

POSTER PRESENTATION

3. MODERN PATHOLOGY

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The effects of furfural upon endocrine insular B-cells of the pancreas

KEYWORDS: Furfural; Endocrine pancreas; B-cells, Insulin

INTRODUCTION

Furfural ($C_5H_4O_2$), unsaturated cyclic aldehyde, is a hepatotoxic substance (1-4), whose oxidative product, pyromucic acid ($C_4H_3O_5COOH$) (5), is responsible for the liver damage. Among others (6), the liver plays a significant role in the synthesis of various compounds (7,8). The synthesis of pancreatic peptide hormones proceeds due to the substrates from the liver, which suggests a close functional interrelationship between the two organs. It is for this reason that liver damages result in functional and structural alterations in the pancreas (9).

MATERIAL AND METHODS

White male Wister rats (150-200g b.w.) were used. The animals were treated with furfural dissolved in drinking water for two months, while progressively increasing the dose from 20 mg/kg/b.w. at the start, up to 40 mg/kg/b.w. at the end of the experiment. For the purpose of examining B-cells, the immunohistochemical PAP method (monoclonal antibodies in solution 1:1200) was used.

RESULTS

In the control group, B-cells were localized only within the insula and spread in the medial part. Their nuclei were round and small, and there were dark brownish, grain-like, dense deposits of insulin in the cytoplasm. Immunocytochemical polymorphism of B-cells of the insula was not detected. In the animals treated with furfural, the examined cells were hypogranulated, with less cellular deposits when compared with the same cells in the control group. Namely, the number of the cells increases in the exocrine pancreas, resulting in nesidioblastosis. This alteration is made evident by the increase in the cell number periductular and periductal, as well as by the presence of B-cells between epithelial cells of small and large pancreatic ducts.

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CONCLUSION

On the basis of the results obtained for the obvious hypogranulation of B-cells, a conclusion can be made that furfural significantly reduces the synthesis and deposition of insulin in these cells. Furfural induces compensatory, non-significant hyperplasia of B-cells and their nesidioblastosis, i.e., differentiation of endocrine cells in the exocrine pancreas.

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Clear cell sarcoma - A case report

KEYWORDS: Clear cell sarcoma; Neural crest; Immunohistochemistry

INTRODUCTION

Clear cell sarcoma (CCSa), or melanoma of the soft part, is a rare and uncommon tumor of the neural crest origin, which Ensinger first described in 1965. This tumor tends to affect the tendons and aponeuroses, distal parts of the extremities, mainly of young adults, but visceral origin of the tumor was described, too (1). The tumor has an indolent growth, frequently low score of microscopic prognostic parameters for malignancy, but possesses a high potential for local relapses and metastasizing in regional lymph nodes and distant organs, most frequently the lungs (1,2). Melanotic and amelanotic forms of these tumors may cause diagnostic dilemmas towards melanoma, peripheral nerve sheath tumors (PNST) and synovial sarcomas.

CASE REPORT AND METHODS

A male at the age of sixty, presented with a subcutaneous mass on the right thumb of his lower extremity, which was growing slowly for many years. The tumor was spherical and covered with intact skin. On cut sections it was multinodular, white coloured, hard in consistency, with the main diameter of 15cm. Histologically, the tumor had irregular anastomosing islands with dense fibrocollagenous tissue between them. The reticulin fibers were present around the nests of tumor cells. More cellular areas of the tumor had reticulin delineated individual cells. On serial sections, there was no connection of the tumor with the epidermis, neither were the nerve fibers found in or around the tumor. Below the tumor there was the cellular connective tissue of the tendon. There was no tumor necrosis and hemorrhage. Neoplastic cells ranged from fusiform to oval with clear or palely staining eosinophilic cytoplasm. The amount of clear cell cytoplasm was moderate with indistinct cell border. Most cells with eosinophilic cytoplasm were fusiform. While the tumor cells possessed minimal to moderate pleomorphism, its nuclei were round, vesicular, with prominent basophilic nucleoli and regular nuclear border. Occasional cells were multinuclear, with blend nuclei which formed rings. Mitoses were scarce but most of them were pathological. A varying amount of intracellular melanin pigment was demonstrable in some cells by Fontana-Manson stain-

ing technique as well as fine glycogen granules by PAS reactions. The tumor tissue was examined immunohistochemically with different monoclonal antibody. Vimentin was demonstrated in all cells with moderate reaction, while reactions with Melan A, HMB 45 and S-100 protein were strong but more focal. The tumor cells didn't possess (smooth muscle actin, cytokeratin and epithelial membrane antigen).

DISCUSSION AND CONCLUSION

It appears that CCSa is a slowly growing tumor since there is frequently a long period from the first symptoms until diagnosing, as well as from diagnosing to death. Microscopic features of CCSa have been firmly established: 1) intimate association of small solid aggregates round to fusiform in shape, pale-staining cells with the dense connective tissue of tendon and aponeurosis, 2) a fine reticular stroma surrounding these cellular aggregates, 3) relative uniformity of the cells without evidence of biphasic differentiation, 4) prominence of nucleoli, 5) scattered multinucleate giant cells, 6) low mitotic figure count, and 7) focal necrosis with or without evidence of hemorrhage appears to be a variable feature³. It is well known that S-100 protein is an excellent marker of neural crest origin tumors, and its presence in soft tissue tumors derived from Schwann cells and melanocytes has been reported. The presence of specific markers for melanoma in the tumor (Melan A and HMB 45) raise the question of a melanoma but the absence of the junction activity of tumor cells in the epidermis differentiates CCSa tumors from melanomas (4). Some melanotic types of PNST tumors don't possess nuclei like CCSa and usually have different growth characteristics in peritumor stroma. In case of amelanotic CCSa, a suggestive diagnosis may be synovial sarcoma. The finding of intracellular glycogen granules and the absence of cytokeratins in tumor cells are parameters for differentiation between these two sarcomas (2,4). The ability of CCSa to produce melanin and the fact it possesses the antigenic profile of melanocytes and Schwann cells point to its origin from neural crest derived cells. Diverse neoplasms may share a common microscopic presentation with clear cell morphology and melanin productions. Careful microscopic examination with specific groups of histochemical and immunohistochemical methods and correlation with clinical symptoms may resolve these problems.

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Correlation of histopathologic and genetic prognostic factors in neuroblastoma

KEYWORDS: Neuroblastoma; Genetic factors; Prognosis

INTRODUCTION

Neuroblastomas are the most common extracranial solid tumors of childhood with diverse behavior ranging from a rapid malignant progression to a spontaneous regression. As a result, both prognosis and response to therapy can vary widely. In view of selection appropriate therapeutic groups, several great studies have made extensive efforts to collect the data about all patients and with clinical criteria to define the significance of biologic, especially morphologic and genetic features of tumors. Shimada et al. introduced a classification system based on the amount of schwannian stroma, degree of differentiation, mitotic-karyorrhectic index (MKI) and age at diagnosis which divides tumors into favorable and unfavorable histopathologic groups (survival rate 87% vs. 7%) (1). The System of Modified Risk Groups introduced by Joshi et al. including presence of calcification also divides tumors into prognostic favorable and unfavorable histopathologic groups (survival rate 85% vs. 40%) (2). Many genetic abnormalities characterize the cells of NB and several of them are indicators of poor prognosis: diploidy or tetraploidy, deletion of 1p, MYCN amplification and 17q gain (3). At the beginning of the more extensive study of neuroblastoma in the Institute, the aim of this analysis is to identify the correlation between histopathologic prognostic criteria and the most important genetic predictors without analyzing the association of these tumor characteristics with clinical features, therapy and survival rate.

MATERIAL AND METHODS

Analyzed tumors are NB from the patients that were treated in the Institute from 1 June 1996 to 31 May 2002. In this period 50 primary tumors (NB 37, ganglioneuroblastoma (GNB) 7, ganglioneuroma 6; localized in the abdomen 36, in the other sites 14; age of diagnosis <1 year 7, >1 year 43; male 32,

female 18) as well as 15 relapsed or residual tumors were operated or biopsied. The histopathologic diagnosis was established based on the precise criteria (1,2,4) after standard histopathologic procedure of tumor samples. NB and GNB were divided into favorable and unfavorable histopathology according to two classification systems (Shimada and Yoshi) and 31 tumors (22 primary and 9 relapsed) underwent genetic analyses using a fluorescence-in-situ hybridization; the samples for genetic analyses were imprints of tumor tissue and paraffin embedded tumor samples in fewer cases. The following genetic abnormalities were determined: deletion of 1p, MYCN amplification and 17q gain. The analyses of all three genetic abnormalities were made at 9 tumors, two genetic abnormalities (1p deletion, MYCN amplification) at 12 tumors, only MYCN amplification at 3 tumors. The genetic analyses were not possible at 7 tumors due to technical reasons. The cytogenetic and part of molecular genetic analyses were made at the Institute and the part thanks to kindness of Prof. P. Ambros from St. Anna Kinderspital, Vienna, Austria. Statistical analysis of correlations between all histopathologic and genetic prognostic factors at 31 tumor samples were made using the Pearson's test of linear correlation with significance at the 0.05 level. Presence of unfavorable histopathology and genetic abnormalities was marked with 1, on the other hand, presence of favorable histopathology and lack of genetic abnormalities was marked with 0.

RESULTS

The genetic finding of 1p deletion and MYCN amplification correlate with high statistical significance ($R=0,603$, $p<0,01$). Statistically significant correlation ($p<0,05$) exists between prognostic groups in Shimada's classification and finding of deletion of 1p ($R=-0,524$) and MYCN amplification ($R=-0,436$) respectively, and also between Yoshi's histopathologic groups and MYCN amplification ($R=-0,529$). Statistically significant correlation has been revealed neither in comparison between findings of two histopathologic prognostic classifications nor between each of them and 17q gain.

DISCUSSION

The comparative analysis of histopathologic and genetic prognostic factors in our cases of NB and GNB shows highly significant association of 1p deletion and NMYC amplification which is well known fact (3,5). Despite the fact that 17q gain is the most important single unfavourable prognostic factor often associated with previous two factors (3,6) we have not found this correlation. This could be consequence of having relatively small number of cases in which all three genetic analyses were applied. At first sight, the surprising result of our analysis presents significant correlations between unfavourable Shimada histological type with 1p deletion and MYCN amplification respectively, and also between unfavourable Joshi histological type with MYCN amplification, all three of them with negative direction. Correlation of histological and genetic prognostic factors in NB has been analysed in few studies in which positive correlation between unfavourable histology and 1p deletion (7) and MYCN amplification (8) was found. However, the regression analysis in recent comprehensive study of NB in 108 children showed that histopathologic risk systems by Shimada and Yoshi did not yield results of prognostic significance for survival (3).

CONCLUSION

This study showed that only classification according to new INPC had independent prognostic significance. In this classification criteria for assessment of both tumor differentiation and MKI depending on the cell population density were defined (9). This conclusion suggests that we should reclassify cases of NB according to INPC for more precise analyses in future.

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The prognostic significance of tumor-induced angiogenesis in colorectal cancer

KEYWORDS: Angiogenesis; Colorectal neoplasms, Immunohistochemistry

INTRODUCTION

The colorectal cancer is a leading malignant tumor of the gastrointestinal tract, and second major cause of cancer-associated mortality. Angiogenesis (neovascularisation) is the formation of new vessels from the preexisting vasculature network. Angiogenesis is a complex process involving multiple steps and pathways dependent on the local balance between positive and negative regulatory factors (1). Tumor-induced angiogenesis is a central pathogenic step in the process of tumor growth, invasion and metastasis. The tumor growth can be divided into prevascular (avascular) and vascular phase. In the prevascular phase tumor is very small (1-2 mm) and principally never gives metastasis. Transition from prevascular to vascular phase is followed by rapid tumor growth and appearance of metastasis. Beyond its effects on tumor expansion, perhaps the most important way in which angiogenesis can facilitate tumor metastasis is by providing an efficient route of exit for tumor cells to leave the primary site and enter the blood stream. The obvious correlation between angiogenesis and tumor metastasis first was showed by Weidner who found a direct correlation between the microvascular density (MVD) and likelihood of metastasis in human breast cancer patients. The quantification of intratumoral MVD in histological specimens has been found to be of prognostic value in several cancers, such as in colorectal carcinoma (2). The differences in the survival rate of patients with the same stage of the colorectal cancer disease induced a search for new diagnostic methods with prognostic relevance. Advances in diagnosis and treatment and modern aspects of tumor biology introduced other prognostic factors, such as tumor-induced angiogenesis, with prognostic significance. The aim of this study was to analyse the quantitative expression of angiogenesis in colorectal carcinoma and determine how angiogenesis correlates with other clinicopathologic factors and prognosis. We hypothesized that the intratumoral vascular density in colorectal cancer correlated with other histopathological prognostic factors and the patients with a hypervascular tumor showed a higher metastatic potential and worse prognosis.

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PATIENTS AND METHODS

This study included 40 patients who underwent a curative resection of the colorectal cancer at the Department of Surgery in the General Hospital Senta in the period from 1990-1995, with a complete follow-up of 5 years or until death. Among 40 patients, 20 were without and 20 were with regional lymphonodal metastasis at the time of operation. The identification of microvessels was performed immunohistochemically, using the monoclonal antibodies CD31 (DAKO) against endothelial proteins. The microvessel count was assessed by means of stereology with the ocular test grid M42, as the vascular surface density in the volume portion of the stroma at the invasive front of the colorectal cancer. The statistical analyses were performed using the computer program MedCalc for biomedical investigations. A value of $p < 0,05$ was considered statistically significant.

RESULTS

The microvessel count ranged from 4.77 to 19.64, with average 11.47 ± 3.71 (standard deviation), median 11.16. The patients were divided at the mean value (11.47) of the microvessel count into two groups, the patients with hypovascular (< 11.47) and hypervascular (> 11.47) tumors. In the Dukes B stadium of the colorectal cancer disease, the mean of MVD was 10.16 ± 3.13 , median 9.55. In the Dukes C stadium of the colorectal cancer disease, the mean value of MVD was 12.77 ± 3.86 , median 11.75. There were statistically significant differences in the MVD between Dukes stage B and C ($t = 2.345$, $p = 0.024$). A significant difference existed with respect to the histologic grade, with a trend of MVD to become significantly higher as the histologic differentiation became poorer ($t = 3.902$, $p = 0.0028$), as in the mucinous tumor ($t = 3.902$, $p = 0.0018$). In the subgroup of patients without recurrence of the disease ($n = 22$), the average MVD was 9.01 ± 2.23 , median 8.93. In the patients with recurrence of the disease ($n = 18$), the mean MVD was 14.20 ± 3.24 , median 14.80. The difference of MVD in patients with and without recurrence of the disease was statistically significant ($t = 5.989$, $p < 0.0001$). In the patients with hypovascular tumor ($n = 23$), the mean survival was 53.78 ± 12.82 months. Seventeen (73.91%) outlived the 5-year follow up. The mean survival in the patients with hypervascular tumor was 32.82 ± 19.72 months. Five (29.41%) patients outlived the 5-year follow up. The difference between the survival rate of the patients with hypovascular and hypervascular tumor was statistically significant ($t = 4.072$, $p = 0.0002$).

DISCUSSION

The count of tumor-induced angiogenesis of colorectal carcinomas correlated with other classic histopathologic prognostic parameters such as a stage of the disease and histological grade of the tumor. There were no statistically significant associations between MVD and sex and age of patients and localization of the tumor. Patients with recurrence of the disease were with statistically significantly higher MVD. The five-year survival rate in patients with hypervascular colorectal tumor was statistically significantly lower than in patients with hypovascular tumor. The microvessel density in colorectal cancer was an independent prognostic factor with significance behind the stage of the disease and histological grade of the tumor. The quantification of MVD in histological specimens of colorectal carcinoma truly reflects biological malignant potential of colorectal carcinomas (2).

CONCLUSIONS

The assessment of MVD in colorectal carcinomas may be a useful surrogate predictive marker in cases for which pathological specimens are not available, such as in locally excised rectal cancers. Another application of MVD might be to supplement pathological stage when the role of adjuvant therapy is in question, such as for Astler-Coller B2 lesion. The MVD in tumor specimens would be valuable in identifying patients at high risk for recurrence

and, consequently, in stratifying patients for planning appropriate adjuvant and antiangiogenic therapy after surgery.

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Cardioprotective efficiency of amifostine in rats treated with doxorubicin: A morphometric analysis

KEYWORDS: Heart; Pathology; Morphometry

INTRODUCTION

Therapeutic use of the antineoplastic drug doxorubicin is mainly limited due to its pronounced cardiotoxicity (1). Single administration of the high doses of the drug causes intracellular oedema, nuclei piknosis and mitochondrial oedema in the myocytes. Apoptosis of the cardiomyocytes damage is successfully reduced with preventive administration of compounds with antioxidative action (2). Cytoprotector amifostine (WR-2721) offers protection against toxic effects of both ionizing radiation and many radiomimetic anti-neoplastic drugs. Recently, it has been shown that amifostine may ameliorate the adverse effects of a number of cytotoxic drugs with various of mechanisms of antitumor action (3). However, scarce data exist about its protective efficiency against toxic effects of doxorubicin. Accordingly, the aim of this study was to perform the morphometric evaluation of cardiomyocytes damage in rats treated with doxorubicin, alone or along with amifostine.

MATERIALS AND METHODS

Male Wistar rats 6-8 weeks old, weighing 200-250 g, were used in the experiment. Twenty-five animals were randomly allocated to five groups, each of them consisting of 5 animals. Their treatments were: (1) saline (the control), (2) doxorubicin, 6 mg/kg *iv*, (3) doxorubicin, 10 mg/kg *iv*, (4) amifostine 300 mg/kg *ip*, 20 minutes before doxorubicin, 10 mg/kg *iv* (5) amifostine 300 mg/kg *ip*. Animals were sacrificed 48h after treatment with doxorubicin and/or amifostine. The hearts of all rats were fixed in 10% neutral formalin. After the process of fixation the tissue samples were dehydrated in graded alcohol, xylol and paraffin wax. Finally, 5 µm thick paraffin sections were stained by hematoxylin and eosin (HE). Morphometric analysis was performed using digitized microscopic slides of the rat hearts with "image-analysis" program Mikro v1.1. produced in the Laboratory IMP-Belgrade. ANOVA and Student's t-test were used for statistical analysis of the results.

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RESULTS

The hearts of intact rats had normal histological structure. In the group of rats sacrificed 48h after single administration of amifostine pathohistological alterations were not found, neither was the number of the damaged myocytes significantly increased in comparison with the control group. In the hearts of rats treated with doxorubicin 6 or 10 mg/kg *ip*, normal myocardial fibres were seen. In minority of the myocardial cells a granular cytoplasm and small vacuoles, without a nuclei, were present. Careful morphometric analysis revealed a significant, dose dependent, increase in number of the damaged myocytes in comparison with the control group. Pretreatment with amifostine in doxorubicin-treated rats (10 mg/kg), significantly ameliorated the cardiotoxic effects of doxorubicin reducing the number of altered myocytes on the level achieved with the dose of 6 mg/kg. Amifostine itself did not produce any changes in the structure of the rat hearts.

CONCLUSIONS

Our results suggest that cytoprotector amifostine (WR-2721) produces good protective effect against acute cardiotoxicity of doxorubicin in rats.

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Quantitative nuclear image analysis of lung carcinoma

KEYWORDS: Image analysis; Squamous cell lung carcinoma; Small cell lung carcinoma

INTRODUCTION

Carcinomas of the lung are classified histologically into four major types: small cell carcinomas, squamous cell carcinomas, adenocarcinomas and large cell carcinomas. However, the histological phenotype is the result of multiple differentiation potentialities of individual tumor cells and, as a result, different cell types can occur in the same tumor. Therefore, it is not surprising that a slight interobserver variation is present in diagnoses made by different pathologists. In difficult cases additional classification methods may be helpful, particularly karyometry (1). The aim of this study was image analysis of nuclear variables in squamous cell and small cell carcinoma of the lung.

MATERIAL AND METHODS

At the Institute of Pathology, University of Niš, formalin-fixed, paraffin-embedded bronchoscopic mucosal samples from 48 patients with squamous cell carcinoma and 41 patients with small cell carcinoma of the lung were retrieved from pulmonary pathology archives. Serial histologic sections of 4 μm thickness were prepared for staining with hematoxylin and eosin and analyzed by image analyzer Lucia M 3.51 ab (Nikon, Tokyo, Japan), using objective x40 (NA=0.65). The binary images were manually edited. Seven nuclear variables were estimated: nuclear area, equivalent diameter ($\sqrt{(4 \cdot \text{area} / \pi)}$), volume of equivalent sphere ($\pi E_q \text{ diameter}^3 / 6$), perimeter [$\pi (Pr_0 + Pr_{45} + Pr_{90} + Pr_{135}) / 4$], mean chord [$4 \text{ area} / Pr_0 + Pr_{45} + Pr_{90} + Pr_{135}$], circularity ($4\pi \text{ area} / \text{perimeter}^2$) and integrated optical density [$-\log(\text{pixel gray value} + 0.5) / 62.5$]. In each case a hundred nuclei were measured. Statistical significance was obtained by Student's t test.

RESULTS

The values of the nuclear variables which were assessed (mean \pm standard deviation) are listed in Table 1.

All measured seven nuclear variables were found to be significantly different between squamous cell carcinoma and small cell carcinoma of the lung ($p < 0.001$). The values of nuclear variables (except circularity) of squamous cell carcinoma of the lung were significantly larger than in small cell carcinoma.

Table 1. Nuclear variables in squamous cell carcinoma and small cell carcinoma of the lung

Nuclear variable (mean \pm SD)	squamous cell carcinoma	small cell carcinoma
Nuclear area (μm^2)	35.17 \pm 9.54	20.12 \pm 4.59
Equivalent diameter (μm)	6.53 \pm 0.85	4.98 \pm 0.56
Volume of Eq. sphere (μm^3)	169.61 \pm 73.51	71.54 \pm 24.99
Perimeter (μm)	21.88 \pm 2.74	16.32 \pm 1.83
Mean chord (μm)	4.82 \pm 0.66	3.74 \pm 0.44
Circularity	0.88 \pm 0.03	0.92 \pm 0.03
Integrated optical density	325.02 \pm 103.67	250.03 \pm 52.07

DISCUSSION

Histologic classification of lung cancer is subjective and often difficult to reproduce. The distinction between small cell carcinoma and squamous cell carcinoma of the lung on routine bronchoscopic biopsies sometimes presents diagnostic problems, particularly when keratinization and intercellular bridges are not evident (2,3). For this reason, more quantitative approaches to the classification of these tumors would be desirable. Image analysis permits pathologists to obtain quantitative measurements on histologic preparations, so that visual impressions can be augmented by quantitative morphometry (4). By quantitative nuclear image analysis, the morphology of the nucleus is described by a number of mathematical parameters (5). In order to obtain additional differentiating criteria for these two histological types of lung carcinoma, in the present study, we estimated seven quantitative nuclear image features. We identified significant differences with regard to nuclear size, shape and optical density. The values of all measured nuclear variables (except circularity) of squamous cell carcinoma were significantly larger than in small cell carcinoma of the lung. Our findings are in agreement with other studies (6,7,1.) Quantitative nuclear image analysis can be used to make distinction between small cell carcinoma and squamous cell carcinoma of the lung in the biopsy specimens.

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Karyometric analysis of fetal heart

KEYWORDS: Fetal heart; Myocytes; Karyometry

INTRODUCTION

During embryonic development the heart is required to grow in size and cell number, undergo complex morphologic alterations, and function to circulate the blood. Accelerated cardiomyocyte differentiation is accompanied by a significant increase in trabeculation of ventricular myocardium (1). In the myocardium, myocyte cell division is irreversibly blocked shortly after birth (2). The signal that initiates cell cycle withdrawal is unknown. Cyclin dependent kinase inhibitors are powerful inhibitors of the cell cycle and may play a direct role both in myocyte development and in preventing cell division in the adult. The expression of p21 showed a gradual increase during the heart development in both the rat and man, becoming maximal in adulthood (3). On the other hand, gene activation is often preceded or accompanied by a perturbation of the chromatin structure (4). The aim of this study was to estimate the nuclear size and shape of cardiac myocytes during fetal development.

MATERIAL AND METHODS

At the Institute of Pathology, University of Niš, the autopsy material from January 1988 to December 2001 was analyzed including 1356 standard pediatric autopsies performed. Karyometric analysis was done on cardiac myocytes from 35 babies, divided in 7 equal groups, at 13, 16, 20, 25, 30, 35, and 40 weeks' gestation, on routinely stained (HE) histological sections, using an image analyzer Microlmage 3 (Olympus, Tokyo, Japan). Five nuclear variables were estimated: area, optical density, minor axis, major axis, diameter, and perimeter. The area is determined by calculating the number of pixels encompassed by the shape. The standard optical density determines the amount of matter in a material by measuring the amount of light it transmits (lets pass through it), according to formula:

$$\text{OPTICAL DENSITY}(x,y) = -\log[(\text{INTENSITY}(x,y) - \text{BLACK}) / (\text{INCIDENT} - \text{BLACK})]$$

where: INTENSITY(x,y) is the intensity at pixel (x,y), BLACK is the intensity generated when no light goes through the material, and INCIDENT is the intensity of the incident light. The minor axis reports the length of the minor axis of the ellipse equivalent to the object (i.e., an ellipse with the same area,

first and second degree moments). The major axis reports the length of the main axis of the ellipse equivalent to the object (i.e., an ellipse with the same area, first and second degree moments). The diameter reports the average length of diameters measured at 5° intervals around the centroid of each object. The perimeter reports the length of the outline of each object.

RESULTS

The largest nuclear area was found at 20 weeks' gestation ($35.49 \pm 3.13 \mu^2$). The smallest nuclear area was found at 40 weeks' gestation ($18.58 \pm 4.65 \mu^2$). The highest value of the average nuclear density was found at 28 weeks' gestation (0.43 ± 0.05), and the smallest value was found at 13 weeks' gestation (0.15 ± 0.02).

DISCUSSION AND CONCLUSION

The heart development depends on a dynamic interaction between genetic and epigenetic factors and apoptosis is a method of remodeling the second-trimester fetal heart development (5). Myocyte cell division is irreversibly blocked shortly after birth (2). According to our results, the smallest nuclear size is found at the end of pregnancy. Gene activation is often preceded or accompanied by a perturbation of the chromatin structure (4). According to our results, the highest average nuclear density was found at 28 weeks' gestation.

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Immunohistochemical study of gastrin, p53 protein and PCNA in advanced gastric carcinomas

KEYWORDS: Gastric Neoplasms; p53 protein; Immunohistochemistry

INTRODUCTION

The basic role of gastrin is stimulation of the gastric acid secretion. It is less known that this peptide hormone also exerts growth-promoting effects on normal and malignant transformed gastrointestinal mucosa (1). Recent immunohistochemical studies examined a range of gastric tissue changes, from metaplastic and dysplastic changes, to the intestinal type of the gastric carcinoma. These studies showed that both gastrin and its receptors were activated in all cases with increasing expression from benign to malignant lesions (2). Wild type p53 tumor suppressor gene temporarily stops the cell cycle and allows the reparation of damaged DNA. A lack of possibility for reparation leads the cell (under control of p53) to apoptosis. Because of a very short half-life, the protein product of p53 gene is not detectable by immunohistochemical methods. The accumulation in the nuclei of tumor cells that is immunohistochemically detectable, is the result of production of p53 protein with a longer half life, that became transformed by mutation of the p53 gene (3). PCNA (proliferating cell nuclear antigen) is involved in the cell cycle and represents a marker of cell proliferation. Wild type p53 inhibits PCNA in a non transformed cell, while the tumor tissue shows an increased expression of PCNA. The aim of this study was to determine gastrin, p53 protein and PCNA presence in advanced gastric carcinomas and correlation with involvement of the regional lymph nodes, type and grade of tumor.

MATERIAL AND METHODS

The retrospective analysis included 35 unselected patients with gastric carcinoma who had undergone partial or radical gastrectomy. By Lauren, carcinomas have been classified into the intestinal $n=21$ (60%), and diffuse type $n=14$ (40%). The patients were divided in two groups according to the presence of metastatic regional lymph nodes: the group without involved lymph

nodes pN0; $n=10$, and the group with verified metastatic regional lymph nodes pN1, N2; $n=25$. According to the degree of histological differentiation (HG), the tumors have been divided in three groups: well differentiated G1 ($n=7$), moderately differentiated G2 ($n=11$), and poorly differentiated G3 ($n=17$). Samples of the tumor tissue 4 micrometer in thickness, fixed in formalin, paraffin embedded, were used to determinate immunohistochemical reactivity of gastrin, p53 protein and PCNA. LSAB+ method was used for detection of the p53 protein and PCNA, and Envision for gastrin identification. To perform this we needed to use the monoclonal antibodies in optimal concentrations (DAKO, Denmark), and AEC+ Substrate-Chromogen. According to the percentage of nuclei that were positive for p53 and PCNA, the tumors were classified in two groups: tumors that were negative - if less than 25% of the nuclei had a positive reaction, and tumors that were positive- if more than 25% of the nuclei had a positive reaction. Statistical analysis was performed using the X^2 and Fisher tests.

RESULTS

We found a positive reaction in 71.4% of gastric carcinomas. There was a difference in the intensity of immunohistochemical reaction in tumor cells cytoplasm. We did not find a statistically significant difference between the intestinal (57.1%) and diffuse (50%) type of carcinoma regarding the presence of gastrin in tumor cells. The intestinal type showed a higher frequency of p53 protein nuclear reactivity than the diffuse tumor type. This difference was statistically significant, $p<0.05$. The presence of p53 protein has been detected in all poorly differentiated tumors and only in 29% as well differentiated. PCNA was found in the nuclei of all diffuse carcinomas (100%), and in 85.7% of intestinal type. That difference was not statistically significant. According to the level of histological differentiation, there was no statistically significant difference in the frequency of gastrin, p53 protein and PCNA expression.

DISCUSSION

Our study confirms the immunohistochemical expression of the peptide hormone gastrin in gastric carcinomas, in both the intestinal and the diffuse type. On the other hand, no statistically significant difference was established regarding the frequency of gastrin presence in these two histologic types. According to the histological type of the tumor, the frequency of p53 presence is statistically significant. Our results are compatible with previous studies, which pointed to a higher frequency of this protein in gastric adenocarcinomas of intestinal type. It has been considered that the presence of p53 protein in tumor cells is in correlation with pure prognosis (3). In most advanced gastric carcinomas PCNA was present in a high percentage which points to a high level of the tumor cell proliferative activity, regardless the histological type of the tumor.

CONCLUSION

High frequency and poor prognosis of the gastric carcinoma require that more effective and precise biomarkers are introduced in the diagnostic, prognostic and even therapeutic evaluation of this disease. We showed that the p53 protein could be an important prognostic parameter in the intestinal type of gastric adenocarcinoma.

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Significance of pathohistologic classification of precancerous lesions of vocal cord mucosal

KEYWORDS: Classification; Precancerous lesions; Immunohistochemistry

INTRODUCTION

Until nowadays, microlaryngoscopy with light-microscopy examination of the biopsy material, is still the only reliable method for diagnosing laryngeal cancer. The ground of each classification is categorizing epithelial lesions in accordance with their malignant potential. Considering different histomorphological parameters to be relevant for malignant alteration of precancerous lesions, until nowadays more than 20 different classifications have emerged. Among them, we find the Ljubljana classification (LjC) as a useful and acceptable one. Three general principles of that classifications are: epithelial thickening, preserved basement membrane and no importance of surface keratin layer (1). Considering epithelial hyperplasia, delayed maturation, degree of nuclear and cellular atypia, mitotic activity, number of dyskeratotic cells and infiltration of immunocompetent cells in the subepithelial stroma; this classification differs simple hyperplasia and abnormal hyperplasia as benign ones with 0.9% chances for malignant transformation on one hand, and atypical hyperplasia with 11% chance for developing invasive cancer on the other. The LjC considers carcinoma in situ as a separate entity and treats it as an intraepithelial carcinoma.

MATERIALS AND METHODS

Our study includes 35 patients with clinically suspected precancerous lesions of the vocal fold mucosa. All patients were submitted to biopsy sampling with standard histologic procedures to establish the histopathologic diagnosis. All biopsies were reexamined applying the LjC. It excluded six biopsies with unclear degree of dysplasia, i.e. malignant alteration and a huge cell infiltrate. These biopsies were histochemically (PAS, trichrome Mason) and immunohistochemically (CD 3, CD4, CD 8) treated in order to analyze the epithelium and cell infiltrate.

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RESULTS

Examining 35 biopsies and introducing the LjC, 10 simplex hyperplasia, 8 abnormal, 9 atypical hyperplasias and 8 carcinomas in situ were found. Although the biggest problem was to distinguish abnormal and atypical hyperplasia, three biopsies from each group were taken for histochemical and immunohistochemical analysis. Analyzing them, we noted that it is easier to recognize the changed epithelial cells which are most frequently in the cell infiltrate, that were analyzed separately.

DISCUSSION

Therapeutic procedure depends on pathologic diagnosis and that's why it is important to make the pathological diagnosis correctly, in order to avoid therapeutic procedures which are either too conservative or too destructive, both equally harmful for the patient. For that reason, the prime concern of each classification is to make a clear distinction between the lesions, which are at no risk for malignant alteration, and the lesions which carry a substantial risk for it. Comparing the dysplasia grading method with the LjC, Michaels re-evaluated some lesions which had been designated to lower degrees of dysplasia into 'risky' hyperplasia according to the LjC (2). Also, the LjC unlike other classifications makes sharp distinction between atypical hyperplasia as a form of the most pronounced dysplasia from carcinoma in situ, which represents an intraepithelial neoplasia. Using the LjC these two entities are exactly separated and this is in accordance with the clinical data which suggest a more radical treatment for carcinoma in situ - surgery or radiation and a conservative treatment for atypical hyperplasia. Comparing the lesions which progressed to a carcinoma and those which remained stable or resolved, Blackwell found five histomorphologic parameters to be significantly different: abnormal mitotic figures, mitotic activity, stromal inflammation, maturation level, and nuclear pleomorphism (3). Surface morphology, nucleolar prominence, and koilocytosis were not significantly different when comparing the two groups. Hellquist found the Ljubljana system to be more precise for routine diagnostic work than the others (4). Also, he showed that the LjC significantly corresponded with the results obtained by quantitative morphometry and immunohistochemistry. The LjC allows the prediction of conditions at risk for neoplastic progression and enables a clinician to choose the treatment modality that promises the best results. Considering simple and abnormal hyperplasia benign, we do nothing more than excisional biopsy. Atypical hyperplasia is considered 'risky' and beyond stripping of the vocal cord mucosa it requires close follow-up and repeated histologic assessment. Carcinoma in situ represents an actual malignant state, and requires surgical treatment or radiotherapy. Histochemistry and immunohistochemistry are very helpful supplementary techniques, especially in marginal cases, when it is difficult to make a clear distinction just on the ground of light microscopy. Still, none of them can replace histopathologic evaluation, but they are useful adjunctive methods in marginal and difficult cases, especially when laryngeal dysplasia is joined with infection or marked cellular immune reaction.

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Planimetric-karyometrical analysis of irritated seborrheic keratosis and squamous cell carcinoma grade 1

KEYWORDS: Irritated seborrheic keratosis; Squamous cell carcinoma; Karyometry

INTRODUCTION

Numerous squamous cells, which overwhelm in number the basaloid cells, and the presence of many squamous whirls - "squamous eddies", which resemble the poorly differentiated "horn pearls" of squamous cell carcinoma (SCC), characterize irritated seborrheic keratosis (ISK). These "squamous eddies" are different from the horn pearls of SCC, because they are smaller, more numerous, and more circumscribed. Besides this, ISK can show regions of inverted proliferation, passing horizontal demarcation, which is present at "non-active" seborrheic keratoses, although mitotic figures are rare, and the base of this lesion is sharp toward dermis (2). SCC is true, invasive cancer of epidermis. It is the most common malignant lesion in Caucasians, and represents 20% of all malignancies of the skin. Often, it appears on actinically damaged skin, primary or on terrain of solar keratoses, and its development on normally appearing skin is rare. It could develop on burn scars and stasis ulcers, as well as in immunosuppressed patients, where it shows more aggressive course (1-3). This cancer is composed of irregular masses of epidermal cells, which proliferate toward dermis. Invasive tumor masses are built up, in different percentages, of more differentiated and atypical squamous cells (1). Well differentiated squamous cell carcinoma - grade 1 (SCCG1) is characterized by bulbous cords and nests of squamous cells. Atypia is relatively limited in one or two peripheral layers of each nest. More centrally placed cells have uniform characteristics, enlarged nucleoli and moderate eosinophilic cytoplasm (4). These cells show progressive keratinization toward the center of the nest, which gives a pattern of horn pearls in SCCG1 (1,4). ISK due to its inverted growth, and "squamous eddies" can be a differential-diagnostic problem toward early invasive forms of SCC (2). It seems that this problem can be solved in the histopathology by a sufficient number of morphologic criteria, supported by pathologists' experience. The criteria for differentiation have never included morphometrical analysis of the nuclei in these lesions, and the available literature lacks the comparative data on karyometrical prop-

erties of the lesions. Concerning the existing differential-diagnostic problem between ISK and SCCG1, the aim of this work is to compare ISK and SCCG1 on the basis of their karyometric properties.

MATERIAL AND METHODS

Groups of ISK and SCCG1 had 14 lesions each (equal number of both sexes) obtained from 28 patients, aged 35-90 years. Both groups underwent routine histological processing and were stained by hematoxylin-eosin. Computerized analysis of digitized microscopic pictures was performed on an Olympus Micro Image v.3.0.1. Using the method of interactive separation, 100 nuclei were chosen from each lesion. In karyometric analysis the following parameters were taken: cross-section area (AR), average diameter (AD), perimeter (PER), circularity (CY), and optical density (OD) of nuclei. The data were calculated with descriptive statistics, and Student's t-test.

RESULTS

There is a statistically significant difference between ISK and SCCG1 for all quantified parameters except for optical density ($p > 0.05$). The ISK nuclei were smaller than the nuclei of SCCG1, according to the values of dimensional parameters (ISK: $AR = 34.4 \pm 7.5$, $OD = 0.3 \pm 0.03$, $AD = 6.4 \pm 0.7$, $PER = 22.6 \pm 2.3$; SCCG1: $AR = 50.0 \pm 7.8$, $OD = 0.2 \pm 0.07$, $AD = 7.7 \pm 0.7$, $PER = 27.6 \pm 2.3$) ($p < 0.001$), and had more regular contour (ISK: $CY = 1.2 \pm 0.05$; SCCG1: $CY = 1.3 \pm 0.04$) ($p < 0.01$).

DISCUSSION AND CONCLUSIONS

It can be very difficult to differentiate the irritated seborrheic keratosis from squamous cell carcinoma. From the results obtained, an evident difference has been noticed between the ISK and SCCG1 groups in regard to all quantified nuclear characteristics except optical density. ISK nuclei are smaller and show regular contours as opposed to the SCCG1 nuclei, which is confirmed by statistically significant difference between the values of nuclear dimensions and circularity.

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Detection of occult colorectal cancer micrometastases using immunohistochemistry

KEYWORDS: Micrometastases; Colorectal Neoplasms; Immunohistochemistry

INTRODUCTION

The prognosis of patients with colorectal cancer is closely linked to the stage of the disease at the time of diagnosis. Those without lymph node metastases (stage II disease) at diagnosis have 5 year survivals of 70-80%. Currently, it is difficult to identify those 20-30% of patients who are likely to have recurrence of the disease. For stage III patients with an involvement of regional lymph nodes, 5-year survival decreases to 30-50%, but a significant improvement in survival can be achieved with adjuvant therapy with 5-fluorouracil and leucovorin. Survival decreases to less than 5% for patients with distant metastasis at diagnosis. Macroscopic metastases are not, however, always evident during primary resection. Liver metastases are clinically evident at initial presentation in only 10-25% of patients, yet 60-70% of those who die of colorectal cancer develop distant metastasis. This is caused by micrometastatic disease at local and distant sites that is not detected at "curative" resection of the primary tumor. The ability to detect such micrometastases may help to better determine the prognosis and identify the subgroup of patients with a higher-risk disease that would benefit from adjuvant chemotherapy (1,3,5). Standard testing of colorectal cancer specimens for lymph node metastasis involves examining a single H&E-stained section through each lymph node for the presence of malignant appearing cells. The method of routine pathological examination for lymph node metastases may vary between different institutions, and efforts have been made to standardize the number of nodes resected and analyzed to improve accuracy. The accuracy of finding malignant cells in lymph nodes can be improved by examining multiple layers through each node and by using modern techniques as: immunohistochemistry, highly-sensitive molecular biological techniques and RT-PCR techniques (1,3-5). This study was undertaken to identify the subgroup of stage II patients with micrometastatic disease that would benefit from adjuvant chemotherapy.

MATERIALS AND METHODS

We examined 147 lymph nodes from 18 patients with colorectal cancer

who had no evidence of lymph node metastases on routine H&E staining (TNM stage II disease).

The paraffin embedded lymph nodes were sectioned on five micron serial sections. one section was stained with HE for detailed histological analysis. Adjacent sections were stained with anti-cytokeratin antibody (CK18), epithelial specific antigen (ESA) and carcinoembryonic antigen (CEA). Additional sections were used as negative control. immunohistochemistry (IHC) was performed using the avidin-biotin immunoperoxidase complex technique of Hsu et al.

RESULTS

A total of 147 lymph nodes from 18 patients were examined. the initial pathologic examination of lymph nodes from two patients showed no evidence of metastases (H&E-negative), yet a detailed histological analyses of serial H&E stained sections revealed the presence of tumor deposits in 18 lymph nodes. All of them contained CK18, ESA and CEA immunoreactive cells. The remaining 129 lymph nodes (16 patients) showed no evidence of tumor deposits by routine histology (H&E-negative), but IHC analyses of the serial sections revealed the presence of malignant immunoreactive cells in 21 (16%) lymph nodes from 7 (37.5%) patients. All 21 lymph nodes demonstrated cytokeratin immunoreactive cells, while epithelial specific antigen immunoreactive cells were found in 13 (10%) of these lymph nodes. IHC evaluation by CEA was less sensitive technique. It was able to detect tumor cells in only 6 (4.6%) of these lymph nodes. The pattern of immunoreactivity in tumor cells was typical of that seen in epithelial cells. Most of the malignant immunoreactive cells were found in the subcapsular sinuses and only a few in the medullar sinuses.

DISCUSSION

The results presented here demonstrate that micrometastases not detectable by routine histology can be identified with immunohistochemistry. The usage of cytokeratin 18 gave best results. Immunohistochemistry for CEA failed to identify malignant cells (had a limited tissue distribution and was less sensitive). The detection and significance of micrometastases in patients with cancer have been subject of interest recently. Most of the work was focused on colorectal cancer. It is well established that the presence of micrometastases in stage II disease identifies a group of patients with significantly greater risk of recurrence and death from the disease. Survival of these patients has been improved with adjuvant treatment. Those patients with no evidence of micrometastases had survival rates similar to stage I patients.

CONCLUSION

The results of this and other similar studies show the necessity of detecting micrometastases by IHC in order to make the staging of the disease more precise (2-4).

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Acute lymphoblastic leukaemia (ALL) with intracellular inclusions - A case report

KEYWORDS: Leukaemia; Ultrastructural analysis; Immunohistochemistry

The occurrence of intracellular inclusions is a very rare phenomenon in ALL, described in only a few cases. Most often inclusions are seen on the ultrastructural level as granules or viral-like particles. We are presenting a case of a patient with ALL with very distinct intracellular inclusions. A 49-year old patient was admitted due to systemic complaints. At admission he had marked hepatosplenomegaly. In the blood count he had WBC $24 \times 10^9/L$ with 46% blasts. In the bone marrow aspirate 91% were blasts of an irregular nuclear outline, often clefted with pale rose inclusions of 3-5 μm in diameter, placed paranuclearly in the area of Golgi or in the nuclear pouches. Cytochemically, the blasts were Sudan B, MPO, PAS and AcP negative, while the inclusion bodies were PAS positive. In the bone marrow core biopsy, the marrow was heavily infiltrated in the leukaemic pattern, with the blasts of similar morphological features and inclusions placed not only intracellularly but also extracellularly, intensively PAS+. The immunohistochemical analysis revealed TDT+, CD34+, CD74+, CD10+, CD20+, CD43+, $\lambda^{+/-}$, and MPO-, GpA-, CD38-. The inclusions were markedly CD74, CD10 and λ positive. Cytogenetics revealed Philadelphia chromosome /46XY/ 46XY,t (9;22)/. The ultrastructural analysis showed that the inclusions consisted of membranous structures with small vesicles inside and within the inner side of the membrane, and a partially amorphous nucleus. The patient received chemotherapy according to ADE 3+5+8 schedule (doxorubicine 45 mg/m², VP16 100 mg/m² and AraC 200 mg/m²). He developed the bone marrow aplasia and died due to complications. Comparing the appearance of inclusions in our study with previously published findings as well as with the immunophenotype, it can be concluded that these inclusions are derived from membranous structures as a part of the antigen processing apparatus because some similar structures were found in some antigen presenting cells and also the molecules CD74 (part of HLA DR molecule), CD10 and λ chain are parts of the antigen binding complex of B cells. As far as we know, no similar case of acute lymphoblastic leukaemia has been reported yet. The immunohistochemical typing was necessary to confirm the real lineage of the leukaemic clone.

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Myeloblastoma as initial extramedular presentation of acute myeloid leukemia - Report of two cases

KEYWORDS: Bone Marrow; Neoplasms; Immunohistochemistry

Myeloblastomas (chloromas) are extramedullary tumors of myeloid precursors which can occur during the dissemination of acute myeloid leukemia (AML) or as a relapse. De novo myeloblastomas may be a sole form of AML or precede AML several months. We report two cases of extramedullary myeloblastomas as initial presentations of AML with maturation, M2. The first patient, a 19-year old male, had a painless axillary lymphadenopathy. In 6 months, lymphadenopathy involved the cervical and submandibular region. Multiple biopsies were diagnosed as reactive lymphadenitis. A biopsy of the submandibular lymph node showed a sinusoidal and paracortical diffuse infiltrate composed of blasts and promyelocytes. A detailed immunophenotypic analysis excluded the lymphoproliferative disease and secondary deposits. As the tumor cells were strongly positive for CD34, CD117 and MPO, we diagnosed the lymph node myeloblastoma. The bone marrow was normocellular without immunophenotypic signs of AML. The patient is well 5 months after the diagnosis. Another patient, a 54-year old male, had a subcutaneous infiltrate in the parietal region of the head that occurred around the wound made by a wooden thorn sting. The biopsy was diagnosed as a small lymphocytic lymphoma. After 6 months he developed AML M2 with the multilineage dysplasia in the blood and bone marrow. The immunophenotypic revision of the subcutaneous infiltrate with the positivity for CD34, MPO, CD15 and CD117 confirmed the diagnosis of extramedullary myeloblastoma. The patient died soon after the diagnosis. Extramedullary myeloblastoma as the only presentation of AML has a good prognosis and may enter the spontaneous remission. Myeloblastoma that precedes leukemia in the bone marrow has a poor prognosis with the same course as AML.



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Composite lymphoma of mediastinum - A case report

KEYWORDS: Lymphoma; Mediastinum; Immunohistochemistry

Lymphomas composed of two morphologically and immunophenotypically different components (composite lymphoma) are rare. Hodgkin's lymphoma may occur simultaneously with non-Hodgkin's lymphoma, usually of the B cell type. We report the composite lymphoma of the mediastinum in an 11-year old boy. The disease started as pharyngitis. Soon the respiratory symptoms appeared and were diagnosed as asthma. During the course of the disease the subcutaneous tumor infiltrate appeared in the region of the sternum. The patient was operated and a part of the huge tumor localized in both lungs, pericardium and thymus was taken out. One of the biopsy specimens was the lung tissue with moderate interstitial fibrosis, partly replaced by collagen bands and the tumor tissue with a nodular growth pattern. The nodules contained numerous Reed-Sternberg cells, lacunar cells, mononuclear cells, small lymphocytes, numerous eosinophils, histiocytes and few plasma cells. The morphology, together with the immunophenotype, was of the HL, nodular sclerosis type. Another specimen contained the thymus tissue partly replaced by the tumor with both diffuse and nodular growth pattern. The neoplastic cells were large, with abundant pale cytoplasm with variably dense compartmentalizing fibrosis. The nuclei were vesicular, centoblast-like. The immunophenotype was of the mediastinal large B-cell NHL: TdT-, CD79 α +, CD20+, HLA-DR-, IgM-. Some remains of the epithelial spindle thymus cells (Cytokeratin+, S-100+) were observed between the lymphoid cells. By detailed morphologic and immunofenotypic analyses diagnosis of composite lymphoma, composed of HL and mediastinal large B cell NHL, was confirmed.

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Molecular analysis of γ t-cell receptor gene- valuable tool in diagnosis of cutaneous T-cell lymphoma

KEYWORDS: Cutaneous Lymphomas; T-cell receptor gene; Molecular analysis

Cutaneous T-cell lymphomas (CTCL) account for 60-65% of all primary cutaneous lymphomas (CLs), the second most common extranodal non-Hodgkin lymphomas (NHL). The diagnosis of CLs is based on clinical and histomorphologic features, and lately, on immunophenotypic or molecular-genetic analysis. Although, the vast majority of T-cells and malignant T-cell lymphomas express the α/β heterodimer on their surface, the γ chain of TCR is also rearranged in all T-cells early during their development. Therefore, we analyzed clonality within the TCR γ locus by polymerase chain reaction (PCR) to confirm the diagnosis of CTCL. Seven skin and blood samples from patients with known CTCL were employed in the study. Blood and skin samples of four patients (one with drug-induced erythroderma, one with lymphocytopenic lymphoma and two with B-CLs) were used as negative controls. Monoclonality detection was based on multiplex PCR amplification of different regions between V and J segments of TCR γ gene. Monoclonal T-cell populations were detected in all 7 CTCL skin samples and in two CTCL blood samples. In all control samples the polyclonal pattern was found. Monoclonality detected in our samples was in concordance with the diagnosis of CTCL and although it is not an independent diagnostic method, this analysis can be very useful, especially in cases with uncertain clinical and morphologic diagnosis.



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Prognostic impact of bone marrow angiogenesis in idiopathic myelofibrosis

KEYWORDS: Myelofibrosis; Angiogenesis; Vascular endothelial growth factor

Idiopathic myelofibrosis (IMF) is a clonal proliferation of bone marrow cells associated with deposition of connective tissue and extramedullary hematopoiesis. Clinically, patients with IMF manifest anemia and hepatosplenomegaly that may be associated with constitutional symptoms. We report the extent and prognostic significance of bone marrow angiogenesis in patients with IMF. Biopsies of 35 bone marrow were evaluated for cellularity and subsequently graded for the presence of reticulin fibrosis (I-IV). Vascular structures were visualized by immunohistochemical staining for factor VIII, CD31 and CD34. We counted the number of vessels per 400x microscopy field (HPF). All samples were further analyzed for the expression pattern of vascular endothelial growth factor (VEGF) in bone marrow cells. 21(60%) patients were in cellular phase and 14 (40%) were in hypocellular phase of IMF. In the majority (30) we found a marked increase of reticulin fibrosis (III-IV). All patients have some degree of anemia (Hb<93 g/l), more pronounced in hypocellular bone marrow. Significant splenomegaly, median 20.1 cm (15-32), was observed in all. The mean vessel count per HPF was 7.6 (±2.7). The number of VEGF-positive bone marrow cells was increased as well as the extent of VEGF expression. In multivariate analysis, increased angiogenesis correlated significantly with the number of VEGF positive cells. Increases in marrow angiogenesis strongly correlate with phase of the disease (p=0.001) and degree of bone marrow fibrosis (p=0.027). The significant correlation exists between the degree of anemia and VEGF expression (p=0.043). In contrast, there was no correlation between increased vascularity of bone marrow and increased spleen size. Our findings suggest that in bone marrow in IMF may be a marker of the disease activity. The density of microvessels and expression of VEGF may provide useful prognostic information and basis for the therapeutic investigation of anti-angiogenic agents.

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High grade multiple myeloma morphologic and immunophenotypic characteristics

KEYWORDS: Multiple myeloma; Plasma cell neoplasms; Immunohistochemistry

High grade multiple myeloma (MM) or plasmablastic myeloma (PM) is a rare form of the plasma cell neoplasm. PM is characterized by atypical morphology and poor prognosis. We present morphological and immunophenotypic characteristics of PM.

We analyzed 4 bone marrow biopsy specimens of PM for morphological details and the presence of reticulin fibrosis. The immunophenotypic analysis was performed by the panel of monoclonal antibodies: EMA, CD79 α , CD38, CD20, CD3, kappa, lambda, IgA, IgG, IgM. We further analyzed the expression pattern of CD10, CD138, cyclin D1 and vascular endothelial growth factor (VEGF) in tumor cells as well as the density of microcirculation (CD34). The proliferative activity of tumor cells was labeled by Ki-67. The bone marrow was hypercellular (>80%) in all, with marked reticular fibrosis (II-III) and high percent of tumor cells (70%-90%). Plasmablastic morphology dominate with oval or spindle cells with dispersed nuclear chromatin, high nuclear/cytoplasmic ratio and prominent nucleoli. All tumors showed weak positivity for EMA, strong for CD38 and CD79 α and were monoclonal. The expression of Ig-associated antigens was weak. CD138, CD10 and VEGF were strongly positive. Proliferation was high (>30% Ki-67+) with prominent angiogenesis (18-25 blood vessels per HPF). Cyclin D1 was negative in all. PM span immature morphology with high degree of fibrosis. The high expression of CD10 and CD138, together with high proliferation rate are associated with poor prognosis. Pronounced angiogenesis is the basis for the therapeutic investigation of anti-angiogenic agents.



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Bone marrow angiogenesis, CD44 and cyclin D1 expression in hairy cell leukemia

KEYWORDS: Hairy cell leukemia; Cyclin D1; Immunohistochemistry; Angiogenesis

The significance of neoangiogenesis and vascular endothelial growth factor (VEGF) for tumor growth and progression is well established. CD44 is a nonintegrin receptor for cell homing especially to the bone marrow, the main localization of hairy cell leukemia (HCL). Increased levels of cyclin D1 mRNA have been found in HCL. We investigate the correlation between the bone marrow angiogenesis, expression of VEGF, CD44 and cyclin D1 and the degree of bone marrow infiltration with HCL. The bone marrow biopsy specimens from 29 patients with de novo HCL were analyzed for cellularity and the percent of hairy cells (DBA44+). We counted the number of CD34+ vessels per 10 400x microscopy fields (HPF). All samples were analyzed for the expression pattern of VEGF, CD44 and cyclin D1. Bone marrow was hypercellular in all patients (74.4%±11.4). The average degree of HCL infiltration was 64.1% (31%-95%). The mean vessel count per HPF was 7.2(±2.4). In multivariate analysis, increased angiogenesis correlated significantly with the number of VEGF positive hairy cells ($p=0.0001$). These two features strongly correlate with the degree of HCL infiltration. CD44 was ubiquitously present throughout HCL, mainly strong positive (58.5%). The extent of CD44 expression significantly correlate with the percent of hairy cells ($p=0.006$). Cyclin D1 was overexpressed in 29/29 specimens, usually weak, in subpopulation of tumor cells. Expression of cyclin D1 was inversely proportional to the degree of HCL infiltration. Our findings suggest that neoangiogenesis in the bone marrow is extensively increased in HCL. CD44 and cyclin D1 may be markers of the disease activity and may serve as useful prognostic parameters in HCL.

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Abdominal neuroenteric cysts in adults

KEYWORDS: Abdominal cyst; Adults; Immunohistochemistry

It is postulated these cystic masses arise after a failure of complete separation of the mesodermal notochord from the primitive (endodermal) foregut or hindgut during the third week of embryogenesis and most probably represent its communication with the spinal canal (a persistent neuroenteric canal). In adults, these (semi)cystic lesions are more often composed of endodermal elements and, contrary to pediatric cases, are rarely associated with overt vertebral dysplasia, spina bifida or neurological disorders and so referred to as enteric, endodermal, enterogenous or teratomatous cysts. Generally, neuroenteric cysts are less often (1) presented as abdominal than mediastinal masses, (2) in adult than in pediatric or fetal pathology, and (3) clinically overt with tumoral compression or complications than incidentally found. Five cases from the Department of Histopathology Registry are presented, including 2 male and 3 female patients (19-38 years old) operated at the Clinical Centre of Serbia during the period 1991-2002. The gross findings varied from simple 3.5 cm unilocular thin-walled sac filled with clear serous liquid to more complex, septated or semi-solid / multilocular irregular masses up to 11.5 cm in the greatest diameter, sometimes containing milky or gray-white or yellowish viscous, even mucinous fluid. Microscopically, there was a regular finding of the simple cuboidal or cylindrical enterogeneous lining (Type 1 cysts, 1 case) sometimes admixed with the foci of squamous stratified epithelium. In some cysts more specialized enterocyte-like, foveolar-like or ciliated epithelia associated scanty gastric, intestinal or mucus glands and the smooth muscle wall (Type 2 cysts, 2 cases) focally with tracheobrochial elements or salivary/pancreatic glands (1 case). In addition, there were admixed abundant neuro-ganglionic or neuro-glial elements (Type C cysts, 2 cases). Unspecialized enterogeneous/endodermal epithelia more consistently showed immunoreactivity to anti-cytokeratin 8,17,18 and anti-CEA, and rarely and focally to anti-cytokeratin 7, 10/13, 19, 20, as well as anti-CA 19-9 and anti-CA 15-3 antibodies. None of the patients expressed histopathological or clinical signs of a malignant alteration during the follow-up and long-term recurrence of a suprapancreatic multilocular cyst in one patient was clinically interpreted as a result of the incomplete primary resection. Clinical significance was obvious in differentiation from other cystic lesions and tumors in the posterior abdominal and pelvic/retroperitoneal regions, especially from teratomas, hernias, bronchogenic cysts, gastrointestinal duplications, anterior meningocele and cystic mesothelial lesions as well as exclusion of possibly associated vertebral, gastrointestinal or other malformations.



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Histomorphological and immunohistochemical findings in human vein grafts

KEYWORDS: Vein graft; Histopathology; Immunohistochemistry

The fate of an aortocoronary saphenous vein bypass graft appears to be dependent on several factors relevant to the time interval from bypass grafting to graft obstruction. Graft occlusion developing within one month of bypass graft insertion is usually due to graft thrombosis, related to intraoperative vein trauma, or stenosis at aortic or coronary anastomotic sites. In the first year following the graft insertion, graft stenoses are characterized by intimal thickening, histologically composed of cellular or acellular fibrocollagenous tissue. Saphenous vein graft standing longer than one year, and generally after three years following the graft insertion, usually consists of atherosclerotic plaque in addition to intimal fibrous thickening. The aim of this paper is to present autopsy, histomorphological and immunohistochemical findings in aortocoronary saphenous vein bypass. Aortocoronary vein grafts removed at autopsy of five patients (1998-2001) who died within one month up to 2 years after the bypass operation were examined. Histomorphological analysis was performed on paraffin sections stained with hemalum eosin, Van Gieson elastica and Trichrome Mason. Immunohistochemical analysis for smooth cell actin, desmin, vimentin, CD68, Mac387, LCA, CD20 and CD43 (DAKO products) was also done. A technique of LSAB+ immunoperoxidase (DAKO) was used with prior microwave antigen retrieval in citric acid buffer (pH 6,0). The analysed group consisted of one female and four males at the mean age of 66 years. The mean heart weight 600 grams. In four cases three levels of saphenous vein grafting were found, and in one there were three saphenous vein grafts while one was taken from the mammary artery. According to the time interval from bypass grafting to graft complications, there was one case with early complications (10 days after surgery, the patient died), and three cases with late complications (died 13 months-, 15 months-, 17 month- and 23-months after surgery). In the first case three early aortocoronary vein grafts and one aortocoronary arterial graft were found. The excised grafts were free of calcium deposits, but a graft thrombosis in two vein and one arterial grafts were found, beginning at the proximal anastomotic sites. The main histomorphological finding was the presence of intraluminal thrombotic masses, endothelial damage with slight hyperplasia of tunica intima. Immunohistochemically, we found a few smooth cell actin and vimentin positive cells in the intima. No inflammatory cells were found, except at the anastomotic sites (CD68+, Mac 387+ and a few LCA+ cells). One of the other three cases died 15 months after the operation, presented by the clinical signs of congestive heart failure after the surgical treatment of a prostatic gland tumor. There were three vein grafts found with thrombosis of the right aortocoronary vein graft and left descendent aortocoronary vein graft for the major

complication. The analysis showed a significant increase of smooth muscle cells in the graft's intima, with the presence of atherosclerotic plaques. We found positivity for smooth muscle cell actin and vimentin in the thickened intima. In the atherosclerotic plaque, there was strong positivity for histiocytic markers. We couldn't demonstrate the presence of lymphocytes in these lesions. One of the cases (died after 13 months), besides described changes in the previous case, showed the presence of amyloidosis in the myocardial tissue as well as in the blood vessels and graft wall. The other two patients who died 17 and 23 month after implantation of the graft, showed significant stenotic lesions of the vein grafts due to severe atherosclerotic damages accompanied by cholesterol clefts and calcium salt deposits. On the immunohistochemical staining the presence of histiocytic cells (CD68+, Mac387+) was revealed in the atherosclerotic lesions, as well as areas of smooth muscle cell hyperplasia in the rest of the intima. In the other layers there was medial and adventitial fibrosis. Morphologic changes in aortocoronary vein bypass grafts have been already reported. We present a short list of patients with the implanted aortocoronary vein graft, that have undergone autopsy after their death. The analysis showed that the fate of an aortocoronary saphenous vein bypass graft is dependent on several factors relevant to the time from bypass grafting to graft complications development. In one case an early graft complication - thrombosis, was found, with minimal changes of the vessel's intima. Thus, the surgeons always should have in mind the early technical factors that may limit the function of the grafts, and should act to supervise them. The other cases showed significant atherosclerotic changes in addition to the intimal hyperplastic thickening, that could imply continuous control of the patients and application of vein graft angioplasty. Positivity for the muscle cell actin in the intima suggests the presence of adhesive molecules and cytokines that influence migration and hyperplasia of these cells, as well as formation of atherosclerotic changes that occur in the wall of the vein grafts.



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Klinefelter's syndrome with a pericentric inversion of chromosome 9, 47,XXY, inv(9)(p11q13) - A case report

KEYWORDS: Klinefelter's syndrome; Mosaicism; Down's Syndrome

Klinefelter Syndrome is the most common disorder among males, which is associated with sex chromosomes. It is characterized by an extra X chromosome, which is the result of nondisjunction in parents' gametes. In 80% of all cases the karyotype is 47,XXY, while in 15% of cases there is a mosaicism, 47, XXY/46,XY. The syndrome is associated with hypogonadism and azoospermia (except in rare cases of mosaicism), and with a tall stature and long extremities. Some data indicate disorders in behaviour and speech and lower IQ. The incidence of this syndrome is 1 in 600-1000 newborn males. From some literature data, it is associated with a higher incidence of some malignant diseases, especially with breast cancer. The use of exogenous androgens during and after puberty can improve psychosexual development. The incidence of inversion of the chromosome 9, inv (9) (p11q13), in normal population is very small and it seems that this inversion doesn't have pathological phenotypic expression. It occurs rare in Klinefelter's Syndrome. We report a patient with a hypogonadism in whom Barr bodies were detected on smears of buccal mucosa. The cytogenetic analysis was done with chromosomes from T lymphocytes after 72h of cultivation in the presence of PHA mitogen and GTG banding technique. 16 mitoses were analysed. The presence of an extra X chromosome and pericentric inversion of chromosome 9, inv(9)(p11q13) were detected. This inversion is confirmed by the C banding technique, which stains specific high repetitive sequences of secondary constriction of chromosomes. Population studies in Japan indicate a higher incidence of inversion of chromosome 9 in normal female group than in normal male group. The incidence is lower in Down's Syndrome patients. In Klinefelter syndrome the incidence is slightly higher in the male than in female group. Why is there a difference in the incidence of this inversion among sexes, and what is the genetic effect of this inversion are still the questions.

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Uterine myxoid leiomyosarcoma arising in a leiomyoma - A case report

KEYWORDS: Uterine Neoplasms; Leiomyosarcoma; Immunohistochemistry

Myxoid leiomyosarcoma of the uterus (MLU) is a very rare neoplasm. We report a case of MLU arising from a pre-existing leiomyoma. Between 1989 and 2001, 45 uterine leiomyosarcomas were diagnosed in our laboratory and MLU was established in one case only (2.1%). Subtotal hysterectomy was performed on a 56-year woman with clinical diagnosis of a uterine myoma. The histopathological processing included hematoxylin-eosin, histochemical and immunohistochemical staining of selected specimens. Macroscopically, the uterus measured 15 x 12 x 11 cm, with an indistinctly circumscribed multi-nodular tumorous mass 10.5 cm in diameter, and infiltrative satellite nodules in the surrounding myometrium. The morphology of the neoplasm showed a leiomyoma with distinct degenerative changes, necrosis and hemorrhage. In some areas the cells were round or oval with a vacuolated cytoplasm, slight atypia and rare mitoses. The mitotic count was 1-3 cells/10 HPF. Due to the presence of an abundant mucoid substance, these areas appeared as hypocellular. The additional processing confirmed the smooth muscular origin of the neoplasm (Masson trichrome, azan, desmine, alfa-smooth muscle actin and vimentine positive). The areas of the mixomatose nodules were characterized with hormone independence (estrogen and progesterone receptors negative), high proliferative activity (Ki-67 - 30%) and the presence of p53 protein product (45%). The areas of the pre-existing leiomyoma showed hormone dependence, low proliferative activity and absence of p53 protein product. Additional immunostaining is useful in supporting a diagnosis of MLU in myxoid uterine smooth-muscle tumors with a low mitotic rate.



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Detection of K- and H-ras mutations in bronchoalveolar aspirate

KEYWORDS: Lung Neoplasms; K-ras; H-ras gene

Lung cancer is the most common cause of cancer deaths and cancer-related deaths worldwide. Rapid assays for the detection of gene mutations in various types of human tumors and body fluids could determinate whether molecular genetic assays could augment conventional clinical and laboratory diagnostic procedures. To study mutations of K- and H-ras oncogenes in patients who underwent a routine bronchoscopic procedure and to assess the relation between the gene mutations and cytogenetic findings. Alterations in codons 12 and 13 of K- and H-ras genes in bronchoalveolar aspirates of 53 patients (pts) were examined by polymerase chain reaction and SSCP analysis. Mutations in K-ras gene were identified in 18/53 (34%) specimens of bronchoalveolar aspirates, out of which 25 % were from pts with adenocarcinoma, 48% squamous cell carcinoma, two pts with large cell carcinoma and two pts with chronic lung disease. The same samples were examined by conventional cytologic analyses providing the findings opposite to ours in 6 specimens of malignant tumors where cytological analyses were positive in two cases and K- and H-ras negative, while four specimens were cytologically negative but K-ras positive. Five specimens were inappropriate for cytological assessment. A presence of mutated K-ras gene may prove useful as an adjunct to cytological analysis and also could serve as an additional criteria for early diagnosis in patients with bronchogenic carcinoma.

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Metastatic tumors in the bone marrow

KEYWORDS: Bone marrow Neoplasms; Metastasis; Immunohistochemistry

Hematological abnormalities are frequent in patients with malignant tumors. Small round cell tumors and atypical proliferation of megakaryocytes induce major difficulties in the diagnosis of metastatic tumors in the bone marrow. The aim of our study is to determine the clinico-pathological characteristics of bone marrow metastases. We analyzed clinical parameters of 44 patients, treated for hematological abnormalities as a consequence of the dissemination of an unknown primary tumor in the bone marrow. Bone marrow biopsy specimens were analyzed morphologically and immunohistochemically with a wide panel of monoclonal antibodies in determining the site of origin of the metastatic tumor. Anemia was present in 95.83%, thrombocytopenia in 83.3%, left shift in formula in 54.2%, erythroblasts in the peripheral blood in 37% and hepatosplenomegaly in 33% pts. In 21.7% of the patients the bone marrow aspirate showed "dry tap". In 30 pts tumor deposits were adenocarcinoma, in 8 small round cell tumor, in 2 squamous cell carcinoma. The rest were a hepatocellular carcinoma, carcinoid tumor, papillary carcinoma and sarcoma. The bone marrow fibrosis was present in all specimens: grade IV in 45.45%, grade III in 31.81%, grade II in 20.45%, grade I in 2.29%. Osteoblastic reaction was seen in 20 pts. The primary site of the tumor was determined in 54.54% pts: the lung in 9, gastrointestinal tract in 7, breast in 6, prostate in 1, thyroid gland in 1 and liver in 1 patient. Pancytopenia accompanied with "dry tap" is highly suggestive for the bone marrow metastatic tumor. The origin of an unknown primary tumor can be determined in more than a half of these patients.



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Neoangiogenesis in myeloproliferative diseases

KEYWORDS: Angiogenesis; Myeloproliferative diseases; VEGF

The chronic myeloproliferative diseases (CMD) are clonal hematopoietic stem cell disorders. Malignant hematopoietic diseases depend on neovascularization like solid tumors. The aim of our study was to determine the extent of bone marrow angiogenesis in various CMD: polycythemia vera (PRV), essential thrombocythemia (ET), chronic idiopathic myelofibrosis (CIMF) and chronic myelogenous leukemia (CML). We compared the neoangiogenesis in 131 bone marrow biopsy specimens from de novo CMD (32 PRV, 32 ET, 32 CML, 35 CIMF) with angiogenesis in 10 normal bone marrow samples. Vascular structures were visualized by immunohistochemical staining for factor VIII, CD31 and CD34. We counted the number of vessels per 400x microscopy field (HPF). All samples were further analyzed for the expression pattern of the vascular endothelial growth factor (VEGF) in bone marrow cells. The average number of blood vessels in the normal bone marrow was 5.5 (± 2.1), with weak expression of VEGF. In all CMD the number of blood vessels was increased to 11.4 (± 6.5) per HPF. In the multivariate analysis a significant correlation in the vascular density ($p < 0.01$) exists between CIMF 7.6 (± 2.7), ET 10.1 (± 5.3), PRV 13.9 (± 6.5) and CML (15.1 ± 7.5). The number of VEGF+ cells was markedly increased in CML and CIMF. The significant correlation exists between the vessel number and VEGF expression ($p < 0.05$). We showed that neoangiogenesis is significantly increased in CMD and dependent on the disease type.

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Comparison of Streptavidin biotin complex, Envisiontm+ and LSAB+ as visualization systems in immunostaining to HMB-45 and Melan A antigens

KEYWORDS: HMB45; Immunohistochemistry; LSAB

Immunohistochemical stainings are very selective and specific, but they also have some limits. As a contribution to standardisation attempts in immunohistochemistry, we analysed three visualization systems (Strept ABC, EnVision TM+ and LSAB+) in immunohistochemical detection of HMB-45 and Melan A antigens. We use tissue samples from patients with malignant melanoma for our experiments. Samples were fixed in 4% buffered, neutral formaline and routinely processed in SAKURA V.I.P. We performed microwave antigen retrieval procedure in 0.5M citrate buffer pH 6.0. Monoclonal mouse anti-human HMB-45 (DAKO Code No M 0634) and Melan A (DAKO Code No M7196) are used as primary antibodies, Strept ABC, EnVision TM+ and LSAB+ as systems for visualization and DAKO AEC+ as chromogen. Staining results are analysed on Leica DMLB microscope and with CAMIA software. The Mean and Maximum optical density and Stained area were measured and compared. The statistical analysis of the uncorrected data for all three detection systems showed significant differences between EnVISION TM+ and LSAB+ on one side and Strept ABC system on the other. The corrected data for the Mean and Maximum OD are also significantly different for Strept ABC system. The image analysis is more useful and objective in standardisation attempts in immunohistochemistry than comparative observations judged by the human eye.



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Morphologic and morphometric analysis of alterations in the oral cavity caused by *Candida albicans* - Experimental work I

KEYWORDS: Gingiva, *Candida*, Morphometry

Our knowledge on the presence, that is spread of fungi as well as on their toxic effects on living organisms dates back to distant past. Defensive mechanisms of the host play a vital role in the protection of organisms against the fungal invasion. Numerous and well known are the factors, both local and general that may disturb the integrity of mucous membrane and cause fungal infections. Having all this in mind the authors intended, in the experiment on rats, to find out pathomorphologic alterations caused by blastospores of the *Candida albicans* as well as morphometric alterations. The experiment was carried out on rats 2.5 months of age, weighing 110-130g and divided into the experimental and control groups. The rats of experimental group were infected with blastospores of *Candida albicans* in a dosage of 400.000 in the 0.5ml physiologic solution for one animal. After the sacrifice over the definite time intervals, cuts of gingiva were stained by HE and PAS methods and tested for histomorphological purposes. Blastospores of *Candida albicans* were found to be pathogenic for the gingiva; after the fourth day pseudohyphae were found, while the stereological investigation established an acute increase in the volume of nuclei as a consequence of the toxic effects of *Candida albicans* and Candidine, the metabolic product of *Candida albicans*.

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Telecytology - quadrennial experience at the Department of pathoanatomy, Military Hospital of Niš: analysis of 62 hematological cases

KEYWORDS: Hematology; Telecytology; Imaging

Digital imaging has many applications in pathology and cytology including training and education, image analysis, diagnosis, report documentation and archiving, and telecommunications. Telepathology/telecytology involves the use of telecommunications to transmit pathology/cytology images for the purposes of diagnosis, consultation or education. Primary aim of this paper is the presentation of our diagnostic experience with 62 hematological cases regarding their accuracy and quality assurance through the specificity of the static consultation system used. There are two basic systems for telepathology or telecytology: dynamic and static. In the dynamic system, a telepathologist or telecytopathologist can control a robotic microscope at the remote site, while in the static system a telepathologist or telecytopathologist makes the diagnosis based on the microscope images that have been selected and transmitted by someone in a remote hospital. We have developed the static system for remote consultation and diagnostics based on the relation between the relational databases model and WWW. Over the last four years, the Pathology Division of the Military Hospital in Niš, 250 km away from the Military Medical Academy in Belgrade, has been sending via POTS, multimedia documented requests for remote consultations and diagnostics in cytological cases. All cases were documented by clinical, laboratory and radiological data and imaged using the Sony DXP 1-CCD camera, Philips 490 1-CCD camera and Pixera digital camera, at a resolution from 768x574 to 1024x768 pixels. The average number pictures/case was 10.9. Only the cytological slide stained with May-Grunwald-Giemsa method was presented under the telemicroscope. We analyzed 62 hematological cases, which were clinically evaluated, in the period 1998 - 2001. They are characterized with a high level of accuracy and quality assurance. 78% of all cases were definitely resolved in the first remote consultation. 19% of cases were also definitely resolved after a repeated sampling procedure and additional clinical data or images provided. Only in two cases the definitive diagnosis was established by glass slide observation. Definitive concordance between telecytologic and glass slide diagnosis was 88%. 14 (22%) cases were malignancies. Our study proved the efficiency and high diagnostic accuracy of static telecytology for the diagnostic consultations in the hematological cytology. The high-level agreement between the telecytologic and glass slide diagnosis, in our experience, is due to adequate education and training for telepathology services of both the referring pathologist and telecytologist, and specific configurations of our teleconsultation system. Telecytology is an area in particular need of studies of good quality to provide data on factors affecting accuracy.



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Morphologic and morphometric analysis of alterations in the internal organs caused by *Candida albicans* - Experimental work II

KEYWORDS: Gingiva, *Candida*, Morphometry

Well known are the factors that may cause disturbances in the integrity of the skin and mucous membrane resulting thus in fungal infections. The defensive mechanisms play an important role in the protection of the organism against the fungal invasion. The authors have investigated the hematogenous dissemination of *Candida albicans* in internal organs of rats from the moment of infection as well as pathomorphologic alterations caused by blastospores of *Candida albicans* in the brain, stomach and kidney. Also, a morphometric analysis has been done. The experiment was carried out on rats 2.5 months of age, weighing 110-130 g, divided into the experimental and control groups. The rats of the experimental group were infected with blastospores of *Candida albicans* in a dosage of 400.000 in the 0.5 ml physiologic solution for one animal. After the sacrifice over the definite time intervals, cuts of internal organs (brain, stomach, kidney) were made by HE and PAS methods and tested for histomorphological purposes. Blastospores and pseudohyphae were found in the brain, stomach and kidney after the sixth day from the inoculation. An acute increase in the nuclei volume was the consequence of the toxic effects of blastospores and Candidine, the metabolic product of *Candida albicans*.

Molecular mechanisms involved in induction of apoptosis of thymocytes with microtubular poisons

KEYWORDS: Apoptosis; Microtubular poisons; Molecular mechanisms

A disturbance of the microtubular cytoskeleton produced by the action of the microtubule disruptive agent (Colchicine) or microtubule stabilizing agent (Paclitaxel), induces apoptosis. Although caspases are involved in molecular mechanisms of the executional phase of apoptosis, there are little data about their involvement in apoptosis induced by microtubular poisons. In this study the effects of specific inhibitors of caspases (50 $\mu\text{mol/L}$) on apoptosis of rat thymocytes induced by Colchicine (2 $\mu\text{mol/L}$) and Paclitaxel (10 $\mu\text{mol/L}$) were investigated. The effect of inhibitors of caspase-3, caspase-9, caspase-1 and z-VAD after 4, 8 and 12 hours of incubation of thymocytes with microtubular poisons were analysed. After incubation, the cells were fixed, stained with Türk solution and the percentage of apoptotic cells (apoptotic index) was determined. General inhibitor of caspases z-VAD led to a significant decrease of the apoptotic index, that dropped from 28.34% to 18.54% after 8 hours of incubation with Colchicine, and from 29.03% to 19.73% when incubated with Paclitaxel. Inhibitors of caspase-3 and caspase-9 had similar effects. While all inhibitors of caspases lowered the apoptotic index also in thymocytes cultured without microtubular poisons, the inhibitor of caspase-1 did not affect the on apoptotic index in controls, but it lowered the apoptotic index from 69.93%, to 52.33% and from 64.34% to 44.23% after 12 hours of incubation with Colchicine and Paclitaxel respectively. These results show that apoptosis induced by microtubular poisons is caspase dependent and that it, due to the effect of the inhibitor of caspase-9, probably involves mitochondria. Our results suggest that caspase-1 activity might be also involved in the mechanisms of induction of apoptosis of thymocytes with microtubular poisons.



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Correlation between expression of p53 oncoprotein and histological grade and stage of disease in squamous carcinomas in oral cavity

KEYWORDS: p53 protein; Squamous carcinoma; Oral cavity

An alteration in the p53 tumor suppressor gene is the most frequent genetic abnormality in human cancers. The overexpression of the p53 oncoprotein and histological differentiation were investigated in 28 routinely formalin-fixed and paraffin-embedded squamous cell carcinomas of the oral cavity. The P53 oncoprotein was detected in all specimens with the monoclonal antibody DO-7. Expression of the p53 oncoprotein was scored in the following way: score 0 >10%, score 1 10-50%, score 2 < 50% positive cells. Using the histological differentiation, all specimens are divided in three groups: group I - well differentiated carcinomas (6 cases), group II - moderately differentiated carcinomas (12 cases) and group III - poorly differentiated carcinomas (10 cases). In the first group 2/6 (33.3%) specimens were negative, and 4/6 were highly positive (<50%) for the mutant p53 protein. In the second group, positive detection of the p53 oncoprotein was observed as low in 66.6% of the cases, moderately positive in 25% and highly positive in 8.3%. In the third group 6/10 (60%) expressed low positivity for the p53 oncoprotein and 20% of the cases had moderate and high positivity respectively. Mutation in the p53 tumor suppressor gene is highest in the well differentiated squamous cell carcinomas, and its expression decreases in poorly differentiated tumors.

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Adult extrarenal rhabdoid tumor - A case report

KEYWORDS: Rhabdoid tumor; Immunohistochemistry; Extrarenal rhabdoid tumor

Rhabdoid tumor known to be a paediatric renal tumour is rarely present in other localisations in adults. In adults, it has very aggressive clinical course with the mortality rate greater than 75% in the first year of the clinically manifested disease. We present a female patient, 54 years old, whose first symptoms were oedema and unclear compression in the left inguinal region, perineum and labia. Punctional biopsy of the ill defined subcutaneous mass was performed in the local hospital where the primary diagnosis of subcutaneous liposarcoma was established. Two months later, the patient was admitted in the Institute of Oncology and Radiology of Serbia, where a minor tumour mass on the opposite side of the perineum was also detected, as well as additional bigger tumour masses of retroperitoneum and obturator fossa. The patient was submitted to the maximal tumour reduction, but the complete excision of necrotic paraaortic lymph nodes failed. The gross finding of the tumour showed solid, soft, grey and necrotic, partly myxoid mass with ill defined borders. Microscopically, the tumor consisted of oval and epitheloid cells with eosinophilic cytoplasm, prominent nucleoli in ovoid to round nuclei and rare PAS positive hyaline inclusions. The majority of tumour cells showed strong anti-vimentin reaction and also significant positivity to anti-cytokeratins 5, 6, 8, 17 and 19, EMA and anti-actin antibodies, while there were no reactions with antibodies to HMB45, CA125, CA 19-9, CEA and S-100. Immunoreactivity to anti-myosin antibodies was focally positive but some of the examined tumour cells showed significant anti-CA15-3 reactions. Immunohistochemical analysis was performed with highly sensitive LSAB+ method and AEC as chromogen. In our opinion, a series of extrarenal rhabdoid tumours is probably larger and should be more often considered in the differential diagnosis of these tumour localizations. We support a viewpoint that rhabdoid tumor is a special clinical entity of unknown histogenesis rather than it is a subtype of Wilms' tumour or other types of sarcomas.



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Gene alterations in lung carcinoma

KEYWORDS: Lung Neoplasm; Gene alterations; Polymerase chain reactions

Lung cancer is the leading cause of cancer deaths among men and women worldwide. In the spite of extensive efforts to detect lung cancer at the early stage and introduce treatment with newly developed anticancer drugs, the prognosis of lung cancer patients has not been remarkably improved. We determined the *p53*, *K-ras* and *H-ras* mutations in 40 resected primary Non-Small-Cell Lung Cancer (NSCLC) patients of different histological types: adenocarcinoma, squamous cell carcinoma and large cell carcinoma. DNA was isolated from frozen tumors by standard phenol-chloroform extraction. *K-ras*, *H-ras* and *p53* mutations were analyzed by PCR-SSCP method. *K-ras* (codon 12 and 13) activating mutations were present in 30% of the tumors, with a higher incidence detected in squamous cell carcinomas. Mutations in the *p53* gene were found in 19 of 40 cancers (47.5%) (more frequent in squamous cell carcinomas). Mutation analysis showed that 5% of the cancers contained two *p53* mutations and 12.5% cancers had mutations in both *K-ras* and *p53* genes, all in advanced squamous cell carcinomas. The incidence of both *H-ras* (codon 12, 13) and *p53* mutations was 7.5%, all detected in early-stage squamous cell carcinomas. *H-ras* mutations were detected only in squamous cell carcinomas and appear to be less frequent than *K-ras* mutations. *K-ras* and *p53* mutations are common in early-stage of NSCLC, suggesting a possible role of these genes as early events in lung cancerogenesis.

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Analysis of work of the immunohistochemical service of the Institute of Oncology Sremska Kamenica

KEYWORDS: Immunohistochemistry; K-67; Hormonal receptors

Although immunohistochemical processing of the preparations for experimental purposes was initiated as early as in 1988 in our laboratory, we are of the opinion that December 12, 1991 was the day when our laboratory was capable of its routine work and it should be regarded as the date of the beginning of the work of this service. As it coincided with the years when our country was under the international sanctions, it was quite understandable that the scope of work of this laboratory was limited and twice even completely discontinued for long time intervals. In the 12-year period cited, a total of 58,838 histologic preparations were histologically processed and stained by the HE method. Out of that number only 665 (1.14%) biopsies were immunohistochemically processed by the methods such as: PAP, APAP and Avidin Biotin. In accordance with our oncologic issues, a total of 53 markers were used, which amounted to 3 markers per biopsy on the average. Markers of lymphocytic population in general and general T and B markers in particular were most often used, then Ki-1 and Ki-67 markers and in a considerably less number the remaining 20 diagnostically significant markers for lymphomas. Of other markers, the most often used were the following markers (cited with the falling number of cases): S-100, CK, Vimentin, EMA, CEA, LCA, NSE, Alpha fetoprotein, Factor VIII, Alpha-1 antihimotripsin and other 40 markers (in which the above-cited auxiliary markers for lymphomas were also included). From the cited list of markers it could be concluded that immunohistochemistry was most often used for lymphoma differentiation which was accompanied by poorly differentiated tumors of the soft tissue, mesenchymal tumors and tumors of melanogenetic origin. A great number of immunohistochemical analyses included both the consultative cases from the centres which did not have the immunohistochemical laboratory and the cases where the oncologic commission of the Institute sought revision of the existing histologic diagnoses. The least number of analyses referred to the cases from the Department of Experimental Oncology of the Institute. Ninety-three breast carcinomas, in which hormonal receptors and the P53 marker hadn't been determined, were not included in the total of 665 immunohistochemical analyses. At the beginning of 1991 our laboratory was entirely equipped for the determination of hormonal receptors by the methods of ERI-CA and PRI-CA "Abott" and even the first (experimental) analyses were performed at that time. However, a deficiency of some necessary chemicals did not make it possible for us to use this method in a routine way. As late as around the middle of 2000 we reinitiated the determination of hormonal receptors and P53 markers by the method of LSAB Peroxidase "DAKO", at the beginning in the routine

way. However, it was of a very short duration and only 93 cases were analysed. Fortunately, enzymoimmunological determination of hormonal receptors by the methods of ER-EIA and PqR-EIA has been also performed in our Institute beginning from December 1, 1994. For enzymoimmunological analyses sections have been taken by the pathologist who prepares the material for the "ex tempore" analysis. The sections are taken routinely in all patients with carcinoma except for the cases where the section is so small that it is by all its volume within the diagnostic section. The hormonal status has been so far determined in 2411 patients enzymoimmunologically, so that our number of 93 (3.85%) cases is irrelevant in respect to the enzymoimmunological one. Our report has predominantly been aimed at analysing the 12-year work of the immunohistochemical laboratory which is incorporated in the routine work despite a very small number of cases. It also points to the fact that in addition to the personnel reinforcement this laboratory is entirely ready and equipped to perform immunohistochemical analyses in a far greater scope than it has been the case so far.

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Prognostic relevance of BCL-6, Caspase 3, PARP, vascular endothelial growth factor (VEGF) expression and international prognostic index in diffuse large B cell lymphoma

KEYWORDS: Large B cell lymphoma; BCL; Immunohistochemistry

Diffuse large B cell lymphomas (DLBCL) represent a heterogeneous entity that includes centroblastic (CB), immunoblastic (IB) and B-cell large anaplastic lymphomas. The aim of our study was to confirm the prognostic relevance of highly heterogeneous DLBCL from both a clinical and histopathological point of view. We analyzed 65 pts with de novo DLBCL and categorized them clinically by the international prognostic index (IPI). Biopsy specimens (47 lymph nodes and 18 bone marrows) were diagnosed by the histomorphology and by immunohistochemistry. The paraffin embedded samples were analyzed for the expression pattern of bcl-6 protein, Caspase-3, PARP and protein for VEGF. All results were statistically confirmed. The histopathological analysis showed 55 pts with CB and 10 pts with IB DLBCL. The IPI was low in 36.92%, low intermediate in 29.23%, high intermediate in 27.69% and high in 6.15% of the patients. The IB pts had high intermediate and high IPI. During the 48 months follow-up period 38/65 pts (58.46%) are alive and under control (ten IB pts died). All analyzed samples were bcl-6+, but in 48 CB we noticed statistically significant strong positivity (>75% cells), $p=0.04$. The similar results were obtained for Caspase-3. PARP was strongly positive in 45 pts, mainly of CB type. There was no statistically significant differences between CB and IB in VEGF expression but we observed 10 completely negative reactions in CB pts only. Together with bcl-6 and caspase-3, IPI can be used as a clinical marker to differentiate CB and IB DLBCL, and they can serve as valid prognostic markers in DLBCL.



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Extranodal marginal zone B-cell lymphomas of mucosa associated lymphoid tissue (MALT NHL) outside the gastrointestinal tract

KEYWORDS: MALT lymphoma; Gastrointestinal Neoplasms; Immunohistochemistry

MALT NHL is the most common extranodal lymphoma. The gastrointestinal tract (GI) is the usual site; 85% of MALT NHL are localized in the stomach. Other sites are rare. Systemic dissemination does occur, usually in other extranodal sites or in the bone marrow. The aim of this study was to confirm the clinical, morphological and immunophenotypic characteristics of MALT NHL outside the GI. We analyzed 84 de novo MALT NHL localized outside the GI. The biopsy specimens of all patients were analyzed immunohistochemically with the panel of monoclonal antibodies: CD79(, CD20, CD10, CD3, CD5, CD43, kappa, lambda, IgA, IgG, IgM and bcl-2. The patients' median age was 59.2 years (28-82). The majority of them were presented with stage I and II of the disease. 28% patients had bone marrow infiltrations. The international index was low or low intermediate in 80%. The sites of tumors were: Waldeyer's ring (25%), salivary glands (21%), ocular adnexae (21%), nose and oral cavity (9,52%). 6 tumors were in the lung, 4 in the thyroid gland, 3 in the skin, 2 in the uterus and 1 the gall bladder, kidney, testis and breast. Morphologically, the monocytoid B-cells and centrocytoid cells dominated, with plasmacytic differentiation. Lymphoepithelial lesions were seen in the majority of tumors. All tumors strongly express CD20 and CD79 α , with a high proportion of CD43+ cells. Expression of bcl-2 was moderate. The differential diagnosis of extranodal MALT NHL outside the GI is a problem due to its unexpected localization and indolent course. The distinction from reactive processes is based mainly on immunophenotyping or molecular genetic analysis.

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Lipoleiomyoma of the uterus: Immunohistochemical analysis of 11 cases

KEYWORDS: Leiomyoma; Uterine neoplasms; Immunohistochemistry

The histogenesis of uterine lipoleiomyomas (UL), which are commonly considered to be rare tumors, has not been fully clarified. The purpose of this study is to ascertain the actual incidence of UL, and to establish the origin of the lipomatous component in UL. Out of the total of 812 uterine smooth muscle neoplasms diagnosed over the period between May 2001 and May 2002, 11 were UL (1.4%). The tissue samples from all cases were routinely processed, and the lipid content was histochemically demonstrated with Oil-red-O. This method was performed in areas, which were macroscopically suspected to contain fatty tissue. Selected sections were immunostained. Ten leiomyomas and one smooth muscle neoplasm of uncertain malignant potential, with various amounts of the lipomatous component were identified. In three tumors the lipomatous component consisted of mature lipocytes. In eight tumors, the perivascularly localized focal areas of cells with pale abundant cytoplasm similar to smooth muscle cells or to fibrocytes containing cytoplasmic lipid droplets were found. Immunohistochemically, the cells in the perivascular areas were positive for S-100 protein, desmin, vimentin, neuron specific enolase, alpha 1-antitrypsin, CD 34 and collagen IV and were negative for alpha-smooth muscle actin. Additionally, these cells and mature lipocytes were negative for estrogen and progesterone receptors. Our results suggest that UL are not rare neoplasms and that lipomatous differentiation in leiomyomas can be found more frequently than expected. Immunohistochemical results indicate that lipomatous cells, as well as smooth muscle cells, derive from multi-potential undifferentiated mesenchymal cells.



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The effect of microtubule poisons intraventricularly injected on apoptosis of cells in the hippocampus of rats

Tissue clearing as a morphometrical measurement feature in evaluating the renal and bone marrow toxic damage

KEYWORDS: Apoptosis; Hippocampus; TUNEL technique

KEYWORDS: Bone marrow; Tubular necrosis; Morphometry

Microtubule poisons, Colchicine (a microtubule disrupting agent) and Paclitaxel (a microtubule stabilizing agent) induce apoptosis in a number of cell types. Directly applied into various brain regions they have neurotoxic effects. In this study, we have investigated the effects of intraventricular injections of microtubule poisons on the hippocampus cells. Wistar rats were anaesthetized with Chloral hydrate and stereotaxically injected with Colchicin (2 μ Mol/L) or Paclitaxel (10 μ M/L) into the left lateral ventricle 48h before sacrifice. Control animals (injected with artificial CSF) and test animals (ingested with microtubular poisons) were anaesthetized with Chloral hydrate, perfused through the ascending aorta with 0.5% NaNO₂, followed by fixation with 4% paraphormaldehyde, or 2.5% glutaraldehyde. The brains were rapidly dissected and immersed in the same fixative. The tissue was later processed and embedded in paraffin for light microscopy and EPON for electron microscopy. We have also characterized the mode of neuronal cell death, induced by microtubule poisons by detecting single DNA strand breaks using the TUNEL technique. Many hippocampal cells, 48h after a single intraventricular injection of microtubule poisons, exhibited a positive TUNEL reaction. Hematoxylin-eosin staining of hippocampal sections reveals the features fitting the morphological criteria for apoptosis. Electron microscopy analysis of ultra thin sections also reveals features of apoptosis in these cells. Apoptotic morphology was also observed in the ependymal cells. No apoptotic cells were observed in brain sections obtained from control rats, except in the area around the place of injection. Our study shows that intraventricularly injected Colchicine or Paclitaxel induced apoptosis of cells in hippocampus.

The quantitative valuation of the morphological features and changes represent revival of the many old unrealized ideas enabled with new digital techniques and many image analysing softwares. The purpose of this study was to find morphometrical measurement feature in evaluation of the tubular necrosis and hypoplastic bone marrow changes after cisplatin (CDDP) and 5-fluorouracil (5-FU) chemotherapy. The study was performed on the series of thirty rats divided in three groups treated with: I.CDDP, II.5-FU and III-the control group. All medications were injected as a single doses: CDDP 6 mg/kg, 5-FU 150 mg/kg. The rats were killed on days 1,2,4,6,8,10,14 and 21. Autopsy, standard histological procedure and morphometrical analyses were done. The difference between the measured area and the area occupied by tissue structures was named the "tissue clearing". With the tissue clearing many morphological changes were integrated and their quantification was enabled. This morphometrical feature offers the opportunity to determine standard values for normal human tissues, quantification of the tissue damages and also their comparative analysis.



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Histological evaluation of sentinel lymph nodes at breast cancer

KEYWORDS: Brest Neoplasms; Lymph Node; Immunohistochemistry

The use of the Sentinel Concept as the newest approach in the surgical treatment of breast cancer, apart from cancerectomy, means the extirpation and analysis of one or more regional lymph nodes which receive the lymph from a primary tumor (guardian lymph nodes - sentinel nodes). The presence or the absence of secondary deposit in them is considered the most important predictor of the positive secondary deposit status of other axillary glands. Depending on the histological analysis of sentinel gland, the operation is finished with or without the evacuation of the fat tissue ipsilateral to the axilla. Sentinel concept in surgical treatment of the breast cancer is applied only in the cases where the largest dimension of the tumor does not exceed 2cm (pT1). Marking of the sentinel node is conducted preoperatively by a combined method, the usage of Patent Blau colour and Technicium 99m as a radio marker. Sentinel lymph nodes identity, before sending to a histopathological analysis, is checked by a gamma camera. Two groups of prospective tests (A and B) were formed with 20 cases each. Sentinel lymph nodes from group A in the first stage of the testing were treated by the histopathological method (one HE preparation) and then microscopied. The cases where a secondary deposit was not noted, were subjected to a serial cutting and immunohistochemical analysis by the EMA markers, specific for intraductal epithelium of the breast, manufactured by "DAKO". Sentinel nodes from group B were immediately delivered for the *ex tempore* analysis, where they were approached with a series of cuttings and immunohistochemical analysis by the EMA markers. The nodes in which no secondary deposits were detected were then paraffin-embedded and, with the application of immunohistochemistry (EMA), submitted to serial cutting (similar to the second phase in group A) as a precaution. The authors obtained significant data in both groups of testis and determined the sensitivity, specificity and efficiency of the applied methods. The acquired data suggested the implementation of one of the methods from the Sentinel Concept. The application of the suggested method would spare a considerable number of patients with pT1 breast cancer from subsequent extirpation of the fat tissue from the axilla and reduce the number of postoperative complications.

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p53 mutations and distribution of high-risk HPV types in women with premalignant lesions of the cervix

KEYWORDS: Human papilloma virus; p53 protein; Polymerase chain reactions

Human Papillomviruses are accepted as etiologic agents for premalignant cervical lesions as well as cervical carcinoma. The purpose of this study was: a) to determine the frequency of high-risk human papillomavirus (HPV16, HPV18, HPV31 and HPV33) infection among women with SIL (squamous intraepithelial lesions), which are considered as preinvasive stages of cervical cancer and b) to investigate the status of p53 tumor suppressor gene in HPV positive and HPV negative women. The histopathological analysis of 98 cervical swab samples classified patients into two groups: 92 were LSIL (low-grade SIL) and 6 were HSIL (high-grade SIL). DNA was isolated by phenol/chloroform/isoamylalcohol extraction. HPV genome was detected by PCR, and the analysis of p53 (5, 6, 7 and 8 exon) was performed using the SSCP method. 36 (36.7%) patients were HPV positive and eight of them were double positives. HPV31 was the most frequent HPV type (11.2%). 83.3% of HSIL and 33.4% of LSIL patients were HPV positive. Point mutations of p53 were detected in only two cases, both HPV positive and diagnosed as HSIL. Our study showed that high-risk HPV types are important for the transition of LSIL to HSIL. The fact that both p53 mutations were detected in HSIL patients suggests that the loss of wild-type p53 function is important for etiology of HSIL and cervical cancer. In contrast to some authors, we did not find inverse correlation between HPV infection and p53 mutations.



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Molecular detection of B-lymphocytes clonality in patients with "Hairy cell" leukemia

KEYWORDS: "Hairy cell" leukemia; B-lymphocytes; Polymerase chain reactions

"Hairy cell" leukemia (HCL) is found in only 2% of leukemias. The median time of survival had been about four years before the new therapies were introduced. Detection of "hairy" cells with the characteristic morphological shape in the peripheral blood or bone marrow is essential for the diagnosis of the disease. However, besides histopathological, cytogenetic and immunophenotype analyses, detection of the dominant clone of lymphocytes on the molecular level is sometimes essential to diagnose lymphoproliferative disorders. Persistence of a minimal residual disease (MRD) is usually detected by polymerase chain reaction (PCR) using the uniformity of rearranged heavy chain of the immunoglobulin gene (IgH). The most important aim of therapy is to provide the minimal risk for patients. That's the reason why detection of MRD is important, especially in the patients with a total clinical remission. In this study MRD was determined in 19 patients with HCL who were submitted to different therapies. DNA was isolated by phenol extraction from mononuclear cells of the peripheral blood. The segment of IgH gene between FRIII and JH was amplified by PCR. The products of PCR reaction were analysed by 10% PAAG electrophoresis. The positive result (monoclonality) was found in 7/19 (36%) samples from patients with HCL. In all other samples polyclonality was found. Also, polyclonality was found in all samples from healthy persons.

Epithelioid vascular tumor of the upper cava vein

KEYWORDS: Vascular Neoplasm; Immunohistochemistry; Bone Neoplasms

Diffuse large B cell lymphomas (DLBCL) represent a heterogeneous entity that includes centroblastic (CB), immunoblastic (IB) and B-cell large anaplastic lymphomas. The aim of our study was to confirm the prognostic relevance of highly heterogeneous DLBCL from both a clinical and histopathological point of view. We analyzed 65 pts with de novo DLBCL and categorized them clinically by the international prognostic index (IPI). Biopsy specimens (47 lymph nodes and 18 bone marrows) were diagnosed by the histomorphology and by immunohistochemistry. The paraffin embedded samples were analyzed for the expression pattern of bcl-6 protein, Caspase-3, PARP and protein for VEGF. All results were statistically confirmed. The histopathological analysis showed 55 pts with CB and 10 pts with IB DLBCL. The IPI was low in 36.92%, low intermediate in 29.23%, high intermediate in 27.69% and high in 6.15% of the patients. The IB pts had high intermediate and high IPI. During the 48 months follow-up period 38/65 pts (58.46%) are alive and under control (ten IB pts died). All analyzed samples were bcl-6+, but in 48 CB we noticed statistically significant strong positivity (>75% cells), $p=0.04$. The similar results were obtained for Caspase-3. PARP was strongly positive in 45 pts, mainly of CB type. There was no statistically significant differences between CB and IB in VEGF expression but we observed 10 completely negative reactions in CB pts only. Together with bcl-6 and caspase-3, IPI can be used as a clinical marker to differentiate CB and IB DLBCL, and they can serve as valid prognostic markers in DLBCL.



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Her2 protein as diagnostic aid in Paget`s disease of breast

KEYWORDS: Breast Neoplasms; HER2-Neu; Immunohistochemistry

HER2 is a member of tyrosine kinase growth factor receptor family. It has prognostic and predictive value and represents the target for monoclonal antibody based therapy. About 25-30% of breast carcinomas show overexpression of the HER2 protein. Almost all breast carcinomas with epidermotropism are HER2 positive. Paget`s disease of the breast may represent a diagnostic problem, especially in cases with no detectable tumor. By the use of histochemical (e.g. PAS-Alcian blue) and immunohistochemical methods (e.g. cytokeratin 7) the diagnosis can be reached, but it can be difficult in some cases. Ten biopsies of Paget`s disease of the breast were analyzed for the expression of the HER2 protein. The immunohistochemical reaction ranging from moderate to strong intensity was present in all cases. The reaction is evident in atypical cells as well as in the cells which can not be appreciated as malignant solely on the morphological basis. In one case the diagnosis was reached by means of IH detection of the HER2 protein. The immunohistochemical analysis of the HER2 protein can be used as adidiagnostic aid in Paget`s disease of the breast.

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Imunohistochemical analysis of neural markers (GFAP and NSE) in normal salivary gland tissue and pleomorphic adenoma

KEYWORDS: Salivary gland Neoplasms; Pleomorphic adenoma; Immunohistochemistry

Numerous immunohistochemical studies were performed in order to explain the cellular characteristics of pleomorphous adenoma (PA). Biological behaviour of salivary glands is variable and PA with benign histological characteristics can give recurrences, locally aggressive behaviour and malignant transformation. 20 cases were selected. These were foxed in 5% formalin, and then set in formalized blocks. Polyclonal antibodies DAKO Products were used (Glostrup Denmark). The results were obtained through a CCD camera visual information quantification system. Ten areas were photographed for every individual case at a magnification level of 400X. In the normal salivary gland tissue the NSE positivity was registered in the striate and excretory ducts, while the acinuses, myoepithelial and basal cells were negative. Reactivity to GFAP in the normal tissue was recorded only in the myoepithelial cells. Our study showed an irregular NSE positivity in tumour cells that produced solid areas. The cells of the outer luminal layer showed a clear positivity. The mixoid structures were less positive while the chondroid gave a strong immunosensitivity. The modified myoepithelial cells showed a mediocre positivity. After GFAP staining positivity was registered in mixomatous and chondromatous areas. Luminal cells were negative, while the outer cells were positive, as were the spindle-shaped. NSA and GFAP positivity gives us an argument more for further investigation and determination of the neuroectodermal origin of the pleomorphic adenoma.



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Cytogenetic follow up of the patients treated by autologous stem cell transplantation

KEYWORDS: Stem cell transplantation; Cytogenetic aberrations; Hematologic neoplasms

Malignant transformation of the cell is often associated with nonrandom chromosomal aberrations. Hematologic malignancies comprise about 70% of all described cytogenetic aberrations. Cytogenetic aberrations are significant for diagnosis and prognosis in this group of malignancies. The new therapeutic approach which has been in use in recent years includes high dose chemotherapy with autologous stem cell transplantation (SCT). After the application of this therapeutic treatment chromosomal aberrations, detected at diagnosis, are markers of a minimal residual disease. In this study we present the group of 7 patients (chronic myeloid leukemia (CML)-1, acute myeloid leukemia (AML)-3, acute lymphoblastic leukemia (ALL)-1, Hodgkin disease (HD)-1 and myelodysplastic syndrome (MDS)-1) with a pathologic karyotype detected at diagnosis, treated by autologous SCT. Cytogenetic analysis were done on bone marrow chromosomes after 24h culture and direct preparation by HG banding method. At least 20 metaphases were analysed. The interval of cytogenetic follow up was from one month to three years. In 6 patients we found only the normal karyotype after SCT and cytogenetic remission was achieved. In the patient with HD we found multiple chromosomal aberrations after SCT. One patient relapsed after the cytogenetic treatment. The patient with CML exhibited a "minor" cytogenetic answer of 35%. The cytogenetic analysis is a useful method for monitoring a minimal residual disease. Introduction of more sensitive methods of molecular pathology is necessary for this group of patients.

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Detection of B lymphocyte clonality in the samples of salivary gland tissue of patients with Sjogren syndrome

KEYWORDS: Sjogren Syndrome; B Lymphocyte; Polymerase Chain Reactions

Sjogren's syndrome is a systematic chronic autoimmune inflammatory disorder of exocrine glands, primarily manifested through reduced or absent secretion of saliva and tears. The altered function of exocrine glands is caused by continuous activity of infiltrated lymphocytes and plasmacytes. Although B lymphocytes represent about 20% of a mononuclear infiltrate, they have a dominant role in pathogenesis characterized by local auto-antibody production and by regulation of autoimmune response mediated by T lymphocytes. Additionally, these patients demonstrate about 40 times higher risk of developing lymphoma originating from malignantly transformed B lymphocytes that infiltrate exocrine glands. Consequently, determination of dominant B lymphocyte clones in the salivary gland tissue could have a great predictive value in monitoring the patients with Sjogren's syndrome. The aim of our study was to demonstrate clonality of B lymphocytes infiltrating the salivary glands tissue of Sjogren's syndrome patients. DNA samples were isolated from minor labial gland biopsies from 32 Sjogren's syndrome patients hospitalized in the Clinic for rheumatology, MMA. After PCR amplification of the CDR3 immunoglobulin heavy chain gene region, B lymphocyte clones were identified as dominant bands on polyacrilamide gel electrophoresis. The presence of a single dominant B lymphocyte clone (monoclonal) was identified in 37.5% of the patients, the presence of several clones (oligoclonal) was identified in 25%, while a polyclonal type of the B lymphocyte infiltration was identified in 37.5% of the patients' samples. The monoclonal infiltration of B lymphocytes in almost 1/3 of the samples identifies Sjogren's syndrome patients at the high risk for lymphoma development.

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Application of immunohistochemical methods and CAMIA software in the analysis of dendritic cells (DCs) and interstitial tubular epithelial cells (ITEC) - New prognostic factors for acute and chronic allograft rejection

KEYWORDS: Nephropathy; Immunohistochemistry; Image Analysis

The intensity of an inflammatory infiltrate is already in use for Banff classification of acute renal allograft rejection and chronic renal allograft nephropathy. The severity of the damage of blood vessels has a good correlation with bad prognosis of transplanted kidney, yet when blood vessels are severely damaged, it is too late for any efficient therapy. The aim of this study was to find new parameters that may serve as new prognostic factors in the stage before terminal rejection, when the therapy may have better results. We decided to analyze the occurrence of DCs in different types and stages of acute and chronic kidney allograft rejection. DCs in the interstitial space of transplanted kidney originate from a recipient and as a professional antigen presenting cells (APC) they have an important role in the control of immune reactions at the level of the graft. For visualisation of DCs we chose antibody against S-100 protein. In normal kidney there are no DCs but they are present in the interstitial inflammatory infiltrate in some kidney diseases. The only elements that are expected to be stained in normal kidney were nerve fibers. However, the distinction between DCs and nerve fibers was easy. For counting, CAMIA programme (Computer Aided Medical Images Analyzer) has been used. This programme is supreme in semiautomatic counting and morphometric measurement of cells stained with immunocytochemical methods. Fine needle biopsies from renal transplants of 17 patients as well as from 7 patients with different forms of GN (FGS, IgAGN, MPGN, RPGN) as a control group were 4% formaldehyde fixed and paraffin embedded. All PAS stained cases of rejection were classified according to the Banff system. All cases from experimental and control group were stained with antibody against S-100 protein (1: 1000), LSAB+ kit and DAB (DAKO). Three microphotographs (848x640 pixels) were taken for each patient from the control and experimental group to provide the surface of 0,83 mm² for each of the following renal

elements; glomeruli, tubules, interstitium and blood vessels. For morphometric study of digitized pictures a specialized software CAMIA has been used. For statistical analysis we used 2 WAY ANOVA test from the statistical software of the Institute for Medical Statistics, School of Medicine, University of Belgrade. While we were counting S-100 positive DC in the renal interstitial space we found some tubules with cytoplasmic and nuclear S-100 positive staining of tubular epithelial cells (TEC). Additionally, in the interstitial space there was yet another cell population of S-100 positive cells in the interstitial space with the same staining pattern as those cells that were a part of tubular structures. We hypothesized that in the interstitial space there were two different S-100 positive cell populations; DCs and TEC that have changed their localization and morphology with the process of epithelial to mesenchymal transition that is often called transdifferentiation. We found medial mathematical surface area for interstitial TEC to be 332.29nm² while for DC it was 73.75nm². We found a statistically highly significant difference in the size of these two cell populations with interstitial TEC being always much bigger. Furthermore, with our aforementioned criteria for discrimination of these two cell populations, we were able to calculate the relative number of DCs and ITEC for each case. For the control group we did not find significant difference in the number of DC but we did find a significant decrease in the number of DC during the process of acute and chronic rejection. We compared the occurrence of DC and ITEC in acute and chronic rejection because of the possible interplay between recipient's DCs and donor's interstitial TECs that may act as a kind of non-professional APC in the control of the immune reactions at the level of the graft. In acute rejection, both cell populations were simultaneously disappearing. On the contrary, there was a statistically highly significant difference in the number of these two cell populations with the progression of chronic rejection. It is important to note that the decrease of DCs is statistically highly significant event during a chronic rejection. We report for the first time that S-100 protein could be a useful marker for transdifferentiation of tubular epithelial cells. Until now, this epithelial to mesenchymal transition or the process of transdifferentiation of TEC has been reported by other authors but with alpha-SMA. We suggest for the first time DCs as well as the ratio between DC and interstitial TEC as new prognostic factors in chronic allograft nephropathy. Finally, we recommend CAMIA and S-100 immunostaining as potent tools in assessment of these new prognostic factors for kidney allograft rejection.