biochemistry and pancreas for morphological examinations were collected in 2^{nd}, 6^{th}, 24^{th} hour after the injection of sodium taurocholate. There was an increase in serum levels of amylase and lipase in all the three groups, which developed AP comparing with the controls. The increase was lower in group III treated with dipiridamole and IV treated with dilazep as compared to group II. In all three groups after the administration of sodium taurocholate, we observed the development of acute pancreatitis with the presence of necrosis and hemorrhages. The morphological results were similar; the administration of substances that had increased the level of endogenic adenosine decreased the intensification of inflammatory changes. These results could suggest a positive effect of adenosine transport inhibitors in the course of experimental model of acute pancreatitis.

TISSUE MICROARRAY TECHNOLOGY IN THE ASSESSMENT OF PROTEIN EXPRESSION OF SOME CELL CYCLE REGULATORY GENES IN LARYNGEAL CARCINOMAS

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Tissue microarray (TMA) technology facilitates fast and cost-effective simultaneous assessment of the protein expression in large number of different tumor tissues in one pathologic slide. The aim of this study was the use of TMA for assessment of expression of p21/WAF1, p27 and p53 proteins in laryngeal cancer. On the one TMA containing 200 samples of laryngeal squamous cell carcinomas immunohistochemical reactions with monoclonal antibodies to p21/WAF1, p27 and p53 proteins were performed and computerized image analysis system Quantimet 600S (Leica) was used to evaluate the expression of protein products. The results were correlated to tumor location, histological grading according to Glanz criteria and status of regional lymph nodes.

PROGNOSTIC VALUE OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) EXPRESSION IN COLORECTAL CARCINOMA

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Angiogenesis is a central point in biology of cancers. This process is responsible for tumor growth and metastases formation. The theory of angiogenesis reaches 1945 when Algire et al. showed, that vascularization increases the rate of growth of implanted tumors in mice. In 1971 Folkman et al. demonstrated that tumor cells in culture can grow in absence of vascularization only up to nodule limited to 1 – 2mm^3. A few years later Weidner et al. demonstrated a correlation between a number of microvessels in the primary tumor and prognosis, especially lymph nodes status in human breast carcinoma. Since then the intratumoral microvessel density (IMD) has become a new and strong prognostic factor. Vascular Endothelial Growth Factor (VEGF) is one of the most important angiogenic factor. It is specific for endothelial cells. It also increases the permeability of vessels and its activity exceeds the activity of histamine 50,000 times. We decided to examine the relationship between classical prognostic factors, microvessels density and immunohistochemical expression of VEGF in 122 cases of colon carcinoma. Our study did not reveal any statistically significant correlation between IMD and most of clinical data. Although the incidence of hepatic metastases was more likely in tumors with high microvessels density, but this correlation was not statistically significant (p=0.06). When analysed the VEGF expression a statistically significant correlation between IMD (p=0.0022), tumor size (p=0.001), lymph node status (p=0.004) and the Dukes' stage (p=0.0095) was shown.

IMMUNOHISTOCHEMICAL DIFFERENTIAL DIAGNOSIS OF METASTATIC MALIGNANCIES OF UNKNOWN PRIMARY ORIGIN

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The aim of this study is to assess the application of contemporary immunohistochemistry in determining the primary site or cell lineage of metastatic malignancies of unknown origin. Tissue and cytological specimens were obtained from 120 patients with metastatic malignancy of unknown primary origin. One hundred twenty patients with malignancies of known diagnosis formed the "control" group. The patients' ages ranged between 28 and 78 years. All were diagnosed and treated in the Department of Oncology, Karol Marcinkowski University of Medical Sciences, and in the Wielkopolska Oncology Center in Poznań. Immunohistochemistry was performed using the two-step EnVision+^{TM}/HRP system (DakoCytomation, Denmark). Monoclonal and polyclonal antibodies were supplied by DakoCytomation (Denmark) and Novocastra (United Kingdom) companies. The immunohistochemical battery consisted of site-specific associated antibodies, a selection of cytokeratins and tumor markers including: CK–7, CK–20, CK(CKMNF–116), CK AE1/AE3, CEA, Vimentin, HMB–45, FP, LCA, Hepatocyte marker OCH1E5, GCDFP–15, PSA, PSAP, THY, CALC, Chromogranin, TTF–1, CD10, ER, PGR, S–100, DES, EMA, CAM5.2, and mesothelial marker HBME–1. The immunohistochemical results correlated with pathomorphological patterns to establish a diagnosis and identify a primary in 76% of patients in the unknown group. The diagnosis was not possible in 10% of cases. In 9% of cases the primary origin was narrowed down to two organs, whereas in 5% of cases the primary origin was narrowed down to 3 organs. The use of immunohistochemistry established a diagnosis in 76% of cases while narrowing the assessment of a primary site in 14% of patients. The remaining 10% of patients need further investigation to correctly evaluate the primary site of the origin of the malignancies.

PROFILES OF CYTOKERATINS AND THE EXPRESSION OF HER–2 AND STEROID RECEPTORS IN BREAST CANCER

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The application of microarray techniques has revealed the existence of several groups of breast cancers, with the CK5/6 positive breast cancer group, with a high mitotic index and generally ER and PR negative. Furthermore, it has been proven that molecular profiling is an important prognostic tool. The aim of this study was to estimate the expression of cytokeratins, steroid receptors, HER–2, Ki–67 and selected oncogenes using immunohistochemistry and Quantitative RT-PCR in breast cancer, and subsequently to investigate the correlation between such profiles and the prognosis. All the tumors were associated with a more aggressive clinical outcome and shorter overall survival. The second part of the research was aimed at establishing the importance of such profiling, and at verifying the relationships among molecular profiles and the expression of genes. The material comprised 197 tumors (N0 and N1) originating from patients operated on between 1997 and 2001. The tumor tissues were processed by forming paraffin blocks and deep freezing. The paraffin slides served as material for numerous immunohistochemical reactions: ER, PR, HER–2, CK5/6, CK17, CK8/18, and Ki–67. We found the most important correlation between basal cytokeratin expression and both steroid receptors and HER–2. The Her–2(–)CK5/6(–) group accounted for 41.1%; Her–2(–)CK5/6(+) – 23.9%; Her–2(+)CK5/6(–) – 24.9%, and Her–2(+)CK5/6(+) – 10.1%. Her–2(–)ER(–) accounted for 31.5%; Her–2(–)ER(+) – 33%, Her–2(+)ER(–) – 23.3%; Her–2(+)ER(+) – 11.7%.

ER(–)CK5/6(–) accounted for 25.38%; ER(–)CK5/6(+) – 31.47%, ER(+)CK5/6(–) – 40.61%; ER(+)CK5/6(+) – 2.54% We noted that Her–2 and ER usually existed separately, similarly as in the case of HER–2 and CK5/6 expression. CK5/6(+) tumors were commonly ER(–). Further immunohistochemistry research and QRT-PCR investigations have been continued to analyze the correlation between the expression and prognosis.

PRIMARY MALIGNANT MESENCHYMAL TUMOR OF THE LUNG WITH BONE-FORMING OSTEOBLASTIC ELEMENTS – AN IMMUNOHISTOCHEMICAL STUDY

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Primary malignant mesenchymal tumors of the lung are very rare. A correct preoperative diagnosis is difficult. In a 56-year-old female computed tomography of the chest revealed a well-defined mass with a maximal diameter of 15cm in the left lower lung lobe. There was no previous pathological diagnosis established by either bronchoscopic biopsy or computed tomography-guided percutaneous needle biopsy. The pathology report on the frozen section was malignant mesenchymal tumor. The lower lobe and mediastinal lymph nodes were excised (complete resection). Histopathologically, the lesion was characterized by a composition of hypocellular and hypercellular areas with cells showing focally moderate to marked cytological atypia, and numerous mitoses (> 30/10hpf). There were areas with thin-walled branching vessels having staghorn configuration and bone-forming osteoblastic elements. Immunohistochemical analysis was performed using a panel of several antibodies including cytokeratins, WT-l, EMA, bcl–2, CD31, CD34, CD99, vimentin and Factor XIIIa. Strong expression of WT-l, CD99, vimentin and bcl–2 was found. Tumor cells were negative for cytokeratin, EMA, CD31, CD34 and Factor XIIIa. The obtained allow as diagnosing definitively the neoplasm as primary malignant mesenchymal tumor of the lung with bone-forming osteoblastic elements.

HEMORRHAGIC MYOCARDIAL INFARCTION

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Hemorrhagic myocardial infarction is one of the rarest kinds of myocardial necrosis. It results from thrombolysis, hemorrhagic disturbances or mechanical trauma. The aim of the work was the characterization of the morphology of myocardial hemorrhagic infarction and its differences when compared to ischemic myocardial necrosis (myocardial infarction). The material consisted of 30 cases of macroscopically hemorrhagic myocardial infarctions. The comparative group consisted of 20 cases of ischemic, pale myocardial infarctions. The clinical history of the disease was 48 hours. The studies were based upon histology and confocal laser scanning microscopy. Hemorrhagic myocardial infarction was characterized by the bulk of interstitial erythrorrhages, paucity of inflammatory infiltration, preserved continuity of cardiocytes, hyperemia of the coronary veins, compression of the coronary arterioles and nerve trunks. In confocal laser microscopy, the myocardial fibers showed no increased autofluorescence and fractures, but the dominant feature was their thinning by interstitial hemorrhages. Conclusion: hemorrhagic myocardial infarction is a diverse morphological entity, closely resembled with hemorrhagic complications.

Parametr (median)	Curve lenght [ ]	Curve width [ ]	Mean grey level	Aspect	Fullnes
Group					
Control	6.923	1.538	87.16	2.333	0.876
Diabetes	5.845	3.361	15.819	3.090	0.967
P	**>0.000001**	**>0.000**1	**>0.0001**	**>0.0001**	**>0.0001**

AORTIC ELASTIC FIBERS IN DIABETIC MACROANGIOPATHY

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The basis of diabetic macroangiopathy consists in biochemical disturbances in the vascular wall – collagen and elastin glycosylation. Modified elastic elements diminish the vascular function and its elasticity, leading to the progression of atherosclerosis and hypertension. The aim of the study was to evaluate the aortic elastic components by means of morphometric studies in diabetic patients undergoing coronaro-aortic bypass grafting (CABG). The aortic tissue was collected from twenty randomly selected diabetic patients undergoing CABG (Group I, age 58.9±5.8, diabetes duration 11 4 years). Cases with microscopic evidence of atherosclerosis were disregarded. Analogous tissue fragments from heart donors served as the controls (Group II). In Group I, 3566 elastic fibers were analyzed and in Group II – 4689 fibers. The autofluorescence of the elastic fibers was induced in a confocal microscope by an Argon laser (490nm), and using epifluorescence (520–560nm HBO 100). Morphometric measurements included the curve length, curve width, aspect, fullness, mean grey level. The aortic elastic fibers in diabetes were wider, shorter, with focal irregular thickening. A significant alteration of fiber linearity was also observed in diabetic patients.

Conclusions: 1. Disturbed metabolism in diabetes type 2 leads to a disturbed architecture of the aortic elastic fibers. 2. The differences in elastic fiber autofluorescence indicate changes in elastic fiber composition in diabetic patients.

MORPHOLOGICALLY DISTINCT NEOPLASTIC C-CELL HYPERPLASIA OF THE THYROID

GLAND IN PATIENTS UNDERGOING PROPHYLACTIC THYROIDECTOMY FOR FINDING *RET* GENE MUTATION

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Medullary carcinoma is a malignant tumor, arising from thyroid C-cells and occurs in two variants: sporadic (75%) and hereditary (25%). Genetic *RET* protooncogene analysis is necessary to find a mutant gene carrier or exclude the carrier status in a families with hereditary MTC. A germ-line mutation is found in 90–95% of MEN2A/FMTC patients and it occurs most frequently in exon 10 or 11 (codons: 609, 611, 618, 620 and 634). Point mutations in codons 768 and 804 have been found in some families with hereditary MTC and 98% of patients with MEN2B have a point germ-line mutation in exon 16 (codon 918). Hereditary MTC is the on1y inherited cancer syndrome, in which surgical therapy is accepted when the responsibility mutations are found, even without clinically evident disease. Aim of the study: histopathological, immunohistochemical and quantitative analysis of the thyroid gland tissue obtained from patients who underwent prophylactic thyreoidectomy for *RET* gene mutation. Forty patients (21 females and 19 males), aged between 3 and 72 years (mean 25.02±16.24), underwent prophylactic total thyreoidectomy. The whole thyroid was embedded in paraffin and next serial 5-mm sections of each block were used for routine stains HE. In each case, slides were stained for calcitonin and next computerized quantitative image analysis was performed. Morphological features of cellular nuclei were examined in two groups: hyperplastic C-cells and medullary carcinoma of thyroid cells. In the first step digital image acquisition of standard HE slides was performed, using Sony 3CCD color camera and Axioplan 2 research microscope. Original magnification of objective was x40. Morphological measurements were made with KS400 image analysis system. Features connected with dimensions and shape of nucleus and whole cells were measured: area of filled region, maximal and minimal dimensions, a ratio of minimal and maximal dimensions and form. Standard descriptive statistical analysis and Student t-test were used for obtained values. We found C-cell hyperplasia or medullary thyroid carcinoma in all the 40 gene carriers. Twenty six patients (F-M ratio: 12:14, mean age: 30.4y) had a MTC, with neoplastic CCH. Histopathological examination revealed 18 multifocal MTC (69.23%) and nodal metastases in 5(19.2%) patients. Fourteen men (mean age: 15y) had neoplastic CCH on1y. Neoplastic CCH was characterized by clusters of polygonal, round or spindle atypical cells, which were easily recognizable on HE sections. Statistical analysis of nuclear and cells morphological parameters revealed that C-cells in hyperplasia do not differ from medullary carcinoma cells. Conclusion: neoplastic transformation of C-cell to MTC in patients with *RET* gene mutation is not quantitative but a qualitative chance. New morphological criteria for diagnosis of C-cell hyperplasia and medullary carcinoma in patients with *RET* mutations are necessary.

	Nuclear area [ ]	Lenght [ ]	Breadth [ ]	Perimeter [ ]	Round-ness	Aspect	Mean grey level
Chronic cholecystic	32.26	8.20	5.57	25.08	1.41	1.43	98.67
Chronic hyperplastic cholecystitis	31.35	8.19	5.41	23.93	1.35	1.48	93.03
Probabiliy	0.18	0.67	p>0.01	p<0.01	p<0.01	p<0.01	P<0.0001

MORPHOLOGY OF THE GALLBLADDER MUCOSA IN VARIOUS TYPES OF CHRONIC CHOLECYSTITIS

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Chronic inflammation of gallbladder comprises a simple form with slightly atrophic mucosa and rarely a chronic hyperplastic form with arboriform hyperplasia of mucosa, suggesting neoplasia. The aim of this study was the comparison of the morphology, proliferative activity and karyometry of mucosa cells in both types of cholecystitis. Twenty-five cases of simple chronic cholecystitis and 30 of chronic hyperplastic cholecystitis were collected. The morphological analysis of mucosa architecture was done using confocal laser scanning microscopy, the proliferative activity was assessed with Ki-67 reaction, the karyometric measurements included the nuclear area, length, breadth, perimeter, roundness, aspect and mean grey level. Simple chronic cholecystitis was characterized by monolayered glandular epithelium, in cases of hyperplastic chronic inflammation, multilayered epithelium was additionally observed, Ki-67 was positive in almost all epithelial cells, whereas in simple chronic inflammation only single cells were Ki-67-positive. The results of karyometric comparisons were given in the table below.

Conclusion: Chronic hyperplastic cholecystitis shows a different architecture of the epithelium, an increased proliferative activity and different karyometric characteristics, including rounded and widened nuclei with the predominance of euchromatin.

PINEOCYTOMA – A RARE NEOPLASM OF CENTRAL NERVOUS SYSTEM. HISTOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL ANALYSIS

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We present here a case of a 65-year old woman hospitalized due to a slight right hemiparesis. A CT brain scan showed three-ventricle hydrocephalus and a spherical, focally calcified tumor in the pineal region, compressing and modeling the third ventricle. The patient underwent surgery. Microscopic examination revealed a neoplasm composed of small, monomorphic, densely packed cells with slightly acidophilic cytoplasm and round or oval nuclei filled with a few granules of chromatin. Large pseudorosettes were easily seen. The mitotic figures were absent. Almost all neoplastic cells showed strong immunoreactivity for neuron specific enolase, and many of them were synaptophysin-immunopositive, while some showed the expression of neurofilament proteins and chromogranin.

EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IN VULVAR SQUAMOUS CANCER AND VIN

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Vascular endothelial growth factor (VEGF) is one of important angiogenic factors produced by neoplastic cells, which shows a high promitotic activity almost entirely for endothelial cells (paracrine activity). We present the results of VEGF immunoexpression in precancerous lesions (vulvar intraepithelial neoplasia) and in squamous carcinoma of the vulva. The material included 31 cases of vulvar squamous cancer, 28 cases of VIN III, 10 cases of VIN II and 12 cases of VIN I. The diagnosis was established according to the WHO criteria on the ground of postoperative histopathological examinations supplemented with the proliferation index estimated by the use of MIB-1 antibody. Immunohistochemical examinations were performed on paraffin-embedded material, using MIB-1 antibody (Immunotech), VEGF antibody (Santa Cruz), Goat serum Normal (DAKO), DAKO StreptABComplex/HRP Duet, Mouse/Rabbit DAKO DAB Chromogen Tablets, TBS (SIGMA). Positive cytoplasmic expression of anti-VEGF polyclonal antibody (diffuse and/or focal and of various intensity) was observed in almost all samples from precancerous and cancerous lesions. The expression was especially strong and diffuse in all cancer cases; in cases of VIN it was mainly focal and weak.

HISTOLOGICAL, IMMUNOHISTOCHEMICAL AND ULTRASTRUCTURAL CHARACTERISTICS OF RENAL CELL TUMORS

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Renal cell tumors are lesions of a great biological diversity and often overlapping morphology, which is the source of occasional diagnostic difficulties, as in the case of differential diagnosis of chromophobe renal cell carcinoma and oncocytoma. The aim of this study was to assess the utility of light microscopy, immunohistochemistry and electron microscopy for diagnosis of specific renal cell tumor types. Fifty-seven renal cell tumors were evaluated by means of light microscopy and immunohistochemistry – all the tumors were immunostained with antibodies against CD10, cytokeratin 7 (CK7) and vimentin; 20 selected cases were additionally stained with anti-CD117 antibody. All the tumors were studied by transmission electron microscopy. Histological features of the tumors rendered the diagnosis of 6 oncocytomas and 51 cases of renal cell carcinoma (RCC) – 40 cases of clear cell RCC, 6 cases of papillary/chromophilic RCC, 4 cases of chromophobe RCC and 1 unclassified RCC. In the clear cell RCC group (n=40), vimentin was expressed in all the cases, CD10 in 37 cases, CK7 in 13 cases. Chromophilic/papillary RCCs (n=6) were characterized by the expression of vimentin in 5 cases, CD10 in 2 cases and CK7 in 3 cases. All chromophobe RCCs (n=4) were CD10 and CD117-positive, whereas only 2 of them expressed vimentin and CK7. Oncocytomas (n=6) consistently expressed CD117, while vimentin and CD10 were detected in 2 cases and CK7 in 1 case. CD117 staining was negative in clear cell RCCs (n=3) and papillary/chromophilic RCCs (n=6). The ultrastructural features corresponded with the histological diagnosis in most cases. One oncocytoma did not present typical ultrastructural characteristics and the unclassified RCC presented features of a poorly differentiated carcinoma. We conclude that immunostaining with antibodies against vimentin, CD10, CK7 and CD117 might be helpful in the differential diagnosis of specific renal cell tumors. Electron microscopy might be of a supplementary diagnostic value in these cases and it plays a decisive role in the differential diagnosis of chromophobe RCC and oncocytoma.

PROGNOSTIC AND PREDICTIVE FACTORS IN RECTAL CARCINOMA AFTER PREOPERATIVE RADIOTHERAPY

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Neoadjuvant therapy causes the regression and improves the prognosis in rectal cancer. The correlation of the histopathological findings with the clinical response to the therapy remains doubtful. Forty patients with rectal adenocarcinoma with no distal metastases were studied. The patients' age ranged from 44 to 77 years. In all the patients, uTNM (UICC/AJCC) was evaluated. All the patients underwent hyperfractionated radiotherapy (42Gy), 15 patients additionally received chemotherapy and thereafter surgical resections were performed. The following histopathological prognostic and predictive parameters were evaluated: the macroscopic features, tumor size, ypT, ypN, ypTNM, Dukes, Astler-Coller, Jass, histological type, grade, "budding" index, perineural invasion, vascular invasion, tumor border, margins, lymphocytic infiltration, lymph nodes, the degree of tumor regression, pCR-pPR-pSD-pPD, cellular degeneration, the amount of mucus, necrosis, the Nasierowska-Guttmejer's index and radiation-induced changes, inflammatory infiltrates, fibrosis, the presence of keloid-like collagen and myxoid stroma, nuclear abnormalities, vacuolization, crypt distortion, erosions and calcifications. The patients were then divided into two groups according to tumor response to radiotherapy – a decrease in the T status between uT and ypT. The following tests were used in the statistical analysis: the Fischer's exact test, Pearson's chi-squared test with the Yates correction and the U-test. Tumor regression was observed in 11(27.5%) cases. Statistically significant differences were observed in: ypT (ypT1+2 vs. 3) – $p<0.00000001$; ypTNM (ypTNM1 vs. 2+3+4) – $p=0.0000002$; Dukes (A vs. B+C) – $p<0.0000002$; Astler-Coller (A vs. B1+B2+C1+C2) – $p=0.0007$; Jass (1+2 vs. 3+4) $p=0.000014$; the degree of tumor regression (1+2+3 vs. 4+5) $p=0.0243$; pCR+pPR1 vs. pPR2+pSD+pPD $p=0.0167$; cellular degeneration (0+1 vs. 2+3) $p = 0.00086$; the amount of mucus (absent vs. present) $p=0.0053$; the Nasierowska-Guttmejer's index (1+2 vs. 3) $p=0.0093$; neutrophilic infiltrate in peritumoral tissue (absent vs. present) $p=0.00054$; and mucosal vacuolization (absent vs. present) $p=0.0496$. Conclusions: a comparison of uT and ypT values may be used in the evaluation of tumor response to the preoperative radiotherapy. There is a correlation between uT – ypT and the

degree of tumor regression, pCR, pPR1, pPR2, pSD, pPD, and the Nasierowska-Gutmejer's index.

EXPRESSION OF ESTROGEN RECEPTORS IN INVASIVE BREAST CANCER

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In this study we evaluate the expression of and estrogen receptors in neoplastic tissues of patients with invasive breast cancer. Moreover, we compare the expression of and estrogen receptors with expression of other markers. Paraffin embedded tissues from 67 patients with breast cancer were used in this study. Monoclonal antibodies against estrogen and progesterone receptors (DakoCytomation) and polyclonal antibodies against estrogen receptors (CHEMICON) were used. The EnVision detection system (DakoCytomation) was applied. Expression of á estrogen receptors was demonstrated in 57% of all patients, while in patients older than 50 years it was higher – 71%. Expression of estrogen receptors was demonstrated in 49% of patients and this percentage was not dependent on the age of the patients. In tumors expressing estrogen receptors, expression of P53 and Ki67 was less common. In addition these tumors were of a lower grade of malignancy. Our results demonstrate a negative correlation between the expression of estrogen receptors and expression of P53 and Ki67. The expression of estrogen receptors may be a good (positive) prognostic indicator.

USEFULNESS OF HISTOLOGICAL EXAMINATIONS IN FOLLOW-UP OF INTESTINAL POUCHS

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In gastrointestinal surgery, there is sometimes a necessity to translocate one part of the gastrointestinal tract into the place of another, resected part with the formation of an intestinal pouch. It is applied to the large intestine and the stomach. A total of 338 restorative proctocolectomies were done within the past 18 years: 218 because of ulcerative colitis and 120 because of familial polyposis syndrome. The postoperative follow-up was performed in 110 patients, 3–84 months after the ileostomy closure. There were also 34 patients with gastric carcinoma in whom the Hunt-Lawrence-Rodino procedure was done. In the latter group of patients, the follow-up was done 1–34 months after the surgery. The clinical, endoscopic and microscopic features of the pouch were determined. The microscopic changes were evaluated according to the Moskowitz Index. The modified by the authors Pouchitis Disease Activity Index, taking into consideration microscopic features of chronic inflammation, was used to summarize the analysis. In microscopic examinations, the following features were evaluated: inflammation, dysplasia, the degree of the mucosa atrophy and expression of some immunohistochemical markers (Ki-67, bcl-2, ICAM 1, p53) and mucin. The postoperative follow-up revealed inflammatory symptoms, endoscopic changes and microscopic changes in 25.5%, 32.7% and 43.6% of patients, respectively. In 41.7% of cases, inflammation had features of chronicity. In 4 patients, chronic distal pouchitis was accompanied by low-grade dysplasia. Adaptive changes (colonization) were significantly common in distal pouches, but not in proximal pouches. The appearance of microscopic changes did not require pharmacological or surgical treatment. Dysplasia and the increased risk of neoplastic transformation were related to chronic pouchitis. The patients with clinical symptoms and endoscopic changes in the pouch (confirmed by microscopic examinations) are regarded as a high-risk group. They undergo regular follow-up. Ki–67, ICAM 1 ($p<0.05$) and sulfo/sialomucins ratio ($p<0.05$) are very useful immunohistochemical markers in monitoring the inflammatory intensity, while p53 and Ki-67 are helpful in the evaluation of dysplasia.

BRESLOW'S THICKNESS MEASUREMENT OF MELANOMA USING TRADITIONAL METHOD AND DIGITAL IMAGE ANALYSIS

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Breslow's measurement plays an essential role in malignant melanoma diagnostics. A relationship between Breslow's measurement using scale in light microscope and digital image analysis was explored. A group of 50 cases was examined by four pathologists with a different experience. Breslow's measurement using traditional measurement is less accurate but sufficient for estimation of the tumor thickness. It was observed, that measurement differences were caused by subjective factors and resulted from pathologist' experience. The measurement differences appeared more often in cases with irregular shape, inflammatory infiltration and superficial ulceration.

CYTOLOGICAL AND HISTOPATHOLOGICAL PICTURE OF METASTATIC MYXOFIBROSARCOMA IN THE LUNG – A CASE REPORT

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We present a case of 69-year old female, who was diagnosed because of a tumor located in the right popliteal region, lasting for four months. In histopathological examination the diagnosis of myxofibrosarcoma was established. During one year after the primary operation the patient was several times hospitalized because of the local recurrences, which appeared despite radiotherapy and distant metastases to the lungs and the breast. We present the changes of cytological and histological picture of the tumor along the progression of the disease, especially, when comparing the primary tumor and the endobronchial metastasis. As the disease progresses the proportion of low-grade to high-grade myxofibrosarcoma changes, and the light microscopy pictures resemble just pleomorphic/storiform pattern of MFH. The endobronchial metastasis of myxofibrosarcoma is especially interesting because in this way the primary MFH of the lung can manifest. Light microscopy

picture of myxofibrosarcoma metastasis is rarely described because these sarcomas frequently give local recurrences but distant metastases occur in advanced stages of disease. In this case distant metastases occurred during one year after the primary operation simultaneously to subsequent local recurrences; during the second year of follow-up metastases to the breast appeared.

DIFFERENTIAL DIAGNOSIS OF NEUROENDOCRINE AND ENDOCRINE TUMORS – APLICATION OF ELECTRON MICROSCOPY METHODS

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Histological differential diagnosis of neuroendocrine tumors and tumors of endocrine system can be difficult because there is no apparent correlation between morphological features and clinical behavior in these tumors. Frequently, the histological grade of the malignancy at the light microscopy level does not correspond to the biology and endocrine activity of the neuroendocrine neoplasm. Sometimes heterogenic differentiation in these tumors may be the reason that an endocrine tumor may be capable of secreting more than one hormone. Neuroendocrine tumors can present significant diagnostic problems in poorly differentiated lesions. Some of these problems can be solved on the electron microscopy level, especially by implementing the immunogold techniques to supplement the examination of neuroendocrine and endocrine tumors. Our material consisted of more than 400 tumors, including pituitary adenomas, medullary carcinomas of the thyroid and neuroendocrine lung tumors. All the cases were diagnosed by routine histological examinations and an immunohistochemical panel of the expected, tissue-specific markers was implemented additionally. Electron microscopy was performed in all the cases. In selected cases, immunoelectron microscopy was done using the post-embedding immunogold technique. In the majority of cases, our findings support a role of electron microscopy in the evaluation of endocrine and neuroendocrine tumors. Electron microscopy analysis was of value and provided useful information regarding the diagnosis. Ultrastructural methods, including immunogold electron microscopy, play the key role in pituitary tumors diagnosis. For practical implementation of these methods, a diagnostic postoperative algorithm must be used for better pathological management.

ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL DIAGNOSIS OF PITUITARY TUMORS IN PATIENTS WITH CLINICAL SYMPTOMS OF HYPERPROLACTINEMIA

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The term "clinically nonfunctioning pituitary adenoma" includes: 1) gonadotroph adenomas that may be caused without endocrine symptoms, 2) *null cell* adenomas, detected by electron microscopy, and 3) immunopositive, but not functioning *silent* adenomas. Because of the mild elevation of prolactin levels, nonfunctioning adenomas are sometimes erroneously diagnosed as prolactinomas. On the other hand, among tumors with clinical symptoms of hyperprolactinemia, aggressive, poorly prognosing mixed GH- and PRL-cell adenomas may be found, as well as metastases and other, non-neoplastic lesions. An accurate pathomorphological diagnosis of such tumors with the application of immunohistochemistry and electron microscopy allows for establishing an appropriate prognosis and postoperative management (e.g. discontinuation of dopamine agonist therapy). The aim of the study was the evaluation of the practical employment of electron microscopic techniques in the diagnosis of pituitary adenomas. The material consisted of 33 consecutive cases of surgically resected pituitary tumors. Among the diagnosed pituitary adenomas, there were clinically 10 patients with hyperprolactinemia and 14 clinically nonfunctioning tumors. All the cases were diagnosed by routine histological examinations, by immunostaining for all pituitary hormones (growth hormone (GH), prolactin (PRL), ACTH, TSH, FSH, LH and alpha-subunit) and by electron microscopy. In selected cases, immunoelectron microscopy was done using the post-embedding immunogold technique. In our ten patients with hyperprolactinemia, 8 cases of sparsely granulated PRL-cell adenoma, 1 mixed GH- and PRL-cell adenoma and 1 case of lymphocytic hypophysitis were diagnosed. Double labeling immunogold electron microscopy greatly facilitated the diagnosis of mixed adenoma. Among nonfunctioning tumors, 2 cases of silent lactotroph adenoma were revealed. Conclusions: electron microscopy analysis was valuable in providing useful information regarding the diagnosis. Our results confirmed the practical value of the WHO recommendations for a five-tier evaluation of pituitary adenomas. In our material there were 4 out of 24 cases, in which immunohistochemical and ultrastructural features were not concordant with the preoperative clinical diagnosis.

PROGNOSTIC AND POTENTIALLY PREDICTIVE FACTORS IN EARLY GASTRIC CANCER

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No abstract available.

IN VITRO EXPRESSION OF ICAM–1, VCAM–1 AND P-SELECTIN ON THE ENDOTHELIAL CELLS CULTURED WITH c-erbB2 POSITIVE AND c-erbB2 NEGATIVE BREAST CANCER CELLS

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We have examined expression of ICAM-1, VCAM-1 and P-selectin on the endothelial cells cultured with c-erbB2-positive (SK-BR–3 cell line) and c-erbB2-negative (MCF-7 cell line) breast cancer cells. Expression of ICAM-1, VCAM-1 and P-selectin was assessed immunocytochemically and measured by laser scanning cytometer. Significant increase of ICAM-1, VCAM-1 and P-selectin expression was found (1) on endothelial cells cultured with either MCF-7 or SK-BR–3 tumor cells versus endothelial cells cultured without cancer cells ($p<0.001$), and (2) on endothelial cells cultured with SK-BR–3 tumor cells as compared with MCF-7 cells ($p<0.001$). The results suggest that the experimental system used may serve as a model for testing the effect of chemotherapeutic agents on the

expression of adhesion molecules on endothelial cells cultured with cancer cells from individual patients.

PULMONARY SURFACTANT INFLUENCE ON THE CELLS OF RESPIRATORY TRACT – IMMUNO-ELECTRON MICROSCOPY

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Hydrophilic surfactant proteins SP-A and SP-D have an unique molecular structure. Each of them is composed of four molecules, including collagen-like and group C lectin-like chains. SP-B and SP-C (hydrophobic) organize the lipid structures. SP-A and SP-B are found in the "maltanian crosses" of tubular myelin. Lectin-like parts of SP-A and SP-D react with hydroxyl groups of sugars connected to the structural proteins of cell membranes. This is important for the incorporation of surfactant microsomes into biological membranes. Hydrophobic SP-B and SP-C are connected by ester junctions to lipid molecules, and after -OH groups stimulation, segments of surfactant can be incorporated into cell membranes. In the treatment of RDS, exogenous surfactants have been used for more than 20 years; their employment in ARDS has had a somewhat shorter history. The present study was done in young male Wistar rats. After anesthesia, the animals received intratracheally Curosurf (150mg lipids/kg b.w.) marked by synthetic SP-B. The controls received a comparable amount of 0.9% NaCl. After 1, 3, 6, and 24 hours, the lungs were collected for further studies. Histological studies and transmission electron microscopy (including post-embedding immunogold) were performed. Earlier studies (including our reports) have demonstrated that instillation of exogenous surfactant into a mature "dry" lung increases degranulation of type II pneumocytes, increases surfactant recirculation and increases the number of alveolar macrophages (which phagocyte the surfactant). Observations made a short time after surfactant instillation into a healthy lung revealed the mixing of endo- and exogenous surfactants in the lung alveoli. This was performed by type II pneumocytes degranulation and incorporation of segments of endo- and exogenous surfactants into those cells. Moreover, the disintegration of surfactant-laden macrophages, as well as incorporation of endo- and exogenous surfactants into cells other then type II pneumocytes was observed. It is still an open question how long exogenous surfactant can persist within the alveolar cells and if commercially available surfactant preparations do differ in refer to this parameter.

NUCLEOLIN EXPRESSION AND ITS INTRANUCLEAR DISTRIBUTION IN ESTROGEN RECEPTOR-POSITIVE AND ESTROGEN RECEPTOR-NEGATIVE BREAST CANCER CELLS AS ASSESSED BY LASER SCANNING CYTOMETRY

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The aim of this study was to evaluate expression of nucleolin (NU) and its intranuclear distribution in 98 ductal and 11 lobular invasive breast cancers. Cells were double-stained with antibodies against NU and estrogen receptor (ER) conjugated with FITC and APC, respectively. NU-green and ER-red fluorescence was measured by laser scanning cytometry (LSC). The following parameters were recorded: NU fluorescence over nuclei and over NU aggregates (NUA), number and area of NUA, ER fluorescence and area of nuclei. A high correlation was found between ER-bound fluorescence and NU-bound fluorescence within nuclei ($r=0.68$, $p<0.001$), NUA ($r=0.62$, $p<0.001$) and within karyoplasm beyond NUA ($r=0.42$, $p<0.001$). An increase in nucleolin fluorescence was observed among the cells in G_1-phase, S-phase and G_2M-phase. Ductal and lobular carcinomas differed in NU expression within nuclei and in the karyoplasm beyond NUAs. No significant differences were found between ER-positive and ER-negative breast cancers.

MYELOID SARCOMA – A TUMOR IMITATING A LYMPHOMA

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Myeloid sarcoma is an extramedullary tumor composed of immature cells of granulocytic lineage. It appears de novo, preceding the symptoms of acute or chronic myeloid leukemia, in the course of leukemia or other myeloproliferative disease. Occasionally, it presents as an isolated tumor, which may or may not progress in the course of time. The tumor may be located in various sites: the lymph nodes, skin, but also bones, breast, testes, uterine cervix, etc. Sometimes tumors imitating metastases are present in several locations in the same patient. Morphologically, the tumor appears as: 1. well differentiated, composed of cells representing various stages of granulocytic development; 2. poorly differentiated, consisting of large cells with vesicular nuclei and distinct nucleoli; 3. blastic – monotonous population of small cells with evenly distributed chromatin and invisible nuclei. The diagnosis is difficult, particularly in the case of isolated tumors, because the histopathological pattern resembles lymphoma and the cells express LCA. The awareness of the pathologist and his high index of suspicion are essential for a correct diagnosis of myeloid sarcoma. Our material involved 22 cases of myeloid sarcoma. The tumors were studied with a wide panel of antibodies (LCA, CD20, CD3, CD30, CD15, CD117, elastase, Tdt, CD34, myeloperoxidase and CD43). The last two were most specific for myeloid lineage, while CD117 and CD15 showed lesser specificity. In 8 cases, the tumors were isolated, in 3 cases they developed in patients in CR (with previously diagnosed AML and PBS), and in 11 in the course of AML. The analysis of the method of treatment indicates that all cases of myeloid sarcoma, including isolated tumors, should be treated with intensive chemotherapy protocols, as in acute leukemia. When followed by bone marrow transplantation, both methods considerably prolong the survival.

HISTOPATHOLOGICAL PATTERN OF GASTROINTESTINAL STROMAL TUMORS (GIST) AFTER TREATMENT WITH IMATINIB

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Mesenchymal neoplasms derived from the stroma of the gastrointestinal tract (GISTs) are resistant to classic chemotherapy. These tumors arise from Cajal cells and show immunopositivity for tyrosine kinase receptor – CD117. This reaction is a basis for recognizing them and allows for treating such neoplasms with imatinib (Glivec). To-date, there have been no reports about the results of curative efforts. Two patients (the first patient with primary GIST of the small intestine, the second one with primary GIST of the stomach) were treated by imatinib because of dissemination of neoplasm into the peritoneum. A few months later, a partial response was present. At this time, the metastatic tumors were removed. The resected specimens were fixed in 10% formalin and additional tissue sections were routinely stained with hematoxylin and eosin. The immunohistochemical analysis for CD117 (Dako) was also done. On gross examination, tumors of the diameter from 2 to 6 cm, with a gelatinous consistency were present, showing numerous hemorrhages and necrotic slough. Microscopically, residual neoplasms were seen, what was confirmed by the CD117 positive reaction in their cells. The histological pattern showed a partial response. The results of the therapy were necrosis, hemorrhage and foci of fibrosis and hyaline changes. The tumor cells showed a low degree of damage (about 30% of neoplasm volume). Conclusion: a partial regression of neoplasm may be expected in patients treated with imatinib. This situation allows for an earlier resection of non-operable GISTs within the healthy limits.

HISTOLOGICAL FINDINGS IN LIVER BIOPSIES FROM PATIENTS INFECTED WITH HIV

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Seventy-two liver biopsies from 65 patients with HIV infection were studied (seven patients had 2 biopsies). The indications for liver biopsy were abnormal liver function tests and/or HBV or HCV infections. Most patients (49) had HIV and HCV co-infection. Histological lesions in these biopsies were compared with histological findings of untreated chronic hepatitis C without HIV infection. The histological patterns were similar in both groups. In patients with HCV/HIV co-infection, a nearly normal liver was observed more often and cirrhosis was seen more rarely. We found a strong correlation between the mean count of CD4 lymphocytes and the inflammatory activity assessed according to the METAVIR classification.

ALBUMIN AND FIBRINOGEN/FIBRIN COLOCALIZE WITH CD55 AND CD59 AT THE SURFACE OF PLACENTAL VILLI

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The understanding of the phenomenon of fetal defense against maternal anti-allogenic immune response is not complete. A well-known element of the feto-maternal barrier is the presence of complement inhibitors CD55 and CD59 at the surface of the placental villi. According to some hypotheses and in vitro experiments, fibrinogen/fibrin and albumin, derived from plasma, are able to form a complex that could cover and protect the surfaces of various cells. For example, such complex might prevent the access of immune-competent cells and molecules. In this report, we present the results of immunohistochemical studies addressing the above-mentioned hypothesis with regard to the surface of the villi in the human placenta. In addition, we have investigated whether there is a correlation between the distribution of CD55, CD59 and the putative fibrinogen/fibrin-albumin complex. By means of both peroxidase-based and fluorescent techniques, we have immunolocalized fibrinogen/fibrin and albumin in paraffin sections of normal term-placentas, obtained upon delivery from 11 women. We found marked deposition of both fibrinogen/fibrin and albumin around the villi, in the form of dense layers covering respectively 78.4±1.4% and 82.4±1.2% of the villous surface. By double-label immunofluorescent confocal microscopy, at those sites, both immunoreactivities strictly colocalized with each other, as well as with complement inhibitors. The analysis of fluorescence-resonance energy transfer (FRET) between fluorophores indicated that a substantial portion of fibrinogen/fibrin and albumin molecules on the villous surface were separated from each other by the distance smaller or equal to 30nm. Our results suggest the existence of an inter-molecular complex between fibrinogen/fibrin and albumin, which, similarly to CD55 and CD59, might constitute an element of a protective barrier at the feto-maternal interface.

ROLE OF PATHOLOGISTS IN THE DIAGNOSIS OF RECTAL CANCER

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1. Standardization of the oncological treatment. Randomized, multicenter trials are an accepted method of treatment in oncology. One of the concerns associated with classical rectal cancer surgery is the recurrence rate (25–30% in the B and C stage, according to Dukes). Well-performed total mesorectal excision (TME) surgery has now become the golden standard in rectal resection. TME with or without preoperative radiotherapy (5x5Gy) is employed to reduce local recurrence and increase the survival of patients. **2. The role of pathologists in the quality control of diagnosis and treatment of rectal cancer.** Multidisciplinary teams are important for the achievement of optimal treatment planning of rectal cancer patients. It has been concluded that the histopathological type, grade and stage of cancer according to the depth of cancer invasion (pT), status of lymph nodes (pN) and metastases (pM) are the most important prognostic factors. The new role of pathologists in the quality control of treatment is associated with the evaluation of predictive factors in the course of a macroscopic study of TME and microscopic analysis of circumferential margin involvement. The cancer protocol according to the UICC classification of 2003 includes both parameters. The first one concerns the examination of the non-peritonealized surface on the fresh specimen. The completeness of the mesorectum is scored as incomplete, when defects in the mesorectum reach down to the muscularis propria, as nearly complete, when irregularities of the mesorectal surface are noted, with defects greater than 5mm, but none extending to the muscularis propria, and complete, when only minor irregularities, less than 5mm in depth, are seen. The circumferential margin involvement is the second powerful predictor of local recurrences in rectal cancer. A margin smaller than or equal to 2mm should be regarded as associated with an increased risk of local recurrence (16% versus 6% for patients with the margin

over 2mm). Margins of 1mm or less are predictive of an increased risk of developing distant metastases (37% versus 15% for patients with the margin over 1mm) and shorter survival times (70% versus 90% 2-year survival rates, respectively). **3. Conclusions.** The macroscopic examination of TME and microscopic evaluation of the circumferential margin involvement are the most powerful predictors of local recurrences in rectal cancer and they are the factors of surgical treatment quality control in the rectal cancer patients.

ACTIVITY OF METALLOPROTEINASE 2, METALLOPROTEINASE 9 AND THEIR INHIBITORS IN ANEURYSMAL AND ATHEROSCLERIC AORTA

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The aim of the study was to estimate the activity of metalloproteinase 2 (MMP2), metalloproteinase 9 (MMP9) and tissue metalloproteinase inhibitor 2 (TIMP2) in tissue samples of the aortic wall obtained from patients with aortic abdominal aneurysm (AAA) and arteriosclerosis. Aorta samples were taken during reconstructive surgery of the aorta. The activity of MMP2, MMP9 and TIMP2 was estimated by morphometric analysis. The number of positive cells was counted in microscopic slides after immunohistochemical reactions with appropriate antibodies. The results were evaluated and compared in 4 groups: I – AAA, operated electively (N=49), II – symptomatic and ruptured AAA (N=19), III – aorto-iliac occlusion (N=17), IV – the controls – aorta samples obtained from organ donors (N=10). Metalloproteinase expression was significantly higher in aneurysms than in other groups. A statistically significant difference was revealed also in MMP9 activity in ruptured AAA. MMP2 expression was higher in electively operated AAA, but the difference was not significant.

PARAGANGLIOMA OF UNUSUAL LOCATION AND CLINICAL MANIFESTATION. A CASE REPORT

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Paragangliomas are uncommon neuroendocrine tumors. In the head and neck, they are most commonly seen in the carotid body, the temporal bone or along the vagal nerve. Many of them present with symptoms related to catecholamine excess. A 77-year old woman was operated on because of a large subcutaneous tumor located in the occipital region, destructing the adjacent bone and forming an intracranial mass. Neurosurgeons suspected a meningioma. Ten years previously, this woman had been diagnosed to have a small subcutaneous mass situated behind the ear, but at that time the patient refused her consent to be operated on. Frozen sections revealed monomorphic cells without atypia organized in a lobular pattern. The lobules were divided with tiny blood vessels. The patient was suspected of a paraganglioma. Paraffin sections and immunohistochemical studies confirmed the initial suspicion of paraganglioma.

THE PRESENCE OF ENDOGENOUS BIOTIN AS A POTENTIAL SOURCE OF THE DIAGNOSTIC FAILURE – PERSONAL EXPERIENCE

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Among the immunohistochemical methods, which are in use today the most popular are those, which base on high affinity, which biotin has to avidin. They are the most often recommended methods in the literature, because they are very sensitive and they are more desirable than the previously described methods such as PAP or APAAP. They are recommended by the producers but using the methods based on avidin-biotin affinity we have to remember about the endogenous occurrence of these substances in tissues. We present same examples from the literature as well as our personal experience and we describe in which way the presence of endogenous biotin can influence upon the occurrence of false positive reaction and subsequently on the diagnostic failure. We try to elaborate the practical model to correctly resolve this technical problem using the adequate methodology and quality control system which in everyday laboratory diagnostics we have to remember about.

EFFECT OF rhTNF-alpha ON NEOANGIOGENESIS ACCOMPANYING THE GROWTH OF MORRIS 5123 HEPATOMA

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The study presents the ultrastructural characteristics of endothelial cells (EC) in the development of passaged Morris 5123 hepatoma, modified by intratumor (i.t.) administration of human recombinant TNF-alpha (rhTNF-alpha). The enhancement of angiogenesis (fields of microvessels and single EC) was evaluated using immunohistochemical methods in a light microscope. Ultrastructural examinations were performed under a transmission electron microscope (TEM). In the primary focus of the neoplasm, the i.t. rhTNF-alpha injections caused hemorrhagic necrosis, as well as thromboembolic changes and fibrinoid necrosis of the walls of the host vessels supplying the tumor and capillaries formed by the neoplasm. Tumor areas where hemorrhagic necrosis was removed by macrophages showed the inflow of a varied population of EC, formation of buds and capillary canaliculi, and proliferation of fibroblasts. The TEM analysis revealed differences between EC, which participated in the local repair, forming normal vascular structures enclosed by the basement membrane and pericytes, and immature EC, which were dispersed or present in abnormal capillary structures and occurred in the texture of naturally growing control hepatomas. The TEM analysis confirms that rhTNF-alpha in the primary focus of hepatoma not only induces extensive hemorrhagic necrosis of the neoplastic tissue, but also stimulates vasculogenesis and fibroplasia in the repair processes observed in purified necrotic areas, and inhibits proliferation and migration of immature EC stimulated by neoplasm growth. We assume that EC proliferation and development of capillaries in the tumor may depend not only on the effect of the cytokine

on the impairment of the circulation in the primary focus, but also on the direct local action on EC, which may be reflected both in the high EC activity noted in the study and in the ultrastructural characteristics of vessels formed by these EC.

ANGIOGENESIS AS A RISK FACTOR FOR RECURRENCE AND DEATH IN WOMEN WITH NODE-NEGATIVE, GRADE II INVASIVE DUCTAL BREAST CANCER

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Prognostic significance of angiogenesis (number-MCV, area-MVA, and perimeter-MVP of microvessels) has been assessed in the group of 120 women with node-negative, Bloom and Richardson grade II invasive ductal breast cancer. Microvessels were detected immunohistochemically with CD31 monoclonal antibody and computerized image analysis (Quantimet 600S Leica) according to Weidner et al. All three parameters of angiogenesis were significantly higher in breast carcinomas with subsequent recurrence as compared to those without them (MCV – 78.1 vs. 54.8, p=0.00005; MVA – 7992.3 vs. 5738.9, p=0.0004; MVP – 3586.7 vs. 2482.9, p=0.0002). Similarly, higher values of the parameters of angiogenesis were found in cancers of women who died of the disease as compared to those who survived 5 years (MVC – 87.1 vs. 57.1, p=0.0001; MVA – 9145.7 vs. 5963.8, p=0.001; MVP – 3910.1 vs. 2593.4, p=0.0002). No recurrence was found if the number of microvessels was less than 44. Significantly lower percentage of women with high intensity of angiogenesis survived 5 years as compared to those with low angiogenesis (MVC – 83.1% vs. 98.4%, p=0.003; MVA, MVP – 83.3% vs. 98.3%, p=0.004). Similar associations were found in relation to recurrence-free survival (RFS) (MVC, MVA, MVP – 71.9% vs. 96.5%, p=0.0003). In the group of 93 women with node-negative, grade II less than 20mm in diameter breast carcinomas probability of 5-year survival and RFS was lower for those with high intensity angiogenesis as compared to those with low intensity (respectively: MVC – 88.1% vs. 100%, p=0.01; MVA – 88.9% vs. 100%, p=0.02; MVP – 88.4% vs. 100%, p=0.01; and for RFS: MVC – 71.4% vs. 100%, p=0.00007; MVA – 73.3% vs. 100%, p=0.0002; MVP – 72.1% vs. 100%, p=0.00009). Conclusion: The parameters of angiogenesis may stratify prognostically a heterogeneous group of node-negative grade II invasive breast ductal cancers into subgroups with better and worse prognoses with obvious therapeutic implications.

COMPARISON OF ER AND PG RECEPTOR EXPRESSION WITH HER2 STATUS BY IMMUNOHISTOCHEMICAL METHODS IN BREAST CANCERS

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Modern breast cancer diagnostics, except well-established prognostic and predictive factors (e.g. histological type, histological grade, pTNM) employs the assessment of other parameters in order to make the diagnosis sufficient and useful. These factors are estrogen, progesterone and HER2 receptor status. They enable to predict the patient's reaction to the treatment (hormono- and chemotherapy). The aim of the study was the description of dependency between HER2 and estrogen/progesterone status. The study was performed on 1776 consecutive cases of breast cancer. The estrogen, progesterone and HER2 receptor status were evaluated immunohistochemically (IHC) in each case. ER/PR positive cases were classified by presence of 10% or more positively stained cancer cell nuclei, regardless of strength of the stain. ER/PG status was stated positive, when the presence of both hormone receptors was detected. A mixed group consisted of cases, in which only one steroid hormone receptor was present. HER2 positive cases were classified by the presence of cancer cell membrane staining of 3+, using four-tier, semiquantitative DAKO system (0, 1+, 2+, 3+). The group of cases described by IHC as "0", "1+" and "2+" showed the presence of ER/PG expression in more than the half of all cases (52%, 63% and 57%, respectively), while this group was negative in 31%, 18% and 17%. Among the tumors exhibiting very strong HER2 receptor expression (3+), the majority of them were negative, considering the expression of EG/PG receptors. Only one third of them (29%) showed the presence of EG/PG receptors. The remaining groups consisted of cases with mixed ER/PG status. The results indicate the difference between the expression of estrogen and progesterone receptors and HER2 status. and suggest a necessity of independent evaluation of both steroid receptors and HER2 status.

CORRELATION BETWEEN THE LIVER HISTOPATHOLOGICAL PICTURE AND SELECTED BIOCHEMICAL PARAMETERS IN CHRONIC TYPE B AND C VIRAL HEPATITIS

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Viral B and C hepatitis infections are serious global clinical problems. The aim of this study was to evaluate the relationship between biochemical parameters and leptin concentration and the progression of morphological changes of the liver in patients with chronic viral hepatitis type B (CHB) and chronic viral hepatitis type C (CHC). The studies were carried out in 15 patients with CHB (4 females and 11 males, aged 16–49 years) and 15 patients with CHC (6 females and 9 males, aged 20–56 years), all of whom were anicteric and not treated. The histopathological evaluation was based on the commonly used criteria according to the international expert group "Metavir" classification, which includes necro-inflammatory activity (G) and the progression of fibrosis (S). Biochemical parameters – enzymes: AST, ALT, AP, GGT, LAP, NT and serum total bilirubin, leptin, total protein and electrophoresis were estimated. In both groups, the rise of AST, ALT and GGT activity depended on the observed grade of necro-inflamma-

tory change progression. GGT activity also depended on the stage of liver fibrosis, fatty change and biliary duct damage. In 73% of patients with CHB, an increased leptin level was found, but it diminished together with necro-inflammatory activity progression. In patients with CHC, this change was less characteristic. Conclusions: 1. Progression of necro-inflammatory changes is accompanied by increased AST, ALT and GGT levels. 2. GGT level rises both in fibrosis and in other morphological liver damage. 3. It seems that leptin level is a more sensitive indicator of inflammatory progression in CHB patients than in CHC individuals.

EVALUATION OF SELECTED HISTOPATHOLOGICAL AND CLINICAL PARAMETERS IN PATIENTS WITH CHRONIC TYPE B HEPATITIS

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Goal of study: evaluation of preliminary treatment results with Encortolon/TFX of patients with chronic type B hepatitis treated with interferon alpha (IFN). Material and methods: forty (40) patients with chronic type B hepatitis, matching the criteria of HBsAg(+), HBeAg(+), pDNA(+), ALT100V/L, were divided into two (2) groups. Group I was treated with IFN , while Group II was preliminarily administered Encortolon/TFX and then – IFN . Biochemical tests of hepatocyte activity and markers of HBV infection were done in blood serum. In all the patients, histopathological verification of liver biopsy was performed before, and – in the majority of individuals – after the treatment, taking into account the necrotic-inflammatory activity (G) and the degree of fibrosis (S), following the scale designed by the "Metavir" international group of experts. Also immunohistochemical reactions were performed for HBV presence in the liver and the percent of immunopositive HbsAg and HbcAg cells. Results: in Group I, after six (6) months of therapy, two (2) patients (10%) recovered, while remission was observed in 6 patients (30%). In Group II, seven (7) patients (35%) recovered, while 11(55%) entered remission. The clinical picture was identical with the histopathological picture. The HBcAg expression rate did not depend on G-positive values. HBsAg expression was absent in some G1 cases. In 2 out of 7 patients from Group II, who recovered, the S value decreased. Conclusions: preliminary treatment with glucocorticosteroids/TFX, administered prior to IFN treatment in patients with chronic HBV, increases the chances for recovery and remission. It may also positively influence the regression of hepatic fibrosis. HbcAg expression in the liver of patients with HBV always accompanies G positivity, but does not always depend on its values. No HbcAg expression has been observed in the hepatic tissue at G=0.

CORRELATION OF ESTROGEN AND PROGESTERONE RECEPTORS EXPRESION WITH PROLIFERATION INDEX MIB–1 IN MENINGIOMAS OF THE CENTRAL NERVOUS SYSTEM

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Meningiomas are more frequent in women than in men. The female to male ratio ranges from 3:2 to 2:1. These data suggest the effect of sex hormones in the genesis of meningiomas. The aim of the study was to estimate the correlation between the expression of estrogen and progesterone receptors in some histological types of meningiomas (G1 WHO) and atypical meningiomas (G2 WHO) with the MIB–1 proliferation index and the sex of patients. Tissue material was obtained from 68 surgically removed meningiomas (46 benign – meningothelial, fibrous and transitional, 21 atypical and 1 anaplastic). The expression of estrogen and progesterone receptors and the expression of MIB–1 were examined in paraffin sections of tumors after immunohistochemical reactions with appropriate antibodies. A very weak immunoexpression of estrogen receptors was present in 33% of atypical meningiomas and in 35% of benign tumors, and it did not correlate with sex. Immunoexpression of progesterone receptor was present in the majority of benign meningiomas, and in 38% of atypical ones, and was more frequent in women. Conclusion: immunoexpression of progesterone receptors in meningiomas is more frequent in women than in men and it is more rare in atypical than in benign meningiomas. The expression of estrogen receptors does not correlate with the MIB–1 index, nor with histological malignancy (G) and with the sex of patients.

EVALUATION OF PORPHYRIN LEVELS IN PATIENTS WITH SKIN AND SYSTEMIC SCLERODERMA

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Porphyrins are compounds mainly produced in the liver and bone marrow. Their biosynthesis is controlled by enzymatic processes, and the inhibition of such biosynthesis results in accumulation of undesired compounds in very high levels in multiple locations, e.g. in urine. By absorption of visible light they become photodynamically active; their photoactive action is based on the accumulation in the skin. In hepatic porphyria, specific skin alterations have been observed, such as collagen overproduction, and pseudoscleroderma as the final outcome. The development of scleroderma-like lesions may be dependent on collagen synthesis under the influence of porphyrins accumulated in the skin, what has been confirmed by *in vitro* studies. The pathogenesis of scleroderma is rather complicated. The main feature is progressive sclerosis and fibrosis. In the present study we investigated whether blood and urine porphyrin content may correlate with clinical symptoms. The study included 25 patients with skin and systemic scleroderma. The concentration of porphyrins was measured in 24-hour urine collection. A characteristic feature of porphyrins is red fluorescence, which allows for their macro- and microfluoroscopic visualization. Urine samples were studied under a Wood lamp and spectrophotometrically. Quantification was also done with relation to delta-aminolevulinic acid, uroporphyrinogen and coproporphyrinogen concentrations. Our results revealed an increase in porphyrin concentration in the urine. The most important alterations were found with regard to porphyrin precursor, i.e. delta-aminolevulinic acid and porphobilinogen. Less important shifts were found in phase II of porphyrin synthesis, i.e. uroporphyrin. The studies on porphyrin accumulation in the blood of patients with scleroderma have also been commenced. Our

preliminary results indicate that coproporphyrin is elevated in such cases.

ENDOTHELIAL NEOPLASMS IN THE LUNGS

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Within 20 years, we had an opportunity to observe 10 cases of endothelial neoplasms in the lungs. The group included 7 women and 3 men, aged from 18 to 65 years. These cases were divided into two groups: low-grade malignancies – an epithelioid hemangioendothelioma (8 cases – seven women and one man, aged between 18 and 65 years), and highly malignant endotheliosarcomas, seen in two men, 28 and 47 years of age. The patients with epithelioid hemangioendotheliomas did not manifest any clinical signs, or else the signs were discrete. The individuals reported to hospitals because chest X-rays accidentally revealed nodular lesions in both lungs. Histological examination of the material collected from the bronchi and the sputum was negative for neoplasm. These patients were suspected of tuberculosis or sarcoidosis and in some cases the therapy was even initiated. The correct diagnoses were established based on the histological examination of lesion samples collected in the course of thoracotomy or thoracoscopy. The progression of the disease in these cases was slow and the patients were in good condition for many years. In one case (a 27-year old woman), the diagnosis was possible after autopsy. Highly malignant hemangiosarcomas were diagnosed in two men with poor clinical status and one patient died shortly after the histological diagnosis based on material taken during thoracotomy. In the other case, the diagnosis was possible on autopsy. The morphological appearance of these tumors is very characteristic. In case of any doubt, we can use immunohistochemical tests, using endothelial markers, mainly CD34, eventually CD31 or factor VIII. Because in all cases multiple separate lesions are seen in both lungs, there is some doubt whether it is a primary multifocal neoplasm or rather a metastatic one. In our opinion it is a metastatic neoplasm arising from an unknown primary lesion. In some cases it can be a primary liver tumor. In highly malignant cases, primary tumors were situated in the liver and soft tissues of the scrotum. The prognosis for patients with highly malignant tumors is very poor. Epithelioid hemangioendothelioma progresses slowly and the patients live for many years. No successful therapy for this neoplasm is available. In one case, chemotherapy has slightly improved the state of a patient.

LATE PULMONARY METASTASES OF SMOOTH MUSCLE TUMORS

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The authors present a series including 5 cases of metastatic pulmonary smooth muscle tumors diagnosed in the Department of Pathology, Collegium Medicum, Jagiellonian University, in the years 1991–2001: 3 cases of metastatic high-grade and 1 case of metastatic low-grade leiomyosarcomas and 1 case of benign metastasizing leiomyoma. In all the cases, immunohistochemical examinations were performed to show smooth muscle differentiation (desmin) and the mitotic activity (Ki–67 index). All the materials were obtained from women ranging in age from 38 to 62 years. In 4 cases, there was a previous history of a primary smooth muscle neoplasm of the uterus (in one case the clinical data were unavailable). All the patients remain in follow-up. The group of metastatic pulmonary smooth muscle tumors can be divided into leiomyosarcomas and benign metastasizing leiomyomas. The most frequent site of origin of these neoplasms is the uterine corpus. Benign metastasizing leiomyoma is a rare neoplasm presenting as single or more frequently multiple tumors consisting of smooth muscle cells with histological characteristics of leiomyoma. The tumors are composed of spindle, uniformly benign-appearing smooth muscle cells with a low mitotic activity (usually below 1/10hpf). Necrosis is not observed. Within the smooth muscle nodules there are entrapped epithelial-lined spaces, which are remnants of small bronchi. The follow-up in these cases usually shows a very slow progression of lesions and long-term survival. Some cases with an increased mitotic activity and/or cellularity and/or atypia represent metastatic low-grade leiomyosarcomas rather than benign metastasizing leiomyomas.

PRIMARY AMYLOID TUMORS OF THE LUNGS – FIVE CASES

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Primary amyloidosis may occurs in the lungs in three forms: tumor-like lesions located in peripheral parts of the lungs, disseminated tumor-like or confluent lesions of the bronchi and trachea, and in the most rare form, diffuse amyloid deposits in the lung stroma. We present material of five cases of lung amyloidosis in the form of tumors that were diagnosed between 1996 and 2002. These were cases, in which because of tumors having been noted in chest X-ray, neoplastic processes were suspected. Since cytological and histological examination of the samples did not confirm the diagnosis of neoplasm, in each case a total tumorectomy was performed. After the surgery, the condition of the patients was good. The location of the lesions and the results of immunohistochemical staining in the tumor samples will be presented in the table. The series included 3 women aged 46, 63 and 69 years and 2 men, both aged 58 years. The location of the tumors was as follows: 3 in the right and 2 in the left lung. Amyloid AA was absent in all the cases, AL amyloid was found in all the patients and transthyretin in one case. Doubtful expression of beta–2-microglobulin was observed in 3 tumors and surfactant A in one. In all the presented cases, positive AL immunohistochemical staining was observed and it is probably associated with inflammatory processes and the secretion of immunoglobulin in the lung. In one case, amyloid tumor occurrence was associated with marginal-zone lymphoma (BALT) in the lung. Because the reports on primary tumoral amyloidosis in the lungs are rather scarce, and the described groups are not numerous, the presentation of the above five cases with immunohistochemical assessment of the amyloid type seems to be worthwhile.

COMPARATIVE ANALYSIS OF MICROSATELLITE INSTABILLITY AND EXPRESSION OF MLH1, MSH2, P21(WAF1), P53 PROTEINS IN

COLORECTAL CANCER AND ESTIMATION OF THEIR CLINICO-PATHOLOGICAL SIGNIFICANCE

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On the basis of clinico-pathological and molecular data three hypotheses regarding colorectal carcinogenesis were established. There are: adenoma-carcinoma sequence, cancers with microsatellite instability and cancers associated with ulcerative colitis. In cancers originating at the base of adenoma or colitis ulcerosa *APC*, *K-ras*, *DCC*, *P53* are mutated and secondarily expression of some other proteins related to passage through the restriction point S/G1 is changed, for example P21 (WAF1). Instead microsatellite instability arises as a result on mutation of one of mismatch repair genes: MSH2, MLH1, PMS1, PMS2, MSH3, MSH6 (GTBP), and mutations of MSH2 and MLH1 are responsible for 80% of cases of the disorder. A group of 110 colorectal cancers from an equal number of patients was investigated for P21(WAF1), P53, MSH2, and MLH1 protein expression by immunohistochemical techniques. Analysis of microsatellite instability (MSI) was established in polymerase chain reaction (PCR) with 10 pairs of primers. The results were correlated with various clinico-pathological parameters. Statistical analysis revealed a statistically significant inverse correlation between MSI and MLH1-immunopositivity ($p=0.004$) and P53 and P21(WAF1)-immunopositivity ($p=0.00008$) and a correlation between P53 and MSH2-immunopositivity ($p=0.0003$), P53 and MLH1-immunopositivity ($p<0.000001$). Furthermore, it revealed a correlation between MSI and right-sided location of a tumor ($p<0.000001$), high grade of malignancy ($p=0.006$) and the presence of lymphocytic infiltration surrounding a tumor ($p=0.000006$); between MLH1-immunopositivity and right-sided location of a tumor ($p<0.000001$), high local stage ($p=0.00006$), lack of liver metastases ($p=0.05$) and vasal emboli ($p=0.0004$), high grade of malignancy ($p=0.000002$) and the presence of lymphocytic infiltration surrounding a tumor ($p=0.00001$). Conclusion: These data suggest that two basic types of colorectal carcinogenesis exist: one is connected with *P53* mutations and secondary lack of P21(WAF1) expression and another is connected with MSI, most often occurring at the base of changes of MLH1 expression. Cancers demonstrating MSI and lack of MLH1 expression differ in biology from the others: most often they are right-sided, have higher local stage and higher grade of malignancy, instead unusually vasal emboli and liver metastases are revealed, what may be related to the presence of abundant lymphocytic infiltration surrounding a tumor.

EXOGENOUS SURFACTANT (SURVANTA) EFFECT ON LUNG PARENCHYMA (AN ANIMAL MODEL)

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Morphologic studies on lung parenchyma after exogenous surfactant administration have been published since the end of the 70-ties. The majority of publications address the lung of the newborn. Pulmonary surfactant forms an insoluble film at the surface of the alveolar lining fluid and modifies the surface tension in a manner that depends on alveolar surface area. In tissue cultures of lung epithelia, the supplementation of the media with low concentrations of surfactants led to their recirculation. Increasing concentrations of surfactant caused the cell membranes lysis. To evaluate the effects of therapeutically useful surfactant, experimental studies were done. Young, healthy male anesthetized Wistar rats received surfactant intratracheally, while the controls were administered an equivalent amount of saline. From anesthetized animals whole lungs were removed after 30' and 6h and 24h and 5 and 10 days. Light and electron microscopic examinations were performed. Thirty minutes after administration of Survanta hypopneumatic spots were irregularly distributed. In those areas changes in epithelia, endothelia, stromal cells and macrophages included an increased number of micropinocytic vesicles, intracytoplasmic edema, enlargement or elongation of all cellular membrane systems and additionally the presence of coiled or foiled surfactant lipid material in the stroma and capillaries lumen. Focally, epithelial cell lysis, erythrorrhages with the change of shape of red blood cells were noted. At the some time, single macrophages laden with exogenous and endogenous surfactants were seen. Parallel, in some alveoli, numerous lamellar bodies and tubular myelin were present, being in contact with cell membranes. In consecutive observation periods, the above-described changes were maintained and the number of lipid-laden macrophages was increasing in the alveolar lumina. In some alveoli, numerous macrophages, also with interrupted cell membranes and liberated to alveolar lumen whorls of surfactant material were visible. Starting on the first day up to the 10^{th} day, in addition to the aforementioned changes, aggregates of platelets and fibrin depositions in capillaries and fibrin in alveolar lumen were present. Exogenous surfactant administered to a healthy lung causes alveolar damage, intraalveolar bleeding, also with red blood cell changes of shape, an increased number of alveolar macrophages and lysis of cellular membranes of some cells.

COMPARATIVE ASSESSMENT OF THE INFLUENCE OF BONE IMPLANT MATERIALS ON THE TISSUE REACTION AND INFLAMMATORY MEDIATORS LEVEL

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No abstract available.

A CORRELATION OF ESTROGEN AND PROGESTERONE RECEPTOR EXPRESSION IN FINE NEEDLE ASPIRATION BIOPSIES AND SURGICAL SPECIMENS

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Actually, the semi-quantitative evaluation of expression of estrogen (ER) and progesterone (PR) receptors in breast cancers is a routine test. In this study, we evaluated correlation of ER and PR receptor expression determined by immunocytochemistry on cytological material from FNAB and by immunohistochemistry on

postoperative specimens from 46 women with primary breast carcinoma, aged 33–75 years. Fourteen patients received neoadjuvant treatment after FNAB. The same expression of ER and PR receptors in cytological and tissue samples was observed appropriately in 30/46 (65.21%) and in 28/44 (63.63%) patients; including 10 cases after neoadjuvant therapy. Four patients after neoadjuvant therapy had different receptors expression in surgical specimens comparing to cytological material: in 2 women ER expression decreased, in 1 increased and PR expression increased in 3 patients. Receptor expression changes in tissue samples in the remaining patients were as follows: in 9 women ER and PR expression was higher comparing to cytological material; lower expression of ER was in 3 patients and PR in 4. In postoperative specimens, ER expression changes occurred in 36.36% (16/46) of cases and PR in 34.78% (16/44) of cases. The changes having a predictive value occurred in 10 patients (21.73%): positive in 6 (13.04%), negative in 4 (8.69%). Significant correlation of ER and PR receptor expression in cytological and tissue samples indicates clinical usefulness of determination of hormonal steroid receptors in cytological material.

GASTRIC LYMPHOMAS

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The stomach is one of the most common sites of extranodal lymphoma (30–40%), while primary lymphomas account of about 5% of gastric neoplasms. Lymphomas may affect the stomach as primary tumors or as secondary involvement by disseminated nodal lymphomas. Most low-grade B-cell gastric lymphomas are marginal zone lymphomas (MALT type), which have a unique appearance and behavior. Most gastric marginal zone lymphomas arise as an immunological reaction to an infection by Helicobacter pylori, what leads to chronic follicular gastritis with activation and clonal expansion of B cells. There are two main chromosomal aberrations in gastric MZL. Trisomy 3 is found in 60% of cases and these patients react well to treatment. In 30% of cases there is t(l1;18)(q21;q21) connected with a change of expression of API-2-MLT-1 gene. These tumors are frequently in advanced stages and do not respond to Helicobacter pylori eradication. On endoscopy, gastric MZL appears as a flat, infiltrative or ulcerated lesion. Histologically, it is characterized by cellular heterogeneity, including small lymphocytes, centrocyte-like cells (marginal zone cells) and/or monocytoid B cells, often with plasmacytic differentiation and accompanied by lymphoepithelial lesions. In small routine gastrobiopsy sometimes it is very difficult to distinguish MZL from reactive lymphoid hyperplasia. MZL cells typically express IgM and show light chain restriction. They are CD20+, CD5-, CD10-, CD23-, CD43+/–. MZL usually has a very indolent course and excellent prognosis (5-year survival >70% of cases). High-grade lymphomas may arise in the stomach de novo or from MZL and most of them are classified as diffuse large B-cell lymphoma (DLBCL). They present as exophytic tumors and the patients have symptoms similar to those of gastric carcinoma (pain, weight loss and bleeding) and require the same treatment as that employed in DLBCL. Mantle cell lymphoma (MCL) presents as multiple lymphomatous polyposis. The cells are small or medium-sized, with cleaved nuclei and IgM+, IgD+, CD5+, CD43+, cyclin D1+, CD23–.The stomach is a common extranodal location of highly aggressive Burkitt's lymphoma (BL). BL occurs in children, young adults and HIV+ individuals. Histologically, monomorphic medium-sized cells (CD20+, c-myc+, bcl2–) with numerous mitotic figures (MIB1 in nearly 100% cells) infiltrate the stomach wall.

MARGINAL ZONE B-CELL LYMPHOMA (MALT TYPE) OF THE SALIVARY GLANDS AND SCLERODERMA – AN ACCIDENTAL COEXISTENCE OR A PATHOGENIC RELATION

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The occurrence of neoplasms (mainly lung cancer) in the course of scleroderma, as well as salivary gland lymphoma (MALT type) in Sjögren's syndrome (SS) is very well known. However, the association of scleroderma and non-Hodgkin's lymphoma is uncommon and its pathogenic relationship is a much debated subject. We described a previously unreported association of scleroderma and a marginal zone B-cell lymphoma (MALT type) of both parotid glands, which developed simultaneously. A 22-year old woman suspected of scleroderma and secondary SS was referred to our department for a second opinion because of unsuccessful treatment. Scleroderma was confirmed by clinical presentation and laboratory examinations. The specimen of the parotid gland was re-examined to verify the previous diagnosis of lymphoepithelial sialadenitis, characteristic of SS. The parotid gland biopsy revealed the picture of marginal zone lymphoma (broad strands of monocytoid B-cells that surrounded and invaded the epithelial-myoepithelial islets and the presence of monotypic immunoglobulin in these cells). Tumor cells were CD20+, kappa+, CD5–, CD43–, cyclin D1–, CD23–. A possible paraneoplastic nature of scleroderma could be taken into consideration in this case. Lymphoma can induce endothelial damage and the sclerosis process by secretion of pro-inflammatory factors: TGF , Il–2, –4, –6 and endothelial cell cytotoxic factors. Independently of the pathogenic mechanism of these two diseases, it seems very important to emphasize that scleroderma may be the first manifestation of lymphoma.

AL AMYLOIDOSIS – AN INTERDISCIPLINARY DIAGNOSTIC PROBLEM

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Primary AL amyloidosis is plasma cell dyscrasia characterized by fibrillar deposits of monoclonal immunoglobulin light chains in various organs, especially in the heart, causing its rapid dysfunction and death. Despite of the fact that cardiac amyloidosis is a well-known disease, it is not taken into diagnostic consideration because of its rare occurrence, different clinical picture and sometimes difficulties in diagnostic management. We report a case of a 60-year old man with symptoms of cardiac refractory arrhythmia, but no response to conventional treatment and many ablation procedures. Echocardiography revealed thickening of the left ventricular wall and abnormal myocardial texture (granular sparkling). The electrocardiogram showed low

voltage. On the basis of these features, amyloidosis was suspected. Congo-red positive amyloid deposits and -light chains restriction (immunoperoxidase) were observed in abdominal fat pad biopsy. Immunohistochemistry for kappa light chains, complement A, transthyretin and 2-microglobulin was negative. A low level of monoclonal light chain protein was revealed in the serum by immunofixation. A trephine bone marrow biopsy showed -light chain restricted plasmocytosis (15% of the nucleated cells) without evidence of amyloidosis. Taking into consideration all these findings, a monoclonal gammapathy of uncertain significance associated with systemic AL amyloidosis was diagnosed. Unfortunately, the patient did not receive new therapy (high-dose chemotherapy and bone marrow autotransplantation), because amyloidosis was diagnosed too late and the patient was in a very advanced stage of disease. Congestive heart failure developed rapidly and the patient died one month after the diagnosis. The presented case is remarkable as it demonstrates that cardiac amyloidosis could be a symptom of plasma cell dyscrasia.

MORPHOLOGIC CHANGES IN THE COURSE OF ACUTE PANCREATITIS AFTER STIMULATION OF ADENOSINE A2a RECEPTORS

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The aim of this study was to assess the role of adenosine A2a receptors in the course of acute pancreatitis (AP) induced with sodium taurocholate at the dose 0.3ml/100g b.w. The experiment was performed on Wistar male rats divided into four groups: I – control healthy animals, II – control animals with AP, III – animals with AP, which were injected with A2a receptor agonist CGS 21680 at the dose of 3mg/kg b.w., IV – animals with AP, which were injected with A2a receptor antagonist ZM 241385 at the dose of 3mg/kg b.w. Blood and pancreas samples for biochemical and morphological examinations were collected 2, 6, and 24 hours after the injection of sodium taurocholate. There was an increase in serum amylase and lipase in all the three groups of AP animals comparing with the control group. The increase was the lowest in the group receiving receptor A2a agonist CGS 21680. In all the three groups after the administration of sodium taurocholate, we observed the development of acute pancreatitis with the presence of necrosis and hemorrhages. The morphological results were similar; the administration of CGS decreased the intensification of inflammatory changes. These results could suggest a positive effect of A2a receptor in the course of the experimental model of AP.

MOLECULAR DIAGNOSTICS OF SOFT TISSUE AND BONE TUMORS

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Soft tissue and bone tumors comprise a very heterogeneous group of tumors and frequently cause diagnostic difficulties. Immunohistochemical studies have become a very helpful tool in classifying tumors histogenetically. Cytogenetic studies of these tumors have been performed for several years and the discovered cytogenetic rearrangements have been studied using molecular methodologies. Many of these cytogenetic alterations are already characterized on the molecular level. Since many genetic rearrangements have proved to be specific for the tumor type, molecular determination of these rearrangements can be used in the diagnostic process. Recently, microarray studies have been introduced; they allow for examining the expression of many genes at the same time. The authors will present molecular characteristics of one of the rare soft tissue tumors, extraskeletal myxoid chondrosarcoma, and review the application of molecular methodology in the soft tissue and bone tumors diagnostics.

DIFFUSE PULMONARY OSSIFICATION – A CASE REPORT

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The author describes the case of diffuse pulmonary ossification in a 67-year old man, admitted to the Pulmonary Diseases Ward because of inspiration dyspnea. The patient had suffered from chronic coronary disease for some years. He underwent a myocardial infarction two years previously and had an artificial mitral valve implanted because of mitral valve stenosis (hemodynamic stage III/IV according to NYHA) and plastic surgery of tricuspid valve done four months earlier. The patient died after three days of hospitalization. The autopsy revealed that the death was caused by acute left ventricle failure with a massive pulmonary edema. Moreover, in both lungs multiple diffuse foci of bone formation were revealed.

P53 PROTEIN EXPRESSION AND HPV INFECTION IN CONJUNCTIVAL AND EYELID PAPILLOMAS AND CANCERS

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Squamous cell papilloma (SCP) and cancer (SCC) as well as basal cell carcinoma (BCC) of the conjunctiva are common epithelial tumors. P53 protein is present in normal cells and is assumed to induce G1 arrest or apoptosis in the presence of DNA damage. In many tumors *p53* gene mutation leads to stabilization of this protein, which can be detect immunohistochemically. It was shown recently, that HPV infection can take part in the development of conjunctival and eyelid benign and malignant lesions. The aim of our study was an evaluation of P53 protein expression and HPV detection in 45 papillomas and 38 squamous and basal cell carcinomas of the conjunctiva and eyelid. P53 protein accumulation was detected immunohistochemically using polyclonal antibody (DAKO). HPV was detected by PCR-RFLP method. We observed P53 protein expression in 30 out of 45 squamous cell papillomas (66.6%) and in 31 out of 38 squamous and basal cell carcinomas (81.6%). Papillomas with dysplasia revealed P53 overexpression in 5 out of 7 cases however, there was no relationship between the presence of dysplasia and P53 protein overexpression ($p=0.44$). The study showed that in SCC group P53 expression increased with G grade – every case of G3 SCC was characterized by P53 protein overexpression. In nodular type of BCC all cases were P53 positive also, as well as 9 out of 10

mixed type BCCs. Moreover, there was a statistically significant difference between P53 protein expression in squamous and basal cell carcinomas (p=0.03). HPV infection was observed in benign and malignant lesions of the conjunctiva and eyelid. Preliminary analysis showed the presence of HPV infection in about 30% of papillomas. Such observations might suggest an influence of HPV infection and P53 protein accumulation on carcinogenesis of the conjunctiva and eyelid.

ANALYSIS OF HER–2/neu EXPRESSION IN TISSUE AND OF ITS CONCENTRATION IN SERUM AND NEOPLASTIC EFFUSIONS IN OVARIAN CARCINOMA PATIENTS

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HER–2/neu is a tyrosine kinase receptor associated with signal transduction pathway. The aim of the study was the comparison of HER–2/neu expression in tissue sections and respective cyst and/or ascitic fluid cells with the concentration of antigen in serum and neoplastic effusions in patients with ovarian carcinoma of various histological types. The over-expression of HER–2/neu was evaluated immunohistochemically and the level of the antigen using a commercial enzyme immunoassay (ELISA). The heterogeneity in HER–2/neu expression in the tissue and different concentrations of the circulating marker in individual patients were revealed. The higher values of HER–2/neu level in tumor effusions than in corresponding sera were detected. In comparison to the sera the level of HER–2/neu in tumor effusions appeared to be significantly related to its expression in the tumor tissue. Our results indicate, that HER–2/neu evaluation in the place of its generation seems to be more helpful in planning of therapy blocking HER–2/neu receptor in ovarian carcinoma patients.

MOLECULAR STUDIES OF REARRANGEMENT OF GENES CODING FOR TCR-GAMMA AND IgH IN THE ROUTINE DIAGNOSTICS OF LYMPHOPROLIFERATIVE LESIONS

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Modern histopathological diagnostics requires integrated approach, involving study of histology, cytological features, immunophenotyping and a clinical picture. More effective lymphoma therapy results mostly from an entity-specific therapeutic approach, and this requires an accurate classification of lymphomas by the pathologists. In some cases the exact diagnosis has to include search for the entity-specific mutations or assessment of the rearrangement of genes coding for lymphocytic antigen receptors (immunoglobulin heavy chains: IgH, immunoglobulin light chains: IgL, T-cell receptors: TCR). The molecular biology methods are also useful in the differential diagnosis between lymphomas and reactive lymphoproliferative disorders. In the Department of Pathomorphology, Collegium Medicum, Jagiellonian University, we routinely analyze several cases a month, using PCR-based studies of TCR and IgH rearrangement. We can also analyze common lymphoma-specific translocations, including t(11;14), t(14;18) and t(11;18). These methods may be applied to fresh/freshly frozen tissues and to paraffin-embedded archival materials. The diagnostic utility of ancillary molecular studies was proved in numerous cases of lymphoproliferative diseases, with a significant therapeutic impact. We present selected cases illustrating the usefulness of molecular diagnostics in routine evaluation of the lymphoproliferative lesions. Additionally we offer a brief overview of technical and interpretation problems, as well as a summary of our material in the last few years.

COMPARISON OF DIAGNOSTIC VALUE OF HISTOPATHOLOGY AND IMMUNOHISTOCHEMICAL REACTION WITH FLOW CYTOMETRY IN LYMPHOMA DIAGNOSTICS

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Estimating the phenotype of lymphomas is most commonly based on immunohistochemical reactions (IH) in paraffin sections, less frequently on flow cytometry (FCM) of cell suspensions. Histopathology evaluations (HP) with IH constitute the basis for the diagnosis of lymphomas. Flow cytometry (FCM) has been used only as an ancillary method in lymphoma diagnosis. In 2000, the WHO lymphoma classification was published. Unlike previous classifications, it emphasizes the role of evaluating the phenotype and genetic changes (GEN) of various lymphoma subtypes. At the Institute of Oncology, only a part of the material provided – original slides or paraffin blocks – meet the technical requirements for reliable HP and IH diagnosis. In case of material insufficiency, fine needle aspiration biopsies (FNAB) were usually carried out to obtain cells for FCM and possibly GEN. During the period between 1997 and 2003, the flow cytometry laboratory carried out over 1200 cytometric lymphoma examinations. Initially, in case of doubtful diagnoses, the FCM method served to specify HP and IH diagnosis. Nowadays, FNAB/FCM is considered a fully independent diagnostic method. Our objective was to present the diagnostic utility and limitations of both HP/IH and FNAB/FCM methods with reference to various lymphoma subtypes. Based on about 450 cases of lymphomas diagnosed both using HP/IH and FCM methods and a wide range of antibodies, we concluded that the amount of unreliable or incorrect HP/IH diagnoses was the highest in the group of MCL and MZL lymphomas and accounted for 58% and 64%, respectively. Our experience allows us to believe that in order to establish a reliable diagnosis and evaluate some of the prognostic factors in many lymphomas and leukemias, it is necessary to perform a FCM examination, especially in case of lymphomas from precursor T and B lymphocytes, as well as lymphomas of the so-called “small B-cell peripheral lymphocytes”, in case of distinguishing Burkitt lymphoma from diffuse large B-cell lymphomas and some reactive nodal hyperplasia from follicular lymphomas and finally in some peripheral T-cell lymphomas. In highly specialist oncology and hematology centers, the FCM analysis associated with a cytological examination is a recognized and quick method, which is more and more widely used in lymphoma diagnosis and reduces the need for HP examination. Pathology departments dealing with leukemia and lymphoma diagnostics should be equipped with flow cytometry as a necessary diagnostic tool.

COMPARISON OF PRIMARY AND HEPATIC AND PULMONARY METASTATIC FOCI OF COLORECTAL CARCINOMA

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The aim of the study was to analyze the infiltration of tumor-surrounding tissues and the quantity of fibrous stroma in primary and secondary foci of colorectal carcinoma. The study group included 42 patients with colorectal adenocarcinoma and metastases to the liver and lungs (42 primary foci, 35 hepatic and 7 pulmonary metastases). The analysis of each studied tumor included the degree of fibrosis, type of tumor growth (infiltrative, pushing) and peripheral infiltration activity (tumor budding). No correlation was found between the amount and distribution of fibrous tissue and the type of growth and tumor budding in primary tumors. Hepatic metastases were mostly characterized by a pushing growth pattern and marked peripheral fibrosis. The formation of connective tissue stroma was observed mostly in central parts in some cases of pulmonary metastases. No correlation was found between the investigated parameters in primary and metastatic foci.

A CASE OF WHIPPLE'S DISEASE WITH CHANGES SUGGESTING SARCOIDOSIS

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A 43-year-old man suffered from transient rash. Several weeks later, abdominal pain, vomiting and weight loss appeared. The patient was admitted to one of the local hospitals, where endoscopy was performed and a cervical lymph node was removed for the histological examination. Histopathology (made in another histopathological laboratory) revealed changes similar to those observed in sarcoidosis (Besnier–Boeck–Schaumann's disease) in both the mucosa of the stomach and lymph node. Because of the lack of improvement after treatment and deterioration of the patient's status, he was referred to the Department of Gastroenterology, Human Nutrition and Internal Diseases of the University School of Medical Sciences in Poznań. During his stay in the ward, another episode of the rash occurred. A skin specimen was collected for histological examination. The sample was evaluated in the histopathological laboratory outside the Department of Clinical Pathology. The same changes as those in the stomach and lymph node were observed. During the subsequent two endoscopies, specimens of the mucosa of the stomach and duodenum were taken. The endoscopic examination revealed that the mucosa was irregularly thickened with scattered ecchymoses. Subsequently, the histological examination of 3 specimens from the mucosa of the stomach confirmed the presence of non-caseating granulomas. Additionally, in 2 specimens, groups of large cells with slightly eosinophilic, strongly paS-positive cytoplasm were present. In one of the specimens, there were only scattered, isolated groups, in the other one there was more diffuse infiltrate. Similar changes were observed in the duodenal mucosa. The diagnosis of Whipple's disease was established. After one week of treatment (antibiotics, biseptol) the status of the patient distinctly improved. Within the next 3 weeks he gained ten kilograms in weight.

IMMOTILE CILIA SYNDROMES – CHANGES WITHIN THE RESPIRATORY TRACT MUCOSA

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Among the risk factors for recurrent infections of the respiratory airways are immotile cilia syndromes. The cilia lack one and/or two arms of dynein, as well as manifest concomitant abnormalities in the microtubule structures. Often, complexes of microtubules for some cilia are covered by a common cellular membrane. In the present study we used samples of epithelial lining-covered bronchial mucosa biopsies collected from 66 patients (aged from 2 months to 49 years) with recurrent respiratory tract infections. The most commonly observed abnormality was the lack of inner arms of dynein. In 24 patients, the lack of both dynein arms, and in 2 cases – a lacking inner arm were observed. In those patients, in whom the lack of both arms was diagnosed, some cilia were also found without the outer dynein arm only. In more then 50% of patients, there were multiple complexes of cilia microtubules found under a common cytoplasmic membrane. Moreover, in more then 50% of patients the cilia were irregularly located on the cell surface and interspersed with several microvilli. In one patient, all the lining cells of the bronchial mucosa demonstrated microvilli found only on the free surface. In the majority of patients, chronic inflammatory changes were present with fibrosis of the mucosa and a decreased number of capillaries displaced from the epithelium. In 15% of cases, there were also cells of the acute phase of inflammation, as well as edema in the lamina propria. In 2 patients, fibrosis of the bronchioli and lung parenchyma was found. In 2 patients squamous metaplasia, and in one patient – low-grade dysplasia of the bronchial epithelium were diagnosed. Conclusions: the consequences of the immotile cilia syndromes include fibrosis of the bronchial mucosa and metaplasia and dysplasia of the bronchial epithelium.

PRESENCE OF SARCOID-LIKE GRANULOMAS IN THE THYROID GLAND AS A POSSIBLE CONSEQUENCE OF FINE NEEDLE ASPIRATION BIOPSY

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We present the case of a 56-year old man who underwent thyreoidectomy due to multinodular goiter. The specimen of thyroid gland displayed the presence of granulomas similar to granulomas observed in sarcoidosis. The granulomas were present in only one lobe, near the site of fine-needle aspiration biopsy performed a few months previously. The granulomas consisted of epithelioid cells and multinucleated cells of the Langhans' type, surrounded by a dense lymphocytic infiltration. The granulomas were not found in the remaining parts of the thyroid. We know from clinical data that the patient has never suffered from sarcoidosis. Furthermore, there was no clinical suspicion of thyroiditis. Preoperative physical and laboratory

examinations, as well as chest radiography were normal. In fine-needle aspiration biopsy, neither epithelioid cells nor Langhans' cells were determined. The patient had been treated for nodular goiter. The case seems to be interesting because of the rarely described type of lesions observed in the thyroid gland, as well as in other organs, as a consequence of fine-needle aspiration biopsy.

ANALYSIS OF THE IMMUNOHISTOCHEMICAL EXPRESSION OF TUMOR NECROSIS FACTOR-ALPHA (TNF-) IN THYROID FOLLICULAR CELLS AND INTERSTITIAL CELLS FROM PATIENTS WITH NON-TOXIC MULTINODULAR GOITER AND TOXIC MULTINODULAR GOITER

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The role of tumor necrosis factor alpha (TNF-) in thyroid gland diseases is still being discussed. The participation of cytokines in neoplastic pathogenesis and autoimmune disorders is partly identified, whereas to-date the relations between cytokines and nodular goiters have not been clarified. Hence, we tried to analyze the immunohistochemical expression of tumor necrosis factor (TNF-) in thyroid epithelial and interstitial cells in non-toxic nodular goiter and toxic goiter. The studies were carried out in samples of the thyroid gland obtained from sixty patients (fifty women and ten men) undergoing strumectomies. Immunohistochemical staining was performed by means of goat monoclonal anti-human TNF- antibody. The presence of TNF- expression in thyroid epithelial cells and interstitial cells was identified in 87% of non-toxic multinodular goiters and in 93% of toxic multinodular goiters. The results of our studies may suggest the participation of TNF- in the development of both non-toxic and toxic multinodular goiters.

DIFFICULTIES IN DIAGNOSTICS OF NULL CELL TYPE PITUITARY ADENOMA

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No abstract available.

TWO CASES OF SALIVARY DUCT ADENOCARCINOMA

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No abstract available.

CARDIAC MYXOMA, NUMEROUS LUNG INFARCTS AND BRONCHIOALVEOLAR CARCINOMA IN A 55-YEAR OLD PATIENT

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No abstract available.

LYMPHOEPITHELIOMA-LIKE CARCINOMA OF THE UTERINE CERVIX – A REPORT OF TWO CASES

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No abstract available.

ANALYSIS OF ANGIOGENESIS IN TREPHINE BIOPSIES FROM PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKEMIA

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Angiogenesis plays a significant role in the growth, dissemination and metastasis of solid tumors and may be involved in the pathogenesis of hematopoietic malignancies. Because of contradictory data concerning vascularity in CLL, we decided to evaluate the degree of angiogenesis in trephine biopsy sections of CLL by measuring the microvessel density and comparing it to normal bone marrow sections. Twenty-four trephine biopsies obtained from patients treated in the Department of Hematooncology and Bone Marrow Transplantation Center of Lublin were estimated histologically and immunohistochemically. Twelve patients aged 46–72 years (mean, 53 years) had typical CLL defined morphologically and immunophenotypically. The control group consisted of trephine biopsies taken from 12 patients aged 20–71 years (mean, 48 years) and negative for infiltrative lesions. Bone marrow biopsy specimens used in this study were prepared from paraffin blocks. Immunohistochemical staining using antibodies to CD34, and CD105 revealed more microvessels in bone marrow trephine biopsy sections with CLL involvement (35.74±9.41, 39.11±8.87, respectively), as compared to control biopsies (26.88±9.72, 27.42±9.01) ($p<0.05$). These results indicate that angiogenesis may be involved in the pathogenesis of CLL.

CHRONIC LYMPHOCYTIC LEUKEMIA/SMALL LYMPHOCYTIC LYMPHOMA – THE CLINICAL AND HISTOPATHOLOGICAL PICTURE AND DIFFERENTIAL DIAGNOSIS

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Chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) is a neoplasm of monomorphic small, round B-lymphocytes in the peripheral blood, bone marrow and lymph nodes, admixed with prolymphocytes and paraimmunoblasts, usually expressing CD5 and CD23. The term SLL, consistent with CLL, is restricted to cases with the tissue morphology and immunophenotype of CLL, but which are non-leukemic (WHO). SLL/CLL comprises 6.7% of non-Hodgkin's lymphomas. The

majority of patients are > 50 years old (median age 65), with the male:female ratio of 1.5–2:1. The patients are asymptomatic or present with constitutional symptoms, fatigue, susceptibility to infections, or occasionally autoimmune hemolytic anemia. Most patients have bone marrow and peripheral blood involvement at presentation, with or without lymphadenopathy or hepatosplenomegaly; a variety of extranodal sites may also be involved. The lymphocyte count is > $5x10^9$/l. The clinical course is indolent. The median survival is 5–8 years .Transformation to large B-cell lymphoma (Richter's syndrome) occurs in 3–10% of cases. The lymph nodes typically show a characteristic diffuse infiltrate with scattered, vaguely delineated pale areas, creating a pseudofollicular pattern (proliferation centers). The predominant cell is a small lymphocyte. Variable numbers of larger cells – prolymphocytes and paraimmunoblasts – are also present. They are clustered in pseudofollicles or distributed evenly throughout the node (a diffuse pattern). In exceptional instances, the involvement is interfollicular. In the spleen, white pulp involvement is usually prominent, but red pulp is usually involved. Bone marrow involvement appears as nodular, interstitial or diffuse infiltrates of small lymphoid cells or a mixture of these patterns. The neoplastic cells express weak sIgM or sIgM and sIgD, CD19+, CD20+ (weak), CD22+ (weak), CD79a+, CD5+, CD23+, CD43+, CD11c+(weak). CD10 and cyclinD1 are negative. Differential diagnosis: mantle cell lymphoma, follicular lymphoma, nodular lymphocyte predominance Hodgkin's lymphoma, lymphoplasmacytic lymphoma, B- and T-cell prolymphocytic leukemia, lymphoblastic lymphoma, hairy cell leukemia.

CLINICOPATHOLOGICAL STUDY OF 12 CASES OF CASTLEMAN'S DISEASE

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Castleman's disease (CD) is a heterogeneous group of lymphoproliferative disorders of an uncertain cause. Three histological variants (hyaline vascular, plasma cell, and mixed) and two clinical types (localized and multicentric) of CD have been described. Localized disease generally presents with a single enlarged lymph node or widening of the mediastinum, whereas multicentric disease is a systemic lymphoproliferative disorder characterized by lymphadenopathy, hepatosplenomegaly, constitutional symptoms, anemia, hypoalbuminemia, and hypergammaglobulinemia. Unlike the localized type, for which surgical excision is curative regardless of the histological type, multicentric disease often necessitates aggressive systemic therapy and portends a poorer outcome. We report the clinical features and histopathological findings of 12 cases of CD diagnosed at the Chair and Department of Clinical Pathology in Lublin in the years 1996–2003. Nine patients were female and 3 male; their age ranged from 5 to 67 years. Eleven patients with localized CD (8 with hyaline vascular type, 1 with plasma cell and one with mixed type) had no systemic symptoms. One patient with multicentric CD developed anemia, fever, hypergammaglobulinemia and manifestations of multisystem involvement.

THREE CASES OF CHRONIC LYMPHOCYTIC LEUKEMIA TRANSFORMATION TO DIFFUSE LARGE B-CELL LYMPHOMA (RICHTER'S SYNDROME)

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The transformation of 3–10% of chronic lymphocytic leukemia (CLL) to high-grade lymphoma is commonly referred to as the Richter's syndrome. The development of diffuse large B-cell lymphoma is the most frequent form of this syndrome. It may represent a second neoplasm or a transformation from the same clonal population. The Richter's syndrome occurs mostly in the lymph nodes, however few extranodal (digestive) cases have been described. We report three cases of diffuse large B-cell lymphoma, which developed in patients treated for chronic lymphocytic leukemia. Two of these occurred in the lymph nodes and one was an intestinal lymphoma.

TYPE 1 GAUCHER'S DISEASE IN A 43-YEAR-OLD MAN – A CASE REPORT

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Gaucher's disease is an inherited (autosomal recessive) metabolic disorder, in which glucocerebroside accumulates in the spleen, liver, lungs, bone marrow and, in rare cases, the brain. It is a rare disease and occurs once in 40,000 to 65,000 persons in the whole world. The patients exhibit a deficiency of -glucocerebrosidase that catalyzes the first step in the biodegradation of glucocerebroside, which is stored in lysosomes of macrophages (Gaucher's cells). Three types of Gaucher's disease are commonly recognized. Type 1 is the most common. Patients in this group usually bruise easily and experience fatigue due to anemia, low blood platelets, hepatosplenomegaly and bone pain. There are no signs of brain involvement. The onset of clinical manifestations may be early in life, or delayed until adulthood. In type 2, hepatosplenomegaly is apparent by 3 months of age. In addition, there is an extensive and progressive brain damage. These patients usually die by 2 years of age. In type 3, liver and spleen enlargement is variable, and signs of brain involvement gradually become apparent. A highly effective enzyme replacement therapy is available mainly for patients with type 1 Gaucher's disease. We present the case of type 1 Gaucher's disease in a 43-year-old man. The main clinical signs that we observed were splenomegaly, hepatomegaly, jaundice, hemorrhagic diathesis and also bone pain. The diagnosis was based on histology showing the Gaucher's cells in bone marrow and hepatic biopsy, and confirmed by enzymatic tests.

THE COEXISTENCE OF MANTLE CELL LYMPHOMA WITH COLON ADENOMA AND ADENOCARCINOMA

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The coexistence of primary lymphoma and adenocarcinoma of gastrointestinal tract is an extremely uncommon event and only a few cases have been documented. We present the case of co-occurrence of mantle cell lymphoma in the form of multiple lymphomatous polyposis and colon adenocarcinoma in a 68-year-old man. Additionally a tubular adenoma was noted in the colon. The lymph nodes were involved by malignant lymphoma, but not by metastases of adenocarcinoma. Representative tissue samples were submitted for routine histopathological diagnosis. Immunohistochemically, the lymphoma infiltration revealed

expression of CD20, CD5 and cyclin D1, whereas CD3 and CD23 were negative.

PLEOMORPHIC (SPINDLE/GIANT CELL) CARCINOMA OF THE LUNG

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Pleomorphic carcinoma (PC) of the lung is a well defined pathologic entity in the spectrum of non-small cell carcinomas (NSCLC). PC is composed of dual cell population of epithelial and sarcomatoid cells, with features of differentiation to squamous cell carcinoma, adenocarcinoma (AD) or large cell carcinoma, intermingled with spindle cells and/or giant cells, or presents as carcinoma composed only of spindle or giant cells. The pleomorphic component should comprise at least 10% of a neoplasm for exclusion of tumors with occasional sarcomatoid cells. The terms spindle cell carcinoma (SCC) is restricted to NSCLC composed exclusively of spindle cells, and giant cell carcinoma (GCC) to tumors composed of highly pleomorphic giant cells. Tumors with malignant heterologous elements are called carcinosarcomas. SCC and GCC are very uncommon in their pure forms (0.3% of all lung malignancies), most often occur in association with SCC, GCC and AD, therefore mainly built the category of PC. The male preponderance and high correlation with cigarette smoking of PC patients is observed. PCs were associated with more advanced disease stage at presentation and more aggressive clinical course. The percentage of pleomorphic cells may have clinical relevance in more advanced stage of PC. An epithelial origin of PC with divergent mesenchymal differentiation is currently accepted. The differential regulation at the cellular level of several markers suggests two cell components – epithelial or sarcomatoid with variable phenotype expressions of epithelial markers, which may indicate an metaplastic origin of biphasic PC. A broad spectrum of epithelial markers is necessary to disclose the epithelial nature of the tumor (e.g. MNF116, AE1/AE3, EMA, CEA), however the epithelial markers might be negative on the spindle cell component. Moreover, several studies support the view, that epithelial cells of PC may acquire the expression of VIM and other mesenchymal markers. PC tends to be large peripheral tumors ranging in size from 2.2 cm to 18 cm, with predilection for upper lobes, however some reports on endobronchial involvement were published. Overall survival of PC patients is poor, median survival is about 10 months; 10–12% of the patients survive 5 years. Predictive factors include: size > 5 cm, distant metastases, any lymph node metastases. Spectrum of differential diagnosis: sarcomas vs. SCC, biphasic PC containing glandular and spindle cell components vs. pulmonary blastoma, carcinosarcoma and carcinoma with desmoplastic reactive stroma.

EXPRESSION OF p53 PROTEIN IN IRRADIATED GLIOMA CELL LINES

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The expression of p53 protein plays an important role in radiation-induced apoptosis and may affect the efficacy of radiotherapy. The aim of the study was to assess p53 expression in irradiated glioma lines. Eight human glioblastoma lines were gamma irradiated (Co^{60}) with 2 and 10Gy doses. The p53 expression was studied immunocytochemically (antibody DO-7, DAKO), and the labeling index (LI) was calculated. The radiosensitivity of cells was estimated with a micronucleus assay. The basic and radiation-induced p53 expression was very heterogeneous. The mean LI in control lines was 21.7% (range from 1.8 to 100%). In GAMG and GOS–3 lines, a prominent increase of p53 expression after irradiation was observed. In DK-MG, a strong decrease of expression was seen, which could be responsible for radioresistance of the cell line, as confirmed by the micronucleus assay. An extremely high p53 expression in LN–405 correlated with its radiosensitivity. The assessment of p53 expression provides useful radiobiological information and may be a valuable tool in the planning of radiotherapy.

PROLIFERATION OF GLIOMAS *IN VITRO*: MOLECULAR AND MORPHOLOGICAL ASSESSMENT

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Assessment of glioma proliferation *in vitro* may be used as a prognostic factor and predictor of response to radiotherapy. The aim of the study was to assess the usefulness of quantitative analysis of histone genes as a proliferation marker in gliomas *in vitro*. Eight established human glioblastoma cell lines were studied. The expression of genes coding for histones H1, H2A, H2B, H3 and H4 was assessed with quantitative RT-PCR (TaqMan). After a cytokinesis block, induced with cytochalasin B and DAPI staining, the cells with one, two or more nuclei were counted under a fluorescent microscope. The percentage of binucleate cells (%BNC) and nuclear division index (NDI) were calculated. A high heterogeneity of the examined lines in respect to the proliferative rate was seen. The percentage of BNC ranged from 8.13 to 62.5, NDI from 1.11 to 2.90. Amongst histone genes, H2B correlated most strongly with morphological parameters (Spearman R for %BNC=0.69, for NDI=0.74, $p<0.05$). The histone H2B gene expression is a useful proliferation marker of gliomas *in vitro*.

SYNAPTOPHYSIN IMMUNOREACTIVE STROMAL CELLS IN COLORECTAL CANCER OBTAINED FROM THE POSTOPERATIVE MATERIAL. PRELIMINARY STUDIES

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According to some authors, the perivascular mesenchyma of newly formed thin-walled vessels appears to be the source of myoid cells (myofibroblasts), from which cancer cells arise. The morphogenesis and the role of stromal cells with features of myofibroblastic differentiation in colorectal cancer (c.c.) is a subject of interest of many investigators. The aim of the study was a semiquantitative estimation of synaptophysin (Syn–38) positive stromal cells in c.c. in postoperative material.

Formalin-fixed (10% buffered formalin) and paraffin-embedded sections from the postoperative material obtained from 60 patients (28 men; 32 women) were subjected to immunohistochemical (IHC) analysis for the presence of Syn–38 (Syn – an integral membrane glycoprotein; 38kDa) using anti Syn–38 standard protein (DAKO, clone SY–38, M 0776). Immunohistochemically, the stromal tissue found on the periphery of the tested tumors (in the head of cancer invasion) displayed thin-walled vascular channels. We observed that Syn–38 positive cells, which seem to be the source of cancer cells, were particularly well visible in perivascular mesenchyma of some newly formed vessels. We also found these cells to be dispersed in the stromal tissue on tumor periphery. Other parts of the tumors were only weakly positive or negative for this marker. The studied cells were accompanied by damage to the Auerbach intramural ganglia located in the proximity of the head of cancer invasion, whose neurons showed strong cytoplasmic expression of Syn–38 and features of disintegration. The additionally performed IHC research demonstrated that these cells were alpha-SMA-positive. Conclusion: preliminary results of our study suggest that perivascular stromal cells of thin-walled newly formed channels with Syn–38 expression could be the source of myoepithelial cells. A relationship may also exist between the presence of Syn–38-positive stromal cells with myofibroblastic differentiation and the damage degree of neurons in the intramural Auerbach ganglia, c.c. morphogenesis and/or spreading of neoplastic infiltration, which requires further studies.

EVALUATION OF THE EXPRESSION OF MARKERS ASSOCIATED WITH RESISTANCE TO CHEMOTHERAPY IN OVARIAN CARCINOMAS

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The aim of the study was the evaluation of P53, HER–2/neu, P-gp, LRP and GSTpi expression in ovarian neoplasms taking into account the conventional clinicopathological variables and the determination of prognostic value of the studied proteins in ovarian carcinomas. The expression of P53, HER–2/neu, P-gp, LRP and GSTpi was assessed immunohistochemically on frozen tissue sections and respective cyst and/or ascitic fluid cells of individual patients. In multivariate analysis, the P53 expression appeared to be an independent prognostic factor in ovarian carcinomas determining a shorter disease free and overall survival time. Considering the immunophenotype of ovarian carcinomas, it was shown that a shorter disease free survival time, as well as a higher percentage of deaths occurred more frequently in groups with the phenotype of $P53^+/P\text{-}gp^+$, $P53^+/LRP^+$, $P53^+/GSTpi^+$ as compared to subgroups with undetectable expression of these markers. The increase of the expression of P53, P-gp and GSTpi in individual patients during or after chemotherapy was observed and it could indicate that the cells acquired selective growth advantage during treatment and resistance to chemotherapy.

DESIRED METAPLASIA OF ADULT STEM CELLS *IN VIVO*

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The purpose of this study is the presentation of desired metaplasia and practical consequences of this phenomenon. Desired metaplasia is the transformation of one fully developed tissue into another in the process of metaplasia, in the situation when this kind of change is morphologically, anatomically and functionally needed at the particular site. In the final stage, it is responsible for the neo-regeneration of tissues and organs. The following conditions are necessary for desired metaplasia: 1. Colonized/grafted adult stem cells and the recipient tissue system/organ derived from the same germ layer during embryogenesis. 2. Adult stem cells and the recipient tissue system/organ should develop in an anatomical, physical neighborhood during embryogenesis. 3. Adult stem cells are pluripotential, i.e. they have the ability to differentiate into another kind of cell lineage in vivo. 4. The presence of the adult stem cells in the new anatomical tissue system/organ is achieved by surgery. 5. The new compatible environment is essential to initiate the differentiation and proliferation of cells. 6. The new conductive functional demand stimulates the differentiation and proliferation of cells in a specific place. 7. The superficial receptors of adult stem cells must match the morphogenetic cytokine signals of their extracellular matrix

AMYLOID GOITER – A CASE REPORT

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Amyloid goiter can be defined as a thyroid enlargement due to deposits of amyloid with no coexisting C cell involvement. Amyloid goiter occurs most often in secondary generalized amyloidosis. In such cases, the thyroid gland usually enlarges rapidly (weeks–months) till it reaches the mass of 300 grams. In consequence, the patients may manifest hoarseness, dysphagia and dyspnea. Usually there are no disturbances in the thyroid gland function. The prognosis for the patients with amyloid goiter depends mainly on the condition, which led to the disease. In amyloid goiter in secondary generalized amyloidosis, it depends on internal organs (kidneys, liver, heart) impairment. Problems associated with amyloid goiter diagnostics are illustrated by our case report of a 60-year old woman with amyloid goiter.

CYTOGENETIC ABNORMALITIES IN MULTIPLE MYELOMA

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Multiple myeloma is one of several cancers categorized as "plasma cell dyscrasias". They account for 10% of all blood system malignancies. Multiple myeloma is a B-cell neoplasia characterized by the expansion of a malignant plasma cell population within the bone marrow, often associated with a low mitotic index leading to difficulties in the cytogenetic detection of the malignant clone. Cytogenetic studies were performed on bone marrow specimens of 84 myeloma patients prior to chemotherapy. The analysis was performed in cells from stimulated and non-stimulated short-term cultures. Twenty-seven per cent (23) of patients had abnormal

karyotypes with various numerical and structural aberrations. Numerical abnormalities usually involved chromosomes 1, 5, 7, 9, 13, 14, 19 and 21. In the above group, in 30% of cases (7 patients), monosomy of chromosome 13 was detected, as well as the loss of sex chromosome in 48% (11 patients). Structural aberrations were present in 70% (16 patients) and preferentially involved chromosomes 1, 3, 11, 13, 14 and 17. Multiple myeloma patients with chromosomal aberrations had more advanced disease at diagnosis than those with the normal karyotype. The overall survival of patients with abnormal karyotypes was significantly shorter, particularly in the case of patients with monosomy of chromosome 13. Cytogenetic studies of patients with multiple myeloma are of a great prognostic and diagnostic importance.

C-C CHEMOKINES AND CHEMOKINE RECEPTORS IN IgA NEPHROPATHY (IgAN) AND IN NON-IgA MESANGIAL PROLIFERATIVE GLOMERULONEPHRITIS (MPGN). AN IMMUNOHISTOCHEMICAL COMPARATIVE STUDY

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Chemokines are a large family of low-molecular-weight pro-inflammatory cytokines. The C-C family of chemokines is a class of major mononuclear-cell chemoattractants. The specificity of chemokine action is mediated through a selective expression of chemokine receptors by different leukocyte subpopulations. The experimental data and studies on human renal tissue indicate that C-C chemokines play a main role in the resolution and progression of inflammatory processes. The objectives of the present study were to compare immunoexpression of C-C chemokines and their receptors in IgA nephropathy (IgAN) and in non-IgA mesangial proliferative glomerulonephritis (MPGN), and moreover, to find any relationships between immunoexpression of chemokines and chemokine receptors and renal interstitial lesions. Using immunohistochemistry, we analyzed immunoexpression of monocyte chemoattractant protein-1 (MCP-1), monocyte inflammatory protein–1 (MIP-1), regulated upon activation normal T-cell expressed and secreted (RANTES) and C-C chemokine receptors: CCR1 and CCR5 in renal biopsy specimens from 19 patients with IgAN and in 23 patients with MPGN. Our study revealed an increased immunoexpression of tubulointerstitial MCP-1 ($P<0.02$), RANTES ($P<0.03$) and interstitial CCR5+ cells ($P<0.05$) in IgAN as compared with MPGN. There were no significant differences in glomerular MCP-1 immunostaining, tubulointerstitial immunoexpression of MIP-1 and interstitial CCR-1+ cells between IgAN and MPGN. In renal tissue in patients with IgAN, the intensity of tubulointerstitial immunostaining of MCP-1 and the number of CCR1+ cells and CCR5+ cells were significantly correlated with renal interstitial cortical volume; meanwhile in biopsy specimens from patients with MPGN, only interstitial CCR5+ cells were positively correlated with interstitial cortical volume. In conclusion, these observations suggest that in renal tissue in IgAN patients, MCP-1, RANTES and CCR5 are up-regulated as compared with renal biopsy specimens in patients with MPGN. Moreover, MCP-1, as well as CCR5 and CCR1 positive cells may play a role in interstitial processes leading to fibrosis in IgAN. CCR5 positive cells may participate in interstitial lesions of renal tissue in MPGN patients.

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IMMUNOEXPRESSION OF MONOCYTE CHEMOATTRACTANT PROTEIN-1 (MCP-1), TRANSFORMING GROWTH FACTOR -1 (TGF- -1) and CD68+ CELLS IN LUPUS NEPHRITIS. A CORRELATIVE STUDY

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Monocyte chemoattractant protein-1 (MCP-1) is not only an important mediator of monocyte recruitment, but also has profibrogenic effects. Moreover, monocytes infiltrating the renal tissue through the release of cytokines and growth factors may play a role in the pathogenesis of kidney damage. An important regulator of extracellular matrix formation in nephropathies is transforming growth factor -1 (TGF- -1). The present study investigated the immunoexpression of MCP-1 and its correlation with monocyte/macrophage infiltration and the immunoexpression of TGF- -1 in biopsy renal tissues in lupus nephritis. Paraffin-embedded biopsy specimens from 17 patients with IV class of lupus nephritis were studied by immunohistochemistry using antibodies anti-MCP-1, anti-TGF- -1 and anti-CD68. MCP-1 protein was weakly distributed in the glomeruli (mean value: 0.11±0.16) in renal biopsy specimens in patients with lupus nephritis. MCP–1 immunoexpression was moderate to intense in the tubular epithelium, interstitium and interstitial infiltrating cells (mean value: 1.55±0.83). TGF- -1 was detected in the renal tubular epithelial cells and interstitium (mean value: 1.02±0.76), and to a lesser extent within the glomeruli (mean value: 0.04±0.07) of patients with lupus nephritis. The mean number of glomerular CD68+ cells was 3.31±2.36, and the mean count of interstitial monocytes/macrophages was 68.91±28.08. Tubulo-interstitial MCP–1 immunoexpression was significantly correlated with monocyte/macrophage interstitial infiltrates ($P<0.03$) and with the immunoexpression of TGF- -1 in the tubuli and interstitium ($P<0.001$). There were no correlations between glomerular MCP-1 immunoexpression and glomerular CD68+ cells ($P=0.79$, NS), as well as the intensity of TGF- -1 immunostaining within the glomeruli ($P=0.52$, NS). In conclusion, these data suggest that in lupus nephritis, MCP-1 may play a role in modulating the interstitial inflammatory process and in tubulointerstitial renal damage via transforming growth factor -1.

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ANTIOXIDANT STATUS AND LIPID PEROXIDATION IN COLORECTAL CANCER, CORRELATION WITH ANATOMO-CLINICAL FEATURES

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Reactive oxygen species (ROS) are involved in quite many events during carcinogenesis. Apart from their mutagenic properties, they enhance cell proliferation and angiogenesis, leading to metastases. Significant disorders of oxidative-antioxidative homeostasis have been reported in our previous findings. They occurred in primary tumors of colorectal cancer and were accompanied by an

increase of lipid peroxidation products in neoplastic cells. The aim of the study was to analyze correlations between the parameters of the antioxidative system and the selected anatomo-clinical features of the cancer. We carried out homogenization of samples, derived from 81 primary tumors. In 10% of homogenates, we measured the activity of the following enzymes: superoxide dismutase (Cu,Zn-SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSRG-R). We also studied the level of non-enzymatic antioxidants (glutathione, vitamin C and E). An insight into lipid peroxidation was achieved by quantification of its terminal products: malondialdehyde (MDA) and 4-hydroxy–2-nonenal (4-HNE). In case of antioxidative enzymes and glutathione, we used spectrophotometry as a quantification tool. The HPLC method was applied to determine the level of vitamin C and E and products of lipid peroxidation. We made an attempt at correlating these data with the histological type, as well as stage and grade of the tumors. Eighty-one specimens of macroscopically unchanged colon comprised the control group. Results and conclusion: The investigated parameters of the antioxidative system and the level of lipid peroxidation products matched the analyzed anatomo-clinical features of the cancer in a statistically significant manner. The activity of antioxidative enzymes: superoxide dismutase (Cu,Zn-SOD), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSG-R) were significantly ($p<0.001$) increased in cancer tissues, especially in G3-grade adenocarcinoma and mucinous adenocarcinoma, as well as in pT4 colorectal cancers. Increasing, statistically significant differences [($p<0.05$) – ($p<0.001$)] between enzyme activity and all staging grades (II-III, II-IV and III-IV) of the cancer, as well as between the activity of Cu, Zn-SOD in G2-grade adenocarcinoma and mucinous adenocarcinoma were observed. On the other hand, catalase (CAT) activity was significantly ($p<0.001$) decreased in cancer tissues and was the lowest in mucinous adenocarcinoma and pT4-stage cancer group. The levels of vitamin C and vitamin E in cancer tumors were also decreased ($p<0.001$). Our results confirm that a gradual advancement of oxidative-antioxidative disorders is followed by the progression of colorectal cancer.

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ANTIOXIDANT STATUS AND LIPID PEROXIDATION IN COLORECTAL CANCER

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Reactive oxygen species (ROS) can induce carcinogenesis via DNA damage. Both enzymatic and non-enzymatic mechanisms participate in cell protection against the harmful influence of oxidative stress. The aim of our study was to assess the activity of antioxidative enzymes, particularly superoxide dismutase (Cu,Zn-SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and glutathione reductase (GSSRG-R) in colorectal cancer. Moreover, we focused on quantification of non-enzymatic antioxidants (glutathione, vitamin C and E) and lipid peroxidation products, such as malondialdehyde (MDA) and 4-hydroxy–2-nonenal (4-HNE). The investigations were conducted in 81 specimens of primary tumors. As the controls, the same number of samples was collected from macroscopically unchanged colon regions of the most distant location to the tumor. The homogenization of specimens provided 10% homogenates for our evaluations. The activity of antioxidant enzymes and the level of glutathione were determined by spectrophotometry. HPLC revealed the levels of vitamin C and E and served as a method for detecting terminal products of lipid peroxidation in colorectal cancer. Results: Our studies demonstrated a statistically significant increase of Cu, Zn-SOD ($p<0.001$), GSH-Px ($p<0.005$) and GSSRG-R ($p<0.001$) and a decrease of CAT activity ($p<0.001$) in primary tumors as compared with normal colon. In case of non-enzymatic antioxidants, we discovered a decreased level of reduced glutathione ($p<0.001$) and vitamins C and E ($p<0.001$). We also observed an increase in the level of lipid peroxidation products in cancer homogenates ($p<0.001$). The above-mentioned parameters varied significantly according to patients' age and sex. Conclusions: our results show that colorectal carcinogenesis is associated with serious disorders of oxidative-antioxidative homeostasis. The direction of presented changes remains to be defined. Nevertheless, augmentation of lipid peroxidation indicates the predominance of oxidative processes that seem to be far more advanced and favored in colorectal cancer than in normal colon.

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EXPRESSION OF COX-2 IN BREAST CARCINOMA CELLS IS A NEGATIVE PROGNOSTIC FACTOR

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Cyclooxygenases (COXs) are enzymes involved in the conversion of arachidonic acid to prostaglandins (PGs). The expression of COX-2 in physiological conditions is very low and often undetectable. An increase of this isoform is inducible by mitogens, cytokines and in neoplastic tissues. The latest reports have shown an elevated COX-2 expression in several neoplasms, including carcinomas of the breast, ovary, lung, stomach, esophagus and colon. It has been also demonstrated that an elevated COX-2 expression is an independent negative prognostic factor. COX-2 could be the aim of adjuvant therapy using specific inhibitors. The purpose of the study was COX–2 prognostic value estimation in a varied cohort of female patients with ductal breast carcinoma. The analyses were performed in tissue samples obtained from 106 female patients operated on in the Lower Silesia Oncology Center in Wrocław in the years 1993–1994. Immunohistochemical reactions using COX-2 monoclonal antibodies were performed in paraffin embedded tissue samples taken from the examined cases. The statistical analysis of immunohistochemical reactions results and clinical data showed that COX-2 expression was characteristic of cases with shorter overall and disease free survival. The current data suggest that COX-2 inhibition in case of breast cancer could be an interesting form of adjuvant therapy.

PREDICTIVE VALUE OF IMMUNOCYTOCHEMICAL STAINING OF MRP2/cMOAT/ABCC2 EXPRESSION IN OVARIAN CARCINOMA CELLS

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The diagnosis of ovarian carcinomas in advanced disease stages renders chemotherapy as very important part of treatment. The so-called cytostatic multidrug resistance phenomenon is the main reason of therapeutic failures. MRP–2/cMOAT/ABCC2 is one of the ABC-transporters and is responsible for cellular resistance to alkylating drugs, methotrexate and vinblastin. Immunohistochemical analysis using MRP–2 antibodies was performed in paraffin-embedded tissue samples obtained from 44 female patients operated on in the Department of Gynecology and Obstetrics in Poznań. Tissue samples were taken during the first operation and second-look intervention. The investigated patients were treated with paclitaxel-ciplatin or cisplatin-cyclophosphamide protocols. We observed not only cytoplasmic/membranous (MRP–2c), but also the nuclear location (MRP–2n) of MRP–2. The statistical analysis proved that an elevated MRP–2n expression in specimens obtained during the first and second operation was characteristic for cases with shorter overall and disease free survival.

VALUE OF BRCA-1 EXPRESSION IN SPORADIC DUCTAL BREAST CARCINOMAS

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BRCA-1 is a suppressor gene, whose mutations are often associated with inherited cases of breast cancer. The analyses carried out in cell lines and breast carcinoma tissue specimens have proved that a decreased BRCA-1 expression plays an important role in sporadic cases of breast carcinoma. The goal of the study was BRCA-1 prognostic value estimation in a varied group of sporadic ductal breast carcinoma cases. The analysis was performed on breast carcinomas tissue samples taken from 106 female patients operated on for ductal breast carcinoma in the Lower Silesia Oncology Center in Wrocław in the years 1993–1994. The examined group of patients was diversified as to the stage of disease, tumor differentiation, administered treatment and age. Immunohistochemical staining with monoclonal antibodies against BRCA-1 was carried out in paraffin-embedded tissue samples from the examined cases. The statistical analysis proved that cases demonstrating lower BRCA–1 expression were correlated with shorter overall and disease free survival. Our current investigations showed that in cases of sporadic breast carcinoma, a decreased BRCA-1 expression turned to be an independent negative prognostic factor.

HER-2 EXPRESSION IN BREAST CARCINOMA CELLS DECREASES DURING INVASION THROUGH THE MYOEPITHELIAL BARRIER

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About 30% of patients with invasive breast carcinoma and up to 80–90% of ductal carcinomas in situ (DCIS) reveal the amplification of *HER-2* gene or overexpression of HER-2 protein. Such a frequent occurrence of HER-2 overexpression was also reported in cases of carcinoma *in situ* as in *in situ* components of invasive carcinomas. The aim of the study was to investigate the expression of HER-2 in ductal invasive carcinoma cells and their *in situ* components, and an *in vitro* examination of the correlation between HER-2 overexpressed breast carcinoma cells and myoepithelial cells. The investigations were performed in two stages. In the first one, immunohistochemical analyses using antibodies against HER-2 and p63 and smooth muscles actin (SMA) were performed in tissue samples obtained from 60 cases of breast ductal carcinoma. Subsequently, breast carcinoma SK-BR–3 cell and myoepithelial cell (Hs 578Bst) lines coculture was carried out. Reactions using HER-2 and SMA antibodies were performed in acetone and methanol mixture-fixed specimens. The analyses performed in paraffin sections revealed that HER–2 expression in the component *in situ* cells was considerably higher in comparison with invasive breast carcinoma cells. *In vitro* experiments demonstrated that the initially high HER–2 expression in breast carcinoma cells significantly decreased as a result of direct contact of these cells with myoepithelium. The current data suggest that HER-2 expression decreases significantly because of an interaction between breast carcinoma and myoepithelial cells during neoplastic invasion.

EVALUATION OF PEPTIDE YY IMMUNOREACTIVITY IN COLORECTAL CARCINOMAS

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Peptide YY (PYY), as other peptides of the GLI group, is produced by the intestinal endocrine type L cells. Apart from other functions, it probably acts as a growth factor for the intestinal epithelium. According to a few reports, some colorectal carcinomas express PYY. In 57 randomly selected carcinomas of the large intestine, immunostaining for PYY was performed. The reaction was positive in 5 cases (8.8%). In three

of them, the cells with the expression of PYY were numerous, distributed irregularly in groups, nests, or individually. In two other carcinomas, there were a few such cells in each section. A portion of the cells with PYY expression (especially the individually dispersed cells) resembled normal endocrine cells of the intestinal epithelium in terms of their shape and location, but luminal processes were not observed. The average size of the tumors with PYY expression was smaller than that of other tumors (2.9cm±1.14 cm versus 5.07cm±2.51 cm). The difference was statistically significant ($p<0.05$). The expression of PYY was more frequent in less differentiated tumors (G1–0%, G2–8.9%, G3–14.3%) and it was more common in the cases with metastases (stage C+D according to Dukes; 13.6%) than in the cases without metastases (A+B; 5,7%) but these differences were not significant. The importance of the expression of PYY in colorectal carcinomas remains unclear. This phenomenon is revealed in a small percentage of tumors and it requires further studies on a large series of cases to make a more accurate statistical analysis possible.

EVALUATION OF BETA-HCG IMMUNOREACTIVITY IN COLORECTAL CARCINOMAS

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Various non-trophoblastic tumors, such as carcinomas of the breast, lungs and the alimentary tract, including the large intestine, may express the beta subunit of human chorionic gonadotropin (HCG). It has been reported that the expression of beta-HCG in those tumors is an unfavorable prognostic factor. It is suggested that beta-HCG acts as an autocrine/paracrine stimulator of tumor growth and as an immunosuppressor. In 57 randomly selected colorectal carcinomas, immunostaining for beta-HCG was performed. The reaction was positive in 11 cases (19.3%). Neoplastic cells with the expression of beta-HCG tended to form irregularly distributed concentrations among other tumor cells. The expression of beta-HCG was much more frequent in carcinomas with metastases (stage C+D according to Dukes; 36.4%) than in carcinomas without metastases (A+B; 8.6%). The difference was statistically highly significant ($p<0.01$). The incidence of beta-HCG expression was higher in less differentiated tumors (G1 – 0%, G2 – 17.8%, G3 – 42.9%) and that was also highly significant ($p<0.01$). The incidence of beta-HCG expression was more than twice as high in tumors originating from men (26.9%) than in tumors from women (12.9%). This difference was statistically significant ($p<0.05$). In comparison to the expression of other endocrine markers evaluated in the same series of cases (chromogranin A, serotonin, somatostatin, glucagon, peptide YY and gastrin), only the expression of beta-HCG was significantly more frequent in more advanced and less differentiated tumors. In contrast to neoplastic cells expressing other markers, the cells with beta-HCG expression were not morphologically similar to normal endocrine cells of the digestive tract. We suppose that beta-HCG may prove to be more useful as a prognostic indicator in colorectal carcinomas than endocrine markers characteristic of the normal intestinal epithelium.

SUSPICIOUS BREAST LESIONS IN FNA BIOPSY AND HISTOPATHOLOGIC CORRELATION

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The aim of the study was to correlate cytological and subsequent histopathological intraoperative and postoperative (paraffin-embedded surgical material) pictures of suspicious breast lesions. FNAB smears, intraoperative and postoperative tissue sections from 38 patients (37 females, 1 male, aged 22–78, medium 51 years) with suspicious breast lesions were reviewed. Smears were assessed for cellularity, cellular arrangement, nuclear features – size, shape, pleomorphism, chromatin distribution, nucleoli, nuclear/cytoplasm ratio, background. FNAB results were divided into two groups: suspicious probably malignant (SPM) – 20 cases and suspicious probably benign (SPB) – 18 cases. Cytological findings of SPM were as follows: high (10) and low to moderate (8/20) cellularity, loosely cohesive groups and small syncytial tissue fragments, small single cells (7), nests (7), papillary pattern (1), increased nuclear/cytoplasm ratio (10), small (13) or medium-size (7) nuclei with minimal nuclear pleomorphism (10) and slightly irregular nuclear envelope (10), hyperchromasia (5), nucleoli present (7), finely granular (18) or coarse (2) chromatin, cytoplasm scant (10), intracytoplasmic inclusions (2), fibrous and fatty stroma in the background (4). Seventeen out of 20 SPM lesions were described as malignant in histopathology: ductal invasive carcinoma (10), tubular carcinoma (3), intraductal carcinoma (2), intracystic papillary carcinoma (1), atypical ductal hyperplasia(1). Three of SPM were benign: fibroadenoma, fibrocystic changes, gynecomastia with component of usual ductal hyperplasia. Cytological findings in SPB were: high (13/18) or moderate (5) cellularity, groups and sheets with or without branching (18), with modeling, crowding and overlapping nuclei (13), small single cells (11), nests (3), papillary pattern (4), small (18) or medium-size (2) nuclei with minimal pleomorphism (9), finely granular (16) or dispersed (4) chromatin, hyperchromasia (3), high N/C ratio (12), nucleoli (12), bare nuclei (9), myoepithelial (13) and apocrine (3) cells, protein fluid, fibrous stroma, lymphocytes and macrophages in the background. Seventeen out of 18 SPB lesions were described as benign in histopathology: fibroadenomas (10), including fibroadenoma in axillary location, fibrocystic lesions (5), inflammation (2), although they had the component of usual ductal hyperplasia. One SPB change was diagnosed malignant (invasive squamous cell carcinoma) with flat epithelial atypia in adjacent gland ducts. Conclusions: malignant cytological diagnosis must not be made when the aspirate consists of small cells and there is lack of large single malignant cells. Intraductal proliferative lesions can cause diagnostic problems on cytology.

A CASE OF LYMPHOMA DURING IMMUNOSUPPRESSIVE TREATMENT OF A PATIENT AFTER HEART TRANSPLANTATION. A CASE REPORT

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The last WHO Classification of Tumors of Hematopoietic and Lymphoid Tissues presents Post-Transplant Lymphoproliferative Disorders as a specific group of changes in the lymph nodes. The authors present the case of a 56-old male with a lymphoma diagnosed during 24-month immunosuppressive treatment after

heart transplantation. The disease progressed despite of treatment modification and radio- and chemotherapy, the patient died 10 months after the diagnosis of lymphoma.

THE USEFULNESS OF USG MONITORED BIOPSY OF FOCAL LIVER LESIONS IN THE DIAGNOSIS OF CARCINOMA OF UNKNOWN PRIMARY ORIGIN ("FOCUS PRIMARIUS IGNOTUS")

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We evaluated 109 fine-needle aspiration biopsies (FNAB) performed in patients presenting as "focus primarius ignotus" with a tumor mass in the liver, clinically suspected of constituting metastatic liver tumors and seen in the period of July, 2002 – December, 2003. In all the cases, ultrasound was used to help to guide the needle. All the specimens were fixed routinely in 96% alcohol and stained with hematoxylin and eosin for cytological examinations. The diagnosis of neoplastic cells was confirmed in 73/109 cases (66.97%), 36 (33.03%) of samples proved to contain benign material, including satisfactory levels of hepatocytes, blood or paucicellular non-diagnostic material. Among the cases with malignant neoplastic cells, the large number (30/73, 41.09%) of primary hepatocellular carcinomas was confirmed. A surprisingly low number of cases (20/73; 25.35%) was diagnosed as adenocarcinoma, including mucinous carcinoma – 1 case, and metastatic colorectal adenocarcinoma, which constituted only 2 cases. Small cell carcinomas were diagnosed in 6 cases, non-small cell carcinoma and dispersed carcinoma in 2 cases, poorly-differentiated carcinomas in 4 cases, 8 samples of neoplastic cells were misclassified, 2 cases were mixed tumors, 1 case represented malignant lymphoma, and 1 case contained a few neoplastic cells. Our findings showed that FNAB studies of liver neoplasms clinically suspected of constituting metastatic tumors with unknown primary sites, disclosed a surprisingly high percentage (41.09%) of primary hepatocellular carcinomas, whereas a low percentage of cases, in comparison with the literature, constituted metastatic colorectal adenocarcinomas.

RECTAL ADENOCARCINOMA WITH OSSEOUS METAPLASIA

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Osseous metaplasia is a very rare finding in gastrointestinal malignances. Its incidence in rectal adenocarcinomas has been estimated at less than 0.4%, but to-date only about 20 cases have been reported. We present a case of osseous metaplasia in rectal carcinoma in a 79-year-old man. It was a moderately differentiated stage III adenocarcinoma with a lymphatic invasion. Numerous irregular bone spicules surrounded by a rim of osteoblasts were distributed within the tumor. A clear transition between the stromal cells and osteoblasts was focally noted. Some of the spicules were calcified. Nodal metastases showed no evidence of bone formation. The pathogenesis of osseous metaplasia is unclear. It is probably a lesion without any clinical significance, but it could be misdiagnosed as carcinosarcoma or an osseous invasion of carcinoma, especially in small biopsy specimens.

FINE-NEEDLE ASPIRATION BIOPSY MONITORED BY CT IN THE DIAGNOSIS OF PULMONARY FOCAL LESIONS

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The delay in the diagnosis of lung cancer makes the radical treatment of this disease impossible, leading to its becoming generalized. X-ray detection of pulmonary focal lesions ought to conclude in establishing the histopathological diagnosis. It is necessary to collect the material from the lesion for the microscopic investigation. The results determine the introduction of an appropriate treatment. Many pulmonary focal lesions are inaccessible by bronchoscopy, and a biopsy through the chest wall is required. Our aim was to show the possibilities created by the application of FNAB monitored by CT in the diagnosis of pulmonary focal lesions. In the period between 1995 and 2003, in the CT Laboratory of the Radiology Department, Military Hospital in Bydgoszcz, 592 fine-needle aspiration biopsies of pulmonary focal lesions were performed. Cytological diagnosis based on the obtained material was possible in 491 cases (82.9%). In 47 cases, normal pulmonary cells were found in the material. Of the above mentioned number of cases, as well as in 54 cases suspected of a tumor, where non-diagnostic material was obtained (blood, necrosis), 39 repeated biopsies were performed. In the group under investigation, 538 patients did not report any ailments or complaints. Pneumothorax was one of the most serious complications after biopsy. It required surgical intervention (22 cases – 4%). Mildly bloody sputum was observed in 24 cases (4%). CT-monitored FNAB of pulmonary lesions is an effective diagnostic method of low invasiveness, allowing for establishing the cytological diagnosis. This method substantially shortens the time between the X-ray detection of the lesion and the introduction of an appropriate treatment.

600 STEREOTACTIC BRAIN TUMOR BIOPSES – AN ESTIMATION OF DIAGNOSTIC POSSIBILITY OF PATHOMORPHOLOGICAL EXAMINATION

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The clinical-diagnostic and clinical-therapeutic role of stereotactic biopsies depends in a major part upon a precise pathological examination of the specimen taken in the stereotactic way. From the neuropathological point of view, a morphological evaluation of a small tissue sample and a cytological intraoperative diagnosis pose serious problems. Based on the analysis of 600 stereotactic biopsies performed between 1996

and 2003, the authors focused on the specific features and difficulties of the cytological diagnosis of intracerebral tumors and tumor-like lesions. Stereotactic biopsy is a serial biopsy, what means that several tissue samples are collected for classic histopathological and cytological examinations. Apart from objective problems associated with the size of the sample and a short time required to establish the diagnosis in 11% of the performed biopsies, major problems were posed by the lack of correlation between cytological and histopathological results, caused by collecting a specimen which was impossible to evaluate. In these cases, the result was established based on one of the examinations, either cytological or histopathological. The second problem was the differential diagnosis between reactive gliosis and low-grade gliomas. It was impossible to make a cytological diagnosis in tumors commonly regarded as difficult to examine, e.g. central neurocytomas, DNT or germinomas. There also appeared a tendency to overgrade the neoplasms in cytological examination – 1.5% cases. In 10% of the examined tumors, the grade of the tumor increased from the margin to the center. In few cases, samples were taken outside the tumor. Thus, the presence of the pathologist while this procedure was performed and a possible correction of the specimen collection were essential. Despite the objective difficulties, cytological evaluation had a high sensitivity (70%) as confirmed by histopathological identification; additionally, in correlation with histo- and immunohistochemical evaluation it allowed for a nearly 90% rate of successful morphological diagnoses of tissue samples obtained from brain tumors.

QUANTITATIVE ANALYSIS OF MUC1 (CD227) EXPRESSION ON LUNG CARCINOMA TISSUE ARRAYS

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This study was carried out to characterize the MUC1 (CD227) expression pattern and association with outcome and patient response to antigen-specific therapy. A tissue microarray containing 343 lung cancer samples was utilized. The available data included a detailed histological type, tumor stage, grade and follow-up. Staining intensity was scored as the percentage of positive cells. In addition, automated quantitative measurements were performed with the evaluation of 7 classes of digital picture attributes. Based upon all the data, a final rating was assigned to each sample. A strong staining intensity of MUC1 was significantly associated with tumor type (adenocarcinoma), advanced stage and higher grade. No clear-cut association was found with the presence of lymph node or distant metastases. To date, 17 cases of MUC1-positive patients were qualified and treated with recombinant vaccine virus containing sequences coding for human MUC1 antigen and interleukin-2. The evaluation of the response to this therapy and outcome is in progress.

INTERMEDIATE CELLS IN REGENERATION AND PROLIFERATION PROCESSES IN CHRONIC PANCREATITIS

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In the search for therapeutic methods to treat type I diabetes (insulin-dependent), great expectations are associated with the possibility of implantation or stimulation of proliferation of endocrine progenitor cells. Endocrine and exocrine cells originate from the pool of omnipotential endodermal cells present in the ductular wall or in the cells, which acquire the embryonic potential during proliferation and metaplastic lesions. The aim of the study was to assess the role of various types of intermediate cells in the processes of cellular regeneration and proliferation in chronic pancreatitis. The study was conducted using samples obtained from 30 patients with chronic pancreatitis operated on in BiaŹystok hospitals. The following investigations were performed: histological, immunohistochemical with the use of monoclonal antibodies against nestin (progenitor cell marker), insulin and glucagon (islet cell markers), immunofluorescence staining using anti-amylase (acinar cell marker), and ultrastructural. The chronically affected pancreas, apart from progenitor cells with nestin expression, showed the presence of intermediate cells, including acinar-islet, duct-acinar and duct-islet cells. The role of intermediate cells in pathology is not clear and requires further studies. It has to be elucidated whether intermediate cells are a transient stage in differentiation of precursor cells or rather abnormal forms of cells generated during regeneration and proliferation.

MORPHOMETRIC BIODEGRADATION ANALYSIS OF EARLY PERIOD OF CARBON FIBRE IMPLANTS INSERTED INTO THE TRACHEAL WALL

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Tracheal stenosis can be treated by a resection followed by the reconstruction by alloplastic materials. Biocompatible implants should enhance the healing of tissue defects, finally undergoing biodegradation, while filling the defect with biocompatible tissue or creating an effective prosthesis for a part of the tracheal wall. The aim of the study was a morphometric evaluation of carbon implants inserted into the tracheal wall of the experimental animals (rams and sheep) in the early healing period. Healthy experimental animals (sheep), under general anesthesia, were subjected to surgical procedures. After the resection of approximately 70% of the trachea circumference, sparing the entire membranous part and fragments of tracheal cartilages, a carbon cloth patch was implanted and sutured in situ. After 1, 2 and 3 weeks, the animals were sacrificed under general anesthesia and the tracheas with the implanted carbon cloth fragment were routinely histologically processed for further microscopic image analysis with a Quantimet Leica. This analysis included: 1. the breadth responding to the shortest diameter of carbon fibers, 2. the curve length of carbon fibers, 3. the fullness factor coefficient, 4. the mean gray level,

reflecting the density of the material – optic density. The results were analyzed statistically. The breadth showed a significant increase of the mean value despite slight differences in the median value. The curve length of carbon fibers behaved similarly. The fullness factor decreased significantly, showing distinctive resorptive pits in the 3rd week after the implantation. The mean grey level manifested a progressive and significant decrease. The correlation analysis showed the strongest correlation of the shortest breadth with the time of carbon fiber implantation.

TISSUE REACTION AFTER THE TRACHEAL IMPLANTATION OF CARBON CLOTH

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Tracheal surgery is associated with a risk of complicated and prolonged healing. An ischemic necrosis of the sutured tissues and inflammatory complications can occur at the suture sites. A chronic inflammatory reaction with granulation tissue both weakens the tracheal wall and can lead to its stenosis. Biomaterial implantation can be associated with the same complications. The aim of the study was an evaluation of the tracheal wall healing process after carbon fiber patch implantation in experimental animals. Experimental healthy animals were implanted under general anesthesia with carbon fibers in the form of a patch following the resection of a trachea fragment including 70% of its circumference, preserving the cartilage fragments and the entire membranous part. After 1, 2 and 3 weeks, the animals were sacrificed and the tracheas with implant fragments were diagnosed histologically. No dehiscence of the sutures connecting the implant with the trachea was observed in all cases. The presence of heaped-up elevation with tissue necrosis, granulocytic infiltration and the presence of Aspergillus mycelium were observed at the margin of the area of the carbon fiber anastomosis with the tracheal wall, and metaplastic stratified squamous epithelium was noted here and there. In the third week, the proliferation of fibrous connective tissue with perivascular inflammatory infiltrations, consisting mainly of plasma cells, was noted. The number of granulocytes increased with a decreasing distance to the trachea lumen, extensive fibrosis was also noted, while granulation with histiocytes, and polymorphonuclear inflammatory infiltration, a few giant cells and fibroblasts were found in place of the mucosa. The inner edge was encompassed with necrosis. Fragments of ciliated epithelium with disturbed architecture were visible among necrotic tissues. Attention was drawn by intensified epithelium pseudostratification and focal squamous metaplasia. Transverse trachea cross-sections, in the first week, disclosed mucosa covered with wrinkled, relatively thin ciliated epithelium with a presence of mucus-secreting cells. Also, numerous neutrophiles, plasma cells, small lymphocytes and granulocytes were visible directly under the epithelium. Infiltrations in the deeper layers of the mucosa and muscular membrane were grouped around the vessels. In the second and third week, the mucosa showed focally features of squamous metaplasia. Inflammatory infiltrations were located also in deeper submucosa layers. No damage of the tracheal cartilage was observed. Carbon fibers were visible in the preparations in the form of simple black bands, focally fragmented.

HISTOLOGICAL STRUCTURE OF THE SOFT PALATE IN OBSTRUCTIVE SLEEP APNEA PATIENTS

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The histology of the soft palate and uvula in snorers and patients with OSA syndrome has been a subject of investigations performed by many authors. In the majority of specimens, hypertrophy of the salivary glands, as well as congestion and dilation of the thin-walled vessels were observed. Some of the samples presented atrophy of the muscle bundles. Also, inflammatory changes appearing in the form of lymphocytic infiltrations were demonstrated. In each case adults served as the control group. In this study, the histological analysis of the soft palate and uvula samples from patients suffering from snoring and OSA syndrome was performed. Uvula samples from newborns that died on the first day of their lives were chosen as the control group. The choice of such a group excluded the influence of vibration force on the soft palate structure. The intergroup comparisons were made by the Fisher exact two-tailed test. Muscular atrophy was observed only in patients with airway disturbances. No case of neonatal tissue sample with this pathology was found. Less dilation and congestion of the blood vessels were observed in the newborn group. Superficial salivary glands situated between the muscle bundles and the epithelium were found significantly more frequently in the OSA and snoring patients. Our results showed distinct differences between the tissues of the patients with airway disturbances and the control group. These differences may be caused by the influence of the vibration on the soft palate and uvula, but on the other hand, they may be the reason for an excessive flaccidity of these structures and disturbances occurring during sleep.

EXPRESSION OF IMMUNOLOGICALLY/CONFORMATIONALLY DIFFERENT FORMS OF P53 PROTEIN IN COLON ADENOCARCINOMA, ADENOMA AND ULCERATIVE COLITIS

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The aim of the study was to evaluate overexpression of P53 protein, detected by two different antibodies DO7 and PAb240 in 27, 15 and 12 colon adenocarcinomas, adenomas and ulcerative colitis, respectively, considering clinico-pathological parameters. Expression

of P53 was examined prospectively by using EnVision test. The comparative analysis of both MoAbs revealed 4 phenotypes. In adenocarcinomas and adenomas the phenotype DO7+PAb240+ predominated (67% and 53%, respectively). In ulcerative colitis the phenotype DO7-PAb240- predominated (92%). The phenotype DO7-PAb240+ was present only in adenomas (7%). In adenocarcinomas DO7 react more frequently than PAb240. In preneoplastic diseases the frequency of positive reaction with DO7 and PAb240 is equal. The data obtained reveal that P53 expression depends on the applied antibody. No significant relation between DO7 and PAb240 expression and tumor histological type and grading was found.

IMMUNOHISTOCHEMICAL LOCALIZATION OF METALLOTHIONEIN (MT) AND p53 PROTEIN IN PANCREATIC SEROUS CYSTADENOMAS

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Metallothionein (MT) is a low-molecular protein (6–7kDa), which binds metals (Zn, Cu, Cd) and is present in the nucleus and in the cytoplasm of epithelial cells. MT participates in the anti-oxidant protection of the organism. Metallothionein overexpression was detected in cancers and its level closely correlated with the tumor size and the bad prognosis. Pancreatic serous cystadenomas are composed of microcysts lined with glycogen- rich cells. Incidence peak of the tumors was noted among fifty- year old women. Diagnosing serous cystadenomas, a rare pancreatic neoplasm, is difficult at each stage of the disease. The aim of this study was to determine the level of metallothionein and p53 protein expression (known neoplastic transformation markers) in pancreatic serous cystadenomas. The study material was obtained from 5 women (age range: 39–69) hospitalized in the Chair and Department of the Gastroenterological and General Surgery of the Wrocław Medical University between 1992 and 2001. Neoplastic pancreatic tissue was taken during surgical intervention while control pancreatic tissue originated from healthy persons who had died in car accidents. Sections were stained with HE. In all pancreatic sections of ill women pancreatic serous cystadenomas were detected. Immunohistochemical localization of MT and p53 protein was carried out by means of the LSAB2-HRP using specific antibodies directed against MT and p53. The immunohistochemical reaction for metallothionein antigen was observed only in the epithelial cells of the neoplastic tissue. The metallothionein expression in cystadenomas was weak when compared to the MT expression in healthy pancreatic tissue. No tissue was found to feature p53 protein expression. Conclusion: weak metallothionein expression and no p53 protein expression in pancreatic serous cystadenomas confirm a benign character of the neoplastic lesion.

PROGNOSTIC VALUE OF sTNF-R AND sICAM–1 LEVELS IN SEROUS OVARIAN CANCER

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Tumor necrosis factor (TNF-alpha) is a cytokine possessing antitumor and immunomodulatory properties. It has been recently suggested that in malignant neoplasms, soluble TNF receptors (sTNF-Rs) and the soluble intercellular adhesion molecule (sICAM–1) may represent prognostic factors. The aim of our study was to measure the levels of serum concentrations of sTNF-R in 29 patients with serous ovarian cancer at stage I/II according to the FIGO classification (Group I) and in 17 patients with stage III/IV (Group II) and to determine whether these levels correlated with those of sICAM–1 and were involved in disease progression. Significantly raised sTNF-R levels were detected only in Group II compared with normal controls ($P<0.002$; $P<0.001$), whereas sICAM–1 levels were increased both in Group I and II vs. controls ($P<0.001$). A correlation between sICAM–1 and sTNF-R1 ($r=0.646$; $P=0.002$) and sTNF-R2 ($r=0.518$; $P=0.016$) concentrations in patients' sera was observed in Group II. In this group, a correlation between sTNF-R1 and sTNF-R2 levels ($r=0.78$; $P=0.001$) was also demonstrated. In conclusion, we have shown that in patients with serous ovarian cancer an increase in the serum concentration of sTNF-R and sICAM–1 seems to be related to tumor stage and may contribute to cancer progression.

HER-2 GENE AMPLIFICATION AND PROTEIN p185 OVEREXPRESSION IN INVASIVE DUCTAL BREAST CANCER

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The purpose of the study is to evaluate p185 protein and *HER-2* status by an immunohistochemistry (IHC) and by hybridization *in situ* (FISH), respectively in invasive ductal cancers of the breast. Histologic slides from 392 invasive breast carcinomas were tested by IHC (*Herceptest*) with anti HER-2 protein monoclonal antibody. Cancers assessed as score 2+ and 3+ were further evaluated by computerized image analysis and also tested by FISH with unique probe HER–2 and satellite probe CEP17. Fluorescent parameters were measured using computer program *Analysis*^{R}. In 25% of cases with score 2+ and 95% with score 3+ HER-2 amplification was found. Cancers with *HER-2* amplification revealed various amount of protein p185 as assessed by computerized image analysis.

FLUORESCENCE HYBRIDIZATION *IN SITU* (FISH) IN THE DIAGNOSIS OF TRANSITIONAL CELL CARCINOMA OF THE BLADDER

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The incidence of carcinoma of the urinary bladder (TCC) is increasing all over the world. Cystoscopy and urinary cytology are considered the gold standard in the diagnosis of TCC. Numerous studies have demonstrated that routine cytology is

highly specific but is limited by low sensitivity (40%). A number of genetic alterations have been observed in TCC. Particularly common are chromosome 9 monosomy and deletions, deletions of 17p and alterations of many other chromosomes (1, 7, 11). Interphase FISH uses fluorescent labeled DNA probes to centromers or unique loci to detect cells with numerical and/or structural changes indicative of malignancy. A multicolor multitarget interphase FISH (UroVysion) consisting of probes to the centromers of chromosomes 3, 7, 17 and to the region 9p21 has been used to establish the diagnosis in 28 patients with clinically diagnosed TCC (32 urine samples). The visualization of hybridization results was performed using fluorescence microscopy. The study proved that the sensitivity of FISH for the detection of TCC is superior to that of routine cytology. FISH analysis may be useful to screen urine for early detection of a primary tumor or recurrence of the disease.

WEGENER'S GRANULOMATOSIS – AN AUTOIMMUNE DISEASE?

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Wegener's granulomatosis (WG) is a clinicopathologic disease of unknown etiology characterized by: (i) necrotizing granulomatous inflammation of the upper and lower respiratory tracts, (ii) glomerulonephritis, and (iii) systemic vasculitis. Despite of 70 years of the WG history, a significant progress in diagnostic procedures, treatment and prognosis, WG is still a mysterious and strange disease with many unexplained pathogenic problems. Moreover, microscopic pictures are very diverse and cause many diagnostic problems. Opinions are expressed increasingly frequently that diagnostic morphological criteria for WG are too restrictive, especially in the initial phase of the disease (or the limited form of the disease), when the most common biopsy sites are the lesions in the upper respiratory tract. As it follows from the experience of the pathologist, the presence in the upper respiratory tract of the classic microscopic lesion triad: necrosis, granulomas and vasculitis in the first biopsy is seen in 3–15% of cases only, but in lung biopsy this triad is present in about 90% of patients with WG. Despite extensive research efforts, the nomenclature of many subtypes of WG has not been established. The lack of standardization of terms and definitions constitutes a genuine problem: different terms are used for the same type of disease and there are various interpretations of particular terms. For example, the term "limited" WG has different clinical descriptions. The complete morphological picture of this disease includes two quite different types of lesions: granulomas and vasculitis. It seems that these two lesion types result from different independent or quasi-independent pathogenic mechanisms. The discovery of anti-neutrophile cytoplasm antibodies (ANCA), particularly c-ANCA with anti-proteinase–3 specificity, and their use for monitoring of disease activity and relapse prediction has not only improved the diagnostic procedures of WG, but also formed the basis for considering an autoimmune background of this disease. The role of ANCA in the pathogenesis of tissue lesions, as well as the role of autoimmunity in tissue destruction through their reactions with granulocytes and endothelial cells seem to be unquestionable. The role of autoreactive T cells and their possible relation with autoantibodies in this disease is a separate problem. In recent attempts at the classification of vasculitis, the significance of ANCA antibodies in WG has been strongly emphasized. It has been decided that the presence of ANCA antibodies should be one of the diagnostic criteria of WG. To summarize, the diagnosis of WG as a clinicopathologic process has to be the result of a common effort of pathologists, as well as clinicians. It should be noted that an early diagnosis, especially in the initial phase of the disease, is very important for effective treatment. However, in the majority of cases, the first biopsy of the upper airway mucosa does not form a reliable basis for the diagnosis of WG. Hence, the biopsy should be repeated and the ANCA antibodies presence and titer should be investigated. The presence of autoantibodies (anti-neutrophile cytoplasm, antibodies with rheumatoid factor specificity), as well as the negative results of tests aiming at detection infectious etiological agents constitute a very important index of autoimmunity in Wegener's granulomatosis.

EFFECTIVENESS OF 4-HYDROXY-TEMPO AND VITAMIN E IN EXPERIMENTAL MODEL OF CAERULEIN-INDUCED ACUTE PANCREATITIS. A HISTOPATHOLOGICAL STUDY

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Oxygen free radicals are involved in the development of tissue damage in all forms of acute pancreatitis. The efficacy of scavenger treatment was evaluated in an experimental model of caerulein-induced acute pancreatitis. This study aimed at evaluating morphologic disorders in the pancreas. Male Wistar rats (260–290g) were used (n=36). The rats were randomized to 6 groups. Acute pancreatitis was induced by four intraperitoneal injections of caerulein (15 g/kg) at 1-hour intervals (n=18). Group I was treated with saline, Group II with Vit. E (200mg/kg), Group III with TEMPO (100mg/kg), Group IV with caerulein, Group V with caerulein+ TEMPO, Group VI with caerulein+Vit.E (i.p.). After 6 hours, the animals were sacrificed and the pancreas was removed for morphological studies. The material was fixed in 10% formalin and stained by routine HE. The degree of edema, inflammatory infiltration of neutrophiles and necrosis was evaluated using a semiquantitative method. In Group I, no morphological changes were noted, in Groups II and III only focal neutrophile infiltrations in the adipose tissue were observed in some rats. In Group IV, severe and moderate interlobar and interlobular edema of the pancreas, intensive neutrophile infiltrate in both the adipose (+++ – 5/6 rats) and the gland tissue (+++, ++), steatonecrosis in 3/6 rats and necrosis of the pancreas (3/6) were observed. Group V treated with TEMPO demonstrated moderate interlobar and interlobular edema of the pancreas, neutrophiles infiltrate in the adipose tissue (+++ – 1/6; ++ – 3/6 and + – 2/6 rats), as well as in the glandular tissue (++ – 2/6; + – 4/6 rats) of the pancreas. Fat necrosis was absent and focal necrosis of the pancreatic cells was observed only in 1 out of 6 cases. In Group VI treated with Vit. E, moderate lobar edema of the pancreas, neutrophilic infiltrate in the adipose tissue (+++ – 1/6; ++ – 2/6 and + – 3/6) and in the pancreas (+ – 6/6 cases) and focal steatonecrosis in 3/6 cases was observed. Necrosis of the pancreas cells was not observed. These results suggest that 4-hydroxy-TEMPO and Vit. E decrease the intensity of inflammation in experimental acute pancreatitis. Vit. E is more effective than TEMPO in decreasing the intensity of inflammation in the pancreas and it seems to prevent the occurrence of necrosis of pancreatic cells, but in a half of cases steatonecrosis has been observed.

EFFECTIVENESS OF ADRIBLASTINE IN EXPERIMENTAL MODEL OF ACUTE PANCREATITIS. A HISTOPATHOLOGICAL STUDY

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In acute pancreatitis, the mechanisms involved in auto-amplification of inflammation can be suppressed by inhibiting the pancreatic secretion. The administration of cytostatic drugs causes a decline in the synthesizing activity of cells in acinar parenchyma, but also may induce intracellular lesions. The purpose of this study was to evaluate the effectiveness of cytostatic treatment of rats with acute pancreatitis. Male Wistar rats (260–290g) were used. Acute edematous pancreatitis was induced by four intraperitoneal injections of caerulein (15 g/kg) at 1-hour intervals. Rats (n=18) were randomized to control or caerulein-induced pancreatitis groups, and treated with saline or adriblastine (3mg/kg). After 6 hours, the animals were sacrificed and the pancreas was removed for morphological studies. The material was fixed in 10% formalin and stained by routine HE. The degree of edema, inflammatory infiltration of neutrophiles and necrosis was evaluated using a semiquantitative method. In the first group, severe and moderate interlobar and interlobular edema of the pancreas, intensive neutrophile infiltrate in both the adipose and gland tissue, steatonecrosis (3/6) and necrosis of the pancreatic cells (3/6) were observed. In the second group treated with adriblastine, we observed moderate interlobar and interlobular edema of the pancreas, neutrophile infiltrate in the pancreas – (++) in 4/6, (+) in 2/6 cases, as well as in the adipose tissue – (++) in 3/6 and (+) in 3/6 cases. Steatonecrosis and necrosis of the pancreatic cells were not observed. These results suggest that adriblastine does not considerably influence the decrease of inflammatory infiltrations in acute pancreatitis. Steatonecrosis and necrosis of the pancreas cells were absent.

IMMUNOEXPRESSION OF PERFORIN AND GRANZYME B ON INFILTRATING LYMPHOCYTES IN HUMAN RENAL ACUTE ALLOGRAFT REJECTION

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Graft destruction can be effected by a direct cell-to-cell contact between an activated effector T-cell and a target graft, resulting in a delivery of cytotoxic molecules. Perforin and granzyme B can be used as activation markers for cytotoxic cells in allograft tissue. The aim of the study was to determine the immunoexpression of perforin and granzyme B by immune cells infiltrating the renal tissue during acute allograft rejection and to evaluate any correlation between the phenotype of infiltrating lymphocytes and cells expressing cytotoxic granules, as well as the severity of graft damage as defined by the Banff 97 criteria. Immunoperoxidase staining was carried out using monoclonal antibodies anti-perforin, -granzyme B, -CD3 and -CD8 on renal allograft biopsy specimens from twenty-one patients with acute renal transplant rejection: Banff 97 IA (n=11) and Banff 97 IB (n=10). As the controls, 11 biopsy specimens of renal transplant patients without any signs of rejection were used. All allograft biopsy specimens with acute renal transplant rejection contained a high number of CD3+ T cells (Banff IA: 437.4±154.4 and Banff IB: 825±339.9 vs.123.4±52.5 in the controls) and CD8+ T lymphocytes (Banff 97 IA: 177.6±89.2 and Banff IB: 293.2± 112.4 vs. 64.2±37.1 in the controls). Immunostaining for granzyme B and perforin was negative in the controls. The immunopositivity for perforin was similar in Banff IA and Banff IB acute allograft rejection (1.5±0.6 vs. 1.8±0.8, respectively). Granzyme B+ cell count was significantly higher in the severe rejection group Banff IB (128.3±74.3) than in the Banff IA group (48.2±18.3). Moreover, in acute allograft rejection Banff IB, the number of granzyme B+ cells and perforin+ cells was correlated with the number of CD8+ T cells. In conclusion, our results suggest that in acute tubulointerstitial allograft rejection, activated cytotoxic T lymphocytes play a major role. The strong immunopositivity for granzyme B on infiltrating cells in renal transplant tissue is suggested as a marker of severity of graft damage.

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PRIMARY CULTURE OF COLORECTAL CARCINOMA METASTASIZING TO THE LIVER – A STUDY OF COMPLEMENT INHIBITORS

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Metastatic liver tumors represent a major challenge for clinical oncology, and there is a need for continuous research aiming at developing improved treatment modalities for this disease. One of those is immunotherapy with monoclonal antibodies, the efficacy of which depends on the tumor cell expression of complement inhibitors. The latter is highly variable among patients and should be evaluated before the treatment. Thus, we attempted to establish a system of primary cultures of metastatic liver tumors, to correlate a range of morphological and molecular features of living cancer cells obtained from patients, with their expression of complement inhibitors. Here, we present the preliminary results of such an analysis performed in a single individual. A tumor sample was obtained upon surgical procedure performed in a male patient, aged sixty-two, with colon adenocarcinoma metastasizing to the liver. The specimen was dispersed using collagenase and the cells were cultured for several weeks. The immunocytochemical studies were performed using multiple label fluorescent confocal microscopy. Electron microscopy and cytogenetic analysis were performed routinely. The mutation analysis of p53 gene was done by direct sequencing. Virtually all cells in the culture were cytokeratin-positive. They were poorly differentiated, with fibroblast-like morphology, and grew as a monolayer. The cytogenetic analysis showed complex chromosomal abnormalities with a nearly triploid karyotype. The sequencing data of p53 gene revealed two single nucleotide substitutions (846C T; 216G C) in coding regions, which are supposed to result in the expression of abnormal protein. The immunofluorescence assay revealed moderate-to-weak CD55 and CD59 immunoreactivities, located predominantly in the plasma membrane. The immunoreactivity of factor H concentrated on the surface of cell processes, where it was closely colocated with sialic acid residues. Conclusion: our approach might be a valuable strategy to investigate the correlation between the expression of the complement inhibitors and unique molecular properties of the tumor in a given patient. The clinical relevance of such an analysis remains to be elucidated.

HISTOPATHOLOGICAL FEATURES OF THE LUNGS IN PROGRESSING SYSTEMIC SCLERODERMA

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No abstract available.

ULTRASTRUCTURAL EXAMINATION OF *CHLAMYDIA PNEUMONIAE* IN AORTIC ABDOMINAL ANEURYSMS

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The aim of this study was the ultrastructural examination of the presence of *Chlamydia pneumoniae* in aortic abdominal aneurysms. Seven patients with high levels of specific antibodies class IgG, IgM, IgA, were classified for ultrastructural examination. In light microscopy the material resected during surgery manifested typical atherosclerotic changes. In three cases, the presence of macrophages, foamy cells, inflammatory cells and collagen and elastic fibers was seen. We observed the presence of intracellular inclusions of *Chlamydia pneumoniae* containing elementary and reticulate bodies EB, RB, as well as extracellular EB. The inclusions were mainly seen in the cytoplasm of macrophages. In one case, the macrophage was filled with inclusions. Morphologically, components of thrombi and ulceration, as well as fragments of dead cells were seen. In the subsequent two cases, the inclusions and elementary bodies were seen in or among the cellular remnants or fragments. We found single macrophages and foamy cells, but without the presence of inclusions.

EXPRESSION OF E-CADHERIN IN PROSTATE ADENOCARCINOMA

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In the androgen-independent, metastatic prostate adenocarcinoma there is currently no successful therapy. Therefore we need to improve our understanding of markers which can be helpful in determining the metastasizing potential of prostate adenocarcinoma. It has been proposed in recent research studies that progression may be related to the presence of neuroendocrine differentiation, proteins from the cyclin-dependent kinase inhibitors family (p21, p27) and adhesion molecule E-cadherin. Decreased expression of this protein may be associated with invasion potential of prostate carcinoma. The aim of our study was to evaluate if E-cadherin is valuable to identify individuals at risk for biologically active prostate cancer. Archival prostate tissues from 75 patients with prostate adenocarcinoma after total prostatectomy and 75 controls with benign nodular hyperplasia and with a 10-year prostate cancer-free follow-up were labeled for E-cadherin by immunohistochemistry. Immunoreactivity was quantified using morphometric computer analysis. Light-microscopic patterns were saved (mean 30 in every case) and the percentage of cells with positive membranous reaction per 1000 cells was then calculated. The results obtained were compared with the histological grade, Gleason score, staging system. Benign nodular hyperplasia cases from controls strongly and widely expressed membranous positive reaction for E-cadherin in all areas of studied sections. Regions of adenocarcinomas were less intensively stained and the percentage of cells with positive membranous reaction was significantly lower. Additionally, correlations between E-cadherin immunostaining pattern and histological malignancy were found. Conclusions: the results obtained confirm the importance of E-cadherin as strong biomarker for prostate cancer. Decreased expression of this adhesion protein is associated with aggressive phenotype in prostate adenocarcinomas and may provide additional information to decide, which patients need more intensive monitoring or treatment (chemoprevention).

USEFULNESS OF ULTRASTRUCTURAL AND IMMUNOHISTOCHEMICAL STUDIES IN CHILDREN WITH IMMATURE GLOMERULI

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In the biopsy material we examined, 44 cases of children were encountered, in whom all, or most of the glomeruli were immature. In such cases light microscopic evaluation is difficult, because hypercellularity masks the pathological changes. Thus, we decided to examine those cases in electron microscopy and to carry out an immunohistochemical study of immature glomeruli to compare the results with those obtained in renal material from laboratory animals and stillborn fetuses or neonates who died immediately after birth. The study group consisted of 44 children aged from 2 to 56 months (at this age immature glomeruli occur sporadically and only a few of them are present), hospitalized due to the first episode of nephrotic syndrome (NS). In 35 cases, an ultrastructural evaluation was performed (in 9 cases no glomeruli were present). In all the cases, the immunohistochemical pattern of -SMA, CD31, CD34 and vimentin was studied. Histological examination documented minimal change disease (MCD) in 36 cases, mesangial hypercellularity (MES) in 5 cases and focal segmental glomerulosclerosis in 3 cases. In electron microscopic study the diagnosis of MCD was confirmed in 24 cases. In 9 children the diagnosis of MES was established and in 2 – FSGS. In all the cases, the mesangial cells exhibited focally and weakly -SMA (as in immature glomeruli during glomerulogenesis), the visceral glomerular epithelial cells expressed vimentin throughout the cytoplasm. The CD31 immunostaining pattern of glomerular capillary endothelial cells was positive (similarly to normal adult and immature glomeruli). The loss of CD34 immunostaining, typical for adult glomerular endothelial cells, was not evident. The clinical course in children with immature glomeruli as compared to the control groups was unfavorable. Conclusions: the results confirm the importance of glomerular immaturity as a biopsy finding and the necessity of electron-microscopic examination in such cases. Immunostaining patterns are not useful in the diagnosis, as they only confirm glomerular immaturity.

TWO CASES OF PRIMARY PHEOCHROMOCYTOMA OF THE PANCREAS

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No abstract available.

COMPARATIVE STUDY OF NUCLEAR MORPHOMETRY, CELL PROLIFERATION MARKERS EXPRESSION AND APOPTOSIS IN THYROID TUMORS

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The majority of thyroid tumors are not homogeneous histologically, what creates problems in histological interpretation and evaluation of prognostic factors. The aim of this study was the analysis of the usefulness of cell proliferation markers expression (Ki-67 antigen, argyrophilic nuclear organizer regions – AgNORs, insulin receptor – IR), apoptosis (caspase–3 and caspase-8) and nuclear morphometry using a computer assisted image analysis system (SAMBA 2005 and Multiscan II). Surgical specimens of the thyroid obtained from 196 patients were processed (57 multinodular goiters, 24 follicular adenomas, 30 follicular carcinomas, 81 papillary carcinomas, 4 medullary carcinomas). Four nuclear morphometric parameters (the area, perimeter, major axis length, minor axis length) demonstrated a statistically significant correlation with the histological type of tumor, whereas roundness did not show any correlation. Cellular proliferation patterns correlated with tumor differentiation and supported the morphological classification. Significant differences of the stromal cell proliferation potential were observed among the analyzed tumors. The expression of caspase-3 and caspase-8 showed marked heterogeneity in all the analyzed groups.

EXPRESSION OF E-CADHERIN AND -CATENIN IN GASTRIC ADENOCARCINOMA AND ADJACENT MUCOSA

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E-cadherin is an epithelial transmembrane glycoprotein. Together with cytoskeleton proteins, such as -catenin, it forms the adhesion complex, responsible for intercellular adhesion. It is believed that a dysfunction of both these proteins may play an important role in tumor genesis and progression. The study was aimed at comparing the expression of E-cadherin and -catenin in gastric adenocarcinoma and tumor-adjacent mucosa with the morphologically intact gastric mucosa of patients without any neoplastic diseases of the stomach. The study material comprised 21 cases of cardiac and corporeal adenocarcinomas. The control group included 5 cases of microscopically normal cardiac mucosa biopsied on gastroscopy. In normal gastric mucosa, E-cadherin and -catenin showed a membrane staining pattern, while in gastric adenocarcinoma both demonstrated a strong cytoplasmic reaction with only weak focal membrane staining of E-cadherin. In tumor-surrounding epithelial samples, both proteins showed a strong membrane reaction. Additionally, a weak cytoplasmic reaction for -catenin was observed in the foci of intestinal metaplasia.

MUTATIONS IN E-CADHERIN GENE IN SPORADIC GASTRIC CARCINOMAS IN POLAND

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E-cadherin protein plays a crucial role in epithelial cell-cell adhesion. The protein is encoded by CDH1 gene, which is located on chromosome 16q22. The gene is composed of 16 exons spanning region of approximately 100kbp. Therefore, we screened 81 sporadic gastric carcinomas for CDH1 mutations, by SSCP analysis of all 16 exons and flanking sequences. We compared DNA obtained from normal and tumor tissue. Reamplification of DNA extracted from additional bands and direct sequencing were performed. In sporadic diffuse gastric cancers CDH1 mutations are evenly distributed along exons 7–9, however we also observed mutations in exons 10 and 14. We revealed mutations in exons: 4–6 in mixed type gastric carcinomas, and a splice site mutation preceding exon 4 in one out of six high-grade gastric cancers. The first Polish mutational analysis of the CDH1 gene reveals that mutation frequency in our country (approximately 12% of mutations in sporadic gastric cancers) is placed between Western European and Scandinavian results.

ASSOCIATION OF ALPHA-V INTEGRIN IMMUNOEXPRESSION AND SOME CLINICO-PATHOLOGICAL PARAMETERS IN NON-SMALL CELL LUNG CARCINOMA

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Integrin alpha-v receptor (vitronectin receptor or CD51 antigen) belongs to a major family of cell surface receptors that recognize the Arg-Gly-Asp sequence. Integrins are heterodimeric glycoproteins that mediate interactions between cells and the extracellular matrix (ECM) and participate in cell migration, tissue organization, cell growth, hemostasis, angiogenesis, inflammation and the differentiation of many cell types. Integrins also play an important role in tumor cell growth and metastasis through their interactions with ECM and endothelial cells. In this study we investigated the expression of alpha-v integrin in a series of patients with NSCLC to asses their distribution and correlations with some clinico- pathological parameters and to determine the significance of this factor in the prognosis. We performed a retrospective study of alpha-v integrin expression in resected tumor tissues from 55 patients with NSCLC using immunohistochemistry. The integrin alpha-v expression was significantly associated with the age of patients, size of tumor and lymph nodes status. A high level of integrin alpha-v expression was observed in 37 tumors (67%). The overall 5-year survival rate was 27%. Arranging the survival of 55 patients with NSCLC according to their integrin alpha-v expression status at the beginning of the 10^{th} month after the operation revealed that the patients with a higher expression of this integrin had a significantly shorter survival than patients with a lower expression. These results suggest that alpha-v integrin expression can be useful in predicting tumor behavior in NSCLC patients and can be regarded as a prognostic factor.

INDUCTION CHEMOTHERAPY IN NON-SMALL CELL LUNG CARCINOMA – AN ANALYSIS OF THE PROLIFERATION ACTIVITY AND THE EXPRESSION OF p53 VS. SURVIVAL TIME

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Locally advanced (stage IIIA) non-small cell lung carcinoma (NSCLC) has a poor prognosis following surgery alone because of a high rate of local and metastatic relapses. Neoadjuvant chemotherapy may be used for lung cancer to improve the complete resection rate and to treat non-detectable metastatic disease. The *p53* gene and p53 protein play an important role in the process of neoplasia, as well as sensitivity and resistance to chemotherapy. In the current study we evaluated the associations between p53 protein expression and PCNA expression and age of patients, histopathological type of carcinoma, size of tumor, lymph nodes and pleural status and survival time before and after chemotherapy. Forty patients and 38 patients with NSCLC without and after induction chemotherapy, respectively, were entered into the study. Tumor samples were analyzed immunohistochemically for p53 protein accumulation and PCNA expression. All the patients were in clinical IIIA stage. Their age ranged from 39 to 72 years in both groups (mean age, 60 and 58 years, without and with chemotherapy, respectively). There was a statistically significant association between the histological type and size of tumor, lymph nodes status and survival time in the group with chemotherapy, but there was no significant relationship between p53 expression, PCNA expression and survival time. In the group without chemotherapy, the size of the tumor, lymph nodes status and PCNA index had a significant influence on survival time. The comparison of patients with and without induction chemotherapy in the analysis of potential prognostic factors showed that only the tumor size was a significant unfavorable prognostic factor. The overall 5-year survival rates were 25% and 34% in groups without and with chemotherapy, respectively. Moreover, in the beginning of the 10^{th} month after operation, the differences in survival time of patients with and without chemotherapy were statistically significant. These results indicate that induction chemotherapy in NSCLC with stage IIIA is effective, resulting in longer survival times of patients.

LIVER BIOPSY AFTER ORTHOTOPIC LIVER TRANSPLANTATION IN ADULTS – OUR EXPERIENCE

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In the period from IX2000 till XII2003 we diagnosed 117 liver biopsies, obtained from 83 adult patients after orthotopic liver transplantation (OLT). In 56 patients the liver biopsy was performed once, in 21 patients twice, in 5 – three times, and in one patient – four times. In analyzed group the most frequent cause of OLT was liver cirrhosis related to autoimmunological disorders – 34.9% (primary biliary cirrhosis – PBC, primary sclerosing cholangitis – PSC, autoimmunological hepatitis – AIH). Nearly the same number of patients (32.5%) had the liver cirrhosis of viral etiology: viral hepatitis B and/or C alone, or accompanied by other diseases (alcoholism, AIH, hepatocellular carcinoma, Wilson's disease, hemochromatosis, Budd-Chiari syndrome). In 8 patients the etiological factor of liver cirrhosis was alcoholism. Other rare causes of OLT were: paracetamol, Tibet herbs and mushroom toxicity, polycystic liver disease, Wilson's disease, alpha–1-antitrypsin deficiency, secondary biliary liver cirrhosis, carcinoid metastases, renal clear cell carcinoma metastases. In 9 patients cryptogenic cirrhosis was diagnosed. Liver biopsies were performed only in the patients with some clinical disturbances. 29 liver biopsies were obtained in early posttransplant period (one month), 67 – between 1 to 12 months after OLT, and 21 – later than one year after OLT. Because of a necessity of immediate diagnosis, related to the decision concerning additional immunosuppressive treatment in the cases of acute liver rejection, the histological material was processed in the shorter time than routinely – 4 hours. The biopsies were considered to be satisfactory, when they contained minimum 7 portal tracts (110). Seven of them were estimated as normal. In 62 cases one lesion was diagnosed: 21 – extrahepatic cholestasis, 21 – viral hepatitis, 8 – acute rejection, 5 – functional cholestasis, 4 – liver blood flow disturbances, 2 – macrovesicular steatosis, 1 – secondary biliary cirrhosis. In 41 cases the diagnosis was more complicate, because there was overlapping of minimum 2 disorders. Most often we diagnosed the coexistence of viral infection with other pathologies (26 cases); mainly with acute rejection (8 cases) and with extrahepatic cholestasis (13 cases). Extrahepatic cholestasis accompanied acute rejection in 6 cases. In 5 patients the suspicion of chronic rejection was posed, in 3 cases coexisting with viral infection, and in 2 – with extrahepatic cholestasis. In the early posttransplant period (1 month) acute rejection was the most frequently diagnosed pathology (10 cases – 34.5%). Between 1 and 12 months after OLT we described 28 cases of extrahepatic cholestasis (41.8%) and 27 cases of viral infection (40.3%). Acute rejection was diagnosed in 15 patients (22.4%), and in one – chronic rejection. In the period later than one year after OLT, viral hepatitis was most frequently diagnosed (14 cases – 66.7%). We described chronic rejection in 4 patients. The aim of this study is to present typical and atypical histopathological pictures of transplanted liver biopsies. We would like to underline the diagnostic difficulties in the cases of coexistence of two or more pathologies and also the morphological resemblance between many of them. The proper histological diagnosis of liver biopsy is very often possible only when clinical data (biochemical and serological) are known. The necessity of close cooperation of pathological and clinical teams is obligatory.

POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER (PTLD) – MORPHOLOGICAL PICTURE AND DIAGNOSTIC DIFFICULTIES – OWN EXPERIENCE

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PTLD – posttransplant lymphoproliferative disorder – is a well-known complication of both solid organ and bone marrow transplantation. It includes a wide spectrum of pathological proliferative changes ranging from reactive polyclonal hyperplasia, borderline lesions to highly malignant lymphomas. PTLD develops in 1 – 10% of transplant recipients, taking the third place after skin carcinomas and Kaposi's sarcoma. Risk factors for the development of PTLD include first of all: type and

intensity of immunosuppressive therapy, type of organ transplanted, antiviral prophylactic therapy and primary EBV infection after transplantation. The main causes of PTLD are chronic infections, which because of continuous antigen stimulation, leads to the proliferation of B lymphocytes. At the beginning it is polyclonal, reactive, but with the passage of time it escapes from the control of suppressor T lymphocytes and becomes monoclonal – neoplasmatic. The main etiological factor of PTLD (especially for lymphomas of B-cell origin) is Epstein-Barr virus infection. According to the newest WHO classification, 4 morphologic categories of PTLD are defined: 1. early lesions (reactive plasmacytic hyperplasia and infectious mononucleosis-like); 2. polymorphic PTLD; 3. monomorphic PTLD – B and T cell origin, classified according to lymphoma classification; 4. Hodgkin's lymphoma and Hodgkin's lymphoma-like PTLD. We present here 7 cases of PTLD. Although it is a rather small group, they represent the wide variety of morphological pictures and are the examples of all groups described above. Four of them developed after renal, three after liver transplantation. Among early lesions we diagnosed 2 cases of reactive plasma cell hyperplasia of lymph nodes, which occurred 4 and 11 months after liver transplantation and 1 infectious mononucleosis-like PTLD in lung biopsy obtained from renal transplant recipient 22 months after operation. A polymorphic lesion was diagnosed in the transplanted kidney 6 months after surgical procedure. Among lymphomas we described 2 DLBCL (diffuse large B-cell lymphoma). One of them, immunoblastic variant, developed as the multifocal lesion in the abdominal cavity, 6 years after renal transplantation. The second one, centroblastic variant, was diagnosed in the tonsil, 27 months after liver transplantation. The last case, Hodgkin's lymphoma-like PTLD was diagnosed only on the base of bone marrow biopsy in a recipient of a renal transplant 9 years after operation. The early, reactive PTLD has a good prognosis, and antiviral therapy and the reduction of immunosuppression usually lead to regression of the lesions. Contrary to this statement, one of our patients with plasmacytic hyperplasia presented with a rapid course and died because of sepsis. Morphological pictures of 5 PTLD cases (DLBCL, polymorphic PTLD, infectious mononucleosis-like PTLD, 1 case of plasma cell hyperplasia) were typical and were not a diagnostic problem. In the second case of plasmacytic hyperplasia the lymph node morphology was atypical, with atrophy of lymphoid components accompanying plasma cell proliferation. The decreased number of lymphocytes was probably the cause of patient's susceptibility to infections, and when the generalized infection occurred, the patient died. The most difficult case, presented by us during XI Congress of European Association for Hematopathology in Siena (2002) and during VI Congress of Polish Transplantation Society in Jachranka (2003), was very rare Hodgkin's lymphoma-like PTLD. Because of not characteristic immunophenotype it was primarily diagnosed as anaplastic cell lymphoma of T-cell type. After immunohistochemical studies with the newest antibodies BOB and OCT2 the final diagnosis of Hodgkin's lymphoma-like PTLD was established. Because of still increasing number of solid organ and bone marrow transplantations, the patomorphologist may more and more often face the transplant complication known as PTLD. The knowledge of this problem is necessary in everyday practice of our profession.

TRIAL EVALUATION OF THE DAMAGE TO EPIDERMAL BASAL CELLS AND PHENOTYPING OF INFLAMMATORY CELLS IN THE DERMIS IN PSORIASIS

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The exponents of the basal layer damage and the cells presenting antigens and macrophages can play an important role in the psoriasis pathogenesis. Aim the study: evaluation of the damage exponents of the basal layer in psoriasis (metallothionein, collagen IV, laminin), proliferation of keratocytes (Ki-67) and a number of CD68 cells in the inflammatory infiltration. The study included 29 patients with regular psoriasis (18 individuals) and 11 patients with pustular psoriasis. The reagents supplied by DAKO were employed. All the immunohistochemical procedures were performed according to the manufacturer's instructions. The semiaxial evaluation was done at least in 5 high power fields (HPF) – magnification 400x. In the study of Ki-67 antigen, the index ranged from 113 to 760 (per 1000 cells). In the analysis of the CD68 cells, the absolute value ranged from 0 to 118 color reactions in the power field; the percentage of color reactions ranged from 0 to 373 per 1000 cells. Two groups were selected: one with the number of CD68 cells up to 20 (HPF); with numerous CD68 cells, more than 50 CD68 cells (HPF. Laminin stained positively in all the cases in the spinous layer of the epidermis and in a few cells in the basal layer (16 segments). Metallothionein was stained positively in 22 cases; a few microgranular reactions were obtained in the basal layer. Conclusions: 1/ There are at least 2 phenotype groups of psoriasis, with an intensive and weak lymphoid reaction of CD68 cells located subepidermally. 2/ The Ki-67 antigen can be helpful in the diagnosis of psoriasis. 3/ Our study did not confirm any significance of the antigens of basal layer damage (metallothionein, collagen IV, laminin).

MODIFICATION OF SAMPLING TECHNIQUES IN LARYNGEAL CARCINOMA AND HISTOPATHOLOGICAL EVALUATION AT THE TUMOR FRONT

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In the biology of laryngeal carcinoma, attention is paid to the role of inflammatory infiltration in the tumor front grading and the co-occurrence of epithelial dysplasia close to and at a distance from the carcinoma and pre-carcinoma states. Aim of the study: -modification of sampling techniques in laryngeal carcinomas; -antigens CD15, CD68 evaluation in the cells of inflammatory infiltration at the tumor front grading. Twenty- three postoperative specimens collected from men at the age of 43–70 years and treated for primary laryngeal cancer were studied. The patients were operated on at the Otolaryngological Ward, Silesian Medical University in Zabrze. The modification of sampling from laryngeal cancers was based on sampling from all the laryngeal levels. The procedure of immunohistochemical reactions was compatible with the manufacturer's instructions.

G-differentiation was mostly the same in all the samples of the analyzed cases. The precursors of a lesion in 11 patients were a “papilloma” and “basaloid carcinoma”. In 17 cases, a co-occurrence of dysplasia of different severity, regardless of the main tumor mass was found. Positive color reactions with CD15 antigen in the immunohistochemical studies were obtained in 87%, which included about 5% of the lymphoid cells in the tumor front grading. CD68 stained positively in 96% – about 5–10% of the lymphoid cells. Conclusions: 1/ In 1/2 cases, a “papilloma” (of a suspected viral etiology) was indicated as a precursor of the lesion; 2/ Concomitant dysplastic lesions in the epithelium outside the tumor indicate a multifocal neoplastic process in the larynx. 3/ Tumor front grading includes a small number of lymphoid cells of the CD68 phenotype. 4/ Our study did not confirm the significance of cells of the CD15 phenotype. 5/ There is a necessity of introducing into common practice the system of segmental (sectional) larynx sampling in the case of carcinoma.

IMMUNOHISTOCHEMICAL ANALYSIS OF GASTRIN EXPRESSION IN CHRONIC THYROIDITIS. A PRELIMINARY COMMUNICATION

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Chronic diseases of the thyroid are usually associated with inflammatory processes of autoimmunologic origin of other organs. The aim of the study was to mark immunohistochemically gastrin expression in thyreocytes in the course of inflammatory changes in the thyroid gland. Tissue samples were obtained from 26 women aged 21–66 years (mean age 50 years) treated surgically at the Chair of General Surgery, Silesian Medical University, Bytom. The Mizukami’s histopathological classification of chronic thyroiditis was applied and the intensity of inflammatory infiltrations was

marked according to Waterhouse and Doniach. The following groups of patients were defined: oxyphilic and mixed inflammation (Hashimoto – 14 cases), focal inflammation (5), proliferative inflammation (4) and parenchymatous goiter without inflammatory infiltrations (3). Positive gastrin staining was found in 62% of all the bioptates, which includes 86% of mixed and oxyphilic inflammations, 25% of proliferative inflammations and 20% of focal inflammations. In the biopsies of the goiter without inflammation positive staining was visible in one case. In the patients with Hashimoto's goiter the percentage of the stained thyreocytes ranged from 10 to 20%, in the another ones the intensity of the staining was fairly low and no significant differences were noticed in the subgroups. The staining intensity increased in biopsies, in which the damage to thyreocytes was found. In the group with focal inflammation the expression of gastrin protein accompanied intensive inflammatory infiltrates of nodular character. Conclusions: 1) thyroid gland shows gastrin expression of different intensity depending on the type of chronic thyroiditis; 2) in the patients operated on due to Hashimoto's goiter show gastrin expression is found more often than in other types of chronic thyroiditis; 3) further studies of gastrin expression in different types of thyroiditis are need.

IN VITRO PHOTODYNAMIC DIAGNOSIS OF ATHEROSCLEROTIC WALL CHANGES WITH THE USE OF MONO-L-ASPARTYL-CHLORIN

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The photodynamic diagnosis (PDD) and therapy (PDT), a new method evaluated for tumor treatment, is a modern approach to detecting and treating atherosclerosis. The aim was to assess *in vitro* the efficacy of PDD with the use of chlorin e6 to detect atherosclerotic plaques. Thirty specimens of human aorta and 15 of human coronary arteries were examined. The samples were soaked with chlorin e6 for 15 minutes and then washed out. The luminescence spectra were then collected. The samples were also examined in light microscopy. Tissue fluorescence was seen as green light. We observed a very strong red fluorescence of chlorin e6 originating from lipid-rich plaques in comparison to normal tissues. Conclusion: this study confirmed that chlorin e6 accumulated within atheromatous plaques. It may be a specific tool for atheromatous and normal or calcified segments discrimination.

DETERMINATION OF VEGF AND bFGF LEVELS IN THE SERUM FROM TUMOR-BEARING BALB/c MICE TREATED WITH PDT

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Photodynamic therapy (PDT) is a well-known method of tumor treatment, during which some biologically active proteins are induced. In the present study we checked whether PDT influenced the concentration of basic-Fibroblast- (bFGF) and Vascular-Endothelial-Growth Factor (VEGF). The tumor – BFS1 fibrosarcoma – was implanted into BALB/c mice, and then they were given peritoneally a photosensitizer, a phthalocyanine, BON–6 at the dose of 2.5mg/kg b.w. After 24 hours, the tumors were irradiated at 680nm and the light dose of 64J/sq.cm. Subsequently, the blood was collected from mice and in thus obtained sera cytokines were determined using the ELISA method. BON–6 was found to be effective in PDT. This feature was accompanied by low levels of both cytokines in the sera from mice after PDT, prolonged survival and – in single cases – a complete tumor regression. This was statistically significant. Conclusion: decreasing VEGF and bFGF serum levels, PDT may influence the capability of tumor tissue to form new vessels.

VASCULAR CHANGES IN ULCERATIVE COLITIS AND LEŠNIOWSKI-CROHN'S DISEASE

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Considerable attention of both clinicians as pathologists has been paid in the literature for years to the Lešniowski-Crohn's disease (LC) and ulcerative colitis (UC). Different issues are discussed. One of them is the problem of the role of vessels in both inflammatory bowel diseases. The aim of the current study was to elucidate and compare the character of vascular changes in LC and UC. The archival postoperative material from 42 patients with UC and 30 with LC was evaluated. Histological and immunohistochemical analysis was performed (reactions with antibodies against ICAM-1, VCAM-1, CD34, FVIII and UEA-1 were done). The results were then statistically analysed. There was evidence, that in both diseases studied vascular changes occur and are similar. They differ, however, quantitatively. Additionally, the presence of inflammatory infiltrates surrounding vessels in less involved or even uninvolved segments of intestines indicates the leading role of vascular changes in both inflammatory processes. Interestingly perivascular inflammatory infiltrates occurred in all layers of intestinal wall, not only in LC, but also in UC. Differences in immunostaining pattern of studied vascular markers were present. Among others in UC the higher expression of ICAM-1, CD34 and UEA-1 was found, when in LC the increase in VCAM-1 and FVIII immunostaining was observed. Conclusions: the obtained results indicate, that some histological differences (especially with regard to the status of mucosa vessels), as well as differences in the immunostaining pattern may be helpful in differential diagnosis of studied diseases especially during evaluation of the postoperative material.

Zasady procesu licencjonowania zakładów (pracowni) patomorfologii przez Polskie Towarzystwo Patologów

Dbając o właściwy poziom diagnostyki histopatologicznej, cytologicznej i autopsyjnej Zarząd Główny Polskiego Towarzystwa Patologów (ZG PTP) powołał Komisję Akredytacyjną Polskiego Towarzystwa Patologów, która dokona procesu licencjonowania zakładów patomorfologii. Licencja, w zależności od spełnienia wymaganych kryteriów, udzielana jest oddzielnie na wykonywanie:

— cytologii złuszczeniowej,
— cytologii aspiracyjnej,
— histopatologii,
— autopsji.

Proces uzyskiwania licencji ZG PTP ma charakter dobrowolny. Mogą do niego przystępować zakłady/pracownie publiczne i niepubliczne. Proces ten ma charakter niezależny od uzyskiwania certyfikatów jakości PN-EN ISO 9001, dotyczących sytemu zarządzania.

ZG PTP uważa, że wszystkie zakłady/pracownie posiadające akredytację Ministerstwa Zdrowia do prowadzenia specjalizacji w patomorfologii automatycznie uzyskują licencję PTP do wykonywania badań patomorfologicznych (histopatologia, cytologia, autopsja).

Zarząd Główny Polskiego Towarzystwa Patologów będzie rekomendował placówki licencjonowane do Narodowego Funduszu Zdrowia jako zapewniające odpowiedni standard świadczonych usług.

Proces licencjonowania będzie realizowany w dwóch etapach

Etap pierwszy (licencja tymczasowa)
Będzie miał na celu:

- udzielenie tymczasowej licencji na prowadzenie diagnostyki patomorfologicznej na okres trzech lat zakładom, które uzyskały wcześniejszą akredytację Ministra Zdrowia do prowadzenia specjalizacji w dziedzinie patomorfologii;
- udzielenie tymczasowej licencji zakładom, które indywidualnie wystąpią o jej uzyskanie pod warunkiem spełnienia podanych poniłej kryteriów i zweryfikowaniu ich przez Komisję Akredytacyjną Polskiego Towarzystwa Patologów;
- dokonanie szczegółowej ewidencji zakładów (pracowni) patomorfologicznych w kraju świadczących usługi na rzecz:
  1) własnego szpitala, którego są częścią składową;
  2) innych placówek służby zdrowia np.:
     — zakład uczelni czy szpitala świadczący usługi dla innych placówek służby zdrowia,
     — samodzielny, państwowy lub prywatny zakład patomorfologii świadczący usługi na podstawie umów cywilnoprawnych.

Zakłady, które uzyskają tymczasową licencję zostaną wpisane na **listę Zakładów licencjonowanych Polskiego Towarzystwa Patologów**, a tym samym rekomendowane do Narodowego Funduszu Zdrowia.

Etap drugi (licencja pełna na okres 5 lat)
Po spełnieniu wszystkich warunków uzyskania licencji opartych na szczegółowych danych zgodnych z założeniami europejskimi, które są przedstawione poniżej, zakład/pracownia uzyskuje licencję pełną na okres 5 lat.

Warunki uzyskania licencji

A. Licencja tymczasowa w zakresie

I. Cytologii złuszczeniowej
Zakład/pracownia występujćca o przyznanie licencji w tym zakresie musi wykonywać diagnostykę przynajmniej w zakresie dwóch dziedzin (cytologia ginekologiczna, płyny, plwocina, wymazy szczoteczkowe, mocz), przy czym liczba badań musi gwarantować pełne obciążenie roczne dla co najmniej jednego cytotechnika (skrinera). Dla cytologii ginekologicznej jest to minimum 7000 badań na jednego cytotechnika rocznie, a w pracowni powinno się wykonywać co najmniej 20 000 badań cytologicznych rocznie.
Skryning w cytologii złuszczeniowej (ginekologicznej i nieginekologicznej – płyny z jam ciała, wymazy szczoteczkowe, etc.) powinien być wykonywany przez odpowiednio wyszkolonych cytotechników pod kontrolć specjalisty patomorfologa.
Ostateczne rozpoznanie cytologiczne ustala i autoryzuje specjalista patomorfolog. W przypadku wcześniejszego skryningu wynik badania jest sygnowany również przez wstępnie oceniającego skrinera.

II. Cytologii aspiracyjnej
Zakład/pracownia występujćca o przyznanie licencji w tym zakresie musi wykonywać diagnostykę przynajmniej dwóch narządów lub układów w liczbie co najmniej 300 badań rocznie dla każdego z nich, wykonywanych przynajmniej przez okres dwóch lat kalendarzowych poprzedzających datę wystąpienia o licencję.
Ocena rozmazów z biopsji cienkoigłowej musi być wykonywana tylko przez specjalistów patomorfologów. Lekarze specjalizujący się w patomorfologii mogą wykonywać biopsję aspiracyjną cienkoigłową i dokonywać wstępnej oceny rozmazów.
Autoryzacja wyników jest identyczna jak w pkt. I.

III. Histopatologii
Opracowanie materiału makroskopowego (pobieranie wycinków) wykonuje patomorfolog lub osoba specjalizująca się w patomorfologii, opisując oceniany materiał. Autoryzacja tego postępowania musi byç podana w wyniku badania szczególnie w przypadku, gdy rozpoznanie histopatologiczne ustala inny patolog/lekarz niż ten, który ustalił ostateczne rozpoznanie. **Oceny mikroskopowej preparatów dokonuje specjalista patomorfolog, który autoryzuje** wynik. Wstępną ocenę może wykonać patomorfolog z I stopniem specjalizacji (wg starego systemu) lub osoba specjalizująca się.
Zakład/pracownia oczekująca na licencję powinna wykonywać co najmniej 3000 badań histopatologicznych rocznie.

IV. Autopsji
Oceny materiału autopsyjnego dokonuje specjalista II stopnia z zakresu patomorfologii lub lekarz z I stopniem specjalizacji (lekarz specjalizujący się) pod nadzorem lekarza specjalisty patomorfologa, po wcześniejszym zapoznaniu się z pełną dokumentacją kliniczną (nie tzw. kartą sekcyjną).

Protokół autopsyjny będący zbiorem ocen makroskopowych i mikroskopowych wraz z ustosunkowaniem się do otrzymanej dokumentacji i rozpoznania klinicznego **autoryzuje specjalista II stopnia z patomorfologii wraz z lekarzem wykonujćcym autopsję (jak wyżej).**

Kadra

Warunkiem przyznania licencji jest wykazanie, iż zakład (pracownia):

- jest kierowany przez specjalistę II stopnia z zakresu patomorfologii zatrudnionego w nim na pełnym etacie;
- posiada co najmniej dwóch techników histopatologicznych zatrudnionych w wymiarze pełnych etatów do wykonywania preparatów histologicznych;
- posiada technika sekcyjnego (laboranta) zatrudnionego w wymiarze odpowiednim do potrzeb (jeżeli wykonywane są autopsje).

Funkcjonowanie zakładu (pracowni)

Zakład musi spełniać warunki zgodne z zasadami zarządzania i kontroli jakości podanymi w opracowaniu opublikowanym w Pol. J. Pathol. 1999, supl. 2.

Wewnętrzna kontrola jakości

Wewnętrzna kontrola jakości w pracowniach zgodna z rekomendacją ekspertów Unii Europejskiej, obejmuje:

I. Cytologia ginekologiczna

1. Procedurę laboratoryjną, na którą składa się:
   a. rejestracja materiału,
   b. barwienie rozmazów metodą Papanicolaou, najlepiej w zautomatyzowanych aparatach gwarantujćcych odpowiednią jakość,
   c. nakrywanie rozmazów odpowiednio dużymi szkiełkami nakrywkowymi (o wym. 50 × 22 mm),
   d. archiwizowanie rozmazów i wyników badań.
2. Procedurę diagnostyczną, na którą składa się:
   a. skryning pierwotny wykonywany przez cytotechników;
   b. konsultacja wszystkich dodatnich i podejrzanych rozmazów przez specjalistę patomorfologa z doświadczeniem w cytodiagnostyce ginekologicznej;
   c. konsultacja przez patomorfologa rozmazów ujemnych w przypadkach gdy:
      - istnieją zmiany klinicznie podejrzane w szyjce macicy,
      - wiadomo z wywiadu, że uprzednio rozpoznano i leczono pacjentkę z powodu raka lub neoplazji śródnabłonkowej (CIN);
   d. skryning wtórny wykonywany przez starszego cytotechnika:
      - ocena 10% losowo wybranych rozmazów „ujemnych", lub
      - tzw. szybki przegląd wszystkich rozmazów „ujemnych" metodą „schodkową";
      - podwójny skryning rozmazów pochodzących od pacjentek z grup szczególnego ryzyka, np. HIV+;
   e. ponowna ocena rozmazów uprzednio badanych (archiwalnych), w przypadku gdy:
      - w badaniu cytologicznym bieżącym stwierdza się zmiany patologiczne,
      - w badaniu histopatologicznym stwierdzono zmiany patologiczne, natomiast w rozmazie ich nie stwierdzono,
      - obecność nieprawidłowych komórek w rozmazie nie została potwierdzona w badaniu histopatologicznym;
   f. korelacja rozpoznań cytologicznych z histopatologicznymi;
   g. formułowanie wyników badań metodą opisową wg systemu Bethesda.

II. Cytologia złuszczeniowa nieginekologiczna

opiera się na następujących zasadach:

1. Procedura laboratoryjna jak w cytologii ginekologicznej (z wyjątkiem tego, że w cytologii nieginekologicznej nie obowiązuje barwienie metodą Papanicolaou).
2. Skryning wykonują cytotechnicy.
3. Rozpoznanie w każdym przypadku (zarówno „dodatnim" jak i „ujemnym") ustala specjalista patomorfolog.
4. Korelacja rozpoznań cytologicznych z histopatologicznymi.

III. Cytologia aspiracyjna

opiera się na następujących zasadach:

1. Oceny dokonuje i rozpoznanie ustala wyłącznie specjalista patomorfolog.
2. Korelacja rozpoznań cytologicznych z histopatologicznymi.

IV. Histopatologia

obejmuje:

1. kontrolę adekwatności wykrawania materiału stosownie do potrzeb właściwej diagnostyki histopatologicznej;
2. kontrolę zabezpieczenia materiału operacyjnego co najmniej miesiąc od wydania wyniku badania histopatologicznego;
3. kontrolę zastosowanych metod:
   - utrwalania materiału tkankowego w zależności do potrzeb diagnostycznych,
   - jakości technicznej preparatów,
   - procedur barwienia rutynowego i technik specjalnych,
   - badań immunohistochemicznych,
   - badań np. molekularnych, cytogenetycznych i innych.

V. Autopsja

obejmuje:

- weryfikację ocen makroskopowych m.in. na podstawie ocen mikroskopowych zmian w narządach;
- udział w konferencjach kliniczno-patomorfologicznych, których celem jest dociekanie ewentualnych niezgodności między obrazem klinicznym a morfologicznym.

Niezbędne pomieszczenia

Dział cytopatologii

Pracownia cytologiczna powinna składać się z:

- laboratorium (wyposażonego w wyciąg, instalację ciepłej i zimnej wody) do barwienia rozmazów cytologicznych,
- pomieszczenia do prowadzenia diagnostyki cytologicznej,
- archiwum dokumentacji cytologicznej i preparatów cytologicznych,
- węzła sanitarnego.

Wyposażenie pracowni powinno obejmować: stanowisko do automatycznego lub ręcznego barwienia preparatów, wirówkę, cytowirówkę, mikroskop badawczy dobrej jakości umożliwiający uzyskanie powiększenia 400×, komputer z procesorem tekstu i odpowiednimi bazami danych.

W zakładach, w których lekarz patomorfolog wykonuje samodzielnie biopsje aspiracyjne cienkoigłowe, powinno być wyodrębnione pomieszczenie do wykonywania tych czynności i wyposażone zgodnie ze standardami gabinetu zabiegowego.

Dział histopatologii

Zakład (pracownia) musi spełniać minimalne warunki do pracy z materiałem biologicznym, a zatem posiadać wydzielone pomieszczenia:

- do opracowania (pobierania – wykrawania) materiału biologicznego z odpowiednim systemem wentylacyjnym; w pomieszczeniu tym mogą znajdować się również procesory do przeprowadzania wycinków, mrożak (kriostat) oraz urządzenie do zatapiania wycinków;
- do krojenia bloczków parafinowych i barwienia;
- dla lekarza/y prowadzącego/cych diagnostykę morfologiczną;
- sekretariat;
- zaplecze socjalne;
- zaplecze sanitarne.

Dział autopsyjny

W Zakładach prowadzących diagnostykę sekcyjną powinny znajdować się:

- chłodnia do przechowywania zwłok;
- sala sekcyjna;
- sala przygotowawcza i ewent. oddzielna do wydawania zwłok;
- pomieszczenie dla technika (laboranta) sekcyjnego;
- zaplecze sanitarne.

Dokumentacja (niezbędne minimum)

Zakład musi prowadzić pełną dokumentację działalności w następującym zakresie:

- księga główna zakładu (pracowni)/komputerowa baza danych;
- opracowanie (w formie pisemnej) procedur stosowanych w zakładzie/pracowni, a dotyczących postępowania z materiałami, począwszy od otrzymania przez zakład/pracownię aż do ich archiwizacji. Obejmują one:
  - — sposoby utrwalania,
  - — sposoby postępowania technicznego (procesory, stosowane odczynniki, okres wymiany itd.),
  - — sposoby krojenia (mrożarki, kriostaty, mikrotomy),
  - — sposoby barwienia standardowego i uzupełniającego,
  - — sposoby technik specjalnych, w tym badań immunohistochemicznych.

  W każdej z wymienionych procedur oczekuje się określenia algorytmu postępowania (np. w przygotowaniu utrwalacza), przypisania określonej odpowiedzialności wykonującym procedury i in.
- zbiór kopii wyników badań mikroskopowych (histopatologicznych i cytologicznych) opracowywanych wg przyjętego schematu (*vide* raport z badania histopatologicznego/cytologicznego/BACC – Pol. J. Pathol. 1999, supl. 2);
- zbiór protokołów autopsyjnych opracowanych zgodnie z przyjętymi założeniami (*vide* protokół autopsyjny Pol. J. Pathol. 1999, supl. 2).

Podana powyżej dokumentacja musi byç przechowywana co najmniej przez okres 20 lat.

Autoryzacja wyników

Wszystkie wyniki badań morfologicznych (cytologia, histopatologia, autopsja) muszą być autoryzowane przez specjalistę II stopnia z patomorfologii i dodatkowo przez osoby współuczestniczące w diagnostyce (specjaliści I stopnia z patomorfologii, lekarze w toku specjalizacji, cytotechnicy). Celowe jest wdrożenie symboli techników wykonujących badania w celu oceny m.in. ich zaangażowania w procesie diagnostyki różnego typu oraz wynikającej z tego odpowiedzialności.

Wykonywanie kopii wyników jest dopuszczalne zgodnie z przepisami dotyczącymi dokumentacji medycznej z podaniem instytucji/osoby, która zwróciła się o wykonanie kopii. W takim przypadku na egzemplarzu/kopii niezbędne jest naniesienie słowa „KOPIA". Wynik taki musi byç autoryzowany przez osobę przeprowadzającą (oceniającą) badanie, a w przypadku jej nieobecności przez kierownika zakładu. Polecane jest zachowywanie kopii nie tylko w postaci elektronicznej, ale również kopii pierwotnego wydruku, który powinien być sygnowany podobnie jak oryginał.

W zakładach/pracowniach prowadzących elektroniczną ewidencję wyników badań niezbędne jest przechowywanie skierowań do badań wraz z rękopisem/maszynopisem wyniku z autoryzacją osób/osoby wykonujących/wykonującej.

W przypadku konsultowania preparatów i wspólnego ustalania rozpoznania patomorfologicznego na wyniku niezbędna jest autoryzacja osób uczestniczćcych w konsultacji.

Wyniki konsultacji zewnętrznych powinny być dołączone do archiwizowanego wyniku badania morfologicznego i udostępniane w całości osobom zwracającym się o ponowne powtórzenie diagnostyki.

Archiwum

Zakład musi prowadzić:

- archiwum preparatów histopatologicznych (okres minimum 10 lat);
- archiwum preparatów cytologicznych (preparaty „dodatnie" i „podejrzane" przez okres przynajmniej 10 lat, a preparaty „ujemne" przez okres przynajmniej 5 lat);
- archiwum bloczków parafinowych (okres minimum 10 lat).

Wymienione zasoby archiwalne powinny być dostępne dla kontrolujćcego w czasie nie dłuższym niż 1.0 godz.

B. Licencja pełna

W procesie akredytacji przewiduje się sprawdzenie jakości diagnostyki na podstawie wyrywkowo wybranych przypadków przez zespół ekspertów Komisji Akredytacyjnej

PTP. Ocena powyższa niezależnie od oceny poziomu diagnostyki będzie miała na celu wycinkowe sprawdzenie przestrzegania procedur związanych z opracowaniem materiału morfologicznego.