

The 1st Annual Scientific Meeting of the College of Pathologists, Academy of Medicine Malaysia & 10th Combined Malaysia-Singapore Pathology Meeting was held at the Paradise Sandy Bay Hotel, Penang from 1st to 3rd October 1999. Abstracts of the free paper communications follow:

Oral presentations:

1. Fine needle aspiration cytology and serum alpha feto-protein level as diagnostic tools for hepatocellular carcinoma: an experience at Hospital Tengku Ampuan Afzan, Kuantan

*Sunita Ahlawat, R Kalavathy and *Sharmila Sachithanandan*

*Departments of Pathology & *Medicine, HTAA, Kuantan, Malaysia*

Hepatocellular carcinoma (HCC) is one of the most common malignancies among males in Malaysia. Radiological guided FNA and serum alpha feto-protein (AFP) are the main diagnostic tests used in this hospital. Both these investigations are reported to be relatively insensitive and not very specific. In this study we present retrospective data with correlation of FNA and serum APP to find out the specificity and sensitivity of these tools. A total of 59 FNA from liver were reported from December 1996-June 1999. 51 were satisfactory for review. Diff-Quik, MGG and PAP stains were available. Cellblocks were available in 17 cases and immunostains in 21 cases. Serum AFP levels were available in 44/59 cases. AFP was measured on automated immunoanalyser Abbott **IMX** using microparticle immunoenzyme assay kits from same source.

38/51 (74.50%) were malignant and 13/51 (25.4%) were benign. 23/38 (60.52%) were diagnosed to have HCC (18 moderately, 2 each well and poorly differentiated & 1 case of fibrolamellar HCC). 11/12 (>90%) showed **AFP** positivity on immunostaining. Cellblock preparation were extremely useful for immunostaining and studying the architecture of tumour. Other primary tumours of liver were a case of NHL and a case of cholangiocarcinoma. 13 other tumours were diagnosed including a case of carcinoid, small cell carcinoma and sarcoma each. 10 cases of adenocarcinomas were diagnosed. 7 cases of liver abscess and 6 cases of benign liver lesions including cirrhosis, nodular **hyperplasia** and regenerative nodules were diagnosed. One case of hemangioma of liver yielded blood on FNA.

Serum AFP levels showed that >90% of HCC had a level of >100 ng/ml. Only the case of fibrolamellar HCC and a case of poorly differentiated HCC had AFP levels of 3 and 37 ng/ml respectively. Both these cases showed positive immunostaining for AFP while none of the metastatic or other primary **tumors** of liver had this high level of AFP. All the benign lesions had AFP levels below 50 ng/ml except a case of cirrhosis with a level of 65 ng/ml.

Conclusions: (1) FNA with cellblock preparation and judicious use of immunostaining is quick, safe, sensitive and specific for the diagnosis of HCC. (2) The only complication was bleeding in the case of hemangioma hence FNA should be deferred in a suspected case of hemangioma. (3) Both serum **AFP** and FNA are sensitive and specific in distinguishing benign from malignant lesions and for specific typing of tumour.

2. Spectrum of malignant lymphomas from Klang General Hospital

*N THANALETCHIMY, *SC PEH, *LH KIM AND *SP CHAI*

*Departments of Pathology, Klang Hospital and *University of Malaya*

Background: Malignant lymphoma is a common group of neoplasm seen in Malaysia. This study aims to elucidate the pattern of disease encountered in a government **service** hospital. **Material and methods:** A total of 97 biopsies from 94 patients were reviewed. They were retrieved from the archives of Klang Hospital, from January 1993 to June 1999. The diagnosis were agreed upon by both the pathologists, and classified based on the WHO proposed scheme. Immunohistochemical staining

was routinely performed, with a panel of antibodies in aid of sub-typing. **Results:** 10 cases were excluded due to inadequate tissue or reconfirmed as non-lymphoma. The remaining 87 biopsies were from 54 male and 30 female patients, **male:female** ratio of 1.8:1. There were 66 Malays, 5 Chinese, 13 Indians. Patients' ages ranged from 3 to 86 years. There were 15 Hodgkin's lymphomas, HL (5 nodular-sclerosis, 6 mixed-cellularity, 3 lymphocyte-predominant, 1 lymphocyte-depleted) and 69 non-Hodgkin's lymphomas, NHL patients. The **HL:NHL** ratio was 1:5. Of the 69 cases of **NHL**, 9(13%) were T-cell, 59(86%) B-cell and 1(1%) null-cell types. The commonest NHL encountered was diffuse-large-B-cell type. Follicular lymphoma constitutes 16% of NHL. **Conclusion:** There is relatively small proportion of T-cell lymphoma in this series. The reason may be related to the small numbers of Chinese patients. The ethnic distribution of patients reflects the utilization of the hospital facilities by these populations.

3. Cytological and histological features of papillary-cystic variant of acinic cell carcinoma: a case report

Hasnan J and Meor Zamari MK.

*Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia.
16150 Kubang Kerian, Kelantan. Malaysia*

A case of papillary cystic variant of acinic cell carcinoma arising from the parotid gland is described. The tumour measured **60x55x30mm** and was a circumscribed mass composed of greyish white tissue with cystic spaces and a large central haematoma measuring **20x15mm**. On fine needle aspiration cytology, the following features were of interest: (1) oncocyte-like cells which were likely to lead to the impression of Warthin's tumour or oncocytoma; and (2) occasional calcified cells and **psammoma** bodies, which may be construed as metastatic papillary carcinoma of the thyroid. On histopathological observations, the following features were of interest: (1) prominent cleft artefacts and dilated haemorrhagic cystic spaces that appeared like anastomosing vessels of angiosarcoma; and (2) scattered psammoma bodies, papillary structures and spaces filled with 'colloid-like' materials. A brief literature review of the entity is also discussed.

4. Fine needle aspiration cytology, a sensitive diagnostic tool for malignancies: experience at Hospital Tengku Ampuan Afzan, Kuantan.

K RAMACHANDRAN and S AHLAWAT

Hospital Tengku Ampuan Afzan, Kuantan

Fine needle aspiration is a popular tool for initial evaluation of palpable lumps. The aim of this study is to evaluate the efficacy of this procedure in the diagnosis of malignancies. A total of 1485 FNAs were performed in 17 months (Jan 1998 - May 1999). **192/1485** (12.9%) were diagnosed as malignant. Histopathologic correlation **and/or** immunohistochemical/clinical correlation was available in 109 (53.1%) cases. On review, 7 cases were false negative on cytology of which 4 cases were from the thyroid gland which were reported as **Hurthle** cell adenoma, follicular adenoma and multinodular goitre. On histology, all 4 cases had in addition microscopic papillary carcinoma. There were 2 cases from the breast and 1 from the lymph node where the aspirates were not representative of the malignancy. There were 2 cases, both from the lymph node, which were suggestive of Hodgkin's lymphoma on FNA. On histology, one had Kikuchi's lymphadenitis while the other was diagnosed as atypical reactive lymph node. These **formed** the 2 false positive cases in our study. There were 35 (18.2%) cases of radiologically guided FNAs in which immunohistochemistry helped to differentiate the primary from the metastatic lesions in 16 of the cases

5. Fine needle aspiration cytology of lymph nodes in H N infected individuals

Gita JAYARAM

Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur

Lymphadenopathy is one of the earliest manifestations of opportunistic infections occurring in

human immunodeficiency virus (HIV) positive individuals. The utility of fine needle aspiration (FNA) cytology in the diagnosis of neoplastic and non-neoplastic lymph node lesions is well established. Recent reports have documented the value of cytology in the evaluation of lymph nodes in HIV infected individuals (HIVII). Thirty-nine HIVII presenting with lymphadenopathy to the University Hospital, Kuala Lumpur were subjected to FNA cytology. Cytological smears were routinely stained with May-Grunwald-Giemsa. Special stains and immunostains were used where necessary. In nine cases, the cytological appearance was compatible with HIV type-A and in one case with HIV type-C lymphadenopathy. In 21 cases acid fast bacilli (AFB) were demonstrated in the cytological smears, enabling a diagnosis of mycobacterial lymphadenitis. In one of these cases there was a concomitant infection with *Penicillium marneffeii* that was overlooked on initial cytological examination. The cause of granulomatous lymphadenitis could not be ascertained in one case where neither AFB nor any other organisms were demonstrable. Two cases of Histoplasma and one case of cryptococcal lymphadenitis were diagnosed as was also one high-grade non-Hodgkin's lymphoma that could be immunophenotyped on cytological material. In three cases the aspirates were inadequate for cytological opinion. In conclusion, it can be said that lymph node FNA is a valuable investigative modality in HIVII. Most opportunistic infections (bacterial and fungal) can be correctly identified and high-grade lymphoma can be diagnosed and also phenotyped.

6. Rare mesenchymal liver tumour: a report of two cases

Razmin G, Arni T, Noor Laili M, Wahidah A, K.K.Foo and Krishnan R.

Department of Pathology, Hospital Kuala Lumpur

Two cases of rare hepatic angioliipoma are reported. The first patient presented with sudden onset of right hypochondrial pain and CT scan revealed a ruptured liver abscess. The second patient presented with chronic epigastric pain for five years. An epigastric mass was detected on physical examination and CT scan revealed a large pedunculated mass arising from the left lobe of the liver. Histologically, both tumours varied in their proportion of mesenchymal components. Immunohistochemistry confirmed the presence of myoid cells in one of the cases. Resection of the tumours were carried out in both cases and patients were well post-operatively.

7. Malignant peripheral nerve sheath tumor: a clinicopathologic study of 7 cases seen in 10 years.

M. Madhavan, Hasnan J, Nor Hayati O, Gurjeet K, Mutum Samarendra S

*Department of Pathology, School of Medical Sciences, Universiti Sains Malaysia
16150, Kubang Kerian, Kelantan, Malaysia*

Malignant peripheral nerve sheath tumor (MPNST) is a spindle cell sarcoma arising from nerve or neurofibroma or showing nerve sheath differentiation. It is a preferred term over the 'malignant Schwannoma'. MPNST is one of the most difficult and elusive diagnoses in soft tissue tumors because of the lack of standardised criteria. The aim of this study is to highlight the clinical, histological and immunohistochemical (IHC) features observed in those cases of MPNST reported in Hospital Universiti Sains Malaysia (HUSM) over a period of 10 years. A total of 7 cases were retrieved from our pathology registry dating from 1990 to August 1999. All the slides were reviewed and a panel of IHC staining was performed. All the patients were Malays between 14 years to 79 years of age with an Male: Female ratio of 1:1.3. The chief complaint was painless lump (86%) involving predominantly the thigh (42%). Two cases (28%) primarily involved the bones. There was no clinical evidence of neurofibromatosis in any of these patients. Though grossly appearing circumscribed, almost all the tumors (86%) had infiltrative borders under microscopy. The histological features include a mixture of hypo- and hypercellular areas, haemorrhage, necrosis and pleomorphism. The degree of vascularity appears variable and roughly corresponds with the mitotic activity. On IHC study, NSE and CD 68 were found to be positive in 100 % of cases, S 100 and vimentin in 86% of cases and desmin and myoglobin were positive in 28% of cases. Synaptophysin, chromogranin and cytokeratin were negative in all the cases. In this series of 7 cases, we conclude that MPNST can occur in both children and adults and can affect bone and soft tissue. Pain was not a significant

symptom and the tumors were usually more than 5 cm. in size. The IHC markers S 100, vimentin, CD 68 and NSE were significant and comparable to other studies. NSE positivity was noted mainly in ovoid or epithelioid cells in earlier studies whereas it was observed even in spindle cells in our cases.

8. Utility of CD79a and TdT in the diagnosis of B lymphoid malignancies in comparison to CD19, CD20 and CD22.

Veera S Nadarajan and TAN Chai Din

Department of Pathology, Penang Hospital, Pulau Pinang

Multi parametric flow cytometric analysis plays an invaluable role in the diagnosis and monitoring of leukaemias. In order to optimise the use of monoclonal antibodies in the analysis, we sought to compare the use of intracytoplasmic **CD79a** and **TdT** as compared to the more traditional surface markers **CD19**, **CD20** and **CD22** for the classification of B lymphoid malignancies into the various types.

Nine cases of suspected B lymphoid malignancies were analysed by flow cytometry using a combination of B surface markers (**CD19**, **CD20**, **CD22**, **CD10**, kappa, lambda, **CD23**) and intracytoplasmic **CD79a** and **TdT**. All cases were **CD19+** of which 7 were also **CD22+** and 5 **CD20+**. **CD79a** was also unequivocally positive in all cases analysed. **TdT** was positive in 2 cases, both of which were also **CD10+** and diagnosed as B-precursor ALL. Two cases were **CD79a+**, **TdT-** and **CD10-**, which turned out to be a pre-B ALL and B-ALL. The remaining 5 cases were all **CD79a+** and **TdT-**. Using an extended panel of monoclonal antibodies, 2 of them were diagnosed as B-CLL, 1 hairy cell leukaemia, 1 follicle centre lymphoma and 1 diffuse large cell non-Hodgkin's lymphoma.

We believe that combined intracytoplasmic staining of **CD79a** and **TdT** is useful in the diagnosis of B lymphoid malignancies and allows the distinction of B-precursor ALL from the more mature ALL subtypes and from non-Hodgkin's lymphoma. **CD79a** and **TdT** may offer more information than the surface analysis of **CD19**, **CD20** and **CD22** and should be considered in the primary panel for immunophenotyping, and can replace the use of **CD19**, **CD20** and **CD22**.

9. Phyllodes tumour of the breast

Samarendra S Mutum, M Marthavan, Hasnan Jaafar, Aziina Abd Rahman and Nor Hayati Othman

Universiti Sains Malaysia, Kubang Kerian, Kelantan

A total of 7 cases of phyllodes tumours of the breast were identified in the histopathology files of Universiti Sains Malaysia, Kelantan, during the period 1987-1999. Two of the cases presented as bulky ulcerating tumours with histological features of malignant phyllodes tumours (**MPT**). During the same period 2 cases were diagnosed as stromal sarcomas (**SC**) and 144 cases as primary breast carcinomas. Case files, available slides, new sections stained by **H&E** and special stains including immunostains, were examined.

It is probable that cases labeled as **SC** may represent **MPT** with massive overgrowth of stromal cells and destruction of the epithelial component. The rarity of **MPT**, presentation with skin ulceration, lymph node metastasis and the need for extensive sampling before labeling a tumour as **SC** are stressed.

10. The pattern of TARC, a CC chemokine, in Hodgkin's lymphomas, ALCL, TcRBcL and other lymphoproliferativedisorders

Suat-cheng PEH, Lian-hua KIM, Siew-pin CHAI

Department of Pathology, University of Malaya

Background: Thymus and activation-regulated chemokine (TARC) is a recently identified lymphocyte-directed CC chemokine that specifically chemoattracts T helper type 2 (**Th2**) **CD4+** T cells in human. Hodgkin's lymphoma (HL), anaplastic large cell lymphomas (ALCL), T-cell rich B-cell lymphoma (**TcRBcL**) are some lymphoproliferativedisorders which mimic Hodgkin's lymphoma.

This study is aimed to establish the pattern of TARC positivity in these diseases. **Methods & Materials:** A new commercially available polyclonal goat anti-human TARC antibody (R&D System, USA) was used to stain a random series of previously confirmed HL, (12 cases), of which 8 were classical HL (5 mix **cellularity**, 2 nodular sclerosis and one lymphocyte rich) and 4 lymphocyte predominant; 5 ALCL, 5 TcRBcL and one EBV-driven lymphoproliferative disorder cases. **Results:** All classical HL cases showed positive TARC expression in the Reed-Stenberg cells. The expression is found present in the membrane and cytoplasm. The number of RS cells stained positive and the intensity of staining varied from case to case. Cases of lymphocyte predominant Hodgkin's lymphoma, ALCL, TcRBcL and EBV-driven lymphoproliferative disorder did not express TARC in the tumour cells and the abnormal B blasts in the latter. **Conclusion:** The characteristic TARC positive staining in the RS cells of classical Hodgkin's lymphomas suggest that TARC can be a useful antibody in aid of diagnosis in difficult cases of classical Hodgkin's lymphoma. **Acknowledgement:** This study was supported by IRPA 26-02-03-0586

11. Comparison of three different methods for the presumptive detection of extended-spectrum beta-lactamase production in multi-resistant strains of *Klebsiella pneumoniae*

S Palasubramaniam and N Parasakthi

Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

Strains of *Klebsiella pneumoniae* may be resistant to the third and fourth generation cephalosporins by the production of extended-spectrum beta-lactamase (ESBL) of the molecular class A type. It is important for laboratories to be able to identify ESBL producing strains so that alternative therapeutic regimens may be used to treat infection caused by them. Three different methods for the presumptive detection of extended-spectrum beta-lactamase production in multi-resistant strains of *Klebsiella pneumoniae* were evaluated using 28 strains of multiresistant *Klebsiella pneumoniae* (MRKP) isolated from blood cultures of in-patients of University Hospital, Kuala Lumpur. Resistance to ceftazidime by the disc diffusion test was used as a marker of ESBL production.

In the DDST method an **amoxicillin/clavulanate (AMC/Clav)** disc and a B-lactam disc were placed on a Mueller-Hinton (MH) agar plate lawned with the test strain. An enhancement of the ceftazidime zone of inhibition near the **AMC/Clav** disc indicates the presence of ESBL enzymes in the test organism. The inhibitor-potentiated disc diffusion test involves the placing of four B lactam discs (**CAZ, CRO, CJX and AZT**) on MH agar plates with and **without clavulanate** and their zones of inhibition were compared. An augmentation zone width of **>= 10mm** was considered positive for ESBL production. The E-Test ESBL method uses an E-Test strip impregnated with ceftazidime (**0.125mg/L-8mg/L**) on one end and both ceftazidime (**0.125mg/L-8mg/L**)/clavulanate (**4mg/L**) on the other. The strip was placed on a MH agar plate lawned with the test strain. A greater than four fold reduction of **ceftazidime/clavulanate** MIC when compared with ceftazidime MIC alone was taken as positive for ESBL production. The DDST method could detect all strains as ESBL producers only when a combination of either CAZ and CRO with clavulanate respectively or CJX and AZT with clavulanate respectively were used simultaneously. Likewise using the combination of CJX and CRO with clavulanate respectively or CRO and AZT with clavulanate respectively enabled the detection of all strains as ESBL producers. The E-Test ESBL method was able to identify all the strains as ESBL producers. The log reduction of the strains ranged from 16 to 256 with 50% of the strains having a log reduction of > 64 of the MIC of CAZ. The inhibitor-potentiated disc diffusion method could detect ESBL enzymes in all strains by using a minimum of two b-lactam discs relating to any of the combinations except a combination of either ceftazidime and aztreonam or cefotaxime and ceftriaxone.

In conclusion the E-Test ESBL screen is a simple and reliable method to detect ESBL enzymes produced by MRKP strains from our centre. However, as **ESBLs** vary in their substrate profile, the recent NCCLS recommendations suggesting the use of one of the above b-lactams including cefpodoxime as initial ESBL Screen test may have to be considered. In addition to this, as a phenotypic confirmatory test, the use of both cefotaxime and ceftazidime, alone and in combination with clavulanate is also required.

12. A pathologist's dilemma

*Roziana Ariffin, Abu Dzarr Abdullah, *M Ramanathan*

Departments of Pathology and * Medicine, Hospital Taiping, Perak

Infectious mononucleosis (IM) is traditionally described to affect the young, with exudative pharyngitis, lymphadenopathy and the presence of atypical lymphocytes and heterophil antibodies. We present a 39-year-old lady (single, University lecturer) with none of the above mentioned features. Instead she had prolonged fever (for 1 month), leucopenia with marked neutropenia, **thrombocytopenia**, haemophagocytic syndrome (from bone marrow examination) and positive biochemical and histological features of hepatitis. Liver biopsy showed intact liver architecture with marked macrovesicular steatosis, spotty necrosis and collections of lymphoid cells within the parenchyma. Some perivenular areas showed more extensive necrosis although there was no bridging. In situ hybridization for **Epstein-Barr** Virus (EBV) was negative. However, her **IgM** EBV viral **capsid** antibody was positive and at the same time dengue **IgM** were positive twice. Arguably due to epidemiological consideration dengue was the more probable diagnosis. However this would only further enhanced our prejudicial tendency of diagnosing dengue for almost every viral infection. This case illustrates the typical presentation of infectious mononucleosis in the middle age which deviates from our understanding of classical infectious mononucleosis.

13. In vitro susceptibility of *Chlamydia trachomatis* clinical isolates to azithromycin, erythromycin and ciprofloxacin

V HEMA, T ESWARY, S BAHNU, YF NGEOW

Department of Medical Microbiology, University of Malaya

Chlamydia trachomatis causes genital tract infections with important sequelae. Azithromycin, an **azalide** antibiotic, has been recommended as a single dose treatment for chlamydial infections. In this study, *C. trachomatis* isolates from cervical and endometrial specimens were tested for their susceptibility to azithromycin and two other antichlamydial drugs, erythromycin and ciprofloxacin.

Minimum inhibitory concentrations (MIC) were obtained by inhibiting the propagation of isolates in **McCoy** cell cultures containing 0.03-8 **ug/ml** of antibiotic. An inoculum of about 10^3 **IFU** per **ml** was used. After 48 hours of incubation, chlamydial inclusions were stained by a *C. trachomatis* - specific monoclonal antibody. The MIC was determined as the lowest drug concentration at which no inclusions were detected. To obtain the minimum chlamydiacidal concentrations (MCC), the infected monolayers showing no inclusions in antibiotic-containing medium were harvested and reinoculated onto antibiotic-free cells. The concentration at which no inclusions were detected after one pass was taken to be the MCC. The MIC ranges obtained were 0.125 - 1.0 **ug/ml** for azithromycin and erythromycin and 1.0 - 4.0 **ug/ml** for ciprofloxacin. The MICs obtained after one pass were similar to the MICs. There was no difference in the MICs between cervical and endometrial strains. Azithromycin and erythromycin appear to be more active than ciprofloxacin against *C. trachomatis* clinical isolates.

14. Spermatozoa in 'rape' examinations: comparison of two "rape and murder" cases with previous study on clinical rape examinations

George PAUL

Forensic Unit, Department of Pathology, Faculty of Medicine, University of Malaya

Two cases of "rape- and- murder" are presented. In the first case from New Delhi, India, the victim, a 15-year-old girl was abducted by her father, raped and strangled to "teach his wife and daughter a lesson" for "interfering" in his extramarital affair with a neighbour. The body was discovered on the fourth day, and showed a fairly well preserved appearance due to the severe December winter. In the second case from Kuala Lumpur, the victim of rape and murder by the alleged "serial rapist" was kept alive in hospital for nearly four days, with the autopsy being conducted on the fifth day from the day of assault. In both the cases, besides evidence of assault in the form of injuries on the body,

there were plenty of sperm heads seen in the vaginal smears examined, with an occasional intact spermatozoa on H & E examination, even after an interval of 4-5 days since the incident. There were also signs corroborative of recent sexual assault. On comparison with earlier data from a 7-month study in New Delhi of 50 alleged rape victims, examined in the **A&E**, Maulana Azad Medical College, the findings in this case conform to the findings observed there. In that study, the alleged sexual assaults were also associated with crimes of kidnapping (50%) or abduction (40%) by offenders **known/related** to the victims (80%); being assaulted usually in the evenings (afternoon if the victims were children). Most of the victims (i.e. 94%) were unmarried; 58% were from poor socioeconomic background; and minors (62% - below 16 years; while 52% were below 15 years). While 90% appeared to be able to put up some resistance, only 14% had evidence of injuries elsewhere on their bodies. 80% had no injuries to their genital parts; tears of fourchette and superficial **perineal** tears were seen in 4% and vestibular injuries were seen in 6% of <10 years age group. 76% had old hymenal tears, 12% had fresh tears and 2% showed an elastic hymen. 36% of cases reported within 2-4 days of the act; 30% within 24 hours. Innumerable intact well defined spermatozoa were seen within 12 hours of the act; morphologically intact spermatozoa were present up to 2-4 days from the act and recognizable intact sperm heads were evident in the vaginal smears up to 10 days from the act and medical examination. Early degradation of spermatozoa was seen in those victims who reported late for examination and had evidence of infection. Conclusion to the two cases being reported, there were evidence of other injuries to the rest of body, tears to the vagina and the hymen, and the cause of death was from other injuries, not directly related to the sexual act. The overall nature of corroborative evidence matches the earlier clinical study. This paper seeks to highlight that timely preparation and examination of the vaginal smear by the Forensic Pathologist is of great value to establish sexual intercourse for determining the nature of the offence i.e. Sec 375-376 Penal Code. Such examinations can still establish the basic requirement of the offence of "rape" i.e. presence of seminal discharge, as in these reported cases, even after a lapse of 4-5 days from the incident and examination irregardless of whether the victim was alive or dead in the intervening period.

15. Distinction of contaminating T lymphocytes from abnormal cells in multiparameter flow cytometric analysis using CD45 and SSC gating

Veera S Nadarajan and Vanitha K.

Department of Pathology, Penang Hospital, Pulau Pinang

Accurate gating is crucial in flow cytometric analysis to isolate abnormal cells from contaminating cells in bone marrow specimens. We find that T lymphocytes are a frequent contaminant during flow cytometric analysis. Three cases were identified where 2 equally distinct population of cells were noted on the scatterplots on multiparameter analysis using FSC and SSC for gating. In all 3 cases, one population appeared to express CD7, CD5 or CD3. The use of CD45 and SSC for gating was however able to resolve the populations into CD45 intermediate and CD45 bright cells which allowed better discrimination of the population of interest. We seek to highlight in this paper the importance of proper gating using CD45 together with FSC and SSC in 2 colour analyses and to be wary of the presence of contaminating T lymphocytes which may mislead the diagnosis.

16. Mortality from hanging in University Hospital, Kuala Lumpur - A 5-year retrospective study

Beng Beng ONG and George PAUL

Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

A 5-year retrospective study of hanging was undertaken to study the pathology of neck injury in hanging and to compare the differences if any, with other series. Post-mortem reports of hanging cases were extracted from 1994 to 1998. There were a total of 83 cases. The age ranges from 16 to 86 years with a majority between 20 to 39 years (60.2%). All cases were suicides. In the majority of cases, the nature of ligature used were rope (41.0%) and cloth (26.5%). 56 of cases had asphyxial signs. Out of these cases, 16 cases were fully suspended while the rest were partially suspended. The

knot was in the usual posterior position in 53 cases, lateral position in 24 cases and the anterior position in 6 cases.

23 (27.8%) cases had injury to the soft tissue and muscles of the neck. Out of these, only 2 cases used soft ligature (cloth) to hang themselves. 5 cases showed fractures of hyoid bone only, 14 cases showed fractures of the superior **horn** of thyroid cartilage and only 1 case showed fractures at both structures. One case showed an incomplete fracture of the cricoid cartilage. When the fractures were compared with age, 75.0% were 60 years old and above. When compared with type of ligature, only 2 cases were of soft type. 5 cases with asphyxia signs were fully suspended and 6 cases were partially suspended.

The findings revealed that neck fractures increase with advancing age. Injuries on the neck may also be related to the type of ligature used. These results are compared with other studies and discussed.

17. Telomerase enzyme activity in human breast cancer and benign breast lesions

Angela MH NG, Lai-Meng LOOI and Phaik-Leng CHEAH

Department of Pathology, Faculty of Medicine, University Malaya.

One major characteristic of tumorigenesis is the process of immortalization of the cancer cell where it acquires the ability to proliferate indefinitely. In normal somatic cells, the telomeric region of the chromosomes shortens in every round of cell replication. As the shortening process progresses and reaches a critical stage the cells then undergo senescence. The cells may escape this stage and continue dividing due to the **workings** of some mutational genes but, will still reach another critical stage where most cells die. Therefore to acquire immortality, cells will have to bypass these two stages. Telomerase, an RNA-containing enzyme that restores the telomere length is proposed to be one of the essential components in the immortalization process. In our study, fresh tissue from 18 breast carcinomas, 14 fibroadenomas and 10 non-neoplastic breast tissue (normal controls) were investigated for telomerase activity using a **kit** combining both the PCR and ELISA techniques (TRAP-ELISA). A positive control (a transformed cell line) and a negative control (an RNA treated sample) were included in each test. Telomerase activity was found in 6 (33%) of 18 breast carcinomas, 1 (7%) of 14 fibroadenomas and in none of the controls. It appears that neoplastic tissue possesses a higher telomerase activity than non-neoplastic tissue. However, the positivity rate does not appear to be high enough to justify using telomerase assay as a diagnostic marker of malignancy. Nevertheless, this finding points to a contributory role for telomerase in tumorigenesis. Telomerase activity in our study appears lower than those reported by other workers. One of the reasons for this difference may be related to difference in methodology used in telomerase assay.

Poster presentations

P1. Detection of *Chlamydia pneumoniae*-specific markers in patients with coronary heart disease

S Bahnu, A Matsumoto, YF Ngeow

Department of Medical Microbiology, University of Malaya

Chlamydia pneumoniae, a common cause of respiratory tract infections, has been implicated in the pathogenesis of atherosclerosis and coronary heart disease (CHD). A chlamydial heat shock protein **HSP60** is believed to play a major role in the initial stages of the development of atherosclerosis. Other *C. pneumoniae*-specific proteins are potential virulence factors and antibodies to these proteins may serve as markers for chronic *C. pneumoniae* infection.

In this study, sera from subjects with and without CHD were examined by a microimmunofluorescence (MIF) test for chlamydial **IgG**. MIF-positive sera were then examined for antibodies to chlamydial proteins by immunoblotting, using partially purified *C. pneumoniae* TW 183 elementary bodies as the source of protein. The results showed that MIF-positive sera contained antibodies to chlamydial **HSP60**, genus-specific proteins of molecular weights 46- and 70- **kDa**, as well as species-specific proteins of 98-, 53- and 42- **kDa**. Antibodies to the 53- and 46-**kDa** proteins were present at a higher frequency in CHD patients than in non-CHD subjects.

P2. *Stenotrophomonas Maltophilia*: a comparison of antibiotic susceptibility testing by disk diffusion and E test with the agar dilution method.

Rina N Karunakaran and *N Parasakthi

Department of Pathology, Hospital Kuala Lumpur and *Department of Medical Microbiology, University Hospital Kuala Lumpur.

Stenotrophomonas maltophilia is a gram-negative bacillus which has emerged as an important nosocomial pathogen over the past decade. Infections with this organism often present management difficulties due to antibiotic resistance, including inherent resistance to imipenem. Antimicrobial susceptibility test results are variable and is methodology dependent. For in-vitro susceptibility testing, the NCCLS recommends that the agar or broth dilution methods be used as disk diffusion methods are deemed inaccurate with poor reproducibility.

We compared the antimicrobial susceptibility of 30 strains of *S. maltophilia* from patients in UHKL using the NCCLS agar dilution method (the reference method) with the disk diffusion and E test methods. The antimicrobial agents evaluated were ceftazidime cefoperazone, **trimethoprim/**sulfamethoxazole, ciprofloxacin, **gentamicin**, amikacin, **amoxicillin/clavulanate** and **imipenem/**cilastatin. We found that agreement between the methods was very good (> 97%) only when testing certain antibiotics, namely **trimethoprim/** sulfamethoxazole, **imipenem/cilastatin** and **amoxicillin/**clavulanate. For all the other antibiotics tested, the E test and disk diffusion method had less than 90% agreement with the agar dilution method, and in the case of cefoperazone, ticarcillin/clavulanate, ciprofloxacin and amikacin, the disk diffusion method had 59% or less agreements, whereas the E test had < 50% agreements for ciprofloxacin and amikacin. There was also a high percentage (>20%) of 'very major' discrepancies for both the E test and disk diffusion methods with respect to susceptibility testing to amikacin, **gentamicin**, ceftazidime and ciprofloxacin. For cefoperazone, although there were only 40% agreements by the disk diffusion method and agar dilution method, most discrepancies were minor.

In conclusion, we found that susceptibility testing of *S. maltophilia* by the disk diffusion and E test methods were only reliable for **trimethoprim/** sulfamethoxazole, **imipenem/** cilastatin and **amoxicillin/** clavulanate. For the other antibiotics evaluated in this study the results are unreliable, especially with regards to the aminoglycosides, ceftazidime and ciprofloxacin. **Trimethoprim/** sulfamethoxazole had the highest in-vitro activity against this organism by all three methods.

P3. Malignant, benign and infective ocular adnexal tumours - a pathologic perspective

TV Kumar, K Ramachandram and S Ahlawat

Hospital Tengku Ampuan Afzan, Kuantan.

Ocular adnexal tumours pose a special problem to the ophthalmologist at initial presentation. A biopsy **ensues** and surprises are in store sometimes. Often the absence of a pre-operative biopsy makes the final histological diagnosis an even bigger surprise. We present here four such surprising encounters of ocular adnexal tumours.

CASE 1: A Merkel cell tumour of the eyelid managed until the demise of the patient 24 months later.

CASE 2: Ossifying fibroma involving the paranasal sinuses, ocular adnexa and orbital apex in a 9-yr-old Malay child.

CASE 3: **Syringocystadenoma papilliferum** of the eyelid presenting in a 25-yr-old Malay lady.

CASE 4: Rhinofacial Zygomycosis involving the left periocular adnexa in a 4-yr-old female child.

We attempt to present here the clinician's dilemma when the histological diagnosis differs from the pre-operative diagnosis.

P4. B-lymphoid CML blast transformation: coexpression with myeloid or otherwise? A case discussion

*.Azizon Othman, *Khalid Hassan, **Alan Teh, Norhanim Asidin and Roshida Hassan*

*Pathology Department, Kuala Lumpur Hospital, *Tawakkal Hospital, Kuala Lumpur and **University Hospital, Kuala Lumpur*

SS, a 28-year-old Malay man, a known case of **CML**, presented with low grade fever, jaundice, LOW, LOA, shortness of breath and abdominal distension for several weeks. Clinical examination confirmed the presence of gross splenomegaly and a slightly enlarged liver. Initial investigations showed **Hb:7g/L, TW:109.6x10⁹/L, platelets:24x10⁹/L**. Urea, alkaline phosphatase and conjugated bilirubin were high. Hypoalbuminaemia was also noted.

FBP showed presence of 66% "plasmablast-like" blasts which were peroxidase negative. Immunophenotyping by flow **cytometry** using primary-panel markers showed positivity for CD 19, 34, 38 and 33. Further markers could not be performed as the patient discharged himself against medical advice. These results are indicative of B-lymphoid transformation which accounts for 20% of CML blast transformation. However, the more common myeloid **CML** transformation is still a possibility because peroxidase may be undetectable in early myeloid precursors.

Proper diagnosis of the case is critical as the treatment and prognosis of the differentials, i.e. B-lymphoblastic, myeloblastic and plasmablastic CML blast transformations are diversified. Further markers e.g. **cTdt, 79a, BB4, immunoglobulins and cMPO** could have thrown more light on the diagnosis.

This case illustrates the limitations of morphology alone in the diagnosis, which otherwise, could have been diagnosed as plasmablastic transformation. It also illustrates the potential difficulty faced in immunophenotyping when only a limited number of markers are available.

P5. Comparative analysis of three permeabilization methods for cytofluorometric evaluation of cytoplasmic myeloperoxidase.

SP TAY, SK CHEONG, NH Hamidah and O Ainoon.

Haematology Unit, Department of Pathology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

A comparative study was conducted to evaluate three different permeabilization methods: FACS Permeabilizing Solution (FPerm), **CytoFix/CytoPerm Kit (CFP)** and Paraformaldehyde-Tween 20 (**PFT**) reagents, in cytoplasmic labeling of myeloperoxidase (MPO). Peripheral blood cells from 23 healthy subjects were fixed and permeabilized according to the proposed procedures, prior to direct immunofluorescence staining with **CD14, CD45, IgG1, IgG2** and MPO monoclonal antibodies (**MoAb**). Subsequent flow cytometric analysis was performed on **FACSCalibur** flow cytometer (Becton Dickinson, BD). As far as the antigenic expression of **MPO** in normal samples is concerned, FPerm and **CFP** demonstrated better cytoplasmic staining by inducing minor effects on **light-scattering** properties of the cell populations, whereas **PFT-treated** samples showed a diminished ability to distinguish the cell types. However, the simple and rapid FPerm method required an earlier processing of samples since the stored whole blood samples (for more than 8 hours) tended to show a significant decrease of fluorescence intensity. We also have demonstrated that **P/N** ratio possesses added value in evaluation of cell reactivity in immunophenotyping, based upon the apparent nonspecific **cytoplasmic-staining** of **MPO** in the lymphocyte population.

P6. CD34 cell quantitation by single platform assay

*SK CHEONG, K SIVAGENGEI, *KH LEONG, NH HAMIDAH, O AINOON.*

*Haematology Unit, Hospital Universiti Kebangsaan Malaysia and *Becton-Dickinson (M) Sdn Bhd, Kuala Lumpur, Malaysia.*

Haemopoietic progenitor cells have been widely used as part of the treatment for a variety of blood disorders. The source of haemopoietic progenitor cells can be autologous or allogeneic, harvested

from the bone marrow, peripheral blood or cord blood. Expression of CD34 is commonly used to determine the progenitor cell content as this marker is expressed on primitive and committed stem cells. Transplant physicians use the number of CD34 cells as a guide to the adequacy of the stem cell graft. A typical threshold dose is $2-5 \times 10^6/\text{kg}$ patient weight. Quantitation of CD34 cells can be performed in a variety of ways, the most common being those based on flow cytometric methods. More recently, single platform assay for CD34 cells has become commercially available. This approach does not require a leucocyte count from a separate instrument, thus improving the accuracy of the measurement. We describe our experience with the **ProCount** system (Becton-Dickinson, San Jose, CA, USA) based on fluorescence reference microparticles and using the **FASCalibur** flow cytometer. This system of CD34 cell enumeration is rapid, reproducible and suitable for routine use.

W. Positive direct antiglobulin test in a patient with valvular heart disease treated with Unasyn
C-F LEONG, S-K CHEONG and S FADILAH

Department of Hematology & Transplantation, Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia.

A 56-year-old Chinese lady with valvular heart disease and atrial fibrillation was referred to us from a private hospital for further management of autoimmune hemolytic anemia. Physical examination and laboratory investigations did not support the diagnosis of hemolytic anemia. However, direct antiglobulin test (DAT) was strongly positive with anti-IgG and negative with anti-C3d. There was also mild anaemia and reticulocytosis, which was attributable to persistent hematuria. The DAT became positive after commencing Unasyn and cessation was associated with decreasing reactivity of the positive DAT. We believe that the positive DAT in this patient was most likely due to the Unasyn therapy and constituted a positive DAT, induced by the drug.

P8. G6PD mutations in Malays with G6PD deficiency

O AINOON, YH YU, JA JABAMONEY, ZA ZAINAL, SK CHEONG, NH HAMIDAH and *NY B O O

*Departments of Pathology and *Paediatric, Faculty of Medicine, HUKM, Kuala Lumpur.*

G6PD deficiency is the commonest disease-causing enzymopathy and is estimated to affect 200 million people worldwide. In Malaysia, the overall incidence of **G6PD** deficiency is 3.1%, a disorder which is more common among the Chinese, the Malays and less so among the Indians. In this part of the world, **G6PD** deficiency is known to have an important association with severe neonatal jaundice. To date at least 120 different mutations in the **G6PD** gene have been identified and they have been shown to be population-specific. The objective of this study is to determine the molecular abnormalities in Malay **G6PD** deficiency. We present here our preliminary data on the study we carried out to detect known mutations in 58 consecutive Malay **G6PD-deficient** neonates. We used established PCR methods based on the use of specific primers and restriction enzymes to detect four common Chinese mutations and the Mahidol variant, a common Thai variant. Common Mediterranean mutations were detected by sequencing **exon 6** of the **G6PD** gene. We found 26% (15 of 57) were mutation C->T at nt 563, (**G6PD** Mediterranean), 14% (8 of 57) were mutation G->A at nt 487 (**G6PD** Mahidol), 10.5% (6 of 57) were mutation G->T at nt 1376 (**G6PD** Canton), 3.5% (2 of 57) mutation G->A at nt 1388 (**G6PD** Kaiping) and 3.5% (2 of 57) mutation C->T at nt 592 (**G6PD** Coimbra) also a variant seen among the Mediterraneans. In conclusion, this study shows that the **G6PD** deficiency in the Malays, as in other populations, is genetically heterogenous and that 57.8% of the Malay **G6PD** deficiency is caused by at least 5 alleles. In the remaining 42.2% of the cases, **G6PD** deficiency may be caused by other known mutations or novel mutations. Further studies are being carried out to fully characterise **G6PD** mutations in the Malays and to establish its clinical relevance, especially in relation to severe neonatal jaundice.

P9. Precore mutations in chronic Hepatitis B carriers

Yap SF, Chen YC and Wong PW

Department of Pathology, Faculty of Medicine, University of Malaya

HBV variants due to mutations in nearly all parts of the HBV genome have been described. The most prevalent mutations involve the precore and core genes of the virus. Many of these mutations interfere with the production of the hepatitis B e protein - through the introduction of stop codons, elimination of the start codon or mutations involving the core promoter. The net result is production of HBe minus virus variants. Such mutant viruses have been found to be associated with active infection and with HBe seroconversion.

The present study was carried out to determine the frequency of precore mutations in our chronic HBV carriers and to relate the emergence of the mutant virus with progress of the chronic infection. Detection of the precore mutation(s) was performed using allele specific hybridisation of amplified viral DNA to specific oligonucleotide probes. The mutations detectable in this assay were C to A change at nucleotides 1896 and 1899. Specificity of hybridisation was confirmed using sequence analysis. Analysis was carried out using specimens from 23 HBeAg positive and 43 HBeAg negative patients. Seventeen out of 23 HBeAg subjects harbour the wild type (WT) virus only. The remaining 6 patients had mixed virus population comprising both WT and mutant virus. The reverse appears to be the case for the HBeAg negative patients. 27 out of 43 cases (66.8%) carry the mutant virus either alone or in combination with the WT virus. The remaining 16 patients (37.2%) were infected with WT virus only. These data suggests that precore mutant virus is prevalent in chronic HBV carriers. The emergence of the mutant virus precedes HBe seroconversion, but dominance of the mutant strain appears to increase with seroconversion.

Sequence analysis was conducted to determine the genotype of the WT virus. The target region is the precore sequence that comprises a set of nested inverted repeats which are predicted to form a stem loop structure. This region contains the encapsidation sequence (Epsilon) of the virus and the site for initiation of minus strand DNA synthesis. Virus genotype that carry a thymine (T) at nucleotide I 858 (located at the lower stem of the putative stem loop structure) will carry a TG mismatch at the 1858-1896 pair. A C to A mutation at nucleotide T896 will confer increased stability to the lower stem. This may explain the frequency of the G to A mutation at nucleotide 1896 in genotypes that carry a Tat 1858. Our sequence data supports this hypothesis - 75% of our WT virus indeed carry a T at 1858.

P10. Molecular typing of *Mycobacterium tuberculosis*

YAP SF, CHEN YC, *KUPPUSAMY I and NGEOW YF.

*Department of Pathology Faculty of Medicine, University of Malaya and *Institute of Respiratory Medicine, Kuala Lumpur.*

The primary objective of this study was to determine the distribution of genotypes in local isolates of *M tuberculosis* including background strains and resistant strains. Genotyping analysis was carried out using RFLP based on the insertion sequence IS6110, and spoligotyping based on the polymorphisms residing in the DR region of the *M tuberculosis* complex group. Spoligotyping results of 72 unselected isolates showed that there are 3 major patterns encompassing 61 isolates. The predominant group comprising 31 isolates (43.1%) is characterised by deletions of DVRs 29 to 32. Twenty-three isolates (31.9%) are characterised by deletions spanning DR1 to DR34 (Beijing strain) and 7 isolates (9.7%) are distinguished by deletions of DVRs 33 to 36 inclusive (Type 38). Of the remaining isolates, 3 are *M bovis* and 8 are heterogeneous. Overall, 47 of the 72 isolates have unique spoligo patterns. All except one of the isolates within the "Beijing" group are indistinguishable. Analysis of typing results of 76 resistant strains also demonstrated 3 predominant groups. The largest group comprising 35 isolates (46.1%) show deletions of DVRs 29 to 32. Type 38 represents the next largest group with 19 isolates (25.0%). The Beijing strain makes up the third group of 13 isolates (17.1%). The remaining isolates are heterogeneous. All the Beijing strains are indistinguishable. Clustering of the resistant isolates is evident; 7 clusters can be identified, each making up a group of 2 to 5 isolates. RFLP analysis was carried out on a number of the Beijing strains. All isolates typed

showed unique IS611 0 typing patterns, indicating that spoligotyping is not discriminatory for the Beijing strain.

In summary, the majority of local isolates can be assigned to 3 spoligotypes. Excluding the Beijing strains, 46/49 unselected isolates (93.9%) are unique. In comparison, only 40/63 resistant isolates (63.5%) have unique typing patterns. The remaining 23 fall into 7 clusters, suggesting the presence of transmission among the resistant organisms.

P11. The pattern of Castleman's disease: the University Hospital, Kuala Lumpur experience

SC PEH, J SHAMINIE and LH KIM

Department of Pathology, University of Malaya

Background:Castleman's disease (CD), also known as angiofollicular hyperplasia, is an uncommon disease. It can present at any age, as localised or systemic (multicentric) disease. There are 2 histomorphological variants, hyaline-vascular (HV) and plasma-cell (PC). This study aims to investigate the clinicopathological and immunophenotype profile. **Material and methods:** A total of 10 patients with CD were retrieved from files in the University Hospital, in a period of 16 years. Archive materials were reviewed. Immunohistochemical staining using a panel of antibodies to lymphoid antigens and oncogenes bcl-6 and bcl-2 were performed. The clinical presentation was extracted from patients' case files. **Result:** The ages of patients ranged from 8 to 60 years (median, 30 yr.). There were 4 male and 6 female patients, 3 PC and 7 HV variants. All patients presented with lymph node enlargement. 2 of the PC had multicentric lesions associated with systemic disease: one had POEMS, the other had hypoalbuminaemia and raised ESR. The third patient had localized disease. The plasma cells were polyclonal. All RV patients had localized lesions. The follicle centres are atrophic in CD, with relatively few CD57 positive T-cells and rare bcl-6 expressing follicle-centre-cells present, when compared to reactive lesions. Bcl-2, but rarely CD5 is expressed by the mantle cells. **Discussion:** CD is an uncommon disease. Both variants are present in Malaysian patients. PC is more likely to present with systemic and multicentric disease. Immuno-profile can help to distinguish CD from reactive lesion.

P12. Double labeling of EBER (ISH) and L26 or CD3 (IH)

SC PEH, SP CHAI and LH KIM

Department of Pathology, University of Malaya, Kuala Lumpur, Malaysia

Introduction: Epstein-Barr virus infection (EBV) is known to be a causative agent in lymphoproliferative lesions. The virus genome can be detected in the tumours of B-, T- and NK/T-cell lymphomas, as well as the B-cell blasts of infectious mononucleosis of post-transplant lymphoproliferative lesions. In the investigation of the role of EBV in these diseases, it is important to determine accurately the exact cell populations which are infected by the virus. The performance of double stain on biopsy material allows detection of co-expression of markers of information. **Methodology:** Immunohistochemical staining for B-cell (CD20) and T-cell (CD3) antigens using clones L26 and polyclonal CD3, both were from DAKO were optimised by manipulating the retrieval method and dilution factor. Epstein-Barr virus early RNA (EBER) -ISH was found to work best using proteinase K digestion. We performed different sequence of double staining steps, and vary the staining conditions until we obtained the optimal results. We also compared different combination of substrates and chromogens for the best staining effects (AEC- red or DAB-brown with NBT/BCIP - dark blue). **Result & Discussion:** We found that both ISH-IH or IH-ISH staining sequence gave acceptable results, but more superior with ISH-IH. When comparing the colour contrast between red (AEC)/dark blue(NBT/BCIP) and brown(DAB)/dark blue(NBT/BCIP), we found that red/dark blue gave better colour contrast than brown/dark blue. The important principle features for double staining are employment of two different visualisation systems that do not show cross reactivity and two different chromogen used have the maximum colour contrast. **Conclusion:** The best result of double-stain is by performing EBER-ISH using NBT/BCIP as substrate follow by immunohistochemistry using 15% AEC as substrate. **Acknowledgement:** This project is supported by IRPA 06-02-03-0576 (1999).

P13. CD44 expression in primary breast infiltrating ductal carcinoma: a comparison study on cases with or without lymph node metastasis

W.R. ZHAO, L.M. LOOI, M.H. NG and P.L. CHEAH

Department of Pathology, Faculty of Medicine, University of Malaya, 50506 Kuala Lumpur

Metastasizing to distant lymph nodes represents the life-threatening property of malignant tumours, but the mechanisms of metastasis are still largely unsolved. CD44 is a widely expressed, multistructural cell surface adhesion molecule, which participates in cell-cell and cell-matrix interactions. Many cancer cell types as well as their metastasis express high levels of CD44. The expression of CD44 in human breast cancer has been previously showed to be associated with metastasis. To investigate the correlation of CD44 expression in breast cancer with metastasis to lymph nodes, deparaffinized sections from 73 female primary breast infiltrating ductal carcinoma, in which 46 cases have lymph node involvement and 27 cases have no lymph node involvement, were stained with MoAb for CD44. The positivity rate for CD44 in the cases with and without lymph node metastasis is 20% and 22%, respectively. Statistical analysis (chi-square test) shows that there is no significant difference ($p=0.07$) between these two groups of breast cancer. Unlike previous reports, our results suggest that the metastatic behavior of breast infiltrating ductal carcinoma may not be explained by the expression of CD44, or at least that CD44 expression is not the dominant event in breast cancer metastasis. Other mechanisms involved remain to be investigated.

P14. Telomerase in cervical carcinoma

Phaik-Leng CHEAH, Min-Hwei NG and Lai-Meng LOOI

Department of Pathology, University of Malaya Medical Centre, Kuala Lumpur

Telomeres are distal ends of human chromosomes composed of tandem TTAGGG repeats which protect chromosomal ends against degradation, end-to-end-fusions, re-arrangements and loss. Progressive loss of telomeres with cell division results in chromosomal instability and cellular senescence. One possible reason for telomeric shortening is due to repression of telomerase, a ribonucleoprotein polymerase which directs synthesis of telomeric repeats. Telomerase thus appears to be essential in maintaining cellular immortality and derepression of telomerase may play an important role in malignant change. A pilot project was initiated at the Department of Pathology, University of Malaya Medical Centre to study the role of telomerase in cervical carcinoma. 17 cervical carcinoma diagnosed clinically were subjected to frozen section examination on receipt of the fresh specimen. Only cases in which the frozen section confirmed the presence of tumour were assayed for telomerase using a commercial photometric enzyme immunoassay kit (Telomerase PCR ELISA:Boehringer-Mannheim) based on the Telomeric Repeat Amplification Protocol (TRAP) on fresh tissue sections. Cervical tissue from 11 hysterectomies performed for benign conditions in the cervix and other parts of the female genital tract were similarly subjected to frozen section confirmation of diagnosis and served as normal controls. The absorbance range of the normal controls measured at 450 nm wavelength was -0.013 to 0.035. 11 (64.7%) cervical carcinoma showed telomerase activity above the normal range and were interpreted as showing derepression of telomerase expression. The findings of this study thus indicate an elevation of telomerase activity in the majority of cervical carcinoma supporting the possibility of telomerase derepression being an important step in cervical malignant transformation. Nevertheless, the mechanism triggering this derepression remains unclear.

P15. Placenta histology in prolonged rupture of membranes

HAYATI AR AND *CHEAH FC

*Department of Pathology and *Department of Paediatric, Hospital Universiti Kebangsaan Malaysia, Kuala Lumpur*

This is a morphological study on 30 placentas from women who presented at Hospital UKM with prolonged leaking liquor of 19 hours to four days duration. Their gestational ages ranges from 33

weeks to term. Thirteen cases showed the presence of acute choridamnionitis while 11 cases had deciduitis. Villitis was seen in 6 cases and there were 5 cases of each of microscopic abruptions acute atherosclerosis and infarctions. Two placentas had fetal artery thrombosis while one had meconium laden macrophages. From the 30 cases studied, histological changes were present in 48, out of which 18 had overlapping pathology

P16. Massive haemorrhage due to polyarteritis nodosa causing death: A case report

Ong BB

Department of Pathology, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia

A 31-year-old man was found dead one morning. He had been apparently unwell for the past 3 months. Autopsy findings include massive retroperitoneal haemorrhage and haematoma which appeared to have been originated from both **kidneys**. Both **kidneys** showed multiple areas of cortical infarction. Multiple thrombi were noted. The other organ systems appeared to be unremarkable. Histology showed necrosis consistent with infarction in the renal cortex. In addition, some medium size arteries showed vasculitis with fibrinoid necrosis while others showed intimal proliferation with surrounding chronic inflammatory infiltrate. Some renal arteries were not affected. Similar changes in the medium-size arteries were noted within the liver and heart. The arteries of other organs were unremarkable. Cause of death was massive retroperitoneal hemorrhage due to polyarteritis nodosa (PAN).

PAN is an uncommon vasculitides which is well-known to cause sudden cardiac death. The genitourinary tract is involved in 80% of the cases but usually presents with loin pain, dysuria or microscopic haematuria. Hypertension is also a well-known complication. This case illustrates an uncommon presentation of PAN, i.e. massive haemorrhage due to involvement of the genitourinary system.

P17. Squamous cell carcinoma of the vulva: a report of 9 cases

Nor Salmah Bakar and Siti Aishah M.A.

Department of Pathology, Faculty of Medicine, Hospital Universiti Kebangsaan, Kuala Lumpur

Squamous cell carcinoma (SCC) of the vulva is a rare disease, accounting for 0.4% of malignant tumours of the female genital tract in our material. We present a study of 9 cases. Patient's age at presentation ranged from 32 to 77 years (mean 63.3 years). There were 5 Malay (55.6%) patients, 2 (22.2%) each of Chinese and Indian patients respectively. The common symptom was pruritus. The common site was labia **majora** or **minora**. The gross appearance was of a mass or an ulcer. Seven patients (77.8%) had radical vulvectomy done and two cases (22.2%) with lymph node metastases. Six cases (66.7%) were of grade I. In this small series, SCC occurred mainly in elderly women (88.9%). It also showed that the occurrence of this carcinoma is lower compared to Caucasian population.

P18. Arteriovenous malformation (AVM) of the mesosalpinx - a case of a vanishing ectopic pregnancy - an insight into its pathogenesis

Nor Hayati Othman, M Madhavan and Wan Himratul Azna,

Pathology Department, Universiti Sains Malaysia, Kelantan

Arteriovenous malformation (AVM) of the female genital organs is very rare. To date there are 73 cases reported in the literature, of which 70 cases were seen in the uterus. Only 2 cases of AVM of mesosalpinx reported thus far. A 21-year-old Malay lady Gravida 1 Para 0 was admitted through the casualty department with a complaint of left iliac fossa pain. The urine pregnancy test was positive. Ultrasound showed features suggestive of an ectopic pregnancy. No intrauterine pregnancy was noted. The patient underwent an emergency laparotomy. On gross examination, the fallopian tube and the ovary was haemorrhagic. A piece of blood clot was seen in the peritoneum. There was

neither fetus nor placental tissue seen. The histopathological examination showed typical feature of **arteriovenous** malformation. No trophoblasts, chorionic villi or decidua of the fallopian tube was seen. It is postulated that an involucional ectopic pregnancy induced the rapid growth of abnormal vessels. We believe this is the first case of AVM of the mesosalpinx reported in Malaysia.

P19. Primary hepatic carcinoid tumour

Arni T, Subathra S, *Harjit Singh and Wong CM

*Departments of Pathology and *Surgery, Hospital Kuala Lumpur.*

Primary carcinoid tumour of the liver is very rare. To date only 44 cases have been reported. **A** case of primary carcinoid tumour arising in the liver of a 46-year-old Malay man with no endocrine symptoms is reported. Radiological examination revealed a tumour at segment IV of the liver with intraductal infiltration. Histologically, the tumour was composed of uniform small cells arranged in trabecular and anastomosing ribbon-like nests. Immunohistochemically, the tumour cells stained positively for **chromogranin A**, neuron specific **enolase** and cytokeratin. Intensive and careful searches pre- and post-operatively revealed no other source of tumour other than the liver. The patient however succumbed post-operatively due to septicemia.

P20. Ovarian lymphoma with mixed epithelial carcinoma arising in the same ovary - case report.

K. Ramachandram and A. Jalaluddin.

Hospital Tengku Ampuan Afzan, Kuantan.

Primary malignant lymphoma in the ovary is not a common tumour. We report a case of lymphoma coincidentally found with a cystic, mixed epithelial carcinoma of the same ovary. A 62-year-old Chinese lady who was found to have an ovarian tumour had a total hysterectomy. At operation, the right ovary was enlarged. There were no omental seedlings nor any enlarged lymph nodes. On gross examination, this was a cystic ovary with a solid nodule at one end. Histological examination revealed a high grade, non-Hodgkin's B-cell lymphoma with a mixed epithelial carcinoma, clear cell and serous carcinoma side by side. She also had a leiomyoma and adenomyosis of the uterus. The patient is doing well after 5 courses of a combination chemotherapy for lymphoma and carcinoma.

P21. Status of HPV infection in cervical carcinoma in a Malaysian population

Phaik-Leng CHEAH, Min-Hwei NG, Lai-Meng LOOI and 'Valipuram SIVANESARATNAM

*Departments of Pathology and *Obstetrics and Gynaecology, University of Malaya Medical Centre, Kuala Lumpur*

The human papillomavirus (HPV) has been aetiologically linked to human cervical carcinomas. Little data is however available regarding its prevalence in the Malaysian setting. A study was conducted at the Department of Pathology, University of Malaya Medical Centre to determine the prevalence of HPV infection in histologically confirmed cervical carcinoma. PCR analysis was conducted on cases of cervical carcinoma by first using commercially synthesised primers to HPV 6, 11, 16 and 18. For each case, both a fresh and formalin-fixed, paraffin-embedded piece of confirmed tumour tissue were subjected to **PCR** amplification using the respective primers. In the event, the case did not demonstrate any HPV sequences, PCR analysis was repeated on the same samples using commercially acquired consensus primers to the conserved L1 region which amplify a wide spectrum of HPV types. Using this design, HPV sequences were found in 33 of 40 (82.5%) cases. Of these, 18 (45.0%) were HPV 16, 3 **HPV** 18 and 1 HPV 6. 11 of the 18 cases which were negative for **HPV** 6, 11, 16 and 18 showed positive sequences with the L1 consensus primers indicating the presence of other **HPV** types. 52.5% of the cases showed HPV 16 or 18, the two most commonly encountered types in cervical carcinoma. Unlike what has been previously reported and more in line with findings from more current studies, HPV 16 and 18 are important but are not the

only culprits involved in cervical carcinogenesis. Other and new types of HPV have been identified in cervical carcinomas. This is also evident in our study when consensus primers were employed in cases that were negative for HPV 6,11,16 and 18.

P22. Paediatric papillary thyroid carcinoma: a case report

*Noraini MD, Arni T, Wahidah A and *Zakaria Z*

*Department of Pathology, HKL and * Department of Paediatric Surgery, Institute of Paediatrics, HKL.*

A case of papillary thyroid carcinoma in a 10-year-old boy is reported. This patient presented with left neck swelling for 10 months' duration which was non-tender and progressively increasing in size. Physical examination revealed an enlarged, mobile and firm left cervical lymph node measuring 3.0 X 3.0 cm. Thyroid scan showed heterogenous left lobe of the thyroid without enlargement. An incision biopsy of the cervical lymph node was carried out. Histological examination revealed metastatic papillary thyroid carcinoma. Subsequent left hemithyroidectomy and lymph node clearance was performed. Patient was well post-operatively and on regular follow up.

P23. Pedunculated fibrolamellar hepatocellular carcinoma : a case report

*Wan Muhaizan WM, *M.Rohaizak, MJ Julia Munchar, *FA Meah and *A Haron*

*Departments of Pathology and *Surgery, Hospital University Kebangsaan Malaysia, Kuala Lumpur*

Fibrolamellar hepatocellular carcinoma (**FL-HCC**) is an uncommon variant of hepatocellular carcinoma with distinct histological and clinical features from ordinary hepatocellular carcinoma. It is usually seen in young adults, not associated with cirrhosis and carries a better prognosis. We report here a very rare case of pedunculated fibrolamellar **subtype** in a 15-year-old girl. She underwent a complete surgical resection and she was well without recurrence of the tumour one year post surgery.