3. HRVATSKI KONGRES PATOLOGIJE I SUDSKE MEDICINE

3. HRVATSKI KONGRES KLINIČKE CITOLOGIJE

1. HRVATSKI SIMPOZIJ CITOTEHNOLOGIJE

s međunarodnim sudjelovanjem

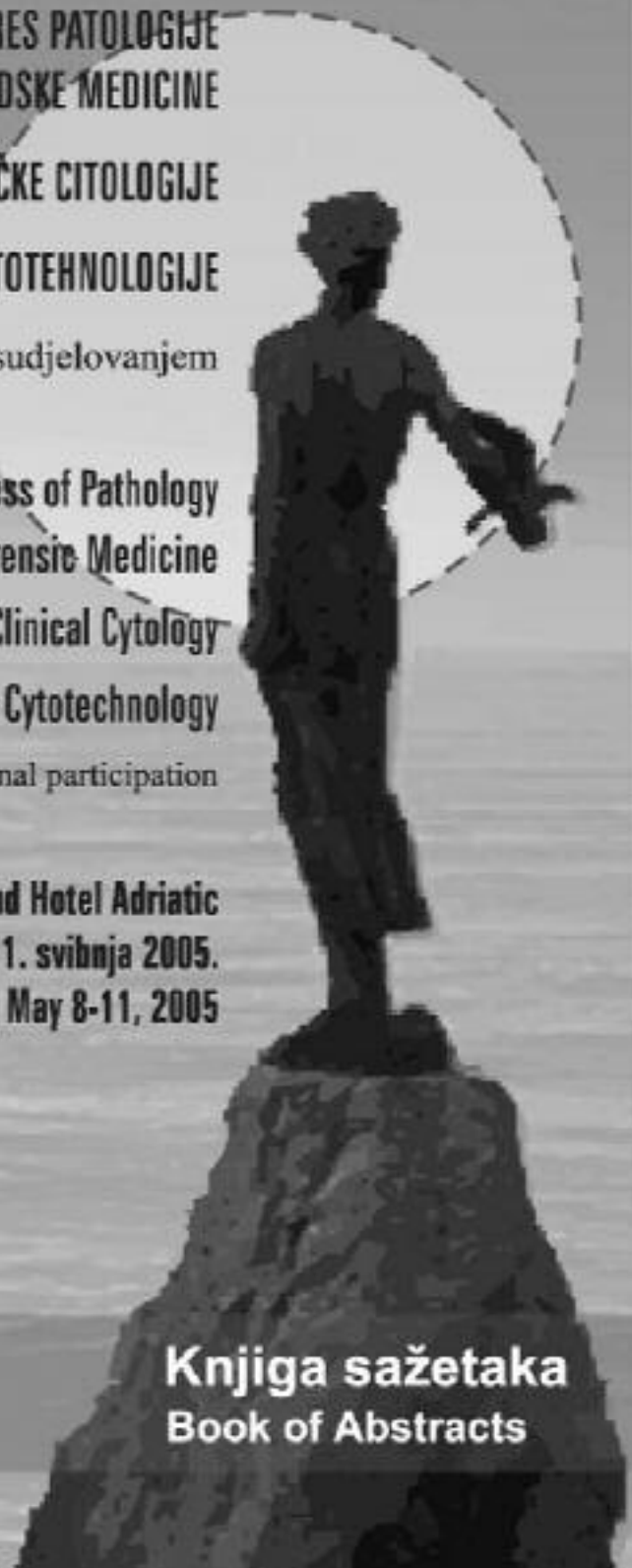
3rd Croatian Congress of Pathology and Forensic Medicine

3rd Croatian Congress of Clinical Cytology

1st Croatian Symposium of Cytotechnology

with international participation

OPATIJA, Grand Hotel Adriatic
08.-11. svibnja 2005.
May 8-11, 2005



Knjiga sažetaka
Book of Abstracts

Urednici / ***Editors***

Prof. dr. sc. Nives Jonjić
Mr. sc. Ika Kardum-Skelin

Grafičko oblikovanje i priprema za tisak / ***Design and prepress***

Studio Hrg d.o.o., Zagreb

Tisak / ***Press***

MB tisak d.o.o., Karlovac

Naklada / ***Printing***

300 primjeraka / copies

svibanj 2005. / May 2005

SADRŽAJ / *TABLE OF CONTENTS*

Cijenjene kolegice i kolege, dragi prijatelji,

Velika nam je čast i zadovoljstvo pozdraviti vas u ime Organizacijskog odbora na 3. Hrvatskom kongresu patologa i sudskih medicinara, 3. Hrvatskom kongresu kliničkih citologa i 1. Hrvatskiom simpoziju citotehnologa s međunarodnim sudjelovanjem, u Opatiji od 8. do 11. svibnja 2005.

Neobično nam je drago što je po prvi puta Kongres organiziran kao most između tri srodna morfološka društva, čime nam je pružena prilika za razmjenu znanstvenih istraživanja, novih ideja i tehnologija te njihova primjena u svakodnevnoj dijagnostičkoj praksi.

Vjerujemo da je odabir tema i aktivno sudjelovanje vrlo uglednih svjetskih i domaćih stručnjaka pobudio iznimno zanimanje liječnika različitih specijalnosti koji se bave morfologijom ili njene rezultate koriste u svakodnevniom radu. Rad Kongresa odvija se kroz plenarna predavanja, simpozije, seminare, usmena izlaganja i poster sekcije.

Drago nam je što boravak u Opatiji možemo koristiti za ugodno druženje tijekom Kongresa, obilazak Grada, izlet u unutrašnjost Istre te zajedničku večeru.

Opatijska rivijera s više od 160 godina tradicije u turizmu, predstavlja jedno od najpoznatijih turističkih mjesta na obali. Za vrijeme Austro-Ugarskog carstva bila je ekskluzivno ljetovalište europskog plemstva. Raskošni hoteli i vile, okružene prekrasnom prirodom, ugošćavale su brojne goste iz najviših europskih krugova, i to tijekom cijele godine, zahvaljujući svojoj blagoj klimi. Predivni vrtovi, puni mirisnog sredozemnog raslinja, palmi i kamelija, učinili su Opatiju nezaboravnim ljetovalištem.

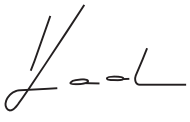
Izlet u centralni dio Istre, na pola sata vožnje od Opatije, pruža nam mogućnost da upoznamo ljepote vinskih cesta i seoskog turizma ovog kraja.

S nadom u ispunjenje vaših očekivanja, napominjemo da uspjeh Kongresa ovisi i o vašem aktivnom sudjelovanju.

Dobrodošli u Opatiju.

Prof. dr. sc. Nives Jonjić
Predsjednica Hrvatskog društva za patologiju i sudsku medicinu

Mr. sc. Ika Kardum-Skelin
Predsjednica Hrvatskog društva za kliničku citologiju



Veronika Anić
Predsjednica Hrvatske udruge citotehnologa

Dear colleagues and friends,

It is our pleasure to solute you, on behalf of the Organizing Committee, on the 3rd Croatian Congress of Pathology, Forensic Medicine, Clinical Cytology, and the 1st Croatian Symposium of Cytotechnology with international participation, in Opatija, Croatia, 8 -11 May 2005.

In our attempt to achieve the Congress objective and meet the scientific and professional standards in different fields of pathology, cytology, and forensics, we shall be joined by invited speakers, prominent foreign experts. The Congress is organized in plenary sessions, slide seminars, oral presentations and poster sections.

We are particularly happy that it is for the first time that Congress in organized by three related professional societies, which offers us a chance to integrate our interests and exchange experience resulting from our scientific and professional work.

We would like you to use the participation at the Congress for pleasant socializing, sightseeing of Opatija, trip to Istrian inland, and a dinner organized for all the participants.

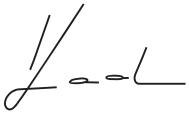
The Opatija Riviera has over 160 years long tradition in tourism, and Opatija is one of the most famous coastal tourist resorts. In Austro-Hungarian era it was an exclusive summer resort for the European aristocracy. Exquisite hotels and villas located in beautiful locations have hosted numerous visitors from the creme of the European societies in all four seasons, due to the mild climate. Charming gardens of Mediterranean plants, palms, and camellias have made Opatija a particularly attractive holiday resort.

An excursion to the central part of the Istrian Peninsula, half-an-hour drive from Opatija, is a pleasure trip to learn about the wine roads and rural tourism of the region.

We hope we shall meet your expectations, and the success of the Congress and Symposium shall certainly depend on your active participation.

Welcome in Opatija.

Professor Nives Jonjić, PhD *President, Croatian Society of Pathology and Forensics*	*Ika Kardum-Skelin, MSc* *President, Croatian Society for Clinical Cytology*	*Veronika Anić President,* *Croatian society of Cytotechnology*



ORGANIZATORI / ***ORGANIZERS***

Hrvatski liječnički zbor / *Croatian Medical Association*
Hrvatsko društvo za patologiju i sudsku medicinu / *Croatian Society of Pathology and Forensic Medicine*
Hrvatsko društvo za kliničku citologiju / *Croatian Society for Clinical Cytology*
Hrvatska udruga citotehnologa / *Croatian Society of Cytotechnology*

POKROVITELJI / ***UNDER THE AUSPICES OF***

Predsjednik Republike Hrvatske / *President of the Republic of Croatia*
Akademija medicinskih znanosti Hrvatske / *Academy of Medical Sciences of Croatia*
Ministarstvo zdravstva i socijalne skrbi Republike Hrvatske / *Ministry of Health and Social Welfare of the Republic of Croatia*
Hrvatski liječnički zbor / *Croatian Medical Association*
Hrvatska liječnička komora / *Croatian Medical Chamber*
Sveučilište u Rijeci i Medicinski fakultet sveučilišta u Rijeci / *University of Rijeka and University of Rijeka School of Medicine*

POČASNI ODBOR / ***HONORARY COMMITTEE***

I. Črepinko, D. Danilović, M. Devčić, R. Dobi-Babić, E. Hauptmann, V. Hlavka, B. Krstulović, M. Petyo-Dembić, M. Roglić, Z. Singer, V. Šeparović, Đ. Vranešić, A. Zimolo, Ž. Znidarčić

ORGANIZACIJSKI ODBOR / ***ORGANIZING COMMITTEE***

Predsjednici / ***Presidents*****:** V. Anić, A. Bosnar, N. Jonjić, I. Kardum-Skelin
Dopredsjednic / ***Vice Presidents*****:** B. Grahovac, J. Jakić-Razumović, B. Molnar-Stantić, K. Rubić, I. Seili-Bekafigo
Tajnici / ***Secretaries*****:** O. Dutković, I. Fabijanić, M. Krašević, S. Ries
Rizničari / ***Treasures*****:** A. Čobanov, D. Kovač, L. Škopljanac-Mačina
Članovi / ***Members*****:** A. Dekanić, B. Dmitrović, G. Đorđević, D. Fučkar, G. Fumić, Lj. Gavranović, M. Golemac, A. Lakošeljac, M. Marcikić, K. Matušan, I. Peharec, V. Stemberga, S. Štemberger-Papić, C. Štemberger, M. Šimat, S. Štifter, S. Tomić, D. Verša-Ostojić, D. Vrdoljak-Mozetič, G. Zamolo

ZNANSTVENI ODBOR / ***SCIENTIFIC COMMITTEE***

Š. Anđelinović, S. Audy-Jurković, S. Behrem, I. Borić, S. Boljkovac, D. Cuculić, I. Damjanov, M. Dominis, J. Forko-Ilić, M. Halbauer, T. Jeren, S. Jukić, M. Krstulja, K. Lučin, M. Marković-Glamočak, E. Mustać, M. Nola, M. Pajtler, T. D. Strinović, T. Stanković, M. Sučić, J. Škavić, A. Vince, K. Žarković

POZVANI STRANI PREDAVAČI / ***FOREIGN INVITED SPEAKERS***

Prof. dr. A. Verhest, Belgija / *Belgium*
Dr. G. Kocjan, Velika Britanija / *UK*
Doc. dr. Ž. Pohar-Marinšek, Slovenija / *Slovenia*
U. Fricke, CFIAC, Njemačka / *Germany*
Prof. dr. W. Chan, SAD / *USA*
Prof. dr. M. Cook, Velika Britanija / *UK*
Prof. dr. sc. N. Gale, Slovenija / *Slovenia*
Prof. dr. sc. A. Dei Toss, Italija / *Italy*
Prof. dr. J. Hayes, SAD / *USA*
Prof. dr. W.T. Olszewski, Poljska / *Poland*
Prof. dr. P. Vielh, Francuska / *France*
Dr. A. Pogačnik, Slovenija / *Slovenia*
Prof. dr. R. Kurman, SAD / *USA*
Prof. dr. V. Eusebi, Italija / *Italy*
Prof. dr. Z. Gatalica, SAD / *USA*
Prof. dr. I. Damjanov, SAD / *USA*
Prof. dr. C. Hitchco, SAD / *USA*
Prof. dr. R. Bollmann, Njemačka / *Germany*
Prof. dr. Kessler, Austrija / *Austria*
Prof. dr. V. Schneider, Njemačka / *Germany*

ADRESE ZA KONTAKT / ***CONTACTS***

Patologija i sudska medicina / ***Pathology and Forensic Medicine***

Medicinski fakultet u Rijeci
Zavod za patologiju
Prof. dr. Nives Jonjić
Ul. braće Branchetta 20
51 000 Rijeka
Hrvatska / *Croatia*
tel. : +385 51 32 58 22
fax.: +385 51 32 58 10
e-mail: nives@medri.hr www.kpms2005.medri.hr

Citologija i citotehnologija / ***Citology and Cytotechnology***

KBC Rijeka
Klinike za internu medicinu, Citološki laboratorij
Mr. sc. dr. Irena Seili-Bekafigo
Krešimirova 42
51 000 Rijeka
Hrvatska / *Croatia*
tel.: +385 51 32 58 24
e-mail: Irena.seili-bekafigo@ri.htnet.hr www.hlz.mef.hr

Kongresni servis / ***Congress Service:***

Studio Hrg d.o.o.
Hrvatske bratske zajednice 4
10 000 Zagreb
Hrvatska / *Croatia*
Phone: +385 /0/1 6183 140, 6110 449
Fax: +385 1 6110 452
e-mail: kongres@studiohrg.hr
www.studiohrg.hr

Kongresna web stranica / **Congress web site:**
www.studiohrg/pathology-cytology2005

PATOLOGIJA I SUDSKA MEDICINA
PATHOLOGY AND FORENSIC MEDICINE

Pozvana predavanja / *Invited Lectures (01-10)*

Pathology and Forensic Medicine / Invited Lectures / 01

DIAGNOSTIC VALUE OF QUANTIFICATION IN CLINICAL PATHOLOGY

Reinhard Bollmann, Germany

The present paper deals with problematic issues in diagnostic pathology related to subjectivity of descriptive histopathological terms and semiquantitative assessment of adjuvant methods.

Traditionally, pathology has been a science critically dependant on subjective interpretation of tissue sections, with well documented problems of inter- and intra observer disagreement. Since tumor typing and grading have major therapeutic impact in malignant diseases, there is a need to increase the objectivity and reproducibility of diagnostic work. Computer-aided morphometric analysis of tissue structures may help in overcoming diagnostic standardization problems generally inherent in diagnostic histopathology.

Diagnostic accuracy has dramatically increased during the last decade by the introduction of adjuvant methods, e.g. immunohistochemistry, DNA-image cytometry, chromosomal fluorescence in-situ hybridization (FISH) and polymerase chain reaction (PCR). Immunohistochemistry has become a powerful tool for reproducible tumor typing and the image analytic measurement of the Ki67 positive proliferative tumor cell fraction will take the place of mitosis counting, with its well-documented inter- and intraobserver variability. Our own preliminary results strongly support this idea, as we found a certain number of breast cancers apparently under-graded by just estimating the mitotic count. The quantitative immunohistochemical detection of molecules, which are targeted by molecular therapy (HER2, EGFR etc.) is inevitable for adequate selection of patients who may respond to these new treatment modalities.

Finally, a combination of adjuvant methods, as presented by our multimodal cervical screening protocol (the Bonn-Scheme) significantly increases the positive predictive value of cytology and reduces the number of unnecessary invasive diagnostic procedures in women with benign cervical lesions.

Pathology and Forensic Medicine / Invited Lectures / 02

THE IMPACT OF GENE EXPRESSION PROFILING STUDIES ON THE DIAGNOSIS OF MALIGNANT LYMPHOMA

Wing C. Chan, USA

Gene expression profiling in the past 5 years has generated a large amount of data on a variety of malignancies. Unique gene expression signatures have been identified for the more common types of non-Hodgkin's lymphoma (NHL) including clinically and biologically important subsets that have not been defined before. In addition, molecularly defined prognosticators have also been constructed for the major types of NHL and these prognosticators provide added value to the widely used International Prognostic Index. The new information should be included in our evaluation of NHL patients especially when conducting clinical trials. Studies are ongoing to validate and refine these diagnostic and prognostic signatures and to develop platforms that are suitable for routine clinical applications. Similar studies will be performed on the less common types of NHL to complete the molecular classification of NHLs. It is also anticipated that gene expression profiling studies will lead to the identification of novel targets for the development of new therapeutic agents for NHL.

Pathology and Forensic Medicine / Invited Lectures / 03

PROGNOSTICALLY IMPORTANT SUBTYPES (PATTERNS) OF MELANOMA

Martin G. Cook, UK

The classification of cutaneous melanoma has for many years been based on the descriptive pathological basis originated by Clark et al (1967). This classification was adopted by an international conference in Sydney (McGovern et al 1973) and amplified subsequently by Reed (1976). The resulting nomenclature included lentigo maligna, superficial spreading, nodular and acral lentiginous melanomas. This use of a so-called histogenetic typing has continued despite later work suggesting it had no prognostic significance (Koh et al, 1984). Prognosis was demonstrated to be more closely related to growth phases (Clark et al 1986) which in some respects is another way of more precisely describing the earlier classification. More recently, large studies have re-emphasised the prognostic value of some subtypes of the original histogenetic classification (Chang et al, 1998). There is also a suggestion that the development of some subypes of melanoma is influenced by the interplay of skin type and variations in sun exposure (Bastian et al, 2003). This more recent work is beginning to suggest that some subtypes of melanoma have distinctive molecular genetic changes. Whether these observations are either self fulfilling or the beginning of a new genetic definition of melanoma subtypes remains to be seen.

The Histogenetic Classification of Melanomas

This system comprises superficial spreading, lentigo maligna, acral lentiginous and nodular melanomas and combines clinical details with histological features. It is essentially a classification based on the features of the pre invasive component of melanoma.

Superficial spreading melanoma occurs on any part of the skin and is characterised clinically by a flat irregular area of variably pigmented skin within which there may evolve thickened areas or nodules which histologically correspond with invasive melanoma in the dermis. The flat areas histologically comprise melanoma in situ with or without superficially invasive foci. The in situ component may be irregularly nested or lentiginous or pagetoid and usually has a combination of two or all of these patterns.

Lentigo maligna melanoma is characterised by flat dark brown irregular areas, usually on the face. It has a long pre invasive phase from ten to fifty years. Histologically it is an atypical lentiginous proliferation of melanocytes in atrophic severely sun damaged skin. Invasion is preceded by a progression of palisaded basal melanocytes to a more disorderly pleomorphic junctional proliferation with downward buds of atypical cells. When finally invasive, the dermal component may show a more frequent spindle cell pattern and occasionally desmoplasia.

Acral lentiginous melanoma is seen on the soles of the feet and less commonly on the palms and is also noted in subungual melanomas and other glabrous sites. Mucosal melanoma has many similarities. Histological correlates are a lentiginous proliferation of small atypical epithelioid melanocytes in a hyperplastic epidermis. It often shows intraepithelial extension of atypical melanocytes into sweat ducts. When invasive the dermal component is not distinctive.

Nodular melanoma, like other types of melanoma is characterised by its in situ component. In this case it is defined by the absence of an adjacent junctional component. Clinically it appears as a raised nodule without adjacent pigmentation. By definition the lesion is always invasive and the melanocytic atypia in the overlying epidermis does not extend more than 3 rete ridges beyond the invasive component.

LMM represents a response to chronic continual ultraviolet exposure in individuals who have a low to medium susceptibility in terms of skin type. SSM represents a reaction to intermittent high exposure to UV light in people of medium to high susceptibility e.g. those with pale skin and red or blonde hair. ALM is not known to be related to sun exposure and is noted to be most common in Asians. Nodular melanoma is also not clearly related to sun exposure or to skin type.

Work by Bastian et al (2003) suggests that there is some support for this traditional histogenetic classification. Melanomas on an acral site in which sun exposure appears unimportant show an increase in Cyclin D1 and CDK 4 among other changes. It may be of considerable relevance that the Cyclin D1 amplification is not only seen within the clinically observable tumour but in the adjacent skin. This may be an explanation for the tendency to local recurrence in acral lesions.

LMM and SSM show a spectrum of chromosomal changes which are less marked than those seen in ALM and do not include the typical changes of Cyclin D1 and CDK 4 seen in ALM.

Any specific changes associated with nodular melanoma remain to be established.

Patterns Associated with Progression of Melanoma (levels and growth phases).

The histogenetic subtypes of melanoma are histologically recognised by the features of an in situ component or its absence. Once invasive, these melanomas tend to follow similar patterns. With the exception of nodular melanoma, infiltration of the papillary dermis begins with single or small groups of atypical melanocytes scattered loosely in the papillary dermis. These patterns equate with Clark's level 2.

Clark et al (1986) have also described progression on the basis of growth phases. An in situ component with early invasion to level 2 would be said to be in radial growth phase. This is synonymous with microinvasion and is an indication that the melanoma, although invasive, is unlikely to have metastatic potential.

Nodular melanoma, by definition, is only recognisable when invasive and already in a so called vertical growth phase. This vertical growth phase is recognised by a sheet or nodule of cells in the dermis, usually larger than 20 cells in width and bigger than any nests in the overlying junctional component and often with mitoses. Although a small vertical growth phase may still be classified as Clark's level 2, many of these lesions are Clark's level 3 or 4.

Growth phases are most important as a means of identifying metastatic potential. Metastases are not seen in the radial growth phase even when invasion is present but may occur in the vertical growth phase. It is, therefore, more relevant to confine the detailed prognostic microstaging procedures to the vertical growth phase component.

Variants of growth patterns with prognostic significance

Most melanomas which have metastasised have a clear vertical growth phase pattern, have invaded to Clark's level 3 and have a thickness of more than 1 mm. Occasionally, however, metastases are seen in association with melanomas without an obvious vertical growth

phase as conventionally defined are not in level 3 and are less than 1 mm in thickness. The most common explanation for these unexpected metastases is the presence of regression in the primary lesion. The presumption is that a more advanced melanoma had been present before regressing. However in a smaller number of cases, metastases can be seen in thin melanomas without regression. These show either a confluent sheet of junctionally situated atypical melanocytes (a so called junctional expansile nodule) or with atypical melanocytes involving and destroying hair follicles in a folliculocentric pattern with little involvement of the intervening epidermis (a folliculocentric melanoma). Both these patterns are usually mitotically active and have been shown to metastasise (Cook et al 2002). Neither of these patterns would conventionally be regarded in vertical growth phase and the folliculocentric pattern would be excluded from Breslow's thickness measurement.

Other Types of Melanoma with Prognostic Significance

So far the melanomas described progress from dysplasia in the junctional component of a pre existing melanocytic lesion through in situ melanoma and microinvasion to an invasive melanoma capable of metastasis. There are, however, some melanomas which do not fit well in this concept of progression and since they also have a completely different biological behaviour, they need to be separately identified as distinct entities. The melanocytic lesions in these categories include desmoplastic melanoma and lesions derived from blue naevi.

Desmoplastic Melanoma:

This is an uncommon melanoma occurring with a peak incidence in the elderly on the head and neck but which may involve any site and any adult patient. It is a diagnostic problem because it often presents with little or no overlying pigmentation which can be a confusing problem for the clinician. The histologist will I note that there is often only a scanty non atypical junctional proliferation of melanocytes or there may be no junctional component at all (Jain & Allen 1989).

The lesion is characterised by a poorly circumscribed, usually deeply infiltrative tumour composed of narrow spindle cells associated with dense collagen production. The irregular infiltration of spindle cells far beyond the main bulk of the tumour, sometimes in a perineural or intraneural site, is highly relevant to the management of the patient and creates problems of recognition to the pathologist. These subtly infiltrating cells are, like the rest of the tumour, usually S100 positive and HMB 45 negative but occasionally staining even with S100 can be weak, putting great emphasis on clinicopathological correlation.

The tumour in its pure form is very late to metastasis and, therefore, can be cured by complete local excision. For the same reason sentinel lymph node biopsy is not appropriate. When a desmoplastic component is mixed with a more conventional melanoma, the metastasising potential of the latter assumes a greater importance.

Melanomas related to blue naevi:

The criteria for malignancy in blue naevi are not well established. Atypical cellular blue naevi may show occasional mitoses and an irregular margin. Numerous mitoses, including occasional atypical forms, and marked pleomorphism, particularly in an infiltrative component, and necrosis should raise suspicion of progression to malignancy. Metastases are said to occur in blue naevi without necessarily proving the malignant nature of the lesion since some of these blue naevi with metastases do not appear to progress any further.

Severely atypical variants of blue naevi with epithelioid features have been described, occasionally with bizarre features (Youngberg et al 1986). Some of these lesions have been noted in association with the Carney complex and behave in a benign way (Carney & Ferneiro 1996) whilst others, histologically indistinguishable, have occasionally metastasised (Zembowicz et al 2004). This group of lesions has been recently termed pigmented epithelioid melanocytoma. Criteria for recognising aggressive behaviour in this type of lesion have not been established. They are, for the time being, examples of uncertain malignant potential.

Melanocytic Lesions of Uncertain Malignant Potential

Many melanocytic proliferations do not conform to classic patterns and their behaviour is therefore difficult to predict. Perhaps the most common example of this dilemma occurs with atypical Spitzoid lesions, deep penetrating naevi and cellular and epithelioid blue naevi. There has been much work attempting to define criteria of malignancy in atypical Spitzoid lesions but it remains a problematic area. The resolution of the recent confusion associated with atypical blue naevi awaits further study. In the meantime the wise course of action with such cases is to seek support by consultation with colleagues and do not hesitate to support uncertainty of the biological behaviour of a lesion. It is much better for the clinician dealing with the patient to be aware of the uncertainty of the classification of a lesion than to be misled by a confident but possibly wrong diagnosis.

References:

Clark WH A classification of melanoma in man correlated with histogenesis and biological behaviour
In: Montagna W Hu F eds Advances in the Biology of the Skin Vol VIII 621 - 647 Pergamon New York 1967

McGovern VJ Mihm MC Bailly C et al
The classification of melanoma and its histologic reporting
Cancer 1973 32 1446 - 57

Reed RJ Acral lentiginous melanoma
In: New concepts in surgical pathology of the skin p 89 - 90
Wiley, New York 1976

Koh HK Michalik E Sober et al
Lentigo maligna melanoma has no better prognosis than other types of melanoma
J Clin Oncol 1984 2 994 - 1001

Clark WH Elder DE Van Horn
The biologic forms of melanoma
Hum Pathol 1986 17 443 - 50

Chang AE Karnell LH Menck HR
The National Cancer Data Base report on cutaneous and non cutaneous melanoma Cancer 1998 83 1664 - 1678

Bastian BC Olshen AB LeBoit PE et al
Classifying melanocytic tumours based on DNA copy number changes
Am J Pathol 2003 163 1765 - 70

Cook MG Spatz A Bröcker EB Ruiter D
Identification of histological features associated with metastatic potential in thin (<1.0 mm) cutaneous melanoma with metastases
J Path 2002 197 188 - 93
Jain S Allen PW
Desmoplastic malignant melanoma and its variants
Am J Surg Path 1989 13 358 - 73

Younberg GA Rasch EM Douglas HL
Bizarre blue naevus
JAAD 1986 15 336 - 41

Carney JA Ferreiro JA
The epithelioid blue naevus
Am J Surg Path 1996 20 259 - 272

Zembowicz A Carney JA Mihm MC
Pigmented epithelioid melanocytoma
Am J Surg Path 2004 28 31 - 40

Pathology and Forensic Medicine / Invited Lectures / 04

STEM CELLS OF TESTICULAR TUMORS

Ivan Damjanov, USA

Most of the testicular tumors are of germ cell origin and are classified as seminomas or nonseminomatous germ cell tumors (NSGCT). Seminomas are composed of a single population of stem cells which phenotypically correspond to spermatogonia and/or fetal primordial germ cells. So far it has not been possible to culture in vitro seminomas long-term wise and no seminoma cell-lines are available for in vitro studies. The stem cells of NSGCTs are in most instances classified as embryonal carcinoma cells (ECC). ECC can be developmentally unable to differentiate (nullipotent), as in pure embryonal carcinomas, or developmentally pluripotent, as in mixed germ cell tumors. These cells correspond to early embryonic cells from preimplantation stage embryos. Several developmentally pluripotent ECC lines have been established as permanent tissue culture cell lines. These cell lines express some surface markers, which are lost when the ECC differentiate into somatic cells types. ECC isolated from testes have the same properties as the ECC isolated from ovarian or extragonadal germ cell tumors. ECC share some properties with human embryonal stem cells (ESC) that have been derived from human embryos explanted in vitro. Several ESC lines have been established so far as permanent cell lines in vitro. In contrast to ECC, these ESC lines do no give rise to malignant tumors when injected into nude mice. Malignant transformation of human embryonic cells is thus not a prerequisite for the propagation of these cells in vitro.

Pathology and Forensic Medicine / Invited Lectures / 05

EVOLVING CONCEPTS IN THE DIAGNOSIS OF SOFT TISSUE SARCOMAS

Angelo Paolo Dei Tos, Italy

MSoft tissue tumours are relatively rare and account for less than 1% of adult malignancies [1]. Progress in the treatment of these rare tumours has been based on multi-institute prospective phase III trials. This is especially true for treatments concerning a multidisciplinary approach including (experimental) chemotherapy. Since rapid scientific progress is being made in immunophenotyping, the genetics underlying the different disease processes, and drug targeting, a continuous adjustment of criteria used within the field of bone and soft tissue tumour pathology is necessary together with their subsequent communication to the world of clinical oncology. These developments include (in the order they are discussed below): A) the launching of the new WHO classification of bone and soft tissue tumours [2], B) the international acceptance of the updated French grading system and the exclusion of its use for certain tumour types, C) developments in immunophenotyping and D) genetics of tumours, E) and finally immuno-identification of drug targets.

A. The 2002 WHO-Classification of soft tissue tumours

The previous WHO-classification of soft tissue tumours dates from 1994 [3]. Since then, considerable new insights have been gained into the molecular pathogenesis and classification of soft tissue tumours. Hence, the new 'blue book' expanded and concentrated more on genetic data and prognostic factors [2]. Also the epidemiology, macroscopic & microscopic descriptions are more elaborated. A number of chapters have disappeared from the soft tissue issue, since they are, -or will be-, handled in other volumes. These include neural tumours, paraganglionic tumours and mesothelial tumours. A large number of vascular, and so-called fibrohistiocytic tumours will be covered in the volume on skin tumours. The following paragraphs highlight the changes in classification in the 2002 WHO-classification per group.

I. Adipocytic tumours

Amongst the benign tumours, myolipoma and chondroid lipoma represent two well-defined new entities. The rare fibrolipomatosis of nerve has been renamed as lipomatosis of nerve. Angiomyolipoma and myelolipoma disappeared from the soft tissue fascicle and are discussed in the urogenital, resp. endocrine volumes. Liposarcomas are the most common soft tissue sarcomas, of which 4 types are now well characterized:

Atypical lipomatous tumour/well differentiated liposarcoma. These terms are synonyms, and the latter term is justifiable for lesions that occur in the retroperitoneum/mediastinum. At this specific site, they almost always recur due to surgical irresectability, and hence have a higher morbidity/mortality than extremity lesions. In the 1994 version of the classification, the term 'atypical lipoma' was used for a subcutaneous well-differentiated liposarcoma [3].

Dedifferentiated liposarcoma. Dedifferentiation occurs in about 10% of well differentiated liposarcomas of any subtype especially in deep seated tumours esp. retroperitoneal. Dedifferentiated liposarcoma tends to recurr locally in about 40% of cases. Originally

dedifferentiation was per definition of high-grade morphology. In the new WHO classification also low grade dedifferentiation is recognised. Overall about 15-20% of cases show distant metastases. Interestingly, dedifferentiated liposarcoma exhibits a less aggressive clinical behaviour that other types of high grade pleomorphic sarcomas.

Myxoid liposarcoma. Originally, myxoid liposarcoma was split from round cell liposarcoma. It became clear that both 'entities' represent the ends of the spectrum of one disease, the round cell type being the poorly differentiated variant.

Pleomorphic liposarcoma.

The rare combinations of the aforementioned subtypes of liposarcoma are obviously labelled as mixed-type liposarcoma.

II. Fibroblastic/myofibroblastic tumours

Keloid disappeared from the scene as it is no longer regarded as true neoplasm, and a large number of new benign entities were included: Ischaemic fasciitis, desmoplastic fibroblastoma, mammary-type fibroblastoma, angiomyofibroblastoma, cellular angiofibroma, Gardner fibroma, inclusion body fibromatosis, calcifying fibrous tumour, giant cell angiofibroma and lipofibromatosis. An important conceptual change is represented by the inclusion of 'hemangiopericytoma' into the chapter on solitary fibrous tumour, since the border between those lesions became increasingly blurred. The formerly labelled 'myxoid variant of malignant fibrous histiocytoma' has now been definitively allocated to the fibroblastic category and has been renamed as myxofibrosarcoma. Low-grade fibromyxoid sarcoma, (acral) myxoinflammatory fibroblastic sarcoma, sclerosing epithelioid fibrosarcoma, and low-grade myofibroblastic sarcoma represent new malignant entities.

III. So-called fibrohistiocytic tumours

Tenosynovial giant cell tumors were formerly covered in a separate chapter on synovial tumors [3]. Since they have descriptively more in common with 'fibrohistiocytic tumors, these lesions are now allocated to this chapter. The concept of 'malignant fibrous histiocytoma' (MFH) has been challenged and is still debated. Pleomorphic MFH is now synonymous with high-grade undifferentiated sarcoma. In addition, the morphological features of giant cell MFH and inflammatory MFH are shared by a variety of other tumour types. This will have major impact on classification of pleomorphic sarcomas and thus in comparing trial results

IV. Smooth muscle tumours

Smooth muscle tumors occurring in the skin, genital system and GI-tract, - most of the latter 'smooth muscle tumors' representing CD117 positive gastro-intestinal stromal tumors -, are covered in the respective volumes. It is of note that soft tissue leiomyosarcoma is more frequent than its benign counterpart and that smooth muscle tumors occurring in immunocompromized patients are often Epstein-Barr virus related.

V. Pericytic/perivascular tumours

Only glomus tumors and myopericytoma are retained in this category. The latter forms a morphological continuum with myofibroma, angioleiomyoma and so-called infantile hemangiopericytoma.

VI. Skeletal muscle tumours

Three malignant types are included: embryonal (encompassing the spindle cell, botryoid and anaplastic subtypes), alveolar, and pleomorphic rhabdomyosarcoma.

VII. Vascular tumours

Since 1994, various new entities have been characterized, particularly in the intermediate malignancy category, including the kaposiform, retiform and composite types of hemangioendothelioma. Epithelioid hemangioendothelioma is the only hemangioendothelioma classified as malignant, due to its considerable metastatic rate. Endovascular papillary angioendothelioma has been renamed as papillary intralymphatic angioendothelioma.

VIII.Chondro-osseous tumours

Only soft tissue chondroma and extra skeletal osteosarcoma are retained. Myositis ossificans and fibro-osseous pseudotumor are regarded as variants of nodular fasciitis (see group III) and fibroplasia ossificans progressiva seems to be a non-neoplastic lesion. Since extra skeletal myxoid chondrosarcoma does not show convincing cartilaginous differentiation, this entity is now placed in the 'tumors of uncertain differentiation' category. Well-differentiated and mesenchymal chondrosarcoma, though occurring in the soft tissues as well, are discussed in the bone tumour section [2].

IX. Tumours of uncertain differentiation

This category contains tumors without a clear line of differentiation or without a normal cellular counterpart. Obviously several new entities have been described since 1994, including pleomorphic hyalinising angiectatic tumour (PHAT), mixed tumour/myoepithelioma, and neoplasm's with perivascular epithelioid cell differentiation (PEComas). Clear cell sarcoma was originally put in the peripheral nerve tumour section, but the line of differentiation is still unknown. Angiomatoid fibrous histiocytoma, formerly present in the fibrohistiocytic tumour category, is now also allocated to this section. Since we now know more about divergent differentiation in various sarcomas, the category of malignant mesenchymoma is gradually leaving the stage, while intimal sarcoma is introduced as a new entity. Finally, Ewing sarcoma (ES)/peripheral neuroectodermal tumour (PNET) including its soft tissue variant is discussed in the bone tumour section.

B. Grading

Soft tissue sarcomas are aggressive tumours which metastasise in a large percentage of cases. Tumour size, location, depth and histological type are all prognostic factors in terms of metastatic risk and overall survival. Grading systems based on histological parameters were introduced to provide more accurate information on the degree of malignancy of tumours. They are nonetheless of poor predictive value regarding local recurrence, which is mainly correlated with suboptimal surgical procedures.

Many grading systems were developed in the past, in order to increase the discrimination between low-grade tumours (with good prognosis) and high-grade tumours (with poor prognosis). Two systems are mentioned in the third edition of the WHO classification of soft tissue tumours [2], and currently used : the National Cancer Institute (NCI), and the French Federation of Cancer Centres Sarcoma Group (FNCLCC) systems. Both are three-tiered

systems. The FNCLCC system was chosen for use in EORTC trials and advocated in the 2002 version of the WHO classification of international standard. It offers slightly better discrimination between low and high-grade sarcomas, the intermediate group being smaller. Additionally, it seems to be more easily reproducible between pathologists, as it is based on three histological parameters, two of which are measurable (necrosis amount and mitotic rate), while the last one, differentiation, is more subjectively assessed. These parameters were selected after multivariate analysis performed in a large series of patients. The FNCLCC system was created in 1984 [4], then updated in 1997 [5].

Performing accurate grading requires awareness of some limitations. Only untreated primary soft tissue sarcomas may be graded, thus excluding recurrences. Also excluded are tumours, which have been previously treated by radiotherapy or chemotherapy. These treatments can alter parameters like necrosis extent or mitotic count. Visceral sarcomas must also be excluded. Gastro-intestinal stromal tumours yield to other prognostic factors, mainly location, size and mitotic activity. The FNCLCC grading system has not yet been tested in other visceral sarcomas. In a number of entities grading is no longer recommended according the new WHO classification: angiosarcoma, extraskeletal myxoid chondrosarcoma, alveolar soft part sarcoma, clear cell sarcoma, and epithelioid sarcoma).

Grading should be performed on representative material. One sample should be taken for every centimetre in the largest diameter of the tumour. Needless to say that in heterogeneous tumours there can be differences in grade within one tumour. Therefor biopsies are recommended from those parts on MRI which argue most for a high grade compartment. The histological diagnosis of sarcoma must be unquestionable. Grading is not a substitute for diagnosis: it cannot distinguish between benign lesions and malignant tumours.

Performing grading on needle core biopsies is controversial. It raises the problem of representativity of small samples, even though they provide sufficient material for diagnosis. High-grade tumours can often be accurately recognized and graded, while the pathologist is at great risk of missing one or more components in a seemingly low-grade tumour.

Grading provides strong information on metastasis risk and overall survival. Nevertheless when using this information for therapeutic management, clinicians must be aware of its limits. Patient selection for adjuvant therapies is based not only on grade, but also on histological type or subtype, and on staging [2], which includes various criteria such as size, depth, regional lymph node involvement, and distant metastatic spread.

Grade is the most important prognostic factor for sarcomas when studied as a whole. But further analyses have shown that the prediction was more accurate for STS which showed greater histological variation from tumour to tumour, e.g. high grade pleomorphic sarcomas (such as the in the past so popular diagnosis of malignant fibrous histiocytomas) or leiomyosarcomas [6]. In some tumour groups, histological diagnosis is more informative than grade in terms of prognosis, e.g. various subtypes of liposarcomas. Another study showed that grade has no prognostic value in malignant peripheral nerve sheath tumours, or rhabdomyosarcomas [7]. Paediatric tumours are better prognosticated by other parameters, such as age or resectability, which emphasizes that the FNCLCC grading system must be restricted to adult STS. Lastly cytogenetics and molecular biology will probably soon provide new factors for predicting the clinical course and therapeutic response of STS.

C. Immunophenotyping:

Immunohistochemical characterization plays a key role in the diagnostic workup of STS. The determination of the line of differentiation is not only crucial in order to ensure proper classification, but also to provide prognostic and/or predictive information. A detailed description of the immunophenotype of soft tissue tumours is beyond the aims of this manuscript [8]. We will therefore focus upon those differentiation markers showing major clinical relevance. Importantly, as the vast majority of immunoreagents are very sensitive but not much specific, immunohistochemical characterization should always be performed using a panel of markers (Tables 1-3). Secondly, in order to avoid diagnostic pitfalls, immunostains should be evaluated strictly in context with morphology.

Myogenic differentiation markers.

Demonstration of myogenic differentiation is important not only in order to differentiate between rhabdomyosarcoma (RMS) and non-RMS paediatric soft tissue tumours, but also to recognize within the undifferentiated (ex-MFH) pleomorphic sarcoma category both pleomorphic leiomyosarcoma (LMS) and RMS, which represent prognostically unfavourable subtypes [9].

Smooth muscle markers are basically represented by smooth muscle actin, desmin and h-caldesmon. Smooth muscle actin immunopositivity is observed in most LMS. Desmin as well as h-caldesmon immunoreactivity is observed in approximately 70% of cases [10]. Focal smooth muscle immunoreactivity can be seen in a variety of STT as well as in non-sarcomatous lesion (e.g. sarcomatoid "spindle cell" carcinoma) and therefore should not interpreted "per se" as unequivocal evidence of smooth muscle differentiation.

Desmin and muscle specific actin also represent very sensitive markers of striated muscle differentiation staining up to 90% of RMS of all subtypes (embryonal, alveolar and pleomorphic). However, all these markers are overshadowed by myogenin, a nuclear transcription factor involved in striated muscle differentiation, which specifically identify striated muscle differentiation in all RMS subtypes [11]. Importantly myogenin is more abundantly expressed in alveolar RMS than in embryonal as well as pleomorphic subtypes.

Neural markers.

S-100 immunopositivity, despite total lack of specificity still represents the most sensitive marker of neural differentiation. Approximately 50% of malignant peripheral nerve sheath tumours (MPNST) exhibit S-100 positivity, which is usually limited to less than 30% of neoplastic cells. Epithelioid MPNST represents and important exception as most neoplastic cells will express this marker. It has to be remembered that about 30% of monophasic synovial sarcoma may also exhibit S-100 immunopositivity, making differential diagnosis with MPNST somewhat challenging [12]. GFAP (glial fibrillary acidic protein) also decorates up to 30% of MPNSTs. NSE (neuron-specific enolase) and PGP 9.5 are too non-specific to play any useful role.

Epithelial differentiation markers.

Synovial sarcoma (SS), as well as any soft tissue neoplasm featuring epithelioid morphology is characterized by variable expression of cytokeratin and epithelial membrane antigen (EMA). Recognition of SS among spindle cell sarcoma is extremely important as this neoplasm exhibits significant chemo sensitivity. Cytokeratin expression is observed in up to

80% of classic SS and in about half of the poorly differentiated ones [12-14]. Cytokeratin decorates virtually all epithelioid sarcomas [12,14], and is also observed in half of epithelioid angiosarcoma (EAS) [15]. EMA also stains most epithelioid sarcomas, and 90% of SS (including poorly differentiated ones); it therefore represents the most sensitive marker of epithelial differentiation in this context.

Endothelial differentiation markers.

Demonstration of endothelial differentiation appears crucial when dealing with poorly differentiated vascular neoplasm's, in particular epithelioid angiosarcoma, an AS variant than can mimic a carcinoma. Classic markers are represented by CD34, CD31 and Factor VIII-RA. CD34 is very sensitive but is also expressed in half of epithelioid sarcomas [16], in addition to a endless list of spindle cell neoplasm's which include dermatofibrosarcoma protuberans and solitary fibrous tumour. CD31 is far more specific even if CD31 immunopositivity can be detected within intratumoural histiocytes. FVIII-RA is also very specific but generally tends to be less sensitive. A promising marker is represented by Fli-1, a nuclear transcription factor involved with endothelial differentiation, which appear to stain both normal and neoplastic endothelium [17,18]. Fli-1 immunoreactivity is also observed in ES/PNET as a result of FLI-1 gene rearrangement [19]. An important diagnostic adjunct is represented by nuclear detection of HHV-8 in Kaposi's sarcoma [20].

Other useful markers

CD99 represents a powerful diagnostic marker when dealing with the differential diagnosis of small round cell sarcomas. In fact the vast majority of Ewing's sarcoma/peripheral neuroectodermal tumour (ES/PNET) exhibits strong membrane CD99 immunopositivity [21]. However, it has to be underlined that among potential mimics of ES/PNET, CD99 is also expressed in 90% of synovial sarcoma (including the poorly differentiated round cell variant), in 40% of Merkel cell carcinomas [22], in most examples of lymphoblastic lymphomas, and in mesenchymal chondrosarcoma [23].

C-kit (CD117) has recently become one of the most clinically relevant phenotypic markers. In fact its expression in GIST permits the accurate recognition of this once orphan tumour as well as proper selection of patients for treatment with tyrosine kinase inhibitors [24,25]. It should be underlined that widespread and unnecessary application of heat-induced antigen retrieval in this context has led to indiscriminate reports of c-kit immunopositivity in a variety of mesenchymal neoplasm's [26] with potential irrational application of targeted-therapy. In fact, it has to be underlined that c-kit expression "per se" does not predict sensitivity to Imatinib.

HMB-45 not only plays an important role in the differential diagnosis of malignant melanoma but has also proved extremely helpful in recognizing those entities belonging to the recently coined family of PEComas, which include angiomyolipoma, lymphangioleiomyomatosis and other rarer entities [27].

In addition to myogenin and Fli-1, other nuclear transcription factors appear to represent extremely promising phenotypic markers. WT-1 has proved useful in the recognition of desmoplastic small round cell tumour [28], an extremely aggressive neoplasm characterized by polyphenotipic expression of neural, epithelial and myogenic markers [29]. Even more recently it has been shown that TFE3 decorates the neoplastic cell population of alveolar soft part sarcoma [30]. It has to be stressed that, with the exception (until now) of myogenin none

of these markers appears to be entirely specific. However, when evaluated in context with morphology, they not only increase significantly the diagnostic accuracy but also predict the presence of the underlying genetic aberration. The expression of proto-oncogenes and tumour suppressor genes products has also proved useful in the differential diagnosis of soft tissue sarcomas. It has been shown recently that both well-differentiated liposarcoma and dedifferentiated liposarcoma are characterized by amplification and overexpression of mdm2, cdk4 and HMGIC. mdm2 overexpression has been recently proposed as a useful diagnostic tool in distinguishing between dedifferentiated liposarcoma and other retroperitoneal high grade sarcomas [31,32]. p53 over expression may also play a role in the differential diagnosis of atypical fibroxanthoma (AFX), which include malignant melanoma, spindle cell carcinoma and leiomyosarcoma. p53 expression in AFX is due to UV-induced TP53 mutations and represents an almost constant finding [33].

Obsolete Markers.

In parallel with the conceptual evolution of soft tissue tumours classification, several immunohistochemical markers have lost most of their utility. This has proved true for "fibrohistiocytic" markers such as lisozyme and alpha-1-antichimotrypsin, but also for vimentin, whose diagnostic application is minimal. Several differentiation markers may still be valid but have been practically replaced by reagents showing better reproducibility. In this perspective the replacement of MyoD1 by myogenin represents an illuminating example. In general it has to be underlined that tumour immunophenotyping represents a dynamic process. Pathologists need to be aware of the consolidated advances in the field in order to ensure diagnostic accuracy. Participation in external quality control programs is also strongly advised, as technical as well as clinical validation is mandatory, particularly in the context of clinical trials.

D. Genetics

Although traditional morphological and immunohistochemical assessment remain the foundation of clinical decision-making, adjunctive data from genetic studies can improve precision of diagnosis and accuracy of subtyping in clinically important soft tissue tumour areas. Increasingly, genetic data are useful for predicting behaviour and response to therapy, and in some areas such as paediatric small round cell tumors are likely to be mandatory [34]. Cytogenetic and molecular genetic aberrations have been described in many benign and malignant soft tissue and bone tumours. These include chromosomal deletions, duplications and rearrangements, gene amplification by rearrangement or mutation, and oncogene activation and suppression. Examples of the latter include p53 gene located at 17p13, MDM2 gene at 12q, RB1 gene at 13q14, WT1 gene at 11p13, and the NF1 gene at 17q11.2. Mutations in the p53 gene are common abnormalities in human cancers, and loss of the short arm of chromosome 17, point mutation of p53, and homozygous loss of both alleles have been reported in soft tissue sarcomas, including rhabdomyosarcoma, leiomyosarcoma and undifferentiated pleomorphic sarcoma (formerly MFH). Inheritable mutations of germ line p53 (in Li-Fraumeni syndrome) or RB1 (hereditary retinoblastoma) are found in familial sarcomas. Of particular importance, a number of sarcomas have consistent specific translocations, which result in new fusion genes (Table 4). The specific functions of these genes are largely unknown, but their tumour-specific RNA transcripts generally encode proteins which represent transcription factors (derived from one partner gene) or nucleic acid binding domains (from the other); the latter include the EWS gene, which is involved in several different translocations. Most translocations appear to act through

production of abnormal proteins with altered control of cell proliferation. Identifying the rearrangement or the gene products enables precise diagnosis. Techniques for detection of these and other clinically relevant abnormalities such as MYCN gene amplification in neuroblastoma include FISH, PCR, RT-PCR, mutation screening and gene sequencing, and usually require fresh or frozen tissue, but are increasingly applicable to formalin-fixed, paraffin-embedded material. A molecular diagnostic service for sarcomas should be available in all specialist centres. Genetic findings can confirm relationships between morphologic subtypes, improve diagnostic accuracy and prediction of behaviour in specific sarcomas beyond the general features of size, depth and grade, and lead to improved therapy. Thus, in fatty tumours, the morphologic classification has been validated. Abnormalities of 12q13-15 region (which includes MDM2 and CDK4 genes) and giant marker and ring chromosomes are found in both atypical lipomatous tumours and dedifferentiated liposarcomas, and identical translocations are seen in myxoid and round cell liposarcoma (see table 4). Pleomorphic liposarcomas, however, are a distinct subgroup having complex rearrangements with numerous extra chromosomes, as in many pleomorphic unclassified sarcoma. Similarly, the genetic identities of dermatofibrosarcoma protuberans and giant cell fibroblastoma, and of low-grade fibromyxoid sarcoma with hyalinizing spindle cell tumour, have been shown, again confirming morphological suppositions. Extra skeletal myxoid chondrosarcoma, which is probably not a cartilaginous tumour, has translocations, which are not found in skeletal myxoid chondrosarcoma, a separate entity. On the other hand, Ewing's sarcoma/PNET has the same genetic abnormalities irrespective of site of origin. No specific genetic features relating to diagnosis or prognosis have been identified in pleomorphic sarcomas. In diagnosis, the finding of a specific translocation is especially useful for distinguishing small round cell tumours, including PNET, poorly differentiated synovial sarcoma, and desmoplastic small round cell tumour. Additionally, in some tumours with variable translocations, the gene involved in the rearrangement (e.g. whether PAX3 or PAX7 in alveolar rhabdomyosarcoma, or SSX1 or SSX2 in synovial sarcoma) relates to prognosis and potential response to chemotherapy, independent of tumour site, stage, and size. In ES/PNET with t(11:22)(q24;q12), the EWS-FLI1 rearrangements have been found to show great diversity; the so-called type I gene fusion, in which EWS exon 7 is fused to FLI1 exon 6, is reportedly associated with an improved prognosis compared with other fusion types. Mutational analysis is proving particularly relevant in clinicopathological assessment of gastrointestinal stromal tumours (GIST). In most GISTs there are activating mutations in the KIT gene, located on chromosome 4q11-21, which encodes a type-III receptor tyrosine kinase protein (CD117), a diagnostically useful immunohistochemical marker. In GISTs, the KIT gene mutations are mostly in the juxtamembrane domain at exon 11, and in a smaller number at exons 9 and 13. Familial and multiple GISTs are associated with a germline mutation at exon 11. It appears that the type of mutation might determine response to therapy with imatinib (STI 571, Glivec) a selective inhibitor of ABL and KIT tyrosine kinases. Receptors for platelet derived growth factor receptor (PDGFR) belong to the same subfamily, suggesting a potential role for the same drug in therapy of dermatofibrosarcoma protuberans and related fibrosarcoma, in which the PDGFR gene is rearranged.

Gene expression profiling is a recently introduced technique for simultaneous examination of thousands of genes in cDNA microarrays containg hundreds of sarcomas. Some tumour types - synovial sarcomas, gastrointestinal stromal tumours, neural tumours, and a subset of leiomyosarcomas, show distinct gene-expression patterns. Other tumours, such as pleomorphic sarcoma and liposarcoma, share molecular profiles. Marked expression of known genes, such as KIT in gastrointestinal stromal tumours, occurs within gene sets that

distinguish the different sarcomas. However, many uncharacterised genes also contribute to the distinction between tumour types. Analysis of the huge amount of data derived from these studies might reveal further useful markers for diagnosis, specific prognostic factors and identify possible targets for molecular therapy.

E. Immuno-identification of drug targets

Several primary genetic events in tumours, such as translocations or mutations subsequently lead to expression of downstream drugable targets such as C-kit, EGFR-1, or Her-2 / Neu. Given the vast amount of new and unexpected genes expressed, which are nowadays identified as a result of CDNA expression microarrays, this list is most likely to expand rapidly. The expression of these molecules can be monitored either at the RNA level or more simply and maybe more effectively at the protein expression level by immunohistochemistry. Immunohistochemistry however has its limits in specificity, both as a result of the primary antibody cross reactivity as well as detection technique such as antigen retrieval. Currently clinical trials are being performed based on selective interactions with these molecules. In spite of the clinical success of targeting C-kit in GIST, caution should be the guidance for overenthusiastic reactions based upon presumed expression of C-Kit in miscellaneous tumours. Though there are several reports of C-Kit expression in an array of tumours, this is largely based on immunohistochemical techniques using antigen retrieval, rendering false positive results. In a trial comparing GIST and non-GIST sarcomas no benefit on survival was observed for non-GIST patients after the administration of imatinib (Glivec) [35]. Also for instance in osteosarcoma, where despite original reports of HER2 over-expression [36] [37] [38] [39] more and more critical papers appear [40] [41,42], challenging the original observation. Phase II trials however have even found their way in testing the efficacy of Herceptin in patients with relapsed or refractory osteosarcoma [37] [43]. Obviously standardisation in technology is necessary to avoid these caveats presented by overoptimistic interpretation of preliminary results. It opens however a future for pathology not only in careful diagnosing tumours, but also in identifying potentially useful treatment options. EGFR has been found to be expressed in synovial sarcomas using micro-array technology [44-46]. This lead to the rapid design of a trial targeting EGFR-1 by the drug Iressa. It remains to be proven whether this approach is effective since preliminary data show, at least at the immunohistochemical level, that the expression of EGFR-1 is restricted to subsets of tumour cells, leaving others untouchable by the drug. Moreover, preliminary additional gene profiling data somewhat contradict previous study on EGFR involvement in synovial sarcoma, raising the necessity of a more thoughtful approach to clinical application of not yet validated molecular information.

Table 1: Immunohistochenical panel for spindle cell sarcomas.

Tumor Type	Cytokeratin	EMA	Desmin	SMA	h-Caldesmon	S-100
LMS	5%	5%	70%	90%	70%	-
SS	80%	>90%	-	-	-	30%
MPNST	-	5%	-*	-	-	50%

Legend: LMS = leiomyosarcoma; SS = synovial sarcoma; MPNST = malignant peripheral nerve sheath tumour.
* Positive in MPNST with heterologous rhabdomyosarcomatous differentiation

Table 2: Immunohistochemical panel for round cell sarcomas.

Tumor	CD99	Myogenin	Desmin	Keratins	EMA	SYN	FLI-1
PNET/ES	95%	-	-	20%	5%	20%	>90%
ARMS	10%	>90%	>90%	Rare	-	-	-
DSRCT	10%	-	80%	90%	90%	5%	-
PDSS	90%	-	-	50%	90%	-	-
MCHS	80%	-	-	-	-	-	-

Legend: PNET/ES = peripheral neuroectodermal tumour/Ewing's sarcoma; ARMS = alveolar rhabdomyosarcoma; DSRCT: desmoplastic small round cell tumour; PDSS = poorly differentiated synovial sarcoma; MCHS = mesenchymal chondrosarco

Table 3: Immunohistochemical markers for epithelioid sarcomas.

Tumor	Cytokeratin	EMA	CD34	CD31	S-100	FLI-1
ES	>90%	>90%	50%	rare	-	-
EAS	50%	30%	80%	80%	-	100%
EMPNST	20%	-	-	-	100%	-

Legend: ES = epithelioid sarcoma; EAS = epithelioid angiosarcoma; EMPNST = epithelioid malignant peripheral nerve sheath tumour.

Table 4: Chromosomal translocations in malignant soft tissue tumours

Tumour type	Translocations	Involved genes
Synovial sarcoma	t(X;18)(p11.2;q11.2)	SSX1or SSX2, SYT
MRC liposarcoma	t(12;16)(q13;p11)	CHOP,TLS
	t(12;22)(q13;q11-q12)	CHOP,EWS
Ewing's sarcoma/PNET	t(11:22)(q24;q12)	FLI1,EWS
	t(21:22)(q22;q12)	ERG,EWS
	t(7;22)(p22;q12)	ETV1,EWS
	t(2;22)(q33;q12)	FEV,EWS
	t(17;22)(q12;q12)	E1AF,EWS
Desmoplastic SRCT	t(11;22)(p13;q12)	WT1,EWS
Alveolar rhabdomyosarcoma	t(2:13)(q35;q14)	PAX3,FKHR
	t(1;13)(p36;q14)	PAX7,FKHR
Extraskeletal myxoid chondrosarcoma	t(9;22)(q21-31;q12.2)	CHN,EWS
	t(9;17)(q22;q11)	CHN, RBP56
Clear cell sarcoma	t(12;22)(q13;q12)	ATF1,EWS
Alveolar Soft Part Sarcoma	t(X;17) (p11;q25)	TFE3, ASPL
Dermatofibrosarcoma/GCF	t(17;22)(q22;q13)	COL1A,PDGFB1
Infantile fibrosarcoma	t(12;15)(p13;q25)	ETV6, NTRK3
Low grade fibromyxoid sarcoma	t(7;16)(q34;p11)	FUS, BBF2H7

MRC = myxoid/round cell PNET = peripheral primitive neuroectodermal tumour SRCT = small round cell tumour GCF = giant cell fibroblastoma

References

1. Hogendoorn PCW. Pathology of soft tissue sarcomas with emphasis on molecular diagnostic techniques. Eur J Cancer 2003, S205-S210.

2. Unni KK, Fletcher CDM, Martens F. World Health Organization Classification of Tumours. Pathology and Genetics of Tumours of Soft Tissue and Bone. 2002, IARCPress, Lyon.

3. Weiss SW,Sobin LH. World Health Organization. International histological classification of tumours. Histological typing of soft tissue tumours.1994

4. Trojani M, Contesso G, Coindre JM, Rouesse J, Bui NB, De Mascarel A, Goussot JF, David M, Bonichon F,Lagarde C. Soft-tissue sarcomas of adults; study of pathological prognostic variables and definition of a histopathological grading system. Int J Cancer 1984, 33, 37-42.

5. Guillou L, Coindre JM, Bonichon F, Nguyen BB, Terrier P, Collin F, Vilain MO, Mandard AM, Le D, V, Leroux A, Jacquemier J, Duplay H, Sastre-Garau X,Costa J. Comparative study of the National Cancer Institute and French Federation of Cancer Centers Sarcoma Group grading systems in a population of 410 adult patients with soft tissue sarcoma. J Clin Oncol 1997, 15, 350-362.

6. Kilpatrick SE. Histologic prognostication in soft tissue sarcomas: grading versus subtyping or both? A comprehensive review of the literature with proposed practical guidelines. Ann Diagn Pathol 1999, 3, 48-61.

7. Coindre JM, Terrier P, Guillou L, Le D, V, Collin F, Ranchere D, Sastre X, Vilain MO, Bonichon F,N'Guyen BB. Predictive value of grade for metastasis development in the main histologic types of adult soft tissue sarcomas: a study of 1240 patients from the French Federation of Cancer Centers Sarcoma Group. Cancer 2001, 91, 1914-1926.

8. Coindre JM. Immunohistochemistry in the diagnosis of soft tissue tumours. Histopathology 2003, 43, 1-16.

9. Fletcher CD, Gustafson P, Rydholm A, Willen H,Akerman M. Clinicopathologic re-evaluation of 100 malignant fibrous histiocytomas: prognostic relevance of subclassification. J Clin Oncol 2001, 19, 3045-3050.

10. Watanabe K, Kusakabe T, Hoshi N, Saito A,Suzuki T. h-Caldesmon in leiomyosarcoma and tumors with smooth muscle cell-like differentiation: its specific expression in the smooth muscle cell tumor. Hum Pathol 1999, 30, 392-396.

11. Kumar S, Perlman E, Harris CA, Raffeld M,Tsokos M. Myogenin is a specific marker for rhabdomyosarcoma: an immunohistochemical study in paraffin-embedded tissues. Mod Pathol 2000, 13, 988-993.

12. Miettinen M, Fanburg-Smith JC, Virolainen M, Shmookler BM,Fetsch JF. Epithelioid sarcoma: an immunohistochemical analysis of 112 classical and variant cases and a discussion of the differential diagnosis. Hum Pathol 1999, 30, 934-942.

13. van de RM, Barr FG, Xiong QB, Hedges M, Shipley J,Fisher C. Poorly differentiated synovial sarcoma: an analysis of clinical, pathologic, and molecular genetic features. Am J Surg Pathol 1999, 23, 106-112.

14. Hazelbag HM, Mooi WJ, Fleuren GJ,Hogendoorn PCW. Chain-specific keratin profile of epithelioid soft-tissue sarcomas: An immunohistochemical study on synovial and epithelioid sarcoma. Appl Immunohistochem 1996, 4, 176-183.

15. Fletcher CDM, Beham A, Bekir S, Clarke AM,Marley NJ. Epithelioid angiosarcoma of deep soft tissue: a distinctive tumor readily mistaken for an epithelial neoplasm. Am J Surg Pathol 1991, 15, 915-924.

16. Arber DA, Kandalaft PL, Mehta P,Battifora H. Vimentin-negative epithelioid sarcoma: The value of an immunohistochemical panel that includes CD34. Am J Surg Pathol 1993, 17, 302-307.

17. Folpe AL, Chand EM, Goldblum JR,Weiss SW. Expression of Fli-1, a nuclear transcription factor, distinguishes vascular neoplasms from potential mimics. Am J Surg Pathol 2001, 25, 1061-1066.

18. Rossi S, Orvieto E, Furlanetto A, Laurino L, Ninfo V,Dei Tos AP. The utility of the immunohistochemical detection of Fli-1 expression in round cell and vascular neoplasm using a monoclonal antibody. Mod Pathol 2003,

19. Folpe AL, Hill CE, Parham DM, O'Shea PA,Weiss SW. Immunohistochemical detection of FLI-1 protein expression: a study of 132 round cell tumors with emphasis on CD99-positive mimics of Ewing's sarcoma/primitive neuroectodermal tumor. Am J Surg Pathol 2000, 24, 1657-1662.

20. Courville P, Simon F, Le Pessot F, Tallet Y, Debab Y,Metayer J. [Detection of HHV8 latent nuclear antigen by immunohistochemistry. A new tool for differentiating Kaposi's sarcoma from its mimics]. Ann Pathol 2002, 22, 267-276.

21. Ambros IM, Ambros PF, Strehl S, Kovar H, Gadner H,Salzer-Kuntschik M. MIC2 is a specific marker for Ewing's sarcoma and peripheral primitive neuroectodermal tumors: Evidence for a common histogenesis of Ewing's sarcoma and peripheral primitive neuroectodermal tumors from MIC2 expression and specific chromosome aberration. Cancer 1991, 67, 1886-1893.

22. Nicholson SA, McDermott MB, Swanson PE,Wick MR. CD99 and cytokeratin-20 in small-cell and basaloid tumors of the skin. Appl Immunohistochem Mol Morphol 2000, 8, 37-41.

23. Granter SR, Renshaw AA, Fletcher CDM, Bhan AK,Rosenberg AE. CD99 reactivity in mesenchymal chondrosarcoma. Hum Pathol 1996, 27, 1273-1276.

24. Dei Tos AP. The reappraisal of gastrointestinal stromal tumors: from Stout to the KIT revolution. Virchows Arch 2003, 442, 421-428.

25. Graadt van Roggen JF, Van Velthuysen MLF,Hogendoorn PCW. The histopathological differential diagnosis of gastrointestinal stromal tumours. J Clin Pathol 2001, 96-103.

26. Hornick JL,Fletcher CD. Immunohistochemical staining for KIT (CD117) in soft tissue sarcomas is very limited in distribution. Am J Clin Pathol 2002, 117, 188-193.

27. Sturtz CL,Dabbs DJ. Angiomyolipomas: the nature and expression of the HMB45 antigen. Mod Pathol 1994, 7, 842-845.

28. Hill DA, Pfeifer JD, Marley EF, Dehner LP, Humphrey PA, Zhu X,Swanson PE. WT1 staining reliably differentiates desmoplastic small round cell tumor from Ewing sarcoma/primitive neuroectodermal tumor. An immunohistochemical and molecular diagnostic study. Am J Clin Pathol 2000, 114, 345-353.

29. Ordonez NG. Desmoplastic small round cell tumor: II: an ultrastructural and immunohistochemical study with emphasis on new immunohistochemical markers. Am J Surg Pathol 1998, 22, 1314-1327.

30. Argani P, Lal P, Hutchinson B, Lui MY, Reuter VE,Ladanyi M. Aberrant nuclear immunoreactivity for TFE3 in neoplasms with TFE3 gene fusions: a sensitive and specific immunohistochemical assay. Am J Surg Pathol 2003, 27, 750-761.

31. Dei Tos AP, Doglioni C, Piccinin S, Sciot R, Furlanetto A, Boiocchi M, Dal Cin P, Maestro R, Fletcher CD,Tallini G. Coordinated expression and amplification of the MDM2, CDK4, and HMGI-C genes in atypical lipomatous tumours. J Pathol 2000, 190, 531-536.

32. Coindre JM, Mariani O, Chibon F, Mairal A, Saint Aubain SN, Favre-Guillevin E, Bui NB, Stoeckle E, Hostein I,Aurias A. Most malignant fibrous histiocytomas developed in the retroperitoneum are dedifferentiated liposarcomas: a review of 25 cases initially diagnosed as malignant fibrous histiocytoma. Mod Pathol 2003, 16, 256-262.

33. Dei Tos AP, Maestro R, Doglioni C, Gasparotto D, Boiocchi M, Laurino L,Fletcher CD. Ultraviolet-induced p53 mutations in atypical fibroxanthoma. Am J Pathol 1994, 145, 11-17.

34. Graadt van Roggen JF, Bovee JVMG, Morreau J,Hogendoorn PCW. Diagnostic and prognostic implications of the unfolding molecular biology of bone and soft tissue tumours. J Clin Pathol 1999, 52, 481-489.

35. Verweij J, Van Oosterom A, Blay JY, Judson I, Rodenhuis S, van der GW, Radford J, Le Cesne A, Hogendoorn PC, di Paola ED, Brown M,Nielsen OS. Imatinib mesylate (STI-571 Glivec, Gleevec) is an active agent for gastrointestinal stromal tumours, but does not yield responses in other soft-tissue sarcomas that are unselected for a molecular target. Results from an EORTC Soft Tissue and Bone Sarcoma Group phase II study. Eur J Cancer 2003, 39, 2006-2011.

36. Onda M, Matsuda S, Higaki S, Iijima T, Fukushima J, Yokokura A, Kojima T, Horiuchi H, Kurokawa T,Yamamoto T. ErbB-2 expression is correlated with poor prognosis for patients with osteosarcoma. Cancer 1996, 77, 71-78.

37. Gorlick R, Huvos AG, Heller G, Aledo A, Beardsley GP, Healey JH,Meyers PA. Expression of HER2/erbB-2 correlates with survival in osteosarcoma. J Clin Oncol 1999, 17, 2781-2788.

38. Akatsuka T, Wada T, Kokai Y, Sawada N, Yamawaki S,Ishii S. Loss of ErbB2 expression in pulmonary metastatic lesions in osteosarcoma. Oncology 2001, 60, 361-366.

39. Akatsuka T, Wada T, Kokai Y, Kawaguchi S, Isu K, Yamashiro K, Yamashita T, Sawada N, Yamawaki S,Ishii S. ErbB2 expression is correlated with increased survival of patients with osteosarcoma. Cancer 2002, 94, 1397-1404.

40. Maitra A, Wanzer D, Weinberg AG,Ashfaq R. Amplification of the HER-2/neu oncogene is uncommon in pediatric osteosarcomas. Cancer 2001, 92, 677-683.

41. Thomas DG, Giordano TJ, Sanders D, Biermann JS,Baker L. Absence of HER2/neu gene expression in osteosarcoma and skeletal Ewing's sarcoma. Clin Cancer Res 2002, 8, 788-793.

42. Anninga JK, Van de Vijver MJ, Cleton-Jansen AM, Kristel PMP, Taminiau AHM, Nooij M, Egeler RM,Hogendoorn PCW. Overexpression of the Her-2 oncogene does not play a role in high-grade osteosarcomas. Eur J Cancer 2004,

43. Kilpatrick SE, Geisinger KR, King TS, Sciarrotta J, Ward WG, Gold SH,Bos GD. Clinicopathologic analysis of HER-2/neu immunoexpression among various histologic subtypes and grades of osteosarcoma. Mod Pathol 2001, 14, 1277-1283.

44. Nielsen TO, West RB, Linn SC, Alter O, Knowling MA, O'Connell JX, Zhu S, Fero M, Sherlock G, Pollack JR, Brown PO, Botstein D,van de RM. Molecular characterisation of soft tissue tumours: a gene expression study. Lancet 2002, 359, 1301-1307.

45. Nielsen TO, Hsu FD, O'Connell JX, Gilks CB, Sorensen PH, Linn S, West RB, Liu CL, Botstein D, Brown PO,van de RM. Tissue microarray validation of epidermal growth factor receptor and SALL2 in synovial sarcoma with comparison to tumors of similar histology. Am J Pathol 2003, 163, 1449-1456.

46. van de RM,Rubin BP. Gene expression studies on soft tissue tumors. Am J Pathol 2002, 161, 1531-1534.

Pathology and Forensic Medicine / Invited Lectures / 06

DUCT CARCINOMA *IN SITU* OF THE BREAST: MORE CLASSIFICATIONS THAN REAL TUMOURS.

Vicenzo Eusebi, Italy

In 1950 Professor Businco, a pathologist from Bologna Medical School, in a paper on lung carcinomas stated that at the time of writing his paper more than 53 classifications on lung tumours had been published (3)and he commented (personal comunication to Professor A.M. Mancini) that some classifications are more the result of a narcisistic approach to pathology rather than the search of a common language. There is no doubt that some people like classifications more than diseases themselves and it is common experience to attend meetings dealing with classifications and semantic of diseases. Examples of this tendency in the past were provided by the several classifications published on lymphomas. In recent years breast pathology was the most active subspeciality in which a large number of classifications dealing with in situ carcinomas were proposed.

Here the development of the concept of duct carcinoma in situ (DCIS) is discussed, followed by comments on the most recent classifications.

As in all fields of pathology, classifications of tumours are directed either to the histogenesis or to the differentiation of a given neoplasia, or both.

Accordingly since the classical paper of Foote and Stewart (9), *in situ* carcinomas of the breast have been subdivided into lobular (LCIS) and ductal (DCIS) types. The morphological details of lobular neoplasms (LN) were described so finely by these same authors that they are still very much in use today. The belief that lobular carcinomas were the only breast epithelial neoplasms that recognized a lobular in origin was shaken by the demonstration that most of breast carcinomas arise in the same microanatomic structure, i.e. the terminal ductal lobular unit (TDLU) (24). The debate on how to distinguish these two conditions has gone through at least two decades till the work of Voos et al (23)who demonstrated that lesions with the morphology as described by Foote and Stewart(9) lacked e-cadherin while tumours with e-cadherin positive cells were conventionally included in the range of duct neoplastic lesions.

Therefore duct carcinomas are formed by cohesive cells that most of the time arise within TDLU. Occasionally DCIS can be seen within galactophorous ducts only. Neoplastic cells may extend into the epidermis featuring Paget's carcinoma. Finally there are cases that can combine at the same time features of DCIS and LN , defined as coexistent lobular carcinoma in situ and intraductal carcinoma in a single lobular-duct unit (20), carcinomas in situ with indeterminate features(14), solid low grade in situ carcinoma (17) hybrid mammary intraepithelial neoplasia (11).

Traditionally DCIS have been classified on structural grounds as micropapillary, cribriform, solid and comedo subtypes. To this Azzopardi(1) added clinging carcinoma (CC) , a highly debated subtype of DCIS. Azzopardi defined CC as " merely descriptive term to indicate that neoplastic cells are limited to the periphery of the containing structures.....Clinging carcinoma arises sometimes as a variant of comedo cancer". Azzopardi also was convinced that clinging carcinoma was the first morphological sign of carcinoma in a duct. It was not

a tumour entity, but the morphological sign of early carcinoma. This same lesion was found to be of two types: one type had monotonous nuclei, while the other showed pleomorphic nuclei and the cells were consistently Her-2 positive (4). The anxiety for recognizing any sign of incipient carcinoma led to the concept of atypical ductal hyperplasia (ADH) first proposed by Page and Dupont (5) to indicate the existence of a morphological bridge between normal epithelium and frank carcinoma. Although the concept of ADH was highly interesting the lesion was never fully defined including the "inventor" of it who gave at least three different definitions in different times. The lack of morphological definition was highlited by a paper where same lesions were considered and diagnosed differently by five experts of breast pathology (19) .

Holland et al in 1994(13) proposed a novel approach to the classification of DCIS that was mainly cytological(13). Accordingly Grade 1 DCIS had regular, monotonous nuclei; Grade III showed pleomorphic nuclei with prominent nucleoli. Grade II showed nuclei with intermediate changes. This was irrespective of any structure. G1 DCIS were frequently ER and PR positive, had low Ki 67 index, and were negative for P 53 and Her-2. The reverse was found in G 3 DCIS (2). G 1 DCIS had finely granular calcifications (12) in which calcium had precipitated on a substrate of acidic mucosubstances (10). Coarse granular microcalcifications are seen in G 3 DCIS and calcium precipitates above nuclear debris of DNA . Although most DCIS grow within the same breast lobe(16) , G 3 DCIS are unifocal tumours that grow in continuity within the same glandular tree, while G1 are multifocal and grow in different segments of the same gland (8). G1 when invade leads to the formation of G1 invasive duct carcinoma (DCI) , while G3 DCIS leads to G3 DCI in most of the cases(15). Low grade (G1) DCIS had a very low risk of being followed by an invasive carcinoma . This risk was considerably higher in G3 DCIS (6).

Soon other classifications followed : Silverstein et al(21; 18) proposed 2 classes of DCIS high grade and low grade malignancy, the Nottingham classification(7) was mainly based on the presence and extent of necrosis. Tavassoli proposed (22) a new nomenclature introducing Rosai's idea(19) that was easier and more consistent with the nomenclature of other organs to change carcinoma with neoplasia and accordingly the Duct In situ Neoplasia (DIN) nomenclature was generated. This caused a turmoil that, to the opinion of the writer, was caused more as consequence of conflict of personalities than objective reasons. Twenty percent of breast lesions taken all together polarized the attention of different authors and as a consequence about 75% of all clinical papers on breast pathology in 2001 dealth with DCIS and their classifications. This conflict also postponed the publication of the recent WHO blue book for not less that 2 years and the result was that two classifications (traditional terminology vs DIN terminology) were proposed for what has not added very much to our understanding after Holland's paper(13) on the existence of 3 different grades of DCIS.

References

1. Azzopardi J G, Ahmed A and Millis R R. Problems in breast pathology. London,W.B. Saunders Company, 1979.
2. Bobrow LG, Happerfield LC, Gregory WM, et al. The classification of ductal carcinoma in situ and its association with biological markers. Seminars in Diagnostic Phathology 1994;11 (3):199-207.
3. Businco A. Primary carcinoma of the lung. A survey of its pathological anatomy. Scientia Medica Italica 1950;1:656-722.
4. De Potter CR, Foschini MP, Schelfhout AM, et al. Immunohistochemical study of neu protein overexpression in clinging in situ duct carcinoma of the breast. Virchows Arch A Pathol Anat 1993;422:375-380.

5. Dupont WD, Page DL. Risk factors for breast cancer in women with proliferative breast disease. The New England Journal of Medicine 1985;312:146-151.

6. Eusebi V, Feudale E, Foschini MP, et al. Long term follow up of in situ carcinoma of the breast. Seminars in Diagnostic Phathology 1994;11,n 3:223-235.

7. Evans A, Pinder S, Wilson R, et al. Ductal carcinoma in situ of the breast. correlation between mammographic and pathologic findings. Am J Radiology 1994;162:1307-1311.

8. Faverly DRG, Burgers L, Bult P, et al. Tree dimensional imaging of mammary ductal carcinoma in situ: clinical implications. Seminars in Diagnostic Phathology 1994;11 (3):193-198.

9. Foote FW, Stewart FW. Lobular carcinoma in situ. American Journal of Pathology 1941;17:491-500.

10. Foschini MP, Fornelli A, Peterse JL, et al. Microcalcifications in ductal carcinoma in situ of the breast. Hum Pathol 1996;27:178-183.

11. Gocht A, Bosmuller HC, Bassler R, et al. Breast tumors with myofibroblastic differentiation: clinico-pathological observations in myofibroblastoma and myofibrosarcoma. Pathol Res Pract 1999;195 (1):1-10.

12. Holland R, Hendriks JHCL. Microcalcifications associated with ductal carcinoma in situ: mammographic-pathologic correlation. Seminars in Diagnostic Phathology 1994;11 (3):181-192.

13. Holland R, Peterse JL, Millis RR, et al. Ductal carcinoma in situ: a proposal for a new classification. Seminars in Diagnostic Pathology 1994;11 (3):167-180.

14. Jacobs TW, Pliss N, Kouria G, et al. Carcinomas in situ of the breast with indeterminate features. Role of e-cadherin staining in categorization. Am J Surg Pathol 2001;25 (2):229-236.

15. Lampejo OT, Barnes DM, Smith P, et al. Evaluation of infiltrating ductal carcinomas with a DCIS component: correlation of the histologic type of the in situ component with grade of the infiltrating component. Seminars in Diagnostic Phathology 1994;11 (3):215-222.

16. Mai KT, Yazdi HM, Burns BF, et al. Pattern of distribution of intraductal and infiltrating ductal carcinoma: a three-dimensional study using serial coronal giant sections of the breast. Hum Pathol 2000;31:464-474.

17. Maluf HM, Swanson PE, Koerner FC. Solid low-grade in situ carcinoma of the breast. Role of associated lesions and e-cadherin in differential diagnosis. Am J Surg Pathol 2001;25 (2):237-244.

18. Poller DN, Silverstein MJ, Galea M, et al. Ductal carcinoma in situ of the breast:a proposal for a new simplified histological classification association between cellular proliferation and c-erbB-2 protein expression. Mod Pathol 1994;7(2):257-262.

19. Rosai J. Borderline epithelial lesions of the breast. Am J Surg Pathol 1991;15:209-221.

20. Rosen PP. Coexistent lobular carcinoma in situ and intraductal carcinoma in a single lobular-duct unit. Am J Surg Pathol 1980;4:241-246.

21. Silverstein MJ, Waisman JR, Gamagami P, et al. Intraductal carcinoma of the breast (208 cases). Clinical factors influencing treatment choice. Cancer 1990;66:102-108.

22. Tavassoli FA. Ductal carcinoma in situ: introduction of the concept of ductal intraepithelial neoplasia. Mod Pathol 1998;11:140-154.

23. Vos CB, Cleton-Jansen AM, Berx G. E-cadherin inactivation in lobular carcinoma in situ of the breast: an early event in tumori-genesis. Br J Cancer 1997;76:1131-1133.

24. Wellings SR, Jensen HM. On the origin and progression of ductal carcinoma in the human breast. J Nat Cancer Inst 1973;50:1111-1118.

Pathology and Forensic Medicine / Invited Lectures / 07

CURRENT REVIEW OF LARYNGEAL SQUAMOUS INTRAEPITHELIAL LESIONS

Nina Gale, Slovenia

MIntroduction

Histological changes of the squamous epithelium that occur in the process of laryngeal carcinogenesis, are cumulatively designated squamous intraepithelial lesions (SILs). The term SILs has been proposed as an all embracing expression of the whole spectrum of epithelial changes ranging from squamous cell hyperplasia to carcinoma in situ.

It has been widely accepted that the transition from normal mucosa to invasive squamous cell carcinoma (SCC) is a comprehensive and multistage process, causally related to progressive accumulation of genetic changes leading to selection of clonal population of transformed epithelial cells. From six to ten independent genetic events are required for progression to SCC. In their evolution, some cases of SILs are self-limiting and reversible, some persist, and some of them progress to SCC in spite of treatment. Particular interest has been focused on potentially malignant or risky (precancerous) lesions. These lesions have been defined as histomorphological changes of the squamous epithelium from which invasive cancer develops in a higher percentage than from other epithelial lesions.

Aetiology

Laryngeal SILs are most likely related to cigarette smoking and alcohol abuse, and especially to combination of them. Additional aetiological factors are: industrial pollution, chronic infections, voice abuse, obstruction of the upper respiratory tract, vitamin deficiency, and hormonal disturbance. The role of HPV infection in laryngeal carcinogenesis remains unsolved. The overall prevalence of HPV infection in 9 studies of SILs was found to be 12.4 %. However, HPV DNA was also detected in a clinically and histologically normal larynx in 12-25 % of individuals. Definite evidence of an etiologic role of HPV in SIL, at least at present, is lacking, and HPV infection in SIL may represent an incidental HPV colonisation rather than true infection of the laryngeal mucosa.

Histopathology

Traditional light microscopic examination, in spite of certain subjectivity in interpretation, remains the most reliable method for determining an accurate diagnosis of SILs. The clinical validity of any histological grading system depends on the degree of accord with the biological behaviour of the lesions. Worldwide, there are no generally accepted criteria for histological grading system in the head and neck region in relation to severity of SILs and propensity for malignant transformation. World Health Organization (WHO) has recently readopted the dysplasia system for classifying SILs of the larynx. However, due to different standpoints concerning this important problem of laryngeal carcinogenesis, dysplasia system was reviewed simultaneously with two additional classifications: the squamous intraepithelial neoplasia system and the Ljubljana classification. Comparing the dysplasia system and Ljubljana classification, one should be aware that there is no simple relationship and overlapping between both classifications (Table 1). Disagreement starts with presumption of the WHO 2005 classification that each grade of the whole series of dysplasia

is considered to be a precursor or potentially malignant lesion. Histologically, however, there are some similarities between basal and parabasal cell hyperplasia of the Ljubljana classification and mild dysplasia of the WHO 2005.

Table 1. Classification schemas that histologically categorize precursor and related lesions

2005 WHO Classification	Squamous intraepithelial neoplasia (SIN)	Ljubljana classification Squamous intraepithelial lesions (SIL)
Squamous cell hyperplasia		Squamous cell (Simple hyperplasia)
Mild dysplasia	SIN 1	Basal/parabasal cell hyperplasia**
Moderate dysplasia	SIN 2	Atypical hyperplasia (Risky epithelium)***
Severe dysplasia	SIN3*	Atypical hyperplasia (Risky epithelium)***
Carcinoma in situ	SIN 3*	Carcinoma in situ

* The advocates in SIN combine severe dysplasia and carcinoma in situ

** Basal/parabasal cell hyperplasia may histologically resemble mild dysplasia, but the former is conceptually benign lesion and the latter the lower grade of precursor lesions

*** Atypical hyperplasia (risky epithelium), analogy to moderate and severe dysplasia is approxim

Mild dysplasia, in contrast to basal and parabasal cell hyperplasia, was classified as the initial grade within a spectrum of potentially malignant group, whereas in the Ljubljana classification, basal-parabasal hyperplasia is considered a benign lesion with minimum risk of malignant transformation. Atypical hyperplasia of the Ljubljana classification is similar to moderate dysplasia, but partially also includes severe dysplasia. The analogy is, thus, only approximate. Carcinoma in situ is equal to carcinoma in situ of the WHO 2005 classification. However, some cases of severe dysplasia would fall into the category of carcinoma in situ of the Ljubljana classification, and the analogy is again only approximate.

Genetic changes

A genetic progression model with specific genetic alterations for different stages of laryngeal SILs has increased possibilities of recognising potential biomarkers in correlation with histopathologic changes that might signal a stage of carcinogenesis from initiation to invasive growth. This model has revealed that both oncogenes and tumour suppressor genes are involved in tumour progression with a distinct order of progression starting with loss of heterozygosity (LOH) at 9p21 and 3p21 as the earliest detectable events, followed by 17p13 loss. Additional genetic alterations, which tend to occur in severe dysplasia (atypical hyperplasia), or even in SCC, are cyclin D1 amplification, pTEN inactivation, and LOH at 11q13, 13q21, 14q32, 6p, 8q, 4q27, and 10q 23. For some involved chromosomal areas the target genes have been recognised, such as tumour suppressor genes p16 at 9p21, and p53 at 17p13, and cyclin D1 oncogene at 11q13. In terms of prognostic value, genetic events such as LOH of 3p, 9p21 and 17q 13 and DNA aneuploidy are considered substantial risk of malignant transformation. Telomerase reactivation is an early event in laryngeal

carcinogenesis, detectable already in the stage of potentially malignant lesions (atypical hyperplasia). However, other genetic changes appear to be necessary for progression to invasive cancer.

Treatment and predictive factors

The main task of the pathologist dealing with laryngeal SILs is to separate non-risky or a minimal risky from risky changes. Patients with benign hyperplastic lesions (simple and basal-parabasal hyperplasia) do not require as close follow-up after excisional biopsies as those with atypical hyperplasia and CIS, although elimination of known detrimental influences is advised. Diagnosis of atypical hyperplasia in laryngeal lesions requires close follow-up and often repeated histological assessment to detect any possible persistence or progression of the disease. Patients with CIS may require more extensive surgical treatment or radiotherapy, although this is controversial. The histopathologic degree of severity of laryngeal SILs are still used as most reliable predictive factor. The frequency of subsequent malignant alteration markedly increases from squamous (simple) and basal-parabasal (abnormal) hyperplasia (0.9 %), as compared to atypical hyperplasia (11 %). Barnes's review of the literature shows that the risk of SCC developing in mild, moderate and severe laryngeal dysplasia ranges from 5.5 % to 22.5 % and 28.4 %, respectively.

References:

1. Gale, N, Zidar N. Benign and potentially malignant lesions of the squamous epithelium and squamous cell carcinoma (Chapter 1). In: Cardesa A, Slootweg P (eds): Head and neck, soft tissue, retroperitoneum and skin, Springer 2005, Berlin, in press.
2. Gale N, Pilch BZ, Sidransky D, Westra, W, Califano J. Epithelial precursor lesions (Chapter 3). In: Barnes L, Eveson JW, Reichart PA, Sidransky D (eds) World Health Organization Classification of Tumours Pathology and genetics of tumours of the head and neck. IARC 2005, Lyon, in press.
3. Ha PK, Califano JA 3rd. The molecular biology of laryngeal cancer. Otolaryngol Clin North Am. 2002; 35: 993-1012.

Pathology and Forensic Medicine / Invited Lectures / 08

SPSEUDOMYXOMA PERITONEI

Zoran Gatalica, USA

Pseudomyxoma peritonei (PMP) is a clinical term used to describe often dramatic presence of mucinous ascites and mucinous tumor implants diffusely involving peritoneal surfaces. Historically, PMP was used for all cases of mucinous ascites regardless of pathologic features (grade and origin) of mucin-producing tumor. In recent years, it became evident that the vast majority of mucinous tumors presenting with peritoneal dissemination and ascites originate from appendix, and that survival rate was strongly associated with the pathologic features of the neoplasm. Long term follow up studies showed usefulness of pathologic classification of PMP into uniformly low grade adenomatous neoplasm termed Disseminated Peritoneal Adenomucinosis (DPAM) and cytologically atypical, architecturally complex neoplasms termed Peritoneal Mucinous Carcinomatosis (PMCA).

The lecture will focus on criteria for histologic classification of PMP, as well as immunohistochemical, ploidy, molecular and pharmacogenomic characteristics of tumors presenting as PMP.

Pathology and Forensic Medicine / Invited Lectures / 09

USE OF VIRTUAL SLIDE TECHNOLOGY IN DIAGNOSIS AND TEACHING

C.L. Hitchcock, Jewell S.

Department of Pathology
The Ohio State University, USA

Traditional microscopy has evolved into virtual microscopy with the advent of improved computer technology, internet access, and software development for local and remote viewing on the web. Virtual microscopes are capable of digitizing all or a portion of a glass slide into a high resolution image that can be remotely viewed any where in the world. The Department of Pathology at The Ohio State University (OSU) has embraced this technology as both a diagnostic tool and for education. Our department is the national Pathology Coordinating Office (PCO) for the NCI clinical trials cooperative group, Cancer and Leukemia Group B (CALGB). We have used the Aperio ScanScope to digitize 1,702 slides at 20x or at 40x of every research tissue specimen. Pathologists have so far reviewed 373 cases (105 breast and 268 lymphomas) on a website site hosted by OSU. A total of 9/105 breast cases required review of the glass slides due to questionable diagnosis; however, there was a 100% concordance in the diagnosis of the 272 lymphoma case. This same technology is continuously being used to generate image files for both resident and for continuing education. Residents in pathology have built a website dedicated to virtual slides from interesting cases. Residents can also test their diagnostic skills using a quiz mode. Virtual slides are extensively used by OSU faculty during their when presentations at national and international meetings. We have also distributed CDs with viewing software and virtual slides to registrants at our courses.

Pathology and Forensic Medicine / Invited Lectures / 10

SEROUS BORDERLINE TUMORS OF THE OVARY BEYOND THE BORDER OF BORDERLINE

Robert J. Kurman, USA

Clinicopathologic studies have revealed that the "intermediate" behavior of serous borderline tumors (SBTs) is due to the fact that the category is composed of a heterogeneous group of tumors. Most of them are benign and we designate them "atypical proliferative serous tumors (APSTs)" whereas a few are in situ, low-grade carcinomas, which we term "micropapillary serous carcinoma (MPSC)" or "intraepithelial low-grade serous carcinoma". Molecular genetic studies provide compelling evidence that these noninvasive tumors represent intermediate steps in the progression from a serous cystadenoma to an invasive low-grade serous carcinoma. The latter is relatively uncommon compared to the usual type of serous carcinoma, which is a high-grade neoplasm that accounts for the majority of serous carcinomas. Mutations of BRAF and KRAS occur in approximately two-thirds of APSTs, MPSCs and invasive low-grade serous carcinomas whereas mutations of p53 occur in less than 10% of these neoplasms. In contrast, greater than 50% of the usual type of serous carcinoma contains mutant p53 but BRAF and KRAS mutations have not been detected. Based on these findings we have proposed a dualistic model of serous carcinogenesis in which low-grade serous carcinomas develop slowly in a stepwise fashion from cystadenomas, APSTs, and MPSCs (intraepithelial low-grade serous carcinomas). In contrast, high-grade serous carcinomas develop rapidly from surface epithelial inclusion cysts in what has been described as de novo development. Accordingly the classification that we use reflects these advances in our understanding of serous carcinogenesis and is as follows: serous cystadenomas/adenofibroma, atypical proliferative serous tumor, low-grade intraepithelial serous carcinoma (noninvasive micropapillary serous carcinoma), low-grade serous carcinoma (invasive micropapillary serous carcinoma), and high-grade serous carcinoma.

PATOLOGIJA I SUDSKA MEDICINA
PATHOLOGY AND FORENSIC MEDICINE

Usmena izlaganja / *Oral presentations*

Hematopatologija / *Hematopathology (1-6)*

Patologija dojke / *Breast Šathology (7-9)*

Ginekološka i urološka patologija / *Gynecological Pathology and Uropathology (10-16)*

Forensic Medicine (17-21)

Pathology and Forensic Medicine / Oral presentations / Hematopathology / 01

BONE MARROW LYMPHOID AGGREGATES IN MALIGNANT LYMPHOMAS - NEOPLASTIC, REACTIVE?

Dominis M[1], Pešut A[1], Borovečki A[1], Marušić-Vrsalović M[2], Džebro S[1,] Kušec R.[2], [3]

[1]Department of Clinical Pathology and Cytology
[2]Institute of Clinical Chemistry
[3]Department of Internal Medicine, Merkur University Hospital, Zagreb, Croatia

Aim: There is no definitive numerical and morphological "cutoff" between bone marrow lymphoid aggregates (LA) / nodular lymphoid hyperplasia (NLH) and malignant lymphoid aggregates in malignant lymphoma. We examined the usefulness of PCR analysis of CDR3 region of IgH gene rearrangement in assesment of clonality in bone marrow biopsies with LA or NLH in patients with different subtypes of malignant lymphomas.

Methods: 529 samples of bone marrow biopsies were processed routinely. Results were grouped in positive, negative and cases with LA or NLH. In 43 samples with present LA/NLH PCR analysis of the CDR3 region of IgH gene for B-cell clonality test was done.

Results: The bone marrow malignant lymphoma infiltrates were present in 33.8% of lymphoma cases. The incidence of LA/NLH in bone marrow was 8.1%. LA/NLH were more frequently found in patients with extranodal disease and aggressive subtypes of B cell non-Hodgkin's lymphoma, but there was not a significant difference among the incidence according to biological behavior of malignant lymphoma ($p = 0.232$). Results of IgH-CDR3 region PCR analysis have shown a monoclonal pattern in one case of Hodgkin's lymphoma and in one control case, an oligoclonal pattern in two cases of extranodal B-NHL, while allother cases were polyclonal.

Conclusion: It is possible to differentiate LA and NLH by analyzing the characteristics of histotopography and cytology in bone marrow staging procedure, especially in patients withl ow grade NHL. Aggressive B-NHL, nodal and particularly extranodal, have more frequently LA/NLH in bone marrow biopsies than indolent B-NHL.

Pathology and Forensic Medicine / Oral presentations / Hematopathology / 02

ACTIVATED RECEPTOR TYROSINE KINESIS FOR HYPEREOSINOPHILIA

Kušec R.

Department of Medicine and Institute of Clinical Chemistry, Merkur University Hospital Zagreb

Persistent nonreactive hypereosinophilia characterizes both chronic eosinophilic leukaemia (CEL) and idiopathic hypereosinophilic syndrome (HES). Tissue damage by direct infiltration of eosinophils and cytokine release leads to progressive organ failure. Increased marrow blasts or the presence of clonal cytogenetic abnormality distinguishes CEL from HES. Several specific subtypes and cytogenetic aberrations of CEL have been defined in recent years. The most clearly defined entities are CMML-eo with t(5;12)(q33;p13) and CEL with cytogenetic aberrations affecting the 8p11-12 breakpoint. Recently, a novel type of CEL with a distinct recurrent cytogenetic and molecular characteristics, interestitial deletion of 4p and fusion of genes FIP1L1 and tyrosine kinase gene PDGFR" has been described. This disease is responding favorably to Imatinib mesylate therapy. A novel diagnostic and therapeutic algorithm for screening of HES/CEL for FIP1L1-PDGFR" and results of the molecular screening for this marker in our patients will be presented.

Pathology and Forensic Medicine / Oral presentations / Hematopathology / 03

OUTCOME AND PROGNOSTIC FACTORS IN OCULAR ADNEXAL LYMPHOMA

Nola M, Lukenda A, Bollmann M, Kalauz M, Petrovečki M, Blažnović A, Dotlić S, Sičaja M, Prka Ž, Stiglmayer N, Tojagić M, Bollmann R.

Departments of Pathology and Ophthalmology, Zagreb University School of Medicine and Hospital Center, Zagreb; Department of Ophthalmology, Dubrava University Hospital, Croatia; Institute of Pathology, Bonn, Germany;
Zagreb University School of Medicine,Zagreb, Croatia

Aim.To classify ocular lymphomas in patients treated at the Zagreb University Hospital Center, according to the new classification of the World Health Organization (WHO), and determine factors with prognostic significance.

Methods. From 1986 to 2003, histological diagnosis of ocular lymphoma was made in 24 patients. The median age of patients was 62 years, with 2:1 female predominance. The patients underwent staging procedures and clinical evaluations prior to the date of the initial therapy. Histopathologic slides were reviewed and tumors were classified according to the new WHO classification. Additional immunohistochemical studies were performed on 35 available specimens. The antibodies used were CD3, CD5, CD10, CD20, CD43, and bcl-6; and in a few cases cyclin D1, bcl-2, CD23, CD79a, and CD138. The main outcome measures were development of distant recurrence after new presentation with solely ocular adnexal disease, and death attributable to widespread lymphoma.

Results. Ocular adnexal lymphomas were found in orbit in 20 patients, in eyelid in two, and conjunctiva in two patients. Twenty patients had lymphoma stage IE, one had IIE, and three had stage IV. Three patients had prior or concurrent systemic disease and 21 patients had primary lymphoma. The main subtypes of non-Hodgkin lymphoma according to the WHO classification were extranodal marginal zone B-cell lymphoma (n=20), diffuse large cell B-cell lymphoma (n=2), mantle cell lymphoma (n=1), and plasmacytoma (n=1). Six lymphomas were CD43 positive and five of them were extranodal marginal zone B-cell lymphomas. Excision biopsy without additional treatment was performed in two patients. Radiotherapy was given to 11 patients, chemotherapy in 8 patients, whereas radio therapyand chemotherapy were implemented in three patients. Two patients underwent only surgical excision of the tumor. Local relapse was found in three and distant recurrence in four patients. Distant recurrence was found in four patients with stage IE (two of them also had a local relapse). In the group of patients with extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue, the estimated 5-year overall survival was 92.9±6.6% (mean±standard deviation) and the 5-year failure-free survival was 80.1±10.3%. Age, sex, side of involvement, anatomic localization of the lesion, clinical stage of disease, and modeof therapy did not have any prognostic significance during the follow-up period (median 53; range, 9-131 months). Immunohistochemical marker CD43 was the only parameter of prognostic significance (p=0.035). Patients with B-EMZL had almost 14 times higher chance for an unfavorable outcome if the tumor cells expressed CD43 on their surface, than the CD43-negative cases.

Conclusion. Most ocular adnexal lymphomas usually have a B-cell immunophenotype, themorphologic and immunohistochemical features of extranodal marginal zone B-celllymphoma, and a favorable prognosis. Our data suggest that CD43 could be useful toseparate the group of patients with extranodal marginal zone B-cell lymphomas withunfavorable prognosis from those that have a good prognosis. CD43 positive ocularlymphomas are associated with a higher rate of subsequent distant recurrence and the rateof lymphoma-related death.

Pathology and Forensic Medicine / Oral presentations / Hematopathology / 04

APPLICATION OF THE FICTION TECHNIQUE ON ROUTINELY FIXED PARAFFIN WAX-EMBEDDED BONE MARROW TREPHINES

Korać P[1,2], Jones M[1], Dominis M[2], Kušec R[3,4], Mason DY[1], Banham AH[1], Ventura RA.[1]

[1]Nuffield Department of Clinical Laboratory Sciences, University of Oxford, LRF Immunodiagnostics Unit, John Radcliffe Hospital, Headington, Oxford, UK
[2]Department of Clinical Pathology and Cytology
[3]Department of Internal Medicine
[4]Institute of Clinical Chemistry, Merkur University Hospital, Zagreb, Croatia.

Aim: We report a new application of FICTION technique to simultaneously detect both immunophenotype and chromosomal abnormalities in routinely fixed paraffin wax-embedded bone marrow trephines.

Methods: Methods that we used were FISH (used probes were commercial) and immunostainig with antibody against CD 20 that is commonly used in routine analyses.

Results: The FICTION technique was applied to BMTs that had been decalcified with either an ETDA-based or an HCl-based reagent. Attempts to perform FICTION on acid-decalcified BMTs (n=6) had adequate immunostaining but no FISH signals.

In contrast, the EDTA based treatment enabled the successful application of combined immunohistochemistry and FISH. The FISH signals in each of the cases (n=16) were bright, focused and easily interpretable using a x63 objective lens and the immunofluorescent staining in each sample was bright, specific, did not fade and facilitated the rapididentification of neoplastic cells.

Conclusion: For the first time we report the successful application of a modified FICTION technique to allow the study of BMTs. This technique allows the rapid identification of rare CD20-positive B-cells within the bone marrow by immunophenotyping as well as simultaneously identifying any specific genetic aberrations harboured by these cells.

Pathology and Forensic Medicine / Oral presentations / Hematopathology / 05

ANALYSIS OF C-KIT MUTATIONS IN ACUTE MYELOID LEUKEMIA (AML) WITH T(8;21)

Drmić I, Radnić M, Zadro R, Kušec R, Grahovac B.

Split University Hospital and School of Medicine, Split, Croatia; Clinical Hospital Center Zagreb and Zagreb University School of Medicine, Zagreb, Croatia; Clinical Hospital "Merkur", Zagreb, Croatia; Rijeka University School of Medicine, Rijeka, Croatia

Acute myeloid leukemia (AML) has been proposed to arise from the cooperation between abnormalities in genes that encode transcription factors (TF) and tyrosine kinases (TK). Disruption of TFs is often a result of recurring chromosomal translocation with inhibition of heamatopoietic differentiation, whereas the mutations in receptor TK, c-kit and other provide a proliferative signal. The most common chromosomal translocation, t(8;21)(q22;q22), in AML generates AML1/ETO fusion gene. According to recent data, genetic abnormalities that cooperate with AML1/ETO to induce AML remain elusive, as well as clinical significance of c-kit mutations in leukemias with t(8;21).

To estimate the prevalence of c-kit mutations in AML, we analyzed several c-kit mutations in coding sequences and promoter region in AML1/ETO positive patients (n=25).

Obtained results suggest that further studies with extended number of patients at disease presentation, during therapy and follow-up are required to evaluate the significance of c-kit mutations in prognosis and therapy of AML.

Pathology and Forensic Medicine / Oral presentations / Hematopathology / 06

FACTOR V LEIDEN AND PROTHROMBIN G20210A MUTATIONS AS A RISK FACTORSIN PATIENTS WITH VENOUS AND ARTERIAL THROMBOEMBOLISM

Lenz B[1], Dorić A[2], Grahovac M[3], Grahovac B.[4]

[1]Department of Transfusion Medicine, Clinical Hospital Osijek
[2]Polyclinic for Dermatovenerology, Zagreb
[3]Zagreb University Hospital Center, Medical Faculty University of Zagreb
[4]Department of Pathology Medical Faculty University of Rijeka

Aim: We determined the prevalence of factor V Leiden and of prothrombin G20210A mutations in two group of patients referred for suspected deep vein thrombosis (DVT) (n = 94) and arterial thrombosis (AT) (n=36).

Methods: FVLeiden mutation was defined by the method of Bertina et al.(1994) and prothrombin mutation according to Poort et al.(1996).

Results: The prevalence of factor V Leiden was 22.34 % (OR=2.2, p<0,001) in the 94 patients with DVT, and 19.44% (OR= 2.47, p<0.001) in patients with AT, compared with 5.45% in the control group (n=110), matched by sex and age. The prevalence of the prothrombin G20210A mutation did not differ among the control group and DVT patients (5.45% for controls, 4.26% for DVT). For the prothrombin G20210A mutation, significant differences were found between the AT and control group

(6/36, 16.66%, OR=2.24, p<0.01, versus 5.45%), indicating this mutation as a risk factor forAT in our population.

We found that pregnancy/puerperium and substitute hormone therapy in menopausal women, if joined with hereditary thrombophilia, are the most frequent cause of venous thrombosis in female (FVLeden:10/28, 35.71%, OR=4.25, p<0.001 and 2/7, 28.57%, OR=5.8, p<0.001, respectively).

The prevalence of both factors in DVT patients increases from 22.2% to 46.15% and to 61.54%, depending of first, second and, next following episodes of thrombosis.

Conclusion: Carriers of both factor V Leiden and the G20210A prothrombin mutation have an increased risk of recurrent deep venous thrombosis after a first episode, and are candidates for lifelong anticoagulation.

Pathology and Forensic Medicine / Oral presentations / Breast Pathology / 07

HER-2/NEU EXPRESSION IN CROATIAN BREAST CANCER PATIENTS (RESULTS OFONE YEAR MULTICENTRIC PROSPECTIVE STUDY)

Jakić-Razumović J, Šarčević B[1], Šeparović V[1], Kosanović S[1], Boikov J[2], Jonjić N[3], Mustać E[3], Tomić S[4], Bezić J[4], Krušlin B[5], Vučić M[5], Tomas D[5], Dmitrović B[6], Blaičević V[6], Jakovina T[7], Švagelj D[8], Borić I[9], Njirić Z[9], Štitić V[10], Mlinac-Lucijanić M[10], Restek-Samaržija N[11], Predrijevac H[11], Gošev M.[11]

[1]Department of Pathology, Clinical Hospital Zagreb, Department of Pathology Clinic for tumors Zagreb, Croatia
[2]Andrija Štampar School of Public Health, Medical School Zagreb, Croatia
[3]Department of Pathology, Medical Faculty Rijeka, Croatia
[4]Department of Pathology, Clinical Hospital Split, Croatia
[5]Department of Pathology, Clinical Hospital "Sestre milosrdnice", Zagreb, Croatia
[6]Department of Pathology, Clinical Hospital Osijek, Croatia
[7]Department of Pathology, General Hospital Slavonski Brod, Croatia
[8]Department of pathology, General Hospital Vinkovci, Croatia
[9]Department of Pathology, General Hospital Dubrovnik, Croatia
[10]Department of Pathology, General Hospital Karlovac, Croatia
[11]Hoffmann-LaRoche Zagreb, Croatia

Introduction: Breast cancer represents a broad biologic spectrum of disease, for which treatment approaches and prognosis depend on basic prognostic factors and additional markers. Aims: To verify the expression of HER-2/neu in association with other prognostic factors in one year new diagnosed breast cancer population in multicentric study in Croatia. Material and methods: In this study 1446 breast carcinoma patients were analyzed with data of age, menopausal status, histological type, tumor size, grade, axillary lymph node status, intratumoral and extratumoral vascular invasion, estrogen (ER), progesterone (PR) receptor and HER2/neu expression. All traditional prognostic factors were compared with HER-2/neu status.

Results: The results showed that there is statistically significant correlation between HER-2/neu expression and histological grade ($p<0.001$), nuclear grade ($p<0.001$), extratumoral vascular invasion ($p=0.039$), lymph node status ($p<0.001$), and menopausal status ($p=0.040$). Negative correlation was found between HER-2/neu expression and ER and PR positivity ($p<0.001$). Also the correlation between lymph node status and intra ($p=0.038$) end extra-tumoral vascular invasion (0.001) was found.

Conclusion: It seems that measurement of HER-2/neu expression in breast carcinoma patients is valuable prognostic factor in combination other traditional prognostic factors.

Pathology and Forensic Medicine / Oral presentations / Breast Pathology / 08

EXPRESSION OF CD34 AS A VASCULAR MARKER IN INVASIVE BREAST CANCER

[1]Bilalović N, [2]Vranić S.

[1]Department of Pathology, Sarajevo University School of Medicine, Bosnia and Herzegovina
[2]Sarajevo University School of Medicine, Bosnia and Herzegovina

Aim: To explore presence and correlation of CD34 in breast cancer with other clinicopathological and biological parameters and its prognostic value.

Methods: Medical records of 46 patients with invasive breast cancer diagnozed in 1998, from the database of Institute of Oncology, Clinical Center of the University of Sarajevo were analyzed. The mean follow-up was 61.17 monthes (range: 4-103 months).

Routine histopathological analysis was performed for 46 formalin fixed and paraffin embedded tissue samples. Immunohistochemical staining was used for investigation of estrogen (ER), progesterone (PR), bcl-2, CD34 (DAKO monoclonal antibodies), CD10 and cyclin D1 (NovoCastra monoclonal antibodies) expressions.

Results: CD34 expression: Fifteen samples (32.6%) showed expressions 1 and 2, respectively while 16 samples showed expression 3 (34.8%). There was a significant positive correlation of CD34 with lymph node status, tumor grade and tumor size ($p=0.05$, 0.05, 0.04, respectively, Linear-by-Linear Association test). Inverse correlation was found between CD34 and PR expression ($p=0.04$, Linear-by-Linear Association test). Patients with higher CD34 expression had shorter overall survival ($p=0.05$, Kaplan-Meier test). No statistically significant correlation was found between CD34 expression and relapse-free survival. Cox Regression Multivariate Test revelead that only lymph node status and bcl-2 are independent prognosticators in our study of breast cancer specimen ($p=0.01$, 0.02, respectively).

Conclusion: Although CD34 showed its importance on overall survival in breast cancer, it is not an independent prognosticator in invasive breast cancer.

Pathology and Forensic Medicine / Oral presentations / Breast Pathology / 09

VEGF EXPRESSION IS ASSOCIATED WITH NEGATIVE ESTROGEN RECEPTORSTATUS IN BREAST CANCER PATIENTS

Jonjić N, Fučkar D, Dekanić A, Štifter S, Mustać E, Krstulja M, Dobrila F.[1]

Department of Pathology, Rijeka University Medical School
[1]Department of Surgery, University Hospital Center, Rijeka, Croatia

Aim: To investigate the association between vascular endothelial growth factor (VEGF) expression on tumor cells and other well known clinicopathologic parameters in breast cancer, which could give additional information on its prognostic significance.

Methods: Immunohistochemical analysis of the expression of VEGF, estrogen (ER) and progesterone receptor (PR), HER-2/neu and Ki67 was performed in 233 breast cancers. The expression of VEGF estimated semiquantitatively was correlated with all the above mentioned parameters as well as with the clinicopathologic characteristics of breast cancer such as menopausal status of patients, tumor size, histologic and nuclear grade, vascular invasion, and lymph node status.

Results: Most of breast cancers expressed VEGF (95%). In addition to tumor cells, VEGF expression was also observed on stromal components including fibroblasts, macrophages and endothelial cells. A higher percentage of VEGF positive tumor cells was present in premenopausal patients ($p=0.003$) and in ER negative tumors ($rs=-0.195$, $p=0.045$). In postmenopausal patients tumors with a higher expression of VEGF were associated not only with ER negative ($p=0.039$) but also with HER-2/neu positive tumor cells ($p=0.027$). Furthermore, these ER negative tumors were characterized by a higher proliferative activity ($p<0.001$).

Conclusion: Angiogenic switch as well as proliferative activity of breast cancer cells probably is unfavorably dependent on estrogen activity. This negative correlation between VEGF expression and ER status may not only shed more light on tumor biology but may also have future therapeutic implications.

Pathology and Forensic Medicine / Oral presentations / Gynecological Pathology and Uropathology / 10

GYNECOLOGICAL PATHOLOGY AND UROPATHOLOGYANGIOGENIC PROPERTIES OF CERVICAL INTRAEPITHELIAL NEOPLASIAS (CIN):QUANTITATIVE MORPHOMETRIC ANALYSIS

Mrčela M, Ilić-Forko J, Babić D.

Institute for Pathology and Forensic Medicine, University Hospital Osijek, Croatia
Institute for Gynecological and Perinathal Pathology, University Hospital Zagreb, Croatia

Aim. To determine whether the cervical intraepithelial neoplasias are able to provoke neovascularisation - angiogenesis.

Methods. The quantity (the blood vessel count along the basement membrane of the epithelium and the total number of blood vessels of the given microscopic view field) was compared using 30 samples of CIN 1, 30 samples of CIN 2, and 30 samples of CIN 3 and two control groups of which each contained 30 samples of cervical tissue without pathohistological abnormality. The difference between the two control groups was that the control group I consisted of samples taken from the cervix of patients who underwent a hysterectomy due to reasons unrelated with CIN, and control group II consisted of samples taken from the cervix with CIN 3, but from an area without pathohistological abnormality. Samples were analyzed using immunohistochemical method with anti-CD 31 marker.

Results. A statistically significant difference was found in the blood vessel count immediately underneath the normal epithelium of control group I and dysplastic epithelium of CIN 1, CIN 2 and CIN 3 lesions ($P1<0.001$; $P2<0.001$; $P3<0.001$). A statistically significant difference was found by comparing the blood vessels count underneath the CIN 3 and underneath the nonneoplastic epithelium of the control group which consisted of samples of the cervix which was conizated because of the CIN 3, but without pathohistological abnormality in the sense of CIN ($P<0.001$). The blood vessel count immediately along the basement membrane of the epithelium between CIN 1 and CIN 3 ($P=0.022$), and between CIN 2 and CIN 3 were significantly different ($P=0.019$).

Conclusion. Since there is a significant difference in the blood vessel count between the normal and dysplastic epithelium, and as this number increases with the rise of the CIN grade, it was concluded that the cervical intraepithelial neoplasias are angiogenic.

Pathology and Forensic Medicine / Oral presentations / Gynecological Pathology and Uropathology / 11

HPV GENOTYPES DISTRIBUTION IN CERVICAL CARCINOMA AND ITS PRECURSORLESIONS IN REGION OF RIJEKA, CROATIA

Krašević M[1], Šimat M[2], Grahovac B.[2]

[1]Department of Pathology, Rijeka University Medical School, Croatia
[2]Laboratory for Molecular Diagnostics, Department of Pathology, Rijeka University Medical School, Croatia

Aim: To investigate the prevalence and HPV distribution in woman with cervical carcinomas and its precursor lesions.

Material and methods: A retrospective analysis was undertaken on archival cervical intraepithelial neoplasia (CIN III no=43), squamous cell carcinomas (SCCs no=54) and adenocarcinomas (ACs n=40) including 15 adenocarcinoma in situ, from patients treated at Clinical Hospital Rijeka. HPV DNA genotyping was performed by consensus and type specific primers directed PCR.

Results: Ten different HPV genotypes were detected: 6/11, 16, 18, 31, 33, 45, 52, 68, 70, 74 and undetermined type x. The prevalence of HPV infection in CINs, SCCs and ACs was 93.1%, 92.6% and 92.5%, respectively. HPV 16 was the predominant type in CINs and SCCs, detectable in 26/40 (65.0%) and 26/50 (52.0%) respectively, whereas the most prevalent type in ACs was HPV 18 (67.5%). The unexpected high prevalence of HPV 31 accounted for 14.4% of HPV positive squamous cell lesions and 8.1% of glandular lesions, followed by HPV 33, found in 6.7% and 5.4%, respectively. Multiple HR-HPV infections were detected in 25.7%, 29.8% and 21.8% of HR-HPV positive CINs, SCCs and ACs, respectively. No significant correlation was found between specific HPV types and histological subtypes of carcinomas.

Conclusion: Our results indicate that cervical carcinomas and its precursor lesions are largerly associated with HPV types 16, 18, 31 and 33 in study group in Rijeka region. These types should be considered for vaccination program in the future.

Pathology and Forensic Medicine / Oral presentations / Gynecological Pathology and Uropathology / 12

IMMUNOCYTOCHEMICAL DETECTION OF P16INK4 PROTEIN IN NORMAL AND DYSPLASTIC SQUAMOUS CELLS IN CERVICAL SMEARS: A PRELIMINARY STUDY

Kos M[1], Čupić H[1], Kos M[2], Latin V[2], Matić J.[1]

[1]"Ljudevit Jurak" University Department of Pathology, "Sestre milosrdnice" University Clinical Hospital, Zagreb, Croatia
[2]Poliklinika "Vili", Zagreb, Croatia

Aim: To determine the immunostaining properties of squamous epithelial cells for p16 INK4 in cervical smears showing normal morphology, LSIL and HSIL, respectively.

Material and methods: 20 double cervical smears were analysed: the first was stained by Papanicolaou method and the second one by immunostaining kit for p16 (DakoCytomation, Denmark). They were taken from women with previous (according to the 2001Bethesda System) normal findings (5), LSIL(5) and HSIL(10) with positive immunotyping for HR-HPV). These findings were confirmed on Papanicolaou stained smears.

Results: 5/20 smears with normal morphological findings (aside for the mild inflammatory changes) stained negatively for p16. Five smears (5/20) showing LSIL features (even with obvious koilocytes) also showed no immunoreaction. Out of 10 smears showing HSIL features, 8 stained positively for p16. In one of the negative cases, the diagnosed HR HPV was type 31, and in the other, the exact type was not known. In all positive cases the staining was both intranuclear and intracytoplasmic, in roughly 10% of dysplastic cells.

Conclusion: p16 INK4 protein showed to be a useful immunocytochemical marker for dysplastic cervical cells in women at risk of recurrence or further progression of the disease, because it proves the increased HR viral oncogene expression in dysplastic cervical epithelial cells. The preliminary results of this study show that the routine use of immunocytochemical staining in high risk patients can help to distinguish those that are in greater danger of SIL progress and malignant transformation, enabling the clinician to control them more frequently and more carefully than patients not belonging to this group.

Pathology and Forensic Medicine / Oral presentations / Gynecological Pathology and Uropathology / 13

SARCOMA BOTRYOIDES OF THE UTERINE CERVIX IN YOUNG WOMAN

Dasović-Knežević M, Šikić-Lisica N, Dukić B, Hoti-Matešić M.

Department of Pathology and Forensic Medicine, General Hospital, Zadar, Croatia
Department of gineacology, General hospital, Zadar, Croatia

Embryonic rhabdomyosarcoma (RMS), i.e. sarcoma botryoides is a rare malignant mesenchymal neoplasm which originates in the uterine cervix. It is described a case of RMS of 29 years old woman who complains on vaginal bleeding after sexual intercourse. The tumour appeared as a cervical polyp which measured 1cm in diameter. It was smooth, glistening and white. Histologicaly was composed of primitive small spindle basophilic cells with hyperchromatic nuclei and scanty cytoplasm, laying beneath the surface epithelium and encicircling the endocervical glands. Subepithelial condesation of tumour cells was well well developed as a zone of loose stroma as a "cambium layer". There were found a small number of abnormal mitosis and vascular invasion. Immunohistochemical analysis revealed Vimentin, Desmin and SMA strong positivity of tumor cells. Myo D activity was found in rhabdomyoblastic multinuclear poliploid cells too. NSE, S100 and GFAP were positive in tumour cells too. P53 and Ki 67 immunoreactivity was negative. She was treated conservatively with total hysterectomy. The postoperative chemotherapy and radiotherapy are not administrated. The patient is in good condition for three months after gynaecological operation. The main differential problem was to make the distinction towards MPNST and malignant mixed mesoderm tumor.The aim of the case is to stress morphological characteristics of the tumor towards an inflammatory polyp.

Pathology and Forensic Medicine / Oral presentations / Gynecological Pathology and Uropathology / 14

IS QUADRANT BIOPSY ENOUGH IN MEN WITH ADVANCED PROSTATE CANCER?

Brnić Z[1], Gašparov S[2], Džebro S[2], Dominis M[2], Vidas Ž[3], Kučan D.[3]

[1]University Hospital "Merkur", Department of Diagnostic and Interventional Radiology, Zagreb, Croatia
[2]University Hospital "Merkur", Department of Pathology, Zagreb, Croatia
[3]University Hospital "Merkur", Department of Urology, Zagreb, Croatia

We hypothesized that quadrant prostate biopsy (QPB) provides sufficient first-line pathological evaluation of patients with advanced prostate cancer (PC). We studied 84 men which underwent PB, classified in two groups: "H" (high-) and "L" (low likely to have advanced PC). Pathological results of 5-12 cores PB were retrospectively compared with the results of simulated QPB for the presence of PC, PC volume, Gleason score (GS) and the presence of high-grade prostatic intraepithelial neoplasia (HGPIN). PC detection rate was not impaired in H- but dropped significantly in L-group, percentage of positive cores was not significantly changed in H- ($p=0.39$), but was decreased in L-group ($p=0.04$) due to sampling scheme reduction. No HGPIN was missed with QBP in H-group, while 2 HGPIN were missed in L-group. No significant GS change resulted in both groups ($p=0.12$, $p=0.13$) due to reduction to QPB. We conclude that QPB is appropriate first-line scheme in patients likely to have advanced PC as the information lost due to core number reduction is mainly not critical for patient management.

Pathology and Forensic Medicine / Oral presentations / Gynecological Pathology and Uropathology / 15

PERIACINAR RETRACTION CLEFTING IN THE DIAGNOSIS OF PROSTATIC ADENOCARCINOMA

Krušlin B, Tomas D, Čupić H, Odak Lj, Kos M, Belicza M.

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

Aim: Our previous investigation confirmed that periacinar retraction clefts are more frequen tand prominent around neoplastic acini compared to normal glands. The goal of this study was to analyze the presence and extent of retraction clefting in neoplastic glands and glands with prostatic intraepithelial neoplasia (PIN) in needle core biopsies.

Methods: Ninety-three cases with prostatic carcinoma and 28 patients with PIN diagnosed in the year 2004 were chosen for the study. Clefting in neoplastic specimens were analyzed on 10 neoplastic glands in three different high power fields (HPF). Neoplastic glands were classified in three groups; group I with no clefting or no clefts which affected more than 50% of gland circumference, group II with up to 50% of glands showing clefting that affected more than 50% of circumference and group III with retraction clefting affecting more than 50% of circumference in 50% or more glands. Glands with PIN were also analyzed on HPF and classified in three groups: group I with no clefting, group II with clefting, which affected up to 50% of gland circumference and group III glands with clefts, which affected more than 50% of gland circumference.

Results: There were 5 (17.9%) PIN cases in group I, 23 (82.1%) in group II, and non in group III. Twelve (12.9%) neoplastic cases were in group I, 51 (54.8%) in group II, and 30 (32.3%) in group III.

Conclusion: Retraction clefting is significantly more frequent in prostatic carcinoma than PIN suggesting that the clefts could represent reliable diagnostic criterion.

Pathology and Forensic Medicine / Oral presentations / Gynecological Pathology and Uropathology / 16

INTRA-ABDOMINAL OVARIAN-TYPE MUCINOUS CYSTADENOMA ASSOCIATED WITHFALLOPIAN TUBE-LIKE STRUCTURE AND ABERRANT EPIDIDYMAL TISSUE IN A MALE

Rizzardi C, Brollo A, Melato M.

Unit of Pathology of the University of Trieste, Trieste, Italy (RC and MM); Unit of Pathology of the ASS n.1 "Isontina", Gorizia, Italy (BA)

Ovarian-type mucinous tumors may occasionally occur in the retroperitoneum, pancreas and liver, exclusively or almost exclusively in women. In males, only few cases of such neoplasms arising within or around the testis have been reported.We describe a unique case of an ovarian-type mucinous cystadenoma occurring in the peritoneal cavity of a 65-year-old man patient with secondary adrenocortical insufficiency and hypogonadism. Enclosed in the capsule of the tumor there was a typical fallopian tube. Contiguous to that, accessory ectopic epididymal tissue was found. We have interpreted this case as the result of a minor disorder of embryonic development involving structures of both mullerian and wolffian origin. The disproportion of sexual hormones might have had a role in inducing intra-abdominal mullerian remnants to give rise to the mucinous cystadenoma.

Pathology and Forensic Medicine / Oral presentations / Forensic Medicine / 17

THE ETIOLOGY OF SUDDEM DEATH DURING THE PERIOD FROM 2000 TO 2005 FOUND IN CORONERS AUTOPSIES

Pešutić-Pisac V, Aljinović J.

Clinical Hospital Split, Department of Pathology, cythology and Forensic Medicine, Split, Croatia

Aim: The aim of this research was to show etiology of sudden death during the period from 1.1.2000 to 1.1.2005. We wanted to establish correlation between age, sex and occurance of sudden death, as well as, to determine where the death occured and in which month of the year it was the most frequent.

Methods: In this research we hawe analysed data from 160 autopsies of people who died of sudden death in the region of middle Dalmatia. All the autopsies were performed at Department of Pathology, Cythology and Forensic Medicine in Clinical Hospital Split.

Results: Sudden death incidence inclines in last five years. It occured more frequently in males (65%). Incidence is at peak in age between 61 and 70, and in seventh and ninth month of the year. Cardiovascular diseases were the most common causes (59%). Non-cardiac causes were dominated by pulmonary (14%), and neurological (8,1%) diseases. Acute myocardial infarction was the most common post mortem diagnosis. Most people died at their home, and 74,3% had no data of their medical history.

Conclusion: Since the cardiovascular diseases are the most common causes of sudden death, by eliminating the risk factors we could prevent ocurance of sudden death in some cases.

Pathology and Forensic Medicine / Oral presentations / Forensic Medicine / 18

TRAFFIC ACCIDENTS WITH FATALLY INJURED DRIVERS IN PRIMORSKO-GORANSKA COUNTY, CROATIA (1994-2004)

Bosnar A, Čoklo M, Stemberga V, Cuculić D, Cucić T.

Department of Forensic Medicine, Rijeka University School of Medicine, Rijeka, Croatia

Traffic accidents represent a significant cause of death in Croatia, therefore being an important social and public-health problem. More people died in traffic than in the War, in Croatia during the '90-s. The aim of our study was to analyse the characteristics of traffic accidents with fatally injured drivers in Primorsko-goranska county, Croatia, in a 10 year period (between 20. August 1994. and 19. August 2004.). Study population included 277 fatally injured drivers with an autopsy or body examination performed at the Rijeka Institute of Forensic Medicine. Age, gender, level of alcohol intoxication and the cause of death of the drivers were analysed, together with month, day of the weak, time when the accident happened and the type of vehicle involved.

Pathology and Forensic Medicine / Oral presentations / Forensic Medicine / 19

MINORS AS VICTIMS IN 1991-1995 WAR IN CROATIA

Petrovečki V, Mayer D, Čadez J, Gusić S, Simac Kubat G[1], Strinović D.

Department of Forensic Medicine and Criminology, Medical School, University of Zagreb
[1]Outpatient clinic "Sunce" Zagreb, Croatia

Aim: This paper deals with minors which were fatally inflicted by war activities in Croatia.

Background: During military operations of 1991-1995 war in Croatia, certain number of war casualties was of childhood and adolescent age. This vulnerable population suffers different damages of war, including lethal consequence.

Subjects and methods: We reviewed autopsy records of all war victims processed at our Department between 1995 and 2004. Out of 2848 cases, in 1979 (70%) the identity was established. In that group of identified persons, there were 51 (4%) minors. Description of their forensic workup is presented.

Results: The age distribution of cases in our sample revealed the presence of 3 infants (age within 2 yrs), 2 preschool children (age 2-5 yrs), 5 children (age 6-12 yrs) and 41 adolescent (age 13-19). The bodies were mostly (42 cases) recovered from mass graves, while 9 bodies were buried individually. Majority of the bodies (46) were in considerably well preserved state in terms of skeleton integrity. Formation of adipocere was prominent in 15 cases, the others being completely or partly skeletonized. Regarding territorial distribution, bodies were predominantly exhumated in the region of Eastern Slavonia (41 cases), and the rest was exhumated in other regions of Croatia which were temporarily occupied by aggression in

1991. Most of the victims (47) parished in years 1991-1992. That time was initial and also the most extensive period of aggression. The evidence of antemortal trauma was notable in 47 cases. Forty-two individuals were fatally injured by firearms missiles. In two, death followed after explosive trauma. In the remaining 3 cases, the nature of the trauma was not specified in autopsy report.

Conclusion: In recent decades, the proportion of civilian casualties in armed conflicts has increased dramatically. Child war victims, as subpopulation of civilian victims, are the most tragic consequences of war. Despite the many international instruments on the rights of the child, children continue to suffer extreme consequences from the wars.

Pathology and Forensic Medicine / Oral presentations / Forensic Medicine / 20

IDENTIFICATION OF THE HUMAN REMAINS FROM THE PLANE CRASH

Kubat M[1], Furač I[1], Masić M[1], Jakovski Z[2], Nikolova K[2], Karija Vlahović M[1], Marketin S[1], Raguz I[1], Duma A[2], Škavić J[1].

[1]DNA Laboratory, Department of Forensic Medicine and Criminology, School of Medicine University of Zagreb, Zagreb, Croatia
[2]Institute for Forensic Medicine and Criminology, Medical Faculty, Skopje, Macedonia

In February 2004 there was a plane crash in the area of Mostar. Plane belonged to Macedonian government and Macedonian President Boris Trajkovski was on the board. The plane was heading from the Macedonian capital, Skopje, for the town of Mostar, where Mr. Trajkovski and the group of advisers were to attend an investment conference. The crash happened in the area of the mine fields, so the search took more time than expected. None of the eight people on board the plane survived the crash. Human remains were found two days after accident occurred. They were very badly burned, which made classical identification impossible. Samples from remains (tissue and bone) were taken for DNA analysis to lab. To determine identity of each body, blood samples from relatives of missing persons were taken. DNA from bones was isolated by organic extraction method and from tissue using QIAamp kit. DNA from blood stains was isolated with Chelex-100 reagent and QIAamp kit. PowerPlex 16 System, PowerPlex Y System and AmpFlSTR Identifiler kits were used for PCR amplification of STR loci.

Pathology and Forensic Medicine / Oral presentations / Forensic Medicine / 21

RESPONSIBILITIES AFTER A PATIENT'S DEATH

Marcikić M, Dumenčić B, Matuzalem E.

Medical Faculty, Department of Pathology and Forensic Medicine, Osijek, Croatia

We look at ethical and legal issues that arise after a patient has died. Aspect of the management of deceased patients generate frequent queries to the medical associations from doctors and medical students. Some of the most common questions are:

Who should certify and confirm death?
Which deaths should be referred to a medical examiner or investigative judge?
Who gives consent to a legally required postmortem investigation?
Who should be informed about retained human material?
Can members of the public attend an autopsy? How important is confidentiality after death?
Is there a role for minimally invasive autopsies?.

Discusion will be focused on the general ethical principles applicable to this sphere of practice that include:

- The duty to show respect for people, living and dead.
- The need to have clear and effective communication with people who were close to the deceased person.
- The obligation to offer relatives as much information as they need about any medical procedure.
- The duty to balance this openess with the duty of confidentiality owed to the dead patient. The responsibility of demonstrating cultural awareness and sensitivity in relation to the existence in the community of differing attitude to death.
- The duty to involve the public more in informed debate on matters pertaining to death and its management.
- The need to have concern for justice
- The duty to bear in mind the public good and to promote ethical ways of maximising knowledge.

PATOLOGIJA I SUDSKA MEDICINA
PATHOLOGY AND FORENSIC MEDICINE

Posteri / *Posters*

Hematopatologija / *Hematopathology (1-3)*

Dermopatologija i meka tkiva / *Skin and Soft Tissue Pathology (4-6)*

Patologija dojke i ginekološka patologija / *Breast Pathology and Gynecopathology (7-14)*

Gastrointestinalna patologija / *Gastrointestinal Pathology (15-18)*

Uropatologija / *Uropathology (19-26)*

Neuropatologija / *Neuropathology 27-31)*

Eksperimentalna patologija / *Investigative Pathology (32-36)*

Obdukcija i sudska medicina / *Authopsy and Forensic Medicine (37-44)*

Pathology and Forensic Medicine / **Posters** / **Hematopathology** / **01**

CASE REPORT: THYMOMA WITH PSEUDOSARCOMATOUS STROMA AND HODGKIN LYMPHOMA

Dotlić S, Nola M, Šćukanec-Špoljar M, Sičaja M, Križanac Š.

Department of Pathology, Clinical Hospital Center Zagreb, Zagreb, Croatia

Biphasic histological pattern is very rare in thymomas and is usually demonstrated in highly aggressive carcinosarcomas and sarcomatoid carcinomas. Only three papers have been published, describing an extremely rare histological subtype of low-grade thymic epithelial tumour, which defies categorizing into any of the histological types according to the current WHO classification.

A 58-year-old woman presented with a mediastinal mass on the chest roentgenogram. Grossly it was an encapsulated tumour with solid cut surface, measuring 5:5:2 cm and no signs of local invasion. Histologically, the tumor showed a biphasic pattern with solid epithelial and a spindle cell component. Immunohistochemical stains showed diffuse positivity for pancytokeratin and EMA within the round cell-epithelial component and spindle cell elements were positive for vimentin, with only focal immunoreactivity for keratin and EMA. Stains for actin, S-100 are CD 30 were negative. Electron microscopic examination confirmed a biphasic tumour with evidence of epithelial and neural differentiation.

After 8 months the patient presented with cervical lymphadenopathy. Microscopical examination of enlarged lymph nodes demonstrated Hodgkin lymphoma, nodular sclerosis type I with no evidence of bone marrow involvement. Follow up has been done for 2 years after the surgical excision of thymoma and the patient has been free of disease during that period.

Although no increased risk for malignancies following thymoma has been demonstrated, subsequent malignant tumours reported in these patients most frequently were non Hodgkin lymphomas of B-immunofenotype and digestive system cancers.

This is a first reported case of thymoma with pseudosarcomatous stroma with concomitant

Hodgkin lymphoma, suggesting a benign nature of this rare thymic neoplasm.

Pathology and Forensic Medicine / **Posters** / **Hematopathology** / **02**

POST-TRANSPLANT LYMPHOPROLIFERATIVE DISEASE (PTLD) - CASE REPORT

Gašparov S[1], Džebro S[1], Korać P[1], Šitić S[1], Borovečki A[1], Kušec R[2], Dominis M.[1]

[1]Department of Clinical Pathology and Cytology
[2]Department of Internal Medicine, Merkur University Hospital, Zagreb, Croatia

Post-transplant lymphoproliferative disease (PTLD) is a form of lymphoproliferation wich follows solid organ or bone marrow transplantation. This disorder develops as a consequence of immunosuppresion and the majority of PTLD are associated with EBV infection (60-80%). The incidence of PTLD for liver transplant recipiens is 2-4%.

The 61 years old male was transplanted due to cirrhosis of unknown ethiology. After the transplantation the patient was treated with immunosupressive therapy-cyclosporine, but he subsequently developed diabetes. Seven months after the transplantation he was admitted to the hospital due to painfull sensations in the upper abdomen. Clinical examination, including ultrasound and CT scanning detected a solitary tumour in the right upper and middle abdomen largest diameter 5,5 cm. Laparotomy was performed and the tumour was located in the mesenterial radix of the small intestine. Since the tumour was inoperabile, two surgical specimens were taken. Histologically, larger necrotic and solid areas were found. The morphological features of solid part of the tumour consist of full range of B-cell maturation, from immunoblasts to plasma cells, with small and medium-sized lymphocytes with irregular nuclei, resembling centrocytes, CD20 and CD 79a positive. In situ hibridisation

(DAKO, K 5201) detected nuclear EBV expression and PCR method confirmed clonal B lymphocyte population. Bone marrow biopsy was in normal range. Diminishing the dose of immunosuppresive therapy did not result with the tumour regression, therefore, the treatment was continued only with immunotherapy (Rituximab).

Pathology and Forensic Medicine / Posters / Hematopathology / 03

THE API2-MALT1 FUSION PROTEIN AS INDUCER OF AN INDEPENDANT MECHANISM IN PATHOGENESIS OF GASTRIC MALT TYPE LYMPHOMA - CASE REPORT

Borovečki A[1], Korać P[1], Gašparov S[1], Katičić M[2], Džebro S[1], Dominis M.1

[1]Department of Clinical Pathology and Cytology
[2]Department of Internal Medicine, Merkur University Hospital, Zagreb, Croatia

A 46 year old male patient was observed during 29 months due to H. pylori infection and MALT lymphoma. During this period he become H. pylori negative, clinical and endoscope findings regressed almost normal, however morphological features of MALT type lymphoma persist. Molecular analysis of IgH CDR3 region did not confirm clonal rearrangement. All samples were Bcl10 negative, while FISH detected translocation t(11;18)(q21;q21) but not the translocation t(14;18)(q32;q21). Pathogenesis of GI MALT type lymphoma is connected to three specific translocations: t(11;18)(q21;q21) results in a chimeric API2-MALT1 gene fusion, t(1;14)(p22;q32) juxtaposes Bcl10 to an IgH gene locus and t(14;18)(q32;q21) includes MALT1 and IgH gene. These translocations are, on molecular level, the part of NF?B activation through physiological role of Bcl10 and MALT1 genes. The Bcl10/MALT1 complex represents the most frequent mechanism for NF6B activation and is considered responsible for development of MALT type lymphoma. Alternative mechanism is believed to be Bcl10 independent and induced by API2-MALT1 fusion protein. Although t(11;18)(q21;q21) is often accompanied by nuclear Bcl10 expression, the results of our case indicate the alternative pathway of NF6B activation.

Pathology and Forensic Medicine / Posters / Skin and Soft Tissue Pathology / 04

PLEOMORPHIC LIPOSARCOMA OF THE FOOT: A CASE REPORT

Jakovčević A, Brčić L, Batelja L, Orlić D[1], Seiwerth S.
Institute of Pathology, Medical Faculty, University of Zagreb, Croatia
[1]Department of Orthopedics, Medical Faculty, University of Zagreb, Croatia

Liposarcomas are among the most common soft tissue sarcomas of adult life. They often appear in lower parts of extremities and retroperitoneal, but are rarely found in the foot or ankle.

Liposarcomas clinically presents as palpable, poorly defined, slow growing tumor mass. Their morphologic hallmarks are lipoblasts. Immunohistochemicaly those cells are positive for S-100 protein.

Pleomorphic liposarcoma is rarely found in the foot or ankle. Only 2,7% of all liposarcomas occur in the foot. To our knowledge only one case of pleomorphic liposarcoma of the foot is reported (Nishimoto et al.). Of 16 reported cases of liposarcoma, the most common type was myxoid (6 cases), not reported histological type (8 cases), well differentiated (1 case), and poorly differentiated (1 case).

We present a case of a pleomorphic liposarcoma of the foot in a 71-year old female. She presented with a large mass on the dorsum of her right foot that had been gradually increasing in size over the past months, after a reported injury two years before. On physical examination firm, palpable tumor mass was 6-7 cm in diameter, poorly-defined from surrounding tissue and attached to underlying structures and skin. The biopsy specimen revealed bundles of tumor cells with pleomorphic, bizarre nuclei showing cytoplasmic vacuoles and scattered lipoblasts. Mitotic figures were frequent. Immunohistochemicaly tumor cells were positive for vimentin and S-100 protein, negative for desmin, myoglobulin and smooth-muscle actin. These findings indicated that the tumor was a pleomorphic liposarcoma. The surgeon performed below the knee amputation.

Pathology and Forensic Medicine / Posters / Skin and Soft Tissue Pathology / 05

SUBCUTANEOUS DIROFILARIASIS IN DALMATIAN REGION OF CROATIA

Bezić J, Kuzmić-Prusac I, Vrbičić B[1], Njirić Z[2], Puizina-Ivić N[3]

Department of Pathology, Clinical Hospital Split, Split, Croatia
[1]Department of Pathology, Regional Hospital Šibenik, Šibenik, Croatia
[2]Department of Pathology, Regional Hospital Dubrovnik, Dubrovnik, Croatia
[3]Department of Dermatovenerology, Clinical Hospital Split, Split, Croatia

The zoonotic filariae in humans are found most commonly in the subcutaneous tissue, and the vast majority of them belongs to the genus Dirofilaria. The new cases of human dirofilariasis due to Dirofilaria repens have been increasingly reported in past few years, and this zoonosis has become the new emerging zoonosis in Mediterranean parts of Europe. Dirofilaria repens is habitually parasite of dogs, cats, and wild carnivores, transmitted by several species of mosquitoes. The nematodes penetrate the body of the host in the form of infecting filariform larvae, following a bite by an infected mosquito. In the human tissues microfilariae die before maturation probably due to the immunological rejection, with the inflammatory reaction in the surrounding tissue induced by the dead worm. In the majority of the human cases the nodules occurred singly in subcutaneous tissue of the upper half of the body, or subconjuctivaly. We present here the five cases of human Dirofilaria repens subcutaneous nodules discovered in Dalmatian region of Croatia, with the emphasis on the pathohistological characteristics of parasite and host reactions. It is important that histopathologists familiarize themselves with the histological aspects of Dirofilaria repens infestation, taking it in differential diagnosis during examination of solitary nodules of uncertain nature in the subcutaneous tissue.

Pathology and Forensic Medicine / Posters / Skin and Soft Tissue Pathology / 06

EPIDEMIOLOGY OF HEAD AND NECK MELANOMA IN RIJEKA AREA, CROATIA, 1999-2003

Zamolo G[1], Čoklo M[1], Štemberger C[1], Dekanić A[1], Batinac T[2], Bosnar A[3], Grgurević E.[4]

[1]Department of Pathology, Rijeka University School of Medicine, Rijeka, Croatia
[2]Department of Dermatovenerology, Rijeka University Hospital, Rijeka, Croatia
[3]Department of Forensic Medicine, Rijeka University School of Medicine, Rijeka, Croatia,
[4] Institute of Public Health, Rijeka, Croatia

We made a retrospective study on various epidemiological features of all patients with primary cutaneous melanoma diagnosed at the Departments of Pathology and Dermatology, University Hospital Rijeka, Croatia, during the 1999-2003. period, focusing on the head and neck localized melanomas.The study included only the cases of CM in which clinical diagnosis was confirmed histopathologically. During this period 318 cases of CM were diagnosed in Rijeka district. 140(44%) were males and 178 (56%) were females. The average age of the patients was 55.83 (1999: 58.9; 2003: 56.9). The average time period between the patients observed the suspectable change to diagnosis was 10 months. 45 out of total 318 melanomas (14.2%) were localized in the head and neck region. Out of that, 27 (60%) were males and 18(40%) were females. The average age at the moment of diagnosis was 66.7. In 1999. 82% of the head and neck melanomas were diagnosed as nodular and 18% as lentigo maligna, while in 2003. only 17% were nodular, 50% were diagnosed as lentigo maligna and 33% as melanoma in situ. As for the Breslow, in 1999. 18% were < 1.5 mm (B1) and 45% >4 mm(B5) and in 2003: 58% were < 1.5 mm(B1) and 0%>4 mm(B5). The increased incidence, of both head and neck and total cutaneous melanoma is observed during the last five years, but in the "head and neck" group the stage was earlier, with thinner Breslow, probably due to more visible localization and increased patient awareness of the risk factors involved, most importantly ultraviolet radiation.

Pathology and Forensic Medicine / Posters / Breast Pathology and Gynecopathology/ 07

PATOHISTOLOGIC CHARACTERISTICS AND SIZE OF URGICALY REMOVED BREAST LESIONS IN ZADAR GENERAL HOSPITAL 2002-2004.

Krvavica A, Vukelić-Baturić[1], Lozo P[1], Bačić I[2], Bubičić J.[2]

General Hospital, Department of Pathology and Forensic Medicine, Zadar, Croatia
[1]Health Centre "Lozo", Zadar, Croatia
General Hospital, Department of Thoracal Surgery, Zadar, Croatia

Aim: To show that high resolution Ultrasonography can be very useful in the differential diagnosis between benign and malignant lesions of the breast, to investigate occurrence and patohistologic characteristics carcinomas in our material from 2002-2004, to specificate and descript ultrasonographic characteristics of little carcinomas, because there are no relevant literature report nor accepted criteria for distinction them from benign lesions, to invent the possibly correlation between tumor size with patohistologic characteristics carcinomas in our material, as though ultrasonographic depiction.

Material and method: 332 cases of surgicaly removed breast lesions with complete medical documentation were retrospective examinated by pathologist, gynaecologist (who had a practice in breast ultrasonography for seven years) and surgeons. From medical reports we extracted currently repeated ultrasonographic features for certainly benign and malignant breast lesions and compared them with patohistologic caracteristics.

Results: Among the 332 cases, 189 were malignant, 143 benign (mastopathia fibrosa cystica, fibroadenoma, papilloma, abscessus). Mostly patients with malignant lesions were womens in the age 30-84 (median 57 years); and 4 male in the age 54-71 years (median 62).

7/189 malignant lesions were in 0 stage (3,7%), 61/189 in the stage I (32,27%), 84/189 in the stage II (44,44%), 32/189 stage III (16,93%), and stage IV 5/189 (2,65%).

Conclusions: Because of relative little number of carcinomas which were diagnosting in stages 0 and I (35,97%) in front of 64,02% in the invasive stages II-IV showed that we must doing more on motivation womens for screening.

We are expressing agreement with Croatian Senology Program for screening and prevention and trying to iniciate it in Zadar County with disposable medical experts and tehnical capacities.

Pathology and Forensic Medicine / Posters / Breast Pathology and Gynecopathology/ 08

A COMBINED HISTOLOGICAL AND IMMUNOHISTOCHEMICAL APPROACH IN EVALUATION OF DISCORDANT RECEPTOR BREAST CARCINOMAS

Mustać E, Zamolo G, Petković M[1], Đorđević G, Čoklo M, Modrčin D, Žauhar G[2], Batinac T, Sonnenschein I, Mokrović H, Jonjić N, Dobrila F.[3]

Department of Pathology, Rijeka University School of Medicine, Rijeka, Croatia
[1]Department of Radiotherapy and Oncology, Rijeka University Hospital, Rijeka, Croatia
[2]Department of Computer Science, Rijeka University School of Medicine, Rijeka, Croatia
[3]Clinic of Surgery, Clinical Hospital Center Rijeka, Rijeka, Croatia

Aim: To reconsider discordant receptor breast carcinomas with probably dependent (ER+PR- or ER-PR+ subgroup profile) hormonal status in ductal invasive breast carcinomas not otherwise specified (CDI NOS).

Methods: For the present study 90 cases were considered and grouped into three categories according to hormonal status: dependent (D) (ER+PR+), probably dependent

(PD) (ER+PR- or ER-PR+) and not dependent (ND) (ER-PR-), evaluated and compared with some established prognostic parameters in breast carcinomas.

Results: There was statistically significant difference between tumor receptor status distribution and menopause (p=0,0235), age of the patients (p=0,000467), histologic grade

(p=0,000003), vascular invasion (p=0,006), HER-2 status (p=0,0039) and Ki-67 proliferation rate (p=0,000311). D tumors were found exclusivelly at postmenopausal patients, average age 68,9 years, mostly (63,3%) had intermediate (II) grade, with no vascular invasion, HER-

2 status score was predominantly 0 and 1+ with lower Ki-67 proliferation rate. PD tumors were found predominantly at younger postmenopausal patients, average age was lower,

57,5 years, vascular invasion was assesed at 23% of cases. ND tumors mostly (60%) had higher histologic grade, showed the highest percentage of the Ki-67 positive tumor cells and vascular invasion in 30% of cases.

Conclusions: The patients with PD breast carcinomas were younger postmenopausal women with the tumors moderately differentiated, HER-2 score 0 and 1+, with lower Ki-67 proliferation rate.

Pathology and Forensic Medicine / Posters / Breast Pathology and Gynecopathology/ 09

PREDICTING SENTINEL LYMPH NODE METASTASES IN INFILTRATING BREAST CARCINOMA WITH VASCULAR INVASION

Jonjić N, Mustać E, Dekanić A, Marijić B, Gašpar B, Kolić I, Čoklo M.

Department of Pathology, School of Medicine, University of Rijeka, Croatia

Aim: To compare the clinical and pathologic characteristics of women with positive and negative sentinel lymph nodes (SLN) in order to identify features predictive for SLN metastasis.

Patients and methods: Hundred patients with breast cancer with T1 and T2 stage and clinically negative axillary's nodes were preceded for SLN analysis. One hundred and fifty-four SLN was localized using 99Tc sulfur colloid. Protocol which included analyzing of routine hemalaun eosin (HE) and immunostained sections with cytokeratin (CK) was used. Vascular invasion was evaluated with immunostained sections with anti-CD31. Hormonal status was determined by immunohistochemistry using LSAB system and HER-2/neu by Hercep Test (Dako, Glostrup, Denmark). All mentioned parameters as well as known clinicopathological characteristics of breast cancer such as tumor size, histological and nuclear grade were compared with SLN status.

Results: Positive SLNs were found in 29% of patients with pT1 and pT2 breast cancer. Nineteen (19/29) had metastases detected by HE staining and ten (10/29) had micrometastases detected by immunohistochemistry (IHC) using CK antibodies. Nine (9/29) metastases in SLN were confirmed on frozen sections. Positive SLN were present in larger carcinomas ($p<0.03$) with positive PR status ($p<0.037$) and evident vascular invasion ($p<0.002$). Moreover, vascular invasion was associated with breast cancer of higher histological ($p=0.011$) and nuclear grade ($p=0.039$).

Conclusion: Tumor size and the presence of vascular invasion is a significant predictor of pathologically positive SLN in T1 and T2.

Pathology and Forensic Medicine / Posters / Breast Pathology and Gynecopathology/ 10

CARCINOMA OF SURFACE EPITHELIUM OF THE OVARY IN PATIENTS OPERATED IN THE CLINICAL HOSPITAL SPLIT IN TEN YEAR PERIOD FROM 1989.-1999.

Tot T, Tomić S.[1]

Medical University Split, Split, Croatia
[1]Department of Pathology, Clinical Hospital Split, Split, Croatia

Aim: To determine most common clinical signs of presentation, stage of disease in time of diagnosis and rate of positive family history in ovarian cancer patients operated in ten year period in Clinical Hospital Split.

Methods: Data from medical histories of 215 patients operated in Department of Gynecology and treated in Department of oncology were analyzed. We analyzed age of patients, first symptoms of presentation, positive family history, and clinical stage in time of diagnosis.

Results: In time of diagnosis 60,5% patients were in advanced clinical stage (FIGO stage III and IV). Most patients were postmenopausal and older than 50 year (55%). Although 15,4% of patients were without any symptoms, and disease was discovered accidentally, largest number of patients had symptoms due to the presence of tumor mass such as: abdominal pain (27,5%) and enlargement of the abdomen (31,2%). Ascites was found in 71% of patients. First degree relatives with breast and/or ovarian cancer had 7% of patients.

Conclusion: Ovarian cancer tends to present mostly in postmenopausal woman in insidious manner with symptoms produced by presence of tumor mass or ascites. Early symptoms were subtle and easily confused with various benign entities. Lack of specific symptoms is reason why majority of patients present when cancer is in advanced stage of disease with unfavorable outcome.Althought genetic susceptibility has not been so extensively studied as in breast cancer like in another studies we found familiar clustering.

Pathology and Forensic Medicine / Posters / Breast Pathology and Gynecopathology/ 11

CASE REPORT: SMALL CELL UNDIFERENTIATED CARCINOMA OF THE OVARY - HYPERCALCAEMIC TYPE.

Jandrić D, Petrović Z, Stanić G, Babić D, Ilić-Forko J, Jukić S, Stilinović K, Sparac V.

Genaral Hospital " Sveti Duh ", Department of Pathology, Zagreb, Croatia
Clinical Hospital for Gynaecology and Births, Department of Pathology, Zagreb, Croatia
Genaral Hospital "Sveti Duh", Clinic of Gynecology and Obstetrics, Zagreb, Croatia

20 years old woman, gravida 0, presented to the physician for lower abdominal pain radiating to the left. The pelvic and ultrasound exam revealed tumor in the position of the left ovary and laparotomic surgery was performed. On surgery, the tumor of the left ovary was found, uterus, omentum and the right ovary were normal. The tumor measured 100 x 60 mm, with smooth outer capsule, on cut surface it was solid, with some cystic spaces, moderatly soft and gray-yellow, with some haemorrhage. Frossen sections diagnose on ex tempore was possible granulosa cell tumor, could not rule out carcinoma. On parafin embeded HE sections tumor was composed of small cells with little citoplasm lying in sheets. The nuclei were round and polygonal with some clumping of chromatin and a small nucleolus. Mitoses were also found. Imunohistochemistry showed tumor cells positive for Vimentin, focaly positive for Citokeratin and 70% positive for EMA, and negative for Inhibin, Synaptophisin and Chromogranin.

Cytology revealed malignant cells in the abdominal washings.

Small cell carcinoma of the ovary generaly behaves in a very aggressive manner, most of the patients die within a year. Our patient undervent 3 cycles of chemotherapy, and 3 months after the diagnose presented with enlarged left paraaortal lymph nodes and cystic tumor of the other ovary.

Pathology and Forensic Medicine / Posters / Breast Pathology and Gynecopathology/ 12

UTERINE TUMOUR RESEMBLING OVARIAN SEX-CORD TUMOURS

Šitić S[1], Džebro S[1], Borovečki A[1], Zovko G[2], Gašparov S.[1]

[1]Departement of Clinical Pathology and Cytology
[2]University Clinic for Gynecology&obstetrics, Department of Gynecological Oncology, University Hospital Merkur, Zagreb, Croatia

Uterine tumours resembling ovarian sex-cord tumours were first described by Clement and Scully in 1976 who classified the neoplasms into two groups. Group I tumours chiefly comprise typical endometrial stromal neoplasms but with focal areas resembling ovarian sex cord elements. Group II tumours are composed predominantly or exclusively of sex cord like elements. Only 55 cases have been reported so far.

A 76-year-old woman was admitted to the hospital due to vaginal bleeding. Total abdominal hysterectomy and bilateral salpingo-oophorectomy were performed. The uterus measurred

14:10:7 cm. A fairly well-circumscribed, yellow, firm tumour, measuring 7,5 cm was found in the myometrium partially protruding in the cavum of the uterus. The tumour exhibited predominantely patterns of sex-cord-like elements. The tumour cells formed anastomosing trabeculae and tubular formations with scant stromal elements among them. There were no mitotic activities. The tumour cells were negative for CD117, CD34, S100, CK-AE1/AE3, EMA and positive for vimentin, CD10, CD99 and SMA.

According to the morphollogy and immunohistochemistry we diagnosed the tumour as Clement and Scully's group II UTROSCT.

Pathology and Forensic Medicine / Posters / Breast Pathology and Gynecopathology/ 13

PRESENCE OF HIGH RISK HUMAN PAPILOMAVIRUSES (HPV) IN THE LOW GRADE CERVICAL LESION

Iljazović E, Mustedanagić-Mujanović J, Čičkušić E, Karasalihović Z, Avdić S.

Policlinic for Microbiology, Pathology, Immunology and Molecular Medicine, University Clinical Center Tuzla, Bosnia and Herzegovina.

Low grade squamous intraepithelial lesion (LGSIL) and atypical squamous cells undetermined significance (ASCUS) are the most frequent verified cellular abnormalities. Their menagement are still highly controverse mostly caused by uncertainty about their histology and nature of originate.

Detection of HPV DNA in the absence of cytological abnormalities can also indicate presence of high-grade cervical intraepithelial neoplasia (CIN). The aim of this study was to show the association of the benigne cellular changes, ASCUS and LGSIL with oncogenic types of HPV and to prove the necesity of more intensiv screening of this group of patients. Citology and pathomorphology analises were performed first. Identification of the presence of human papiloma virus was carried out by the Digene Hybride Capture II test for all patients. Identification of different HPV types for the particular number of patients was carried out by RFLP (Rsetriction Fragments Length of Polymorphism). Out of the 101 patients in the first group 92 (91,08%) were HPV positive, and 41, 58% had no cellular abnormalities, ASCUS or LGSIL. Out of 509 patinets of the second group 26,92% were positive for HRHPV, and 78,97% of them had no cellular abnormalities, ASCUS or LGSIL.

Conclusion: The combination of smears with the detection of high risk HPV types increases the triage sensitivity before colposcopy to the detriment of specificity and predictive value, and, therefore, of cost.

Pathology and Forensic Medicine / Posters / Breast Pathology and Gynecopathology/ 14

CARCINOSARCOMA UTERI: REPORT OF THE TWO CASES IN TWO DIFFERENT STAGES OF DISEASE

Dasović-Knežević M, Dukić B.

Department of Pathology and Forensic Medicine, General Hospital, Zadar, Croatia
Department of Gineacology, General Hospital, Zadar, Croatia

Carcinosarcomas (CS) of the uterus are mixed epithelial-nonepithelial tumours which appear in less then 5% of all cancers of the uterine corpus. They are highly aggressive and make very early lymphatic and hematogeneous distant metastases. The patient favourable outcome and overall survival depends on early tumour stage, low myometrial invasion and late oneset of the menopause. Here are described two uterine CS-as in 59 and 72 year old menopausal women. Grossly, the both were polypoid tumours which infiltrated deeply the myometrium and uterine cervix.Hystologicaly are composed of spindle cell component with admixed endometroid cancer type of tumour. Immunohistochemically, tumour cells were positive with the skeletal muscle markers, SMA and Myo D. The epithelial markers, MNF 116 and EMA were positive in the spindle cell component of the tumour, too. Epithelial component of tumour showed strong MNF 116 and EMA positivity. CD 117 (c-kit) was strongly positive in the younger womans tumour while the other tumour of the older woman was negative. The metastases in regional lymph nodes were of epithelial origin of the younger and myxed type in the older one. One year after initial operation the CS of the older woman metastased to abdominal cavity as a large tumour mass that measured 30 cm in long diametar.The radio and chemotherapy was administrated to the both of patients. They are still alive and in almost good condition. It is stressed the possible administration of inhibitors of the c-kit tyrosinase in c-kit positive tumours, evan some recent studies showed that survival was not significantly better in c-kit positive than that of c-kit negative cases.

Pathology and Forensic Medicine / Posters / Gastrointestinal Pathology / 15

AGE AND SEX DISTRIBUTION AND LOCALIZATION OF COLORECTAL CANCER IN HOSPITAL CANCER REGISTRY (1989-2004)

Baličević D, Brezovečki-Biđin D, Tomas D, Leniček T, Rajačić N[1], Rožanković V.[1]

Ljudevit Jurak University Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia
[1]Medical School, University of Zagreb, Croatia

Aim: Colorectal cancer generally shows left-sided predominance, but recent epidemiological studies found an increase in the incidence of right-sided cancers, which might have clinical implication for the use of diagnostic or screening tools. The aim of study was to examine this trend of colorectal cancer in our database during a 16-year period.

Material and methods: We analysed 3075 patients diagnosed with colorectal cancer during the period 1989-2004. Patients were assessed according to age, sex and localisation of tumor.

Results: From a total 3075 patient, 57.2% were male and 42.8 % female. A trend of increase in the male sex was observed. In both sexes the average age of patients increased from 63.5 years at the start of the studied period to 67.0 years at the end. The mean age of patients with right-sided carcinoma was slightly higher (66.3) than in patients with left-sided cancer (65.3). When observing the distribution of cancer, the most common localisation was the recto-sigmoid (77.3%), folloved by ascending colon with 7.1%, cecum and transverse colon with 6.6% and 6.1 % and finally descending colon with only 3%. We found a definite increase in the trend of right-sided colorectal cancer (from 16% to 22%) in both sexes, especially during the last 6 years.

Conclusion: Our study shows a male predominance in patients with colorectal cancer and an increase of average age in both sexes. Right-sided cancer is more frequently now than in the past that corresponds to studies done in other western European countries.

Pathology and Forensic Medicine / **Posters** / **Gastrointestinal Pathology** / **16**

MORPHOLOGIC-CLINICAL ASPECTS OF CONGENITAL EPULIS - CASE REPORT

Horvatić E, Krapinec S, Bardek M, Gabaj M, Odobašić Ž, Vondraček N.

"Dr. T. Bardek" General Hospital, Koprivnica, Croatia

Case report of congenital epulis from morphological and clinical point of view with short case history of pregnancy, clinical features with macroscopic and microscopic views of tumor. Congenital epulis is unique and rare tumor that occurs on anterior alveolar mucosal ridges of newborn. The real nature of this tumor is not known. It is also known as congenital gingival granular cell tumor, congenital granular cell epulis, and congenital epulis of the newborn. Methods: The lesion was cut to several thick sections for macroscopic examination. Specimens were routinely stained with hematoxylin and eosin and, where appropriate, were taken for immunohistochemical investigation using S-100 protein, desmin, vimentin, NSE etc.

Results: Microscopic examination showed nests of granular histiocyte-like cells with oval and small nuclei. Cytoplasm was eosinophilic. Tumor cells were rounded and spindle. In colagenic tissue were polymorphic and mostly congestive vascular channels with thick walls and hemosiderin matrix. The overlying epithelium was atrophic.

Conclusion: Congenital epulis is a rare tumor of the newborn. To date, 217 cases have been described in the literature. To diagnose this tumor the histopathology verification with usual histochemical and immunohistochemical investigations is necessary.

Pathology and Forensic Medicine / Posters / Gastrointestinal Pathology / 17

ACINIC CELL CARCINOMA-CASE REPORT

Fuštar-Preradović Lj, Đanić D, Jakovina T, Jakovina K.

Department of Pathology and Cytology, Department of Otorinolaryngology and Cervicifacial Surgery, General Hospital Slavonski Brod, Croatia

Acinic cell carcinomas account for only 2-3% of salivary gland tumors and 90% of them are located in the parotids. They are sometimes bilateral or multicentric and 10%-15% of these tumors metastasize to lymph nodes.

47-old women had tumor in upper lobe of the parotid gland. Aspirate smears was contains cells larger than acinar cells than normal salivary gland.The cytoplasm appears either finely vacuolated or dense gray. The nuclei was uniform, round or oval with small, central nucleolus. Stroma was with lymphoid infiltrates.There were no regional metastasize to lymph nodes.

Parotid gland was surgicaly removed. Microscopically tumor form well-circumscribed masses. Stroma was scanty and few lymphocytes infiltrate the stroma. Tumor was consists of large basophilic, granular cells, sometimes in an acinar configuration. These cells was stain with PAS (periodic acid-Shiff). Usually there were round clear spaces which may result from acumulation of dammed-up secretion or from rupture of cells and coalescence of intracytoplasmatic vacuoles. Diagnose was Acinic cell carcinoma.

One lymph node without tumor invasion was found near to parotid gland.

Pathology and Forensic Medicine / Posters / Gastrointestinal Pathology / 18

PATHOHISTOLOGICAL ASSESSMENT OF CHRONIC VIRAL HEPATITIS IN LIVER BIOPSY MATERIAL WITH ESTIMATION OF THE REPRODUCIBILITY OF ISHAKS SCORING SYSTEM FOR ACTIVITY AND FIBROSIS

Ščukanec-Špoljar M, Čorić M, Anzulović D[1], Črne N[1], Kolenc D, Vucelić B[2], Ivanković D[3].

[1]School of Medicine, University of Zagreb, students
[2]Department of Gastroenterology of the Clinic Medicine, Clinical Hospital Center Zagreb
[3]Institute of Statistics, School of Medicine, University of Zagreb

Aim. The aim of this study was to estimate intraobserver and interobserver variations in the assessment of features, classification, and numerical scoring of chronic viral hepatitis B i C among 2 trained pathologists.

Methods. Two pathologists indipendently reviewed 50 liver biopsy specimens of viral hepatitis B and C, taking into account separately every component of activity in grading (interface hepatitis-confluent necrosis-lobural activity - portal inflammation) and everyone of six steps in staging.

Results: For each variables of inflammation we found intraobserver agreement of 60-74%, and reproducibility increased when a tolerance of +/- 1 was accepted - (82-100%). Kappa scores ranged from 0,39-0.59, and weighted kappa scores from 0,49 - 0,73. For staging we noted 62% agreement, and 98% for tolerance of +/- 1. appa score was 0,58, and weighted kappa score 0,73. For the intraobserver agreement the range of the pairwise percentage agreement was 60-66%, and if a deviance of 1 categorical level was accepted, the percentage was 74-100%. Kappa scores ranged from 0,35-0.63, and weighted kappa scores from 0,44-0,62. For staging we noted 72% agreement, and 100% for tolerance of +/- 1. Kappa score was 0,63, and weighted kappa score 0,77.

Conclusion. The results of this study demonstrate that the Ishak scoring system is associated with good inter and intraobserver reliability, especially if the deviance of one categorical level is accepted and not very different from the findings of some other authors.

Pathology and Forensic Medicine / **Posters** / **Uropathology** / **19**

UNCLASSIFIED RENAL CELL CARCINOMA-REPORT OF TWO CASES

Begić J, Bobonj D, Tomić K, Muić B, Trnski D, Krušlin B.

Department of Pathology and Urology, General Hospital Bjelovar, Croatia; Department of Pathology, General Hospital Slavonski Brod, Croatia; Department of Pathology and Urology, Sestre milosrdnice University Hospital, Zagreb, Croatia

Aim: Unclassified renal cell carcinoma is a diagnostic category which includes renal cell carcinomas that do not fit in any other category, and comprises about 4-5% of all renal cell carcinomas.

Materials and methods: We described 2 patients who underwent nephrectomy due to primary kidney tumors. First one was a 70 years old male patients who presented with polyuria, without hematuria. CT showed solid, expansive tumor placed on the lower pole of the left kidney, measuring up to 5 cm in largest diameter.

Second one was a 61 years old female patient in whom ultrasound examination showed incidental finding of suspected tumorous mass which was confirmed on CT a solid, inhomogeneous mass, measuring also up to 5 cm, located on the upper pole of the left kidney.

Specimens of both tumors were formalin fixed, paraffin embedded, cut and routinely stained with hematoxylin-eosin. Antibodies used were primary antibodies to pan-cytokeratin and vimentin.

Results: Microscopically both tumors showed the same histological pattern and consisted of irregular, partly cystic glandular formations lined by atypical uniform epithelial cells with prominent nucleoli. Glandular formations were embedded in loose fibrous stroma and focally densely packed.

Immunohistochemistry showed positive staining of the tumor cells with pan-cytokeratin and vimentin.

Conclusion: Six months after surgery both patients are alive and without signs of tumors spreading or metastatic disease.

Pathology and Forensic Medicine / **Posters** / **Uropathology** / **20**

PROSTATIC STROMAL PROLIFERATION OF UNCERTAIN MALIGNANT POTENTIAL (STUMP) - CASE REPORT

Tomić S, Bezić J, Projić P, Samija I, Vilović K, Pešutić-Pisac V.

Department of Pathology, Clinical Hospital Split, Split, Croatia

Aim of the study is to present a patient with rare case of prostatic stromal proliferation of uncertain malignant potential.

71 old men presented with acute urinary retention. At digitorectal examination prostate was enlarged and elastic. Ultrasound examination was consistent with diagnosis of prostatic hyperplasia, and serum preoperative values of PSA were 1,2 ng/ml.

After suprapubic prostatectomy two nodules from prostatic tissue with greatest diameter of 6 and 5,8 cm were send to patohystological analysis

At light microscopic analysis we found proliferation of stromal cells showing focal cytological atipia associated with benign prostate gland. Stromal cells varied from round and plump to spindled and had clear to pale eosinophylic cytoplasm. Cytologically atypical cells showed nuclear enlargement and polymorphism, occasional multinucleation and nucleolar prominence. Immunohistochemicaly stromal cells were CD34, vimentin and actin and PR positive, and PSA, PAP and estrogen receptors negative.

Flow cytometric analysis found that sample was diploid with low proliferative rate (S phase - 0,67%).

One year after operation patient is well and without symptoms.

Prostatic stromal proliferation of uncertain malignant potential is newly recognized entity which differential diagnosis include prostatic stromal sarcoma, rhabdomyosarcoma, leiomyosarcoma, carcinosarcoma, pseudosarcomatoid fibromyxoid tumor and postoperative spindle cell nodule.

Although most cases of STUMP do not behave in an aggressive fashion occasional cases have been documented to recur rapidly after resection and minority have progressed to stromal sarcoma so we recommended carefully clinical follow-up.

Pathology and Forensic Medicine / **Posters** / **Uropathology** / **21**

THE EOSINOPHIL VARIANT OF CHROMOPHOBE RENAL CELL CARCINOMA

Racar A, Heinzl R, Aralica G, Müller D, Ljubanović D, Manojlović S.

Clinical Hospital Dubrava, Zagreb, Croatia

Objective: Chromophobe renal cell carcinoma (CRCC) composes about 6% of all renal epithelial neoplasms. There are two types of CRCC: eosinophil and typical. The eosinophil variant of CRCC can have similar morphology as eosinophil type of chromophil renal cell carcinoma, the eosinophil variant of clear cell carcinoma and renal oncocytoma.

We present solution of this differential diagnostic dilemma.

Methods: HE colloidal iron and imunohistochemical stain, for EMA, low molecular weight cytokeratin and vimentin was made. Electronic microscope was also performed.

Case report: Seventy five year old man was presented with yellowish brown, well circumscribe tumor within parenhim of the right kidney. The tumor was homogen, lightely under cut surface, without hemorrhage and necrosis, 3.5 cm in the largest diameter. Histologicaly the tumor was composed of large cells with eosinophilic granular cytoplasm and with modestly polymorphic nuclei. Some nuclei had nucleoli and perinuclear halos. The rare mitosis were note.

Results: Tumor cells were positive for EMA, low molecular weight cytokeratin and colloidal iron, and negative for vimentin. Electronic microscope analysis showed numerous microvesicles and rare mitochondria. The diagnosis of the eosinophil variant of the CRCC was made.

Conclusions: In opposite to eosinophil type of CRCC, the eosinophil type of chromophil renal cell carcinoma do not stain with colloidal iron. The eosinophil type of clear cell renal carcinoma characteristically coexpress cytokeratin and vimentin and the colloidal iron stain is negative. Oncocytoma reacts positively for low molecular weight cytokeratin and do not express vimentin, the colloidal iron stain is negative and the cells are filled with mitochondria.

Pathology and Forensic Medicine / **Posters** / **Uropathology** / **22**

RENAL ARTERY CHANGES IN PATIENTS WITH DIFFERENT TYPES OF PRIMARY TUMOR OF THE KIDNEY

Tomić K, Tomas D, Mladinov D, Kos M, Belicza M, Krušlin B.

Department of Pathology, General Hospital Slavonski Brod, Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Croatia

Aim: Different changes, but most frequently atherosclerosis and fibromuscular dysplasia (FMD) could affect renal arteries. Atherosclerotic changes are most frequently seen in proximal segment while FMD usually involves distal third of the renal artery and its branches. Renal artery stenosis can lead to renovascular hypertension and ischemic atrophy of the kidney that could be connected to necrosis which is frequent in kidney tumors. We analyzed changes of renal arteries in three most frequent primary tumors of the kidney including renal cell carcinoma (RCC), urothelial carcinoma of the pyelon (UC) and oncocytoma.

Methods: We analyzed 3 groups of patients (M: F=48:30) who underwent nephrectomy due to primary tumor of the kidney. In the first group there were 57 patients with RCC, in the second 12 with UC and in the third 9 with oncocytoma. Patients were in age 35-89 years. Specimens were routinely fixed, embedded in paraffin, cut and stained with hematoxylin and eosin, Mallory trichrome method and orcein, and examined by light microscopy.

Results: Renal arteries of 26 patients with RCC showed no changes, while 24 had FMD (M: F=10:14). Seven patients with RCC had atherosclerotic changes of renal arteries.

Four patients with UC of pyelon showed no changes of renal arteries and 7 had FMD while only one male patient had atherosclerotic changes.

Seven patients with oncocytoma showed no changes of renal arteries, while 1 patient had FMD and one atherosclerotic change.

Conclusion: FMD is found in significant number of patients, manly in females with RCC.

Pathology and Forensic Medicine / **Posters** / **Uropathology** / **23**

CORRELATION BETWEEN OCCURANCE OF DIFFERENT UROLOGICAL TUMORS AT THE 20 YEARS DISTANCE

Ulamec M, Tomić K, Leniček T, Vinter O, Krušlin B, Belicza M.

Department of Pathology, Sestre milosrdnice University Hospital, Zagreb, Department of Pathology, General Hospital Slavonski Brod, Croatia

Aim: Aim of the study was to compare incidence between different types of urological tumors in two 5-years periods at the 20 years distance.

Patients and methods: We used histopathological data base (Thanatos) from the Department of Pathology "Ljudevit Jurak" during periods 1980/84 (first period) and 2000/04 (second period).

Results: In the first period 61152 tissue samples were analyzed, 2919 samples were obtained from urologic patients (4.8%), while in the second period there were 73245 samples analyzed of which 6599 (9.0%) were from Department of Urology.

In the first period from all urological patients there were 301 (10.3%) kidney biopsies of which 81(26.9%) was due to kidney tumors while in the second period there was 749 (11.4%) kidney biopsies of which 404 (53.9%) were tumors.

In the first period there were 216 (7.4%) testicular biopsies, and in the second 198 (3.0%) of which 39 (18.0%) from the first period was due to malignant neoplasms, while in the second period there were 102 (51.5%) cases of testicular tumors.

From total number of Urology biopsies in 1980/84 there were 1072 (36.7%) prostatic biopsies and in 2000/04 there were 3382 (51%). Prostatic adenocarcinomas represented 15.5% (166) of all prostatic biopsies in the first period and 29.1% (983) in the second period. Conclusion: On the basis of this investigation we can conclude that the total number of biopsies increased, as well as the number of urological biopsies. We observed an increase in the frequency of all diagnoses of urogenital malignancies.

Pathology and Forensic Medicine / Posters / Uropathology / 24

SOLID VARIANT OF ALVEOLAR RHABDOMYOSARCOMA OF THE SPERMATIC CORD SHOWING VASCULAR INVASION

Zamolo G[1], Čoklo M[2], Štifter S[1], Bosnar A[2], Markić D[3], Maričić A[3], Pavlović-Ružić I.[4]

[1]Department of Pathology, Rijeka University School of Medicine
[2]Department of Forensic Medicine, Rijeka University School of Medicine
[3]Department of Urology, Rijeka University Hospital
[4]Department of Radiotherapy and Oncology, Rijeka University Hospital, Croatia

A 20-year-old man visited the Department of Urology complaining of a painless lump in the region of the right testicle, which he noticed some 4 months ago. The lump did not change in size, since. The patient reported no recent trauma. Physical examination revealed a firm, nodular palpable mass in the caudal region of the epididymis. Ultrasound revealed a heterogeneous sounded tumor formation, measuring up to 3 cm, in the projection of the right epididymis tail Exploration of the right hemi-scrotal region was performed. A tumor formation holding to the beginning of the spermatic cord, close to the epididymis tail, with no signs of infiltration of the adjacent structures, was observed. The tumor was encapsulated and presumably completely resected. On cut surface it appeared nodular. The histopathology revealed diagnosis of alveolar rhabdomyosarcoma, solid variant. Lymph node involvement and systemic metastasis survey revealed no signs of metastasis, so the patient was considered clinical stage I. A radical inguinal orchidectomy was performed, combined with chemotherapy. During the second surgery, macroscopically no residual tumor mass was found. The histopathological examination of the surgical specimen, on the contrary, revealed a residual tumor mass in the region of the spermatic cord, showing vascular invasion. The patient is now 24 months without any sign of disease. As many authorities still recommend a routine lymph node dissection, the most appropriate approach to staging and treatment of these tumors seems controversial, especially regarding the stage I and vascular invasion.

Pathology and Forensic Medicine / Posters / Uropathology / 25

EXPRESSION OF OSTEOPONTIN AND CD44 MOLECULE IN PAPILLARY RENAL CELL TUMOR

Matušan K, Đorđević G, Mozetič V[1], Lučin K.

Department of Pathology, Rijeka University School of Medicine, Rijeka, Croatia
[1]Department of Urology, Clinical Hospital Center Rijeka, Rijeka, Croatia

Aim: To analyze the expression of CD44 adhesion molecule and its ligand osteopontin in papillary renal cell tumors, and to assess the possible prognostic significance of CD44 and osteopontin expression in papillary renal cell carcinomas.

Methods: The expression of standard and v6 isoform of CD44 molecule, as well as of its ligand osteopontin, was immunohistochemically evaluated in 43 papillary renal cell tumors, which included 5 papillary renal cell adenomas and 38 papillary renal cell carcinomas. In order to assess their prognostic significance, the results obtained in papillary renal cell carcinomas were compared to usual clinicopathological parameters such as tumor size, histological grade, pathological stage, and Ki-67 proliferative index.

Results: Normal renal tissue was negative for CD44s and v6 isoform, while the expression of osteopontin was found in distal tubular epithelial cells in the form of cytoplasmic granular positivity. CD44s and v6 isoform were upregulated in 22 (58%) and 12 (32%) out of 38 carcinomas, respectively. Among all clinicopathological parameters examined, we only found significant association of CD44s positive carcinomas with lower pathological stage ($p=0.026$). Papillary renal cell adenomas were generally negative for CD44s, except for focal positivity found in one sample. The osteopontin protein was detected in all adenomas and all papillary renal cell carcinomas, except one.

Conclusion: Our results show constitutive expression of osteopontin in papillary renal tumors, including papillary renal cell adenomas. The upregulation of CD44s and v6 isoform, although found in considerable number of papillary renal cell carcinomas, does not have any prognostic value in this type of renal cancer.

Pathology and Forensic Medicine / **Posters** / **Uropathology** / **26**

CORRELATION BETWEEN VASCULAR ENDOTHELIAL GROWTH FACTOR EXPRESSION AND ANGIOGENESIS IN RENAL CELL CARCINOMA

Đorđević G, Mozetič V[1], Vrdoljak-Mozetič D[2], Jonjić N, Licul V, Matušan K, Mustać E, Oguić R[1], Fučkar Ž.[1]

Department of Pathology, Rijeka University Medical School, Rijeka, Croatia
[1]Clinical Hospital Centre Rijeka, Clinic of Surgery, Department of Urology
[2]Clinical Hospital Centre Rijeka, Clinic of Gynecology and Obstetrics, Department of Gynecologic Cytology, Rijeka, Croatia

Aim: To analyze the immunohistochemical pattern of vascular endothelial growth factor

(VEGF) expression, to investigate angiogenesis by average microvessel density (MVD) in clear cell renal cell carcinoma (CCRCC) and to correlate tumor angiogenic properties to clinicopathological parameters.

Methods: Surgical specimens of 93 CCRCC (62 men and 31 women) were examined by immunohistochemistry using CD31 to visualize endothelial cells, pAb for VEGF expression and MIB-1 for assessing Ki 67 proliferative index. Characteristics of VEGF expression was recorded as percentage of positive tumor cells as low (0-75% VEGF positive cells) and high (76-100%VEGF positive cells), VEGF cytoplasmic distribution and VEGF histological pattern. All features of VEGF expression were correlated with MVD, Ki 67 proliferative index and clinicopathological parameters.

Results: There were 74 tumors in pT1 and pT2 stage and 19 in pT3 and pT4. Statistical significant difference was found between groups of tumors with low and high VEGF expression regarding MVD ($p<0.05$), nuclear grade ($p<0.01$) and Ki67 proliferation index

($p<0.01$). Such difference was found between tumors with homogenous and heterogeneous VEGF expression in relation to pT stage, size of tumor and nuclear grade (for all parameters $p< 0.05$). Tumors with the high VEGF expression and heterogenous patohistologic distribution were bigger, with higher nuclear grade and proliferative index and with less number of blood vessels. There were significant differences between diffuse and perimembranous cytoplasmic VEGF expression. Diffuse VEGF expression was in relation with higher pT stage and nuclear grade ($p<0.01$). Ki67 proliferation index was connected significantly with pT stage ($p<0.01$) and nuclear grade ($p<0.05$).

Conclusions: This tumor model did not confirm the simple postulated relation between VEGF overexpression and angiogenesis through high microvessel count, but the character of VEGF expression showed significant correlation to worse histologic prognostic factors.

Pathology and Forensic Medicine / Posters / Neuropathology / 27

4-HYDROXYNONENAL: THE MAJOR LIPID PEROXIDATION PRODUCT EXPRESSION IN EPENDYMOMAS

Jurić-Sekhar G, Žarković K, Žarković N.[1]

Zagreb University Hospital Center, Zagreb, Croatia
[1]Institute "Ruđer Bošković", Zagreb, Croatia

Aim: Oxidative stress plays an important role in both initiation and promotion of multistage carcinogenesis. However, a tumor in itself represents a condition of oxidative stress. Lipid peroxidation is an autocatalytic process caused by oxidative stress, which damages lipids and causes production of highly reactive species as 4-hydroxynonenal (HNE). HNE is not only a cytotoxic mediator of oxidative stress, but also a potent growth modifying factor. The aim of this study was to determine the expression of HNE in ependymomas.

Methods: Paraffin-embedded specimens from 30 ependymomas, WHO grade II, and 10 anaplastic ependymomas, WHO grade III, were obtained dating from 1984 to 2001. Immunohistochemical staining was performed using monoclonal antibody on HNE-histidine conjugates.

Results: HNE immunopositivity was found in all ependymomas. There was variability of expression in each tumor type: HNE revealed a tendency towards lower expression levels in ependymomas, WHO grade II, while HNE immunopositivity was predominantly high in a vast majority of tumor cells of anaplastic ependymomas, WHO grade III. Mostly HNE immunopositive tumor cells of ependymomas, WHO grade II were distributed perivascular, while in anaplastic ependymomas, WHO grade III were predominantly diffuse. The expression of HNE was observed independent of tumor grade in endothelial cells of vessels except in one anaplastic tumor type. HNE immunopositivity was confined to necroses, mitotic figures and scant apoptosis in each anaplastic ependymomas, WHO grade III. Conclusion: The present findings indicate that ependymomas are under persistent oxidative stress and lipid peroxidation may play an important role in their development and growth as well. This observation may have therapeutic implications in the treatment of ependymomas, particularly in an anaplastic tumor type.

Pathology and Forensic Medicine / **Posters** / **Neuropathology** / **28**

CATHEPSIN D IN SOME NEUROEPITHELIAL BRAIN TUMORS

Pigac B, Vuković J, Plazanić D, Dmitrović B.

Department of Pathology, Forensic Medicine and Cytology, Varaždin General Hospital, Varaždin, Croatia
Institute for Diabetes and Metabolic Diseases " Vuk Vrhovac " Zagreb, Croatia
Department of Pathology, Clinical Center Dubrava, Zagreb, Croatia
Department of Pathology and Forensic Medicine, Osijek Clinical Hospital, Osijek, Croatia

Aim of the study: The activity of Cathepsin D and its possible prognostic value in the most common primary tumors of CNS were the main topics of this research.

Methods: The research included 65 humane primary tumors of CNS. When using immunohistochemical analysis diffuse citoplasmic positive immunohistochemical reaction was observed in tumor parenhime cells of the brain, endothelial cells of brain tumor blood vessels and stromal cells, the results were examined both qualitatively and quantitatively. Results: Parametrical correlation and nonparametrical correlations showed that the positive Cathepsin D in brain tumor parenchimal cells citoplasm and stromal cells citoplasm, as well as in citoplasm of brain tumor blood vessels endothelium and the percentage of immunopositive tumor parenhime cells of the brain and stromal cells as well as of endothelial cells of brain tumor blood vessels on Cathepsin D did not correlate with the tumor gradus, while only the percentage of immunopositive stromal cells correlated with the survival rate. Also by using above mentioned correlations, it was shown the gradus correlated with the survival rate. By using multiple regression method, it was revealed that the tumor gradus did not correlate with the positive Cathepsin D in brain tumor parenchimal cells citoplasm and stromal cells citoplasm as well as in citoplasm of brain tumor blood vessels endothelium and the percentage of immunopositive tumor parenhime cells of the brain and stromal cells, as well as of endothelial cells of brain tumor blood vessels on Cathepsin D as well as with survival, while the survival correlated only with the percentage of immunopositive stromal cells. The Kaplan-Meier curve and the Cox regression test revealed that among all of the tested elements only the percentage of immunopositive stromal cells on Cathepsin D and the gradus of the tumor have the impact on the survival prognosis.

Conclusions: From all gathered results, the percentage of immunopositive stromal cells on Cathepsin D was isolated as the independent indicator. The presented results of this analysis of the prognostic value of Cathepsin D in CNS's primary tumors of neuroepithelial origin, reveal the genuine possibility of using Cathepsin D as an independent prognostic factor in the progression of human gliomas and open new options for scientific research.

Pathology and Forensic Medicine / Posters / Neuropathology / 29

INTRACRANIAL SUPRASELAR ANGIOLIPOMA

Žarković K[1], Mihaljević D[2], Kolenc D[1], Radoš M[3], Paladino J.[2]

[1]Department of Neuropathology, Clinical Hospital Center Zagreb, Croatia
[2]Department of Neurosurgery, Clinical Hospital Center Zagreb, Croatia
[3]Department of Radiology, Clinical Hospital Center Zagreb, Croatia

Introduction: Angiolipomas are benign mesenchymal neoplasm composed of abnormally differentiated vessels and mature adipose tissue. These tumors are most commonly found in peripheral tissues. Within the craniospinal axis they are extremely rare and almost all grow in the spinal epidural space. In two patients intracranial angiolipomas have been reported in middle cranial fossa and sphenoid sinus, and eight in supraselar or paraselar region.

Clinical History: The patient, a 63-year-old man, presented with approximately 6-month history of headache and progressive visual loss of both eyes. The laboratory evaluation of endocrine function reveals normal pituitary function with maintenance of adequate target gland hormone level. MRI on coronary, sagital and transferal sections showed selar and left paraselar tumor. Solid tumor lesion ranged in diameter 3 cm extending to the left cavernous sinus. Tumor was clearly demarcated from left parahypocampal brain tissue, and optic chiasm. After contrast media administration tumor showed partial and inhomogeneous gadolinium enhancement and small hemorrhage in tumor tissue. Radiological diagnosis was pituitary macroadenoma with hemorrhage. In medial part of basilar artery blood flow was reduced and acute hemorrhagic infarction was present in both inferior occipital regions. After recovery of brain infarctions and followed myocardial infarction, patient was operated by transsphenoidal approach. Tumor tissue was red and yellowish. A subtotal resection was performed because of profuse bleeding.

Neuropathological findings: The tumor was composed of mature fat cells and numerous congested, thin-walled blood vessels. Closely grouped cavernous vessels were displaying focal angiomatous character of lesion. Fibrous hyaline tissue separated angiolipoma from pituitary gland.

Conclusion: We report a rare case of intracranial angiolipoma. Preoperative detailed analysis of MRI characteristic of the lesion should suggest this diagnosis to neurosurgeon. The general lack of experience using MRI imaging to study intracranial angiolipomas may explain why the diagnosis was never proposed. Because of profuse bleeding only one tumor out of nine reported cases was completely removed. Different signaling because of variable amount of vessels, blood and fat cells confused radiologist in differentiating angiolipomas from pituitary adenomas, meningiomas or even giant aneurysms.

Pathology and Forensic Medicine / **Posters** / **Neuropathology** / **30**

PRIMARY YOLK SAC TUMOR OF PINEAL REGION-CASE REPORT

Talan-Hranilović J[1], Labinac-Peteh L[1], Bedek D[2], Sajko T[3], Vučić M.[1]

[1]"Ljudevit Jurak" University Department of Pathology
[2]Department of Diagnostic and Interventional Radiology
[3]Department of Neurosurgery "Sestre milosrdnice" University Hospital Zagreb, Croatia

Aim: intracranial germ cell tumors include germinoma, teratoma, embryonal carcinoma, yolk sac tumor, choriocarcinoma. This tumors most frequently arise in the pineal and suprasellar region from embryologic germ cell nests after aberrant migration during early development. Pineal germ cell tumors tend to occur in adolescence and young adulthood with male to female ratio 2:1. We present a rare case of yolk sac tumor in pineal region.

Methods: 14-years-old boy was admitted to hospital due to headaches and visual disturbances on a left eye. Magnetic resonance imaging (MRI) showed an oval, expansive lesion of inhomogeneous structure that compress the third ventricle, measuring 37x35 mm. Patient underwent surgery. Pathohistological study of biopsy specimens showed within a mesenchyme-like myxoid background enteric-type glands and anastomosing strands of cuboidal epithelium in some cases covering delicate fibrovascular projections (Schiller-Duval bodies). Eosinophilic hyaline globules are present free in stroma. Immunochistocemical analysis showed a positive expression of PAN-cytokeratin and alpha-fetoprotein, while placental alkaline phosphate (PLAP) reveal negative immunostaining. Three months after surgery the patient is without the symptoms.

Discussion: primary yolk sac tumor of pineal region is very rare tumors. Histologically yolk sac tumors showed various patterns in different combination. Pathohistologic diagnosis is supported by additional immunostainings (PAN-citokeratin, -fetoprotein and PLAP). Differential diagnosis includes other germ cell tumors. Clinicoradiological features are additionally helpful for accurate diagnosis.

Pathology and Forensic Medicine / Posters / Neuropathology / 31

NEOVASCULARISATION IN GLIOBLASTOMA: AN IMMUNOHISTOCHEMICAL ANALYSIS

Krstulja M, Štemberger C, Benčević H, Franko A, Štanfel O.

Department of Pathology, Rijeka University School of Medicine, Teaching Institute of Public Health and Department of Radiology at the Clinical Hospital Center, Rijeka, Croatia

Aim: Glioblastoma paraffin embedded tissue was stained for vascular stromal and proliferation associated markers with the aim to investigate proliferation and density of microvessels.

Methods: Quantitative analysis of glioblastoma growth in the brain cortex microenvironment was performed with endoglin, SMA, CD34 and Ki67 immunohistochemistry. For descriptive analysis tenascin, vimentin, CD68, GFAP, synaptophysin and VEGF were used. Several parameters of vessel proliferation were investigated.

Results: The present approach differs from other authors as analysis of vessel wall composition in vessel proliferation and analysis of proliferation based on a specific type of vessel cells versus semiquantitative analysis of vessel form. High proliferation index was obtained for SMA positive vascular cells (13.76 ± 1.00%) compared to endothelial cells (3.95 ± 0.82%). Pericytes accompanying neovessel maturation in glioblastoma are an abondant target for antiangiogenic approach to glioblastoma therapy.

Pathology and Forensic Medicine / Posters / Investigative Pathology / 32

GASTRIC PENTADECAPEPTIDE BPC 157 - EFFECTIVE THERAPY OF MUSCLE CRUSH INJURY IN RAT, GIVEN INTRAPERITONEALLY OR APPLIED LOCALLY AS A CREAM

Batelja L, Brčić L, Staršinić M[1], Novinšćak T[1], Seiwerth S, Sikirić P.[1]

Institute of Pathology, Medical Faculty, University of Zagreb, Croatia
[1]Department of Pharmacology, Medical Faculty, University of Zagreb, Croatia

Aim: Stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, M.W. 1419 (PL-10, PLD-116, PL 14736 Pliva, Croatia)), accelerates healing of wounds, burns as well as transected Achilles tendon and shows strong anti-inflammatory effects. BPC is effective alone, without carrier, in trials for inflammatory bowel disease, wound treatment, locally or systemically, with no toxicity reported.

We hypothesize that BPC 157 may have clinical relevance as both systemic and local peptide treatment of major muscle crush injury, such as gastrocnemius muscle complex (GCC).

Methods: In crushed rats (GCC, impulse force 0.4653 Ns, kinetic energy 0.7217 J, force delivered 0.727 Ns/cm^2) BPC 157 (without carrier) was applied intraperitoneally (10 μg, 10 ng, 10 pg/kg) or locally (1.0 or 0.01 μg (dissolved in distilled water)/g commercial neutral cream) (given only immediately after injury (sacrifice at 2h) and/or once daily, final 24 hours before sacrifice (at 4, 7, 14, 21, 28, 72 days)). Assessment was functional, macroscopical and histological.

Results: BPC 157 improves muscle healing: (i) functionally (walking test - sciatic functional index, and extensor postural thrust/motor function index show significant improvement), (ii) macroscopically (less hematoma and edema, smaller limb circumference), (iii) microscopically (less inflammation and fibrosis, more regeneration).

Conclusion: Effective at any of the investigated intervals, BPC 157 induces post-injury muscle healing promptly and then maintains the healing with full function restoration.

Pathology and Forensic Medicine / Posters / Investigative Pathology / 33

BPC 157 ACCELERATES HEALING OF TRANSECTED RAT QUADRICEPS MUSCLE

Brčić L, Batelja L, Staršinić M[1], Novinšćak T[1], Seiwerth S, Sikirić P.[1]

Institute of Pathology, Medical Faculty, University of Zagreb, Croatia
1Department of Pharmacology, Medical Faculty, University of Zagreb, Croatia

Aim: Stable gastric pentadecapeptide BPC 157 (GEPPPGKPADDAGLV, M.W. 1419 (PL-10, PLD-116, PL 14736 Pliva, Croatia)), accelerates healing of wounds, burns as well as transected Achilles tendon and shows strong anti-inflammatory effects. BPC is effective alone, without carrier, in trials for inflammatory bowel disease, wound treatment, locally or systemically, with no toxicity reported.

We investigate whether this peptide improves healing and functional recovery of rat right quadriceps muscle following complete transection.

Methods: After complete muscle transection, Wistar male rats received agents once daily, intraperitoneally, BPC 157 (10 ?g/kg, 10 ng/kg or 10 pg/kg) or saline (5.0 mL/kg) (controls), first application 30 min following injury, last 24 h before sacrifice (at 4, 7, 14, 21, 28, 72 days post-injury). Assessment was functional, macroscopical and histological.

Results: Full assessment i.e., walking track analysis, biomechanical analysis, macroscopic assessment of bridging fragment between cut muscle ends, atrophy of distal part of transected muscle and microscopy (HE, Gomori silver stain, Van Gieson staining, vimentin and desmin immunostaining) reveals gastric pentadecapeptide BPC-157 healing and function recovery at all of the tested intervals. Eventually muscle regeneration was observed in BPC 157 treated animals in contrast to no regeneration, followed by complete atrophy of distal fragment evident in controls.

Conclusion: In conclusion, these results indicate that this suitably stable gastric pentadecapeptide BPC-157 heals transected muscle.

Pathology and Forensic Medicine / **Posters** / **Investigative Pathology** / **34**

HISTOLOGICAL ASSESSMENT OF WOUND HEALING DYNAMICS IN DB/DB DIABETIC MICE TREATED WITH PL 14736

Čužić S, Ivetić V, Brajša K, Parnham MJ.

PLIVA-Research Institute Ltd., Zagreb, Croatia

Aim: PL 14736 is a peptide under investigation for use in wound healing. We have studied the efficacy of PL 14736, applied daily directly to excisional wounds in genetically obese, insulin resistant (db/db) diabetic mice, which exhibit delayed wound healing. The test substance was compared to the reference substance platelet derived growth factor (PDGF-BB).

Methods: Female db/db mice each received an 8mm punch biopsy skin wound on the back under anaesthesia. Animals were divided into 3 groups of 25 animals each, treated daily with PDGF-BB, PL 14736 or vehicle (phosphate buffered saline) applied directly to the wound. Five animals per group were each killed on days 6, 9, 12, 18 and 24 post-wounding.

Tissue was formalin-fixed, cut through the centre of the wound and embedded in paraffin "on a side". Slides were stained with hematoxylin-eosin or van Gieson stain for collagen and elastic fibres. Morphometric evaluation of re-epithelialization was performed together with semi-quantitative granulation tissue analysis.

Results: In db/db mice treated with PL 14736, re-epithelialization rate was higher on day 6 after wounding than in wounds treated with vehicle or PDGF-BB (66% versus 44%; not statistically significant). Maturation of granulation tissue on day 12 post-wounding - seen as formation of collagen bundles - was significantly greater in animals treated with PL 14736 than in those treated with vehicle ($p=0.048$ in Mann-Whitney U test). PDGF-BB had little effect.

Conclusion: We conclude that local treatment with PL 14736 enhances granulation tissue maturation in the first 12 days post-wounding in diabetic mice, while in agreement with the literature, PDGF-BB has a negligible effect on wound healing dynamics in this model.

Pathology and Forensic Medicine / Posters / Investigative Pathology / 35

INTERSTITIAL KIDNEY FIBROSIS IN EXPERIMENTAL MODEL

Aralica G, Radović N, Ljubanović D[1], Manojlović S[1], Knotek M.[2]

Department of Urology, Clinical Hospital "Dubrava", Zagreb, Croatia
[1]Department of Pathology, Clinical Hospital "Dubrava", Zagreb, Croatia
[2]Department of Internal Medicine, Clinical Hospital "Dubrava", Zagreb, Croatia

Aim: Interstitial fibrosis is a final outcome of many chronic renal diseases, including obstructive nephropathy. Fibrosis considers fibroblasts number and intercellular matrix deposition increase, leading characteristic tissue changes resulting in chronic renal failure. The aim of this study is to quantify the degree of interstitial fibrosis in rat kidney after experimental unilateral ureteral obstruction.

Material and methods: Wistar male rats, weight 210-300 g, 2-3 months old were used in this experimental model. There were 2 experimental groups, 6 rats each. Left ureter of rats in one group was ligated. Another group was control - sham-operated rats. Rats were sacrificed

10th day after operation. Left kidney of each rat was routinely processed for light microscopy. The interstitial kidney fibrosis quantification was performed on Gomory stain slides using semiquantitative scale (0-5) and morphometric system (ISSA, Vamstec, Zagreb, Croatia). Morphometric quantification was performed using computer grid (13x10 fields) and counting the number of grid fields with interstitial fibrosis.

Results: Interstitial fibrosis was developed in left kidney of operated rats. Mean level of fibrosis was 3,2 and mean number of grid field with fibrosis per 1 HPVF was 34. In opposite, the mean level of fibrosis was significantly lower in left kidney sham-operated rats and it counted 0,5. Mean number of grid field with fibrosis per 1 HPVF was 13.

Conclusion: Interstitial fibrosis develops in ipsilateral kidney after the ureteral obstruction. The level of interstitial fibrosis can be determined using several methods. We used two methods and found that both, semiquantitative and quantitative method is adequate.

Pathology and Forensic Medicine / Posters / Investigative Pathology / 36

BONE MORPHOGENETIC PROTEIN EXPRESSION IN NORMAL AND MALIGNANT PROSTATE AND BREAST

Đorđević G, Mustać E, Bobinac D[1], Zoričić S[1], Španjol J[2], Fučkar Ž.[2]

Department of Pathology, Rijeka University School of Medicine, Rijeka, Croatia
[1]Department of Anatomy, Rijeka University School of Medicine, Rijeka, Croatia
[2]Department of Surgery, Rijeka University Hospital, Rijeka, Croatia

Aim: It has been proposed that BMPs might act as paracrine factors regulating the proliferation and differentiation of tumor cells. Behind this background we studied the expression of BMPs in carcinoma of different origins and their normal counterparts. Methods: BMP expression was detected in 20 samples of prostate cancer, 15 samples of berast carcinoma with bone metastases and 5 normal prostate and breast samples. Tissue sections were analyzed immunohistochemically using the polyclonal anti-BMP-2/4-7 antibodies. Expression of BMPs was expressed as percentage of immunopositively stained cells. The BMP expression was compared with clinocopathological and biochemical parameters.

Results: Immunohistochemical staining showed that bone morphogenetic proteins were present in the cytoplysm in prostate cells whereas breast tissues showed nuclear staining. Expression of BMP proteins was detected in prostate samples and BMP-2/4 and -7 were predominately expressed. BMP-2/4 was also predominantly expressed in prostate carcinoma while the expression of BMP-7 was significantly lower than in normal prostate. In all breast cancers tissues we found nuclear staining only for BMP-7. In normal breast tissue the BMP expression was not detectable. In breast cancer we found significantly higher percentage of BMP-7 positive cells in comparison to prostatic cancer. Comparing BMP expression levels and clinicopathological parameters, we did not find statistical difference. Conclusion: These results suggest that differed BMPs expression in prostate and breast cancer appear to be adjunct marker for osteoblastic/osteoclastic metastases presence.

Pathology and Forensic Medicine / Posters / Autopsy and Forensic Medicine / 37

CARDIAC PERFORATION: AN ADVERSE OUTCOME OF JUGULAR CATHETERISATION

Mayer D, Petrovečki V, Škavić J.

Department of Forensic Medicine and Criminology, Medical School, University of Zagreb
Zagreb, Croatia

Background: The use of temporary or semi-permanent central vein catheterization for the purpose of hemodialysis is an essential component of contemporary dialysis practice. However, the use of these catheters is coupled with substantial rate of mechanical or infectious complications which may result in patient morbidity and even mortality.

Case: A 68-year-old women having polycystic kidneys has developed renal failure. She was treated with hemodialysis in the last 12 years. The availability of peripheral venous access gradually declined in the course of years. Therefore, a decision was made to employ central venous access by jugular catheterization. Catheters were changed on regular basis during the last 4 months. The most recent catheter exchange was performed 3 days before death. The patient was admitted to hospital for routine haemodialysis. At admittance, she complained on weakness and malaise. Shortly after initiating haemodialysis session the patient collapsed. Her health state deteriorated quickly and cardiac arrest followed. Cardiopulmonary resuscitation remained unsuccessful. External examination revealed scant postmortal lividity. Autopsy recovered abundant haemorrhage in soft tissues of anterior mediastinum and the presence of blood in left thoracic cavity. Pericardial sac was found ruptured. Pericardial space also contained blood. Examination of heart discovered the perforation defect in posterior side of right atrium. Tip of the double-lumen central jugular catheter was found in proximity of the perforation site. The cause of death was assigned to exsanguination.

Discussion: Central vein catheterization may have lethal consequences. Malpositioning of the catheter may lead to cardiac perforation and subsequent cardiac tamponade. As with our case, gradual accumulating of blood in pericardial sac may fail to induce conspicuous clinical manifestations. Therefore, regular radiological re-checking of catheter position is highly advisable to practitioners. Ignoring such a practice may lead to professional liability.

Pathology and Forensic Medicine / Posters / Autopsy and Forensic Medicine / 38

MEDICO-LEGAL EVALUATION OF VASCULAR RUPTURE AFTER LUMBAR DISC SURGERY: A CASE REPORT

Gongolo F, Malannino S.

School of Forensic Medicine, University of Trieste, Italy

Aim of the study: lumbar discectomy is often burdened with multiple serious complications; rupture of iliac vein is an unexpected, rarely reported, lately diagnosed lethal event. We report a case of a medico-legal court expertise.

Methods: analysing the clinical records and the judicial documentation, Authors study the features of medical responsibility due to malpractice in a case of iliac vein rupture, followed by death 36 hours after lumbar discectomy. A review of the literature has been carried out to examine the possible unexpected events occurring in diverse surgical approaches, in order to determine the different kinds of malpractice conduct.

Discussion and Conclusion: rarity of this kind of complication, multiplicity of clinical manifestations, lack of massive haemorrhage and difficulty to perform a laparotomic vascular time of rescue, play all a role making iliac vein rupture during lumbar discectomy an event to be feared of, even in case of extremely skilled orthopaedic surgeons. Authors believe the importance of the anaesthetist's role, promptly evaluating the signs of a circulation failure and disposing the reanimation's manoeuvres. The orthopaedic surgeon should dispose the imaging necessary to prepare a vascular time, considering the high risk and the difficult to proceed immediately to a laparotomic access to the vessels to stop the haemorrhage. Accuracy of both clinical records and operation's registry is a fundamental defensive tool in a trial case of medical malpractice.

Pathology and Forensic Medicine / Posters / Autopsy and Forensic Medicine / 39

FATAL LABOUR ACCIDENTS: AN ANALYSIS OF OUR CASUISTRY

Nardi U, Tomasella F[1], Gongolo F[1], Costantinides F[1], Melato M.[1, 2]

[1]School of Forensic Medicine, University of Trieste, Italy
[2]A.S.S. 2 Isontina, Department of Pathology, Italy

The Authors carry out an analysis of all deaths due to labour accidents, occurred in the last twenty years, in the Italian province of Trieste. We have considered cases recognised by the Italian Institute for Work-related Accidents Aid (INAIL) and therefore eligible for compensation. Fatal labour accidents occurred to public employees and military personnel have been taken into study as well.

Aim: the research was performed to recognise the most common patterns of fatal accidents occurred at work in our province, in order to monitor situations of risk and occupational categories particularly exposed to fatalities.

Methods: the Authors have reviewed the data collected during investigation of the scenes of work fatalities, external inspection and autopsy of the victims. The analysis did comprehend both accidents occurred on Work place and on the way to and from work (in itinere). Gender, age, occupational category, type of lesion, cause of death and accident-death time span, are the considered features of fatalities.

Results and conclusions: the most common causes of death are traffic accidents, precipitation and crushing under heavy loads, generally causing skull fractures and thoracic lesions. Our study comprehends a review of the literature and a discussion of varied iconography of the most interesting lesions. The majority of the victims are construction and dockworkers. In most of the cases death has occurred immediately. We have compared our findings with the data from the other provinces of the region.

Pathology and Forensic Medicine / Posters / Autopsy and Forensic Medicine / 40

CORRELATION OF CLINICAL DIAGNOSES AND AUTOPSY FINDINGS IN CLINICAL HOSPITAL SPLIT IN THE PERIOD 2000-2002

Sikavica A, Tomić S, Kardum G.

Department of Pathology, Clinical Hospital Split, Split, Croatia

Aim: To determine discrepancy between clinical diagnoses and autopsy findings. Methods: The study was a retrospective rewiev of 257 autopsies performed at Clinical Hospital Split during the period 2000-2002. Clinical diagnoses and autopsy findings were compared.

Results: The autopsy rate in Clinical Hospital Split is 4,9%. The discrepancies between clinical diagnoses and autopsy findings were found in 33,1% of the cases. A high discrepancy rate was found among pneumonias (50,0%) and gastrointestinal haemorrhages (45,0%) wich failed to be treated due to the wrong diagnosis. Similar clinical features of acute myocardial infarction and pulmonary embolus caused great difficulties when clinically diagnosed. The discrepancies between clinical diagnosis and autopsy finidings were determined in 51,3 % of the cases of cancer.

Conclusion: A low autopsy rate and a high discrepancy rate in the Clinical Hospital Split during the period 2000-2002. point out a need for the medical authorities and personnel to reconsider the merits of autopsy.

Pathology and Forensic Medicine / Posters / Autopsy and Forensic Medicine / 41

MEDICO-LEGAL FEATURES OF AUTOPSY IN CASE OF ASBESTOS EXPOSED WORKERS

Gongolo F[1], Brollo A[2], Tomasella F[1], Melato M.[1, 2]

[1]School of Forensic Medicine, University of Trieste, Italy
[2]A.S.S 2 Isontina, Department of Pathology, Italy

Due to the large industrial employ of asbestos until 1992 in many industrial countries and considering the latency of malignant mesothelioma and lung carcinoma, the autopsy of asbestos exposed workers will continue to represent a primary tool in cause of death definition, being fundamental both in the qualitative and quantitative documentation of asbestos exposure and in definitive confirmation of the cancer diagnosis. The causation criteria acceptable in court are currently flexible and susceptible of various interpretation by medico-legal experts.

Aim of the study: Authors suggest the regular observation of some reliable, simple asbestos exposure indices in case of asbestos related death, and underline the importance of an high quality pathologic diagnosis, considering the long lasting experience of the Department of Pathology, Monfalcone.

Materials and Methods: starting from the analysis of a large autoptic survey of malignant mesothelioma and lung carcinoma cases, Authors have reviewed the literature about "evidences" linking asbestos exposure to the causation of these neoplasms, considering as well the judicial final outcome.

Discussion and Results: the definition of causation appears strongly supported by autopsy evidences particularly in case of malignant mesothelioma, where given a definite diagnosis and a documented exposure there will be rarely doubt on compensation eligibility. Due to the role of other involved carcinogens, the cases of lung carcinoma are of more difficult, and partly arbitrary interpretation. We regard as fundamental the joined involvement of pathologists and experts in forensic and occupational medicine in order to define a disease as work-dependant.

Pathology and Forensic Medicine / Posters / Autopsy and Forensic Medicine / 42

DIC AND AMNIOTIC EMBOLISM: DIFFERENTIAL DIAGNOSIS IN A CASE OF POST PARTUM DEATH

Tomasella F[1], Gongolo F[1], Costantinides F[1], Melato M.[1, 2]

[1]School of Forensic Medicine, University of Trieste, Italy
[2]A.S.S. 2 Isontina, Department of Pathology, Italy

Diffuse intravascular coagulation (DIC) and amniotic fluid embolism are rare and unexpected events, occurring after labor, because of loss of the post-partum peculiar physiological balance of the woman.

Aim: With regard to the medico-legal aspects of possible malpractice. Authors present a review of the literature concerning differential diagnosis between DIC and amniotic fluid embolism with the purpose of understanding the underlying reasons of a birth delivery fatal evolution.

Materials and methods: study of clinical records, legal autopsy, histopathology and immuno-histochemistry are the bases of the medico-legal evaluation. Subsequently, Authors make a literature review of the possible patophysiological reasons of this fatal occurrence. Discussion and results: after delivery with cupping glass, episiotomy and tracheloraffia the patient presented a persistent vaginal bleeding. The surgeon decided to perform a uterine curettage, but having diagnosed a rupture of the uterus, choose rather to perform a hysterectomy. Haemodinamic instability occurred during operation, because of both serious coagulation impairment and increased embolism index, leading thus the patient to death in 5 hours.

The comprehensive analysis of autopsy samples leads to consider as possible cause of death both DIC and amniotic fluid embolism. Both events are consistent with clinical and laboratory data. Authors reviewed the bibliography on differential diagnosis between the two highly dangerous occurrences and give an evaluation of the potential medical responsibility issues of the case.

Pathology and Forensic Medicine / Posters / Autopsy and Forensic Medicine / 43

CAUSES OF PERINATAL DEATH AT OSIJEK CLINICAL HOSPITAL, 2001-2003

Dmitrović B, Marjanović K, Blažičević V, Bartolić K, Ivanović M, Dumenčić B.

Department of Pathology, Osijek Clinical Hospital, Osijek, Croatia
Faculty of Medicine, J. J. Strossmayer University, Osijek, Croatia

Aim: Perinatal mortality and the causes of perinatal death as determined by autopsy at the Osijek Clinical Hospital in the period 2001-2003 are presented.

Methods: The data were collected at the Department of Pathology: the autopsy reports and the placental biopsy reports were reviewed. The causes of death were classified into 9 categories: intrauterine hypoxia and birth asphyxia, congenital malformations, infection, abnormal pulmonary function, intracranial hemorrhage, prematurity, other diseases, macerated fetus and tumors. The asphyxia was later subdivided into 3 categories: failure of blood to become oxygenated in the placenta, obstruction to circulation through the cord, and of unknown cause. Descriptive statistics are presented.

Results: There were 6880 deliveries at the Osijek Clinical Hospital in the period 2001 2003. In this period, there were 141 children deaths, and the autopsy was performed in 139 cases (98,58%). Perinatal mortality values were: 7,97‰ (2001), 13,81‰ (2002), and 13,29‰ (2003). The majority of deaths were in fetuses and infants who showed evidence of asphyxia (52,48%): 71,62% of those cases was attributed to the failure of blood to become oxygenated in the placenta, 6,76% to the obstruction to circulation through the cord, and the rest 21,62% were of unknown cause. Congenital malformations (13,47%) ranked second, immaturity (8,51%) third, macerated fetus (7,81) fourth, and other conditions were far behind. In the group of 19 congenital malformation, the most common were multiple organs malformations (31,58%), followed by heart and large blood vessel malformations (26,31%), and central nervous system malformations (26,31%). In the early perinatal period, the most common cause of death was general immaturity.

Conclusions: Whether death should be attributed to one cause or another is often subject to variable opinion because of the much overlap among groups. Most of the deaths attributed to asphyxia were explained by placental biopsy report. The placental biopsy report must be an integral part of a autopsy report in the cases of the perinatal death.

Pathology and Forensic Medicine / Posters / Autopsy and Forensic Medicine / 44

A CASE OF FATAL MITOCHONDRIAL CARDIOMYOPATHY

Đorđević G, Golemac M[1], Dekanić A, Vranešić Đ, Dessardo S.[2]

Department of Pathology, Medical Faculty University of Rijeka
[1]Department of Pathology, Clinical Hospital Mostar, Bosnia and Herzegovina
[2]Department of Pediatric, Clinical Hospital Center Rijeka, Croatia

Sudden death is known to be possible consequence of hypertrophic cardiomyopathy. Here we present a case of 13 year-old girl, active swimmer, who died suddenly due to myocardial infarction and in absence of previous cardiac problems. The patient was admitted to the hospital because of high temperature, abdominal pain and signs of cardiac failure. The echocardiography examination revealed massive ventricular hypertrophy, left ventricular systolic dysfunction and pericardial effusion. Laboratory analysis showed markedly elevated plasma levels of creatine kinase and ECG findings revealed high voltages and ST depression. Postmortem investigation demonstrated cardiomegaly (190 gr) with marked symmetric hypertrophy of ventricles, mild dilatation (especially of the right ventricle) and dark mottling in the basal part of the left ventricular wall and septum. Histological examination showed coagulation necrosis, rare neutrophilic infiltrates and contraction band necrosis. The ultrastructural analysis of the left ventricular myocytes demonstrated severe reduction of myofibril content. Furthermore, fibrils were separated with a huge number of enlarged, irregular shaped mitochondria with abnormal multiplication of cristae.

The clinical and pathological findings of the present case are consistent with fatal hypertrophic cardiomyopahy associated with acute myocardial infarction as a consequence of mitochondrial abnormalities which were confirmed on electron microscopic examination.

KLINIČKA CITOLOGIJA
CLINICAL CYTOLOGY

Pozvana predavanja / *Invited Lectures (1-22)*

Clinical Cytology / **Invited Lectures** / **01**

ADENOCARCINOMA *IN SITU* OF UTERINE CERVIX - STATE-OF-THE-ART

Audy-Jurković S.

Institute of Gynecologic Cytology, University Department of Obstetrics and Gynecology, Zagreb University Hospital Center, Zagreb, Croatia

A steady increase over years in the prevalence and relative incidence of adenocarcinoma and adenosquamous carcinoma of the uterine cervix, established by epidemiologic investigation, points to the need of intensified detection of the diseases in the intraepithelial stage.

In contrast to squamous intraepithelial lesions with identifiable subgroups, in case of glandular epithelium only adenocarcinoma *in situ* (AIS) included in the NCI Bethesda 2001 cytologic classification has been recognized.

AIS is frequently (>60%) associated with squamous epithelium abnormalities, pointing to identical etiologic factors, among them human papilloma virus (HPVDNA 16 and 18) playing the key role.

The cytomorphological criteria for the diagnosis of AIS refer to changes in the architecture and cells, however, cellular changes may frequently be less pronounced than those in squamous lesions and are difficult to observe unless architectural alterations call for attention. In mixed lesions, the glandular component may be eclipsed in abnormal cell count and intensity by the squamous component.

In addition, the stage of tumor differentiation, differential diagnostic issues as well as specimen and slide quality also influence the identification and recognition of AIS, thus the sensitivity of cytology for AIS rarely exceeds 75%.

At our Institute, the value of cytology in lesion detection and differential diagnosis was assessed in 62 patients with definite histologic diagnosis of AIS (n=12) or AIS associated with squamous component (n=50), taking lesion severity and type of epithelial abnormality in consideration. The lesion was detected by cytologic analysis in all 62 (100.0%) patients, thus indicating additional diagnostic procedure. The differential diagnostic accuracy of cytology was higher in predicting lesion severity (66.1% - 41/62) than type of epithelial abnormality (58.1% - 36/62), and in predicting abnormality of squamous (90.0% 45/50) than of cylindrical (66.1% - 41/62) epithelium. The accuracy of cytology in predicting abnormality of cylindrical epithelium was considerably higher in case of pure (100.0% - 12/12) than mixed (58.0% - 29/50) lesions.

Diagnostic problems are also present in histology, one of the reasons for it being specimen nonrepresentativeness with consequentially different diagnoses in various sample types (34.8% in our study).

The value of novel methods of technical sample preparation (liquid based cytology), staining (immunocytochemical, etc.) and analysis (image analysis) has been investigated to improve the detection and differential cytodiagnosis of glandular lesions.

In routine diagnostic/therapeutic protocol, cytologic prediction of AIS indicates colposcopy for the detection of possible cytologically overlooked associated squamous lesion or recently described colposcopic pattern in glandular lesions. Biopsy and excochleation are recommended according to colposcopy finding. If conization is indicated (e.g., fertile age, nulliparae), classical procedure in the form of a cylinder is preferred to LETZ and laser conization, otherwise hysterectomy is the only appropriate surgical procedure in AIS.

Conclusion: Considering the role of cytology in the detection, diagnosis and follow up of AIS patients, its sensitivity should be constantly upgraded, primarily by improving specimen adequacy and microscopist skills as wells as by introducing novel methods.

Clinical Cytology / Invited Lectures / 02

IMMUNOPHENOTYPING OF ACUTE LEUKEMIA - DIAGNOSTIC APPROACH

Batinić D.

Division of Immunology and National Referral Center for Immunodiagnostics of Hematological and Immunological Diseases, Zagreb Clinical Hospital Center, Zagreb, Croatia

The introduction of immunophenotyping in clinical medicine was based on the need for more precise criteria to assist the morphological diagnosis and classification of hematological malignancies, especially acute leukemia (AL). Currently, a comprehensive immunophenotypic analysis of malignant cells provides valuable information for the classification of leukemia and lymphoma based on cell lineage and maturation stage. However, the clinical impact of immunophenotyping differs between the two major forms of AL. While in acute (especially B-precursor) ALL it significantly contributes to diagnosis, classification and prognostic stratification of the disease, its value in the classification of AML is restricted. The reasons for this are the multilineage cell nature and greater immunophenotypic and cytogenetic heterogeneity of AML, the presence of two or more subpopulations in a number of patients, as well as the lack of specific "early" myeloid-lineage markers. In general, the utility of immunophenotyping for subclassification of AML is limited to the identification of poorly differentiated AML, megakaryocytic and the microgranular variant of acute promyelocytic leukemia, as well as of rare subtypes of dendritic cell neoplasia. In other types of AML immunophenotyping is used to confirm the diagnosis. In addition to these standard indications, the immunophenotyping is increasingly being used for the definition of prognostic entities, screening of genetic abnormalities, measurement of minimal residual disease (MRD) and monitoring of specific therapies. These indications are based on the fact that AL cells usually display unusual or aberrant phenotypes, allowing their identification even at very low frequencies. Future studies should take advantage of powerful technology (i.e. multiparameter flow cytometry) combined with new marker combinations to address the potentials of immunophenotyping in the demanding clinical management of hematological malignancies.

Clinical Cytology / Invited Lectures / 03

ULTRASONOGRAPHY AS A TOOL FOR DETECTING AUTOIMMUNE THYROID DISEASES

Bence-Žigman Z.

Clinical Department for Nuclear Medicine and Radiation Protection, Clinical Hospital Centre Zagreb, Croatia

Autoimmune thyroid diseases (AITD) - (thyroiditis Hashimoto and Graves' disease) are associated with the typical echographic presentation: diffuse reduction in thyroid echogenity, or hypoechogenic areas within normal ehogenity of the thyroid parenchyma, ehogenic stripes within thyroid, irregular contours of the lobes, and slightly enlarged lymph nodes near the thyroid. Sometimes, only some of these typical signs are presented, or the presence of the signs is very discreet. Colour Doppler ultrasonography is an additional tool for studying thyroid vascularity and blood flow parameters for predicting activity of the disease.

Material and methods: The 1228 subjects, referred for ultrasonography of the thyroid, were examined prospectively with regard to detecting AITD. The reason for referral for ultrasonography of 862 patients was diffuse goiter, thyroid dysfunction (TD), or neck discomfort, but 366 of apparently healthy subjects referred for general check-up, were included in this study, also. Determination of T4, T3, TSH, TPOAb and TgAb was performed.

Results: In 319 subjects with normal thyroid echostructure, normal thyroid vascularity and normal values of blood flow parameters, elevated TPOAb levels in 3,4% and elevated TSH levels in 0,9% were observed. In 593 patients with typical echographic presentation for AITD, elevated TPOAb levels were observed in 90%. In the same group TD was found in 450 patients (76 %). In the third group of 107 patients, based on some discreet echographic signs when an AITD can be suspected only, elevated TPOAb levels were observed in 69% and AITD was found in 33%. Thyroid nodules were observed in 34% of patients with AITD.

Conclusion: Ultrasonograpy is a very useful tool for detecting AITD and subclinical TD, even when only some of discreet echograpic signs are presented. Ultrasound guided fine-needle aspiration biopsy is not necessary for diagnosis of AITD but it is helpful in making the diagnosis in thyroid nodules associated with AITD.

Clinical Cytology / Invited Lectures / 04

USEFULNESS OF DOPPLER WAVEFORM ANALYSIS IN DIFFERENTIAL DIAGNOSIS OF CERVICAL LYMPHADENOPATHY

Brnić Z.

Department of Diagnostic and Interventional Radiology, Merkur University Hospital, Zagreb, Croatia
zoran.brnic@zg.htnet.hr

Aim of the study: We compared Doppler spectral parameters in acute inflammatory, reactive, lymphomatous and metastatic lymph nodes, and evaluated pulsed Doppler sonography as a method for distinguishing between different causes of cervical lymphadenopathy.

Patients and methods: Spectral Doppler analysis with measurements of resistence index (RI), pulsatility index (PI), peak systolic velocity (PSV) and enddiastolic velocity (EDV) was performed in 197 patients with cervical lymphadenopathy. Results of Doppler analysis were compared with findings of cytology and hystology or with clinical presentation and follow-up. Student t-test was used to assess statistical significance of differences in Doppler parameters between groups of patients.

Results: Significant differences for RI and PI were shown between all groups of patients except between lymphomatous and reactive nodes. Specificity of 100% for metastatic nodal involvement was shown for cutoff values RI>0.80 and PI>1.80. PPV of 100% for acute lymphadenitis was shown for cutoff values RI<0.50 and PI<0.60. EDV>9 cm/s has 100% NPV for nodal metastasis, and EDV<1 cm/s has 100% specificity and PPV for metastasis.

Conclusions: Although there exist differences in RI, PI, PSV and EDV between different nodal diseases, none of these parameters offers both good sensitivity and good specificity, and only extreme cutoff values may occasionally be helpful in differential diagnosis. Doppler spectral analysis is valuable noninvasive adjunct, which can help in differentiation between metastatic, lymphomatous, acute inflammatory and reactive lymphadenopathy, but cannot obviate biopsy in the majority of cases.

Clinical Cytology / Invited Lectures / 05

VALUE OF TRANSBRONCHIAL NEEDLE ASPIRATION COMBINED WITH RAPID ON-SITE EVALUATION

Chalfe N[1], Pavlović L[1], Smojver-Ježek S[2], Gorečan M[3], Čučević B[1]

Departments of
[1]Pneumology and [2]Cytology, [3]University Department of Thoracic Radiology, University Hospital for Lung Diseases "Jordanovac", Zagreb, Croatia
nabil.chalfe@zg.htnet.hr

Aim of the study: The samples obtained by transbronchial needle aspiration (TBNA) during fiberoptic bronchoscopy (FOB) are suitable for a rapid on-site evaluation (ROSE). Availability of on-site results may increase the efficacy of FOB and modify subsequent patient management. This study prospectively investigated the diagnostic range, yield and practical value of this technique.

Materials and Methods: Consecutive patients with radiologically suitable mediastinal mass lesions or lymph nodes on conventional chest X-rays and CT lung scanning were investigated with FOB. A cytologist stained and evaluated the aspirates on-site and notified the bronchoscopist about the necessity of further sampling. If sufficient diagnostic material was collected with TBNA, the provisional diagnosis was noted and the procedure ended.

Results: Thirty-six mediastinal masses or lymph nodes were sampled in 22 patients. In 16 patients (73%) a diagnosis was made on-site (non small cell lung cancer: 7; small cell lung cancer: 2; metastatic cancer: 2; granulomatous disease: 4 and lymphoma: 1). In 4 patients (18%), TBNA diagnosis was only reactive lymphoid hyperplasia, which required further investigations. Overall, ROSE shortened bronchoscopic sampling in 73% of patients. Mean intervention time was 25 minutes. No side effects of TBNA were observed.

Conclusion: TBNA combined with ROSE is safe and highly effective diagnostic procedure.

Clinical Cytology / **Invited Lectures** / **06**

LYMPHADENOPATHY: DIAGNOSTIC AND CLINICAL CONTROVERSY

Jakšić B.

University Hospital Merkur, Zagreb, Croatia
branimir.jaksic@zg.htnet.hr

Lymphadenopathy is traditionally one of the most intriguing diagnostic and clinical problems. The unprecedented development of new technologies in recent years has tremendously improved the power of diagnostics in hematological clinical practice leading to development of new fields that require expertise, in addition to traditional cytological and/or histological morphology. By the same token, new abilities to classify disorders have considerably contributed to information noise in diagnostic procedures and clinically relevant classification. The potential of an individual technique (such as morphology, immunology, flow cytometry, cytogenetics, molecular characterization, imaging techniques etc.) to reliably classify patients is much higher then is usually converted into clinical benefit. This is due to the fact that systematic clinical research in evaluation of complex approaches by using various diagnostic techniques is as a rule coming later then description of individual technique.

The apparent controversies that are to be expected in this multidimensional network sometimes discourage investigators to confront different approaches and validate the process in methodologically sound clinical research setting. Instead, certain "rivalry" among disciplines emerges, so that evidence based approaches give place to opinion based approaches, causing a number of controversies that we witness today.

Moreover, precise diagnostic procedures are today even more important then before, since smart drugs, and targeted therapies require precise and reliable definition of underlying disorder. Various diagnostic approaches that are available have different risk, cost, reliability, consistency, sensitivity and specificity. Therefore, clinical research of diagnostic procedures of this growing field is to be encouraged to provide more clinical benefit. Thus, with the knowledge gathered already certain traditional diagnostic dogmas could be amended or changed to improve the efficacy of bedside care.

In this session we will discuss experience in diagnostic process by different experts including radiologist, medical biochemists, cytogeneticists, molecular biologists, cytologists and clinicians. We will share their point of view about individual and combined techniques, and will discuss complexity of diagnostic clinical decision making.

Clinical Cytology / Invited Lectures / 07

FINE-NEEDLE ASPIRATION (FNA) OF LYMPH NODES - AN IMMUNOPHENOTYPING APPROACH

Kardum Paro MM[1], Šiftar Z[1], Nazor A[1], Flegar-Meštrić Z[1], Kardum-Skelin I[2], Šušterčić D[2], Jakšić B.[2]

[1]Institute of Clinical Chemistry, Merkur University Hospital, Zagreb, Croatia
[2]Department of Internal Medicine, Merkur University Hospital, Zagreb, Croatia
mariana.kardum@zg.htnet.hr

Aim of the study: The aim of this study was to determine the potential application of flow cytometric immunophenotyping (FCI) in distinguishing reactive benign lymphoid proliferations (BLP) from malignant B-non- Hodgkin lymphomas (B-NHL) and its subclassification by specific and sensitive identification of the malignant cells in lymph-nodes fine-needle aspirates (FNA) with suspected lymphoma.

Material and Methods: In 423 FNA the particular pattern of abnormal antigen expression was used to identify (multiparameter flow cytometer Coulter EPICS-XL) and quantify (Coulter EPICS XL System II software) a malignant clone. Comparison of FCI and cytologic results was done. Results. Only in 18.6% (92) cases insufficient cels were collected. 69 cases (18.3%) were diagnosed as reactive process, but in 258 cases (67.6%) from total of 327 malignant cells were identified. In reactive hyperplasia, polyclonality was disclosed while B-cll lymphomas showed immunophenotypes for B-cell lineages with 6/8 or 8/6 ratio being over 3/1. Obtained diagnostic concordance between FCI and cytomorphology was 93.2% (reactive hyperplasia group) and 94.8% (B-NHL group). Further subclassification of B-NHLs according to The Working Classification of Lymphomas into low-grade NHLs (B-CLL; B-NHL/FCC) and high-grade NHLs (large cells lymphoma) yielded diagnostic concordance of 93.6%, 88.2% and 86.1%.

Conclusion. The advantages of FCM are in the detection of monoclonal cells in a background of reactive cells. FCI permits the separation between reactive processes and lymphoid malignancies even in less frequently used tissue biopsies (FNA) achieving the objective evaluation results at cell concentration of at least 0.5 x 106 cells /mL when FCI routine instrument settings are used.

Clinical Cytology / Invited Lectures / 08

BILIARY BRUSHING CYTOLOGY

Kocjan G.

University College London, UK

Improved methods of tissue diagnosis for obstruction at the hilum of the liver have contributed substantially to the preoperative diagnosis of cholangiocarcinoma. Endoscopic brushing during endoscopic retrograde cholangiopancreatography (ERCP), with sensitivity of 33% to 100%, is the preferred technique for obtaining accurate pathologic results [1-24]. Extensive hepatic resection with curative intent as well as modern approaches to palliative treatment are based on definitive diagnosis.

Cytological features are classified as: benign, reactive, low grade dysplasia, high grade dysplasia or adenocarcinoma[9]. Cytological dysplasia occurs frequently, with high-grade dysplasia being strongly suggestive of malignancy. Likelihood ratios can be used to calculate the probability of malignancy for any diagnosis[25]. Key malignant cytological features are: 3D micropapillae, anisonucleosis, nuclear contour irregularity, prominent nucleoli, and high N/C ratio[24]. Percutaneous cholangiography (PTC) had a significantly lower sensitivity rate (42.8%) and higher rate for unsatisfactory specimens (21%) compared with ERCP-obtained material (100 and 1.9%)[4]. Intraoperative cytology is more sensitive than non-operative examination (80% v 42%)[26]. Sampling error is a major cause of false-negative diagnoses , followed by interpretive and technical errors [10].

The problems encountered in draining the bile ducts endoscopically in patients with malignant obstruction of the mid or distal common bile duct and/or the papilla appear to be due to the failure to insert a stent [27]. Stricture dilation does not improve the sensitivity of brush cytology for the detection of cancer. However, repeat brushing increases the diagnostic yield and should be performed when sampling biliary strictures with a cytology brush at ERCP [3]. Some authors report enhanced sampling with a modified brushing device which consists of a dilator which has an attached pad of Velcro [28]. The sensitivity of fine needle aspiration (67%) is greater than that of brush cytology (40%) or exfoliative cytology (30%)[26] whilst bile cytology appears less sensitive [4 29]. The combination of stricture dilation, endoscopic needle aspiration, and biliary brushing significantly improves diagnostic yield for malignant bile duct strictures and may particularly be of benefit for extrinsic strictures caused by pancreatic or gallbladder carcinoma[5 30]. When standard methods of tissue diagnosis are inconclusive, EUS-guided FNA may have a potential role in the diagnosis of primary cholangiocarcinoma of the hepatic hilum.[6]. Although cytological brushings are used in preference to biopsy, some centers report higher diagnostic accuracy achieved with the tissue biopsy[17]. Reasons for failure are due to the following: Location, predominance of tumor-induced stroma, an extramucosal growth pattern, sampling error, poor cellular preservation, cells obscured by bile and interpretative experience influence the diagnostic evaluation.[1] Experience is necessary to appreciate subtle malignant changes in well differentiated carcinomas. The review of "false-negative cases" reveals four main reasons for the relatively modest sensitivity of BDB in diagnosis of malignancy: poor sampling, lack of diagnostic criteria for dysplasia-carcinoma in situ, difficulties in recognition of special tumor types, and underestimating the

significance of the smear background[31]. While interobserver variability is very low, variability in cellularity obtained from one brush to the next, is very high. This sampling variability may partially explain the low yield in malignant cells in malignant biliary brushing. Multiple brushings in one patient may alleviate part of this problem[32]. Bile duct brushing is a sensitive method of detecting neoplasia in the setting of Primary sclerosing cholangitis when well-defined cytological criteria are applied [33]. Monolayer cytological preparations detect equivalent disease to conventional bile duct brushings, the only difference being a better preservation and cytological detail on Thin prep[34]. Ploidy assessment by image analysis has potential to enhance the sensitivity of diagnosing malignant strictures compared with routine cytology alone[35]. The findings suggest that image analysis may help select patients having atypical cytology who should undergo a more rigorous evaluation for malignancy[36]. In situ hybridization for telomerase RNA in endoscopic brushings may be an important adjunct to cytology for the diagnosis of pancreaticobiliary malignancy[13]. Loss of heterozygosity (LOH) in a panel of microsatellite markers performed on biliary brushings shows high concordance rate with histopathology[37]. The addition of flow cytometry for DNA content to routine cytology, although increases the diagnostic yield of brushings, may lead to false-positive results[38]. Ki-ras analysis is a sensitive method for diagnosing pancreatic but not biliary carcinoma[39].

In addition to bile duct brushings Fine Nedle Aspiration Cytology (FNAC) is commonly performed at the time of endoscopic ultrasound (EUS) to obtain cellular material for cytological examination. In expert hands, EUS-FNAC has a diagnostic accuracy of greater than 70-90% for the detection of malignancy[40]. Immediate review of the aspirated material improves the adequacy of sampling as well as the accuracy of cytological diagnosis, but this service is not readily available in some institutions. In addition, the diagnosis of certain neoplasms such as lymphoma, stromal and neuroendocrine tumours, as well as well-differentiated pancreatic cancer, can be difficult based on evaluation of cytological specimens alone. Recently, a novel 19g trucut-type needle (TNB) has been designed to obtain core biopsies during EUS. The new EUS TNB technique was seen initially as a method that will be superior to those previously used. So far, the reported results of trucut biopsy compared with EUS FNA appear modest[41 42]. As experience with EUS TNB has now shown, pancreatic lesions are harder to biopsy, partly because of the anatomical position of pancreatic tumours and partly due to their fibrotic nature, making it hard for the needle to penetrate and causing the EUS to move reducing the ultrasound interface. Trans-duodenal TNB puncture is very difficult also due to the rigidity of the needle and the fragility of its firing mechanism. Damage to the endoscope can result and tissue yield is reduced. All this makes EUS FNA the preferred sampling modality. Given the technical difficulties associated with EUS TNB, we have resorted to the routine use of combined EUS FNA and TNB so that the results of both techniques could be used to the best advantage. The main benefit of the combined FNA/TNB approach is in the adequacy of the material obtained.

Conclusions: Brush cytology of pancreatobiliary strictures is the most widely used technique in the diagnosis of carcinoma, with a high degree of specificity. Improvements in sensitivity and diagnostic accuracy for cancer of the pancreatobiliary tract can be achieved by optimizing slide preparatory techniques. Also, enhancement of the cytologist's diagnostic skills enables the identification of the morphologic features of premalignant lesions[20]. Repeat brushings are indicated for suspicious or negative results not consistent with the clinical or radiologic findings[10]. Communication between the cytopathologist and the clinician is critical in the accurate interpretation and proper management of the patients[4].

EUS-FNAC achieves high adequacy and diagnostic accuracy rates. Transduodenal EUS TNB of pancreatic lesions is technically difficult and associated with a higher false negative rate than other biopsy sites. EUS FNA achieves high sensitivity and NPV rates and is sufficient for diagnosis in most cases. The added use of TNB helped define some tumours more precisely (e.g. lymphoma, GIST, small cell carcinoma, gastrinoma), through histological staging and immunohistochemistry.

1. Bardales RH, Stanley MW, Simpson DD, Baker SJ, Steele CT, Schaefer RF, et al. Diagnostic value of brush cytology in the diagnosis of duodenal, biliary, and ampullary neoplasms. *Am J Clin Pathol* 1998;109(5):540-8.
2. Davidson B, Varsamidakis N, Dooley J, Deery A, Dick R, Kurzawinski T, et al. Value of exfoliative cytology for investigating bile duct strictures. *Gut* 1992;33(10):1408-11.
3. de Bellis M, Fogel EL, Sherman S, Watkins JL, Chappo J, Younger C, et al. Influence of stricture dilation and repeat brushing on the cancer detection rate of brush cytology in the evaluation of malignant biliary obstruction. *Gastrointest Endosc* 2003;58(2):176-82.
4. de Peralta-Venturina MN, Wong DK, Purslow MJ, Kini SR. Biliary tract cytology in specimens obtained by direct cholangiographic procedures: a study of 74 cases. *Diagn Cytopathol* 1996;14(4):334-48.
5. RJ, Jain AK, Brandwein SL, Wang H, Chuttani R, Pleskow DK. The combination of stricture dilation, endoscopic needle aspiration, and biliary brushings significantly improves diagnostic yield from malignant bile duct strictures. *Gastrointest Endosc* 2001;54(5):587-94.
6. Fritscher-Ravens A, Broering DC, Sriram PV, Topalidis T, Jaeckle S, Thonke F, et al. EUS-guided fine-needle aspiration cytodiagnosis of hilar cholangiocarcinoma: a case series. *Gastrointest Endosc* 2000;52(4):534-40.
7. Glasbrenner B, Ardan M, Boeck W, Preclik G, Moller P, Adler G. Prospective evaluation of brush cytology of biliary strictures during endoscopic retrograde cholangiopancreatography. *Endoscopy* 1999;31(9):712-7.
8. Govil H, Reddy V, Kluskens L, Treaba D, Massarani-Wafai R, Selvaggi S, et al. Brush cytology of the biliary tract: retrospective study of 278 cases with histopathologic correlation. *Diagn Cytopathol* 2002;26(5):273-7.
9. Layfield LJ, Wax TD, Lee JG, Cotton PB. Accuracy and morphologic aspects of pancreatic and biliary duct brushings. *Acta Cytol* 1995;39(1):11-8.
10. Logrono R, Kurtycz DF, Molina CP, Trivedi VA, Wong JY, Block KP. Analysis of false-negative diagnoses on endoscopic brush cytology of biliary and pancreatic duct strictures: the experience at 2 university hospitals. Arch *Pathol Lab Med* 2000;124(3):387-92.
11. Macken E, Drijkoningen M, Van Aken E, Van Steenbergen W. Brush cytology of ductal strictures during ERCP. Acta Gastroenterol Belg 2000;63(3):254-9.
12. Mansfield JC, Griffin SM, Wadehra V, Matthewson K. A prospective evaluation of cytology from biliary strictures. *Gut* 1997;40(5):671-7.
13. Morales CP, Burdick JS, Saboorian MH, Wright WE, Shay JW. In situ hybridization for telomerase RNA in routine cytologic brushings for the diagnosis of pancreaticobiliary malignancies. Gastrointest Endosc 1998;48(4):402-5.
14. Ponchon T, Gagnon P, Berger F, Labadie M, Liaras A, Chavaillon A, et al. Value of endobiliary brush cytology and biopsies for the diagnosis of malignant bile duct stenosis: results of a prospective study. *Gastrointest Endosc* 1995;42(6):565-72.
15. Rabinovitz M, Zajko AB, Hassanein T, Shetty B, Bron KM, Schade RR, et al. Diagnostic value of brush cytology in the diagnosis of bile duct carcinoma: a study in 65 patients with bile duct strictures. *Hepathology* 1990;12(4 Pt 1):747-52.
16. Renshaw AA, Madge R, Jiroutek M, Granter SR. Bile duct brushing cytology: statistical analysis of proposed diagnostic criteria. *Am J Clin Pathol* 1998;110(5):635-40.
17. Rossi M, Lemos A, Bonaiuti P, Amoruso M, Petrone A, Petrozza V, et al. [Instrumental diagnosis of obstructive jaundice: brushing versus biopsy]. *Radiol Med (Torino)* 1997;93(3):230-5.
18. Ryan ME. Cytologic brushings of ductal lesions during ERCP. *Gastrointest Endosc* 1991;37(2):139-42.
19. Sachdev A, Duseja A, Bhalla A, Handa U, Sandhu BS, Gupta V, et al. Efficacy of endoscopic wire guided biliary brushing in the evaluation of biliary strictures. *Trop Gastroenterol* 2003;24(4):215-7.

20. Stewart CJ, Mills PR, Carter R, O'Donohue J, Fullarton G, Imrie CW, et al. Brush cytology in the assessment of pancreatico-biliary strictures: a review of 406 cases. *J Clin Pathol* 2001;54(6):449-55.

21. Trent V, Khurana KK, Pisharodi LR. Diagnostic accuracy and clinical utility of endoscopic bile duct brushing in the evaluation of biliary strictures. *Arch Pathol Lab Med* 1999;123(8):712-5.

22. Vandervoort J, Soetikno RM, Montes H, Lichtenstein DR, Van Dam J, Ruymann FW, et al. Accuracy and complication rate of brush cytology from bile duct versus pancreatic duct. *Gastrointest Endosc* 1999;49(3 Pt 1):322-7.

23. Witte S, Langer J. [Endoscopic cytodiagnosis of the pancreas and bile ducts]. *Med Klin (Munich)* 1991;86(9):449-53.

24. Ylagan LR, Liu LH, Maluf HM. Endoscopic bile duct brushing of malignant pancreatic biliary strictures: retrospective study with comparison of conventional smear and ThinPrep techniques. *Diagn Cytopathol* 2003;28(4):196-204.

25. Lee JG, Leung JW, Baillie J, Layfield LJ, Cotton PB. Benign, dysplastic, or malignant--making sense of endoscopic bile duct brush cytology: results in 149 consecutive patients. *Am J Gastroenterol* 1995;90(5):722-6.

26. Desa LA, Akosa AB, Lazzara S, Domizio P, Krausz T, Benjamin IS. Cytodiagnosis in the management of extrahepatic biliary stricture. *Gut* 1991;32(10):1188-91.

27. Boender J, Nix GA, Schutte HE, Lameris JS, van Blankenstein M, Dees J. Malignant common bile duct obstruction: factors influencing the success rate of endoscopic drainage. *Endoscopy* 1990;22(6):259-62.

28. Parasher VK, Huibregtse K. Endoscopic retrograde wire-guided cytology of malignant biliary strictures using a novel scraping brush. *Gastrointest Endosc* 1998;48(3):288-90.

29. Kurzawinski T, Deery A, Dooley J, Dick R, Hobbs K, Davidson B. A prospective controlled study comparing brush and bile exfoliative cytology for diagnosing bile duct strictures. *Gut* 1992;33(12):1675-7.

30. Kurzawinski T, Deery A, Davidson BR. Diagnostic value of cytology for biliary stricture. *Br J Surg* 1993;80(4):414-21.

31. Kocjan G, Smith AN. Bile duct brushings cytology: potential pitfalls in diagnosis. *Diagn Cytopathol* 1997;16(4):358-63.

32. Silverman WB, Jensen CS, Crook TW, Henke A. Bile duct brushings in a pig model: examination of intraobserver variability and variability in specimen quality obtained in sequential animals and between two different brushes. Int *J Gastrointest Cancer* 2002;32(1):31-4.

33. Lal A, Okonkwo A, Schindler S, De Frias D, Nayar R. Role of biliary brush cytology in primary sclerosing cholangitis. Acta Cytol 2004;48(1):9-12.

34. Siddiqui MT, Gokaslan ST, Saboorian MH, Carrick K, Ashfaq R. Comparison of ThinPrep and conventional smears in detecting carcinoma in bile duct brushings. *Cancer* 2003;99(4):205-10.

35. Rumalla A, Baron TH, Leontovich O, Burgart LJ, Yacavone RF, Therneau TM, et al. Improved diagnostic yield of endoscopic biliary brush cytology by digital image analysis. *Mayo Clin Proc* 2001;76(1):29-33.

36. Krishnamurthy S, Katz RL, Shumate A, Strohlein K, Khanna A, Tucker SL, et al. DNA image analysis combined with routine cytology improves diagnostic sensitivity of common bile duct brushing. *Cancer* 2001;93(3):229-35.

37. Ohori NP, Fowler MH, Swalsky PA, Pal R, Thompson J, Finkelstein SD. Comparative molecular analysis of loss of heterozygosity in adenocarcinoma in bile duct brushings and corresponding surgical pathology specimens. *Cancer* 2003;99(6):379-84.

38. Ryan ME, Baldauf MC. Comparison of flow cytometry for DNA content and brush cytology for detection of malignancy in pancreaticobiliary strictures. Gastrointest Endosc 1994;40(2 Pt 1):133-9.

39. Van Laethem JL, Bourgeois V, Parma J, Delhaye M, Cochaux P, Velu T, et al. Relative contribution of Ki-ras gene analysis and brush cytology during ERCP for the diagnosis of biliary and pancreatic diseases. *Gastrointest Endosc* 1998;47(6):479-85.

40. Ylagan LR, Edmundowicz S, Kasal K, Walsh D, Lu DW. Endoscopic ultrasound guided fine-needle aspiration cytology of pancreatic carcinoma: a 3-year experience and review of the literature. *Cancer* 2002;96(6):362-9.

41. Varadarajulu S, Fraig M, Schmulewitz N, Roberts S, Wildi S, Hawes RH, et al. Comparison of EUS-guided 19-gauge Trucut needle biopsy with EUS-guided fine-needle aspiration. *Endoscopy* 2004;36(5):397-401.

42. Larghi A, Verna EC, Stavropoulos SN, Rotterdam H, Lightdale CJ, Stevens PD. EUS-guided trucut needle biopsies in patients with solid pancreatic masses: a prospective study. *Gastrointest Endosc* 2004;59(2):185-90.

Clinical Cytology / Invited Lectures / 09

MOLECULAR CHARACTERISATION OF B- AND T- CELL CLONALITY IN REACTIVE AND MALIGNANT LYMPHOID LESIONS

Kušec R[1,2], Bobetić-Vranić T[1], Šiftar Z[1], Kardum-Skelin I[2], Dominis M[3], Jakšić B[2]

[1]Institute of Clinical Chemistry, Divison of Molecular Diagnostics,
[2]Department of Medicine,
[3]Department of Pathology, Merkur University Hospital, Zagreb, Croatia
rkusec@irb.hr

Lymphoid cells proliferations, frequently clinically present as persistent lymphadenopathy and occasionally pose serious diagnostic problems in terms of distinguishing benign from malignant processes. In this context molecular analysis of B- and T-cell receptor rearrangements aimed at answering if the process is polyclonal, reactive non-malignant or clonal, accepted as neoplastic, can be useful. We present the results of molecular characterisation of B-cell receptor by PCR based assay for immunoglobulin heavy-chain gene CDR3 region and T-cell receptor by TCR(multiplex PCR.

Materials were 26 fine needle aspirates and 11 samples of paraffin embedded biopsies of lymph nodes from patients with persistent lymphadenopathy and inconclusive morphological and phenotypic diagnosis on the nature of lymphoid proliferation. Molecular findings of clonality were correlated to definitive cytological and histological diagnosis. Of 37 samples 16 were diagnosed as reactive lymphoid proliferations and 21 as malignant process, eg. lymphoma. For cytological samples (N=26) there was a good correlation of clonality with category of reactive or malignant (P^2 p= 0,017). For histological samples (N=11) there was no significance (P^2 p= 0,24). When both types of materials were analysed clonality tests have strong correlation to the type of lymphoid process (P^2 p= 0,0031).

Six out of 8 T-neoplasms and 11 out of 13 B-neoplasms were clonal in tested molecular assays. In one cytological and one pathological case molecular proof of clonality was decisive for the final diagnosis of neoplasm. In conclusion, molecular tests of lymphoid cells clonality are established and valuable part of the diagnostic process of lymphoproliferative nodal lesions.

Clinical Cytology / Invited Lectures / 10

CURRENT THERAPY FOR ACUTE LEUKEMIA

Labar B.

Department of Medicine, Zagreb Clinical Hospital Center and Medical School University of Zagreb, Zagreb, Croatia
boris.labar@inet.hr

Chemotherapy is still the major treatment approach for acute leukemia. The primary goal of chemotherapy is achievement of a complete remission (CR), defined as the eradication of all detectable leukemia cells from the bone marrow and blood and the restoration of normal hematopoiesis. However, once a CR is achieved, therapy must continue for an extended period of time to eliminate the subclinical disease known to contribute to relapse. Postremission therapy has traditionally been categorized as intensification or consolidation treatment, and prolonged maintenance. Postinduction or "remission consolidation" therapy usually comprises one or more courses of chemotherapy (intensification or consolidation treatment) or bone marrow transplantation and prolonged maintenance. It is designed to eradicate residual leukemia, allowing the possibility of cure. The following clinical and biologic features which correlate with favorable long-term outcome have been identified for AML (Karnofsky score >60%, CD34-negative phenotype, MDR 1-negative phenotype, hypocellular leukemia, normal karyotype, t/8;21/, t /15;17/, inv16, absence of changes in chromosomes 5 and 7 and ALL (younger age /<30/, WBC <30,000/μL, the presence of a mediastinal mass, T-cell or TMy immunophenotype, and absence of the Philadelphia (Ph) chromosome and t /4;11/).

Currently CR rate in AML is achieved in about 70-80% of patients with AML.

The EORTC/GIMEMA AML-10 trial compares the efficacy of allogeneic transplantation with autologous transplantation or intensive chemotherapy as a postremission therapy. The relapse rate was highest in the intensive chemotherapy group and lowest in those receiving allogeneic HCT. The projected rate of disease-free survival was 55 percent for allo-HCT, 48 percent for auto-HCT, and 30 percent for intensive chemotherapy (Table 1). However, the overall survivals were similar (Table 1). Patients relapsing after consolidation chemotherapy had a better survival than those relapsing after HCT.

Table 1. EORTC/GIMEMA AML-10 Trial Testing BMT in Adults with AML in first CR

Subgroup	Ages, years	No of Patients	4-year Estimates, +/- SE	
			DFS %	Survival %
Allogeneic BMT	<65	160	55+/-4	59+/-4
Autologous BMT	<60	128	48+/-5	56+/-5
Chemotherapy	<60	126	30+/-4	46+/-5

CR rate was achieved in 75 percent of adult patients with ALL receiving EORTC ALL-4. In this trial patients were randomized either to receive prednisolon or dexametasone together with chemotherapy for induction therapy. CR rate, DFS and survival status are practically identical for both groups (Table2.)

Table 2. Outcome by induction randomization EORTC ALL-4 trial

	dexamethasone	prednisone
CR	125(76%)	120 (74%)
DFS status		
CCR	49 (30%)	55 (34%)
Relapse	58 (36%)	57 (35%)
Death in CR	18 (11%)	8 (5%)
Survival status		
Alive	67 (41%)	72 (43%)
Dead	96 (59%)	92 (57%)

Patients receiving allogeneic transplantation in postremission therapy are doing better compared to patients treated with autologous transplantation or chemotherapy. According to intention to treat analysis, EORTC ALL-4 trial proved that patients with suitable donor have better survival and lower relapse rate. But treatment related mortality (16%) is too high and remain the main problem of allografting (Table 3.)

Table 3. Outcome by donor availability EORTC ALL-4 trial

	No donor	Donor
	(N = 82)	(N = 71)
CCR	36 (44%)	37 (52%)
Relapse	45 (55%)	23 (32%)
Death in CR	1 (1%)	11 (16%)
Survival status		
Alive	42 (51%)	42 (59%)
Dead	40 (49%)	29 (41%)

In conclusion more intensive chemotherapy for acute leukemia (i.e. allogeneic transplantation) increase long term survival for 10-20% in patients younger than 55 years of age. High treatment toxicity and mortality remain the main problem of such therapy. Better understanding of leukemia pathogenesis, recognition of unfavorable prognostic factors especially the monitoring of minimal residual leukemia, new treatment modalities (especially targeted therapy) and individualized treatment approach according to farmacogenomics might further improve the leukemia outcome.

Clinical Cytology / **Invited Lectures** / **11**

RESULTS OF CONVENTIONAL CYTOGENETICS AND FISH ANALYSIS IN PATIENTS WITH LYMPHOMA IN FINE NEEDLE ASPIRATES

Lasan Trčić R[1], Hitrec V[1], Kardum-Skelin I[2], Šušterčić D[2], Fabijanić I[2], Jelić-Puškarić B[2], Jakšić B.[2]

[1]Division of Genetics and Metabolism, Cytogenetic Laboratory, Department of Pediatrics, University Hospital Centre Zagreb,
[2]Merkur University Hospital, Zagreb, Croatia
lasan_ruzica@yahoo.com

Aim of the study: The detection of chromosomal abnormalities characteristic of lymphomas, is important in the diagnostic workup of aggressive lymphomas given its impact on treatment strategies and prognosis. Recently this has been accomplished using FISH. In conformation with other methods for collecting samples makes the fine needle aspiration (FNA) attractive for diagnosis.

Patients and Methods: We report the cytogenetic investigation in series of 59 patients with lymphoma (28 women and 31 men, median age 40, ranged 3-90 years), comprising 42 non-Hodgkin lymphomas (NHL) (32 abnormal) and 4 Hodgkin disease (HD) (2 abnormal).

Results: In our series 91.5 % of the specimens yield sufficient numbers of analysable metaphases. Among the 54 successful karyotiped specimens 41 (75.9%) showed clonal karyotipic abnormalities. Numerical changes in 4 cases, and numerical with structural changes in 17 cases; trisomies 3, 7, 8, 12, X and monosomy 1 were most frequent. The NHL cases were typically characterised by structural rather than numerical aberrations with chromosome arms 1p/q, 3p/q, 6q, 11q and 14q most frequently involved. The expected t (8;14) (q24;q34) and t (14;18) (q32;q21) translocations were present in 11 (34,37%) N-NHL.

Conclusion: The detected abnormal clones in HD were typically very complex and comprised only a small percentage of metaphases. FISH permited to detect loss or gain of genetic material and reveal rearrangements suspected by conventional abnormalities in 14 (25.9%) cases.

Clinical Cytology / **Invited Lectures** / **12**

TARGET BIOPSY OF INTRAABDOMINAL ORGANS LOCALIZED LESIONS

Odak D[1], Kardum-Skelin I[2], Šušterčić D [2]

University Hospital "Merkur", [1]Department of Diagnostic and Interventional Radiology, [2]Laboratory of Cytology and Haematology, Department of Medicine, Zagreb, Croatia

Ultrasound diagnosis of focal intraabdominal lesions and cytology analysis of the material obtained presents a combined - "duplex" diagnostic procedure which is confirmatory of clinical diagnosis and determinative of further therapy.

Generally, the material obtained by fine needle aspiration biopsy is sufficient for diagnosis, and exclusive of other pathomorphological diagnostic procedures (eg. histological analysis, nuclear diagnostic techniques, etc.)

Collecting of material for cytologic examination may be performed using a free hand technique or a guide which introduces the tip of the needle in the middle of the lesion by means of a fixed procedure.

Lesions aspirated may be solid, liquid or "decomposed". Material may be sampled from all solid organs or from the intraabdominal regions which are a free space (eg. subhepatically, paraaortally, or perivesically).

Target aspiration biopsy may also be performed in the hollow intraabdominal organs (the intestines) which contain air if a pathological process invades into the lumen , wall or the surrounding tissue of an organ (eg. perityphlitic abscess).

In the Merkur University Hospital ultrasound-guided target aspiration biopsy has been performed since 1978, and is an established diagnostic procedure in the clinical diagnostic algorithm.

Clinical Cytology / Invited Lectures / 13

CLINICAL CYTOLOGY IN DIAGNOSIS ANDMANAGEMENT OF LUNG TUMORS

Olszewski T.W.

Department of Pathology, Cancer Center and Institute of Oncology, Warsaw, Poland

In spite of significant progress in the tumor biology and genetic studies still the most reliable diagnostic method in tumor pathology is the evaluation of specimen in light microscope. Development of imaging modalities and endoscopy has allowed obtaining ade¹uate material for microscopic evaluation practically from each location in the body in-cluding lung. In the diagnosis of lung malignancies clinical cytology is almost equal in its practical value to histopathology.

Different types of cytological material from lung may be the source of diagnosis (Fig. 1). Considering clinical setting pleural effusion and fine needle aspirates of enlarged supraclavicular lymph nodes should also be included in diagnostic as a source of diagnostic material. Each type of these specimen has advantages and limitations.

Sputum used to be the most important type of specimen in lung diagnosis but that type of diagnostic material in not appropriated for peripherally located lesions. It is also almost always negative for metastatic lesions. Bronchial brush became most reliable material for diagnosing centrally located lesion. Material obtained by that way is usually well preserved and taken from the most suspicious area in bronchoscopy.

Transthoracic fine needle aspiration biopsy is widely used technique for the primary diagnosis of lung malignancies. Cytodiagnostic criteria allows to make a correct morphological diagnosis. False positive diagnoses can be avoided and percentage of false negative ones is very low. The errors are mostly due to scanty cellular content. Cytological features allow to establish firm diagnosis of small cell carcinoma and differentiate them from non-small cell carcinomas. This is the most important requirement for proper management of a patient with primary lung carcinoma.

Metastatic tumors can be also correctly diagnosed cytologically. Considering clinical data and cytological features in numerous cases often not only metastatic nature of tumors can be correctly diagnosed but also the origin of lesion can be con-firmed. Immunocytochemistry is very useful in evaluation of metastatic tumors. This technique may be particularly useful in search for the location of primary tumor in case when the lung metastasis is the first confirmation of malignancy.

Transbronchial fine needle aspiration biopsy may serve both as diagnostic and staging method of lung carcinomas. Fig 2. presents the algorithm of diagnostic and therapeutic approach to lung tumors after transthoracic and / or transbronchial aspiration biopsy have been done for diagnostic and staging purpose. The choice of cytological material for microscopic evaluation of lung tumors depends on clinical setting, location of a lesion and general status of a patient. Figure 3 presents the adequate choice of cytological specimen.

References

1. Koss LG., Woyke S. Olszewski W. Aspiration Biopsy. Cytologic Interpretation and Histologic Bases. Igaku Shoin, New York, 1992

2. Olszewski W. Mediastinum. in J.A. Young (ed.) Fine Needle Cytopathology. Blackwell Sci. Pub. London 1993, pp.l22-133

3. Olszewski W. Aspiration biopsy in intrathoracic lesions. Current Diagnostic Pathology 1995, 2: 146 - 152

Fig. 1

LUNG LESIONS

Types of specimen for cytological evaluation

- SPUTUM
- BRONCHIAL BRUSH
- BRONCHIAL WASH
- TRANSTHORACIC FNAB
- TRANSBRONCHIAL FNAB
- ----------
- PLEURAL EFFUSION

Fig. 2

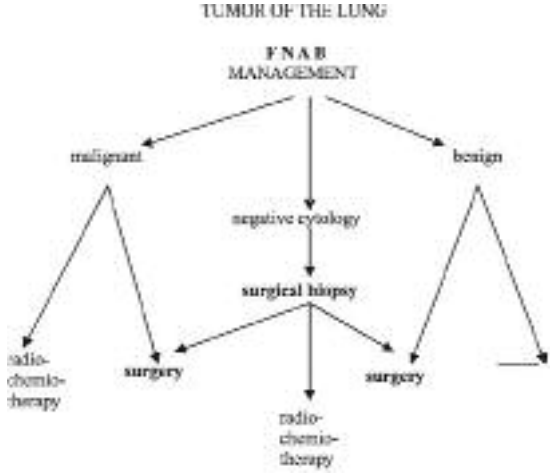


Fig. 3

LUNG TUMORS
INDICATIONS FOR TYPE OF CYTOLOGIC SPECIMEN

CENTRAL	INTERMEDIATE	PERIPHERAL
• SPUTUM	• BRONCHIAL BRUSH	• ASPIRATION BIOPSY
• BRONCHIAL BRUSH	• ASPIRATION BIOPSY	• Bronchial wash
• Bronchial wash	• SPUTUM	
• Aspiration biopsy	• BRONCHIAL WASH	

Clinical Cytology / Invited Lectures / 14

ALKoma: WHAT DO WE KNOW TODAY ABOUT THIS LYMPHOMA ENTITY?

Ostojić Kolonić S[1], Planinc-Peraica A[1], Ljubić M[1], Dominis M[2], Jakšić B.[1]

[1]Division of Haematology Department of Medicine, [2]Department of Pathology and Cytology, Merkur University Hospital, Zagreb, Croatia
ostojic@net.hr

Anaplastic large cell lymphoma (ALCL) presents clinically and morphologically as a heterogeneous disease. Existence of reciprocal translocation t(2;5)(p23;q35) or t(2;5) variants and oncoprotein NPM-ALK expression by tumour cells is frequently found associated with this lymphoma. The results of Falini B. et al. in 1999. showed that patients with primary systemic ALCL expressing NPM-ALK fusion protein or its variants had better prognosis than ALK negative ALCL. The authors proposed the new lymphoma entity: ALK+ALCL or ALKoma and this proposal was widely accepted. Interestingly, since 2002. there were only few new literature reports with clinical informations on this item. At our institution, since 1999. the great interest for this subgroup of lymphoma was present and searching for ALK expression in cases with ALCL and Hodgkin's disease became the standard procedure. Occasionally, additional immunohistochemical studies for the expression of nucleophosmin (NA24) and molecular test for NPM-ALK fusion gene were done. Our first results in 2000 on correlating ALK expression to patients' survival showed that expression of ALK had no prognostic value in patients with ALCL, and that rare patients with morphologic diagnosis of Hodgkin's lymphoma were positive for ALK immunostaining. At that time we could not confirm the importance of ALK expression and definition of ALKoma as a new entity. We present now a new prognostic analysis of ALK expression in the group of adult patients with nodal ALCL and Hodgkin's lymphoma and suggest new tools for differentiation between ALKoma and Hodgkin's disease.

Clinical Cytology / Invited Lectures / 15

ROLE OF FNA CYTOLOGY IN GASTROENTEROLOGY AND HEPATHOLOGY

Papa B.

Merkur University Hospital, Zagreb, Croatia

In the last decades cytodiagnosis has become a method of choice in routine evaluation of patients with gastrointestinal malignancies. Immediate diagnosis of malignancy of the oesophagus, stomach, duodenum, colorectum and biliary tract could be achieved by analysis of imprint or brushing specimens obtained during the endoscopic session.

Early detection and exact estimation of the nature of these tumours is of essential importance for treatment and for prognosis.

US- or CT- guided FNA cytology plays an important role in the diagnosis and classification of malignancies of the liver. Cytology analysis should be subsequently correlated with histopathology which facilitates exact tumour typing and assessment

The role of cytology is crucial in the diagnosis of peritoneal cavity fluids and in the differentiation serous surface carcinoma of the peritoneum from other morphologycally similar tumours including ovarial carcinoma.

Improvement in diagnosis of acute GI diseases such as acute appendicitis and acute pancreatitis could be achieved by using FNA cytology. Diagnosis of infected pancreatic necrosis - by using US guided FNA analysis has become the standard procedure in the treatment of severe acute pancreatitis. US guided FNA is recommended for all patients with necrotizing pancreatitis in whom SIRS persists beyond the first week after onset of symptoms. This approach could substanially improve prognosis for patients with these severe disease.

FNA cytology is of great value in the assesment of pancreatic and peripancreatic malignancies. Preoperative assesment of pancreatic tumour extension allows to choose the best treatment options and to reduce the nummber of unnecesarry explorative laparotomies.

Intraoperative FNA in suspected pancreatic cancer associated with chronic pancreatitis is a suitable method for intrasurgical confimation of pancreatic cancer.It can be performed safely, effectively and rapidly.

Peritoneal washing-cytology at laparotomy is realible method for detecting metastatic spread of pancreatic cancer. Peritoneal metastases are the second most common site of involment, following the liver. Cytologycal diagnosis could be improved by use of immunocytochemical staining methods. The method of perfoming K-ras point mutation has potential to become the standard for peritoneal washing cytology in pancreatic cancer.

It should be pointed out that false negative FNA cytology could postponne diagnosis and treatment of GI malignancies. In the diagnosis of small resectable pancreatic tumours it is not necessary to wait for cytologycally proven diagnosis. When there are enough data from clinical and imaging methods (CT, ERCP) for the diagnosis of pancreatic cancer it is not

necessary to prove the diagnosis cytologycally before performing pancreatectomy

Diagnosis of pancreatic cancer in early stage could be improved by use of cytology and tumor markers analysis in pure pancreatic juice. In the population under increased risk for developing pancreatic cancer (such as chronic pancreatitis patients) cytologycal analysis of pure pancreatic juice combined with elevated levels of CEA and Ca 19.9 in the juice are usefull tools for pancreatic cancer diagnosis even in the early stages.

Clinical Cytology / Invited Lectures / 16

INTRAOPERATIVE IMPRINT CYTOLOGY OF SENTINEL LYMPH NODES IN BREAST CANCER

Pogačnik A, Klopčič U, Grazio-Frković S, Žgajnar J, Vidergar-Kralj B.

Department of Cytopathology, Institute of Oncology, Ljubljana, Slovenia

The status of the regional lymph node (LN) remains the most important predictor of survival in women with invasive breast cancer. As clinical assessment of LNs is unreliable, axillary lymph node dissection (ALND) has been the only precise method for determining LN status. Recent data suggest that as many as 70% of women with early breast cancer have no metastases in axillary LN, therefore unnecessary ALDN should be avoided. As an alternative to routine ALDN sentinel lymph node (SLN) biopsy was recently introduced, so that ALDN is performed only in patients with metastatic deposits in SLN. Two different strategies of intraoperative SLN investigation are in use worldwide, frozen section (FS) and intraoperative imprint cytology (IC). At the Institute of Oncology Ljubljana, FS of SNLs was not introduced because LNs may undergo artefactual changes, e.g. all of the tissue may be used up. On the other hand, imprint cytology provides clear cytological details, rapid diagnosis, and above all preserves tissue for permanent sectioning.

From June 2001 to September 2003, 413 women with fine-needle aspiration biopsy or core biopsy proven carcinomas were included in the study. Mapping of SLN involved injection of ^{99m}Technecium labeled human albumin nanocolloid particles and Patent Blue dye. Excised SLNs were sent to the pathology department to examine intraoperatively. SLN were bisected along the long axis, from each slice imprints were made. All slices were than formalin fixed and embedded in paraffin.

Imprint cytology detected 54/69 macro metastases, 4/57 micrometastases. In the group with negative SLN (254), two cases were "false positives".

Specificity of imprint cytology in our series is 98.8%, which is very important for those women who benefit from having just SLN biopsy, thus avoiding morbidity of axillary LN dissection. Overall sensitivity is only 36.4%, but detection of macrometastases is 77%, which is important for performing ALDN in one session with operation of primary tumor and sparing second operation to a large number of patients.

Clinical Cytology / **Invited Lectures** / **17**

PROBLEMS IN CYTOPATHOLOGY OF CHILDHOOD

Pohar-Marinšek Ž

Department of Cytopathology, Institute of Oncology, Ljubljana, Slovenia
zpohar@onko-i.si

Aim of the study: to investigate accuracy in diagnosing malignant tumors in children from FNAB samples.

Methods: The study included 324 patients younger than 19 years. We compared cytological and histological diagnoses in 72 lymphomas, 69 nephroblastomas, 57 neuroblastomas, 49 osteogenic sarcomas, 33 Ewing/PNET, 37 rhabdomyosarcomas and 20 germ cell tumors. In less frequent entities we investigated cases as far back as 33 years into the period before immunocytochemistry. Results: Percentage of correct specific diagnoses ranged from 66-85%. The overall results were 10-15% better during the period when immunocytochemistry was used. The main problem in lymphomas was differentiating ALCL from Hodgkin's lymphoma. Nephroblastoma was difficult to recognize when only undifferentiated blastema was present in smears. Similarly, neuroblastoma was not recognized in poorly differentiated cases and was mistaken mostly for nephroblastoma. The second problem was differentiating ganglioneuroblastoma from ganglioneuroma due to sampling errors. Ewing/PNETwas mistaken mostly for alveolar rhabdomyosarcoma. There were two main pitfalls in diagnosing osteogenic sarcoma from FNAB. The fibroblastic variant mimicked osteoclastoma, the chondroblastic variant was identical to chondrosarcoma. Embryonal rhabdomiosarcoma simulated not only other malignant round cell tumors but also some benign lesions such as hemangioendothelioma and infantile myofibromatosis. We found cross reactivity for CD99 and desmin in rhabdomyosarcoma and Ewing/PNET. The main drawback in diagnosing germ cell tumors correctly was sampling error due to tumor heterogeneity. Clearly malignant germ cell tumors were not a problem, however 50% of immature teratomas were missed because the immature component was not sampled.

Conclusions: The high percentage of correct specific diagnoses justifies the use of FNAB for obtaining pretreatment morphological diagnosis.

Clinical Cytology / **Invited Lectures** / **18**

EVALUATION OF US-GUIDED THYROID CYTOLOGY

Tomić-Brzac H, Halbauer M.

Department for Nuclear Medicine and Radiation Protection, University Hospital Center Zagreb, Zagreb, Croatia
hrvojka@brzi.net

Aim of the study: Ultrasound (US) guided fine-needle aspiration biopsy (FNAB) is today an affirmative way to perform cytology for thyroid diseases.

The goal of this study was to present the advantage of US-guided FNAB and an evaluation our results over the last 21 years.

Methods: We used standard ultrasound equipment with a 7,5-13 MHz linear probe. FNAB was performed under US guidance with "free hand" technic.

From 1984 to 2004, we performed 150313 US neck examinations, and 75246 FNAB in 41803 patients. The indications for US-guided FNAB were done on the basis of US examination. Physicians who make a US decide which nodule and which place need cytological verification.

Results: Through 1995 we diagnosed an average of 84 thyroid carcinoma per year (operatively proved). In the last 9 years we diagnosed an average of 113 thyroid carcinoma per year. This is half of all new detected malignant thyroid tumors in Croatia per year. About 200 other tumors per year were diagnosed (follicular tumor, Hürthle cell tumor, adenoma, metastases in thyroid, etc.), which also need a operative treatment. Other patients were treated more conservatively.

Conclusion: With US and US-FNAB it is possible discriminate the nodule suspected of a tumor (malignant or benign) which may need surgery and pathohistological verification, and others which can be safely followed up by sonography. The limitation on US diagnosis is tumors less then 0,3 mm. Based on our lengthy experience, clear indications and team work, thyroid diseases can be diagnosed early and precisely. Thyroid tumors can be adequately treated and in a timely manner. In this way the mortality and morbidity of thyroid tumor diseases are very small.

Clinical Cytology / Invited Lectures / 19

IMPORTANCE OF MOLECULAR DIAGNOSIS FOR MONITORING MRD

Zadro R.

Clinical Institute of Laboratory Diagnosis, Zagreb Clinical Hospital Center and University School of Medicine, Zagreb, Croatia

Although many patients with hematologic malignancies achieve a complete clinical remission and remission by morphologic and immunologic criteria, a relatively high proportion of them will ultimately relapse. A persistent malignant cellular population present at low level, below the limit of detection of standard techniques, is the cause of this relapse and is called minimal residual disease (MRD). Several studies have shown that detection and quantification of residual tumor cells significantly correlate with clinical outcome. The quantitative measurement of the decrease in the leukemic cell load during the initial phases of treatment has a high prognostic value.

Methods to detect MRD include technologies designed to detect residual malignant cells beyond the sensitivity of conventional approaches. Ideally, techniques used for MRD detection should have a sensitivity level in the 10^5 - 10^6 range. Only a few commonly used techniques are sensitive enough for detection of MRD in acute leukemias. Currently, PCR based methods represent the most widely accepted technologies for MRD detection. Over the past 15 years, quantitative PCR assays were developed. Competitive RT-PCR employed to monitor patients after transplantation or treatment with specific agents are time-consuming and cumbersome. Quantification of residual disease has been simplified with the introduction of real-time PCR methodologies and machines. Nested PCR and quantitative real-time PCR can be used for disease-associated translocations. If there is not a good translocation target for PCR analysis, patient-specific gene rearrangements may be targeted.

Clinical Cytology / Invited Lectures / 20

CYTOGENETICS - IMPORTANT PROGNOSTIC FACTOR FOR ACUTE LEUKEMIA

Davidović-Mrsić S

Clinical Institute of Laboratory Diagnosis, Zagreb Clinical Hospital Center and University School of Medicine, Zagreb, Croatia

Chromosomal changes found in acute leukemia are specific and are accompanied by characteristic phenotype and have independent prognostic importance.

More than 160 structural chromosomal aberrations have been described in AML. The majority of well characterized abnormalities are translocations and inversions that form fusion genes. The transcribed protein affects leukaemogenesis by changing the control of cell cycle and apoptosis. Deletions, amplifications, and point mutations are rarely found.

Frequent chromosomal aberrations accompanied by specific leukaemic phenotype become a part of new WHO classification and help to distinguish among specific subtypes of AML.

These subtypes are: t(8;21)(q22;q22), inv (16)(p13q22) or t(16;16)(p13;q11) and t(15;17)(q22;q11-12); they have favourable prognosis and translocations involving chromosome 11q23 , deletion -5/5q-, deletion -7/7q-, translocation of 3q and complex karyotype that has poor prognosis. Patients with other cytogenetic abnormalities and patients with ”normal ” karyotype have intermediate prognosis.

Currently, clonal chromosomal changes are identified in 60 - 90% AML patients and their incidence increases with higher age and sophisticated methods used for their detection.

New methods of molecular cytogenetics will in the future help to explain the differences in the course of AML and will help to stratify all AMLs with ”normal ” karyotype into specific genetically defined subsets with similar prognosis, for which it will be possible to find specific gene therapy.

Both chromosome number (ploidy) and chromosome structure changes have been identified as powerful prognostic indicators in ALL. Ploidy classification was first shown to be of prognostic importance in 1978 when it was shown that children with ALL with a hyperdiploid or a pseudodiploid clone had a significantly better or significantly worse prognosis than the rest of patients. Clinical importance of structural changes which identify subgroups is due to their impact on prognosis and to their association with other prognostic variables. Translocations t(1;19), t(4;11) and t(9;22) are associated with poor prognosis.

Clinical Cytology / Invited Lectures / 21

NEW TECHNOLOGY IN CYTOLOGY: SCIENCE OR MARKETING.

Schneider V.

Pathology Laboratory, Freiburg, Germany

Aims: Cytologic samples of the uterine cervix have been used to screen for precursors of cervical carcinoma since 1945. The technique of Papanicolaou has essentially remained unchanged until recently. Since then, a number of new technologies have been developed to improve shortcomings of the cervical smear. Among these are liquid-based/thin layer preparations, computer assisted screening techniques and new generation human papilloma virus (HPV) testing methods. Currently available evidence on these techniques is being reviewed and critically evaluated.

Methods: The available literature on these techniques was systematically searched for. The results of meta-analyses, health technology assessment (HTA) reports and consensus conferences were considered to represent particularly valuable sources of information. Care was taken to discover potential conflicts of interest in studies co-sponsored by manufacturers and to adherence to the guidelines on sponsorship, authorship and accountability (JAMA 2001: 286; 1232).

Results: The quality of publications on new technologies is highly variable with many being useless due to deficiencies in study design, lack of reference standards and bias introduced by manufacturer and user intentions. There is currently no clear cut evidence for an advantage in sensitivity or specificity of thin layer techniques. There are indications for using HPV testing in a number of well defined situations. Wide spread population screening with HPV testing is currently not indicated.

Conclusions: Due to vested interests of corporations and users of new technology more emphasis should be put on independent studies such as HTA's to come to objective conclusions on the value of these new technologies. Uncritical claims as to the superiority of these techniques are premature at this point in time.

Clinical Cytology / Invited Lectures / 22

THE RELEVANCE OF A SECOND READING OF ASCUS + CYTOLOGY WITH HPV TESTING

Verhest A, Querton G, Fayt I, Noël J-C.

Institut Jules Bordet & Hopital Universitaire Erasme
Université Libre de Bruxelles

Introduction: In our practice of liquid based cytology, we took into account an unavoidable learning curve during which time we might expect a significant number of false positives because abnormal rates of lesions may be highly dependent on experience especially in the ASCUS category.

HPV is a sensitive and reproducible test providing a more objective triage for equivocal Pap smears. Surprisingly rates of HPV detection vary widely in the literature, however hrHPV is present in virtually all cervical cancers and precursor lesions.

Therefore we used HPV testing as an external quality control for improving the accuracy of our cytological screening and measuring our performance in diagnosing ASCUS+ cytologies.

Aim of the study: While we introduced LBC in our laboratory in 1998, we submitted our ASCUS+ cases to a second consensus lecture by two experienced cytopathologists. Simultaneously, the aliquot was also assessed for the presence of high risk HPV (Hybrid Capture II,Digene).

The aim was to compare the first to the second diagnoses and correlate both with the detection of HPV.

Adopting the new Bethesda System 2001, we reviewed 680 cases in 2002 and 457 in 2003, the final goal being to improve the correlation between cytological diagnoses and a positive HPV result in SIL. Concurrently we planned to reduce the number of equivocal Pap smears that are followed by excessive repeat cytology, unnecessary colposcopies and avoidable overtreatment.

Results: In the three last years the HPV control of our abnormal smears showed that SIL cases were accurate enough to adopt the recommendations of the ALTS group by restricting the HPV triage to ASCUS and ASC-H situations.

The weak reproducibility of borderline cytology is emphasized by the number of diagnoses that were modified: the downgrading was particularly high in the ASCUS HPV negative group; upgrading occured preferentially in the ASCUS HPV + group.

In both categories the concordance with the presence of HPV in the sample improved through the years.

Discussion: In this study we have tried to clarify the benefits and limits of HPV testing in the triage of ASCUS+ lesions.

The implementation of a second lecture with HPV reflex test has improved our cytologic criteria for producing a more accurate SIL diagnosis.

ASCUS remains a "non-diagnosis" however HPV triage adds a predictive value to this category allowing a more appropriate clinical management of those patients.

Conclusion: The option of HPV triage for ASCUS follow-up can be considered as an educative tool in a team to assess the individual performance in diagnosing equivocal cases and reducing thus far the misinterpretation of former benign cellular changes or ASC favoring SIL categories.

KLINIČKA CITOLOGIJA
CLINICAL CYTOLOGY

Usmena izlaganja / *Oral presentations (1-35)*

Clinical Cytology / Oral presentations / 01

CYTO-HISTOLOGIC CORRELATION FOR MICROINVASIVE SQUAMOUS CELL CERVICAL CARCINOMA

Antulov J*, Audy-Jurković S*, Ovanin-Rakić A*, Babić D.**

Institute of Gynecologic Cytology, University Department of Gynecology and Obstetrics, Zagreb University Hospital Center, **Clinical Institute of Gynecologic and Perinatal Pathology, Zagreb University Hospital Center, Zagreb, Croatia

Aim of the study. To assess diagnostic accuracy of cytology in histologicaly verified microinvasive squamous cell cervical carcinoma.

Patients and Methods. At Department of Gynecologic and Perinatal Pathology, a definitive histologic diagnosis of microinvasive squamous cell carcinoma in a cone and/or removed uterus was made in 257 patients from January 1, 2000 till December 31, 2004. In 112 patients, Pap test was preoperatively analyzed at Department of Gynecologic Cytology. Diagnostic accuracy of cytology testing was determined by comparison with histologic diagnosis for zero degree of correspondence (identical diagnoses) and within-grade, when a cytologic diagnosis of microinvasive carcinoma was associated with the diagnosis of grade III cervical intraepithelial neoplasia, considering the possibly identical therapeutic procedure. The depth of invasion was taken in consideration on the cytology to histology comparison.

Results. The overall diagnostic accuracy of cytology for microinvasive squamous epithelial carcinoma up to 5 mm at zero degree of correspondence was 30.3% (34/112). The rate of accuracy slightly increased with the decrease of invasion depth to 3 and 1 mm to 30.84% (33/107) and 35.16% (32/91), respectively. The cytodiagnostic within-grade accuracy for stromal lesions up to 5 mm was 81.25% (91/112), up to 3 mm 84.11% (90/107), and for invasion up to 1 mm, in our sample histologically detected in 81.25% (91/112) of cases, it was even greater, i.e. 92.30% (84/91).

Conclusion. Cytology is most reliable in microinvasive squamous cell carcinoma with invasion up to 1 mm and within-grade accuracy assessment, i.e. when the cytologic diagnosis of microinvasive carcinoma is associated with the diagnosis of grade III cervical intraepithelial neoplasia. Such an approach is justified by the identical therapeutic procedure required for both lesions, as additionally supported by the clinical stage Ia1 finding in more than 80% of our sample

Clinical Cytology / Oral presentations / 02

RAPID CERVICOVAGINAL SMEAR SCREENING - A QUALITY CONTROL METHOD?

Audy-Jurković S*, Pajtler M**, Škopljanac-Mačina L*, Antulov J*, Barišić A*, Miličić-Juhas V.**

*Institute of Gynecologic Cytology, University Department of Gynecology and Obstetrics, Zagreb University Hospital Center, Zagreb; ** Department of Clinical Cytology, Osijek University Hospital, Osijek, Croatia

Aim of study. To test the method of rapid rescreening (RR) in detection of cervical lesions and false negative results as well as in cytotechnologist's quality control.

Patients and Methods. The RR method was tested on Papanicolaou stained and initially conventionally analyzed vaginal, cervical and endocervical (VCE) swabs. The study included 1640 VCE swabs from laboratory A (Department of Gynecologic Cytology, University Department of Gynecology and Obstetrics, Zagreb University Hospital Center, Zagreb) and 2040 VCE swabs from laboratory B (Department of Clinical Cytology, Osijek University Hospital, Osijek). Histologically verified abnormal cytologic findings accounted for 10% of the samples. The testing was performed by seven cytotechnologists in laboratory A and six cytotechnologists in laboratory B. Each slide was examined by the "step" technique within 1.5 min, the findings were divided into negative and abnormal groups, and the latter were further subdivided according to differential cytologic diagnosis. Results were compared with those obtained by initial screening. Abnormal findings from the group of initially negative findings were analyzed again by the conventional method in order to make definitive cytologic diagnosis.

Results. In laboratory A, of 1640 slides 1459 (89.0%) were correctly (1310 or 79.9% of true negative - TN, and 149 or 9.1% of true positive - TP, and 181 (11%) incorrectly (45 or 2.7% of false negative - FN and 136 or 8.3% of false positive - FP diagnosed by RR. In laboratory B, of 2040 slides 1949 (95.6%) were correctly (1759 or 86.2% of TN and 191 or 9.4% of TP), and 91 (4.4%) incorrectly (21 or 1% of FN and 69 or 3.4% of FP) diagnosed by RR. In laboratory A and B, the RR method showed a sensitivity of 76.8% and 90,1%, specificity of 90.6% and 96.2%, positive predictive value of 52.3% and 73.4%, negative predictive value of 96.7% and 98.8%, and diagnostic accuracy of 88.9% and 95.5%, respectively. According to the initial abnormal differential cytologic diagnosis, the diagnostic value of RR increased with lesion severity. RR detected 30 FN findings in laboratory A and 8 FN findings in laboratory B, most of them in the groups of ASC-US (atypical squamous cells of undetermined significance) and CIN I (cervical intraepithelial neoplasia). The number of FP findings per cytotechnologist ranged from 4.3% to 11.9% in laboratory A, and from 0.9% to 5.0% in laboratory B. The number of FN findings per cytotechnologist ranged from 1.7% to 4.3% in laboratory A, and from 0.0% to 2.1% in laboratory B.

Conclusion. RR can be used as an efficient quality control method to increase the sensitivity of cytologic screening and as method of cytotechnologist quality control.

Clinical Cytology / Oral presentations / 03

CYTOLOGIC FOLLOW-UP OF WOMEN WITH ABNORMAL PAP TEST ACCORDING TO HIGH RISK HPV-DNA

Barišić A*, Audy-Jurković S*, Ovanin-Rakić A*, Žele-Starčević L.**

*Institute of Gynecologic Cytology, University Department of Gynecology and Obstetrics, Zagreb University Hospital Center; **University Department of Clinical and Molecular Microbiology, Zagreb University Hospital Center, Zagreb, Croatia

Aim of the study. The aim of the study is to provide retrospective correlation of the results of cytologic follow-up in women with abnormal Pap-test finding - cervical intraepithelial neoplasia (CIN) and typing for the human papilloma virus DNA (HPV-DNA).

Patients and Methods. At the University Department of Clinical and Molecular Microbiology, Zagreb University Hospital Center, 247 women with abnormal cytologic finding were tested for high risk HPV-DNA by the Digene Hybrid Capture II (HCII) test from June 1, 2000 till May 31, 2001. The Cytologic follow-up of Pap findings was performed at the Department of Gynecologic Cytology, University Department of Gynecology and Obstetrics, Zagreb University Hospital Center over at least a 2-year period on at least 4 Pap findings. The Follow-up outcome was classified into three categories: regression (R), at least 3 consecutive negative cytologic findings over more than a one-year period; stagnation (S), unchanged CIN staging; and progression (P), any higher CIN stage.

Results. Out of 247 women tested for high risk HPV-DNA, 61 (24.7%) women underwent cytologic follow-up at our Department over more than 2 years (mean 34.2, range 24-52 months); two conizations were not included in the analysis. High risk HPV-DNA was identified in 42 (71.2%) of 59 patients with cytologic follow-up. At the end of cytologic follow-up, in the group of patients negative for high risk HPV-DNA (n=17), R was recorded in 10 (58.8%), S in 5 (29.4%) and P in 2 (11.8%) patients. In the group of patients positive for high risk HPV-DNA (n=42), R was recorded in 11 (26.2%), S in 26 (61.9%) and P in 5 (11.9%) patients. The rate of stagnation/progression versus regression of cytologic findings was statistically significantly higher in the group of patients positive than in those negative for high risk HPV-DNA ($c2=4.288$; $p=0.0384$).

Conclusion. Stagnation/progression versus regression of cytologic finding is significantly higher in the group of patients positive for high risk HPV-DNA.

Clinical Cytology / Oral presentations / 04

POSTTRANSPLANT LYMPHOPROLIFERATIVE DISORDER. A CASE REPORT

Fabijanić I[1], Kardum-Skelin I[1], Šušterčić D[1], Cvrlje-Čolić V[1], Naumovski-Mihalić S[1], Škegro D[1], Šiftar Z[2], Kušec R[3], Anić P[4], Jakšić B.[1]

[1]Department of Medicine, [2]Institute of Clinical Chemistry, Divison of [3]Molecular Diagnostics, [4]Department of Diagnostic and Interventional Radiology, Merkur University Hospital, Zagreb, Croatia
iris.fabijanic@zg.htnet.hr

Introduction: Posttransplant lymphoproliferative disorders (PTLD) represent an immunophenotypic, genotypic, and clinical spectrum of diseases seen in patients after bone marrow and solid-organ transplantation (SOT). PTLD are mostly of B-cell origin and are associated with Epstein-Barr virus (EBV). The incidence of PTLD is 2-10% in liver transplant recipients, ranging from 2 to 3 % in adults to more than 10% in some pediatric series.

Patient: A 50-year old man with an antecedent history of type 2 diabetes mellitus underwent an orthotopic liver transplantation (OLT) due to HCV-related end-stage liver disease. Immunosuppressive treatment included cyclosporine and prednisone. Three months after transplant, the patient had jaundice with elevated liver function tests. Color Doppler sonography (US) and computerized tomography (CT) scans showed abdominal lymphadenopathy. US-guided fine needle aspiration biopsy (FNAB) revealed non Hodgkin's large cell lymphoma. Flow cytometry showed these cells to be a monoclonal B-cell population, and a diagnosis of monomorphous/diffuse large B-cell lymphoma (DLBCL) was made. Furthermore, analysis of immunoglobulin heavy-chain gene using polymerase chain reaction (PCR) on cytological samples confirmed a monoclonal population of B-cell lineage. A bone marrow biopsy was negative for lymphoproliferative disorder. All investigations for EBV including immunocytochemical studies on cytological smears, serological tests and PCR were negative. Moreover, cytomegalovirus (CMV) DNA in peripheral blood by PCR showed an insignificant number of copies.

Conclusions: PTLD is a significant and growing problem, due to increase in the number of SOT recipients. Considering the prognosis of PTLD, an early diagnosis and monitoring of transplant recipients will help to identify patients who could benefit from preemptive therapy.

Clinical Cytology / Oral presentations / 05

BREAST TUMOR MORPHOMETRY. A PRELIMINARY STUDY

Hodak I[1], Kardum-Skelin I[2], Šušterčić D[2], Fabijanić I[2], Jakić-Razumović J[3], Šeparović V[4], Manojlović S.[5]

[1]Slavonski Brod General Hospital, Slavonski Brod, [2]Merkur University Hospital, Zagreb, [3]Clinical Institute of Pathology, Clinical Hospital Centre Zagreb, [4]University Hospital for Tumours, [5]Dubrava Clinical Hospital, Zagreb, Croatia

Breast cancer is the most common cancer among women and the second leading cause of cancer mortality in women today. Fine needle aspiration cytology (FNAC) has very important role in diagnosis of breast cancer. In this study economic and simple method was investigated as possible supplement for improve diagnostic accuracy of cytology.

The aim of the study: The aim of the study was to compare morphometric parameters between different types of breast cancers.

Patients and Methods: Image analysis was performed by use of the Sform (Vamstec, Zagreb) software on the May-Grünwald-Giemsa (MGG) stained archive aspiration specimens of breast. 15 patients were selected (5 patients with invasive ductal carcinoma gradus II (IDC II; 5 patients with invasive ductal carcinoma gradus III (IDC III) and 5 patients with invasive lobular carcinoma (ILC). 500 cells and 500 nuclei for each type of cancer were studied, total of 1500 cells and 1500 nuclei. The following parameters were investigated: area, outline, max. radius, min. radius, convex area, breadth, length, nuclear/cytoplasmatic ratio, form factor and elongation. Statistic 6.0, P^2-test were used.

Results: Morphometric analysis showed statistically significant differences ($p<0,05$) between following investigated groups: IDC II/ IDCIII - cell: outline, max. radius; nucleus: area, convex area, outline; IDC II/ILC - cell: area, convex area; nucleus: area, outline, 3. convex area; IDC III/ ILC - cell: area, max. radius, convex area, breadth, length; nucleus: area and convex area.

Conclusions: Morphometric parameters considered individually were highly associated with type and grade of breast tumour.

Clinical Cytology / Oral presentations / 06

FNA ANALYSIS OF JOINT LESIONS: REPORT OF 4 CASES

Ivezić B[1], Trutin Ostović K[2], Kanić R.[1]

[1]Bjelovar General Hospital, Bjelovar, Croatia
[2]Dubrava Clinical Hospital, Zagreb, Croatia
branka.ivezic@bj.htnet.hr

Aim of the study: Synovial tumors and tumorlike conditions are distinctive growths that reproduce many cytological features of normal synovial tissue. Malignant lesions are rare, the only one being synovial sarcoma. Among benign ones there are some common (ganglion) and some rare lesions (villonodular synovitis, giant cell tumor of tendon sheath - nodular tenosynovitis). Our aim was to asses the role of FNAC and to determine cytological findings of different benign and malignant synovial lesions with rather similar cytological appearance.

Study design: We have analyzed four cytologically different synovial lesions, two benign and two malignant. 39-years old female had a swelling on index finger, near postoperative scar. Pathohistological diagnosis of the first lesion was nodular synovitis. Cytological finding of postoperative swelling corresponded to primary process recurrence. 11-years old athlete was treated for a knee sports injury. FNAC of synovial fluid was performed, diagnosis of villonudular synovitis was set, and subsequently pathohistologically confirmed. In 64-years old male cytological imprint was taken from massive exulcerated foot lesion. The cytological diagnosis was malignant mesenchymal tumor of synovial origin, and pathological one was synovial sarcoma-monophasic type. 36-years old male reported for a hand lesion, FNAC with immunocytochemistry diagnosed synovial sarcoma - biphasic type that was confirmed by pathology.

Conclusion: Along with clinical data cytology can usually give accurate diagnosis of some synovial tissue lesions, which can help in planning more adequate surgical treatment.

Clinical Cytology / Oral presentations / 07

MESENCHYMAL TUMOR IN THE SUBLINGUAL AREA: FIBROSARCOMA OR MONOPHASIC SYNOVIAL SARCOMA

Kanić R[1], Ivezić B[1], Kardum-Skelin I[2], Bartoš V[1], Manojlović S.[3]

[1]Bjelovar General Hospital, Bjelovar, [2]Merkur University Hospital, Zagreb, [3]Dubrava Clinical Hospital, Zagreb, Croatia

Synovial sarcoma represents around 3% of all soft tissue sarcomas, but only 3-10% of them are found in head and neck area. They appear mostly as biphasic, whereas monophasic type is rare and represents bigger differentially diagnostic problem because of its similarity, both in histological and in cytological presentation, to some other well differentiated mesenchymal tumors, in the first place fibrosarcoma. It has characteristic chromosomal translocation t(X;18). Adult fibrosarcoma is also a rare tumor, it represents around 3% of all sarcomas, it doesn't have specific genetic abnormality, and is characterized by multiple chromosomal disturbances.

Female patient O.M., 67 years old, called in because of the pain in the throat and temporomandibular area. Ultrasound and computerized tomography revealed expansive formation spreading in the right sublingual and submandibular region, with penetration into vallecula epiglottidis and recessus piriformis, 5cm in diameter. FNA with immunocytochemistry (reaction positive to vimentin and negative to desmin, SMA, NSE, CD31, CD34, CD117, EMA) set the diagnosis of well differentiated mesenchymal tumor: well differentiated fibrosarcoma or MFH. After surgical excision pathohistological finding was following: sarcoma- diff.dg.: monophasic synovial sarcoma or well differentiated fibrosarcoma.

Our conclusion is that the final answer in sub classification of malignant soft tissue tumors cannot always be given by histopathology and immunohistochemistry, but further cytogenetic and molecular analyses are necessary.

Clinical Cytology / Oral presentations / 08

FINE NEEDLE ASPIRATION CYTOLOGY OF HEPATIC AND PANCREATIC TUMOURS

Kardum-Skelin I[1] , Šušterčić D[1], Fabijanić I[1], Jelić-Puškarić B[1], Kušec R[2], Kardum Paro MM[2], Planinc-Peraica A[1], Ostojić Kolonić S[1], Odak D[3], Anić P[3], Škegro D[1], Čolić-Cvrlje V[1], Papa B[1], Jakšić B.[1]

[1]Department of Medicine, [2]Institute of Clinical Chemistry, [3]Department of Diagnostic and Interventional Radiology , Merkur University Hospital, Zagreb, Croatia

Imaging methods (ultrasound-US, computerised tomography-CT and magnetic resonance-MR) have ensured the visualisation of intraabdominal tumours (of liver, pancreas, kidneys, adrenal glands and retroperitoneal nodes) which are very rarely approachable on palpation, and consequently in guided aspiration.

Aim of the study: 1. To analyse the adequacy of cytologic fine needle aspirates from intraabdominal organs and retroperitoneal nodes, 2. To estimate the occurrence rate of malignant lesions, 3. The possibility for typisation of epithelial and nonepithelial tumours by cytomorphology and additional technologies from a cytologic specimen.

Patients and Methods: 1528 intraabdominal aspirates were examined: liver (696), pancreas (289). Fine needle aspiration was performed with the CHIBA needle. The preparations were analysed in standard stained smears by the May-Grunwald-Giemsa method, by determination of cellular markers by immunocytochemical analysis on smears and flow cytometry, cytochemical, cytogenetic and/or molecular analyses (PCR).

Results: In 1480 patients fine-needle aspiration was performed with the US control, in 12 patients with CT, and in only 36 patients without image control. Insufficient material was obtained in 99 patients (6,5%). Malignant cells were found in 648 cases in aspirates of both liver and pancreas. Of a total of 528 liver tumours, based on cytomorphology, completed with immunocytochemical markers, the epithelial, mesenchymal or embryonal cellular origin could be determined (92% epithelial, 6,6% nonepitehelial origin and 1,4% with suspicious features without precise differentiation of origin and/or type of tumours). In the tumors of epithelial group primary epithelial liver tumours numbered 21,4% (101 hepatocellular and 3 cholangiocellular carcinoma), plus 306 metastatic ones, while in the epithelial tumors primary site was recognised in 15,5% cases. In poorly differentiated tumors, the tumor aggressiveness was confirmed by high aneuploidy (DNA image cytometry) and by high proliferation status (AgNOR determination). In nonepithelial tumours the diagnosis of gastrointestinal stromal tumor (GIST) was made in 2, embryonal sarcoma in 1, angiosarcoma in 1, schwannoma in 1, leiomyosarcoma in 1, fibrosarcoma in 3, melanoma in 9 and 15 malignant lymphomas, while others could be diagnosed as sarcomas without precise differentiation. Out of 121 diagnosed malignant tumours of pancreas we found 112 carcinomas, 7 suspicious specimens and 1 mesenchymal tumour.

Conclusion: Cytodiagnostic fine-needle aspiration of the liver and pancreas, completed with immunocytochemical markers on smears and flow cytometry, by cytogenetical and molecular analyses from the specimens obtained by cytologic aspiration, has been proved to be a reliable method in diagnostics and subtypisation of epithelial (primary as well as metastatic) and nonepithelial tumors. However, very often, some tumor markers reveal tissue co-expression and, as a rule, are not specific for only one organ. Despite this, their right combination improves the search for the primary site of the metastatic process or, potentially determine the additional diagnostic procedures (CT, US, MR).

Clinical Cytology / Oral presentations / 09

ULTRASOUND-GUIDED AND STEREOTACTIC FINE NEEDLE ASPIRATION OF BREAST LESIONS MANIFESTED BY MAMMOGRAPHIC CALCIFICATIONS

Kardum-Skelin I[1], Šušterčić D[1], Fabijanić I[1], Jelić-Puškarić B[1], Vidjak V[2], Obad-Kovačević D[2], Kos N.[3]

[1]Laboratory of Cytology and Haematology, Department of Medicine, [2]Department of Diagnostic and Interventional Radiology, Merkur University Hospital, Zagreb, [3]Radiology and Breast Diagnostics Office, Velika Gorica, Croatia

It is known that a number of breast disorder may result in calcifications, most of which are benign. Of note, however, is that one of the early signs of ductal carcinoma in situ (DCIS) is calcification of the ducts. For this reason mammography is used as a screening method for detecting breast cancers before they are clinically apparent. However, mammography has its limitations in women under the age of 35.

Aim of the study: 1) To show the proportion of calcifications in breast lesions; 2) To determine whether high-frequency ultrasound may be used to reliably localize breast microcalcification; 3) To evaluate the cytologic finding of ultrasound (US) guided and stereotactic fine needle aspiration (FNA) in patients with microcalcifications in the breast.

Patients and Methods: A total of 4208 (96,7%) US-guided and 143 stereotactic (3,3%) FNAs were analyzed. FNAs were performed using a 21 G needle. All were analyzed in May-Grûnwald-Giemsa stained smears.

Results: Mammographic calcifications were found in 176/4351 breast lesions. In 90 patients, found to have microcalcifications at mammography, US was able to visualize the calcified particles. Out of 176 aspirates with calcifications, carcinoma was present in 31 (17,6%): 24/90 US-guided and 7/86 stereotactic. For the 145 benign lesions cytology revealed fibrocystic changes, fibroadenomas and atypical hyperplasia. One patient diagnosed as having benign lesion in stereotactic FNA, with suspicious microcalcifications, was advised to undergo core biopsy. Pathohistological diagnosis was carcinoma.

Conclusion: US is an effective method of identifying and localizing breast microcalcifications and may be used as an alternative to hook wire stereotactic localization in the majority of patients. FNA is a reliable minimally invasive diagnostic method for the final diagnosis in patients with mammographic calcifications. However, if malignancy is proven an US-guided or stereotactic core biopsy or open biopsy is necessary.

Clinical Cytology / Oral presentations / 10

CYTO-PATHOLOGICAL CORRELATION OF THYROID GLAND TUMORS

Kojić Katović S, Vasilj A, Ćurić Jurić S.
Sestre Milosrdnice University Hospital, Zagreb, Croatia

Mislav.Katovic@zg.htnet.hr

Carcinoma of the thyroid gland is diagnosed in 5-30% of all patients with nodular changes of thytoid gland referred for examination. Fine-needle aspiration cytology is widely used to distinguish between patients with malignant, or possibly malignant thyroid nodules from those with benign lesions.

The aim of the study was to evaluate the prevalency of thyroid gland tumors and to make correlation with histopathological diagnosis.

The study included smears of the material, obtained by FNAC in 3311 patients during 7-years period (1998-2004) at the Laboratory of Cytology, Department of Internal Medicine, Sestre Milosrdnice University Hospital. The material for cytological analysis was obtained mostly by US guided cytodiagnostic aspiration using a 0,7 mm needle and 10-ccm syringe, in supine position with the head in extension. Cytological smears were stained by standard method by Pappenheim (MGG) and analyzed under a light microscope.

Cytology found tumor in 94/331 (2,8%) nodules, and cytopathological correlation was available in 60 cases. The best correlation with histopathology was in the group of papillary carcinomas: 15/16 (93%), in the group of follicular tumors the correlation was 20/27 (74%) and for medullary carcinomas 2/7 (28%). In the group of the well differentiated tumors the positive correlation was in 7/9 (78%) cases.

FNAC is fast, simple and accurate method in diagnosis of thyroid gland tumors taking into account that the sample is representative and that the same nodule has been analyzed by both cytologist and pathologist. The method can be improved by better team-work with clinicians and also, by using immunocytochemistry.

Clinical Cytology / Oral presentations / 11

MORPHOMETRIC NUCLEAR IMAGE ANALYSIS IN ENDOMETRIAL CYTOLOGIC SAMPLES

Mahovlić V*, Audy-Jurković S*, Ovanin-Rakić A*, Škopljanac-Mačina L*, Barišić A*, Ilić-Forko J**, Škrablin-Kučić S***, Božikov J.****

*Institute of Gynecological Cytology, University Department of Obstetrics and Gynecology, Zagreb University Hospital Center, **Clinical Institute of Gynecologic and Perinatal Pathology, University Department of Obstetrics and Gynecology, Zagreb University Hospital Center, ***Department of Perinatal Medicine, University Department of Obstetrics and Gynecology, Zagreb University Hospital Center, ****Andrija Štampar School of Public Health, Medical School, University of Zagreb, Zagreb, Croatia

Aim of the study. To determine morphometric variables and nuclear DNA content in endometrial cytologic samples by use of image analysis.

Patients and Methods. Feulgen method of staining (Merck, Germany) was used on May-Grünwald-Giemsa stained archival aspiration cytology endometrium specimens with the following histopathologic diagnoses: proliferation, hyperplasia simplex, hyperplasia complex, hyperplasia complex atypica, and adenocarcinoma endometrii grade I (n=5 each). Image analysis was performed by use of the Sform (Vamstec, Zagreb) software. The image (x100 oil) was transferred from a microscope (Olympus BHS) via CCD TV color camera (Sony) to a PC by digitalized image. Integrated optical density (IOD) as a measure of nuclear DNA content was calculated for each nucleus (n$100). Lymphocytes (n$20) were used as internal control, along with the factor of correction. The morphometric parameters of nuclear area, nuclear perimeter, and factors of nuclear regularity and nuclear ellipticity were analyzed. Statistical analysis was done by use of Statistica ver. 6.0.

Results. Multivariate analysis (ANOVA) yielded significant differences ($p<0.01$) in nuclear DNA content (IOD) and all morphometric variables among normal, hyperplastic and malignant endometrium. On group comparison (Scheffe test), the factors of nuclear regularity (form factor) and nuclear ellipticity distinguished only well differentiated endometrial adenocarcinomas according to proliferation and type of endometrial hyperplasia ($p<0.05$), whereas the parameters of nuclear area, nuclear perimeter and nuclear DNA content (IOD) showed statistical significance ($p<0.05$) in all groups.

Conclusion. Morphometric variables and nuclear DNA content (IOD) can provide useful information in the cytologic analysis of normal, hyperplastic and malignant endometrium.

Clinical Cytology / Oral presentations / 12

ULTRASOUND-GUIDED FINE NEEDLE ASPIRATION CYTOLOGY OF THE PAROTID GLAND TUMORS

Markov-Glavaš D[1], Rosso M. [2]

Department of Otorhinolaryngology, [1]Clinical Hospital Center, Zagreb, Croatia
Department of Otorhinolaryngology, [2]Osijek University Hospital, Osijek, Croatia

Aim of the study: The objective of our study was to discuss the value of ultrasound-guided FNAC in the diagnosis and treatment of the parotid tumors.

Patients and Methods: Fifty three patients were included in our study.

They had undergone clinical examination, ultrasound-guided FNAC before parotidectomy.The results of these examinations were compared with histopathological diagnosis.

Results: There were two false positive findings. Cytologic diagnosis of adenoma pleomorphae was made in 32 patients, cystadenolymphoma in 15 patients.Cytologic diagnosis of malignant tumors was made in 6 patients (2 mucoepideromid tumors, 1 acinic cell carcinoma, 1 malignant mixtus, 1 malignant melanoma and 1 NHL).

Conclusion: Ultrasound-guided FNAC is a safe, simple and efficient diagnostic procedure with an accuracy of 96% in diagnosis of the parotid gland tumors. Preoperative recognition of malignat tumors may help to prepare both the surgeon and patient for appropriate surgical procedure.

Clinical Cytology / Oral presentations / 13

CYTOKERATIN 7 AND 20 IN FIBROADENOMA AND MUCINOUS CARCINOMA OF THE BREAST

Marković-Glamočak M*, Sučić M*, Jakić-Razumović J**, Ries S*, Gjadrov-Kuveždić K* and Vrbanus LJ.*

*Division of Cytology, Clinical Institute of Laboratory Diagnosis, **Clinical Institute of Pathology, Zagreb University School of Medicine and Clinical Hospital Center, Zagreb, Croatia
mmglamoc@yahoo.com

Aim of the study: The aim of the study was to establish the value of cytokeratin (CK) 7 and 20 in differentiation between fibroadenoma and mucinous carcinoma.

Patients and Methods: Nine (9) FAs and five (5) mCAs were analyzed in routinely stained smears: imprints of FAs and mCAs by May-Grünwald-Giemsa and histological sections by Hemalaun-Eosin Imunocytochemical analyses of cytokeratins were performed using APPAP technique and antibodies by Dako (DakoCytomation, Glostrup, Denmark). Microscopic examination included intensity and distribution of tumor markers (CK 7 and 20) within imprints and histologic sections of FAs and mCAs. Intensity was scored 0 to 3+ and distribution as diffuse or focal.

Results: Cytokeratin 7 was positive in all FAs and mCAs, while CK 20 was negative in FAs and mCAs. Differences in intensity and distribution of CK 7 expression was found in imprints and pathohistologic sections, respectively. In all Fas, i.e. 9/9 (100%), CK expression ware strong (+3) and diffusely distributed. Expression of CK 7 in mCAs ranged from 0 to 3+: 3/5 (60.0%) were 1+; 1/5 (20.0%) mCAs was 2+ and 1/5 (20.0%) was CAs 3+. A difference was also observed in the distribution of positivity. Focal distribution was found in 2/5 (40%) mCAs and diffuse in 3/5 (60%) mCAs.

Conclusion: Immunocytochemical analysis of CK 7, unlike of CK20, in imprints and/or histologic sections is helpful in differentiation between fibroadenoma and mucinous carcinoma. Intensity and distribution of tumor markers within imprints and histologic sections are important. Strong positivity and diffuse distribution suggest benign lesion, while week expression and focal distribution of CK7 suggest malignant changes.

Clinical Cytology / Oral presentations / 14

CYTOMORPHOLOGICAL CHANGES AND GALECTIN-3 POSITIVITY BY RT-PCR METHOD IN BENIGN THYROID LESIONS

Mateša N, Šamija I, Tabain I, Kusić Z.

Department of Oncology and Nuclear Medicine, University Hospital "Sestre milosrdnice", Zagreb, Croatia
nmatesa@kbsm.hr

Objective: Galectin-3 has been proposed as tumor marker that could improve accuracy of preoperative diagnosis of thyroid lesions. In this study we wanted to find if the presence of macrophages and Hürthle cells in different types of benign thyroid lesions could provide an explanation for false positives by RT-PCR method.

Methods: RT-PCR analysis of galectin-3 expression was performed on RNA isolated from aspirates obtained by ultrasound guided fine-needle aspiration biopsy from 91 unselected patients with benign thyroid lesions. All samples were analyzed for thyroglobulin expression to confirm the presence of the cells of follicular origin. Procedure for RT-PCR analysis was: a) direct RNA isolation from FNA sample, b) reverse transcription, c) PCR for thyroglobulin, d) PCR for galectin-3 (primers: Gal3F and Gal3R). Results of RT-PCR analysis were evaluated against the definitive fine-needle aspiration cytology diagnosis or, in citologically indeterminate or suspicious cases, against the histological findings. The possibilities of presence of macrophages and Hürthle cells in aspirates were: absent (0), few (1) or abundant (2).

Results: Galectin-3 expression was found in: 2 of 8 (25%) follicular adenoma samples (all histologically verified), 4 of 18 (22%) Hashimoto thyroiditis samples, and in 17 of 65 (26%) nodular goiter samples. Macrophages were absent in 77% of galectin-3 negative cases as compared to 12% of galectin-3 positive cases in nodular goiter samples ($p<0.01$). Similar situation was found in Hashimoto thyroiditis samples: macrophages were absent in 86% of galectin-3 negative cases as compared to 25% of galectin-3 positive cases ($0.01>p>0.05$). We did not find a statistically significant relation between the presence of Hürthle cells and galectin-3 positivity in benign thyroid lesions.

Conclusion: We found a statistically significant relation between the presence of macrophages and galectin-3 positivity in nodular goiter and Hashimoto thyroiditis samples. The presence of Hürthle cells does not seem to affect galectin-3 reactivity in benign thyroid lesions.

Clinical Cytology / Oral presentations / 15

SEVERE INTRAEPITHELIAL NEOPLASIA AND GENITAL CANCER AMONG WOMEN WITH CONDYLOMATA ACUMINATA OF ANOGENITAL TRACT

*Milojković M, **Pajtler M, *Rubin M.

*University Department of Gynecology and Obstetrics,
**Department of Clinical Cytology, Osijek University Hospital, Osijek, Croatia

Aim of the study. To determine the occurrence of severe intraepithelial neoplasia and genital cancer among women with condyloma of anogenital tract and how to treat them in the case of not knowing HPV type

Patients and Methods. In the period from 1 Sept 1988 till 31 June 1994 there were 222 women who had colposcopy due to condyloma of anogenital tract (perineal area, perineum, vulva, vagina and cervix). Every woman had a colposcopy and smear tested on malignancy (V, C, E) if they hadn't it taken in the last year. If the result was suspicious, the pathological verification was done. The same group of women was examined again in 2004, 10 till 16 years later after the first diagnosis of condyloma.

Results. Abnormal cytological smear either synchronous or metachronous, suggesting condyloma had 111 (50%) of women. Severe squamous intraepithelial lesion (HSIL) had 31 (14,0%) women and six had cancer (2,7%). There were 14 women (6,3%) who had HSIL with condyloma and for had cancer (1,8%). There were 14 (6,3%) who had HSIL before they had condyloma and two had cancer (0,9%). HSIL appeared in three women (1,4%) after condyloma treatment, but no woman developed cancer. Abnormal colposcopy finding had 152 (68,5%) women. Abnormal histological finding, synchronous and metachronous with condyloma had 80 women (36,0%). Severe intraepithelial neoplasia had 41 women (18,5%) and cancer 7 women (3,2%). There were 8 women who had severe intraepithelial neoplasia histologicaly confirmed before they got condyloma and two cancers (0,9%). There were 28 women (12,6%) who had condyloma and severe intraepithelial neoplasia and cancer three (1,4%). After the condyloma treatment six women (2,7%) had severe intraepithelial neoplasia and two cancers (0,9%).

Conclusion. In spite the fact that Condylomata Acuminata of anogenital tract is caused by HPV of low malignancy (6/11), severe intraepithelial neoplasia and cancer occurs rather frequently among these women. Therefore we think that women with condyloma of anogential tract should belong to the group of women who have a high risk of developing cancer, especially when there is no possibility to determine HPV type among these women.

Clinical Cytology / Oral presentations / 16

MILD TO MODERATE GLANDULAR INTRAEPITHELIAL LESIONS OF THE CERVIX

Ovanin-Rakić A*, Audy-Jurković S*, Mahovlić V*, Babić D**, Barišić D.***

*Institute of Gynecologic Cytology, University Department of Gynecology and Obstetrics, Zagreb University Hospital Center **Clinical Institute of Gynecologic and Perinatal Pathology, Zagreb University Hospital Center, ***Department of Gynecologic Surgery, University Department of Gynecology and Obstetrics, Zagreb University Hospital Center, Zagreb, Croatia

Aim of the study. Cytology findings of patients with histologically verified mild to moderate GIL (grades I and II) were analyzed to achieve the most appropriate and accurate cytologic diagnosis of intraepithelial lesions of endocervical cylindric epithelium.

Patients and Methods. The value of cytology in the detection and differential diagnosis was evaluated in 50 patients with definitive histologic diagnosis of GIL I (n=11), GIL II (n=7), and glandular lesions associated with squamous component, GIL I + CIN (n=14), GIL II + CIN (n=13) and GIL+MIC (n=5), on the basis of lesion severity and/or type of epithelium affected.

Results. In 90% (45/50) of patients, lesions were detected by cytologic analysis that indicated additional diagnostic procedure. In terms of differential diagnosis, cytology showed higher accuracy in predicting lesion severity vs. type of epithelial alteration (40/50; 80% vs. 11/50; 22%), and abnormalities of squamous vs. cylindric epithelium (32/32; 100.0%; vs. 11/18; 61,11%). The accuracy of cytology was higher in pure than in mixed lesions (11/18; 61,11% vs. 11/32; 34,38%).

Conclusion. Continuous improvement in cervical specimens and cytodiagnostic skills, better understanding and definition of intraepithelial adenocarcinoma precursors, and their inclusion in the classification of cytologic and histologic cervical findings are expected to upgrade the detection and diagnosis of GIL, and to reduce the invasive cervical adenocarcinoma morbidity and mortality.

Clinical Cytology / Oral presentations / 17

PAP TEST - WITH OR WITHOUT VAGINAL SMEAR?

Pajtler M*, Milojković M.**

* Department of Clinical Cytology, ** University Department of Gynecology and Obstetrics, Osijek University Hospital, Osijek, Croatia

Aim of study. To assess the value of vaginal smear in primary screening for cervical carcinoma and its precursors.

Patients and Methods. The study included 125,444 subjects with their vaginal, cervical and endocervical (VCE) swabs examined during a 3-year period (2000-2002) at Department of Clinical Cytology, Osijek University Hospital, Osijek. Abnormal findings were compared with the smears found to contain abnormal cells. Diagnosis specific mean age for each differential cytologic diagnosis and overall age structure of the study population were determined. The number of women that could be additionally covered by the screening if vaginal swab be only introduced after the age of 50 was estimated.

Results. Of 125,444 VCE smears, 6283 (5%) were abnormal. The presence of abnormal cells exclusively in vaginal swab was recorded in 441 subjects (7% of abnormal findings and 0.4% of the population examined). Using this smear, 18.5% of invasive squamous carcinoma and only a small part of squamous intraepithelial lesions (6.9% of CIN1, 2.7% of CIN2 and 2.9% of CIN3) were detected. In addition, 22.6% of endometrial adenocarcinoma and 21.8% of endometrial AGCUS, 5.3% of endocervical adenocarcinoma and 2.4% of endocervical AGCUS were detected. The disease specific mean patient age was 55.3, 55.5 and 67.6 years for invasive squamous cell carcinoma, endocervical adenocarcinoma, and endometrial adenocarcinoma, respectively. According to age groups, 76.3% of study subjects were below and 23.7% above age 50. If vaginal smear be introduced in Pap test only for women above age 50, all invasive lesions would be included, while the same number of cytotechnologists could perform screening of additional 47,846 (38%) CE smears.

Conclusion. It does not appear justified to obtain vaginal swab from all women as part of primary screening for detection of cervical carcinoma and its precursors. The inclusion of vaginal swab is only justified in the female population at risk aged >50 and subsequently in the follow up of women with abnormal cytologic findings. In this way, 38% or more women could be included with the same number of cytotechnologists, or the time thus spared could be used for various forms of quality control to upgrade the screening sensitivity.

Clinical Cytology / Oral presentations / 18

THE VALUE AND DIAGNOSTIC PROBLEMS IN FINE NEEDLE ASPIRATION CYTOLOGY OF THYROID NODULE

Pauzar B. Staklenac B, Lončar B, Pajtler M, Dmitrović B, Marić S.

Department of Clinical Cytology, Department of Pathology and Forensic Medicine, Clinical Hospital Osijek, Faculty of Medicine Osijek, Croatia
pauzar.biljana@kbo.hr

Aim of the study: Determine the value and diagnostic difficulties in preoperative cytodiagnostics of thyroid nodule.

Patients and Methods: Cytological smears of aspirates from thyroid gland nodules taken from 273 patients were analized using ultrasound-guided fine needle aspiration biopsy in Clinical Hospital Osijek from January 1, 2003 to December 31, 2004, and compared with the final histopathological diagnosis.

Results: Papillary carcinoma was found in 54 patients, follicular lesions in 50, Hürthe cell neoplasm in 12, medullary carcinoma in 5, Non Hodgkin lymphoma (NHL) in 2 and carcinoma anaplasticum in 2 patients. Benign were cytological smears of 96 patients, and inadequate in 26 patients. Diagnostic accuracy for papillary carcinomas was 77% (47/61), for follicular lesions (including adenomas and carcinomas) 67% (38/56), for Hürthle cell neoplasm (including adenomas and carcinomas) 88,8% (8/9), for medullary carcinomas 100% (4/4), for Non Hodgkin lymphomas 100% (2/2) and for anaplastic carcinomas 100% (2/2). Total diagnostic accuracy for thyroid tumours was 93,28%, and diagnostic accuracy of differential cytology 75,3%. Sensitivity, specificity, positive and negative predictive values were 75,37%, 83%, 80,8% and 78,7%. In the group of histologically verified follicular tumors, only 3.7% (2/56) were malignant, and in the group of Hurthle tumors 22,2% (2/9).

Conclusion: Ultrasound-guided fine needle aspiration cytology is currently the best diagnostic tool for thyroid nodules. The grey area and main diagnostic pitfall is follicular lesion, with differential diagnoses including follicular carcinoma, follicular adenoma and hyperplastic nodules. Therefore there is a need to find clinically reliable tumor markers, which would make this differentiation possible and thus reduce the number of unnecessary surgeries.

Clinical Cytology / Oral presentations / 19

ANALYSIS OF "SUSPICIOUS" LESIONS IN FINE-NEEDLE ASPIRATION CYTOLOGY OF THE BREAST

Ries S*, Jakić-Razumović J**, Gjadrov-Kuveždić K*, Marković-Glamočak M*, Vrbanus LJ*, Sučić M.*

*Division of Cytology, Clinical Institute of Laboratory Diagnosis, ** Clinical Institute of Pathology, Zagreb University School of Medicine and Clinical Hospital Center, Zagreb, Croatia

Aim of the study: The aim was to show the proportion of final pathohistological diagnosis of the breast cancer in a group of cytology reports classified as " suspicious".

Patients and Methods: 6179 ultrasound guided fine-needle aspirations (FNA) were analysed by five cytologists in the 2001-2004 period. Clearly positive FNA were excluded from this study. Biopsy was performed in cases suspicious of malignancy and results were compared.

Results: Biopsy was recommended in 43 of 6179 (0.7%) FNA. Eleven patients were lost from follow-up. Carcinoma were found in 18/32 (56%), fibroadenoma in 6/32 (19%) and fibrocystic disease with adenosis in 8/32 (25%) specimens.

Conclusions:

1. In FNA characterized as "suspicious" biopsy and pathohistological diagnosis are absolutely indicated.
2. Cytological "suspicious" fibroadenoma was not pathohistological diagnosed as malignant lesions.
3. Fibrocystic disease with adenosis remains a "gray" zone in cytology.

Clinical Cytology / Oral presentations / 20

OUR FIRST EXPERIENCES WITH INTRAOPERATIVE TOUCH-IMPRINT CYTOLOGY OF SENTINEL LYMPH NODES IN PATIENTS WITH BREAST CANCER

Seili - Bekafigo I*, Štemberger C*, Rajković K*, Fumić G*, Mustać E**.

* Department of Internal Medicine, Clinical Hospital Center Rijeka, **Department of Pathology, Medical University of Rijeka, Rijeka, Croatia
irena.seili-bekafigo@ri.htnet.hr

Aim of the study: The aim of the study was to investigate the value of intraoperative imprint cytology for detecting metastases in sentinel lymph nodes (SLN) in patients with breast cancer. We carried out a prospective analysis of 52 intraoperative touch-imprints of SLNs from 52 breast-cancer patients aged from 39-78 years.

Patients and Methods: SLNs were bisected. Imprints were made of each cut surface, and stained with rapid, modified May-Gruenwald-Giemsa method. SLNs were further analyzed according to protocol - intraoperative frozen section analysis in most cases, paraffin embedding, hematoxylin and eosin (HE) and, in negative cases, immunohistochemistry (IH).

Results: Out of 52 analyzed touch preparations, we found 10 positive SLNs (19,23%). Frozen section analysis was performed in 48 cases, and revealed 9 positive SLNs (18,7%). Subsequent analysis of paraffin-embedded tissue revealed 13 positive SLNs on HE-stained sections. Immunohistochemical analysis showed malignant cells in 8 more cases. On touch-imprint analysis we had 2 "suspicious" cases, both proven positive after definitive PH and IH analysis. In comparison with definitive PH and IH results of SLNs, intraoperative imprint cytology showed sensitivity of 76,9%, specificity of 100% and accuracy of 94,2%, while intraoperative frozen section analysis showed sensitivity of 69,2%, specificity of 100%, and accuracy of 91,6%. Imprint cytology was concordant with frozen section analysis in 90 % cases.

Conclusion: Intraoperative touch-imprint cytology is a valuable tool in assessing SLN status in patients with breast cancer. It should be used more frequently, since it is reliable and not expensive method.

Clinical Cytology / Oral presentations / 21

SUPRAVITAL STAINING AS A METHOD OF SCREENING IN URINARY SEDIMENT CYTODIAGNOSTICS

Sikirica M[1], Kardum-Skelin I.[2]

[1]Institute of Clinical Chemistry, [2]Department of Medicine, Merkur University Hospital, Zagreb, Croatia
mirjana.sikirica@hi.htnet.hr

Objective: Evaluation of supravital staining of the wet preparation of urinary sediment as a method of screening in urine cytodiagnostics.

Methods: Fresh 82 urine samples were in paralell evaluated with the standard cytological staining according to the May Grunwald-Giemsa method and recommended methods for the analysis of urine samples by the European Urinalysis Group of the European Confederation of Laboratory Medicine (ECLM) and the Approved Guideline by the National Committee for Clinical Laboratory Standards (NCCLS).

Results: Comparison microscopic analyses showed good agreement of the methods in the identification. Agreement of the methods in the identification of morphologically changed erythrocytes, dysmorphic erythrocytes was 93%; in squamous epithelial cells it was 100%; for transitional and renal tubular epithelium was 96%; for neutrophil granulocytes 100%, monocytes 83%, eosinophil granulocytes 86%, and for macrophages it was 93%. Agreement of methods in various nuclear abnormalities (dyscariosis and atypia) was 100%.

Conclusion: O-Toluidin blue is a basic, "nuclear" dye which allows proper visibility of the structure of the nucleus, while eosine is an acidophil, "cytoplasmic" dye. Slight differences in mononuclear white cells (monocytes, eosinophil granulocytes) are due to different staining intensity which may result in their failure to be stained, thus hindering their identification, while slight differences in the identification of dysmorphic erythrocytes are most likely the result of the centrifugation of the samples. The complete agreement in the identification of morphological changes in the transitional epithelial cells supports supravital staining of the urinary sediment as a quick and simple screening method in urine cytodiagnostics.

Clinical Cytology / Oral presentations / 22

CYTO-PATHOLOGICAL CORRELATION OF THE LUNG CANCER TYPING IN BRONCHOSCOPIC SAMPLES

Smojver-Ježek S[1], Gredelj-Šimec Nj[1] , Vrabec-Branica B[1], Križanac Š[3], Peroš-Golubičić T.[2]

Departments of [1]Cytology and [2]Pneumology, University Hospital for Lung Diseases "Jordanovac", Zagreb, Croatia
[3] Institute of Pathology, Medical Faculty, Zagreb, Croatia
ssmojver@pbf.hr

Aim of the study: To investigate cytological-histological correlation of the lung carcinoma typing in the bronchoscopic samples.

Materials and Methods: Cytological records of 3795 slides with lung carcinoma in brochoscopic samples and 697 pathohistological records of the bronchial biopsies taken in a 5-year period in our hospital have been examined. Incidences of the lung carcinoma subtypes in the cytological samples have been established. In the 697 bronchial biopsies with cytological and pathohistological records, lung carcinoma typing was compared.

Results: Incidence of squamous carcinoma was 44%, adenocarcinoma 19%, small cell carcinoma 10%, non-small cell carcinoma 10%, carcinoma without subtype 9%; mixed carcinoma, adenosquamous and large cell carcinoma in 2% each. Bronchoscopic samples for pathohistological analysis were taken in only 19,6% of cytological samples with lung carcinoma diagnosis. Diagnosis of carcinoma was in 621 (89,1%) patohistological samples. Same lung carcinoma type was found in 72,8% of cytologicaly proven squamous carcinomas, 42,4% adnocarcinomas and 63,7% of small cell carcinomas, e.g. 52,5% of samples. Among cytologicaly diagnosed carcinomas, in 39% histological diagnosis was negative. In squamous lung carcinomas with no cytological-histological correlation, carcinoma was pathohistological diagnosis in 39,6%, in 28,3% large cell carcinoma, and in 17% adenocarcinoma. In adenocarcinomas with no correlation, pathohistological diagnosis was only carcinoma in 47,9% of cases, in 34% squamous carcinoma and large cell carcinoma in 10,6% of cases. In small cell carcinomas with no correlation, in 70% pathohistological diagnosis was carcinoma and in 15% squamous carcinoma.

Conclusion: Cytological-histological correlation in lung carcinoma typing was 52,5% and it was possible in 19,6% of bronchoscopicaly taken cytological samples in a 5-year period.

Clinical Cytology / Oral presentations / 23

SIGNIFICANCE OF CERVICOVAGINAL CYTOLOGY IN THE PROGNOSIS OF ENDOMETRIAL CANCER

Stanković T*, Haller H**, Krašević M***, Vrdoljak-Mozetič D*, Štemberger-Papić S*, Verša-Ostojić D*, Rupčić S.**.

*Department of Gynecological Cytology, University Department of Gynecology and Obstetrics, Rijeka University Hospital Center,** Department of gynecology, University Department of Gynecology and Obstetrics, Rijeka University Hospital Center
***Department of pathology, School of medicine, University of Rijeka, Rijeka, Croatia

Aim of the study. To evaluate a prognostic value of abnormal cervicovaginal cytology in patients with endometrial cancer.

Patients and Methods. In 258 patients with endometrial cancer a retrospective analysis of possible correlation between preoperative cervicovaginal cytology and intra/postoperative clinical and pathohistological examination was performed.

Results. From 258 patients with endometrial cancer in 58 (22,5%) of them the preoperative cervicovaginal cytology was negative, while in 200 (77,5%) it was suspect or positive on malignant endometrial cells (classified as positive cytology); 52 (25,2%) of those classified as FIGO stage I were negative and 154(74,8%) positive. All 6 patients in FIGO stage II and all 10 in stage IV were positive, while from those 36 in stage III 30 (83,3%) was positive.

There was statistically significant correlation of positive cervicovaginal cytology with the: 1. depth of myometrial invasion for more than half of the uterine wall ($p=0,0223$), 2. spread of the tumor in the endocervix ($p=0,0344$) I 3. positive lymph nodes ($p=0,0426$). We have also found a correlation of positive cervicovaginal cytology and the grade of tumor differentiation. Statistically significant difference was present between the patients with G1 and G2 ($p=0,0302$), as well as from those with G1 vs. G2 and G3 tumors ($p=0,0207$).

Conclusion. A positive preoperative cervicovaginal cytology correlate with other prognostic factors of endometrial cancer and it can be very valuable in planning of the surgical approach.

Clinical Cytology / Oral presentations / 24

INTRAOPERATIVE CYTOLOGY OF OVARIAN NEOPLASTIC LESIONS

Stanković T*, Haller H**, Krašević M***, Štemberger-Papić S*, Vrdoljak-Mozetič D*, Verša-Ostojić D*, Rupčić S.**

*Department of Gynecological Cytology, University Department of Gynecology and Obstetrics, Rijeka University Hospital Center,**Department of gynecology, University Department of Gynecology and Obstetrics, Rijeka University Hospital Center,***Department of Pathology, School of Medicine, University of Rijeka, Rijeka, Croatia

Aim of the study. To evaluate the importance and reliability of intraoperative cytology of ovarian neoplastic lesions.

Patients and Methods. From 01.01.2000. till 31. 12. 2004. intraoperatively collected samples of 448 ovarian lesions were analyzed. Samples for cytologic analysis were obtained as imprints or scrapings from the cut surface of the tumor. In addition, we also analyzed 186 specimens of peritoneal fluids/ascites and 215 peritoneal washings.

Results. From 448 patients in 255(57%) cases pathohistologicaly were proven benign changes or tumors, in 39 (8.7%) borderline (atypically proliferating) tumors, in 146(32.6%) malignant while 8 (1.7%) were classified as granulosa cell tumors. The cytologic analysis of imprints and scrapings from the cut surface has shown that this method has sensitivity 96%, specificity 99%, positive predictive value 99%, negative predictive value 98% and diagnostic accuracy 97%. In peritoneal fluids/ascites and peritoneal washings of the patients with the malignant neoplasm the sensitivity was 84% and 64%; while in borderline neoplasm it was 80% and 67%. Peritoneal fluids/ascites and peritoneal washings in all cases with benign neoplasm were negative. Diagnostic accuracy of the cytologic analysis of peritoneal fluids/ascites was 91% and for peritoneal washings 96%.

Conclusion. Intraoperative cytology of the ovarian neoplastic lesions is a simple, fast (within 10 minutes) and exact analysis with high diagnostic accuracy which gives to the surgeon reliable information about the nature and staging of ovarian lesions, primarily in the cases when due to small amount of tissue intraoperative pathohistologic analysis can not be made. It is our opinion that intraoperative cytology can help "handling diagnostically difficult" cases.

Clinical Cytology / Oral presentations / 25

CD34 EXPRESSION IN TRANSITIONAL CELL CARCINOMA

Sučić M, Marković-Glamočak M, Ries S, Gjadrov-Kuveždić K, Vrbanus LJ

Division of Cytology, Clinical Institute of Laboratory Diagnosis, Clinical Hospital Center, Zagreb, Croatia
mirna.sucic2@zg.htnet.hr

Ligand for L-selectin (CD34) is expressed on hematopoietic stem cells (HSC), hematopoietic progenitor cells (HPC) and on endothelial cells. Expression of CD34 could also be frequently noticed in some tumors, and is connected with angiogenesis.

Aim of the study: The aim of the study was to analyze the frequency of CD34 expression in transitional cell carcinoma (TCC) and to compare percentages of CD34+ epithelial urine cells between patients with TCC, patients with urine cell atypia (UCA) and patients with normal urine sediment cytology (NUC).

Patients and methods: Immunocytochemical APAAP staining for CD34 was done in 20 patients with TCC, 19 patients with UCA and 19 patients with NUC.

Results: All patients (20) with TCC had CD34+ cells in urine sediment (10-100% CD34+ cells); 13 patients with UCA were negative or had very low CD34 cell expression (0-2% CD34+ cells) and 6 patients with UCA had mild or high CD34 cell expression (20-69% CD34+ cells). All patients with NUC had negative or very low CD34 expression (1-4% CD34+ cells).

Conclusion: CD34 cell expression is negative or very low in NUC and mild or high in majority of urine sediment malignant cells. CD34+ cells in some patients with UCA indicated that CD34 expression is connected with inflammation and repair, but further follow-up of patients is needed to clarify implications of increased cell CD34 expression in urine sediment.

Clinical Cytology / Oral presentations / 26

CERVICAL INTRAEPITHELIAL NEOPLASIA IN PATIENTS AGED SIXTY-FIVE AND OLDER

Škopljanac-Mačina L*, Audy-Jurković S*, Mahovlić V*, Ilić-Forko J.**

*Institute of Gynecologic Cytology, University Department of Gynecology and Obstetrics, Zagreb University Hospital Center, **Clinical Institute of Gynecologic and Perinatal Pathology, Zagreb University Hospital Center, Zagreb, Croatia

Aim of the study. To point to the issue of the patient upper age limit on planning national screening for prevention of cervical carcinoma in Croatia. According to the National Cancer Registry data, in the Croatian female population aged 50-85 the incidence of invasive cervical carcinoma is greater than that of carcinoma in situ.

Patients and Methods. Cases of histopathologically verified intraepithelial neoplasia in patients aged 65 and older with previous cytologic findings, recorded from January 1, 2000 till December 31, 2004, were retrospectively analyzed. Histopathologic analysis was performed at Clinical Department of Gynecologic and Perinatal Pathology, and cytologic analysis at Department of Gynecologic Cytology, University Department of Gynecology and Obstetrics, Zagreb University Hospital Center, Zagreb.

Results. Positive histopathology findings were recorded in 29 patients, mean age 69 (age range 65-79) years, including CIN III (n=17), CIN II (n=7), CIN I (n=4), and CIN III with AIS (n=1). Twenty-five patients had abnormal and four patients negative cytologic findings. Unremarkable clinical diagnosis was made in 20, uterus prolapse was diagnosed in three, and metrorrhagia in six patients.

Conclusion. The unfavorable ratio of the number of newly detected cases of intraepithelial neoplasia and the incidence of invasive cervical carcinoma in older age groups in Croatia calls for additional efforts and investigations concerning the need of including women aged 65 and older in the program of prevention.

Clinical Cytology / Oral presentations / 27

THREE CASES OF THYMOMA DIAGNOSED BY FNAC

Šokčević M, Maričević I, Ćurić-Jurić S.

Department of Cytology, Sestre milosrdnice University Hospital, Zagreb, Croatia
msokcevi@kbsm.hr

Aim of the study: Three cases of the invasive thymoma with cytomorphology and clinical data are presented. In all cases invasive nature of the lesions was clinically evident.

In the first case cytological diagnosis of thymoma was confirmed by immunocytochemistry and DNA PCR analysis, in the second by pathohistology, and in the third case previous histology and subsequent progression demonstrated by CT scans supported the diagnosis of thymoma. Cytomorphology was various: in the first case lymphocytes and in the second the epithelial cells predominated. In the third case scarce cellular smears contained only lymphatic cells. In the cases with epithelial cells these were predominantly round to oval (cortical type of cells) with some anisocytosis and prominent nucleoli (atypia). Lymphocytic population consisted predominantly of small lymphocytes. T-cell immunophenotype was demonstrated in one case by imunocytochemistry and polyclonality by DNA PCR analysis.

Conclusion: Cytological evidence of dual population of epithelial cells and lymphocytes forming cohesive clusters and tissue particles makes the diagnosis of the thymoma reliable in the appropriate clinical setting. Invasiveness of the thymoma can not be evaluated by cytomorphology, but the predominance of the cortical type of epithelial cells with atypia favours it.

Clinical Cytology / Oral presentations / 28

CYTOLOGY AND "FOLLOW UP" OF CERVICAL INTRAEPITHELIAL LESIONS

Štemberger-Papić S*, Krašević M**, Stanković T*, Vrdoljak-Mozetič D*, Verša-Ostojić D*, Mozetič D***, Gobić J***, Lakošeljac A*.

*Department of Gynecological Cytology, University Department of Gynecology and Obstetrics, Rijeka University Hospital Center, **Department of pathology, School of medicine, University of Rijeka,
***Department of Gynecology, University Department of Gynecology and Obstetrics, Rijeka University Hospital Center, Rijeka, Croatia

Aim of the study. Cyto - pathohistological correlation and cytological "follow up" of cervical intraepithelial lesions after the conization/LLETZ (Large Loop Excision of the Transformation Zone).

Patients and Methods. Retrospective study included 232 patients in which conisation or LLETZ were performed in 1998. and 2001. in Clinic for Gynecology and Obstetrics, Clinical Hospital Centre Rijeka. Analyzed parameters included the number of abnormal cytological VCE (Vaginal, Cervical, Endocervical) reports and duration of abnormal cytology before the operative procedure, cyto-pathohistological correlation of those performed, as well as finding and comparation of abnormal cytology after conisation/LLETZ.

Results. 84 conisations and 2 LLETZ were performed during the 1998. and 75 conisations and 71 LLETZ were performed during the 2001. The number of abnormal cytological reports in 1998. ranged from 1-21 (average 3.6) and duration of abnormal cytology from 0-12 years (average 19.6 months). The number of abnormal cytological reports in 2001. year ranged from 1-14 (average 3.1) and duration of abnormal cytology was from 0-20 years (average 23.2 months). Cyto-pathohistological accordance was in 87.1%. Cytological follow up in 1998. was available for 75 (87.2 %) patients and in 2001. for 121 (82.9%) patients. Time period of first abnormal control cytology ranged from 1-58 months (average 14.9) in 1998., while in 2001. ranged from 2-28 months (average 4.7).

Conclusions. Correlation of cytological and pathohistological reports was very high. The period of appearance of the first abnormal control cytology after performed surgery was 9.8 months. Statistically significantly higher number of abnormal control cytology was observed after LLETZ compared to conisation.

Clinical Cytology / Oral presentations / 29

FINE NEEDLE ASPIRATION CYTOLOGY (FNAC) OF GIST: CYTOMORPHOLOGIC FEATURES

Štoos-Veić T[1], Kaić G[1], Ožegović M[2], Buljevac M[3], Trutin Ostović K[1], Kardum-Skelin I.[4]

[1] Department of cytology, [2]Department of Pathology, [3]Department of Internal Medicine, University Hospital Dubrava, Zagreb, Croatia, [4] Department of Medicine, University Hospital Merkur, Zagreb, Croatia

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal tumors arising in the gastrointestinal tract and occasionally in mesentery and omentum. Fine needle aspiration cytology combined with immunocytochemistry has been shown to be quick and reliable diagnostic tool for diagnosis of primary and metastatic GIST.

Patients and Methods: We present five cases of GIST preoperatively diagnosed on MGG stained cytologic slides. Material was obtained by percutaneous US-guided FNA in four cases and by transrectal FNA with Franzen needle in one case. Immunocytochemistry was performed on cytologic smears in three cases and since tumor cells were positive for CD 117 definitive diagnosis was given. In other two cases diagnosis was mesenchymal tumor, probably GIST.

Results: Diagnosis was confirmed by subsequent histology and immunohistochemistry on resected material in four cases and one was considered inoperable. Cytomorphologic features included highly cellular smears with spindle cells in cohesive clusters in all cases. Elongated nuclei with blunt ends were found in all cases with more pronounced anysonucleosis and irregular nuclei in three cases. Chromatin was finely granular and nucleoli incospicous. Cytoplasms were blue-grey, bipolar and delicate. We noticed pink amorphous stroma in most cases. Background was usually bloody with variable number of single cells and naked nuclei. Macrophages and inflammatory cells were found in two cases.

Conclusion: FNA cytology is quick and reliable in the diagnosis of GIST. Combined with immunocitochemistry it enables accurate preoperative diagnosis. It is important for patients with inoperable tumors and metastatic disease especially since new promising therapy with imatinib mesylat was introduced.

Clinical Cytology / Oral presentations / 30

MULTINUCLEATED CELLS IN BRONCHOALVEOLAR LAVAGE (BAL)

Vrabec-Branica B[1], Smojver-Ježek S[1], Peroš-Golubičić T[2], Tekavec-Trkanjec J.[2]

Departments of [1]Cytology and [2]Pneumology, University Hospital for Lung Diseases "Jordanovac", Zagreb, Croatia
ssmojver@pbf.hr

Aim of the study: To determine the frequency, morphology and possible diagnostic significance of multinucleated cells in bronhoalveolar lavage (BAL).

Material and Methods: Retrospectively were examined 50 BAL specimens. Cells with >3 nuclei with described cytomorphologic features were defined as multinucleated cells (MC). All BAL specimens were grouped according to clinicohistologic diagnosis in 10 groups: sarcoidosis (17), tuberculosis (4), idiopathic pulmonary fibrosis (10), asbestosis (1), hystiocytosis X (1), alveolar proteinosis (1), pulmonary eosinophilia (1), pulmonary manifestation of colagenovascular diseases (3), other interstitial lung diseases (6) and other noninterstitial lung diseases (6).

Results: MC were present in all BAL specimens. Based on cytomorphologic features MC have been classified in 3 groups, as alveolar macrophage like MC (AM-MC), Langhans or foreign body type MC (LS-MC) and non-specific MC (NS-MC). AM-MC were present in all BAL specimens, often as the most numerous type of MC. In patients with tuberculosis NS-MC were the most numerous type of MC (55,1%). NS-MC were not present in BAL specimens with hystiocytosis X, alveolar proteinosis and pulmonary eosinophilia. LS-MC were present only in BAL specimens of some clinicohistologic groups (sarcoidosis, tuberculosis, idiopathic pulmonary fibrosis and other interstitial lung diseases). They were the most numerous type of MC in patients with tuberculosis (17,2%) and sarcoidosis (8,7%). We grouped MC also in categories according to the number of their nuclei. AM-MC and NS-MC contained < 10 nuclei in 94,3% and 97,2%. LS-MC contained >10 nuclei in 67%.

Conclusion: MC were present in all BAL specimens. Based on cytomorphologic features 3 types of MC can be distinguished. AM-MC were present in all clinicohistologic groups, and NS-MC were present in 7/10 clinicohistologic groups. LS-MC were specific for some clinicohistologic groups.

Clinical Cytology / Oral presentations / 31

INTRAOPERATIVE CYTOLOGY OF CLEAR CELL CARCINOMA OF THE OVARY

Vrdoljak-Mozetič D*, Stanković T*, Krašević M**, Verša-Ostojić D*, Štemberger-Papić S*, Rupčić S***

*Department of Gynecological Cytology, University Department of Gynecology and Obstetrics, Rijeka University Hospital Center, **Department of pathology, School of Medicine, University of Rijeka, ***Department of Gynecology, University Department of Gynecology and Obstetrics, Rijeka University Hospital Center, Rijeka, Croatia

Aim of the study. To describe the cytomorphology of clear cell carcinoma (CCC) of ovary in intraoperative samples of peritoneal fluid, imprint and scraping of tumor tissue.

Patients and Methods. Fourteen cases of CCC, histologicaly confirmed, were analyzed. Samples were stained by May Grünwald Giemsa (MGG) and Papanicolaou standard procedures and observed by light microscope.

Results. In cytological imprint and scrape samples, beside variable clear cell cellular morphology, two distinct cytological characteristics were observed: eosinophilic, hyaline, extracellular, globular substance with or without forming a <raspberry> body and eosinophilic, intracitoplasmatic, globular substance. In one case lacking eosinophilic, extracellular, globular substance, we observed abundant finding of intracitoplasmatic, eosinophilic globula. These structures were clearly seen only in samples stained by MGG. In peritoneal fluid samples, from nine positive findings only four contained extracellular, eosinophilic, hyaline, globular substance and three contained "raspberry" bodies.

Conclusion. By cytological analysis of intraoperative imprint and scraping samples of ovarian tumors, using the two described cytomorphoplogical features, highly accurate cytological diagnosis of clear cell carcinoma of ovary could be made.

Clinical Cytology / Oral presentations / 32

THE POSSIBILITY OF CYTOLOGICAL DIAGNOSIS OF PROSTATIC INTRAEPITHELIAL NEOPLASIA

Znidarčić Ž, Kaić G, Trutin Ostović K, Stoos-Veić T, Miletić Z, Heinzl R, Bartolin Z, Matulić S, Bedalov G, Savić I, Cvrk B.

Clinical University Hospital Dubrava, Zagreb, Croatia
zznidarc@inet.hr

Aim of the study: Prostatic intraepithelial neoplasia (PIN) is carcinogenic process in the prostate which has not been fully investigated in cytology. Being less invasive and less expensive, cytology should be more used in the early diagnosis of prostatic cancer and its precursors. The aim of this investigation is to examine correlation between cytodiagnosis of prostatic atypical hyperplasia and pathohistological diagnosis of PIN.

Methods: A total of 766 slides (562 FNA and 204 imprints of prostatic tissue) was cytomorphologically analysed, and semiquantitative scores of diagnostic cytomorphological parameters were done. Among them, 175 (22.8%) were diagnosed as atypical hyperplasia. The same tissue specimens, used for cytological imprints, were then pathohistologically examined. Flow-cytometry was made in 237 investigated prostates, and nuclear and AgNOR morphometry was done in 60 specimens. Immunocytochemistry (PSA and PAP) was performed in all investigated prostates, as well as serum PSA. Statistical analysis of all investigated parameters was made in order to get complete information of their correlations.

Results: Not more than 11% of cytologically diagnosed atypical hyperplasias were pathohistologically diagnosed as prostatic intraepithelial neoplasia. Cytomorphological scores, flow cytometry and serum PSA showed statistically significant correlation with pathohistological diagnoses. Morphometric analyses and immunocytochemistry correlated well only with the diagnosis of low differentiated carcinoma.

Conclusion: Our results could not confirm that the cytodiagnosis of atypical hyperplasia corresponds to the pathohistological diagnosis of PIN. However, a further investigation, with experience we have, on a greater group of adequately chosen patients and methods of investigation, can yield different results.

KLINIČKA CITOLOGIJA
CLINICAL CYTOLOGY

Posteri / *Posters*

Citologija dojke / *Breast Cytology (1-2)*

Ginekološka citologija / *Gynecological Cytology (3-5)*

Citologija glave i vrata / *Head and Neck Cytology (6-11)*

Hematološka citologija / *Hematological Cytology (12-15)*

Pulmološka citologija / *Pulmonary Cytology (16-19)*

Slobodne teme / *Free Topics (20-23)*

Clinical Cytology / **Posters** / **Breast Cytology** / **01**

EFFICACY OF FINE NEEDLE ASPIRATION IN THE DIAGNOSIS OF BREAST LESIONS

Maričević I, Ćurić-Jurić S, and Jurić F.

Department of Cytology, Sestre milosrdnice University Hospital, Zagreb
fjuric@mamef.mef.hr

Fine-needle aspiration cytology (FNAC) is widely used in the diagnosis of breast lesions and selection of patients for surgical treatment.

Aim of the study: In our Department of Cytology during six-year period FNAC was performed in 3000 patients, in most cases under ultrasound guidance.

Results: In a group of 202 women who underwent surgical treatment at our hospital, preoperative cytologic findings were malignant in 133 cases, benign in 64 cases and 5 specimens were inadequate. Postoperative histology found 144 carcinomas and 58 benign lesions. Statistical analysis revealed the sensitivity of FNAC for the presence of carcinoma to be 96%, specificity 84,05%, positive predictive value 92,90% and negative predictive value 96,25%.

Conclusion: These results confirm that FNAC is a highly reliable method in the diagnosis of breast lesions.

Clinical Cytology / **Posters** / **Breast Cytology** / **02**

HIGH GRADE MALIGNANT PHYLLODES TUMOR RECIDIVANS AFTER LOBULAR CARCINOMA OF THE SAME BREAST -A CASE REPORT

Staklenac B, Dmitrović B, Pajtler M, Lončar B, Pauzar B, Miličić-Juhas V.

Clinical Hospital Osijek, Osijek, Croatia
staklenac.blazenka@kbo.hr

Aim of the study was to demonstrate diagnostic difficulties of malignant phylodes tumor.

Methods and Results: A 71-year-old woman with two subcutaneous well defined nodes of 1 and 4 cm in diameter on the left thoracic wall in July 2004. was presented.

20 years ago she was admitted in Vinkovci Country Hospital where left mastectomy and axillary lymphadenectomy was performed and diagnosis of invasive lobular carcinoma with axillary lymph node metastases was established. Few years ago patient underwent surgery several times because of the nodules on the thoracic wall.

FNAB of two nodules were made. Monomorphic patterns (MGG) were composed of bizarre oval and spindle shaped cells. Cellular aggregates showed no special organization. Oval, hypochromic nuclei were large, bi- and multinuclear with pronounced nucleoli and nucleolar outline and fine regular chromatin. Cytoplasm were abundant and spindle shaped. Myxomatous part with pale pink mass were also found. Cytological diagnosis of tumor phyllodes was made and patient underwent surgery again.

Reviewing the slides at the Pathology Department, it was observed that the patient has been re-operated 9 times in the last 7 years, and that the slides have been reviewed by 5 different pathologists. The diagnoses were: granulation tissue, fibrosis, fibroma, fibromatosis, and postirradiational fibrosis. Reviewing the 8th specimen in July 2004, the diagnosis of recidive malignant phyllodes tumor was finally achieved. The last recidive excision was performed in A diagnostic accuracy. ugust 2004.

Conclusion: FNAB after mastectomy was very useful in improving

Clinical Cytology / **Posters** / **Gynecological Cytology** / **03**

INTRODUCTION OF P16INK4A BIOMARKER ON FRESH AND ARCHIVAL CERVICAL SMEARS

Audy-Jurković S*, Ovanin-Rakić A*, Jurić D*, Babić D.**

*Institute of Gynecologic Cytology, University Department of Gynecology and Obstetrics, Zagreb University Hospital Center, **Clinical Institute of Gynecologic and Perinatal Pathology, Zagreb University Hospital Center, Zagreb, Croatia

Aim of the study. To introduce immunocytochemical visualization of p16INK4a biomarker on fresh and archival cervical smears.

Method. p16INK4a is a specific inhibitor of the cycline-dependent kinase (CDK4 and CDK6) which plays a crucial role in the regulation of the cell cycle by retinoblastoma protein phosphorylation. Increased expression of the high-risk human papillomavirus E6 and E7 oncogenes through binding to the retinoblastoma protein and release of transcription factor E2F results in a highly specific increase in p16INK4a protein expression in dysplastic and malignant cells of squamous and cylindric epithelium of the cervix, which is detectable by a specific monoclonal antibody. The criteria for patient selection were cytologic and histologic diagnosis of cervical intraepithelial neoplasia (CIN) II and III, and adenocarcinoma in situ (AIS). The expression of p16INK4a was immunocytochemically visualized in fresh fixed cervical smears and reprocessed conventional Papanicolaou stained slides. A fresh exocervical sample was in part smeared onto the slide, and in part placed in saline according to our own modification (Ovanin-Rakić) and centrifuged. Reprocessing of archival slides was performed according to Choi (1991). The specific biomarker was visualized by use of DakoCytomation p16INK4a Cytology Kit containing specific anti-mouse monoclonal antibody (clone E6H4) and EnVision visualization system.

Results. Test result is considered positive if brownish granules are found in the nuclei and/or cytoplasm of at least five dysplastic or malignant cells. The number of positive cells and intensity of nucleus and/or cytoplasm staining vary from case to case.

Conclusion. In future studies, the immunocytochemical analysis of p16INK4a expression will be applied in a greater number of cervical smears to evaluate the method in clinical setting.

Clinical Cytology / Posters / Gynecological Cytology / 04

CONE EXCISION - THE DIAGNOSTIC ACCURACY OF CYTOLOGY

Šentija K*, Krivak-Bolanča I*, Gašparov S.**

*Laboratory for Medical Genetics and Cytology, Department of Gynecology and Obstetrics,
** Department of Pathology and Cytology, Merkur University Hospital Zagreb, Croatia

Aim of the study. To evaluate the diagnostic accuracy of Pap smears in patients that subsequently underwent conization due to abnormal cytological findings.

Patients and Methods. We compare and correlate the cytological findings of 107 patients with follow-up histological findings on the excised cone. Cytological diagnoses were reported according to the modified Bethesda System. The abnormal Pap smears were ranging from LGSIL (2) to most frequent HGSIL (99), microinvasive (1) and invasive carcinoma (5). Histological findings exhibited a wide spectrum ranging from no pathologic abnormality (1), CIN II (6), CIN III (39), CIS (51), MICA (6), and invasive carcinoma (4).

Results. The Pap smear test was fully diagnostic in 94/107 (87.9%) cases: 6/6 (100%) CIN II cases, 38/39 (97.4%) CIN III cases, 47/51 (92.2%) CIS cases and 3/4 (75%) invasive carcinomas. In 8/107 (7.5%) cases cytological and histological findings differed within one degree, and more than one degree in 5/107 (4.7%) patients: in one patient with cytological HGSIL histopathology showed no abnormalities; two patients with cytological LGSIL had CIN III and a CIS lesion; seven patients with HGSIL had MICA (6) and invasive carcinoma (1); three patients cytologicaly overdiagnosed as MICA (1) and invasive carcinoma (2) had CIS.

Conclusion. In our material, we observed high correspondence between the cytological and histological diagnosis of high-grade intraepithelial lesions of the cervix, and, unexpectedly, lower accuracy of cytological smear in the diagnosis of (micro) invasive lesions.

Clinical Cytology / **Posters** / **Gynecological Cytology** / **05**

TWO CASES OF GENITAL ECHINOCOCCUS DIAGNOSED BY INTRAOPERATIVE IMPRINT CYTOLOGY

Vrdoljak-Mozetič D*, Stanković T*, Štemberger-Papić S*, Verša-Ostojić D*, Krašević M**, Haller H***, Rupčić S.***

*Department of Gynecological Cytology, University Department of Gynecology and Obstetrics, Rijeka University Hospital Center, **Department of Pathology, School of Medicine, University of Rijeka,
***Department of Gynecology, University Department of Gynecology and Obstetrics, Rijeka University Hospital Center, Rijeka, Croatia

Aim of the study. To present clinical data and cytological features of two histological proven cases of genital echinococcus.

Patients and Methods. Both patients were admitted to hospital because of abdominal pain and suspected multicystic tumor of ovary by gynecological physical and transvaginal ultrasonographic exam. Both had positive history of liver echinococcosis followed by surgical treatment. In both cases intraoperative cytological samples were taken from enlarged and irregular uterus that was surgically removed. After the uterus has been cut through, the imprint cytology samples were taken from whitish, variably sized, multiple "balls" that were falling out from mother cyst unattached to surrounding tissue or to each other. The samples were stained by quick May-Grünwald-Giemsa method and analyzed by two cytologists.

Results. In both cases the findings of many typical scolices and hooklets, accompanied by noncellular granular debris and elements of granulomatous reaction, enabled us to diagnose the tumor as echinococcal cysts. During the intraoperative procedure cytologists were not aware of data concerning previous echinococcal infections in both patients. In both cases histological reports confirmed the cytological diagnoses.

Conclusion. Although a rare pathology in female genital tract, knowing and recognizing a typical cytomorphology of its elements, echinococcus infection can be diagnosed during surgery by imprint cytology.

Clinical Cytology / **Posters** / **Head and Neck Cytology** / **06**

CYTOLOGICAL DIAGNOSIS OF PATHOLOGICALLY CHANGED PARATHYROID GLANDS

Fuštar-Preradović Lj, Halbauer M, Danić D, Jakovina T, Jakovina K.

Department of Pathology and Cytology, General Hospital Slavonski Brod, Croatia
Clinical Institute of Nuclear Medicine, Clinical Hospital Zagreb, Croatia
Department of Otorinilaryngology and Cervicifacial Surgery, General Hospital Slavonski Brod, Croatia

Hyperparathyreoidism is a syndrome caused by adenoma, hyperplasia or rarely by cancer of parathyroid glands. Medical treatment of hyperparathyreoidism consists of surgical removal of magnified parathyroid glands or percussing inactivity of the tumor of parathyroid glands with alcohol. Both procedures demand preoperative localisation of the tissue of the parathyroid gland. Because parathyroid glands are generally not palpable and are located deeply in the neck region, introduction of ultrasound imagging enabled more precise diagnosis and localisation of parathyroid glands and possibility to perform ultrasound guided feene needle bipsy. The obtained cell material is used for cytologic analyses, for determination of value parathormones in puncture, for cytochemical analyses and imunocytochemical analyses.

Conclusion: Simultaneously using two or more complementary methods provides correct identification and more precise preoperative diagnose pathologychal changes of parathyroid glands

Clinical Cytology / Posters / Head and Neck Cytology / 07

FINE NEEDLE ASPIRATION CYTOLOGY OF PILOMATRIXOMA: REPORT OF 5 CASES

Ramljak V[1], Ranogajec I[1],Novosel I.[2]

[1]University Hospital for Tumors, Zagreb, Croatia,
[2]Dr Ivo Pedišić General Hospital, Sisak, Croatia
vesna.ramljak@kzt.hr

Aim of the study: To evaluate retrospectively clinical and cytological features of five cases of pilomatrixoma, (the cytologic diagnosis confirmed by histology), and to characterize their cytomorphologic features (observed between 2000 and 2004).

Methods: The aspirations were done by cytologist, and the smears were air-dried and stained with May-Grünwald-Giemsa (MGG). In all smears we excluded malignancy but we couldn't confirm diagnosis of pilomatrixoma. Cytological and histological analysis was done by a semiquantitative method. The following features: basaloid cells, shadow cells, inflammatory cells, giant cells, naked nuclei, and debris were semiquantified from 0 to 3 (0 absent, 1+ mild amount, 2+ moderate, 3+ abudant) and then we compared results.

Results: The series included 5 patients undergoing fine-needle aspiration of subcutaneous nodes (2 in the neck region, 1 in the back region, 1 in the facial skin, 1 in the right parotid region). All cellular elements were at least scantly present in each of the examined cytological smears; however, the maximum score was obtained for: basalod cells 9/15, shadow cells 9/15 and giant cells 8/15. The maximum score obtained on the histological sections was for: basaloid cells 15/15 and shadow cells 12/15.

Conclusion: The presence of basaloid cells, ghost cells and giant cells in cytological smears and also clinical information as localization in the head and neck region, and slowly growing tumor of the skin or subcutaneous tissue will allow a conclusive diagnosis of pilomatrixoma by FNA.

Clinical Cytology / Posters / Head and Neck Cytology / 08

FINE NEEDLE ASPIRATION CYTOLOGY OF HEAD AND NECK LYMPH NODES IN 6-YEARS PERIOD

Vasilj A, Kojić Katović S, Ćurić Jurić S, Jurić F.

Department of Cytology, Sestre Milosrdnice University Hospital, Zagreb, Croatia
Mislav.Katovic@zg.htnet.hr

Lymphadenopathy of head and neck is common clinical problem. It can be caused by infections, malignancy, immunological disorders etc. Fine needle aspiration cytology has important role in the diagnosis of enlarged lymph node.

In the Laboratory of Cytology, Sestre Milosrdnice University Hospital, we performed 2196 fine needle aspirations in the 6-years period (1999-2004). 457/2196 (21%) aspirations was performed under ultrasound guidance. 111/2196 (5%) smears were nondiagnostic. 1370/2085 (66%) of lymph nodes were benign and 715/2085 (34%) were malignant. Hematological disease (non Hodgkins lymphoma and Morbus Hodgkin) was diagnosed in 242/715 (34%) and metastatic process in 473/715 (66%) of malignant lymph nodes. Immunocytochemistry was performed in 17 cases (11 lymphomas and 6 metastatic tumors) during the first 5 years and in 24 cases (11 lymphomas and 13 metastatic tumors) during the last year.

Fine needle aspiration cytology was found useful in the management with patients who underwent this procedure. Patients with benign diagnosis were followed up and some of them were serologically tested on infectious diseases. Patients with new detected hematological disease were mostly operated on (node excision) for histopathological diagnosis and patients with metastatic disease were directed to oncological treatment.

Clinical Cytology / **Posters** / **Head and Neck Cytology** / **09**

PAPILLARY THYROID CARCINOMA ASSOCIATED WITH TOXIC ADENOMA

Veselić L, Kopjar A.

General Hospital Varaždin ,Varaždin, Croatia
ladislava.veselic@vz.htnet.hr

Introduction: Thyroid nodules may be found by ultrasound in 50% population over 50 years of age. They are solid or multiply nodules and ultrasound examination demonstrates them like a hipoechoic, isoechoic or hiperechoic nodules. On scintiscan, nodules may be "cold" or "hot". Cold nodules are more frequent benign adenomas and cysts than carcinomas (abt.20% cold nodules are carcinomas). Toxic adenoma in association with thyroid carcinoma are very rare at the same patient. We can't diagnose this case without US-guided needle aspiration. Reviewing foreign medical literature we found only a few reports described patient with toxic adenoma associate with thyroid carcinoma. In Croatian medical literature this case was not reported. Case report: We performed 1023 US-guided fine needle aspiration during the year 2003. In general, 13 aspirates

(1,27%) were diagnosted with papillary carcinoma, 20 aspirates (1,95%) with toxic adenoma. The goal of this report is to show one of three cases (0,29%) with toxic adenoma associate with papillary thyroid carcinoma. Two cases (women patients) were diagnosed with nodules in different lobes, and one case (39 year old man) were diagnosed with toxic adenoma and papillary thyroid carcinoma in the same lobe. By ultrasound examination we found in the same lobe one hipoechogenic and one isoechogenic nodule; at the same time, scintiscan demonstrated cold and hot nodule.

Materials and Methods: The specimen obtained with US guided fine needle aspiration was stained with MGG method and immunocytochemically analysed by thyreoglobulin. Surgical material was embedded in paraffin and 5 μm sections were stained by standard H&E method and immunocytochemically analysed by thyreoglobulin. Results: Cytologic examination in aspiration of isoechogenic nodule, measure 16x13 mm, revealed a clousters like rosetes with no big increase of thyreocits, a colloid and a few hiperactive thyreocits. This examination suggested diagnosis of toxic adenoma. In aspiration of hypoechogenic nodule, measure 12x8 mm, we found a many papillary clousters of epithelial cells with round nucleus with intranuclear inclusions and psammoma bodies. This examination suggested diagnosis of papillary carcinoma. Histologically, first nodule was shelled and made by follicles, overlayed by fibrous capsule without invasion in the capsule and blood vessels. The second nodule was made by papillary fibrovascular stalk, and overlayed by atipical epithelial cells which nucleus shows "ground - glass" phenomenon.

Conclusion: The cases of papillary carcinoma in association with toxic thyroid adenoma are very rare. Cytology has important place in evaluation of thyreoid nodules, as a method for easier decision for surgical therapy.

Clinical Cytology / Posters / Head and Neck Cytology / 10

CYTOLOGICAL DIAGNOSIS OF PATHOLOGICALLY CHANGED PARATHYROID GLANDS

Fuštar-Preradović Lj[1], Halbauer M[2], Danić D[1], Jakovina T[1], Jakovina K[1].

[1]Department of Pathology and Cytology, General Hospital Slavonski Brod, Croatia
[2]Clinical Institute of Nuclear Medicine, Clinical Hospital Zagreb, Croatia
Department of Othorinolaryngology and Cervicifacial Surgery, General Hospital Slavonski Brod, Croatia

Hyperparathyreoidism is a syndrome caused by adenoma, hyperplasia or rarely by cancer of parathyroid glands. Medical treatment of hyperparathyreoidism consists of surgical removal of magnified parathyroid glands or percussing inactivity of the tumor of parathyroid glands with alcohol. Both procedures demand preoperative localisation of the tissue of the parathyroid gland. Because parathyroid glands are generally not palpable and are located deeply in the neck region, introduction of ultrasound imagging enabled more precise diagnosis and localisation of parathyroid glands and possibility to perform ultrasound guided feene needle bipsy. The obtained cell material is used for cytologic analyses, for determination of value parathormones in puncture, for cytochemical analyses and imunocytochemical analyses.

Conclusion: Simultaneously using two or more complementary methods provides correct identification and more precise preoperative diagnose pathologychal changes of parathyroid glands

Clinical Cytology / **Posters** / **Head and Neck Cytology** / **11**

CORRELATION OF CYTOLOGIC AND HISTOPATHOLOGIC DIAGNOSIS OF THE PAROTID GLAND TUMORS

Žoković E., Maričević I., Šokčević M., Jurić F.

Fine needle aspiration cytology (FNAC) is often used in diagnostics of parotid gland lesions. In our Department of Cytlogy, during a 5-years period (1999-2003), FNA was performed in 176 patients, some of them under ultrasound guidance. Cytological analyses found tumor lesions in 85 cases. Correlation with histopathology was available in 50 cases. Of these, 40 were histologically benign (15 Warthin¢s tumors, 23 pleomorphic adenomas, and one basal cell adenoma and possible oncocytoma each) and 10 histologically malignant (4 adenoid cystic carcinomas, 3 mucoepidermoid carcinomas, and one basal cell carcinoma and carcinosarcoma each).

In the group of histological benign tumors (n=40) cytologic diagnosis was also benign tumor in 39 cases and cyst with inflammation in one case.

In the group of histologically malignant tumors (n=10) cytologic diagnosis was malignant in 8 cases and benign tumor in 2 cases.

FNAC is highly efficient in diagnostics of parotid gland lesions, patient selection for surgical treatment and differentiating benign from malignant tumors.

Clinical Cytology / Posters / Hematological Cytology / 12

RHABDOMYOSARCOMA WITH BONE MARROW INFILTRATION-CASE REPORT

Jelić-Puškarić B[1], Kardum-Skelin I[1], Šušterčić D[1], Raić LJ [2], Femenić R [2], Bilić E [2], Batinić D[3], Nola M[4], Konja J.[2]

[1]Department of Medicine, Merkur University Hospital,
[2]Department of Paediatrics, Clinical Hospital Center,
[3]Department of Laboratory Diagnostic, Clinical Hospital Center,
[4]Department of Pathology, Clinical Hospital Center, Zagreb, Croatia
biljana.jelic.puskaric@zg.t-com.hr

Rhabdomyosarcoma is the most common soft tissue sarcoma of children under 15 years of age. According to the WHO classification of soft tissue tumors the following types of rhabdomyosarcoma are subclassified: embryonal rhabdomyiosarcoma (60-70%), affects mainly children younger than 15 years of age, alveolar rhabdomyosarcoma (20-25%), occurs predominantly in adolescents and young adults and pleomorphic rhabdomyiosarcoma (5%), occurs predominantly in adults older than 40 years of age. The most common diagnostic problem is the least differentiated tumors with primitive, undifferentiated, small cells. These tumors are cytologically indistinguishable from other small blue cell tumors in children.

A 14 years old girl was admitted to the hospital in September 2003. She complained about tiredness, myalgia and frequent occurrences of bruises. Physical examination revealed multiple petechiae and hematomas on the skin, an enlarged inguinal lymph node and splenomegaly. The laboratory findings showed increased WBC count- 24.8 x 10^9 /L, low haemoglobin level- 80 g/L and platelet count of 8x10^9 /L. CT scan detected a large retroperitoneal tumor mass and enlarged tracheobroncihal, retroperitoneal and inguinal lymph nodes. The bone marrow aspirate smears showed massive infiltration by tumor cells. The immunocytochemical staining was positive for PAS, vimentin, desmin, CD 68H and negative for LCA, CD 19, CD 20, CD 3, CD 10, NSE, Ber EP 4, OIL RED and ANAE. In regard of morphollogical, cytochemical and phenotype characteristics, cytologist has diagnosed it is rhabdomyosarcoma. The biopsy of tumor was performed and it confirmed the diagnosis of alveolar subtype of rhabdomyosarcoma.

Differential diagnosis of small blue cell tumors in children presents a problem cytomorphologically, especially if they diffusely infiltrate the bone marrow. Additional technologies (cytochemistry, immunocytochemistry, cytogenetics and molecular analysis) are of a significant help and in some cases even crucial for making a diagnosis.

Clinical Cytology / **Posters** / **Hematological Cytology** / **13**

T AND B CLONAL MALIGNANT LYMPHOMA - A CASE REPORT

Hodak I[1], Kardum-Skelin I[2], Šušterčić D[2], Fabijanić I[2], Kušec R[2], Gašparov S[2], Planinc-Peraica A. [2], Ostojić S[2], Šiftar Z[2], Kardum Paro MM[2], Vrhovac R[2], Minigo H[2], Radić D[2], Dejanović M[3], Judin S[3], Jakšić B[2]

[1]Slavonski Brod General Hospital, Slavonski Brod, [2]Merkur University Hospital, Zagreb, [3]Karlovac General Hospital, Karlovac, Croatia
ivanahodak@yahoo.com

Non-Hodgkin lymphomas (NHL) are heterogeneous group of malignant lymphoprolypherative diseases which have different clinical and morphological manifestations. They are generaly classified into T and B lymphomas. B clonality is diagnosed by restricton of 6 and 8 light chains or by rearrangement of heavy chain for IgH-imunoglobulin. T clonality is diagnosed by rearrangement of TCR gene. NHL's are divided into low grade and high grade.

We report an unusual case of a 52 year old patient with lymphadenopathy who was admitted to hospital in July 1999. Cytological diagnosis was Lennert lymphoma and histological examination of lymph nodes revealed anaplastic large cell non T non B lymphoma. Polyclonality of B lymphocytes were found by imunophenotypisation. He was treated with chemotherapy and complete remission was achieved. However, the patient relapsed in March 2004 with enlarged cervical lymphadenopathy. Furthermore, cytology showed Non Hodgkin diffuse large B cell lymphoma, T-rich (DLBCL-T-rich) while histology revealed florid follicular hyperplasia. T and B clonality were determined by polymerase chain reaction (PCR) and flow cytometry.

Conclusion: Disagreement of cytological and histological subclassification and polyclonality of NHL at the begining and clonality of T and B cells in relaps - indicate that new technologies (in this case molecular methods) may contribute (again) to the classification of malignant lymphomas.

Clinical Cytology / **Posters** / **Hematological Cytology** / **14**

RELATION OF C-KIT EXPRESSION AND DUAL ESTERASE ACTIVITY TO FISH PH+ POSITIVITY IN PATIENTS WITH CML

Sučić M, Davidović-Mrsić S, Marković-Glamočak M, Ries S, Gjadrov-Kuveždić K, Vrbanus LJ, Zadro R, Batinić D, Dubravčić K, Juričević M, Labar B.

Clinical Institute of Laboratory Diagnosis, Department of Hematology, Zagreb Clinical Hospital Center, Croatia
mirna.sucic2@zg.htnet.hr

Chronic myelogenous leukemia (CML) is a myeloproliferative disease with t(9;22) and/or BCR/ABL fusion gene. Treatment of CML patients with imatinib mesylate (Gleevec) is based on the inhibition of BCR/ABL and c-kit (CD117) tyrosine kinase activity.

The aim of the study was to analyze immunocytochemical expression of CD117 expression and cytochemical dual esterase activity in bone marrow hematopoietic cells (HCs) of CML patients and Gleevec therapy and compare results with FISH Ph+ positivity.

Patients and methods: Immunocytochemical APAAP CD117 expression and cytochemical dual esterase activity in HCs were evaluated in 22 patients with CML on Gleveec therapy and in 10 patients without hematological disease (control group).

Results: Medians and ranges of CD117+ and dual esterase+ HCs were similar in patients with CML and the control group and without statistical difference. Although medians of CD117+ and FISH Ph+ HCs were highest in CML patients during the first 6 months of Gleveec treatment, correlation of these two parameters was low. FISH Ph+ HCs were significantly lower in CML patients with longer Gleevec therapy. CD117+ HCs were significantly higher in patients with abnormal peripheral blood leukocyte findings.

Conclusion: Results indicated that Ph+ HCs declined in majority of CML patients on Gleevec therapy. Also, slightly increased CD117+ HCs in CML patients could be connected to inferior response to Gleevec.

Clinical Cytology / Posters / Hematological Cytology / 15

THROMBOCYTOPENIA-CASE REPORT

Šušterčić D[1], Kardum-Skelin I[1], Fabijanić I[1], Jelić-Puškarić B[2], Vrhovac R [2], Planinc-Peraica A[2], Minigo H[2], Jakšić B.[2]

[1]Laboratory of cytology and Haematology, [2]Department of Medicine, Merkur University Hospital, Zagreb, Croatia
ikardum@hi.t-com.hr

Thrombocytopenia is defined as a platelet count less than $150x10^9$/L.There are three fundamental mechanisms of thrombocytopenia: decreased platelet production, increased platelet destruction and pooling of a larger than normal fraction of platelets within the spleen. In surveys reported incidence of pseudothrombocytopenia about 0,09 to 0,21 percent when low platelet count reported by automated hematology analyzers, but microscopic examination of the peripheral blood smears show many platelets. The most common artifact causing pseudothrombocytopenia is in vitro clumping of platelets in blood collected into EDTA anticoagulant or attaching platelets to leukocytes and monocytes (rosette). Clamping activity increases over time if samples are left unanalyzed or if a samples are cooled less than 37 C.

A 47 year old woman with thrombocytopenia an unremarkable medical history , without hemoragic diathesis and normal laboratory tests. We made a complete set of laboratory tests, including puncture of bone marrow and peripheral blood smear, all of which were normal. Because of these results, we suspect that it was pseudothrombocytopenia. We determined that the number of platelets in the venous blood with anticoagulant EDTA was $33x10^9$/L and with anticoagulant Na Citrate was $236x10^9$/L, which confirms the diagnosis of pseudothrombocytopenia.

Pseudothrombocytopenia have no clinical importance, no abnormalities of hemostasis or thrombosis and need no any therapy. False diagnosis of thrombocytopenia have led to serious problems and unnecessary glucocorticoid therapy, postponed surgery and even splenectomy.

Clinical Cytology / Posters / Pulmonary Cytology / 16

DETECTION OF ACID-FAST BACILLI IN CYTOLOGIC SMEARS

Kamauf-Balabanić B[1], Smojver-Ježek S[2], Vrabec-Branica B[2].

Departments of [1]Mycrobiology and [2]Cytology, University Hospital for Lung Diseases "Jordanovac", Zagreb, Croatia
blanka-kamauf@net.hr

Aim of the study: The purpose of this review is to present the experience of the combined cyto-bacteriological method for detection of acide-fast bacilli (AFB) in cytological specimens obtained by fiberbronchoscopy and transthoracic fine needle aspiration.

Materials and methods: In our hospital 231 patients were treated for tuberculosis (TB) during one-year period. Only 69 (29,89%) of all TB cases had cytological specimens. All cytological smears were routinely stained with May-Grünwald-Giemsa. Reviewed smears were submitted for bacteriologic auramine staining and examined by fluorescence microscopy.

Results: AFB was detected in 55 smears (79.71%). Cytomorphologically in 26 patients (47.27%) granuloma elements were found, and 21 patients (38.18%) had elements of granuloma with necrosis. All these cases had confirmation of M. tuberculosis by culture. In 9 cases of all TB cases (3.89%), acid fast bacilli were detected in cytological specimens. These cases were the only positive bacteriological finding which suggested the diagnosis of tuberculosis. Positive results of fluorescence microscopy had 118 patients of 231 TB patients. Cytologically investigated smears increased the number of positive smears for 7.62%.

Conclusion: The auramin staining of cytological smears based on the cytomorphological features is a rapid and sensitive method for the presumptive diagnosis of tuberculosis infection.

Clinical Cytology / **Posters** / **Pulmonary Cytology** / **17**

FINE NEEDLE ASPIRATION CYTOLOGY (FNAC) OF METASTATIC LUNG SQUAMOUS CELL CARCINOMA IN THIGH MUSCLE. A CASE REPORT.

Ljubić N[1], Kardum-Skelin I[2], Petrović Z[1], Bago J[1], Grgurević Batinica A[1].

[1]General Hospital "Sveti Duh", Zagreb, Croatia
[2]Clinical Hospital "Merkur", Zagreb, Croatia
nives.ljubic@zg.htnet.hr

Aim of the study: Squamous cell carcinoma of the lung is known as carcinoma that commonly metastasises to lymph nodes, liver, brain, bones and adrenal glands, but one of its well known characteristics is that it can metastasise to almost every organ. In routine diagnostic practice the vast majority of squamous cell carcinoma of the lung is diagnosed on bronchoscopic material.

Methods: In this paper we present a case of a male with diagnosis of metastatic carcinoma of the lung in FNAC of thigh muscle. A 75 year old male was presented with a history of painless tumours on both thighs. Tumours were tender, deep seated and measured 8 cm in diameter. FNAC had been performed.

Results: The air dried smears were stained with May-Grunwald-Giemsa and the diagnosis of the metastatic squamous cell carcinoma has been established. Patient died after 15 days and the autopsy resulted in primary location of the lung carcinoma.

Conclusion: Although the lung carcinoma is one of the most frequent malignancies, FNAC diagnosis of primary tumour that metastases into muscle is extremely rare.

Clinical Cytology / Posters / Pulmonary Cytology / 18

FINE NEEDLE ASPIRATION CYTOLOGY (FNAC) OF METASTATIC LUNG SQUAMOUS CELL CARCINOMA IN THIGH MUSCLE. A CASE REPORT.

Ljubić N[1], Kardum-Skelin I[2], Petrović Z[1], Bago J[1], Grgurević Batinica A[1].

[1]General Hospital "Sveti Duh", Zagreb, Croatia
[2]Clinical Hospital "Merkur", Zagreb, Croatia
nives.ljubic@zg.htnet.hr

Aim of the study: Squamous cell carcinoma of the lung is known as carcinoma that commonly metastasises to lymph nodes, liver, brain, bones and adrenal glands, but one of its well known characteristics is that it can metastasise to almost every organ. In routine diagnostic practice the vast majority of squamous cell carcinoma of the lung is diagnosed on bronchoscopic material.

Methods: In this paper we present a case of a male with diagnosis of metastatic carcinoma of the lung in FNAC of thigh muscle. A 75 year old male was presented with a history of painless tumours on both thighs. Tumours were tender, deep seated and measured 8 cm in diameter. FNAC had been performed.

Results: The air dried smears were stained with May-Grunwald-Giemsa and the diagnosis of the metastatic squamous cell carcinoma has been established. Patient died after 15 days and the autopsy resulted in primary location of the lung carcinoma.

Conclusion: Although the lung carcinoma is one of the most frequent malignancies, FNAC diagnosis of primary tumour that metastases into muscle is extremely rare.

Clinical Cytology / Posters / Pulmonary Cytology / 19

MUCINOUS LUNG ADENOCARCINOMA CYTOLOGY- DIAGNOSTIC DIFFICULTIES (CASE REPORT)

Smojver-Ježek S[1], Vrabec-Branica B[1], Gorečan M[2], Slobodnjak Z[3], Seiwerth S[4].

Department of [1]Cytology, [2]University Department of Thoracic Radiology, [3]Department of Thoracic Surgery, University Hospital for Lung Diseases Jordanovac, Zagreb, Croatia
[4]Institute of Pathology, Medical Faculty Zagreb, Croatia
ssmojver@pbf.hr

Aim of the study: Case report of a rare lung adenocarcinoma variant and diagnostic problems in the transthoracic fine needle aspiration and intraoperative imprint cytology.

Methods: A 59-year old male patient was admitted with peripheral tumor, 6cm in diameter, of the right upper lobe in radiological examination, bronchoscopy was negative. Ultrasound guided transthoracic fine needle aspiration was performed.

Results: Cytomorphological findings in transthoracic fine needle aspiration were mucus and myxomatous background with cuboid epithelial cells and few atypical cells possible of mesenchymal origin. Differential diagnosis was hamartoma and mesenchymal tumour. Radiological finding and inconclusive cytology were indication for surgery. Intraoperative imprint cytology of the resected tumor revealed a large amounts of mucus, macrophages, epithelial cells and a few large atypical cells. Pathohistological diagnosis was mucinous lung adenocarcinoma with small number of peripherally placed malignant epithelial cells. Difficulties and limitations of cytological diagnosis of mucinous lung adenocarcinoma variant due to differences in materials, staining problems and small number of malignant cells were analyzed.

Conclusion: Mucinous lung adenocarcinoma is a rare lung tumor difficult to diagnose in cytological samples.

Clinical Cytology / **Posters** / **Free Topics** / **20**

EMBRYONAL RHABDOMYOSARCOMA OF SOFTPALATE - CYTOLOGICAL DIAGNOSIS - A CASE REPORT

Ivanišin Barač A, Borić I, Petković D.

General Hospital Dubrovnik, Dubrovnik, Croatia
anai@bolnica-du.hr

We report a case of embryonal rhabdomyosarcoma in a 10-year-old girl. She had a swelling in the right side of the soft palate. FNA was performed and cytological analysis demonstrated the presence of malignant undifferentiated cells. The immunocytochemical staining revealed the diagnosis of a malignant mesenchimal tumor - suspected to be an embryonal rhabdomyosarcoma. Subsequent pathohistological examination confirmed the diagnosis of embryonal rhabdomyosarcoma.

Clinical Cytology / **Posters** / **Free Topics** / **21**

DIAGNOSTIC DILLEMAS OF FNAC OF PILOMATRIXOMA IN HEAD AND NECK

Trutin Ostović K.[1], Manojlović S[2], Štoos-Veić T[1], Kaić G[1], Virag M.[3]

[1]Department of Clinical Cytology and Cytometry,
[2]Department of Pathology,
[3]Clinic of Oral and Maxillofacial Surgery, Dubrava University Hospital, Zagreb, Croatia
ktrutin@kbd.hr

Aim of the study: Pilomatrixoma is a rare benign skin appendage tumour. The aim is to determine and list the cytological findings that might mislead the less-experienced cytologists and to give them advice on how to avoid such errors.

Patients and Methods: Within four years we performed fourteen fine needle aspirations of pilomatrixoma out of which eight were located in head and neck. We used 25G needles attached to 20-ml disposable syringes. Smears were stained with Papanicolaou and Pappenheim staining methods. The cytological diagnoses were compared with the histological findings.

Results: Unequivocal benign diagnoses were rendered in six cases. In one case basal cell carcinoma could not be excluded. One case was misdiagnosed. Diagnostic cytological features includes cellular aspirates that consist of keratinized squamous and ghost cells, multinucleated giant cells mainly associated with keratin fragments and pink, fibrillary material enveloping clusters of small, basaloid epithelial cells. Basaloid cells may form rosettes and acini or appear isolated with high nuclear-cytoplasmic ratio, evenly dispersed chromatin, prominent nucleoli and nuclear moulding.

Conclusion: The FNA cytological diagnosis of pilomatrixoma may be extremely difficult especially if there is a predominance of one component over the others. It should be considered in the differential diagnosis of small cell and keratinizing lesions of skin at any age with different clinical presentation.

Clinical Cytology / **Posters** / **Free Topics** / **22**

THE ROLE OF FINE-NEEDLE ASPIRATION CYTOLOGY OF THE TESTIS IN MEN WITH AZOOSPERMIA

Šunjić Stakor M, Čolak B, Vlahović S, Peroš K, Kljajić K.

The Vuk Vrhovac University Clinic, Zagreb, Croatia
msunjic@idb.hr

Aim of the study: Treatment of men with azoospermia is a great diagnostic and therapeutic problem.

This study aimed to determine the presence of spermatogenesis in cytological smears of fine needle aspirates of the testes in patients with azoospermia in the evaluation of male infertility.

Patients and Methods: Testicular samples were obtained by fine-needle aspiration of both testes in 25 men with azoospermia, as determined by laboratory semen analyses, using 10 mL syringes and 23 G needles. The air-dried smears were stained using May-Grünwald-Giemsa method.

The presence of spermatogenetic cells was determined and the number of spermatogonia, primary spermatocytes, spermatids and spermatozoa was counted on 500 Sertoli cells.

Results: A satisfactory production of all spermatogenetic cells was found in 22 of the 50 studied testes (44 %) (sperm/Sertoli index above 0.10). A reduced, but still present spermatogenesis (including spermatozoa) was found in 10 of 50 testes (20%), and only Sertoli cells and no spermatogenetic cells were found in 18 of 50 testes (36%).

Conclusion: Testicular fine-needle aspiration is a very important technique for diagnostic evaluation of male infertility. This method enables identification of sperms and planning of assisted reproduction procedures (TESA -ICSI method).

Pathology / Oral presentation / Education in Pathology / Free topics / 23

WHICH ASPECTS OF THE COURSE PREDICT OR INFLUENCE THE SCHOLASTIC SUCCESS OF MEDICAL STUDENTS?

Nola M[1], Dotlić S[2], Morović A[2], Sičaja M[2], Jukić S[1], Dominis M[1], Damjanov I[3]

[1]Department of Pathology, Zagreb University School of Medicine, Zagreb, Croatia
[2]Department of Pathology, Clinical Hospital Center Zagreb, Zagreb, Croatia
3Department of Pathology, University of Kansas School of Medicine, Kansas City, Kansas, USA

Aim: The pathology course at Zagreb Medical School is based on computer based seminars, with a pretest in the beginning and optional interim examinations during the year. The students were divided into 10 groups, with different teachers for each group.

Aims were: to determine the predictive value of the pretest; to establish whether the students who took and passed the interim examinations were more successful in the final examination than the other students; to determine whether the teachers differed in their teaching success.

Methods: The class of 2000/01 was divided into two groups: P-group, comprising 117 students who passed the final examination, and the F-group, comprising 94 students who failed, or did not take the final examination in the first term. Groups were compared regarding the pretest, interim examinations and the first midterm examination. The highest and the lowest ranked seminar group were compared, too.

Results: Pretest scores revealed no statistical difference between the 10 student seminar groups. Students scoring high on the pretest were more likely to take the interim examinations than students who failed the pretest. There was a significant difference between these two groups of students on all the examinations taken during the year ($p<0.0001$). There was a significant difference between the highest and the lowest ranked seminar group on the midterm ($p=0.030$) and final examination ($p=0.041$).

Conclusion: The pretest has predictive value and could be used to identify students who will have academic problems in the course. At the end of the course some groups performed better than the others, suggesting that some teachers were more effective in preparing students for the final examination than the others.

CITO-PATOLOŠKA KORELACIJA
CYTO-PATHOLOGICAL CORRELATION

Usmena izlaganja / *Oral presentations (1-4)*

Cyto-Pathological Correlation / 01

LONG TERM FOLLOW-UP OF THYROID CANCER METASTASES

Halbauer M.

University Department of Nuclear Medicine and Radiation Protection, Zagreb University Hospital Center, Zagreb, Croatia

Fine needle aspiration cytology is considered as the key method in the evaluation of thyroid nodules. This is a special and unique situation in cytology. The differential diagnostic problem of possible coexistance of two primary carcinomas or a metastatic carcinoma is well known. The management of such cases requires true and tight collaboration of all team members. Fine needle aspiration biopsy using ancillary techniques such as cytochemistry or immunocytochemistry can provide a comprehensive diagnosis.

Hereby, we report on a 47-year-old female patient operatively treated for cystic lump in cervical region followed by liver surgery for cystic tumor. We discuss and analyze in detail the multidisciplinary approach which is very important to clarify differential diagnostic problems.

Cyto-Pathological Correlation / 02

UNUSUAL THYROID TUMOR IN A CHILD PREVIOSLY TREATED FOR NEUROBLASTOMA

Ramljak V[1], Ranogajec I[1], Knežević F[2], Janušić R[3], Došen D[3], Velemir-Vrdoljak D.[4]

[1]Department of Cytology, [2]Department of Pathology, [3]Department of Head and Neck Surgery, [4]Department of Surgery, University Hospital for Tumors, Zagreb, Croatia
vesna.ramljak@kzt.hr

Aim of the study: The report covers a case of Lindsay tumor in a child previosly treated for neuroblastoma, and cytopathological features for the diagnosis..

Patients and Methods: An eight-year-old girl was admitted to our hospital due to a markedly enlarged left thyroid lobe seven years after neuroblastoma treatment.

Thyroid tumor was established by ultrasonography and scintigraphy. Targeted fine needle aspiration was performed and the specimen was stained by standard May-Grûnwald-Giemsa (MGG). Additionally, RT-PCR method was used, and total thyroidectomy with neck dissection based on cytologic diagnosis was performed.

Results: The smears obtained upon targeted FNA showed numerous clusters of malignant cells with occasionally follicles, RT-PCR analysis yielded positive results for galectin 3 and CD44v6.

Pathohistological analysis of standard H/E biopsies revealed the tumor consisted of numerous elongated follicles of various size and also pseudopapillae. Immunohistochemical analysis (thyreoglobulin, FVIII) confirmed cytological diagnosis of papillary carcinoma (follicular variant).

Conclusion: Follicular variant of papillary thyroid carcinoma as a second primary tumor, following neuroblastoma therapy in children, has not been described in the available literature.

Regarding lesions with apparent nuclear changes characteristic of papillary carcinoma and regardless of the follicular architecture, it is recommended to consider the diagnosis of follicular variant of papillary carcinoma.

Thyroid cancer prognosis in children is as favorable as in adults and six months follow-up of our patient did not reveal any relapse or metastatic disease.

Cyto-Pathological Correlation / 03

OMISSIONS AND MISTAKES IN CLINICAL CITOLOGY

Znidarčić Ž.

Zagreb, Croatia
zznidarc@inet.hr

Omissions and mistakes can have significant consequences, and therefore also have to be discussed in clinical cytology. The aim of this report is to reduce their frequency, especially by improving organisation and education in clinical cytology.

Analysis: The causes of mistakes in clinical cytology are analysed according to the steps of cytodiagnostic process - from the indications for cytological diagnosis, followed by the technical procedures and microscopic analysis, then the expressions of cytological findings and their interpretation by clinicians. Modern technologies used in the cytodiagnostic process are also included in this analysis, and the quality control in cytology is discussed. In each part of the cytodiagnostic procedure, possible omissions and mistakes are highlighted, and the way of their reduction is recommended.

Conclusion: The organisation of clinical cytology in the health service as well as the education of non-cytologists in cytology should be improved to reduce the mistakes in cytodiagnosis. Cytologists should be aware of the possibility and danger of their mistakes and make ultimate efforts to make cytological diagnoses reliable and used in the medical practice in the best possible way.

Cyto-Pathological Correlation / 04

ERRORS IN PATHOLOGY

Seiwerth S.

Department of Pathology, Zagreb University School of Medicine, Department of Pathology, Clinical Hospital Center "Šalata", Zagreb, Croatia

1) Errors of the pathologist

Missing the key point: large workload together with insufficient equipment and laboratory environment can easily result in overlooking a feature crucial for correct diagnosis

Insufficient knowledge: "We see what we know" and "Who does not know he does not know is dangerous. Beware of him" are old wisdoms important in everyday life and even more in diagnostic work. Being aware of own limits is of utmost importance. Asking for advice and peer review is not a sign of ignorance but of consciousness. An important anomaly, yielding severe problems is the communist-time formula of "specializing for an outside hospital". Under such circumstances a young specialist in pathology finds himself confronted with overwhelming material from all medical fields with a 10 years book as the only consultation source and the next fellow pathologist at a distance of 150 km.

Misjudgment: Although Pathology produces "final diagnoses" they are often based more on personal judgment, feeling and experience than on exact parameters. Expert does often disagree over particular diagnoses.

Unawareness about circumstances: Morphology is a blessing or a curse yielding disastrous errors. Equal patterns in different clinico-pathological settings sometimes mean divergent diagnoses

2) Errors of pathology as a discipline can more correctly be termed as limitations of the discipline, and a pathologist unaware of these limitations (sometimes under pressure from the clinician) can produce diagnoses which are difficult to support.

3) Laboratory errors are de iure errors of the pathologist who is responsible for all elements of the diagnostic process

4) Errors revealed by the pathologist during his diagnostic work. They are an every day's burden of our work but should not mislead the pathologist to put himself in the position of judging his fellow colleagues.

5) Errors in communication between pathologists and clinicians.

Erare humanum est, but we should beware of demonstrating it as the only sign of our humanity.

OKRUGLI STOL / *ROUND TABLE*

Round Table / 01

PROPOSAL OF NEW PROGRAM FOR SPECIALISATION IN PATHOLOGY, CYTOLOGY AND FORENSIC MEDICINE

Anđelinović Š[1], Jakić-Razumović J[2], Kardum-Skelin I[3], Škavić J.[4]

[1]Department of Pathology, Clinical Hospital Split, [2]Clinical Institute of Pathology, Zagreb Clinical Hospital Center and Medical School University of Zagreb, [3] Laboratory of Cytology and Hematology, Merkur University Hospital and Medical School University of Zagreb, [4] Department of Forensic Medicine and Criminology, Medical School University of Zagreb, Zagreb, Croatia

Croatian Ministry of Health initiated workout of a new residency programs in pathology, cytology and forensic medicine which should be harmonized with national programs in European countries presuming our medical tradition. On grounds of that, the board for clinical morphological sciences (pathology, cytology and forensic medicine) has been established in August 2003 (consisted of: prof.dr.sc. Mladen Belicza, prof.dr.sc. Josip Škavić, mr.sc. Ika Kardum-Skelin, mr.sc. Dražen Švagelj, prof.dr.sc. Jasminka Jakić-Razumović, Igor Borić, dr.med., doc.dr.sc. Jadranka Ilić-Forko, prof.dr.sc. Nives Jonjić, doc.dr.sc. Branko Dmitrović, prim.dr.sc. Mirjana Marković-Glamočak, mr.sc. Vesna Mahovlić, mr.sc. Silvana Smojver-Ježek, doc.dr.sc. Adrijana Vince, prof.dr.sc. Božena Šarčević and president prof.dr.sc. Šimun Anđelinović). Board had five meetings and defined main aims of residency programs, such as: 1) harmonization of the residency program with European praxis and recomendation of European society for pathology, cytology and forensic medicine, 2) availability for residents to choose the length of certain program, and 3) attemption to satisfy the needs of entire Croatian medical society. Unlike recent praxis with three separated specializations in duration of 48 months, new program for residence in clinical morphological sciences would be one education program with three different parts in pathology, cytopathology and forensic medicine in duration of 60 months. The residency program consists of basic and advanced program. After basic program candidates should pass written examination, and after advanced part should pass an oral and practical exam. Pathology and cytopathology will have identical basic residence program of three years duration with 20% possibility of certain program modification in agreement with program supervisor. Basic residence for forensic medicine will last for two years. The aim of all basic residences is over coming the knowledge and skills in general pathology field. After passing the written exam, candidates continue the education in advanced program, which is closely attached for certain morphological science (pathology, cytology, forensic medicine). After passing the final oral examination, depending on choosen program, candidates are becoming: 1) specialist of pathology, 2) specialist of cytopathology or 3) specialist of forensic medicine.

Besides working on specialization program the Board for clinical morphological sciences have consider another occupational questions in these scientific fields. Therefore, some normative suggestions have been made (for staff, space and equipment for pathology, cytology and forensic medicine). One and half years ago the Board members have been changed and new head has been elected (prof. dr.sc. Šimun Križanac), but all goals are still very actual, and new Board is extremely dedicated to implement this proposed program to the practice.

Round Table / 02

POSTGRADUATE STUDY OF CLINICAL CYTOLOGY IN CROATIA 1967-2005

Audy-Jurković S.

Institute of Gynecologic Cytology, University Department of Obstetrics and Gynecology, Zagreb University Hospital Center, Zagreb, Croatia

In Croatia, the postgraduate study (PS) in cytology in human medicine was established in 1967 at School of Medicine, University of Zagreb, incited by the pathologist Ante Zimolo and the hematologist Erik Hauptmann, the first head of the study.[1-3] The study was named Medical Cytology, representing the first form of organized education in cytology in Croatia with 509 periods, 200 of them exercise.3 Required subjects included Introduction in scientific research; Statistical methods; Cell biology; Selected topics in biophysics and biochemistry; Selected topics in general and special pathologic anatomy; and Cytodiagnosis (gynecologic, respiratory, endocrinologic, hematologic, gastrointestinal, cytology of the breast and other regions), accounting for 68% of the time-table.[3] The Institute of General Pathology and Pathologic Anatomy, School of Medicine, University of Zagreb, was the main teaching institution from 1967 till 1970, followed by Ozren Novosel Clinical Hospital, now Merkur University Hospital from 1970. In addition, lectures were also held at Andrija Štampar School of Public Health, Ruđer Bošković Institute, and Zagreb University Hospital Center cytology laboratories Rebro and University Department of Gynecology - Petrova in the afternoon.[3] In his article On Postgraduate Education in Cytodiagnosis and Cytopathology in Croatia, published in 1968 upon completion of the first study course, Ante Zimolo has listed recommendations issued by the World Health Organization in the '60s on the need of appropriate education of medical students, young physicians and pathologists in cytology, whereby cytopathology should be considered a pathologic anatomy subspecialization with firm groundwork in pathology and clinical practice.[3] In Croatia, however, the history of the development of clinical cytology took a different pattern. Clinicians were the first to engage in cytology, to become aware soon that they could not allocate due time and efforts to it along with their primary specialization2, whereas the postgraduate study offered great theoretical knowledge to the account of practical skills needed for independent work in the cytodiagnosis. Therefore, renowned experts in cytology, prominent members of the Section of Cytology, Croatian Medical Association (now Croatian Society of Clinical Cytology), headed by its first president, Professor Inga Črepinko, managed to fight for independent specialization in Medical Cytology, to provide proper education for specialists to work full-time in the profession. This goal was accomplished in 1974, when the postgraduate study was fully integrated in the specialization *curriculum*. Until 1985, the study was headed by Professor Erik Hauptmann, from 1985 till 1988 by Professor Jasna Ivić, and since 1988 it has been headed by Professor Silvana Audy-Jurković.[4] Since 1988, the main teaching institution is University Department of Gynecology and Obstetrics, Zagreb University Hospital Center, with the majority of postgraduate study teaching activities held in the specially equipped lecture hall of the Institute of Gynecologic Cytology (Discussion microscope for 7 observers, TV camera, monitor, 2 videorecorders, LCD projector and laptop), and at almost all university hospitals and Ruđer Bošković Institute in Zagreb.4 At the beginning of the '90s, the study and specialization were renamed into Clinical Cytology. Teaching activities were

continuously performed from 1967 till 1991, then with short interruptions due to the war in Croatia. To date, 30 study courses with 373 students, mostly physicians, residents in cytology and pathology, have been completed. The study was scientifically based since the very beginning, allowing the students scientific and teaching rise. About 70% of the students have acquired MS title[2], a precondition for PhD, which in turn is required for further scientific advancement. The study underwent revision on several occasions, to mention only 1996 when scientific studies turned into professional studies, and 2004-2005, when it was termed specialist study; the latter revision was necessary to adjust to the Bologna Process. According to the latest plan and *curriculum*, the student is obliged to attend 285 periods of required subjects from various fields of cytology (in gynecology, hematology, pulmonology, endocrinology, gastroenterology, urology, pediatric hemato-oncology, and selected topics in clinical cytology), scored 40 ECTS points. By choosing elective subjects at the current study or courses at other postgraduate studies (specialist or scientific-doctoral) and category I courses of continuing medical education, the student can get additional 20 ECTS points, yielding total score of 60 ECTS points. The ECTS score thus acquired is part of the knowledge "dowry" to be further enhanced during active life through a variety of recognized forms of education.

In conclusion, as the Postgraduate Study in Clinical Cytology as organized education within the frame of the same specialization was the first ever encounter with the profession for the students, the offered knowledge, mostly theoretical, was invaluable for their practical training during specialization. At the same time, the study offered the opportunities for scientific-teaching advancement, as best illustrated by the present School of Medicine faculty members in various fields, including pathology and cytology. The university reform launched in this millennium, emphasizing student mobility, as a new aspect of education will certainly also reflect in the Postgraduate Study in Clinical Cytology in the time to come.

References

1. Črepinko I, Ivić J. An Educational Program in Cytology in Croatia. Proceedings of the Citološki dnevi Symposium. Ljubljana: Section of Cytology and Cytodiagnosis, Medical Association of Slovenia; 1969, p. 162-6.
2. Črepinko I, Znidarčić Ž, Audy-Jurković S. Clinical Cytology in Croatia. Zagreb, 2000.
3. Zimolo A. Postgraduate Education in Cytodiagnostic and Cytopathology in Croatia. Ginekol Opstet 1968;8:187-91.
4. Audy-Jurković S. Jasna Ivić and Institute of Gynecologic Cytology, University Department of Obstetrics and Gynecology, Zagreb University Hospital Center. In: Audy-Jurković S, ed. Gynecologic Cytology in Croatia 50 Years After. First International Scientific Symposium of Clinical Cytology "Jasna Ivić". Zagreb: Croatian Society for Clinical Cytology, Croatian Medical Association; 2003, p. 23-61.

Round Table / 03

POSLIJEDIPLOMSKI STUDIJI NA MEDICINSKOM FAKULTETU SVEUČILIŠTA U ZAGREBU

Dominis M, Lacković Z, Božikov J

Medicinski fakultet Sveučilišta u Zagrebu, Zagreb

Glavni ciljevi Bolonjske deklaracije (1999.), koja se temelji na Magna Charta (1988) i Lisabonskoj konferenciju (1997.), jesu:

1. sloboda kretanja studenata
2. sloboda kretanja nastavnika
3. harmonizacija i priznavanje istovjetnih diploma
4. European Credit Transfer System bodova - ECTS - mjera opterećenja studenata u svladavanju određenih zadataka u stjecanju znanja i vještina.

Na temelju tih principa Zakon o znanstvenoj djelatnosti i visokom obrazovanju (NN od 23.07.2003.) kao i Dopune i izmjene tog Zakona od 21.07.2004. u čl. 70. određuju da sveučilišno obrazovanje u Republici Hrvatskoj "obuhvaća slijedeće tri razine:

1. preddiplomski studij
2. diplomski studij
3. postdiplomski studij"

U stavu 5. čl.70 stoji, da" svaka razina studija iz st.1. ovog članka mora biti u skladu s europskim sustavom prijenosa bodova (ECTS) po kojem se jednom godinom studija u pravilu stječe 60 ECTS bodova."

Članak 73. istog Zakona određuje:

1. poslijediplomski sveučilišni studij može se upisati nakon završenog diplomskog sveučilišnog studija.

2. Poslijediplomski sveučilišni studij traje u pravilu tri godine. Ispunjenjem svih propisanih uvjeta i javnom obranom doktorskog rada stječe se akademski stupanj doktora znanosti (dr.sc.) odnosno doktora umjetnosti (dr.art.).

3. Iznimno, osobe koje su ostvarile znanstvena dostignuća koja svojim značajem odgovaraju uvjetima za izbor u znanstvena zvanja, na temelju odluke nadležnog vijeća, utvrđenog statutom sveučilišta, o ispunjavanju propisanih uvjeta te izrade i javne obrane doktorskog rada, a uz suglasnost senata sveučilišta, mogu steći doktorat znanosti.

4. Kratica akademskog stupnja stavlja se ispred imena i prezimena osobe.

5. Sveučilište može organizirati poslijediplomski specijalistički studij u trajanju od jedne do dvije godine, kojim se stječe zvanje specijalista određenog područja (spec.) . naziv specijalista, odnosno njegova kratica dodaje se nazivu iz čl.72.,st.3. ovog zakona

6. Sveučilište statutom može utvrditi da se završetkom poslijediplomskog specijalističkog studija stječe drugačije zvanje od onog propisanog st. 5. ovog članka, u slučaju kada je za određeno stručno područje posebnim zakonom propisano specijalističko usavršavanje.

Članak 83. ovog Zakona govori o uvjetima završetka poslijediplomskih studija, te st. 3. glasi:

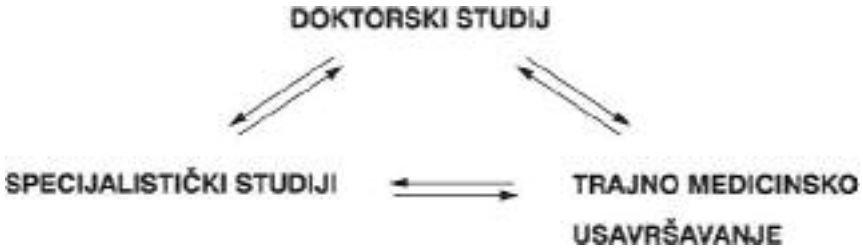
3." Poslijediplomski studij završava polaganjem svih ispita, izradom i javnom obranom znanstvenog ili umjetničkog doktorskog rada (disertacije)", a st. 4.

4. "Poslijediplomski specijalistički studij završava polaganjem svih ispita, izradom završnog rada i/ili polaganjem odgovarajućeg završnog ispita u skladu sa studijskim programom".

Članak 84. ovog zakona govori o ispravama o studiji i u st 2. ovog članka stoji:

2." Po završetku diplomskog, poslijediplomskog i poslijediplomskog specijalističkog studija studentu se izdaje diploma. Diplomom se potvrđuje da je student završio određeni studij i stekao pravo na akademski naziv ili stupanj".

Medicinski fakultet (MF) Sveučilišta u Zagrebu uveo je ECTS bodove 1998. u sustav bodovanja poslijediplomskih studija, a 2003. u sustav bodovanja svih kategorija tečajeva stalnog medicinskog usavršavanja (SMU). Osnovni koncept poslijediplomske edukacije na MF Sveučilišta u Zagrebu je mobilnost studenata i nastavnika unutar sustava poslijediplomskih studija (PDS) kao i SMU, te prijenos bodova unutar sustava edukacije. Shema 1.



Specijalistički PDS je organiziran kao dopunska nastava u tijeku specijalizacije (Zakon o zdravstvenoj zaštiti - NN 1/97 čl.123. i čl. 122. Dopuna i izmjena Zakona o zdravstvenoj zaštiti kao i Pravilniku te dopunama i izmjenama istog o specijalizaciji). Sadrži obvezatne i izborne predmete. Obvezatni predmeti donose 40 ECTS a izborni 20 ECTS bodova. Nakon završenog obvezatnog dijela, student dobije potvrdu o 40 ECTS bodova, a ako sakupi 60 ECTS bodova - diplomu.

Znanstveni doktorski studij Biomedicina i Zdravstvo traje tri godine i završava obranom doktorske disertacije.

Uvjeti upisa jesu:

A.

1. završeni medicinski fakultet ili drugi fakultet iz biomedicinskog i srodnih područja
2. mentor
3. uspjeh u dodiplomskoj nastavi
4. poznavanje engleskog jezika
5. osnovna znanja o primjeni računala

B.

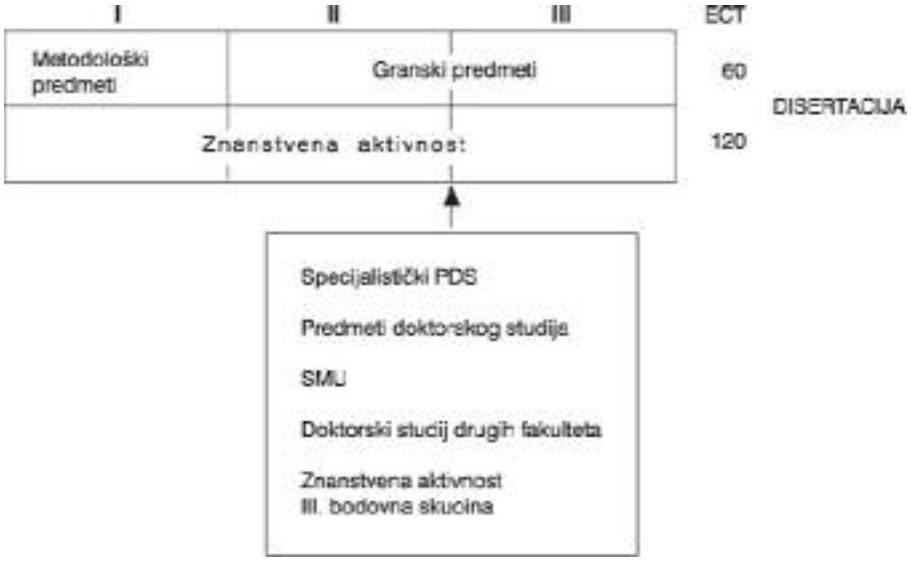
1. "Razlikovnu godinu" upisuju studenti nakon potpuno ili djelomično završenog specijalističkog PDS-a ili prve godine nekog drugog znanstvenog studija, ili ako su na neki drugi način sakupili 50% bodova prve godine studija.
2. mentor
3. poznavanje engleskog jezika
4. osnovna znanja o primjeni računala.

U slučaju prijave ako se prijavi više kandidata od mogućnosti upisa pri sastavljanju liste redoslijeda prvenstva za upis uzimaju se u obzir uspjeh u dodiplomskoj nastavi i rezultati znanstvenog rada prema kriterijima Fakultetskog vijeća.

Doktorski PDS Biomedicina i Zdravstvo koncipiran je da student svake godine sakupi 60ECTS bodova - 20 iz nastave i 40 iz znanstvene aktivnosti. Prva godina obuhvaća Metodološke predmete (I. bodovna skupina) i vođene praktikume, druga i treća godina obuhvaća Granske predmete(II. bodovna skupina) i u svakoj godini se mora sakupiti 60 bodova (isti omjere bodova). Studij završava sa skupljenih 180 ECTS bodova (60 nastava i 120 znanstvena aktivnost (III. bodovna skupina) i obranom doktorske disertacije.

SMU obuhvaća tečajeve I. I II. kategorije koji su bodovani u ECTS bodovima.

Prema tome hodogram poslijediplomskog studija na MF Sveučilišta u Zagrebu bi bio:



Završetak studija je prema principima Bolonjske deklaracije (dopune - Prag, 2001.; Berlin 2003.; Zagreb I, 2004.; i ZagrebII, 2005.; i Bergen 2005.) uspješna obrana doktorske disertacije.

Detaljne obavjesti se mogu se naći na web stranicama:
www.uniri.hr/Bolonski.HTM
www.bologna-bergen2005.no
http://odin.dep.no/filearkiv/238593/041014/Fact Sheet Bologna -process.pd

CITOTEHNOLOGIJA / *CYTOTECHNOLOGY*

Pozvano predavanje / *Invited Lecture*

Clinical Cytology / **Invited Lecture**

FNA CYTOLOGY AS A SOURCE FOR MOLECULAR DIAGNOSTIC PROCEDURES IN THE DIAGNOSIS OF MALIGNANT LYMPHOMA

Fricke U.

Microbiology - Research - FISH -Laboratory, Ulm, Germany
ursula.fricke@medizin.uni-ulm.de

Objective: For diagnosis of malignant lymphomas fine-needle aspiration-cytology is performed. If sub typing is required further investigations are carried out.

Methods: Morphologic examination of May-Grünwald-Giemsa stained specimen - Immunophenotyping - PCR - FISH - technique and CGH (comparative genomic hybridisation)

Results and For detection of distinct genetic aberrations well preserved nucleated

Conclusion: cells are required. Fine needle aspiration supplies sufficient material for further diagnostic procedures.

In conclusion: FNA cytology is an excellent method to link morphologic knowledge and molecular diagnostic procedures.

CITOTEHNOLOGIJA / *CYTOTECHNOLOGY*

Usmena izlaganja / *Oral presentations (1-11)*

Cytotechnology / Oral presentations / 01

DIFFERENTIAL LEUKOCYTE COUNT IN HAEMATOLOGY PATIENTS - AUTOMATIC ANALYSER OF BLOOD CELLS VERSUS ANALYSIS UNDER LIGHT MICROSCOPE

Anić V[1],Knežević G[1], Križaj B[1], Parigros K[1], Harabajsa S[1], Kardum-Skelin I[1], Šušterčić D[1], Fabijanić I[1], Stužić V[2], Jakšić B.[2]

[1]Laboratory of Cytology and Haematology, [2]Department of Medicine, Merkur University Hospital, Zagreb, Croatia
veronika.anic@zg.t-com.hr

A standard analysis of stained peripheral blood smears using a light microscope makes it possible to observe even the most subtle qualitative changes to blood cells and to separate pathological from normal blood cells. Cells are differentiated individually and expressed in percentages. Haematology analysers based on cytometry flow and semi-conductor lasers that measure blood cell volume and density separate particular cell types. This facilitates the speed of the analysis and also includes a large number of cells.

Aim of the study: To analyse the possibility of recognizing pathological blood cells in patients with haematological neoplasm.

Material and Methods: The analysis included 52 samples of peripheral blood in patients with haematological neoplasm at diagnosis, during or immediately after therapy. A light microscope analysis was conducted using standard peripheral blood smears stained using the Pappenheim method (May -Grünwald - Giemsa - MGG), and, at the same time, veinal blood was taken with heparin for an automatic analysis. Samples in which the automatic analyser counter noted the presence of blasts, immature granulocytes, atypical lymphocytes, lymphoblasts, erythroblasts, or abnormal distribution of blood cells were analysed. Peripheral blood samples with changed or pathological forms were also taken from the light microscope analysis even when there was an absence of warning indicators on the haematology counter.

Results: The light microscope indicated the existence of blasts in 28 blood smear samples, whereas the counter identified 13 as containing blasts; in 15 cases the counter specified them as atypical lymphocytes. In 5 cases there were no comments on findings, however, this accounted for only 1% of blasts in blood smears. There was indication of immature granulocytes in 32 samples and in 19 cases they were also noted in the blood smear; whereas there was no shift in the granulocyte order in 13 cases, a shift was noted in 8 blood smear samples, which was not commented by the analyser. Atypical forms of lymphatic cells in both cases were found in 9 samples; in 15 samples the above-mentioned blasts were discovered, and in 10 samples there was no comment. In cases where there was a warning indicating the presence of lymphoblasts in peripheral blood, the samples contained plasma cells, lymphoplasmacytoid forms and other atypical lymphatic cells. A counter warning did not herald the presence of erythroblasts in the smear.

Conclusion: The haematology counter is an invaluable and practical tool for quantifying particular cell types due to its quantitative parameters for blood analyses, and it can analyse a vast number of cells within a short period of time. However, although it cannot completely

replace the light microscope in analysing blood samples from patients with haematological illnesses when there is a presence of various pathological white blood cells during illness, treatment and rehabilitation, it is an aid that provides comments on the presence of atypical forms and helps to separate samples for further analysis.

Cytotechnology / Oral presentations / 02

DEFINITION OF UNSATISFACTORY SPECIMEN AND THEIR FREQUENCY IN CERVICOVAGINAL SMEARS

Gavranović Lj, Krivak-Bolanča I, Šentija K.

Laboratory for Clinical Genetics and Cytology, Department of Gynecology and Obstetrics , Merkur University Hospital, Zagreb,Croatia

Objective: Our aim was to determine the frequency and cause of unsatisfactory specimens of VCE (vaginal, cervical and endocervical) smears in our laboratory during the past year. Specimens are divided into a satisfactory or unsatisfactory category as a means of providing feedback for the improvement of specimen adequacy. The new revised Bethesda classification (BC 2001) offers a standardized approach for reporting whether a specimen is adequate for evaluation.

Methods: The unsatisfactory category includes specimens that do not contain sufficient cells for reliable interpretation, smears with presence of foreign material, poor fixated or preserved cells, excessive cytolysis or autolysis or other quality indicators e.g. excess of inflammatory cells or blood. Any specimen with abnormal cells are described as satisfactory for evaluation, regardless of the number of cells present. Although an unsatisfactory specimen can represent a benign condition, a considerable number of women with unsatisfactory specimens have subsequent histological diagnosis of squamous intraepithelial lesion (SIL) or even cancer.

Results: During the past year 12,83 % of all specimens in our laboratory was unsatisfactory (1594/12424). The majority were unsatisfactory due to an absence of endocervical cells (61,54 %) and an excess of leukocytes or blood (20,63 %). The remainder (17,82%) were unsatisfactory due to a presence of foreign material, such as starch granules, pollen, as well as poor fixation, air drying or overused staining solution (284/1594).

Conclusion: The implications of our findings are that BC 2001 guidelines regarding a satisfactory conventional VCE result in increased health costs (for repeating smears) without identifying a significant number of new epithelial cell abnormalities.

Cytotechnology / Oral presentations / 03

TAKING SAMPLES OF BONE MARROW AND LYMPH NODE FOR POLYMERASE CHAIN REACTION METHOD, IMMUNOPHENOTYPING OF CELLS AND SERVING OF MOLECULAR ANALYSIS

Harabajsa S[1], Parigros K[1], Knežević G[1], Križaj B[1], Anić V[1], Kardum-Skelin I[1], Šušterčić D[1], Fabijanić I[1], Jelić-Puškarić B[1], Stužić V[2], Jakšić B.[2]

[1]Laboratory of Cytology and Haematology, [2]Department of Medicine, Merkur University Hospital, Zagreb, Croatia
suzana.harabajsa@zg.t-com.hr

Aspirational cytodiagnostic puncture of bone marrow (BM) and the lymph node (LN) as part of the haematological evaluation process is usually performed by a thin needle pricked into a fixed part of the organ and aspirated via negative pressure to gain samples containing cells.

Aim of the study: The purpose of this task is to represent the need for a necessary approach according to regulations on bone marrow and lymph node puncture as the only way to present quantity and quality of samples taken. Although this indicates a small possibility of false positive or negative search results, inadequate puncture or the existence of careless sample manipulation would considerably obstruct or totally disable the analysis.

Patients and Methods: At the Laboratory of Cytology and Haematology at the Merkur University Hospital aspiration is used to obtain a cytological analysis that generalizes the morphologic significance of the cells and their disposition, apart from which the above mentioned samples are also taken for certain other diagnostic techniques, such as DNA replication - so called polymerase chain reaction (PCR), immunophenotyphing of blood cells by flow cytometry (IF) and cytogenetic methods (karyogram, FISH).

Results: During an observation period summation was done by 3950 punctures of bone marrow and lymph nodes. In full review of the mentioned aspirations 1526 (38,63%), as a necessity, specimens were taken at other laboratories. For the PCR analysis 21,63% of assays (LN 35, BM 295) were aspirated, for the cytogenetic method the samples taken comprise 17,56% (LN 3, BM 265), while 928 (60,81%) samples were aspirated for the immunophenotyping method (LN 305, BM 623), from among the aspirations completed collectively. Depending on the sampling method, there was a high degree of sample adequacy. All specimens taken for the PCR method were adequate (100%), and results obtained via the karyogram were also extremely satisfying. Only two samples have not been able to be processed because the culture was insufficient for analysis. In both cases it were bone marrow samples. 18% of the lymph node aspiration samples were unsatisfactory, whereas bone marrow samples were 100% usable.

Conclusion: This tests can be used as a supplement to cytological analysis for the purpose of setting an accurate diagnosis. Cytological samples of bone marrow and lymph nodes reveal they are exceptionally adequate for all applied techniques.

Cytotechnology / Oral presentations / 04

"TUMOUR BANK" OF BONE MARROW AND PERIPHERAL BLOOD MALIGNANT CELLS AMONG PATIENTS WITH ACUTE TYPES OF LEUKEMIA AND CHRONIC LYMPHOCYTIC LEUKEMIA

Knežević G[1], Križaj B[1], Parigros K[1], Anić V[1], Harabajsa S[1], Kardum-Skelin I[1], Šušterčić D[1], Fabijanić I[1], Jelić-Puškarić B[1], Stužić V[2], Jakšić B[2]

[1]Laboratory of Cytology and Haematology, [2]Department of Medicine, Merkur University Hospital, Zagreb, Croatia
ikardum@hi.t-com.hr

Bone marrow and peripheral blood are ideal samples for cell suspension and sample preservation in liquid nitrogen at -196°C, in which cells remain viable and are thereby sufficient for subsequent analysis methods.

Aim of the study: To analyse the degree of success in obtaining suspension in patients with acute leukaemia (AL) and chronic lymphocytic leukaemia (CLL) in relation to number of leucocytes in peripheral blood.

Material and Methods: A total of 98 bone marrow and peripheral blood samples were analysed - 40 samples were taken from patients suffering from AL and 58 from patients suffering from KLL. Samples containing 5 ml of bone marrow with 1 ml of heparin and 10 ml of peripheral blood with anticoagulant were diluted in a physiological solution and then washed in a physiological solution after the mononuclears was separated with ficoll. Cells re-suspended in an u RPMI medium, which functions as a form of cell preservation, were divided into aliquots and placed in liquid nitrogen at -196°C after mononuclear concentration had been established.

Results: In 16 patients with ALL and 10 patients with CLL number of leucocytes in peripheral blood reached levels of up to $10x10^9$/L, in 4 patients with AL and 16 patients with KLL levels were up to 10- $20x10^9$/L, in 4 patients with AL and 16 patients with CLL levels were up to 20-$30x10^9$/L and in 16 patients with AL and 16 patients with CLL levels were $>30x10^9$/L. In the first three groups with the highest number of leucocytes in peripheral blood the concentration of mononuclear cells after suspension was constant and varied from 1,7-$3,96x10^9$/L in the AL group, and from 2,9-$4,87x10^9$/L in the CLL group; the number of individual samples was 3-8. A significantly higher concentration was obtained in both groups with bone marrow and peripheral blood suspension in cases where the leukocyte count was higher than $30x10^9$/L: $12,56x10^9$/L with AL and $13,03x10^9$/L with CLL, whereby there was a maximum number of aliquots (10) in all cases.

Conclusion: The concentration of mononuclear cells in suspensions of bone marrow and peripheral blood among AL and CLL samples after separation with ficoll is higher if leukocyte numbers, as well as pathological cells numbers in the bone marrow are higher. However, it is possible to obtain a concentration of cells sufficient for individual samples (aliquots) even when there are smaller numbers of leukocytes.

Cytotechnology / Oral presentations / 05

DIAGNOSTIC VALUE OF INDUCED SPUTUM IN ASTHMA

Krupec A-M[1], Kvaternik M[1], Trutin Ostović K[1], Cvrk B[1], Aleksandrov C[1], Živković V.[2]

[1]Department of Clinical Cytology and Cytometry, [2]Department of Pulmology, Dubrava Clinical Hospital, Zagreb, Croatia
ana-marija.krupec@zg.t-com.hr

Aim of the study: Cytological analysis of induced sputum is a valuable diagnostic tool in asthma. The aim is to find the advantages to induced sputum (IS) in relation to spontaneous sputum and to see the possibilities of using IS instead of BAL.

Methods: We analyzed 14 IS in patients who had no other diseases, were taking no medication and who had been diagnosed with asthma at the time of our study. The patients inhaled aerosoles which generally enabled them to produce a satisfactory amount of sputum for analysis. We measured the weight of sputum and treated it with sputolysin to obtain cell suspension. After removing supernatant, we added HAS with PBS and prepared cytospins. The slides were stained with Pappenheim and Papanicolaou staining.

Results: We analyzed 28 cytospins of IS and 28 smears of spontaneous sputum from 14 patients. The IS was analyzed qualitatively and quantitatively. Differential cell counts were performed by counting 500 cells in each cytospin and expressed the number of lower airway cells in percentages. A contrastive analysis of epithelial and non-epithelial cells showed that, on average, 27,3% are squamous cells, 24% are neutrophil granulocytes, 23% are alveolar macrophages and 18% are eosinophil granulocytes. The spontaneous sputum was analyzed qualitatively, showing mucus and non-cell elements that were not found in IS.

Conclusion: Cytological analysis of induced sputum is a non-invasive and quick method which can replaced BAL in asthma.

Cytotechnology / Oral presentations / 06

SUPRAVITAL STAINING IN THE MICROSCOPY ANALYSIS OF URINE SEDIMENT

Peretić K, Vuković D, Sikirica M.

Institute of Clinical Chemistry, "Merkur" University Hospital, Zagreb, Croatia
mirjana.sikirica@hi.htnet.hr

Aim of the study: To present standardized microscopic analysis of supravitally stained urine sediment

Methods: Urine sediment was prepared using the standardized method and stained with 0,5% liquid solution of 0-toluidine blue in a 1:10 ratio (0,5 mL urine sediment and 50 μL dye). Of urine sediment stained in this fashion 13 μL is taken and mounted on the glass slide for microscopy examination. The preparation is covered with a 18x18 mm cover slip and analysed under a light microscope. The microscope should be equipped with a 10x objective and a 10x eyepiece for small magnification (magnification x100), and a 40x objective and 10x eyepiece for high magnification (magnification x400). Results: This method of urine sediment staining allows good recogniton of leukocytes as round cells with a prominent blue nucleus. Erythrocytes are always lightly stained properly displaying their shape. Squamous epithelial cells are stained at various intensity demonstrating a small dark blue nucleus and a large lightly blue stained cytoplasm. Small epithelial cells also take stain well with the cytoplasm stained lightly blue to violet and the nucleus dark blue. Cylinders are stained immediately and intensively. Bacteria are stained lightly to dark blue, while fungi normally do not stain. Mucus is also dyed intensively blue which makes it welll observable.

Conclusion: The standardized analysis of supravitally stained urine sediment provides good visibility which contributes to the reliability of labortory results in routine urinalysis as one of the most common tests performed at all levels of health care.

Cytotechnology / Oral presentations / 07

THINPREP PAP TEST

Poduje V.

Department of Gynecologic Cytology, University Department of Gynecology and Obstetrics, Zagreb University Hospital Center, Zagreb, Croatia
saudy@kbc-zagreb.hr

Introduction: Liquid based cytology (LBC) is a novel technique of sample preparation in exfoliative and aspirational cytology. The method referred to as ThinPrep Pap Test was initially employed as a substitution for the conventional method of Pap test preparation. Currently, LBC has been widely used in industrialized countries all over the world. The Cytyc Corp. and other companies have developed a highly efficient educational program for cytologists, pathologists and cyto-technicians, enabling most of them to acquire the skills needed for technical preparation and sample analysis in several days. In Croatia, the first Cytyc Corp. organized course in the field of gynecologic cytology took place in 2002.

Method Principle: A sample is obtained from the exocervix and endocervix by a plastic brush and/or whisk broom, which is then immersed in the original bottle containing Preserv Cyt Solution and gently stirred to remove all the material. The bottle is transferred to the laboratory, where the sample is placed into a ThinPrep processor (2000 or 3000) that automatically removes the blood, mucus and cellular detritus, and then mixes the sample thoroughly, pulling the liquid under negative pressure to the TransCyt filter to form a thin layer of diagnostic cellular material. The cellular material is then transferred to a slide glass and mechanically positioned under computer guidance at positive pressure onto the marked circle, 2 cm in diameter, in the form of an evenly distributed thin layer. The slide glass is then placed into a fixing bath, and is ready for staining and cytological analysis. Ten ThinPrep slides of identical cellular content can be obtained from one sample.

Advantages: The LBC method is simple for use. By using a thin layer of evenly distributed cells against a blank background over a circle of only 2 cm, the number of inadequate samples is reduced, the rate of detection of intraepithelial lesions is improved while considerably reducing the screening time and thus increasing laboratory productivity. Furthermore, the fact that a number of identical slides are obtained from a single sample allows for certain additional biologic tests (e.g., HPV, Chlamydia trachomatis, tumor markers) as well as a Pap test to be performed. In this way, a body of data that may prove crucial for patient therapy are obtained without repeat sampling.

Conclusion: LBC improves the slide quality and Pap test sensitivity, allows for various tests to be performed on a single sample, thus reducing patients anxiety associated with unnecessary repeat examinations, and improves day-to-day laboratory productivity.

Cytotechnology / Oral presentations / 08

THE IMPORTANCE OF CYTOLOGICAL BRONCHOSCOPIC SPECIMEN ANALYSIS FOR THE EARLY DISCOVERY OF LUNG CANCER AND ITS DIAGNOSIS

Rubić K, Milanković Lj, Ropar S.

Department of Clinical Cytology, Karlovac General Hospital, Karlovac, Croatia

The early discovery of lung cancer is important in view of its high and increasing incidence and due to the fact that this disease is discovered at an advanced stage in majority of patients, which in turn leads to poor treatment results. In the last few years, significant progress with good results has been made due to a number of early diagnostics methods, including non-invasive diagnostics methods, like sputum analysis, as well as invasive diagnostic methods, like cytological bronchoscopic specimen analysis. The Karlovac district has the highest lung cancer incidence rate. 272 patients were registered in 2004 and 61(22,4%) were diagnosed with cancer. Biopsy material taken from 30 patients resulted in a PHD positive diagnosis in 17 patients. All patients discussed were treated at the Department of Pulmonary Diseases at the Karlovac General Hospital. The results of a cytological analysis have proved that this is not only a valuable and important diagnostic method for the discovery and diagnosis of lung cancer, but also an invaluable potential tool for doctors/cytologists (screening), engineers, medical laboratory diagnosticians and medical cytotechnicians involved in the process.

Cytotechnology / Oral presentations / 09

THE DYNAMICS OF PLEOCYTOSIS AND CYTOMORPHOLOGICAL CHANGES IN CEREBROSPINAL FLUID OF PATIENTS WITH MENINGOCOCCAL MENINGITIS

Stupnišek M.

University Hospital for Infectious Diseases "Dr. Fran Mihaljević", Zagreb, Croatia
stupnisek@hlu.hr

Aim of the study: To present the dynamics of pleocytosis and cytomorphological changes in cerebrospinal fluid (CSF) of patients with meningococcal meningitis. To compare obtained results and investigate any patterns in the dynamics of etiologically same inflammations of the central nervous system (CNS).

Methods: CSF cells were counted in the Fuchs-Rosenthal chamber (in mm^3) immediatelly after lumbar punction (LP), sedimented in a cytocentrifuge at 700 rpm/5 min, stained by the Pappenheim method. At least 100 cells were differentiated (results in %). For easier presentation only two groups of cells were differentiated: polynuclears and mononuclears. The results have been statistically analysed and graphically presented.

Results: A total of 177 CSF samples were analysed. The number of cells in CSF during first LP performed between the first and fourth day of illness was on average higher than 8000/ mm^3, and during second LP (Day 3-5) lower. LP performed during follow-up visit (Day 8 onwards) showed a significant decrease in the total number of cells, so that by the third week of their illness almost all patients had less than 20 cells/ mm^3. Following the dynamics of cytomorphological changes has shown that polynuclears significantly predominated during the first LP (90%), and second LP (70%), while mononuclears predominated from the third LP onwards. In the third week of illness, the number of polynuclears was negligibly small and almost all cells were mononuclears.

Conclusion: By comparing obtained results we observed some patterns in the dynamics of pleocytosis and cytomorphological changes in the CSF of patients with etiologically same CNS inflammations, in our case meningococcal meningitis. A total number of cells in CSF prior to initiated therapy is high. Prior the beginning of therapy polynuclears are detected in high percentages and they predominate until the eight day of illness when the percentage of mononuclears starts to increase. During cytomorphological analysis the causative pathogens of meningococcal meningitis are frequently found intra- and extra-cellularly in the CSF, especially before initiation of therapy.

Cytotechnology / Oral presentations / 10

APAAP IMMUNOCYTOCHEMICAL METHOD: COMPARISON OF TWO DIFFERENT TIME FIXATIONS BY INTENSITY OF ANTIGEN EXPRESSION IN BONE MARROW OF PATIENTS WITH MULTIPLE MYELOMA

Švigir M, Tušek AM, Gavrić Ž, Sučić M.

Division of Cytology, Clinical Institute of Laboratory Diagnosis, Zagreb Clinical Hospital Center, Croatia
mirna.sucic2@zg.htnet.hr

Aim of the study was to analyze whether different time fixations influence the intensity of antigen expression determined by immunocytochemical alkaline phosphatase anti-alkaline phosphatase (APAAP) method.

Patients and methods: The study included 15 patients with multiple myeloma (MM). In all patients bone marrow specimens were analyzed after standard May-Grünwald-Giemsa staining and after immunocytochemical APAAP staining. Immunocytochemical APAAP staining for CD38, CD56, CD45 and cytokeratin was concurrently performed on two bone marrow smears. Both bone marrow smears were fixed in cold acetone (+4 0C), one for 90 seconds and the other for 60 seconds.

Results: Medians of percentages of CD38 and CD45 hematopoietic cells were slightly higher after 60 second fixation, but without statistical difference. Median of percentages of CD56 hematopoietic cells was moderately higher after 90 second fixation, also without statistical difference. Cytokeratin plasma cells in one MM patient (out of 15) were equally positive after both types of fixation.

Conclusion: Results indicated that both 90 and 60 second time fixations were almost equally adequate for antigen preservation. Small variations were probably linked to differences in particular antigen resistance to fixation.

Cytotechnology / Oral presentations / 11

THE RELIABILITY OF TRAINEE CYTOTEHNOLOGISTS IN SCREENING OF VCE SMEARS

Vidranski T, Klen Lj, Pajtler M.

Department of Clinical Cytology, Osijek University Hospital, Osijek, Croatia

Aim of study:To determine the reliability of trainee cytotechnologists in selection of VCE (vaginal, cervical, endocervical) smears and to determine the causes of interpretation difficulties.

Samples and methods: 37422 women, whose VCE smears were examined by 6 cytotechnologists from the Department of Clinical Cytology, Osijek University Hospital during the year 2004, were included in the studies. Two of the cytotechnologists are trainees, with one year and two years experience in screening of VCE smears, respectively. One of them has received formal education, while the other has only had internal education. The number of examined VCE smears was compared with the number of those abstracted, and the number of abstracted smears was compared with the number of abnormal smears, in relation to the cytologist's final diagnosis for all cytotechnologists. In the case of trainee cytotechnologists, the reason for abstraction (differential cytological diagnosis or risk factor) was compared with the cytologist's final diagnosis and the reproductive age of the women.

Results: Trainee cytotechnologists abstracted 10.1% and 11.22% from examined VCE smears, while cytotechnologists with more experience abstracted between 4.2% and 9.25%. From the abstracted VCE samples, the cytologist defined 50.2% and 57.69% as "abnormal cells" for those abstracted by cytotechnologist's beginners, and 52.54 % to 70.17% for those abstracted by more experienced cytotechnologists. The congruence of the differential cytological diagnosis made by trainee cytotechnologists with those made by the cytologist is highest for DG (dysplasia gravis) (100% and 52.6%), CIS (carcinoma in situ) (100% and 71.4%), CAP (carcinoma planocellulare) (100%) and ADCA-E (endometrial adenocarcinoma) (100% and 66.7 %.). The lowest congruence was detected for ASCUS (abnormal squamous cells undetermined significance) (21.7% and 24.6%) and AGCUS (abnormal glandular cells undetermined significance) (24.4% and 32.9%). From among the abstracted VCE smears the cytologist defined 100% of DG, CIS, CAP and ADCA as "abnormal cells" and only 69, 6% and 36.9% from ASCUS and 26.8% and 35.7% from AGCUS. From among 48, false positive (FP) ASCUS, twenty-two (45.8%) were in the atrophic smear (postpartum or postmenopausal), and from among seventy-five FP AGCUS, thirty-one were in the atrophic smear. One hundred and forty eight VCE samples were abstracted without differential cytological diagnoses (needs checking, inflammation, blood? hormones? and endometrial cells), among which the cytologist classified seven (4.7%) as abnormal cells, one (0.7%) as unsatisfactory for evaluation, and one hundred and forty (94.6%) as negative for intraepithelial lesion or malignancy. From among one hundred and forty negative smears, eighty-one (57.9%) were to be found in atrophic smears.

Conclusion: Trainee cytotechnologists abstract more VCE smears, which finally prove positive, in comparison to more experienced cytotechnologists. The best concurrence between trainee and cytologist results, resulting in a higher degree of positive tests, was visible in severe intraepithelial lesions and invasion lesions of both types. Negative findings were mostly in ASCUS and AGUCS diagnoses. Atrophic smears were the main problem for trainee cytotechnologists.

CITOTEHNOLOGIJA / *CYTOTECHNOLOGY*

Posteri / *Posters (1-3)*

Cytotechnology / **Posters** / **01**

TRANSABDOMINAL PLACENTOCENTESIS AND CONTEMPORARY PERINATHOLOGY

Đorđijevski E, Singer Z, Krivak-Bolanča I, Šentija K.

Laboratory for Clinical Genetics and Cytology, Department of Gynecology and Obstetrics , Merkur University Hospital, Zagreb, Croatia

Transabdominal placentocentesis is a simple,quick and rational method in prenatal diagnosis and contemporary perinatology. It may be performed from 11 to 40 weeks of pregnancy. In the last 10 years findings have been obtained in 98% cases. In the last 7 years the risk of abortion has been less than 0,4%. With help of the ultrasound we recommend transabdominal aspiration of the plancental tissue. The most common indications for placentocentesis are risk of an abnormal kariotype of the fetus, DNA abnormalities, biochemical, and microbiological and immunologic investigations. The genetic counsellor must order the type of laboratory investigation that needs to be done. A short term, so-called "over night" cytogenetic technique may be performed using 10 mg of the placental tissue (with cytotrofoblasts). If necessary, a long-term culture from the placental stromal mesoderm may be done. In the last twenty years there have been thousands of cases in which pregnancies have resulted in the birth of healthy babies.

Cytotechnology / Posters / 02

CORRECT CYTOCHEMICAL AND IMMUNOCHEMICAL ANALYSIS OF SMEAR BASED ON SAFE INTERPRETATION

Križaj B[1], Parigros K[1], Knežević G[1], Anić V[1], Harabajsa S[1], Kardum-Skelin I[1], Šušterčić D[1], Fabijanić I[1], Jelić-Puškarić B[1], Stužić V[2], Jakšić B.[2]

[1]Laboratory of Cytology and Haematology,
[2]Department of Medicine, Merkur University Hospital, Zagreb, Croatia
ikardum@hi.t-com.hr

By applying standard morphology it is possible to recognize the morphology of normal cells, inflammatory and degenerative changes, proliferation, malignancy and other states. In addition, it is also possible to define the cell provenance of tumours and the degree of differentiation. The introduction of additional technology increases the safety of not only standard morphology, but also diagnosis accuracy, typification and tumour diagnosis.

Aim of the study: To analyse the frequency of application of additional technology and to stress the importance of technical preparation of smears, fixation and the actual technique of colouring using cytochemical and immunocytochemical methods.

Patients and Methods: Of the 67164 analyses conducted in the period 2002-2004, 9947 analyses were cytochemical or immunocytochemical (approx. 15%). Peripheral blood smears, bone marrow, lymphatic node, tumour biopsies and body fluids were used in the cytochemical and immunocytochemical analyses. In cytochemical analyses the samples were fixed and then stored or processed immediately. In immunocytochemical analyses samples up to 1-7 days old were used (depending on the marker applied) and were coloured immediately.

Results: In cytochemical analyses 5,3% of cases showed a reaction to myeloperoxidase, 2,1% to unspecific esterase, 17,1% to PAS and reaction to iron, and 3,2% to acidic phosphatase. AgNOR (a reaction using silver to mark the region of the nuclear organizer) and the Feulgen reaction for image DNA analysis occurred in 6% of cases. Immunocytochemical analyses using the LSAB method for establishing cell marker was applied in 54,9% of cases.

Conclusions: Morphology is the basis for diagnostics, sub-typisation and grading tumours. Additional methods are planned on the basis of standard morphology. In order to accurately interpret and analyse immunocytochemical and cytochemical analyses, the preparation, analysis, fixation and colouring technique of the blood smear sample is vitally important.

Cytotechnology / Posters / 03

PATIENT KNOWLEDGE OF CYTOLOGICAL DIAGNOSTICS

Parigros K[1], Knežević G[1], Križaj B[1], Anić V[1], Harabajsa S[1], Kardum-Skelin I[1], Šušterčić D[1], Fabijanić I[1], Stužić V[2], Jakšić B.[2]

[1]Laboratory of Cytology and Haematology, [2]Department of Medicine, Merkur University Hospital, Zagreb, Croatia
ikardum@hi.t-com.hr

Cytology is a minimally invasive diagnostic method in which a thin needle is employed for obtaining a cell sample for analysis. Exceptions are bone marrow biopsies where local anaesthetics are used due to the sensitivity of the procedure.

Aim of the study: To evaluate patient knowledge of cytological diagnostics - about the puncture itself and cytological results.

Material and Methods: A survey was conducted among a sample of 30 patients (37 biopsies) from whom cytological biopsies of the bone marrow (10), the lymphatic nodes (12) or other organs (15) were taken. 22 men and 18 women took part in the survey; 7 were under the age of 30, 10 were between 30 and 50 years of age, and 13 were over 50 years of age. The majority had secondary education (53%), whereas 20% had only primary education, and 27% had some form of tertiary education. In 18 cases the patient was undergoing a first puncture, 12 patients were undergoing routine check-up biopsies, whereas 7 patients had biopsies of both the bone marrow and the lymphatic nodes.

Results: The majority of patients knew very little about cytological puncture procedures (17), some were well informed (11), and 9 patients knew nothing about the procedure. 67% were undergoing the procedure due to medical problems, whereas the procedure was a preventive course of action in 33% of patients. Patients were mostly aware of the fact that the procedure is a diagnostic method (93%), but a number of them considered it to be a therapeutic method. Despite the fact that the survey included patients undergoing bone marrow biopsies, 80% of patients described the puncture procedure as being almost painless. The majority received information on the procedure from medical staff (54%); others gained information through medical literature, the Internet, the media or from acquaintances, whereas 20% lacked any kind of knowledge about the procedure. Patients are more afraid of the diagnosis (63%) than the procedure, although it should be noted that patients stated that the procedure itself would have been less stressful if they had had prior detailed information on the procedure.

Conclusion: Despite the subjective experience of discomfort and pain, this survey indicates that pain as such is negligible in comparison to the fear experienced while awaiting diagnoses. In view of this, it is vitally important that patients are informed and knowledgeable about the procedure as such and about details pertaining to the procedure and diagnosis.

CYTOMORPHOLOGY - STANDARD DIAGNOSTIC TOOL FOR ACUTE LEUKEMIA

Sučić M

Division of cytology, Clinical Institute of Laboratory Diagnosis, Zagreb Clinical Hospital Center, Zagreb, Croatia

Acute leukemia (AL) is clonal expression of blasts in bone marrow, blood or other tissue. Morphological French-American-British (FAB) classification of AL is based on cytomorphological and cytochemical blast characteristics according to direction and degree of blast maturation (Table 1) (1976;1985). Old basic FAB criterion for AL diagnosis was at least 30% blasts of all nucleated bone marrow cells.

Table 1. Cytomorphologic FAB classification of AL

AL Type	AL subtype
ALL	L1, L2, L3
AML	M0, M1, M2, M3, M4, M5, M6, M7

ALL are cytomorphologically divided according to blast characteristics in L1 (small uniform blasts) L2 (large heterogenous blasts) and L3 (blasts with basophil and vacuolated cytoplasm).

AML-M2 and AML-M4 subtypes with numerous eosinophils are marked as M2eo and M4eo. Also, AML-M2 and AML-M4 with a high number of basophils are diagnosed as M2baso and M4baso. M3 is AML with numerous atypical promyelocytes with coarse azurophilic granules or Auer rods ("faggot cells") in cytoplasm. In M3 microgranular variant, abnormal promyelocytes have often lobulated nuclei with numerous small azurophilic granules; atypical promyelocytes in M3 basophilic variant have cytoplasmatic basophilic granules. M5a is an immature M5 subtype with numerous monoblasts, and M5b is a more differentiated M5 subtype with at least 20% monoblasts and numerous promoncytes and monocytes. Laurencet FM also proposed M5c subtype with proliferation of abnormal histiocytes. AML-M6 could also be subdivided in M6a, M6b and M6c according to different myeloblast and immature proerythroblast perecentages. M8 is a rare AML subtype with clonal proliferation of basophil blasts, not included in the first FAB classification in 1976.

WHO classification of acute leukemias interrelates morphology, immunologic markers, cytogenetics and molecular genetics in universally applicable and prognostically relevant classification. According to WHO classification, basic criteriaion for AL diagnosis is at least 20% or more blasts of all nucleated bone marrow cells. In WHO group of AL of ambiguous lineage blasts lack morphologic, cytochemical and immunophenotypic characteristics to be classified as myeloid or lymphoid AL (undifferentiated AL-AUL) or have morphologic and/or immunophenotypic characteristics of both myeloid and lymphoid blasts or both B and T lineages (bilineal and byphenotypic AL).

In one of our recent studies, we correlated cytomorphology and immunophenotype of AL. According to FAB classification in 102 AL patients, there were 64 with AML, 35 with ALL and 3 patients with AUL. In AML subgroup, 15 patients had blasts with at least one lymphoid antigen (AML^{Ly+}); in ALL subgroup, there were 2 patients with at least one myeloid antigen (ALL^{My+}) and 4 AL patients with both B and T lymphoid antigens; in AUL subgroup, one patient had blasts with both myeloid and lymphoid antigens. In group of 102 AL, there were 3 biphenotypic AL with M3, L2 and AUL cytomorphology expressing both myeloid and lymphoid antigens with score value 2 or more for both cross lineages.

In another recent study of 15 patients with M3 cytomorphology there were two patients with M3variant cytomorphology (one with M3micro and one with M3baso). In one of our M3 patients both t(15;17) and PML/RAR" were negative, indicating that it could be one of the rare examples of M3-like AL involving RAR gene locus, such as t(11;17) or t(5;17).

EXPRESSION OF CD38, CD45, CD56 AND CYTOKERATIN IN PATIENTS WITH MULTIPLE MYELOMA

Sučić M, Marković-Glamočak M, Ries S, Gjadrov-Kuveždić K, Vrbanus LJ, Labar B, Nemet D

Division of Cytology, Clinical Institute of Laboratory Diagnosis, Department of Haematology, Clinical Hospital Center, Zagreb, Croatia
mirna.sucic2@zg.htnet.hr

Multiple myeloma (MM) is proliferation of monoclonal plasma cells in bone marrow, serum monoclonal protein, osteolytic lesions, hypercalcemia and anemia. Immunophenotype is characterized by monotypic cytoplasmic immunoglobulin, CD38 and CD79 expression. CD56 positivity is also frequent and majority of cases lack CD19 and CD20. Positivity of CD45 in MM is different in various studies. The aim of the study was to evaluate immunocytochemical expression of CD38, CD45, CD56 and cytokeratin in patients with MM.

Patients and methods: Bone marrow specimens of 15 MM patients were analyzed for the presence of plasma cells after May-Grünwald-Giemsa staining. CD38, CD45, CD56 and cytokeratin plasma cell expression was evaluated after immunocytochemical APAAP staining.

Results: CD38 was positive in all 15 patients; plasma cells were CD45 positive in 14 patients and CD56 positive in 11 patients. In one of the 15 patients with MM plasma cells were positive for cytokeratin.

Conclusion: Our results are in concordance with results of other studies. Cytokeratin positivity of plasma cells found in one of the 15 patients indicated that variations in MM immunophenotype can occur and cause diagnostic difficulties.

IMAGE ANALYSIS IN CHRONIC LYMPHOPROLIFERATIVE DISORDERS

Kardum-Skelin I[1], Jakšić B[2]

[1]Laboratory of Cytology and Haematology, [2]Department of Medicine, Merkur University Hospital, Zagreb, Croatia

Chronic lymphoproliferative disorders (CLPD) are defined as a heterogeneous group of malignant monoclonal lymphocyte disorders, sharing their indolence and involvement of primary lymphoid tissue in lymph nodes (LN), bone marrow (BM), spleen and peripheral blood (PB). There are many studies about digital image analysis: morphometry, assessment of argyrophilic nucleolar organizer regions (AgNOR) and image DNA cytometry (ICM) in different benign, suspicious and malignant lesions of various tissue and thus also of leukemic and lymphoma cells.

Aim of the study: To correlate morphometry, DNA ploidy, proliferative activity and the pattern of AgNOR in CLPD between the different compartments of tumor mass (lymph nodes, bone marrow and peripheral blood).

Patients and Methods: Peripheral blood, bone marrow and lymph nodes archive cytology smears from 155 patients were analyzed. Conventional cytology-stained smears (May-Grünwald-Giemsa) were used for morphometric analysis, secondarily stained according to Feulgen for ICM or silver staining for AgNOR, for each. Digital image analysis was performed by use of the SFORM software (VAMSTEC, Zagreb). We analyzed a total of 50 parameters for each individual lymphocytic cell and nucleus: 22 of morphometry, 6 of ICM and 26 of AgNOR.

Results: Morphometric analysis of cells and nuclei has shown statistically significant differences ($p<0,05$) for area and convex area between the groups studied: BM/PB, PB/LN and BM/LN. The most ICM differences (S-phase, % of cells of G_0/G_1 peak, % of cells$<G_0/G_1$ peak and % of cells$>G_0/G_1$ peak) were statistically significant between BM/PB, PB/LN and BM/LN ($p<0,05$). Size of inhomogenic and ringed AgNOR was significantlly different in relationship to nuclei.

Conclusion: The results obtained demonstrate that lymphocytic cells are produced in bone marrow (lower % cells in S-phase and the highest % cells of G_0/G_1 peak). They migrate to lymph nodes, where transformation occurs (higher % cells in S-phase and lower % in G_0/G_1 peak). High S-phase does not necessarily mean a better prognosis, but better survival is seen in patients with higher % of cells in G_0/G_1 peak in lymph nodes. Cells migration from BM to LN and between lymph nodes takes place in PB (cells with high and low proliferative activity: high % of cells in S-phase and in G_0/G_1 peak, as well as present large cells with large nuclei). Prognosis depends on the tumor mass which is a consequence of cells proliferation. Precise typing of the disease is a prerequisite for rational therapy care.

Supplement / **Cytotechnology** / **Oral presentations** / **12**

DIGENE HYBRID CAPTURE 2 - HPV DNA TEST

Valenčić T.

Department of Gynecological Cytology, University Department of Gynecology and Obstetics, Clinical Hospital Centre Rijeka

Aim: To describe sampling and handling of specimens for the DIGENE HPV DNA hybrid capture (hc2) test as well as the method itself. Description of method: The Digene HPV DNA hc2 test is the only standardized commercial assay for clinical use. The test detects group positivity for 5 types of low risk HPV and for 13 types of high risk HPV. The samples are taken with a special cervical sampler (brush or cotton stick) that is immersed in the transporting liquid medium. It could be stored up to two weeks at room temperature or up to three months at -20°C. Digene hc2 test is a hybridization method using signal amplification and detection of chemiluminiscence. First step is the denaturation with denaturation reagent and temperature of 65°C. The target DNA splits and hybridize with a specific HPV RNA probe cocktail. Hybrids are captured onto the microplate and reacted with alcaline phosphatase which reacts with chemoluminiscent substrat.

The light is measured as relative light units (RLU) in the luminometer.

A RLU measurement equal or greater than the <cutoff> value indicates the presence of HPV DNA in the specimen.

Conclusion: Digene hc2 HPV DNA test is a simple and fast procedure that is easy to establish and incorporate in routine laboratory practice.

ZAHVALA / ***ACKNOWLEDGMENT***

Organizacijski odbor i Kongresni servis zahvaljuju sponzorima i pokroviteljima koji su ovaj kongres te njegov stručni i društveni program učinili ostvarivim. / *Organizing Committee and Congressional Service would like to express their gratitude to sponsors and donators who made this Congress and its scientific and social evenets possible.*

SPONZORI / ***SPONSORS***

A & B d.o.o.
Aspecta d.o.o.
Carl Zeiss d.o.o.
Croatia osiguranje d.d.
Grad Rijeka
HBOR
MB tisak d.o.o.
Media d.o.o.
Merck d.o.o.
Ministarstvo znanosti, obrazovanja i športa Republike Hrvatske
Olympus d.o.o.
Plastform d.o.o.
Privatna specijalistička ordinacija dr. Branka Molnar-Stantić
Sole-Mark d.o.o.
Styrocon Handelsgeselschaft mbH, Austria
Sonimed d.o.o.
Tyco
Zaklada Sveučilišta u Rijeci

DONATORI / ***DONATORS***

AutoZubak d.o.o.
Coca-Cola Beverages Hrvatska d.d.
Franck d.d.
Hrvatska turistička zajednica
Instrumentarij d.o.o.
Karlovačka pivovara d.d.
Milsing d.o.o.
Pamučna industrija Duga Resa d.d. u stečaju
PIK Rijeka d.d.
T-MOBILE Hrvatska d.o.o.
Toyota Croatia d.o.o. Predstavništvo u RH
Turistička zajednica Grada Opatije

INDEKS AUTORA / *AUTHORS' INDEX*

PRIVATNA SPECIJALISTIČKA ORDINACIJA
MEDICINSKE CITOLOGIJE

dr. med. Branka Molnar Stantić

spec. medicinske citologije

10000 Zagreb • Kuzminečka 4

tel./fax: 01/3879 528 • GSM: 091/20 188 25

e-mail: branka.molnar.stantic@zg.htnet.hr