

## INTRAOPERATIVE IMPRINT CYTOLOGY OF SENTINEL LYMPH NODES FROM PATIENTS WITH MALIGNANT MELANOMA

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**AIMS:** To evaluate the utility and accuracy of intraoperative imprint cytology (IIC) of sentinel lymph nodes (SLNs) in patients with metastatic melanoma, and to establish this diagnostic procedure in Croatia.

**METHODS:** Seventy-two sentinel lymph nodes from patients with metastatic melanoma were identified by technetium-labeled sulfur colloid and blue dye. SLNs were evaluated intraoperatively by slicing into 3-mm sections. Imprints were made of each cut surface, stained with Hemacolor quick method and examined by a cytologist. Permanent cytologic imprints were stained with Papanheim and immunocytochemical staining for S-100 and HMB-45 proteins. Intraoperative imprint cytology results were compared with final histologic ones.

**RESULTS:** A total of 72 SLNs were excised from 47 patients with malignant melanoma (1.6 SLNs *per* patient). Metastases were identified in 13 (27.5%) patients. The sensitivity of IIC for SNL was 75% (12/16) *per* sentinel node and 69% (9/13) *per* patient. All 4 false-negative SLNs by IIC were detected in patients with isolated cells of melanoma in permanent histopathologic sections. No false-positive results were identified (100% specificity).

**CONCLUSIONS:** IIC of SNLs of metastatic melanoma is a reliable and accurate intraoperative technique. It could be used as an alternative to frozen section, especially because it is rapid, cost-effective and tissue saving.

## REDUCING CLINICAL ERROR AND POOR PERFORMANCE

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We focus on the discussion on the actions that doctors are expected to take to minimize the risk of error, to improve the quality of care, and to address the indicators of potential harm. Patient safety and quality of care are obviously the key concerns for everyone involved in healthcare

provision. In medicine, even quite a minor mistake can have tragic consequences for patients. Mistakes not only harm people but also undermine public trust. They are costly in terms of causing avoidable suffering, generating stress, and draining health service time and money. Medical errors demoralize staff and alarm patients. They often attract high levels of media attention, which can distort public perceptions of relative risks and benefits of certain preventive, diagnostic, and treatment options. Highly publicized errors could affect adversely the uptake of generally beneficial procedures such as screening, for example. Personnel that are suspected of an error or poor performance can be suspended for lengthy periods of time pending investigation, leaving patients anxious and sometimes increasing colleagues' workloads. Even if exonerated in complaint procedures, suspended employees may feel demoralized or that their career or reputation has suffered damage. All of these reasons make it vital that safety nets are in place to minimize the occurrence of mistakes and other adverse incidents.

## ETHICAL PROBLEMS AND MEDICO-LEGAL ISSUES OF NONCONVENTIONAL MEDICINE IN ITALY

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**INTRODUCTION:** Performing nonconventional medicine (also referred to as complementary and alternative medicine) is widespread in Italy since the second half of the last century when the concurrence of different cultural and social factors (end of paternalism in medicine, perception of ineffectiveness of highly technological conventional therapies, new beliefs about health care) have made these practices very common both among the population and the physicians.

**AIM:** After having briefly analyzed common specific areas of nonconventional medicine, the authors present a comprehensive description of their practices in Italy with regard to ethical problems and legal issues involved in these therapies.

**MATERIALS AND METHODS:** Thorough analysis was carried out of nonconventional medicine in Italy (comprehensive of effectiveness, safety and costs, diffusion among physicians), also examining the new law prepared by the Italian Parliament to regulate the practice of these procedures, the position of the Italian Committee of

Bioethics, the standpoint of the Federation of Medical Boards, and the opinion of the Association of the Deans of Medical Schools.

**DISCUSSION AND CONCLUSIONS:** The increasing use of nonconventional medicine raises different problems concerning the necessity of a survey of the clinically appropriate use of these procedures. Regulating these practices, with the help of the Medical Boards regarding the survey and the cooperation of the Schools of Medicine in teaching the fundamentals of these disciplines, will provide assurance of the nonconventional medicine proper practicing, thus minimizing the risks of abuse and malpractice.

### THE DIAGNOSIS OF MESOTHELIOMA IN FORENSIC PATHOLOGY

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**AIM:** The authors define the role of autopsy in the diagnosis of mesothelioma and in the collection of evidence in order to define the cause of neoplasm and consequently its compensation eligibility.

**MATERIALS AND METHODS:** Starting from the analysis of the large casuistics of the Monfalcone Department of Pathology and literature review, the authors carried out thorough exploration of the clinical process leading to the diagnosis of mesothelioma. Attention was paid to the possibilities of mistake regarding these processes, thus outlining an autopsy method that through the collection of evidence can lead to a clear definition of the diagnosis and to corroboration of the cause.

**DISCUSSION AND CONCLUSION:** Given the diffuse industry use of asbestos until 1992, associated with a long latency period of malignant mesothelioma, and considering the fact that the outcome of the clinical diagnosis of such a neoplasm can be influenced in different ways, the autopsy in case of asbestos exposed workers will remain a very important tool, both in confirming the diagnosis and corroborating the causation from occupational exposure for at least two or more decades.

### HINIA RETICULATA POST MORTEM WOUNDS CAUSED BY SMALL SEA FAUNA

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**INTRODUCTION:** Different kinds of fish and sea animals are able to produce typical wounds either on living beings or on corpses. Sea urchin, medusa and moray are some examples. As far as medicine is concerned, there is a great interest for this sort of injury, especially in the fields such as emergency care, surgery, dermatology, and forensic science. Characteristic wounds were observed on the skin of some submerged corpses and they were linked to a common mollusc named *Hinia reticulata*, which usually acts on human body in seawater after death.

**AIM:** This simple analysis is focused on some useful criteria in order to improve the diagnosis of injury caused by fish and small sea fauna.

**MATERIAL AND METHODS:** Bodies were fished out in Trieste Gulf between 1983 and the present days. Investigation on the spot, exterior inspection, autopsy and histologic analysis of the damaged skin were carried out in all cases.

**CONCLUSION:** The knowledge and recognition of *Hinia reticulata* typical wounds on fished out bodies allow the forensic physician to distinguish some pathologic patterns on submerged corpses, which appear to be difficult to interpret.

### RADIOLOGIC INVESTIGATION OF THREE EGYPTIAN MUMMIES BY MULTI-SLICE CT PRELIMINARY RESULTS

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**INTRODUCTION:** The Civic Museum of History and Art of Trieste holds three mummies from Ancient Egypt, which arrived to Italy two centuries ago as a result of trade exchange. The sarcophagus of P3-di-Imn, a God Chons purifying priest of the XXI dynasty (1085-950 BC), contains an unidentified mummy with partially violated bandages, probably of a female individual, dating back to the period between 950 and 663 BC. Traced back to the same XXI dynasty, the mummy of P3-sn-n-Hr an incense bearer in the Amon temple, is still intact in his bandages

and preserved inside its own painted wood sarcophagus. The third Egyptian mummy without bandages, probably a male individual, is contained in a painted wood sarcophagus of the Greek-Roman period.

**AIM:** The authors present preliminary results of a radiologic investigation performed on these mummies and compare them to the previous anthropologic and radiologic studies conducted in the past with traditional methods.

**MATERIALS AND METHODS:** The radiologic investigation was performed using a multi-slice CT Aquilion 16 Toshiba with acquisition of the entire body to high resolution (16x0.5 mm) and subsequent reconstruction both in orthogonal plans and in 3D rendering.

**DISCUSSION AND CONCLUSIONS:** Each mummy seemed to be obtained using a different embalming technique, which allowed the authors to trace back both the appropriate historical period and the social rank. An initial morphological analysis of the bones and articular structures suggested reconsideration of the previous study conclusions and pointed to the necessity of further paleopathologic, microbiologic and toxicologic research.

#### **IMMUNOHISTOCHEMICAL EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR RECEPTOR (VEGFR)-3 WAS CORRELATED WITH AGGRESSIVE TUMOR PHENOTYPE IN INVASIVE BREAST CARCINOMA**

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**AIM:** Vascular endothelial growth factor receptor (VEGF)-3 is considered to be a primary modulator of lymphatic endothelial proliferation and survival but its significance in malignant tumors remains unclear. Our purpose was to investigate the role of VEGFR-3 in invasive breast carcinomas through its correlation with classic clinicopathologic parameters, c-erbB-2, the proliferation marker topoisomerase II $\alpha$  (topoII $\alpha$ ), the members of transforming growth factor- $\beta$  (TGF- $\beta$ ) and mitogen-activated protein kinase (MAPK) signaling pathways, smad2 and ERK2, respectively, and patient survival.

**MATERIAL AND METHODS:** Immunohistochemistry was applied on paraffin-embedded sections from 169 patients with invasive breast carcinomas to detect the expression of the proteins VEGFR-3, c-erbB-2, topoII $\alpha$ , ERK2 and smad2. Results were subjected to monivariate and multivariate statistical analysis.

**RESULTS:** VEGFR-3 was detected in the nuclei of malignant cells and the stromal fibroblasts of the tumor in 41.4% and 65% of cases, respectively. Nuclear VEGFR-3 showed a parallel correlation with nuclear ERK2 ( $p=0.003$ ) and topoII $\alpha$  ( $p=0.03$ ), whereas stromal localization of VEGFR-3 was positively associated with c-erbB-2 ( $p=0.017$ ) and nuclear ERK2 and smad2 of both malignant ( $p<0.0001$  and  $p=0.003$ , respectively) and stromal fibroblasts ( $p=0.023$  and  $p=0.002$ , respectively). Finally, stromal VEGFR-3 was found to be related to a shortened overall survival of postmenopausal patients ( $p=0.058$ )

**CONCLUSION:** In the present study, nuclear VEGFR-3 was found to enhance tumor growth, while stromal VEGFR-3 was related to the emergence of an unfavorable tumor phenotype, affecting negatively the overall survival of postmenopausal patients. The correlation of both VEGFR-3 localizations with ERK2 and smad2 proteins suggests that VEGFR-3 actions may be mediated by TGF- $\alpha$  and MAPK signaling pathway activation, in a possible paracrine manner.

#### **THE EFFECT OF ROFECOXIB, A SELECTIVE COX-2 INHIBITOR, ON EXPERIMENTAL COLON TUMORS**

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**AIM:** Colorectal cancer (CRC) is one of the most common causes of death among human cancers worldwide. In the early 1990s epidemiologic studies showed a 40%-50% reduction in CRC incidence among chronic users of nonsteroidal anti-inflammatory drugs (NSAIDs). Since then, the interest in cyclooxygenase-2 (COX-2) enzyme as a therapeutic target for the initiation and development of CRC has increased because numerous studies in rodent cancer models and in humans have shown NSAIDs to have antineoplastic properties. The aim of our study was to investigate the role of rofecoxib, a selective COX-2 inhibitor, during the initiation and progression stage of chemically induced colorectal carcinogenesis in order to assess its effect on tumor formation.

**METHODS:** Sixty-three ten-week-old male Wistar-Hannover rats were randomly divided into two groups and given subcutaneous injections of 1,2-dimethylhydrazine (DMH) once a week for 15 consecutive weeks. Synchronously with the first injection of DMH the rats from experimental group received rofecoxib orally for 6 months, then autopsy was performed. The number and size of mac-

roscopically visible colorectal tumors were determined, and histologic evaluation of tissue samples for the presence of aberrant crypt foci (ACF), adenomas and adenocarcinomas was performed.

**RESULTS:** The difference in the number of macroscopically visible tumors between the experimental and control groups was statistically significant ( $p < 0.05$ ), and total tumor volume was decreased (by 42%) although the difference was not significant ( $p > 0.05$ ). Histologically, a significantly lower incidence of adenomas and adenocarcinomas ( $p < 0.05$  both) was recorded in the experimental group, however, there were no significant between group differences according to the degree of dysplasia and Dukes stage. In our study no significant differences were found in the number of dysplastic ACF between the experimental and control groups ( $p > 0.05$ ).

**CONCLUSION:** Study results pointed to a conclusion that rofecoxib did not inhibit ACF formation, but did inhibit later steps in the development of experimental colon cancer.

## COMPARISON OF UROLOGIC BIOPSIES IN 2 FIVE-YEAR PERIODS BETWEEN SLAVONSKI BROD COUNTY AND ZAGREB

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**AIM:** Balkan endemic nephropathy (BEN) is a disease of still incompletely known etiology and is associated with an increased frequency of urothelial carcinoma. The aim of the study was to compare urologic biopsies between Dr. J. Benčević General Hospital, Slavonki Brod (SB) and Sestre milosrdnice University Hospital, Zagreb (ZG) in 2 five-year periods 20 years apart. Special point was to compare the frequency of urothelial carcinoma of the kidney and urinary bladder in endemic (SB) and non-endemic (ZG) area.

**MATERIALS AND METHODS:** We used databases from Department of Pathology, General Hospital Slavonki Brod and Ljudevit Jurak University Department of Pathology, Zagreb for the 1980-1984 and 2000-2004 periods.

**RESULTS:** During the 1980-1984 period, a total of 8770 biopsies, 360 (4.1%) of them urologic biopsies, were analyzed in SB, and 61152 biopsies, 2919 (4.8%) of them urologic biopsies, in ZG. During the 2000-2004 period, a total of 27267 biopsies, 1237 (4.5%) of them urologic biopsies,

were analyzed in SB, and 73245 biopsies, 6599 (9%) of them urologic biopsies, in ZG. Out of all urologic biopsies in the 1980-1984 period, there were 106 (30.4%) kidney biopsies in SB and 301 (10.3%) in ZG; 65 (18%) urinary bladder biopsies in SB and 969 (33.2%) in ZG; 81 (22.5%) prostate biopsies in SB and 1072 (36%) in ZG; and 5 (1.4%) testicular biopsies in SB and 216 (7.4%) in ZG. In the 2000-2004 period, there were 248 (20%) kidney biopsies in SB and 749 (11.4%) in ZG; 348 (28%) urinary bladder biopsies in SB and 1299 (19.7%) in ZG; 580 (46.7%) prostate biopsies in SB and 3382 (51.3%) in ZG; and 50 (4%) testicular biopsies in SB and 198 (3%) in ZG. Primary tumors of the kidney were present in 29.2% ( $n = 31$ ) of all kidney biopsies in SB and 27% ( $n = 81$ ) in ZG in the first period, and in 52% ( $n = 129$ ) in SB and 54% ( $n = 404$ ) in ZG in the second period. In SB, urothelial carcinoma of renal pelvis accounted for 83.8% ( $n = 26$ ) of all kidney tumors in the first period and 64.3% ( $n = 83$ ) in the second period. In ZG, urothelial carcinoma of renal pelvis accounted for 22.2% ( $n = 18$ ) of all kidney tumors in the first period and 12.6% ( $n = 51$ ) in the second period. There were 53 cases of urothelial carcinoma of urinary bladder in SB and 630 cases in ZG in the first period, and 170 cases in SB and 939 cases in ZG in the second period.

**CONCLUSION:** Urothelial carcinoma of the renal pelvis accounted for the majority of renal tumors in SB region, probably as a consequence of BEN. The association of BEN and urothelial cancer requires additional, in-depth analysis.

## EXTRALUMINAL GASTROINTESTINAL STROMAL TUMOR SIMULATING OVARIAN TUMOR – CASE REPORT

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Gastrointestinal stromal tumor (GIST) accounts for only 0.1%-3% of all gastrointestinal tumors. It is the most common tumor of mesenchymal origin in the gastrointestinal tract. A case of extraluminal GIST infiltrating the right ovary, uterine tube and urinary bladder is described. Magnetic resonance and routine gynecologic examination suggested a right ovary tumor. Total surgical resection of the ileal extraluminal tumor, hysterectomy with bilateral oophorectomy, and partial resection of the urinary bladder were performed. It measured 20x17x13 cm. A flashy pink tumor mass was present on the cut surface. Histologically

it was composed of fascicles of spindle cells with sporadic storiform formations. Immunohistochemical analysis revealed strong and diffuse CD 117 (c-kit), CD 34 and vimentin expression of tumor cells. Desmin, NSE and SMA immunoreactivity was very weak. Inhibin and calreticulin immunoreactivity as well as differential analysis to primary ovarian tumor of sarcomatoid adult granulosa cell tumor were negative. There were more than 10 pathologic mitoses on 50 HPF. There was wide vascular and perineural invasion of the primary tumor. Multiple mesenteric lymph node and intestinal serosal tumor metastases were found. Because of the high histologic tumor grade and strong c-kit immunoreactivity the use of targeted low toxicity therapy with KIT kinase inhibitors was suggested. Literature data suggest that KIT mutation and activation are important in the GIST pathogenesis and may provide important prognostic information.

#### **HISTOCHEMICAL ANALYSIS OF EXPERIMENTAL APICAL PERIODONTITIS DEVELOPMENT IN DOGS**

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**AIM:** Histochemical assessment of bone resorption kinetics during experimental apical periodontitis in dogs on undemineralized sections using enzymatic histochemical method.

**METHODS:** Experimental model consisted of 7 mongrel dogs (5 experimental and 2 control). Sixteen lower premolar roots served as experimental and 9 as control material. Apical periodontitis was induced by pulp exposure to oral environment for 20, 35, 50 and 65 days. The undecalcified samples of teeth in resected mandibular alveolar bone were embedded in methylmethacrylate and cut in 5- $\mu$ m serial longitudinal sections. Sections were stained with toluidine blue and tartrate-resistant acid phosphatase (TRAP).

**RESULTS:** TRAP activity was detectable already at the pulpitis stage and reached peak at the time of transition from partial necrosis into total pulp necrosis (day 20). In the early stages of total necrosis, while granuloma had not yet formed, a weak TRAP activity could be detected, whereas at the time when granuloma had formed TRAP activity was positive and bone resorption regained momentum.

**CONCLUSION:** Bone resorption is active as long as there is an etiologic factor and inflamed tissue is not eliminated from the root canal. The elimination of etiologic factors creates favorable environment for new bone formation, which is directed towards replacing the lost alveolar bone tissue.

#### **MELANOMA ASSOCIATED VITILIGO – CASE REPORT**

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**INTRODUCTION:** Several clinical observations suggest there is a certain link between vitiligo and malignant melanoma (MM), as the etiology of both diseases evolves around melanocytes. We present a case of a 70-year-old woman with metastatic MM in the lymphatic nodule of the left groin, which developed simultaneously with the “vitiligo-like patches” over the face and extremities.

**CASE REPORT:** The patient was referred to our department after incision skin biopsy of a brown-reddish inflamed nodule in her right groin region. Histopathologic examination showed connective and fatty tissue infiltrated with tumor composed of polygonal cell clusters with prominent nucleoli and substantial quantity of cytoplasmic melanin showing high mitotic activity, indicating metastatic malignant melanoma. At the same time the nodule in the groin appeared, the patient reported emerging of small, multiple, well-demarcated, confluent, depigmented patches over both wrists and dorsal sides of fingers, which had gradually spread over the period of 4 months over the face (starting with forehead, then cheeks), groins and lower legs. Primary melanoma was never detected.

**DISCUSSION AND CONCLUSIONS:** Different clinical studies report on a connection between MM and vitiligo. MM is a cancer of melanocytes, which can, at times, develop simultaneously with vitiligo, a depigmenting disorder with the loss of melanocytes from epidermis and signs of inflammation in deeper layer of the skin. The loss of pigmentation may involve the primary lesion itself or may occur at distant locations (a process that is known as melanoma-associated leukoderma or melanoma-associated hypopigmentation (MAH)). Some authors suggest that the appearance of depigmentation during the course of MM might be considered a good prognostic sign, however, our patient subsequently developed multiple metastases of the lung

as well as in the lymphatic node of the other groin region. This case shows that MM and vitiligo may develop simultaneously, indicating the possibility of similar mechanisms in destruction of both benign and malignant melanocytes.

### **TRICHINELLA SPIRALIS AND BREAST CANCER – CASE REPORT**

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**AIM:** A case is presented of a 47-year-old patient with recurrent ductal invasive breast carcinoma and trichinosis.

**METHODS:** In 1998, the patient underwent mastectomy of the left breast with evacuation of the axilla for ductal invasive breast carcinoma at Department of Surgery in Osijek, followed by radiotherapy of the thoracic wall. Two years later the patient was treated at Department of Infectious Diseases for trichinosis, when she was administered oral therapy with mebendazol and symptomatic therapy. Surgical control showed no signs of local relapse. Six years after mastectomy, a lesion was found on the thoracic wall above the surgical scar, interpreted as local relapse. Excision of the lesion was performed, including pathohistologic examination.

**RESULTS:** Histologic examination showed ductal invasive breast carcinoma with the presence of multiple *Trichinella spiralis* parasites within the muscle as well as tumor tissue.

**CONCLUSION:** A finding of intratumoral parasites indicated infestation that must have occurred in 2000, and the preserved parasite morphology suggested protective effects of the parasite capsule that stopped parasite necrosis.

### **FREQUENCY OF PRIMARY SKIN MELANOMA IN TWO CROATIAN REGIONS**

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**AIMS:** The incidence of malignant melanoma has been constantly rising throughout the world during the last decade. The aim of our study was to analyze primary melanomas among biopsy specimens during a 6-year period (1999-

2004) at two hospitals: one from Zagreb, representative of the inland, and another from Zadar representative of the littoral. Clinical parameters (sex and age distribution) and histologic parameters (type, and Clark and Breslow level of invasion) of primary melanomas were compared between the two regions with different climatic characteristics.

**METHODS:** Thanatos, a computerized tumor registry, was used at Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital from Zagreb, and Tumor Registry was used at Department of Pathology, Zadar General Hospital from Zadar, to obtain relevant biopsy and clinical data on all patients with primary melanoma of the skin.

**RESULTS:** During the 6-year study period, primary skin melanomas were found in 240 patients in Zagreb and 101 patients in Zadar, with the F/M ratio of 1.16 in Zagreb and 0.94 in Zadar. The vast majority of primary skin melanomas were diagnosed on the trunk, followed by extremities, and head and neck region. Histologically, the nodular type was more frequent in Zadar patients, whereas the superficially spreading type was most common in Zagreb patients. The mean patient age was 58 years at both hospitals, ranging from 15 to 90 years in Zagreb and from 10 to 91 years in Zadar. There was a slight (10%) predominance of patients with advanced Clark stages among patients from Zadar.

**CONCLUSIONS:** In our study, the distribution of clinical and histologic parameters of primary skin melanomas among biopsy specimens was similar in the inland and coastal region of Croatia, except for tumor thickness.

### **RENAL CELL CARCINOMA WITH SYNCHRONOUS UROTHELIAL CARCINOMA OF URINARY BLADDER AND NON-HODGKIN LYMPHOMA**

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We report on a case of a 71-year-old male patient with double primary carcinoma involving kidney and urinary bladder, combined with synchronous lymphoma. The patient was admitted to the hospital because of painless gross hematuria. After careful examinations, which included ultrasonography and computed tomography, he was diagnosed with the tumor of the right kidney and papillary tumor of the urinary bladder. Computed tomography of the abdomen and pelvis incidentally revealed a mass of enlarged

lymphatic nodes along the aorta and inferior vena cava. Surgical treatment was performed, including transurethral resection of bladder tumor (TUR), radical nephrectomy of the right kidney, and retroperitoneal lymphadenectomy. Pathohistologic evaluation, combined with immunohistochemistry and electron microscopy, enabled the final diagnosis of renal carcinoma, urothelial carcinoma of the urinary bladder and B-cell non-Hodgkin small lymphocytic lymphoma. So far, no signs of recurrence in urinary bladder or in the kidney have been noted or reported at the time of writing, four months later.

### HER-2/NEU AND OTHER PROGNOSTIC FACTORS IN BREAST CANCER PATIENTS

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**INTRODUCTION:** HER-2/neu (human epidermal growth factor receptor-2)-positive breast cancer, which usually accounts for approximately 25% of cases, has a significantly worse prognosis than HER-2/neu-negative breast cancer, with an increased risk of recurrence and more aggressive disease course. Selection of patients with strongly HER-2/neu-positive tumors is recommended because of the clinical benefit from the HER-2/neu-targeted therapy with trastuzumab (Herceptin<sup>®</sup>) in metastatic breast cancer patients.

**AIM:** The aim of the study was to analyze prognostic features (patient age, tumor histologic grade, pTNM and hormone receptor status) in patients with breast cancer considering HER-2/neu status.

**PATIENTS AND METHODS:** During the 2000-2004 period, 266 patients were tested for estrogen (ER), progesterone (PR) and HER-2/neu status, determined by standard immunohistochemistry tests (DAKO, Copenhagen, Denmark, donated by La Roche Ltd.). All patients were tested irrespective of the spread of the disease, and were divided into two groups according to HER-2/neu overexpression (HER-2/neu 0, 1 negative, and HER-2/neu-2, 3 positive).

**RESULTS:** There were 219 (82.3%) HER-2/neu-negative and 47 (17.7%) HER-2/neu positive cases. The results showed positive association between HER-2/neu expression and grade of tumor. Negative association was found

between HER-2/neu and steroid receptors and mean patient age. T and N tumor status was also found to be in negative correlation with HER-2/neu status, which was the only prognostic factor that did not correspond to the results of a previous multicenter study performed by Croatian authors.

**CONCLUSION:** Evaluation of HER-2/neu status combined with other prognostic markers improves predictive prognosis and should help treat patients, however, its real value should be confirmed by additional studies.

### COMPARISON OF MUCINOUS AND NONMUCINOUS COLORECTAL CARCINOMAS

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**AIM:** Mucinous colorectal carcinomas differ from non-mucinous ones in some clinicopathologic characteristics. In some authors' opinions, these tumors have a predilection for younger age groups, there is no male predominance, and are more advanced at the time of diagnosis. The aim of the study was to test differences in colorectal carcinomas depending on mucinous content.

**MATERIAL AND METHODS:** Patient data were obtained from the computer based colorectal carcinoma registry established at our Department, for the period between January 1, 2000 and December 31, 2004. A total of 1091 patients suffering from colorectal carcinoma were analyzed and divided into 3 groups according to the presence or absence of mucinous content: mucinous carcinomas (>50% of mucin), colorectal carcinomas with mucin (<50% of mucin), and nonmucinous colorectal carcinomas. Sex and age distribution, localization, Dukes stage, and T and N status were observed within all of these three groups.

**RESULTS:** In a total of 117 663 biopsies done between 2000 and 2004 there were 1091 colorectal carcinomas, among which 837 (76.7%) nonmucinous, 169 (15.5%) with mucin, and 85 (7.8%) mucinous carcinomas. The male/female ratio was lower in the groups with mucinous carcinomas (1.1) and carcinomas with mucin (1.0) compared with 1.5 in the group of patients with nonmucinous colorectal carcinoma. The mean age of patients with nonmucinous carcinoma, mucinous carcinoma and carcinoma with mucin was 66.1, 68.1 and 65.4 years, respectively. Mucinous carcinoma and carcinoma with mucin were found to involve more proximal colon (44.4% and 33.5% were right-sided)

than nonmucinous carcinoma (only 18.2% of right-sided). Analysis for Dukes stages revealed 235 (38.3%) C stage cases in the group of patients with nonmucinous cancer, followed by 70 (44.3%) and 46 (56.8%) C stage cases in the groups of colorectal carcinoma with mucin and mucinous carcinoma, respectively. In addition, 85.5% of mucinous carcinomas were in T3 and T4 stage as compared with 73.1% of nonmucinous carcinoma in the same stages. Status N0 was diagnosed in 40.7% of mucinous carcinomas *vs* 57.0% of nonmucinous colorectal carcinoma.

**CONCLUSION:** Mucinous carcinomas showed no male predominance, but showed a higher incidence in the proximal colon as well as higher Dukes stages and T and N status. Predilection for younger age groups was not established, although the mean age of patients with mucinous colorectal carcinoma was found to be slightly lower.

### FIBROMUSCULAR DYSPLASIA OF RENAL ARTERIES IN NONTUMOROUS SURGICAL SPECIMENS

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**INTRODUCTION AND AIM:** Arterial fibromuscular dysplasia (FMD) is a condition that most frequently involves renal arteries, and is bilateral in nearly half of cases. It is more frequent on the right side (3:1), with a female predominance. FMD of renal arteries usually involves distal parts of the artery and its branches. The aim of this study was to analyze renal arteries in patients who underwent nephrectomy due to nontumorous conditions of the kidney.

**MATERIALS AND METHODS:** We analyzed a consecutive series of 30 patients (m:f=14:16) who had undergone nephrectomy in 2003, and whose histopathologic examination resulted in the diagnosis of a benign condition. Eight patients were excluded from the study because specimens of renal arteries were not found. The median age of the patients (m:f=8:14) was 59.4 (range 39-78) years. The patients were divided into the group showing FMD (group 1) and those that showed no pathologic changes of renal arteries (group 2). Specimens were routinely fixed, paraffin embedded, cut at 5 µm, stained with hematoxylin and eosin, and examined by light microscopy.

**RESULTS:** In group 1, FMD of renal arteries was found in 10/22 patients (m:f=4:6), median age 64 (range 39-79) years. In group 2, renal arteries showed no changes in 12/22 patients (m:f=4:8), median age 61 (range 43-70) years. Histopathologic analysis of renal tissue showed chronic pyelonephritis in all 10 patients from group 1 and 7 patients from group 2. Other diagnoses in group 2 were renal calculi, cystic renal disease, hydronephrosis and renal hypoplasia.

**CONCLUSION:** In other studies, the incidence of FMD in renal arteries, found at autopsy, was about 1%, however, we found no data on the incidence *in vivo*. In this study, fibromuscular dysplasia of renal arteries was observed in a significant proportion of patients who had undergone nephrectomy for nontumorous diseases. The significance of this finding should be additionally explored.

### UNDIFFERENTIATED FIBROBLASTIC SARCOMA OF OMENTUM – CASE REPORT

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A 37-year-old female presented to the physician for ascites and abdominal tenderness, without history of any other previous disease. Preoperative examination (CT and ultrasound of the abdomen, colonoscopy and irrigography) showed no pathologic disturbances. Laboratory tests revealed slightly elevated CA 125, CA 15-3 and CA 19. Explorative laparotomy revealed the whole omentum covered with gray-white firm tumor nodules that measured 1-3 cm in largest diameter. The serosa of the colon, ovarian surfaces and uterus were covered with numerous small white nodules, which had not been surgically removed. During laparotomy the omentum was removed and pathohistologically analyzed. The tumor located on the omentum surface was white, firm with infiltrative borders, measuring up to 21 cm. Frozen section was performed and the pathologist suggested the possible mesenchymal tumor or reactive/inflammatory changes. Microscopically, the tumor was composed of sheets and cords of polygonal to round cells with prominent collagen fibers interspersed. Perivascular hyalinization was prominent and necrosis was focally present, with up to 10 mitoses *per* HPF. Immunohistochemical study revealed reactivity for vimentin and bcl-2, focal reactivity for CD68, CD34, CD99, SMA, MSA and



EMA, while CD117, CK AE1/AE3, CD 20, CD30, CD3, CD30, HMB45, desmin, alfa-aktin and s-100 were negative. The patient has currently been on chemotherapy.

### **MAGE-A1, MAGE-A3 AND NY-ESO-1 IN SQUAMOUS CELL CARCINOMA OF THE PENIS**

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**AIM:** To investigate immunohistochemical expression of the MAGE-A and NY-ESO-1/LAGE-1 cancer testis antigens in penile carcinoma tissue.

**METHODS:** The study included 30 penile carcinoma samples from patients undergoing penile amputation at urology departments of the Zagreb University Hospital Center and Sestre milosrdnice University Hospital from 1997 to 2004. Three monoclonal antibodies were used for immunohistochemical staining: 77B for MAGE-A1, 57B for multi-MAGE-A and D8.38 for NY-ESO-1 expression.

**RESULTS:** The mean patient age was 64.7 (range 41-92) years. Expression of MAGE-A1 was observed in 13% of carcinoma samples, whereas both multi-MAGE-A and NY-ESO-1 stained 97% of samples. Immunohistochemical staining was only detectable in the cytoplasm. A significant heterogeneity could be observed within the same tissue sample where areas with strong positivity coexisted with cancer testis antigens negative areas. Cancer testis antigens expression did not significantly correlate with tumor grade.

**CONCLUSION:** This study documented for the first time the expression of cancer testis antigens in penis cancers. Further research is warranted with potential implications for both diagnosis and therapy.

### **MULTIORGAN RESECTION FOR RECTAL CANCER AND MULTIPLE ADENOMA COLI ASSOCIATED WITH PHEOCHROMOCYTOMA AND PANCREATIC TUMOR: CASE REPORT**

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Multiple primary cancers occurring in the same patients are uncommon and have been reported to account for 1.8%-3.9% of all cancers. We describe an extremely rare case of

rectal cancer and multiple adenoma coli syndrome and multiple endocrine neoplasia type 1 (MEN1) related tumors (an endocrine cell tumor of the pancreas and pheochromocytoma). We found only one similar case in the literature, reporting on a patient with genetically proven familial adenomatous polyposis (FAP), and endocrine cell tumor of the pancreas and bilateral parathyroid tumors related to MEN1. It is generally accepted that patients with adenomatous polyposis syndrome such as FAP, or those with hereditary nonpolyposis colorectal cancer (HNPCC) syndrome type II (Lynch II) often have extracolonic malignant tumors or precancerous lesions of various organs. Such cancers include those of the thyroid, stomach, duodenum, duodenal papilla, gallbladder, jejunum, ileum, breast, thymus and skin (melanoma). With improved longterm survival rates, secondary or tertiary cancers have been increasingly found in these patients. A variant of FAP is the so-called multiple adenoma clinically characterized by many but usually fewer than 100 colonic lesions. However, unlike FAP patients, they mostly have no demonstrable germline adenomatous polyposis coli mutation. In our patient, laparotomy was performed for endoscopically proven rectal cancer. During abdominal exploration, additional tumors were detected in the left adrenal gland and tail of the pancreas. Therefore, abdominoperineal rectal resection, left adrenalectomy, and resection of the tail of the pancreas were performed. Pathohistologic examination revealed polypoid adenocarcinoma in the rectum with multiple adenomatous polyps in the rest of resected bowel related to some type of adenomatous polyposis syndromes (FAP or so-called multiple adenoma coli), pheochromocytoma of the adrenal gland, and microcystic cystadenoma of the pancreas. Because of the existence of tumors in two of the four principal tissues (parathyroids, pancreas, pituitary and adrenal glands) affected by MEN1, we concluded this case as a sporadic MEN1 according to previous reports.

### **INTRAOPERATIVE FROZEN SECTION ANALYSIS IN SURGICAL MANAGEMENT OF MAMMARY PHYLLODES TUMORS**

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**INTRODUCTION:** Phyllodes tumors (PT) are rare fibroepithelial lesions that account for less than 1% of all breast neoplasms. Although PT show many features of

much commoner fibroadenomas (FA), they are likely to recur and can be locally aggressive. In addition, a small percent of PT show sarcomatoid features and have a potential to metastasize. Recurrence is directly related to incomplete resection without adequate margin of normal breast tissue. Because many of PT were preoperatively misinterpreted as FA, in many cases initial surgical excision is insufficient and re-excision is required.

**AIM:** To evaluate the influence of intraoperative analysis on surgical management of PT.

**MATERIAL AND METHODS:** Retrospective analysis included all PT diagnosed at University Hospital for Tumors during the 1995-2004 period. Patient age, tumor size, surgical method, number of examined frozen sections, and final histologic diagnosis were analyzed.

**RESULTS:** During the 10-year period, a total of 77 female patients with the diagnosis of PT were recorded. The mean patient age was 44.5 (range 17-81) years, and the mean tumor size was 6.30 (range 1-24) cm. Fifty (65%) cases of PT were diagnosed as benign, 14 (18%) as borderline, and 13 (17%) as malignant. Primary surgical treatment involved lumpectomy in 48 (62%), wide surgical excision in 24 (31%), and mastectomy in the remaining 5 (7%) cases. Intraoperative analysis was performed in 64 (83%) cases. In most cases two frozen sections *per* biopsy were examined, varying from 1 to 4. Intraoperative diagnoses included PT in 50 (78%), FA in 12 (18%) and malignant mesenchymal tumor in 2 (3%) cases. All cases misinterpreted as FA were examined on just one frozen section. As a direct result of intraoperative diagnosis of PT, wide surgical excision was performed in 10 and simple mastectomy in 2 cases.

**DISCUSSION:** Phyllodes tumor is a biphasic tumor composed of benign epithelial elements and a cellular spindle cell stroma. It accounts for approximately 2.5% of all fibroepithelial tumors of the breast. In most cases the pathological distinction from the much commoner FA is straight. Smaller lesions with nonspecific macroscopic features and recurrent tumors may present a diagnostic problem. Leaf-like projections and hypercellular stroma suggest the diagnosis of PT. Other features common in PT include giant cells, foci of pseudoangiomatous stromal hyperplasia, patchy myxoid changes, cystic formations and metaplastic lesions. Most authors classify PT as benign, borderline and malignant. Grading is based on histologic evaluation of stromal cellularity, pleomorphism, mitotic activity, stromal overgrowth, and type of margins (pushing or infiltrative). This classification proved to be correlated with local breast recurrence in patients who did not undergo mastectomy. Af-

ter lumpectomy or local excision, recurrence was observed in 21%, 46%, and 65% of patients with benign, borderline and malignant PT, respectively. Metastases occurred in less than 5% of borderline and as many as 25% of malignant PT. Most authors agree that complete excision with 1-2 cm of normal tissue around the tumor can prevent recurrences in benign PT, and significantly lowers the risk of recurrence in borderline PT. The high risk of recurrence and metastatic potential of malignant PT require radical approach and mastectomy is advised.

**CONCLUSION:** Most histologic features of PT are recognizable on frozen sections. Triple preoperative assessment accompanied by intraoperative analysis helps separate rare PT from FA as well as benign from malignant PT. Adequate primary surgical management with wide excision lowers the risk of recurrence and need of secondary surgery in cases of benign and borderline PT, whereas simple mastectomy is reserved for larger lesions and malignant PT.

#### **INTRAOPERATIVE ANALYSIS OF THYROID LESIONS: A SINGLE INSTITUTION EXPERIENCE**

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**INTRODUCTION:** Frozen section analysis had a great value in the operative management of thyroid lesions in the past. However, with the widespread use of fine needle aspiration biopsy (FNA), the value of frozen sections has been questioned. Although FNA is cost-effective and easy to perform, frozen sections are still preferred by some institutions.

**METHODS:** All patients with thyroid disease operated on between January 2000 and December 2004 at University Hospital for Tumors were identified. Data obtained from pathology files included patient age and sex, number of frozen sections performed, frozen section results, and final histologic diagnosis.

**RESULTS:** A total of 887 patients, 781 (88%) women and 106 (12%) men, were identified. Median age for women was 50 (range 8-80) and for men 52 (range 15-85) years. Frozen section was performed in 675 (76%) patients. The number of examined frozen sections varied from one in cases of macroscopically benign conditions such as nodular goiter to four when malignant follicular lesion was suspected. The intraoperative diagnosis was benign in 135 (20%)

and malignant in 172 (25.5%) patients. In 368 (54.5%) patients frozen section showed follicular neoplasm and definitive diagnosis was postponed until final sections. Intraoperative analysis properly recognized 157 (95%) of 165 papillary carcinomas, 3 (14%) of 21 follicular carcinomas, all 7 cases of medullary carcinoma, and two anaplastic carcinomas. In 3 patients with the diagnosis of malignant tumor, final histologic sections revealed a collision tumor (medullary and follicular carcinoma), insular carcinoma and one squamous carcinoma. In 3 cases papillary carcinoma was misinterpreted as follicular lesion, and another five carcinomas, smaller than 5 mm, were found close to serial sliced follicular encapsulated lesions.

**DISCUSSION:** During the last two decades the benefit of intraoperative frozen section analysis in thyroid surgery has been questioned by many authors. Commonly mentioned disadvantages include a limited number of performed sections, lower quality of intraoperative slides, artifact nuclear clearing, difficult interpretation of post-FNA changes, and identification of vascular/capsular invasion. Our study showed a high sensitivity of frozen sections in the diagnosis of papillary lesions. False-positive results of pseudopapillary hyperplasia in hyperplastic nodules misinterpreted as carcinoma were minimized with the experience of the performing pathologist. Intraoperative diagnosis can be difficult in cases of follicular variant of papillary carcinoma or microcarcinoma which can be incidentally found during evaluation of another thyroid lesion (as was the case in 3 and 5 of our patients). The value of frozen sections in the diagnosis of follicular lesions remains uncertain. Our series showed that only 3 of follicular carcinomas with macroscopically suspect invasion were properly recognized with the use of multiple frozen sections. Therefore, an agreement was reached between the surgeon and the pathologist that all follicular and oncocytic lesions except for those clearly malignant on intraoperative sections should be defined as "follicular" or "oncocytic" tumor, and definitive diagnosis postponed until examination of serial sections. Frozen sections can be used for accurate identification of intrathyroid parathyroid neoplasms, normal parathyroid glands, and lymph nodes that may contain metastatic thyroid carcinoma. Although easy to diagnose, the use of frozen sections in the evaluation of non-neoplastic conditions such as nodular goiter and thyroiditis is not justified, except in cases when neoplasm is suspected on preoperative FNA.

**CONCLUSION:** Intraoperative frozen section analysis has a great value in the diagnosis of papillary lesions and nodal involvement of thyroid primaries, especially in

cases when preoperative FNA was inconclusive or suspicious for malignancy. Frozen sections are of limited value in the diagnosis of follicular and oncocytic tumors and papillary microcarcinoma. With the wider use of FNA and growing experience of cytopathologists, non-neoplastic thyroid diseases as well as clearly malignant neoplasms can be accurately diagnosed preoperatively and definitive surgical treatment can be planned according to FNA results. However, in most institutions final decision which method to use depends on the surgeon's experience and quality of cooperation with the performing pathologist and cytopathologist.

### **ADENOCARCINOMA OF UTERINE CERVIX – PROGNOSTIC SIGNIFICANCE OF CLINICOPATHOLOGIC PARAMETERS**

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**AIM:** To investigate the prognostic significance of several clinicopathologic parameters in patients with adenocarcinoma of uterine cervix.

**METHODS:** The study included 36 patients treated at University Department of Gynecology and Obstetrics, Zagreb University School of Medicine, Zagreb, Croatia, during the 1978-1998 period. Cox proportional hazard analysis was performed to test the prognostic significance of menstrual status, clinical stage, architectural grade, nuclear grade, DNA ploidy, proliferative activity, and mode of therapy.

**RESULTS:** The 5-year survival of patients was 75%. Univariate analysis proved clinical stage ( $p=0.042$ ), architectural grade ( $p=0.009$ ) and nuclear grade ( $p=0.001$ ) to be statistically significant. Multivariate analysis showed that nuclear grade ( $p=0.007$ ) turned out to be the only statistically significant parameter. According to nuclear grade, the 5-year survival was 80% in the prognostically favorable group and 30% in the prognostically unfavorable group of patients.

**CONCLUSION:** Our data showed that in case of adenocarcinoma of uterine cervix, the nuclear grade, clinical stage and architectural grade of the tumor were the most important prognostic parameters. The analysis of DNA ploidy and proliferative activity had no prognostic significance.

## FINE STRUCTURE OF THE RAT EXORBITAL GLAND

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**INTRODUCTION AND AIM:** In the rat, the major lacrimal gland is situated in close proximity to the external ear and the parotid gland. It is called exorbital lacrimal gland or gl. Loewenthalii. Since there is a lack of data on the fine structure of this gland, we examined the morphological features of this organ at various ages of the rat.

**MATERIALS AND METHODS:** Male Fischer rats were sacrificed at various age (2.5 months, 3 months, 6 months and 9 months), their exorbital glands dissected and processed for transmission electron microscopy.

**RESULTS:** Glandular tissue was composed of lobules divided by the interlobular connective tissue. Within the interlobular tissue, interlobular ducts were encountered. Each lobule consisted of numerous acini surrounded by the gentle connective tissue, bearing capillaries and small intercalated ducts. Intercalated ducts were lined with simple squamous epithelium. Acini of the gland were composed of several pyramidal cells. The nucleus of the cell was located centrally or towards the basal part of the cell. Occasionally, 2 nuclei within a single cell could be observed. Each nucleus contained several nucleoli. The cytoplasm of the cells had well-developed rough endoplasmic reticulum, Golgi apparatus and mitochondria with cristae. Some cells had an abundant presence of secretory granules, whereas some were devoid of these organelles. Preliminary, no significant difference in the fine structure of the gland was found concerning the various age of rats.

**CONCLUSION:** It seems that the acini of the rat exorbital gland have 2 types of cells with different appearance of the cytoplasm.

## FROZEN SECTION: HISTOTECHNOLOGIST'S POINT OF VIEW

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Tissues taken from the body for diagnosis of disease processes must be processed at a histology laboratory to produce microscopic slides that are viewed under the microscope by the pathologists. The persons who do the tissue processing and make glass microscopic slides are his-

totechnologists, "the artists of the laboratory". At times, during performance of surgical procedures, it is necessary to get a rapid diagnosis of a pathologic process. The surgeon may want to know whether the excised lesion is benign or malignant, and whether the margins of the lesion, in case of malignancy, are free of disease. Such a diagnosis is accomplished through the use of frozen section. The piece(s) of tissue to be studied are snap frozen in cold liquid or cold environment (-20 to -70 °C), because freezing makes the tissue solid enough to section with a microtome. Frozen sections are performed with an instrument called a cryostat. The temperature inside the cryostat is about -20 to -30 °C.

The tissue sections are cut, preferably at 6-8 µm, picked up on a coated glass slide and stained. The routine stain is that of hematoxylin and eosin (H&E), similar to the normal one, except for its duration of several minutes. Other stains, referred to as "special stains", are employed in specific situations according to the diagnostic need. Frozen sections are stained by hand, because this is faster for one or a few individual sections. The stain is progressive, in which the section is left in contact with the stain until the desired tint is achieved. The stained sections are covered with a thin piece of plastic or glass to protect the tissue from being scratched, to provide better optical quality for viewing under the microscope, and to preserve the tissue section for a long time. They are examined by a pathologist who informs the surgeon about the result. The quality of a frozen section cannot be stressed enough because it provides the basis for the diagnosis to be as accurate as possible.

## DETECTION AND TYPING OF HUMAN PAPILOMAVIRUS IN CERVICAL SWABS BY POLYMERASE CHAIN REACTION

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Genital infection with human papillomavirus (HPV) is very common in sexually active women and can have serious consequences. Persistent infections with high risk HPV are the leading cause of cervical cancer and progression from cervical intraepithelial neoplasia through invasive cervical cancer which is a slow process, estimated to take 10-15 years. Therefore, the necessity for HPV detection and typing is quite obvious. The objective of this paper is to present HPV detection and typing by the method of polymerase

chain reaction (PCR) with commercially available kits, routinely used at our laboratory. After specimen collection, the first step in HPV detection is isolation of viral DNA. The next step is detection of HPV DNA and  $\beta$ -globin as an internal control by PCR reaction of successful sampling and appropriate transporting. If  $\beta$ -globin detection is positive, we perform PCR-RFLP for HPV typing. In case of negative  $\beta$ -globin result, the sampling procedure or sample transport have been questionable and further results are considered doubtful.

### USE OF MONOCLONAL ANTIBODIES IN COCAINE AND HEROIN ADDICTS

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**INTRODUCTION:** Monoclonal antibodies have become a powerful tool in laboratory diagnosis, therapeutic purposes, and experimental medicine. Scientists from the Department of Neuropharmacology, Scripps Research Institute, Los Angeles, CA, USA, have started experimenting with catalytic monoclonal antibodies as potential agents that can be used as a vaccine for treating persons prone to drug abuse.

**AIM:** The aim of this experiment was to create a safe and potent vaccine based on catalytic monoclonal antibodies that can bind to cocaine in the bloodstream and enzymatically break it down into its nonaddictive components, mimicking the body's natural metabolism of cocaine but at a much faster rate. By attacking cocaine directly, this approach could also help reverse some toxic effects of the drug, such as decreased blood flow and reduced oxygen delivery to the brain.

**MATERIAL AND METHODS:** Laboratory rats and mice were used in the experiment. Rats were injected an intravenous dose of the drug until they became addicted. They became hyperactive and snored due to the effect of the drug. After vaccine had been introduced, all of the symptoms disappeared.

**RESULTS:** Thirty seconds of the vaccine administration, the brain concentration of the drug decreased by 30%-63%. The symptoms of drug abuse disappeared.

**CONCLUSION:** The result of this experimental study proved highly promising, the clinical study is under way, yet I believe that further improvement in cocaine addiction therapy will also be achieved through better understanding of the role of neurotransmitters and receptors.

### PRACTICING TRANSFUSION MEDICINE AT CITM

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In the last 10 years, transfusion medicine has been a rapidly developing part of medical science. One of the leading characteristics is its complexity because numbers of different activities have been integrated into one sophisticated system. These activities are presented through current practices of the Croatian Institute of Transfusion Medicine (CITM). Promotion and maintenance of blood donation is the responsibility of the Croatian Red Cross and CITM. Blood or blood components are collected from voluntary non-remunerated blood donors. Informative leaflets and other educational materials are provided at donation sites. Donor selection is carried out in accordance with national standards and relevant regulations. After determining the hemoglobin level, an interview is conducted by a physician using a uniform questionnaire. All donors are subjected to medical examination prior to each donation. Blood is collected using a disposable system into a plastic bag. Platelets and plasma are also collected using apheresis technique. CITM has a fully computerized system and assured traceability using unique donation number (bar-coded). Blood components are produced and stored following the principles of GMP to meet the specific quality requirements. All blood components are produced in a closed system, using automated equipment. The "buffy-coat" depletion method is used during primary production, and all platelet concentrates are subjected to leukodepletion. Quality control has been established for all incoming materials, intermediate products and finished blood products. Statistical process control is in use to monitor collected data over a period of time in a standardized fashion. Routine serologic screening of all donations has to be performed for a range of microbial infections. The following markers are obligatorily tested in Croatia: HBsAg, anti-HIV 1/2, anti-HCV, and syphilis test. Combo HIV Ag/Ab test and HCV Ag test have been recently introduced in CITM. On repeatedly reactive samples confirmatory testing is performed, including PCR techniques. Blood samples taken from blood donors are tested regularly for the determination of blood group AB0, Rh(D) and Kell, and antibody screening. CITM also provides pretransfusion compatibility testing for patients. Laboratory testing is performed according to the principles of GLP, using in-process control, working standards and evaluation of reagents. The performance of testing procedures is regularly assessed by participation in a formal system of proficiency testing.

Quality management system according to ISO 9001:2000 has been established and certified by Lloyd's Register QA. There is a national system of reporting adverse reactions to transfusion therapy. Croatia is member of EHN (European Haemovigilance Network).

### **HEMATOXYLIN BASIC FUCHSIN PICRIC ACID (HBFP) METHOD FOR MORPHOLOGICAL EVALUATION OF FIFTY CASES OF MYOCARDIAL INFARCTION**

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The early stage of myocardial infarction is characterized by a very high mortality rate. The diagnosis becomes difficult not only clinically but also morphologically. Within the first few hours morphological changes associated with myocardial infarction are difficult to detect with certainty by light microscope on standard HE method stained slides. Hematoxylin basic fuchsin picric acid (HBFP) staining method has been used for morphological diagnosis in the early stages of myocardial infarction, however, there are some controversial data on the reliability of this method, probably due to the high sensitivity of the staining procedure, degree of autolysis, fixation time, and section thickness. We applied the HBFP staining method to 50 human heart specimens after autopsy to determine the usefulness of this technique in identifying early myocardial ischemia and extent of early infarcts.

### **A NEW METHOD FOR *LISTERIA MONOCYTOGENES* ISOLATION**

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*Listeria* is a non-sporiferous, non-encapsulated rod with optimum growth temperature of 30-37 °C. In the genus *Listeria*, only *Listeria (L.) monocytogenes* and *L. ivanovii* are associated with disease in humans. Listeriosis can take three clinical forms such as encephalitis, septicemia and abortion, with a high mortality rate. *L. monocytogenes* is an ubiquitous bacterium that can be isolated from soil, vegetation, raw and processed food. The main way of its spread from the environment to humans is contaminated food. *L. monocytogenes* presents a great hazard because of its propagation already at 4 °C (refrigerated temperature).

**AIM:** The aim of the study was to test the results of *L. monocytogenes* isolation obtained by two methods: new immunomagnetic separation (IMS) and traditional culture-based method.

**MATERIALS AND METHODS:** The new IMS detection system has been developed to detect *Listeria* spp. in food. It was introduced in 2000 in our laboratory and has been routinely used since 2001. IMS consists of the following steps: homogenization of 25 g of food sample in 225 ml half Fraser enrichment and 6-h incubation at 37 °C, concentration of *Listeria* by capture onto immunomagnetic particles (Dynabeads anti-*Listeria*, Dynal A.S., Oslo, Norway) and washing with sterile wash buffer, resuspending in 0.1 ml of sterile wash buffer, inoculation of 50 µl of the washed and resuspended magnetic particles on PALCAM agar. Traditional method: in our study we also used the method described in ISO 10560, which includes several steps: homogenization of 25 g of sample in 225 ml UVM (University of Vermont primary enrichment broth) and 24-h incubation at 30 °C, then 24-h subcultivation of 0.1 ml of this pre-enrichment in 10 ml Fraser enrichment at 30 °C. The next step is cultivation on selective agar (PALCAM or Oxford *Listeria* agar) at 37 °C. After 24-48 hours, the possible presence of typical colonies is observed. *L. monocytogenes* colonies on Oxford agar are green-brown with a black halo, whereas on PALCAM agar they are olive-green to black.

**RESULTS AND CONCLUSION:** The traditional method proved to be superior to the IMS method.

### ***MYCOPLASMA PNEUMONIAE*: IMPORTANT CAUSATIVE AGENT OF ATYPICAL PNEUMONIA**

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**INTRODUCTION:** *Mycoplasma M. pneumoniae* is one of the most common etiologic agents of atypical pneumonia. It accounts for 15%-20% of all cases of community-acquired pneumonia in general population and up to 50% of pneumonias in school aged children. The highest attack rates are in children 5 to 20 years old. Infections are endemic throughout the year with outbreaks observed at intervals of 4 to 8 years. The diagnosis of *M. pneumoniae* infection is usually based on serology (complement-fixation test /CF/, enzyme immunoassay /EIA/ and indirect immunofluorescence assay /IFA/).

**AIM:** To determine the incidence and epidemiologic characteristics of atypical pneumonias caused by *M. pneumoniae* in Croatia during the five year period (2000-2004).

**METHODS:** Serum samples from 630 patients with clinical symptoms of atypical pneumonia were tested at the Laboratory of Serologic Diagnosis, CNIPH. The presence of specific antibodies to *M. pneumoniae* was demonstrated using CF test (micromethod). A fourfold or greater increase in antibody titer in paired sera as well as single titer > 1:128 were considered positive finding.

**RESULTS AND CONCLUSION:** *M. pneumoniae* was demonstrated in 108/630 (17.1%) patients. The majority of patients (56/108; 51.9%) were aged 6 to 20 years. The disease occurred sporadically throughout the year in the period from 2000 to 2003, with an epidemic recorded in the autumn and winter 2004 that persisted in 2005. The largest number of patients in the interepidemic years were from the Zagreb County. In the epidemic year 2004, the greatest proportion of cases were recorded in the Zagreb County and in Slavonia.

### THE ROLE OF HLA ANTIGENS IN CADAVERIC KIDNEY TRANSPLANTATION

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Kidney transplantation is the best possible treatment for patients with terminal kidney diseases. Organ donors can be deceased persons (cadaver) or living related donors (LR).

Deceased person is a patient with undected brain damage which results in brain death. Discontinuation of brain circulation and clinically proved brain death identify the person as a potential organ donor. Histocompatibility testing is an essential component of successful kidney transplant programs for two fundamental reasons. Firstly, human leukocyte antigens (HLA) play a central role in the cellular and humoral immune responses that determine the outcome of a transplant. Secondly, the extensive polymorphism of HLA poses a major barrier to successful transplantation. HLA matching is used to choose the best matched kidney candidates from national waiting list. An HLA matching effect was significantly associated with HLA-A, -B, and DR match kidney transplantation and has an overall significant effect on graft survival and/or rejection. Prospective serologic HLA typing of patients and preharvest HLA typing of deceased donor is performed by complement dependent microlymphocytotoxic assay

(MLCT) and PSR-SSP method. The exhaustive study of sera of patients on waiting list for transplantation with respect to the nature, class and specificity of antibodies is important since it is possible that antibodies can bring out events that will have different consequences for the graft survival. Cross match is an essential test, which should be performed before each transplantation event, to prevent positive reaction with potential donor. All assays are performed at National Reference Tissue Typing Center of the Ministry of Health, Republic of Croatia.

### FISH AND IMMUNOENZYMATIC DOUBLE STAINING METHOD ON ROUTINE SINGLE SLIDES AND TISSUE MICROARRAYS

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**AIM:** We tried to develop a method for simultaneous detection of two different proteins in the same tissue sample. We also wanted to try FISH and immunostaining on tissue microarrays (TMAs) to see if they are valid for interpretation of these methods as single slides.

**METHODS:** For double staining, we used Dakocytomation EnVision DoubleStain System (DAKO, Denmark) with different combinations of antibodies. FISH was done by using standard protocol and commercial probes (Vysis). Tissue microarrays were done manually as a modification of the routine paraffin embedding technique.

**RESULTS:** On single slides and on tissue microarrays, both double immunostaining and FISH worked well. There was no difference in the quality of results on single slides or TMA, although samples on TMAs showed better cell divisibility. Double staining gave clear results easy to interpret for the CD20/bcl6, CD20/CD3, bcl6/kappa, CD20/CK antibodies. FISH signals were bright and clear for all probes used (IgH/bcl2, bcl6, IgH/MALT, API/MALT).

**CONCLUSIONS:** By using double staining method it is possible not only to detect the expression of certain proteins in a tumor cell but also to see (co)localization between different antigens. Double staining and FISH work well on single slides and on TMAs. Using a simple technique for making TMAs saves samples, antibodies/FISH probes and time. It also allows for a more precise interpretation of results considering the fact that samples on arrays represent just the tumor without the surrounding tissue.