

## EXPRESSION OF NY-ESO1 PROTEIN IN RENAL ONCOCYTOMA AND CHROMOPHOBE RENAL CELL CARCINOMA

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**AIM:** The cancer-testis family of antigens is expressed in a variety of malignant neoplasms and is silent in normal tissues, except for the testis. Expression of NY-ESO1 member of its family has been described in melanomas, germ cell tumors, certain carcinomas and sarcoma, but there is however little information on expression in renal tumors.

Oncocytoma is the most common benign solid tumor of the kidney, originating from the intercalated cells of the collecting duct. Macroscopically, the tumor usually appears well circumscribed, surrounded with a pseudocapsule, measuring usually up to 7 cm in largest diameter. A fleshy central scar is a characteristic finding in oncocytoma and may be observed in 33-54% of tumors. Necrosis, hemorrhage and calcification are rare findings. Serial cross sections macroscopically show homogeneous mahogany colored tumor in contrast to homogeneously light brown chromophobe renal cell carcinomas (RCC). Microscopically, oncocytomas are composed of oncocytes, large cells with granular eosinophilic cytoplasm that show abundant mitochondria on electron microscopy. The most common appearance of tumor is arranging sheets or a tubulocystic or combined pattern.

The nucleus appears smooth and round, with a minimal degree of nuclear atypia. The main differential diagnosis is chromophobe RCC, which also may show granular eosinophilic cytoplasm.

The aim of the study was investigate the expression of NY-ESO1 in renal oncocytoma and chromophobe RCC detected by immunohistochemistry.

**MATERIAL AND METHODS:** We used computer database (Thanatos) from "Ljudevit Jurak" University Department of Pathology for the period from 1997- 2006 to identify all patients with diagnosed chromophobe RCC and renal oncocytoma. There were 9 chromophobe RCC and 10 oncocytomas.

Male/ female ratio for oncocytoma and chromophobe RCC was 3:7 and 2:7, respectively.

Monoclonal antibody NY-ESO1 was used for immunohistochemical staining.

Melanoma and testicular tissue were used as positive controls throughout the study. Immunohistochemical staining results were semi quantitatively expressed as follows:

- Negative reaction was marked with (-);
- Slightly positive reaction (up to 10% of tumor cells) as (+);
- Moderately positive reaction (>10-50 % of tumor cells) as (++);
- Strong positive reaction (>50% tumor cells) as (+++).

**RESULTS:** The average age of patients with oncocytoma was 66.5 years (ranged 57.0 to 77.0). The size of tumor ranged from 0.9 to 8 cm (mean 3.6 cm).

The expression of NY-ESO1 was observed in oncocytoma tissue with strong reaction in 5 cases (50%), moderately positive in 2 (20%) and slightly positive in 3 cases (30%).

Table 1. Immunohistochemical expression of tumor antigens NY-ESO1 in renal oncocytoma

CASE	AGE (years)	SEX (female/male)	TUMOR SIZE (diameter, cm)	NY-ESO tumor	NY-ESO normal renal tissue
1	62	Female	5.0	+	+
2	57	Male	4.0	+++	-
3	65	Female	3.0	+++	+
4	61	Female	1.7	++	-
5	77	Male	0.9	+++	+
6	68	Female	2.2	+++	++
7	68	Male	3.0	+	+
8	55	Female	8.0	+	+
9	69	Female	6.5	++	+
10	70	Female	1.5	+++	++

Table 2. Immunohistochemical expression of tumor antigens NY-ESO1 in chromophobe renal cell carcinoma

CASE	AGE (years)	SEX (female/male)	TUMOR SIZE (diameter, cm)	NUCLEAR GRADUS	NY-ESO tumor	NY-ESO normal renal tissue
1	47	Female	1.7	G3	-	++
2	60	Female	5.0	G2	+++	+++
3	62	Male	2.7	G2	+	++
4	63	Male	10.0	G2	-	-
5	47	Female	17.0	G4	-	-
6	60	Female	12.0	G2	++	++
7	74	Female	4.5	G2	+	-
8	55	Female	6.5	G2	-	+
9	44	Female	6.8	G2	-	-

The reaction found in surrounding renal tissue was negative or slightly positive. (Table1)

The average age of patients with chromophobe RCC was 57.1 years (ranged 44 to 74). The size of tumor ranged from 2.7 to 17 cm (mean 5.4 cm).

The expression of NY-ESO1 protein was not seen in 5 cases of chromophobe RCC tissues (55.6%), slightly positive in 2 (22.2%), moderately positive in one (11.1%), and strongly positive in one case (11.1%).

Expression correlated with grading of tumor, better differentiated tumors had stronger expression.

The expression found in surrounding renal parenchyma was stronger than in tumor tissue. (Table2)

**CONCLUSIONS:** This study documents for the first time the expression of NY-ESO1 protein in renal oncocytoma by immunohistochemistry, whereas their expression in chromophobe RCC was reported in few cases.

Positive staining was found in all cases of oncocytoma ranging from "slightly positive" to "strong reaction". Surrounding renal tissue performed by lower intensity. The majority of chromophobe RCC specimens showed no expression of NY-ESO1 protein.

NY-ESO1 expression in tumor progressively declined with higher grade. Chromophobe RCC tissue showed slightly lower expression of NY-ESO1 protein than surrounding renal tissue.

Further studies are needed to explore potential value of NY-ESO1 in differentiating oncocytoma from chromophobe renal cell carcinoma.

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## UNCLASSIFIED MIXED GERM CELL AND SEX CORD-STROMAL TUMOUR OF THE TESTIS – A CASE REPORT

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**INTRODUCTION:** Unclassified germ cell and sex cord – stromal tumours of the testis are rare neoplasms containing variable mixtures of neoplastic germ cells and neoplastic sex cord – stromal elements.

**CASE REPORT:** We present a case of 45-year-old men who noticed painless mass in right testis without any other symptoms or history of previous disease or injury. Preoperative ultrasonography showed right-sided testicular hypoechogenic structure and laboratory examination found normal levels of LDH, HCG and AFP. Orchiectomy was performed.

Grossly, there was encapsulated, sharply demarcated, tan soft nodule measured up to 2,5 cm in largest

diameter, in excised testis. Microscopically tumour was composed mostly of quite uniform, oval to spindle cells with enlarged, vesicular nuclei. These cells were immunohistochemically positive for vimentin, S-100 and focally for alpha-inhibin. Also, focally it shows brisk mitotic activity (5 mitoses per HPF). At the periphery, among described cells, small aggregates of large cells with abundant, pale cytoplasm and hyperchromatic nuclei dominate and show no reactivity for antibodies mentioned before, neither for MNF 116 and 7, AFP, HCG and PLAP. Six months after operation the patient is well, without signs of the disease.

### MICROPAPILLARY VARIANT OF UROTHELIAL CARCINOMA OF THE RENAL PELVIS – A CASE REPORT

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**INTRODUCTION:** Micropapillary variant of urothelial carcinoma of the renal pelvis is a rare entity with low incidence (2.8%). According to the literature males are more frequently affected. This variant of urothelial carcinoma is a high grade carcinoma with aggressive behavior that is usually diagnosed in high stage of disease and connected with poor prognosis.

**CASE REPORT:** A 72 years-old female patient presented with lumbal pain in the last 2 months, without haematuria and CT scan showed tumorous mass in the left kidney. Patient underwent radical nephrectomy with lymphadenectomy. Pathologic examination of the left kidney revealed gray tumor in the renal pelvis measuring up to 4 cm in the largest diameter. Microscopically, only small parts of tumor consisted of conventional urothelial carcinoma while micropapillary component was predominant. Tumor tissue infiltrated kidney parenchyma, accompanied with vascular invasion of small intrarenal vessels, perirenal fat tissue, as well as renal vein and ureter with present metastatic disease in regional lymph nodes. Only 2 months after nephrectomy patient developed multiple metastases to omentum that were histologically composed of micropapillary variant of urothelial carcinoma.

**DISCUSSION:** Micropapillary growth pattern in carcinoma is also present in malignant tumors of other

organs (breast, ovary) and although nuclear grade may appear low, patients with this tumor type usually present in advanced stage of disease.

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### PATHOLOGICAL FINDINGS IN KIDNEY NON-TUMOR BIOPSIES: A FOUR YEAR EXPERIENCE

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**AIM:** Retrospective analysis of kidney biopsies at Department of Pathology, Dubrava University Hospital, Zagreb, Croatia was performed to determine frequency of kidney disease entities and to compare it with recent similar studies.

**METHODS:** The pathohistologic diagnosis was made on the basis of the light microscopy (H&E, PAS, Masson trichrome and Jones stains), immunofluorescence (IgG, IgA, IgM, C3, C1q, fibrinogen, albumin, kappa and lambda light chains) and electron microscopy analysis. The data were collected from the Renal Pathology Registry, Department of Pathology, Dubrava University Hospital, Zagreb. The period from April 2003 to April 2007 was analyzed to find frequency of different types of pathohistologic diagnoses.

**RESULTS:** A total of 514 kidney biopsies was analyzed (399 native kidney biopsies and 115 transplant biopsies). In 15 (2,9%) biopsies (8 native and 7 transplant biopsies) there was no kidney tissue. Further evaluation was performed on 499 (391 native and 108 transplant) biopsies. The most frequent diagnosis in native kidney biopsies was IgA nephropathy (20,2%), followed by the focal segmental glomerulosclerosis (16,4%), membranous glomerulonephritis (7,2%), lupus nephritis (7,2%), minimal change disease (5,1%), diabetic nephropathy (3,8%), thin membranes (3,3%), acute tubular necrosis (3,3%), arteriosclerosis (3,3%) and pauci-immune glomerulonephritis (3,3%). The most frequent

diagnosis in transplant kidney biopsies was acute tubular injury (63%), followed by acute cellular rejection (9,3%), acute humoral rejection (6,5%), no pathologic lesion identified (6,5%), focal segmental glomerulosclerosis (3,7%), calcineurin inhibitor toxicity (2,8%), chronic allograft nephropathy (2,8%), interstitial fibrosis and tubulointerstitial atrophy (1,9%), thin membranes (0,9%) and IgA nephropathy (0,9%).

**CONCLUSIONS:** Our data are comparable to the biggest USA study (1), except for the IgA nephropathy that is the most frequent kidney disease in our study and is on fourth place in USA study. These results are most similar to 27-year prospective Western France study (2), where IgA nephropathy was on the first place. There are no similar published data for transplant kidney biopsies.

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## RENAL ARTERY CHANGES IN PATIENTS WITH UROTHELIAL CARCINOMA OF THE UPPER URINARY SYSTEM AND ENDEMIC NEPHROPATHY

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**INTRODUCTION:** Endemic nephropathy (EN) is a chronic tubulo-interstitial disease of unknown etiology, and it is associated with increased frequency of urothelial carcinoma of upper urinary system. Histologically, atrophy of proximal tubules and diffuse cortical fibrosis is the most prominent finding. Hyperplastic arteriopathy of small and middle-sized arteries is also reported as a constant finding in EN cases. Renal artery changes are observed in high frequency in patients with renal cell carcinoma, as well as in patients with urothelial carcinoma of the renal pelvis from non-endemic region. Aim of the study was to analyze renal artery changes in patients with urothelial carcinoma of the upper urinary system and endemic nephropathy.

**MATERIALS AND METHODS:** We analyzed 15 patients (M:F=10:5) with urothelial carcinoma of upper urinary system from Slavonski Brod county who underwent nephrectomy in the period from October 2006 to March 2007, and compared them to 12 patients (M:F=8:4) with urothelial carcinoma of pyelon from Zagreb (data from Ljudevit Jurak University Department of Pathology for the time period 2003-2004). Specimens were routinely fixed, embedded in paraffin, cut and stained with haematoxylin and eosin and Mallory trichrome method, and examined by light microscopy.

**RESULTS:** In group of patients from Slavonski Brod county 12 cases (M:F=8:4) were from endemic region and only 3 cases (M:F=2:1) were from non-endemic regions of Slavonski Brod. Out of 12 patients from endemic region, fibromuscular dysplasia (FMD) was observed in 4 cases (M:F=2:2), 5 (M:F=4:1) patients did not show pathological changes on renal arteries and 3 (M:F=2:1) cases were with atherosclerosis. Two patients from non-endemic region of Slavonski Brod showed fibromuscular dysplasia on renal arteries and one patient was without renal artery changes. Mean value of tumor size in group of patients with FMD was 4.3 cm; 3.5 cm was in group without renal artery changes, and 3 cm in group of patients with atherosclerotic changes on renal arteries. In group of patients from Zagreb 7 (M:F=5:2) had FMD on renal arteries, 1 (male) had atherosclerosis and 4 cases (M:F=2:2) were without renal artery changes. Tumor size in the group of patients with FMD and atherosclerosis was 4 cm, and in the group of patients without renal artery changes mean value of tumor size was 5.1 cm.

**CONCLUSION:** Atherosclerosis was more frequently found in group of patients from endemic region and FMD in non-endemic group. Correlation of renal artery changes and endemic nephropathy as well as urothelial carcinoma should be further analyzed on larger series.

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## STROMAL EOSINOPHIL COUNT IN INVASIVE (pT1) AND NON-INVASIVE (pTa) PAPILLARY UROTHELIAL CARCINOMA OF THE URINARY BLADDER

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**PURPOSE:** Stromal invasion is a key prognostic feature in papillary urothelial carcinoma of the urinary bladder. The inevitable tangential sectioning of papillary tumors and inadequate specimen quantity often make the diagnosis of invasion difficult. Increased numbers of stromal eosinophilic granulocytes were found in some types of human invasive cancers. Aim of this study was to compare the presence of tumor-associated tissue eosinophilia (TATE) in invasive (pT1) and non-invasive (pTa) papillary urothelial carcinoma of the urinary bladder, which could have implications in the diagnosis of lamina propria invasion.

**METHODS:** Eosinophils were counted in the stroma of 18 pT1 and 16 pTa urothelial carcinomas obtained by transurethral resection. Specimens were routinely fixed, embedded in paraffin, cut and stained with hematoxylin and eosin method. The area of the tumor that contained the maximum number of eosinophils was identified by scanning the whole mount section under lower magnification (100X). Eosinophil counts were performed in the previously selected high-power field (HPF, 400X) and in 9 adjacent stromal HPFs. Eosinophil numbers were classified as low (<30/10 HPFs), moderate (30-60/10 HPFs) or high (>60/10 HPFs).

**RESULTS:** The average number of eosinophilic granulocytes per 10 HPFs was 22 in pTa and 92 in pT1 carcinomas. Among pTa carcinomas, the eosinophil count was low in 12 (75.0%) and moderate in 4 (25.0%) specimens, but none exhibited a high number of eosinophils, whereas in pT1 carcinomas the eosinophil count was low in 3 (16.7%), moderate in 4 (22.2%) and high in 11 (61.1%) cases. Also, presence of =20 eosinophils in at least one HPF was not recorded in non-invasive carcinoma, in contrast to 11 (61.1%) of the invasive carcinomas.

**CONCLUSION:** Our results suggest a difference in stromal eosinophil count between pTa and pT1 urothelial bladder cancer. Further investigations on a greater number of specimens are required to assess its usefulness in the diagnosis of invasion.

## CORRELATION OF EXPRESSION OF GROWTH HORMONE RECEPTOR ON DYSPLASTIC NAEVUS AND MALIGNANT MELANOMA OF THE SKIN

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**AIM OF THE STUDY:** To investigate possible differences in expression of growth hormone receptor among normal skin, dysplastic naevus and malignant melanoma.

**MATERIAL AND METHODS:** Twenty cases, ten males and ten females, from each of above mentioned groups were examined to analyse expression of growth hormone receptor. The results of immunohistochemistry were shown in semiquantitative manner and were analysed with help of statistical methods, including  $\chi^2$  – test and Kruskal-Wallis ANOVA test, with the level of significance <0,05.

**RESULTS AND CONCLUSION:** The results showed that there was a higher expression of growth hormone receptor in malignant melanoma, compared with dysplastic naevus and normal skin. This was confirmed with both  $\chi^2$  – test and Kruskal-Wallis ANOVA test. Therefore we conclude that there might be a possibility to intervene the course of malignant melanoma and dysplastic naevus via growth hormone receptor antagonists. We strongly suggest studies on larger number of patients to be made.

## EXPRESSION OF TISSUE ANTIGEN EGFR IN PATIENTS WITH ORAL LICHEN RUBER

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According to WHO criteria oral lichen ruber (OLR) is precancerous lesion of oral mucosis. The aim of this study was to define possible malignant potential of OLR lesions by determining the expression intensity of EGFR antigen. Examination included 60 patients with clinical and pathohistological confirmed diagnosis of OLR. Results were compared with control group of 30 patients diagnosed with oral leukoplakia, verified as leukoplakia simplex. The aim of the examination was to detect the

expression intensity of c-erbB-2 between clinical forms in lichen ruber planus (LRP) and lichen ruber erosivus (LRE). EGFR antigen was detected by LSAB immunohistochemical methods after treatment in microwave oven. The reaction of explore antigen was mosaicly expressed, delicate positive in group of cells in spinous layer and negative in cells of basal layer. In control group reaction was uniform and strong in all epithelium layers. Intensity of this antigen expression was independent to the extent of inflammation, but positively correlated with the extent of the hyperkeratosis in lesion. It can be concluded that such altered expression of EGFR antigen in OLR lesions points to altered nature of these lesions with the potential to undergo malignant transformation.

### PERITUMORAL RETRACTION CLEFTING- USEFUL MORPHOLOGIC FEATURE FOR THE DIAGNOSIS OF DUCTAL INVASIVE CARCINOMA OF THE BREAST

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Microscopic analysis of histological specimen has the most important place in the diagnosis of breast cancer. Because of many different forms of ductal invasive breast carcinoma, along with existing ones, sometimes additional morphologic criteria are needed to distinguish carcinoma from benign breast changes that may resemble cancer. This could be particularly helpful in analysis of tiny specimens obtained by needle core biopsy and the interpretation of intraoperative frozen sections. The change of stroma in presence of tumor is visible by light microscope analysis as clear spaces around tumoral glands, which are named peritumoral retraction clefts. In this study thirty cases of breast carcinoma and thirty cases of benign changes (ten cases of fibroadenoma, fibrocystic breast change and sclerosing adenosis each) were analyzed by light microscope. Results of our study have shown significant difference in the appearance of peritumoral retraction clefting between cancer group and benign breast changes group. This was confirmed by the statistical methods, which included  $\chi^2$  – test and Kruskal-Wallis ANOVA test, with the level of importance  $p < 0.0001$ . Current study has shown that peritumoral

clefting occurs more often in breast cancer group, so further investigation should be performed to analyze stroma degradation products, which can indicate invasion and prognosis of the disease.

### CYTOLOGIC FEATURES OF PULMONARY METASTASIZING LEIOMYOSARCOMA. REPORT OF A CASE DIAGNOSED BY FINE NEEDLE ASPIRATION CYTOLOGY

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**INTRODUCTION:** The differential diagnoses of multiple lung masses are diverse, with metastatic disease being the most common etiologic consideration. Few reports have been published regarding fine needle aspiration cytology of leiomyosarcoma of the lung. We report the cytologic findings of a case of leiomyosarcoma metastasizing to the lung.

**CASE REPORT:** A 56-year-old woman with complaints of increasing dyspnea. Her past medical history showed a hysterectomy for a leiomyosarcoma. The chest radiograph demonstrated opacities. Ultrasound-guided fine needle aspiration cytology was performed using a 22-gauge needle. Aspirate smears were stained by Pap and M.G.G. methods and immunocytochemical stains were performed using commercial available antibodies. **Cytologic Findings:** FNA smears were cellular and showed cellular fragments as well as dissociated cells. The dissociated cells were spindle to plump with scant to moderate cytoplasm. Cellular atypia, pleomorphism and occasionally mitotic figures were noted. Immunocytochemical studies showed the spindle cells were positive for smooth muscle-specific actin and vimentin but were negative for cytokeratin, desmin and S-100 protein. Considering the clinical history, the cytologic findings and the result of the ancillary studies a diagnosis of leiomyosarcoma was offered.

**DISCUSSION:** The reported incidence of spindle cell and mesenchymal lesions encountered in a large series of transthoracic pulmonary FNA specimens ranged from <1-4%. These spindle cell lesions were comprised of a heterogeneous group; therefore, the differential diagnoses of spindle cell lesions are to be expected. A specific diagnosis can be rendered in the majority of these cases by correlating clinical, radiologic and cytologic findings. Ancillary studies will help to narrow down the di-

agnosis. Since this technique is relatively non-invasive, it is very useful in diagnosis of pulmonary lesions.

## PRIMARY AND SECONDARY TUMORS OF LUNG IN COMPARISON WITH EXOGENOUS POLLUTANTS

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**INTRODUCTION:** In Latvia the nature of primary bronchogenic carcinoma (BC) is in 90,7 male per 100000 but autopsy cases prove rather common secondary tumors of the lungs.

**AIM:** Aim of our research was to evaluate primary and secondary tumors in lungs in autopsy cases and to evaluate the significance of exogenous pollutants in the lungs of these patients.

**METHODS:** We have analyzed 96 autopsy cases of primary and secondary tumors of lungs in Latvian Center of Pathology (year 2004.-2006.). We have evaluated epicrisis of case reports, pathology documentation; lung specimens stained with h/e and immunohistochemical reactions with CK7/20, CD20, PSA, vimentin, Pr/Es were done. Density of exogenous pollutants was counted per 1mm<sup>2</sup> and accordingly its amount expressed into 3 degrees. Control cases were taken from children lungs. Results were analyzed statistically.

**RESULTS:** More common metastasis to the lung was from female genital organs (25%), kidney (23%), gut (15%) and the majority of them were adenocarcinoma. From all malignancies in 38% of cases were metastases to the lungs. Primary lung cancer was surgically operated in 17%, but chemotherapy was in 20% of patients. In 14% of cases lung cancer was not diagnosed during patients' life due to short time of hospitalization. To evaluate the role of dust, smoking and others pollutants were have compared amount of black stained exogenous deposits in control lungs of kids, in CHOLD and BC. In last two pathologies pollutants were of 2<sup>nd</sup> degree but exogenous deposits were absent directly in the area of malignant process

**CONCLUSIONS:** 1. In our analyzed period the ratio of secondary and primary lung tumors were 3:1 and lung metastasis were combined with other lymphatic and hematogenous spreading. 2. There were no difference of intensity of exogenous pollutants in cases of chronic obstructive lung disease and primary cancer (p=0,089)

## SURVIVAL OF RAT AND MOUSE FOETUSES AFTER TREATMENT WITH TERATOGENS

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Mouse and rat experimental animals are the most common mammalian models for teratogenic investigation. In our previous work we investigated the teratogenic effect of 5-azacytidine (5azaC) on rat limb development, and discovered the protective effect of aspirin (ASA), although it is known that aspirin given in high doses also acts as a teratogen. In this work, we investigated the influence of these teratogens combination on mouse and rat foetal survival. The goal of these experiments was to determine whether there is any difference in susceptibility to these teratogens between the mouse and the rat. 5azaC was dissolved in PBS and injected intraperitoneally at a concentration of 5 mg/kg body weight to pregnant rat and mouse females on 13<sup>th</sup> and 11<sup>th</sup> day of pregnancy respectively. ASA was dissolved in PBS and administered 2 hr before 5azaC at a concentration of 75 mg/kg. Controls were treated with PBS on the same gestation days. Animals were killed the day before birth. Foetal status (survival, intrauterine death and resorption) was determined. In mouse model 5azaC caused 30% i.u. deaths, no resorptions at all while combination of 5azaC, and presumably, protection agent ASA caused almost 27% i.u. deaths and even 15% resorptions. On the other hand, in rat model neither 5azaC nor 5azaC and ASA combination caused any i.u. deaths or resorptions. Controls of both models showed 100% foetal survival. In conclusion, the mouse model seems to be more susceptible than rat to the influence of investigated teratogens.

## 5-AZACYTIDINE CHANGES VOLUME DENSITY OF PROLIFERATIVE TROPHOBLAST CELLS IN RAT PLACENTA

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In order to investigate the influence of hypomethylation on the development of rat placenta at the periimplantation period we have used the DNA demethylating agent 5-azacytidine (5azaC). We investigated its impact on the potential for proliferation of trophoblastic cells through the quantification of expression of the proliferating cell nuclear antigen (PCNA). Single dose of 5azaC (5 mg/kg) was injected intraperitoneally to gravid female rats at days 4 and 5 of gestation. The animals were killed on the 20th day of gestation. Placentas were prepared for immunohistochemical analysis of the expression of the PCNA. We have analyzed the stereological variable of volume density (V<sub>v</sub>), using Weible's multipurpose system with 42 test points. Statistical analysis was performed using the Student's t test (p<0.01). Application of 5azaC on the day 4 of gestation caused the failure in development of the functionally most important part of the placenta – labyrinth. However the volume density of the PCNA-positive trophoblast cells was significantly higher than in controls (p<0.01). The same result was obtained with placentas treated on the day 5 and the volume density was higher not only in comparison with the controls but also with those treated on the day 4 (p<0.01). Observed changes of proliferative trophoblast cells volume density in the placenta after application of 5-azaC are consistent with our previous results obtained using the stereological variable of numerical density (Serman et al, in press.) and present a further proof of the importance of epigenetics in regulation of development.

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## UROGENITAL CARCINOMAS IN CALIFORNIA SEA LIONS (*ZALOPHUS CALIFORNIANUS*)

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Although historically rare, reported incidences of neoplastic diseases in marine mammals are on the rise. Besides the increased surveillance, it is speculated that there may be a relationship with chemical pollution of the marine environment (organochlorides) and its accumulation within animal tissues of California Sea Lions (*Zalophus californianus*) and Beluga Whales (*Delphinapterus leucas*)<sup>1</sup>. Urogenital carcinomas are one of most frequent malignancies diagnosed in marine mammals submitted for rehabilitation and/or postmortem examination at the Pacific Marine Mammal Center 1996-2007 (51 animals total). The animals are typically presented anorexic, emaciated and lethargic with prominent perineal edema, abnormal tail position, rear flipper paresis/paralysis, and occasional rectal prolapse. The neoplastic mass is typically grossly large in size, multinodular, yellow to light brown colored and commonly necrotic. The masses are composed of nests of neoplastic epithelial cells with prominent squamous metaplasia. Metastatic spread to sublumbar and other lymph nodes and organs in the abdominal, pelvic, and thoracic cavity are frequently encountered. Common sequel is hydronephrosis and hydroureter due to the compression of the ureters. Immunohistology of metastatic carcinomas of genital origin in 10 sea lions of subadult to adult age (up to 15 years of age) revealed the association with a novel *gammaherpesvirus* (Otarine Herpesvirus-1) and suggest venereal transmission, although and underlying immunogenetic (MHC genes) component is suggested.<sup>2,3</sup>

### References

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## EWING SARCOMA, A RETROSPECTIVE STUDY

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**BACKGROUND:** Recently, new concepts in Ewing sarcoma/PNET diagnosis and treatment were introduced. Since 1998, immunohistochemical verification of ES/PNET diagnosis, using CD99, is routinely performed at our Institute. Since end 2003, molecular methods (RT-PCR for EWS/FLI1) were introduced. In 2003, an algorithm was adopted including molecular analysis for residual disease and early recurrence. In order to establish a baseline patient population, we performed a partly retrospective and partly prospective analysis.

**PATIENTS AND METHODS:** 78 Ewing sarcoma/PNET, diagnosed at the Institute of Pathology, Medical School University of Zagreb and treated at the Children's Hospital Zagreb, in the period from 1990 to 2006, were included in this study. Of all analyzed tumors, 8 were extraosseal. Retrospectively, we submitted all collected tumor samples for both immunohistochemical and molecular analysis. Immunohistochemical analysis was successful in all analyzed samples. RT-PCR could not be successfully performed on samples older than 2000. From 2003, together 96 samples – tumor tissue, peripheral blood and 28 bone marrows were analyzed for characteristic translocations.

**RESULTS:** Immunohistochemically, all tumor samples showed moderate to strong positivity, while EWS/FLI1 could not be demonstrated in 6. From the samples submitted according to the new algorithm, 39 were positive by RT-PCR, among them 10 peripheral blood samples. In some cases multiple analyses of blood samples yielded only one positive. In two diagnostically problematic and immunohistochemically equivocal cases of small blue cell tumors molecular analysis fostered the final opinion.

**CONCLUSION:** Introduction of molecular techniques enabled us to solve some diagnostic problems and to detect disseminated disease prior to clinical evidence producing a potent tool for improved patient care and disease management.

## HISTOMORPHOLOGIC ASSESSMENT OF TISSUE SPECIMENS PROCESSED BY CLASSICAL AND SUBSTITUTE FIXATION PROCEDURE

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Over the years, laboratory workers have been alerting to the potential carcinogenicity of formaldehyde and xylene. Although xylene is the most potential carcinogenic agent because of its aromatic chemical combination, formaldehyde is also one of the most toxic components used in staining procedure. As a result, a variety of proprietary fixatives were developed for use in surgical pathology. Several companies started producing proprietary fixatives and clearing agents for surgical pathology. Some of these fixatives contain reduced amount of formalin or none at all and almost all clearing agents are biodegradable and non-carcinogenic, thus reducing the exposure of laboratory workers to the potential carcinogens. Most of them are still hazardous and contain minimal concentrations of chemicals required to preserve the tissues and cell architecture.

The aim of the study was to estimate histomorphology of specimens stained with hematoxylin and eosin fixed in formalin substitute (FS), cleared in xylene substitute (XS) so called "fixation by substitutes" (SF) and fixation in a 10% buffered solution of formaldehyde (F), cleared by xylene (X)- called "classical fixation" (CF).

Five surgical pathologists and residents of pathology examined 5 intraoperative consultation ("frozen section") specimens (follicular adenoma and goiter of thyroid gland, renal adenocarcinoma, metastatic axillary carcinoma of mammary gland and unremarkable adrenal gland) processed by CF and by one proprietary formalin-free fixative advertised as FS (composition of FS: ethanol, glyoxal, 2-propanol, methanol) and cleared by XS (composition of XS: aliphatic hydrocarbon). In a blind study, the pathologists rated 7 criteria including cellular outlines, cytoplasmic details, nuclear detail, erythrocyte integrity, lymphocyte integrity, overall morphology and overall staining in each case. In every category CF received a higher average score except for the estimated cellular membranes where SF performed as well as CF. Discontinued usage of F and X will introduce pathologists to a completely new area of unexpected artefacts they will have to adapt to.

Table 1.

		Criteria							
fixation		ov.morph	ov.st	cell.mem	cpl.det	nc.det	E	lymph	average score
	CF	4.05	3.95	3.35	3.50	4.04	4.50	4.20	3.85
	SF	3.40	3.25	3.35	3.30	3.30	1.70	3.05	3.28

ov.morph=overall morphology, ov.st=overall staining, cell.mem=cellular membrane, cpl.det=cytoplasmic detail, nc.det=nuclear detail, E=erythrocyte integrity, lymph=lymphocyte integrity, CF=classical fixation, SF=fixation by substitutes

Further studies on a larger number of specimens are needed to estimate histomorphology with different fixatives and clearance agents advertised as Formalin Substitute and Xylene Substitute.

## REINKE'S CRYSTALS IN INFERTILE PATIENTS

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**INTRODUCTION AND AIM:** Reinke's crystals are normal constituents of human Leydig cells. The nature and function of these crystals is poorly understood. They can be found in the testis of some primates and the New Zealand white rabbit. However, it should be pointed out that no Reinke's crystals in other species (i.e. rodents etc.) have been detected. The crystals resemble hexagonal prisms of variable size (mostly 2-3 µm). They are composed of parallel 10nm filaments and do not have a unit membrane. Sometimes a single crystal could be found in the cytoplasm and/or the nucleus of Leydig cells. There is a lack of data on number, architecture and composition of Reinke's crystals, especially in infertile patients. The aim of our study was to analyse Reinke's crystals in patients with normal spermatogenesis and in the biopsies of patients with cryptorchidism and non-obstructive azoospermia.

**MATERIALS AND METHODS:** Paraffin blocks have been retrieved from the archive of testicular biopsies (Dept. Histology and Embryology, School of Medicine, University of Zagreb). 12 biopsies with a preserved testicular architecture and spermatogenesis served as controls. The infertile group consisted of 37 paraffin blocks originating from cryptorchid patients and 96 paraffin blocks of testicular biopsies obtained from patients with non-obstructive azoospermia. All tissue samples were fixed in the Gendre fixative. Paraffin blocks were

sectioned in serial sections, 4µm thick. The sections were placed onto slides and stained according to a modified Masson's method. After staining, the slides were observed by a binocular microscope (Nikon Eclipse 200).

**RESULTS:** In control samples, a regular architecture of the testis tissue including full spermatogenesis could be observed. Within Leydig cells, a moderate presence of Reinke's crystals could be seen. The vast majority of the crystals were located within the cytoplasm of Leydig cells. However, the crystals (a much smaller form) could be observed in the nucleus of these cells. In the cases of non-obstructive azoospermia, most of the cases presented a "mixed atrophy" of seminiferous tubules. In these samples, it seemed that the number of Reinke's crystals was somehow reduced. Cryptorchid testes displayed (in the vast majority of cases) a histological picture of "Sertoli cells only syndrome". Interestingly, in these samples, an abundant presence of Reinke's crystals could be found.

**CONCLUSION:** It seems that, most probably due to the different aetiology, cryptorchidism and non-obstructive azoospermia act differently on Reinke's crystals.

## COMPLETE HYDATIFORM MOLE WITH COEXISTING LIVE NEWBORN IN TWIN GESTATION

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A 32-year-old Caucasian woman presented at 18 wks. gestation because on previous ultrasonic examination a lesion that appeared a retroplacental hematoma was discovered. It was her second pregnancy; she conceived during lactation, only 2 months after delivery of an infant that was conceived after hMG-hCG treatment. Ul-

trasonic examination at 18 wk gestation revealed a twin pregnancy, with one viable fetus with normal placenta, while the other placenta showed features of molar tissue. Amniocentesis was performed and revealed normal female karyotype. The pregnancy was managed expectatively. Throughout pregnancy, beta-hCG levels increased steadily and reached the highest level of 199 000 IU/L at 28 wk gestation, followed by a plateau of 140-150 000 IU/L until delivery at 37 wk gestation. A normal female infant weighting 3150 g was delivered and both placentas were sent for histopathological examination. The normal placental tissue weighed 420 g and the molar tissue weighed 540 g; on H-E stained slides the normal placenta showed features appropriate for gestational age, while the molar tissue showed features of a complete hydatiform mole. Immunohistochemical analysis showed intensive intranuclear expression of proliferative antigens anti-Ki-67 (80 % of cells); anti PCNA (90 to 95 % of cells) and strong expression of anti-p53 antigen (60 %) in mosaic pattern in trophoblastic cells. After delivery genotyping was performed; molecular analysis revealed different genotypes of fetal and molar tissue. Molar tissue lacked maternal alleles and showed only homozygous paternal alleles. After delivery beta hCG levels declined, and reached negative level 5 wks after delivery. The follow up lasted for 2 years and was uneventful.

**DISCUSSION:** Twin gestation with one normal fetus and a hydatiform mole is a rarity. In most described cases, the pregnancy ended by an abortion. In this case, it was managed expectatively, and resulted in a birth of a normal, healthy infant.

## **ETHYLENDIAMINE-TETRA-ACETIC ACID (EDTA) DEPENDENT PSEUDOTHROMBOCYTOPENIA - SCREENING AND CONFIRMATORY TESTING**

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Pseudothrombocytopenia (PTCP) is the phenomena of an artificially low platelet count in ethylenediamine-

tetra-acetic acid (EDTA) anticoagulated blood determined by haematology analysers. In EDTA-PTCP platelet autoantibodies directed to the platelet membrane GPIIb/IIIa complex induced platelet aggregate or agglutinate formation. It occurs with incidence of approximately 0.1 % in the general population and is more common in patients with isolated thrombocytopenia (7.5-17%). The aim of our study was to evaluate the incidence of EDTA-PTCP in outpatients with isolated thrombocytopenia who were referred to our laboratory of CITM for platelet antibody testing in six-month period. Platelet count in EDTA and citrated blood samples along with blood smear examination were done in all patients. EDTA-PTCP was present in 18/123 (14.6 %) of cases. The data indicate that PTCP should always be excluded in a newly diagnosed patient with thrombocytopenia to avoid needless evaluations, cancelled surgical procedures and even platelet transfusions in an individual with this form of *in vitro* artefact.

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## **APPLICATION OF HISTOCHEMICAL STAINING METHODS FOR DIFFERENTIATION OF AA AND AL AMYLOID**

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The histopathological diagnosis of AA and AL amyloid is based upon different reactions on histochemical staining methods, firstly modifications of Congo red staining.

Using Congo red and PAS staining it is possible to identify the presence of amyloid in tissues. For the differentiation of AA and AL amyloid, the incubation with

kalium permanganate (KMnO<sub>4</sub>), that changes the intensity of Congo red staining, depending upon the type of amyloid is used. In certain cases, there is an increased

need to differentiate the types of amyloid in tissues, pointing to the importance of standardizing the procedures of staining.