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POSTERS

4-HYDROXYNONENAL IN HUMAN AND ANIMAL CHRONIC LIVER DISEASES

M. Ćorić[1], Ž. Grabarević[2], M. Štroser[3], L. Andrišić[3], G. Waeg[4], S. Seiwerth[5], I. Hrstic[1], L. Brčić[5], N. Žarković[3]

[1]Clinical Department of Pathology and Cytology, Zagreb University Hospital Center, Zagreb, [2]Veterinary Faculty, University of Zagreb, Zagreb, [3]Ruđer Bošković Institute, Zagreb, [4]Institute of Molecular Biosciences, Graz, Austria, [5]School of Medicine, University of Zagreb, Zagreb, Croatia

The aldehyde 4-hydroxynonenal (HNE), a peroxidation product of polyunsaturated fatty acids, is considered the most reliable indicator of endogenous lipid peroxidation *in vivo*. Lipid peroxidation is an autocatalytic mechanism leading to oxidative destruction of cellular membranes. Oxidative stress is an epiphenomenon of hepatitis and a pathogenic factor of liver cirrhosis, while HNE acts as a growth regulating factor and signaling molecule. The aim of the study was to investigate the presence of HNE-modified proteins in human and animal cirrhotic liver by immunohistochemistry. Thirty fixed paraffin-embedded cirrhotic liver samples were retrospectively selected for the study. There were 4 cases of cardiac liver cirrhosis in dogs and 30 cases of various human chronic liver diseases in the stage of cirrhosis. There were 15 cases of chronic hepatitis C and B, 4 cases of hemochromatosis, 4 cases of Wilson's disease, and 3 cases of alcoholic cirrhosis. Slides of paraffin-embedded tissue were prepared for immunohistochemistry with monoclonal antibodies to HNE-histidine conjugate. Positive immunohistochemical reaction to HNE was analyzed semi-quantitatively. Intracellular HNE adduct localized in hepatocyte cytoplasm was detected in 25 of 30 cases (83%). The strongest staining was noticed in cases of Wilson's disease, hemochromatosis, alcoholic liver diseases and cardiac liver cirrhosis in dogs. Weak staining was observed in cases of chronic hepatitis C and B. The results of the study indicated that HNE could be detected in several chronic human and animal liver diseases. Therefore, detection of lipid peroxidation could be used in predicting development of fibrosis and cirrhosis in chronic liver diseases.

REINKE'S CRYSTALS IN BIOPSIES OF CRYPTORCHID TESTES

V. Kozina[1], Lj. Banek[1], I. Weber[2], D. Ježek[1]

[1]Department of Histology and Embryology, School of Medicine, University of Zagreb, [2]Division of Molecular Biology, Ruđer Bošković Institute, Zagreb, Croatia

Reinke's crystals are normal constituents of Leydig cells in humans but their nature and function are poorly understood. The crystals resemble hexagonal prisms of variable size (around 3 μm). They are composed of parallel 10-nm filaments and do not have a unit membrane. Sometimes the crystals cause deformity of the nucleus and infolding of the nuclear membrane of Leydig cells. In some cases, Reinke's crystals are observed like tiny rods within the nucleus. The aim of the study was to investigate the properties of Reinke's crystals in healthy and cryptorchid men. We used 20 biopsies from patients with cryptorchidism and 6 biopsies from men with normal spermatogenesis (age 20-30 years). Tissue was fixed in Gendre, embedded in paraffin and serially sectioned into 7-μm thick sections. After staining with modified Masson's method, specimens were observed and stereologically analyzed. To get closer look of the shape and placement of crystals we used Leica confocal microscope. Stereological analysis revealed a significant increase in the number of Reinke's crystals within cryptorchid testes in comparison with controls. The images obtained on confocal microscope showed a hexagonal form of the crystal. Placement of the crystals was not restricted to Leydig cells only, since the crystals could be found within the rest of the interstitial compartment.

THE INCIDENCE AND PROGNOSTIC VALUE OF HISTOLOGIC CHANGES IN BONE MARROW BIOPSIES FROM PATIENTS WITH NON-HODGKIN'S LYMPHOMA

G. Petruševska[1], A. Stojanović[2], L. Cevrevska[2], S. Stanković[2], B. Georgievski[2], R. Jovanović[1], L. Hagi-Pecova[2], O. Karanfilski[2], M. Jovanovski[1]

[1]Institute of Pathology, [2]University Department of Hematology, School of Medicine, Skopje, R. Macedonia

The aim of the study was to evaluate histologic changes in the bone marrow of patients diagnosed with non-Hodgkin's lymphoma (NHL) and to determine their prognostic value. The study included 189 patients diagnosed with NHL in nodal and extranodal tissue and undergoing bone marrow biopsy for staging of the disease. The patients were followed-up for nine years, with a minimal follow up period of 4 months. Clinical data were obtained from patient histories. Standard histochemical and immunohistochemical staining was done on decalcified, formalin fixed and paraffin embedded bone marrow biopsies. Histologic analysis of bone marrow biopsy specimens showed the presence of lymphomatous infiltration in 79 (41.8%) cases, which yielded no significantly different influence on survival between the groups. The presence of tumor myelopathy was found in 117 (61.9%) cases, associated with worse survival than the rest of study patients. The patients with osteolytic and osteoporotic changes (14.3% and 35.4%, respectively) had worse survival than those with osteosclerotic (5.8%) and normal bone trabecules (44.4%). The probability to survive was better in the group of patients with normal cellularity than in those with aplastic bone marrow and hypercellular marrow spaces. The presence of dysplasia in the three cell lines was an unfavorable prognostic factor: 32.8%, 29.8% and 29.1% of patients with dysplasia survived for 36 months of the disease onset; at longer period these percents fell down to 10.9%, 9.9% and 9.7%. The increased number of eosinophils had no influence on survival. Changes in the stromal tissue had no significant effect on survival in patients with NHL.

TRANSMISSIBLE VENEREAL TUMOR IN MALE DOGS IN THE USA: INCIDENCE AND CHARACTERISTICS

S. Tkalčić[1], M. Blauvelt[2], R. E. Moreland[2]

[1]Western University of Health Sciences, Pomona, [2]Antech Diagnostics, Irvine, CA, USA

Transmissible venereal tumor (TVT) is a round cell tumor primarily of the mucous membranes and skin of the external genitalia in dogs of both sexes. TVT is a typically sexually transmitted neoplasia that can also occur on different sites of the body due to patterns of sexual behavior. They can spontaneously regress, and can be managed either by surgical excision and/or chemotherapy (Vincristine), depending on the location and extent of the disease. In this study, we analyzed Antech diagnostics database for a 3-year period to establish recent trends in the occurrence and distribution, as well as the incidence of this tumor in male dogs across the USA. During the 2006-2008 period, of a total of 224 recorded male dog cases submitted for cytology and biopsy that matched the search for diagnosis of TVT, 108 carried a definitive diagnosis of TVT. In these cases, the predominant location of tumor occurrence included penis and prepuce. Distant sites included nostril, nasal cavity and anus. In the remaining 116 cases, the tumor was diagnosed as a round cell tumor and TVT was considered one of the main differential diagnoses, along with histocytoma, amelanotic melanoma, plasmacytoma, or a mast cell tumor. In these cases, the tumor was less differentiated and excised from various locations of the body.

COMPARATIVE STUDY OF PERITUBULAR TISSUE IN HUMAN AND RAT AGING TESTES

I. E. Plesea[1], O. T. Pop[1], S. D. Enache[2], E. Mandache[3], M. Gherghiceanu[3], B. Stanoiu[1], A. Butaru[1]

[1]University of Medicine and Pharmacy, Craiova, Romania, [2]Emergency County Hospital, Craiova, Romania, [3]Victor Babes National Institute, Bucuresti, Romania

The thickness of peritubular tissue (PT) of human and rat aging testes was assessed and compared. The study material consisted of testicular tissue samples from 28 cases with orchiectomy for prostate adenocarcinoma and from 12 Wistar rats. Tissue samples were processed by the classic histologic technique and stained, on serial slides, with hematoxylin-eosin, Goldner trichrome and immunomarked for smooth muscle actin. Images were acquired and measured with image analysis software. The assessed parameter was thickness of PT (PT-Th). Thirty seminiferous tubules (ST) were randomly selected for each case with X40 objective and 5 random determinations for each ST were performed. The mean PT-Th (M-PT-Th) for each ST, case and age group was calculated. Regression line (RL), slope (m) and slope significance test (P) were calculated for comparison between human and rat PT-Th. Human M-PT-Th was 6.6 μm, with discretely decreasing RL and statistically non-significant P value. The internal LP layer quite commonly revealed areas of collagen focal denseness, with frequent foci of hyaline degeneration, occa-

sionally showing circumferential, collar-like distribution around the ST. Rat M-PT-Th was around 2.5 μm, with no significant trend of variation with age. PT revealed a simpler architectural pattern than the human one and no morphological changes were observed in different age groups. In conclusion, human PT underwent degenerative changes with aging, especially in its internal layer, with a "mosaic", focal distribution and no tendency to advance with aging. In contrast, rat PT showed no morphological changes with aging. Neither human nor rat PT showed any variation in thickness with age.

UROTHELIAL CARCINOMA OF URINARY BLADDER WITH SQUAMOUS, OSTEOSARCOMATOUS AND PLASMACYTOID DIFFERENTIATION: A CASE REPORT

Z. Marušić[1], G. Krajačić-Jagarčec[2], D. Trnski[3], B. Krušlin[1]

[1]Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, [2]Zabok County Hospital, Zabok, [3]University Department of Urology, Sestre milosrdnice University Hospital, Zagreb, Croatia

Urothelial carcinoma has a propensity for divergent histologic differentiation. Divergent differentiation frequently parallels high grade and high stage urothelial cancer. Sometimes a broad spectrum of bladder cancer variants can be encountered in a single bladder tumor specimen. The most common differentiation pattern is the squamous one, which is defined by the presence of intercellular bridges or keratinization and occurs in 21% of urothelial carcinomas of the bladder. Other variants include nested, microcystic, micropapillary, lymphoepithelioma-like, lymphoma-like, plasmacytoid, sarcomatoid, giant cell and undifferentiated variant. We report a case of an 83-year-old man with urothelial carcinoma of the urinary bladder harboring plasmacytoid and osteosarcomatous foci along with a more common pattern of squamous differentiation. According to the literature, the most common heterologous element in the sarcomatous variant is osteosarcoma. It is important to emphasize that divergent differentiation must be noted, as it usually carries a worse prognosis, in part probably due to the fact that it parallels high grade urothelial cancer. Squamous differentiation seems to be an unfavorable prognostic factor, being predictive of a poor response to radiation therapy and possibly also to systemic chemotherapy.

FOLLOWING THE FOOT-PRINTS OF LJUDEVIT JURAK

M. Mlinac-Lucijanić[1], T. Bujas[1], I. Pavić[2]

[1]Department of Pathology and Forensic Medicine, Karlovac General Hospital, Karlovac, [2]Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia

„Show me the manner in which a nation or community cares for its deads and I will measure with mathematical exactness the tender sympathies of its people, their respect for the laws of the land and their loyalty to high ideals". Gladstone

Since the days of Professor Ljudevit Jurak who is the honest man skilled in forensic medicine and death investigation in Katyn Forest Massacre in Poland, until today, investigations of mass death and skeletal remains remain an imperative for providing physical evidence of the crimes committed. Forensic science is a fundamental transitional justice issue as it is an imperative for providing physical evidence of the crimes committed and a framework for interpreting evidence and prosecuting violations according to the International Humanitarian Law (IHL). The evaluation of evidence presented in IHL trials and the outcomes of various rulings by such courts, the accuracy and validity of the methods to be applied in future investigations are necessary to ensure scientific quality. Accounting for biological and statistical variation in the methods applied across population and the ways in which such evidence is used in various judicial systems are important because of the increasing amount of international forensic casework being done globally. The excavation of graves, examination of their contents, and analysis of the remains have an additional purpose, i.e. to collect forensic evidence that would permit prosecution of those responsible for mass graves, crimes against humanity and international human rights violation. Yet, the numbers of unidentified victims remain high due to the lack of ante mortem medical and dental records, or simply because of unavailability of more expensive methods of identification. Answers to these issues are essential to promote reconciliation and justice, give the rights to the dead, and provide moral and emotional satisfaction to the living at any time possible.

NEUROENDOCRINE TUMOR IN LARGE BOWEL BIOPSY

I. Veliki-Dalić[1], I. Pavić[2], A. Demirović[2], D. Baličević[2]

[1]Department of Pathology, University Hospital for Tumors, Zagreb, [2]Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia

Large intestinal carcinoid tumors account for approximately 6% of all neuroendocrine tumors (NETs), with an equal sex distribution and incidence of 0.07-0.11 to up to 0.21 cases *per* 100,000 population *per* year. It is reported at any age from 9 to 83 years, at 64 to 66 years of age on an average. We analyzed gastrointestinal endoscopic biopsies for carcinoid with respect to histopathologic particularities. In this retrospective cross-sectional study, 6500 gastrointestinal endoscopies routinely performed at Department of Gastroenterology from January 1, 2008 till March 31, 2009 were analyzed. The diagnosis was established according to a set of typical histological findings with standard hematoxylin and eosin staining and immunohistochemistry (synaptophysin, chromogranin). Of all gastrointestinal biopsies analyzed, there were 5 (0.08%) cases of neuroendocrine tumor. Three of these 5 cases were found in the stomach, and one in the duodenum and colon each. In addition, five carcinoids from our database were diagnosed after wide intestinal resection, where one specimen was from the small intestine, two specimens were from the ascending colon, one from the appendix, and one was located in the rectosigmoid. Colonic neuroendocrine tumor is associated with worst prognosis among all gastrointestinal carcinoid tumors, with an overall 5-year survival of 33% to 42%. The features such as tumor size and microinvasion tend to be less useful on assessing the prognosis of colonic carcinoid than in other gastrointestinal locations, because at the time of presentation the tumor exceeds 2 cm in size and involves the muscularis propria. Large bowel endoscopic biopsy is a helpful tool in diagnosing NETs, thus regular clinical examinations in the population at risk may improve the overall survival rate.

PROSTATE-SPECIFIC ANTIGEN AND GLEASON GRADING OF PROSTATE CANCER: REVIEW OF COMPUTER DATABASE 1994-2007

M. Mlinac-Lucijanić[1], T. Bujas[1], D. Katušin[2]

[1]Department of Pathology, [2]Department of Urology, Karlovac General Hospital, Karlovac, Croatia

Adenocarcinoma of the prostate is one of the most common malignant tumors in men over 50 years of age. It is characterized by an aggressive clinical course and heterogeneous multimodal histomorphological changes. Prostate-specific antigen (PSA) is the most reliable serum marker in the diagnosis and observation of prostate carcinoma, while Gleason scoring system is a generally accepted method to determine histological grade. The aim of the study was to analyze cases of prostate carcinoma in biopsy material obtained during a 14-year period and to compare PSA level and Gleason score in patients that underwent radical treatment. We used computer database of Departments of Urology and of Pathology, Karlovac General Hospital, for the period from January 1, 1994 to December 31, 2007. During the study period, prostate cancer was diagnosed in 603 patients at Department of Urology, Karlovac General Hospital. Tumors were classified as localized ($T_{1-2}N_0M_0$) in 238 (39.5%), locally advanced ($T_{3-4}N_0$-$_1M_0$) in 244 (40.5%), and metastatic ($T_{1-4}N_1M_1$) in 121 (20.0%) patients. Radical treatment was used in 115 (19.1%), hormonal therapy in 445 (73.8%), and watchful waiting was recommended in 43 (7.1%) patients. This non-controlled study included data on 115 patients with adenocarcinoma located in the prostate that underwent prostate biopsy. All these patients received radical treatment, i.e. radical prostatectomy in 64 (55.7%) and external beam irradiation with curative intent in 51 (44.3%) patients. We compared Gleason score, PSA and pathological features of surgical specimens.

The mean patient age was 65.0 (range 51-75) years in the surgically treated group and 69.2 (range 59-77) years in the radical radiotherapy treated group. T-stage was lower, but not significantly in the surgical group ($p=0.07$), with 53 (82.8%) cases classified as pT_{1-2} and 11 (17.2%) cases as pT_{3-4}. Among irradiated patients, 34 (66.7%) were classified as cT_{1-2} and 17 (33.3%) as cT_{3-4}. Positive lymph node was found in 4 (6.2%) surgically treated patients during pelvic lymph node dissection. The radical prostatectomy group patients were signifi-

cantly younger and had lower PSA value, while there was no significant difference between the radical prostatectomy group and radiotherapy treated group according to pathological grade (Gleason score). In surgically treated patients, Gleason score 2-6 was found in 49 (76.5%) and Gleason score 7-10 in 10 (15.7%) patients. In the group of radiotherapy treated patients, 35 (68.6%) were classified as Gleason 2-6 and 10 (19.6%) as Gleason score 7-10 ($p=0.49$). The preoperative PSA level was significantly lower ($p<0.001$) in the surgically treated group (mean 9.9, range 0.71-33.7 ng/mL) as compared with patients undergoing radiotherapy (mean 20.0, range 1.8-92.0 ng/mL). In both groups, the mean PSA level was generally higher in prostate cancers with lower Gleason score than in cancers with higher Gleason score, except for the years 2002, 2006 and 2007. It was concluded that improvements in diagnostic work-up, PSA testing in particular, resulted in an increased number of cases with early stage disease, and PSA level did not correlate anymore with the degree of tumor cell differentiation as evaluated by the Gleason score. That is why the preoperative PSA level was significantly lower ($p<0.001$) in the surgically treated group (early disease) as compared with patients undergoing radiotherapy (T staging was lower than in the surgically treated group).

COMPARISON OF MAGE-A3/4 AND NY-ESO1 EXPRESSION WITH PROGNOSTIC MARKERS IN CHROMOPHOBE RENAL CELL CARCINOMA AND RENAL ONCOCYTOMA

J. Slipac[1], L. Stemberger[1], A. Demirović[1], S. Bulimbašić[2], M. Milošević[3], Z. Marušić[1], H. Čupić[1]

[1]Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, [2]Department of Pathology, Dubrava University Hospital, [3]Andrija Štampar School of Public Health, School of Medicine, Zagreb, Croatia

The cancer-testis family of antigens is expressed in a variety of malignant tumors. They are not expressed in normal human tissues, except for the testis. Expression of two genes from this family, MAGE-A3/4 and NY-ESO1, has been described in different malignant tumors such as melanomas, germ cell tumors, and uterine neoplasm. To our knowledge, there are no studies on the expression of these genes and their proteins in renal tumors. The aim of this study was to investigate the expression of MAGE-A3/4 and NY-ESO1 in renal oncocytomas (RO) and chromophobe renal cell carcinomas (CRCC) detected by immunohistochemistry and to compare the expression of MAGE-A3/4 and NY-ESO1 with other prognostic markers (size, grade) in CRCC. A total of 35 patients (17 RO and 18 CRCC) treated at Sestre milosrdnice University Hospital and Dubrava University Hospital were included. Monoclonal antibodies for MAGE-A3/4 and NY-ESO1 were used for immunohistochemical staining. Results were classified as follows: no positive tumor cells (-), slightly expressed (+) if <10% of tumor cells were positive, moderately expressed (++) if 10%-50% of tumor cells were positive, and strongly positive (+++) if >50% of tumor cells were stained. Results were analyzed using Mann-Whitney test and Spearman's rank correlation coefficient; $P<0.05$ was considered to be statistically significant. The median of age of RO patients was 65 (range 47-80) years, with tumor size median of 3 (range 0.9-8) cm. The median of age of CRCC patients was 58 (range 34-76) years, with median CRCC size of 6.9 (range 1.7-12) cm. Median MAGE-A3/4 expression was (++) in RO and (-) in CRCC. The difference in MAGE-A3/4 expression between the tumors was significant ($P=0.0013$). Median NY-ESO1 expression was (+++) in RO and (-) in CRCC. The difference in NY-ESO1 expression between the tumors was also significant ($P=0.0008$). RO had a significantly higher expression of both antigens. Comparison of the prognostic markers of CRCC (size, grade) with the expression of MAGE-A3/4 and NY-ESO1 antigens showed that there was no significant correlation between antigen expression and these prognostic markers. The same was found on comparing the expression of MAGE-A3/4 and NY-ESO1 antigens with the size of RO.

However, significant correlation was found between the expression of MAGE-A3/4 and NY-ESO1 antigens in both tumors: $r=0.87$ ($P=0.0003$) for RO and $r=0.93$ ($P=0.0001$) for CRCC. This study demonstrated a statistically significant difference in the expression of MAGE-A3/4 and NY-ESO1 antigens between RO and CRCC. Study results also showed the expression of MAGE-A3/4 and NY-ESO1 antigens not to correlate with other prognostic markers in CRCC, however, additional research is needed to explore their potential diagnostic and therapeutic implications.

MORPHOMETRIC ANALYSIS OF CAPSULE IN CHROMOPHOBE RENAL CELL CARCINOMA AND RENAL ONCOCYTOMA

S. Cesarec[1], A. Demirović[1], S. Bulimbašić[2], M. Milošević[3], Z. Marušić[1], H. Čupić[1], B. Krušlin[1]

[1]Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, [2]Department of Pathology, Dubrava University Hospital, [3]Andrija Štampar School of Public Health, School of Medicine, Zagreb, Croatia

Renal cell carcinoma (RCC) accounts for approximately 3% of adult malignancies and 90%-95% of neoplasms arising from the kidney. These epithelial malignant neoplasms are subclassified in five main types: conventional or common RCC, including clear and eosinophilic cell carcinoma, chromophobe RCC, papillary (or chromophilic) RCC, collecting duct carcinoma, and RCC unclassified. Chromophobe RCC is the third most common carcinoma of renal tubular epithelium and accounts for 5% of cases. This variant of RCC must be differentiated from oncocytoma, which shares a common cellular origin. Renal oncocytoma is an epithelial tumor, considered as benign. It comprises about 2%-5% of renal cell neoplasms. Oncocytoma is usually isolated and unilateral, well circumscribed, non-encapsulated. Pathologic differentiation between an oncocytoma and chromophobe RCC may sometimes be difficult. However, the distinction is essential, due to different biological behavior. The aim of this study was to analyze morphometric parameters of capsule in chromophobe RCC and oncocytoma. The files from Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital and Department of Pathology, Dubrava University Hospital, were searched for cases of histologically confirmed chromophobe RCC and oncocytoma. Eighteen chromophobe RCC (8 male and 10 female) and 19 oncocytomas (7 male and 12 female) slides, stained by hematoxylin and eosin, were analyzed. Attention was directed to capsular characteristics, i.e. presence or absence of the capsule and its thickness. Morphometric measurements of capsule were done by a computerized morphometry system with digital camera and microscope. For each tumor one measurement of capsular thickness was made under higher magnification (X200). Statistical analysis was performed using χ^2-test, Spearman's correlation test, Fisher exact test and Mann-Whitney test. The level of statistical significance was set at $P<0.05$. The presence of capsule showed a statistically highly significant difference between the two tumor types observed; the capsule was present in 12 (66.7%) chromophobe RCC cases and only 2 (10.5%) oncocytomas. The mean capsule thickness was 337.7 ìm in chromophobe RCC and 115.4 ìm in oncocytomas. Although the capsule was less thick, if present, in oncocytomas than in chromophobe RCC, the median was not statistically significant due to the small number of cases. The correlation between tumor size, grade and capsule thickness in chromophobe RCC was not significant. Considering age, patients with oncocytoma were significantly older. The distribution of these two tumor types revealed no significant sex differences. In conclusion, making a correct histological diagnosis of chromophobe RCC and oncocytoma may occasionally be difficult due to the overlapping morphological characteristics. Distinguishing renal oncocytoma from chromophobe RCC is essential, knowing that oncocytoma is a benign tumor, while chromophobe RCC has a potentially malignant biological behavior. The aim of this study was to determine the presence of the capsule in chromophobe RCC as a potential diagnostic tool. Although a statistically significant difference in the presence of capsule between chromophobe RCC and oncocytoma was evident, this study was in part limited by the small number of cases; therefore, studies in a larger number of cases are needed.

LYMPH VESSEL DENSITY IN PROSTATIC CANCER AND ADJACENT NONTUMOROUS TISSUE

M. Ulamec, D. Tomas, H. Čupić, M. Vučić, B. Krušlin

Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital and School of Medicine, University of Zagreb, Zagreb, Croatia

According to some authors, lymphatic invasion in radical prostatectomy specimens is an unfavorable prognostic factor in clinically localized prostatic cancer. Lymphangiogenesis within tumor has been associated with lymphatic invasion and lymph node metastases. In this preliminary study, we analyzed density of lymph vessels in prostatic carcinoma and in adjacent prostatic tissue. Neoplastic glands were analyzed in 25 paraffin sections from radical prostatectomy specimens with prostatic adenocarcinoma diagnosis. Specimens were taken from the archive at the Ljudevit Jurak Department of Pathology, Sestre milosrdnice University Hospital, Zagreb. Each sample contained at least 30% of tumorous

tissue and normal prostatic tissue that was used as an internal control. Specimens were immunostained with D2-40 monoclonal antibody, which specifically recognizes lymphatic endothelium. Each slide was examined at X40 and X100 magnification field and counted for lymphatic spaces on 10 high power fields. The number of lymph vessels within tumor tissue ranged from 2 to 28 on 10 HPF (mean 4.3/10 HPF), whereas the number of lymph vessels in adjacent prostatic tissue ranged from 9 to 63 on 10 HPF (mean 21.5/10 HPF). It is concluded that, in contrast to some other malignancies, density of lymphatic vessels is reduced in the intratumoral tissue compared with normal prostatic parenchyma and does not appear to offer useful prognostic information.

PERITUBULAR CLEFTING – A MORPHOLOGICAL CRITERION OF INVASION IN BREAST CANCER?

L. Labinac-Peteh[1], T. Leniček[2], I. Kučinar[1], B. Krušlin[2]

[1]Department of Pathology and Forensic Medicine, Pula General Hospital, Pula, [2]Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia

Microscopic analysis of histologic specimens has the most important place in the diagnosis of breast cancer. Because of many different forms of ductal invasive breast carcinoma, along with the existing ones, additional morphological criteria are needed to distinguish carcinoma from benign breast changes that may resemble cancer. In the presence of tumor cells, surrounding stroma changes, and this become topic of many different cancer studies. There are only few studies on clefting in breast cancer. Stromal change is visible by light microscopy and by clear spaces around tumorous glands named peritubular clefts. Transformation of stroma is necessary for local and distant spread of tumor cells, so analysis of this change may have diagnostic and prognostic significance. Thirty cases of breast carcinoma and thirty cases of benign changes (ten cases of fibroadenoma, fibrocystic mastopathy and sclerosing adenosis each) were analyzed by light microscopy at X400 magnification. We analyzed thirty glands in each cancer specimen and thirty glands in each specimen of benign breast changes, i.e. 900 glands in either patient group. The expression of peritubular clefts was classified according to gland circumference into three gland groups: 0 – without cleft; 1 – cleft to up to 50%; and 2 – cleft >50% of gland circumference. The presence of peritubular clefting differed between the groups of breast cancer and of benign breast changes ($p<0.05$). Correlation of the results on the presence of peritubular clefting with patient age, histologic grade of tumor, and expression of hormonal receptors and HER2/neu protein in the cancer group yielded no statistical association ($P>0.05$). We believe that peritubular clefts occur as the result of interaction between tumor cells and stroma that enables local and distant dissemination and metastasizing.

NEPHROGENIC ADENOMA OF URINARY BLADDER: A CASE REPORT

K. Tomić[1], I. Mahovne[1], D. Dittrich[2], I. Prvulović[1], T. Jakovina[1], Lj. Fuštar-Preradović[1]

[1]Department of Pathology and Cytology, [2]Department of Urology, Dr. Josip Benčević General Hospital, Slavonski Brod, Croatia

Nephrogenic adenoma, also known as nephrogenic metaplasia or adenomatous metaplasia, is a relatively rare condition mostly affecting adult men with a history of urogenital surgery, trauma, calculi, or renal transplant. Most cases occur in urinary bladder, but the condition may also be found in the ureter or renal pelvis. Histologically, the main differential diagnosis is adenocarcinoma. A 65-year-old man presented with hematuria. Cytologic analysis revealed atypical urothelial cells suspect of malignancy. Magnetic resonance revealed only slightly thickened wall of the urinary bladder. Histologically, the lesion consisted of small tubular and cystic spaces lined with bland, cuboidal and hobnail epithelial cells with no mitosis. The lesion extended into the lamina propria. On immunohistochemistry, tumor cells were CK7 positive and CEA negative. One year after the surgery, the patient is well, without symptoms and with normal cytologic report. The case is presented for being rare and to emphasize the need for an increased awareness of the condition, thus to make an accurate diagnosis and to introduce appropriate treatment.

EXPRESSION OF GST-π IN HUMAN PROSTATIC CARCINOMAS AND NONTUMOR TISSUES

E. Lovrić[1], Z. Gatalica[2], E. Eyzaguirre[3], B. Krušlin[4]

[1]Department of Pathology and Cytology, Čakovec County Hospital, Čakovec, Croatia, [2]Department of Pathology, Creighton University Medical Center, Omaha, Nebraska, USA, [3]Department of Pathology, The University of Texas Medical Branch, Galvestone, Texas, USA, [4]Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia

Glutathione-S-transferase-đ (GST-đ) is an enzyme with tumor suppressor function playing role in eliminating toxins and carcinogens from the body. Polymorphisms in glutathione-S-transferases (GST) could predispose to prostate cancer through a heritable deficiency in detoxification pathways for environmental carcinogens. The aim of this study was to analyze the expression of GST-đ in basal, luminal and stromal cells of human tumor and nontumor samples of prostatic tissues. Formalin fixed, paraffin embedded needle core and transurethral resection prostatic biopsies from 42 patients (34 with carcinoma, Gleason scores ranged from 4 to 9, median age 63 years, and 8 with normal prostate gland, median age 65 years) were evaluated with rabbit polyclonal anti-GSTđ (A8000, Oncor) and anti-cytokeratin antibody clone 34ß E12, DAKO). Standard, automated immunohistochemical procedures were used (LSAB2, DAKO). GSTđ was strongly expressed in basal cells in every case of normal prostatic glands, and was also weakly expressed in luminal cells in a number of cases. GSTđ was also detected in stromal cells (fibroblasts and smooth muscle) of normal prostate. Only one case of 34 prostatic carcinomas showed positivity for GSTđ. Stromal cells surrounding carcinomas expressed GSTđ similarly to the normal stromal cells. Basal cells were identified by strong staining with 34ß E12, while prostatic carcinomas were consistently negative, and it was used as a control and for comparison with GSTđ results. In conclusion, GSTđ is expressed strongly and predominantly in basal cells of human normal prostatic glands. This finding is consistent with its proposed role as a tumor suppressor in malignant transformation of prostatic epithelium. However, rare prostatic carcinomas showed cytoplasmic GSTđ expression. Follow up studies are needed to address the prognostic significance of GSTđ aberrant expression in prostatic carcinomas.

UROTHELIAL CARCINOMA WITH AN INVERTED GROWTH PATTERN: A REPORT OF 3 CASES

G. Krajačić-Jagarčec[1], T. Džombeta[2], O. Kraus[3], B. Ružić[3], B. Krušlin[2,4]

[1]Department of Pathology, Zabok General Hospital, Zabok, [2]Department of Pathology, School of Medicine, University of Zagreb; [3]University Department of Urology and [4]Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia

Urothelial (transitional cell) tumors account for about 90% of all bladder tumors. Their presentation varies from benign lesions that rarely recur to highly malignant tumors. While biological behavior of tumor determines its treatment and prognosis, it is very important to differentiate these variable forms. Inverted (endophytic) growth pattern in urothelial carcinoma is particularly difficult to distinguish from inverted papilloma. Morphological criteria alone are not enough and immunohistochemical assessments must also be done, especially in cases of small size biopsy material or transurethral resections. We present a recent series of three patients diagnosed with a low grade urothelial carcinoma with an inverted growth pattern. All patients were male, aged 37, 61 and 69 (mean 55.6) years. The patients presented with painless hematuria. Histologically, the tumors formed nests of atypical urothelial cells. Very few or no mitotic figures were observed. Invasion of lamina propria was present in two specimens. Immunohistochemically, positive staining for p53, cytokeratin (CK) 7 and CK 20 was observed in all three cases. Proliferative activity was assessed using Ki-67 antibody. The percentage of positive stained tumor cells was 5%, 7% and 12%. Immunohistochemical staining for CK 7, CK 20, p53 and Ki-67, which is positive in most carcinomas but not in papillomas, can help us make the right diagnosis. Urothelial carcinoma with an inverted growth pattern and inverted papilloma have similar morphological features but their biological behavior, treatment and prognosis are different. In order to avoid the urothelial carcinoma to be misdiagnosed as a benign papilloma, we find it very important to make additional investigations besides conventional histology.

UNUSUAL MIXED GERM CELL TUMOR OF THE TESTIS CONSISTING OF RHABDOMYOSARCOMA, MATURE TERATOMA AND YOLK SAC TUMOR: A CASE REPORT

E. Lovrić[1], D. Bobonj-Hižak[2], M. Perić-Balja[3], T. Leniček[4], B. Krušlin[4]

[1]Department of Pathology and Cytology, Čakovec County Hospital, Čakovec, [2]Department of Pathology, Bjelovar General Hospital, Bjelovar, [3]Department of Pathology, University Hospital for Tumors, Zagreb, [4]Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia

Testicular mixed germ cell tumors account for 32%-54% of all germ cell tumors. The various types of germ cell tumors can occur in any combination. The most common combination is teratoma and embryonal carcinoma, and also a variant of embryonal carcinoma, yolk sac tumor and syncytiotrophoblastic cells. Unusual cases of rhabdomyosarcoma arising in mature teratoma and rhabdomyosarcoma arising in mediastinal teratoma have been reported in the literature. We present an unusual case of testicular tumor in a 32-year-old man who complained of left testicular swelling and pain. Physical examination, ultrasonography and CT revealed a tumor in the left testis. Macroscopically, it was a grey, well circumscribed tumor measuring 4.5x7x6 cm in size, showing soft and yellow cut surface. There were white and firm areas, and parts of cartilage, with only marginally visible testicular tissue. Also, there was a nodule of 1.2 cm in size. Paraffin-embedded tissue specimens were routinely processed, cut and stained with hematoxylin and eosin. Additional methods performed on representative slides included desmin. Histopathologic examination revealed a yolk sac tumor and mature teratoma associated with embryonal rhabdomyosarcoma. The largest part of the tumor was yolk sac tumor (80%) with a mycrocystic pattern, and glandular structures lined by cells varying from cuboidal or polygonal cells inserted in mesenchymal stroma with perivascular Schiller-Duval bodies. The second part was mature teratoma (15%) consisting of well differentiated cartilage, and cystic formations lined by pseudostratified cuboidal or flattened epithelium surrounded with dense connective tissue stroma. The third part of the tumor was a sarcoma component of somatic type malignancy (5%), described macroscopically as a nodule. Microscopic examination showed aggregates of atypical small cells with hyperchromatism and marked cellular pleomorphism, and some of them were round or elongated with abundant eosinophilic cytoplasm like rhabdomyoblastic cells with numerous mitoses that showed positivity for desmin. Mixed germ cell tumor of the testis in this combination with sarcomatous component is uncommon. Recognition of this variant is important because it may mimic some other lesions, and the clinical outcome of some variants differs from the typical mixed germ cell tumor of the testis and may call for different therapeutic approach.

TESTICULAR PLACENTAL SITE TROPHOBLASTIC TUMOR

N. Knežević[1], S. Nađ-Škegro[1], T. Kuliš[1], M. Čačić[2], M. Ćorić[2], Z. Kaštelan[1]

[1]University Department of Urology, [2]University Department of Pathology and Cytology, Zagreb University Hospital Center, Zagreb, Croatia

Placental site trophoblastic tumor (PSTT) has been sporadically described in male genital tract. Although it is relatively rare, it has been listed in the last WHO classification of germ cell testicular tumors. Firstly, it was identified as a trophoblastic tumor in the uterus. Most of the knowledge regarding its clinical behavior is based on its appearance in female genital tract. It is considered as an infiltrative tumor with malignant potential and variable response to chemotherapy. To our knowledge, we report on the third case of PSTT in male. A 24-year-old man presented with right testicular enlargement. Preoperative serum levels of tumor markers included elevated ß-hCG (7.0 mIU/mL) and normal AFP (2.29 ng/mL). After radical orchiectomy, serum levels of tumor markers were normalized. Histopathologic examination revealed mature teratoma 65% and PSTT 35%. The PSTT component showed high proliferative activity (Ki 67 index in more than 90% of PSTT component), indicating high DNA replication and proliferation. Furthermore, PSTT component was HPL, cytokeratin and inhibin positive, while ß-hCG and AFP were negative. Computerized tomography of the retroperitoneum did not show enlarged retroperitoneal lymph nodes. We decided to do laparoscopic retroperitoneal lymphadenectomy (RPLND) and tumor was not found in 15 lymph nodes. During the 3-year follow up, the patient has been free from recurrence or metastatic disease. Given the potentially malignant behavior of PSTT, unknown course of disease in male and primary his-

topathologic finding, we opted for RPLND. RPLND is a safe and successful method with low morbidity and mortality, and we think it should be considered in patients with PSTT component of testicular tumor with normal postoperative tumor markers and computerized tomography. In patients with PSTT component in whom chemotherapy was performed for retroperitoneal metastasis, we believe that post-chemotherapy laparoscopic retroperitoneal lymphadenectomy should be considered due to the variable PSTT response to chemotherapy.

LIPOID-CELL VARIANT OF UROTHELIAL CARCINOMA: A REPORT OF TWO CASES

S. Bulimbašić[1], E. Lovrić[2], A. Racar-Pačić[1]

[1]Department of Pathology, Dubrava University Hospital, Zagreb, [2]Department of Pathology and Cytology, Čakovec County Hospital, Čakovec, Croatia

Urothelial carcinomas tend to show unusual morphological appearances. Most urothelial carcinomas exhibit papillary or solid histologic growth pattern, which does not pose diagnostic problem. However, in some variants histological features differ significantly from the usual patterns. Case 1: a 56-year-old man developed painless macroscopic hematuria. Cystoscopic examination revealed a bulky papillary tumor on the posterior bladder wall. Transurethral bladder resection was performed. Case 2: a 75-year-old man with microscopic hematuria developed unilateral obstructive changes. Radiologic studies revealed infiltrative lesion in the lower third of the left ureter. Radical left nephroureterectomy was performed. Gross examination revealed a 1.5 cm long segment of the ureter measuring 1 cm in the diameter with lumen obstructed by hemorrhagic mass. The material from transurethral bladder resection and surgical specimen were processed routinely, embedded, cut and stained with hematoxylin and eosin. Additional methods performed on representative slides included PAS-Alcian stain, and immunohistochemical analysis using cytokeratin AE1/AE3, MUC1 and MUC2 antibodies. Microscopic examination revealed a high-grade papillary carcinoma with invasion of the lamina propria and detrusor muscle in case 1, and muscularis propria of the ureter in case 2. In both cases, 20%-30% of the invasive component showed areas with vacuolated tumor cells resembling lipoblasts. Immunohistochemical analysis showed strong positive reaction for CK AE1/AE3. In addition, tumor tissue from case 1 showed positive reaction for MUC1 and MUC2. According to the latest WHO classification, lipoid cell variant is a rare subtype of urothelial carcinoma which exhibits transition to a cell type resembling signet-ring lipoblasts. In most of the previously reported cases, patients were elderly men and all presented gross hematuria as the initial symptom. Usually, this tumor is mixed with conventional urothelial carcinoma. Foci of lipoid cells tend to be found at the periphery or at the invasive edge of the tumor, and for recognition as a distinct variant they should comprise at least 20% of the neoplasm. Immunohistochemically, tumor cells are diffusely positive for cytokeratins AE1/AE3 and negative for S-100 protein. Lipoblast-like cell differentiation of urothelial carcinoma may occur in the urinary bladder as well as in the upper parts of urinary system. In most cases, the tumors are high stage, either when seen on initial biopsy or on subsequent cystectomy/surgical resection. It is important not to confuse this unusual form of urothelial carcinoma with other urothelial lesions or aggressive sarcoma.

THE ROLE OF ELECTRON MICROSCOPY IN THE EVALUATION OF RENAL TUMORS

S. Bulimbašić, A. Racar-Pačić, A. Bauer-Šegvić, D. Ljubanović

Department of Pathology, Dubrava University Hospital, Zagreb, Croatia

Renal tumors comprise a heterogeneous group of neoplasms derived from the renal tubular epithelium, renal mesenchyma or urothelial layer of the renal pelvis and calyces. Most of them, including the most common "clear-cell" type of renal carcinoma, are easily recognizable by single use of light microscopy. However, to diagnose certain variants of renal tumors, it is necessary to perform additional diagnostic tests. The aim of the study was to evaluate the contribution of ultrastructural analysis in achieving the diagnosis of renal tumors. Furthermore, the reasons for ultrastructural analysis and types of tumors submitted for electron microscopy were analyzed. Data on cases of renal tumors ultrastructuraly analyzed during the period from March 2004 to March 2009 were obtained from the files of the Department of Pathology, Dubrava University Hospital. For the purpose of this study, archived microphotographs and digital images of tumor ultrastructure and hematoxylin-eosin slides of all tumors and immuno stains where available were reviewed. Ultrastructural analysis was performed on a Philips EM400T (March

2004-March 2007) and Jeol JEM 1400 (March 2007-March 2009). All photographs and slides were reviewed by two pathologists (S.B. and D.LJ.) and the original diagnoses were confirmed, or modified according to the latest WHO classification. Results of ultrastructural analysis were classified into four categories: "A" inadequate material (poorly preserved, necrotic, etc.); "B" preserved but non-diagnostic material, inconclusive; "C" supportive features; and "D" specific features. During the 2004-2009 period, a total of 37 renal tumors were evaluated by electron microscopy. This cohort included 12 oncocytomas (RO), 10 chromophobe carcinomas (CHRCC), 5 clear-cell renal carcinomas (CCRCC), 2 papillary renal carcinomas (PRCC), 2 urothelial carcinomas (UC), 2 medullary fibromas, 1 neuroendocrine tumor, 1 angiomyolipoma (AML), and 2 "loopomas" (MTSCTK). The reasons for ultrastructural analysis were: electron microscopy as a routine part of diagnostic algorithm in 22, ambiguous immunohistochemistry results in 4, educational purposes and scientific interest in 10 cases, and confirmation of the neuroendocrine differentiation in one case. Results grouped in "A" and "B" categories, where ultrastructural analysis did not contribute to the diagnosis, comprised 5.4%, while results in the "C" (supportive) and "D" (specific) categories comprised 29.6% and 65% of all analyzed cases, respectively. The best results were seen in the oncocytoma and chromophobe carcinoma subgroups (22/22). Our study has confirmed that electron microscopy is a useful technique for the diagnosis of selected types of renal tumors, especially those with specific ultrastructural features. It may also help solve real diagnostic difficulties where the diagnosis has been narrowed but not achieved by use of immunohistochemistry.

RENAL METASTASES OF PANCREATIC MEDULLARY CARCINOMA: A CASE REPORT.

A. Krvavica[1], M. Ulamec[2], L. Pažanin[2], C. Lež[3], H. Čupić[2]

[1]Department of Pathology, Zadar General Hospital, Zadar, [2]Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, [3]Department of Pathology, Zabok General Hospital, Zabok, Croatia

Medullary carcinoma of the pancreas has been recently described as a distinct subtype of ductal adenocarcinoma that may have a unique pathogenesis and clinical course.

We report on a 69-year-old woman with a tumorous mass in the tail of the pancreas. Fifteen years before, the patient underwent mastectomy for bilateral breast carcinoma. Thirteen years later, bilateral adrenal metastases were found and surgically removed. Two years later, follow-up computer tomography revealed pancreatic tumor. The tumor progressed in three months, resulting in kidney metastases and enlarged para-aortal lymph nodes. Partial pancreatectomy with partial nephrectomy and removal of para-aortal lymph nodes was performed. Histopathologic analysis revealed pancreatic medullary carcinoma with neuroendocrine component and metastatic spread to the left kidney and lymph nodes. Although medullary carcinomas are grouped together with poorly differentiated ductal adenocarcinomas, some authors suggest a distinct pathogenetic course and clinical features. Accordingly, recognition of the medullary variant of pancreatic adenocarcinoma is of high clinical relevance.

PRIMARY INTRACRANIAL PLEOMORPHIC LEIOMYOSARCOMA WITH RHABDOID FEATURES: A CASE REPORT

A. Jakovčević[1], S. Dotlić[1], K. Žarković[1], J. Paladino[2]

[1]University Department of Pathology and Cytology, [2]University Department of Neurosurgery, Zagreb University Hospital Center, Zagreb, Croatia

Primary intracranial leiomyosarcomas are uncommon tumors. The origin of such neoplasm could be smooth muscle cells of the vessels, pluripotent mesenchymal cells or embryonic rests from the inner layers of the arachnoid and pia. They are more common in immunosuppressed and HIV infected patients. In this case, the tumor was not associated with AIDS or immunosuppression. A 58-year-old man presented with a one-month history of headaches and changing mental status. Magnetic resonance images revealed expansive tumor mass 7 cm in diameter in the right frontal lobe with characteristics of high grade glioma. Staging evaluation demonstrated no other sites of the disease. The patient underwent right frontal craniotomy with cranioplasty and gross total tumor removal. Intraoperative specimens referred for pathologic examination were consistent with high-grade sarcoma. Histologically, the tumor was composed of intersecting fascicles of elongated spindle cells with foci of anaplastic areas with collections of rhabdoid cells. Immunohistochemically, tumor cells were positive

for SMA, desmin and vimentin. Stains for GFAP and S-100 protein were negative. Postoperatively, the patient was neurologically intact and started radiotherapy. He is still surviving to date in stable neurological condition. This is a rare case of primary intracranial pleomorphic leiomyosarcoma with rhabdoid features in a previously healthy man. Rhabdoid features are associated with aggressive biological behavior in leiomyosarcoma of external soft tissue.

SPLENIC LITTORAL CELL ANGIOMA

A. Demirović, T. Leniček, M. Ulamec, D. Tomas

Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia

Littoral cell angioma (LCA) is a rare primary tumor of the spleen arising from normal endothelial cells lining venous sinuses of the splenic red pulp (littoral cells). Although considered as benign, LCA has recently been shown to exhibit malignant potential and it may also be associated with other visceral malignancies. Clinical presentation of LCA ranges from asymptomatic to presenting with a constellation of symptoms such as abdominal pain, splenomegaly and hypersplenism. A 78-year-old woman was admitted on routine follow up examination one year after hysterectomy and adnexectomy due to primary extraovarian serous cystadenocarcinoma. Computed tomography of the abdomen disclosed multiple hypodense lesions in the spleen and splenectomy was performed. Grossly, the spleen measured 15x15x5 cm and had a nodular surface. Cut sections showed multiple spongy, soft, brownish nodules ranging in size from 0.8 to 2 cm. Microscopically, splenic tissue was replaced by large dilated, anastomosing vascular channels filled with blood and lined with plump endothelial cells that had vesicular nuclei and clear to eosinophilic cytoplasm. Some of these vascular channels had papillary structures protruding into the lumina. The lining cells exhibited no significant nuclear pleomorphism, atypia, or mitotic activity. Some of the lining cells contained hemosiderin deposits. Immunostaining of the neoplastic cells showed positive reaction for CD31, CD 21 and CD68, and negative reaction for CD8 and CD 34. In conclusion, LCA originates from littoral cells, which are endothelial cells lining the vascular sinuses of the spleen. Immunohistochemically, these neoplastic cells exhibit both endothelial and histiocytic markers. Differential diagnosis mainly includes other splenic vascular neoplasms that may be benign (hemangioma, lymphangioma, hamartoma), indeterminate (hemangioendothelioma, hemangiopericytoma), or malignant (angiosarcoma). Although thought to be benign, LCAs with malignant features have been described. Also, the association of LCA with other malignancies has been reported. These malignancies include colorectal, thyroid, renal, pancreatic, hematologic, testicular and, as in our case, ovarian ones. Therefore, careful investigation in search for a second neoplasm and long term follow up are recommended in these patients.

IMMUNOHISTOCHEMICAL EXPRESSION OF TUMOR ANTIGEN SSX IN PROSTATE CANCER

Lj. Hlupić[1], T. Hudolin[2], Ž. Kaštelan[2]

[1]University Department of Pathology and Cytology, [2]University Department of Urology, Zagreb University Hospital Center, Zagreb, Croatia

The aim of the study was to investigate immunohistochemical expression of SSX, a cancer testis antigen, in prostate carcinoma. The study included 53 prostate samples from patients undergoing surgery at University Department of Urology, Zagreb University Hospital Center, during the 2005-2007 period. All samples were obtained by radical prostatectomy and 20 benign prostatic hyperplasia samples by transvesical prostatectomy. The anti-SSX monoclonal antibody E3AS was used on immunohistochemical staining. Expression of SSX was found in 83% of carcinoma samples. Immunohistochemical staining was only detectable in the cytoplasm. A significant heterogeneity could be observed within the same tissue sample, were areas with strong positivity coexisted with SSX negative areas. The expression of SSX was statistically higher in prostate carcinoma samples with Gleason score 7 than in those with Gleason score 6 ($p=0.008$). Also, the carcinomas that had infiltrated prostatic capsule had higher expression of SSX compared with carcinomas that had not infiltrated the capsule ($p=0.005$). SSX expression did not correlate significantly with either PSA values or tumor size. We also found a weak SSX expression in some areas of some samples of benign prostatic hyperplasia. In conclusion, SSX is a cancer testis antigen that also shows expression in prostate carcinoma tissue. SSX is expressed in more aggressive prostatic carcinomas with higher Gleason score and with infiltration of the prostate capsule. Additional

studies are needed to investigate the potential implications for both the diagnosis and immunotherapy.

PATHOLOGIC FINDINGS IN PROSTATE CANCER PATIENTS SUITABLE FOR ACTIVE SURVEILLANCE THAT WERE TREATED WITH RADICAL PROSTATECTOMY

I. Tomašković[1], D. Trnski[1], O. Kraus[1], B. Ružić[1], B. Spajić[1], A. Reljić[1], J. Katušić[1], D. Tomas[1], H. Čupić[1]

[1]University Department of Urology , [2]Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia

Active surveillance is a valid treatment option for low risk, localized prostate cancer. There are several definitions of low risk prostate cancer. We tested most stringent criteria for active surveillance in our cohort of patients that were treated with retropubic radical prostatectomy. Study population included 586 patients treated with retropubic radical prostatectomy between January 2003 and January 2008. Among them, 106 were eligible for active surveillance according to combined preoperative criteria set by van den Bergh and Carter. These criteria include clinical stage T1c-T2, PSA <10 ng/mL, PSA density <0.20 ng/mL/mL, Gleason score =3+3, =2 positive biopsy cores, and =50% of any core involved with cancer. We analyzed the rate of adverse pathologic findings defined as advanced disease; extracapsular extension (ECE), positive surgical margins (+SM), seminal vesical involvement (+SV), positive lymph nodes (PLN), and intermediate and high Gleason score (7-10). Out of 586 patients treated with radical retropubic prostatectomy, 106 were suitable and could have been selected for active surveillance according to the above criteria. Among them, ECE, +SM and SVI were recorded in 6.6%, 8.4% and 1.8%, respectively. Gleason score 7 was recorded in 39 and Gleason score 8-10 in 0 patients. Overall, unfavorable pathologic findings were present in 41 patients or 38.6% of patients eligible for active surveillance. Accordingly, in our cohort of patients with presumable low risk prostate cancer, 38.6% of patients had unfavorable disease. Although active surveillance is gaining popularity due to the long natural course of prostate cancer and fear of overtreatment, both patients and their doctors need to be aware of the potentially significant disease and possible misclasification into the low risk group based on preoperative parameters.

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Acknowledgments

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