

**The 3<sup>rd</sup> Scientific Meeting of the College of Pathologists, Academy of Medicine Malaysia was held at the Riviera Bay Resort, Malacca from 21 to 23 June 2002. Abstracts of papers presented follow:**

**Guest lectures:**

**Clinical diagnostic virology into the 21<sup>st</sup> century**

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Major advances have been made in clinical diagnostic virology in recent years which greatly influence patient management, disease control and investigation of emerging and re-emerging viral disease. Traditional methods are fast making way for new and innovative technology, especially in the area of molecular-based diagnosis. The increased availability of antiviral drugs offer up the potential of therapeutic and prophylactic management of patients. However, these drugs are not only expensive, but have a narrow range of specificity and quite toxic, thus requiring accurate diagnosis for their usage. There is considerable pressure to provide diagnostic virology which must offer rapid and accurate results with a short turn-around time. In disease prevention and public health, it is necessary to adopt the best technology to address the issue of the causative agents involved so that appropriate control measures can be initiated. Examples based on the enterovirus 71 outbreak and Nipah encephalitis outbreaks will be used to illustrate this. The future of clinical diagnostic virology is looking bright. However, it must be remembered that not all things new are good and there is still a role for traditional methods in our search for an ideal approach in diagnostic virology.

**Update on diabetes mellitus**

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The prevalence of diabetes mellitus is increasing at an alarming rate worldwide, particularly in Asia. This is due to a combination of factors, namely, genetic predisposition, increasing lifespan, sedentary lifestyle, a high fat diet and an increasing number of people who are overweight or obese. The Finnish Diabetes Prevention Study Group found that progression to type 2 diabetes in subjects with impaired glucose tolerance (IGT) was reduced by 58% as a result of lifestyle changes. The Diabetes Prevention Programme in the USA showed that intensive lifestyle changes reduced the risk of developing diabetes in individuals with IGT by 58% whereas taking metformin 850 mg twice a day reduced the risk by only 31%. The Direct Control and Complications Trial (DCCT) on Type 1 diabetics found that tight glycaemic control delayed the onset and slowed the progression of microvascular complications (retinopathy, nephropathy and neuropathy) but did not have a significant effect on macrovascular complications. The United Kingdom Prospective Diabetes Study (UKPDS) on Type 2 diabetics found that tight glycaemic control with insulin or sulphonylureas reduced microvascular complications much more than macrovascular complications. Intensive therapy of obese type 2 diabetics with metformin significantly decreased deaths of combined diabetes-related endpoints and myocardial infarction by about one-third. Tight blood pressure in the UKPDS significantly reduced the risks of virtually all cardiovascular and microvascular outcomes. There is hope for a cure for diabetes in the horizon. Islets neogenesis-associated protein (INGAP) has been found to stimulate the growth of new islet cells and reduce blood glucose levels in animals with diabetes by increasing the production of insulin.

**Oral presentations:****1. Acute lymphoblastic leukaemia presenting as bilateral proptosis**

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Acute leukaemia usually presents as non-specific symptoms due to derangement in the haemopoietic system such as anemia, haemorrhage, infection or infiltration of organs. Ocular manifestations can either be secondary to direct infiltration of leukaemia cells, as a result of abnormal systemic haematological parameters or due to opportunistic infection. The leukaemic infiltrate may range from being asymptomatic to presentation as a space occupying lesion. The patient may have proptosis, ecchymosis, chemosis, diplopia or visual disturbances.

JO is a 33-year-old man who presented with bilateral proptosis, lethargy, intermittent headache, loss of appetite and loss of weight for 3 weeks. On examination he was pale with proptosis of both eyes, sternal tenderness and splenomegaly. Visual and fundoscopic examination was within normal limits. Investigation revealed: WBC  $126 \times 10^9/l$ , Hb 8.6 g/dl, Plt  $52 \times 10^9/l$  with plentiful blasts. Thyroid function test was normal. Bone marrow examination revealed hypercellular marrow, comprising blast cells with high nucleocytoplasmic ratio and inconspicuous nucleoli consistent with acute lymphoblastic leukaemia (FAB L2 type). Immunophenotyping showed positivity for HLA DR, CD34, CD19, CD10, CD13, CD33 and Tdt and negativity for intracellular peroxidase. He was treated with UKALL regime and showed resolution of the proptosis.

**2. Detection of Rifampicin resistant *Mycobacterium tuberculosis* using PCR-SSCP**

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Rifampicin resistance in *Mycobacterium tuberculosis* (Mtb) is due to mutations, dominated by point mutations, within the *rpob* gene. Therefore, polymerase chain reaction-single stranded conformation polymorphism (PCR-SSCP) has been suggested as a rapid screening test for the detection of rifampicin resistance genotype. The aim of the study is to assess the performance of non-radioactive PCR-SSCP in the detection of rifampicin resistant *Mycobacterium tuberculosis*. A total of 39 sensitive, 4 rifampicin resistant and 14 unconfirmed isolates was collected from the National Institute of Respiratory Medicine, Kuala Lumpur. Mtb DNA was extracted using the boiling method. PCR was conducted using TR8 and TR9 primers, which flank the hot spot region of the *rpob* gene. PCR-SSCP was analyzed by electrophoresis using denaturing polyacrylamide gel and stained using silver staining method. Controls were simultaneously examined in the assay. All the sensitive and unconfirmed isolates showed profiles similar to H37Rv's, suggesting the absence of mutation within the amplified region. The specificity of the assay was 100%. However, two out of four rifampicin resistant isolates showed motility shifts, giving a sensitivity of 50%. Failure to identify the other two resistant isolates may be due to technical error and inaccuracy in the *in vitro* drug susceptibility testing, the lower sensitivity of the PCR-SSCP method used and the presence of mutation outside the amplified region. Further work to elucidate the cause of apparent insensitivity is on going.

### 3. Pathological features of Kimura's disease and angiolymphoid hyperplasia with eosinophilia

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There is a paucity of reports on Kimura's disease (KD) and angiolymphoid hyperplasia with eosinophilia (ALHE) from Malaysia. We have identified 10 cases of KD and 4 of ALHE from the pathology files of USM and GHKB, all in the Malays. Sections stained with H&E, Masson trichrome, PAS, toluidine blue, and immunohistochemical stains for L26, UCHL-I and CD31 were examined. Histologically, in KD, dense fibrocollagenous tissue with moderate to marked vascular proliferation surrounding rich lymphoid follicles with prominent germinal centers were seen in all cases. Eosinophilic infiltrate was conspicuous with eosinophilic microabscess but was more prominent in the stroma than in the lymphoid follicles. Vascularisation of the lymphoid follicles was present and was prominent in 2 and mild in 8. Folliculolysis and pink hyaline deposition in germinal centres were present. Polykaryocytes were present in 5 cases but stains for UCHL-I and L26 were negative. Moderate number of mast cells were identified in the inflammatory infiltrate. In the lymph nodes, prominent germinal centres, prominent vascular network and scattered eosinophils, occasional eosinophilic microabscess were seen in the interfollicular areas. Though focal plump endothelial cells with crowding was present solid cords of epithelioid endothelial cells with cytoplasmic vacuolation were absent. Fine needle aspiration cytology in 5 cases suggested inflammatory reactive condition. In the 4 cases of ALHE, presence of fewer lymphoid follicles, eosinophils and prominent endothelial cells forming solid buds and cords differentiate it from KD. Awareness of the condition leads to better diagnosis.

### 4. Detection of *Mycoplasma hominis* and *Ureaplasma urealyticum* in preterm infants by polymerase chain reaction

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To study the role of potential genital tract pathogens in neonatal sepsis, tracheal aspirates and nasopharyngeal swabs obtained from preterm infants (<37 weeks gestation and birth weight < 2500g) admitted to the Special Care Nursery, PPUM, KL. were screened for the presence of *Mycoplasma hominis* (*Mh*), *Ureaplasma urealyticum* (*Uu*), *Chlamydia trachomatis* (*Ct*) and *Neisseria gonorrhoeae* (*Gc*). *Mh* and *Uu* were detected by culture using Mycofast Evolution2 (International Microbio, FRANCE), and a duplex PCR using primers targeting a 16SrRNA gene in *Mh* and the Multiple Banded Antigen gene in *Uu*. *Ct* and *Gc* were detected by another duplex PCR using primers targeting conserved plasmid gene in *Ct* and the *cppB* gene on the 4.2-kb cryptic plasmid in *Gc*. A total of 16 preterm infants were screened. In addition, an endocervical swab was obtained from the mother of one of the infants for similar screening. Among infants, the detection rates were 4/16 (25%) for *Uu*, 1/16 (6.25%) for *Mh* and 0 for *Ct* and *Gc*. One infant was positive for both *Mh* and *Uu*. The mother of this infant was also positive for *Mh* and *Uu*. There was 100% correlation between *Mh/Uu* PCRs and culture as well as between tracheal aspirates and corresponding nasopharyngeal swabs.

## 5. Clinicopathological scenario and surgical outcome of Hirschsprung's disease in a Malaysian population

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*Aim:* To determine the clinicopathological manifestations and surgical outcome of Hirschsprung's disease (HD) in a Malaysian population. *Methodology:* Consecutive cases of HD referred to Paediatric Institute, GHKL from January 1997 to June 200 were evaluated. *Result:* There were 177 (141 male 36 female) patients with ethnic composition of Malay (57%), Chinese (15%), Indians (4%) and Others (24%). 53% patients were diagnosed during neonatal period. 5% had positive family history while 33 (19%) had associated congenital anomalies. 146 had short segment and 23 had long segment aganglionosis. Females were at a significantly higher risk of having long segment aganglionosis [OR=2.83 (95% CI 1.01-7.92)] whereas a male predominance was significant in short segment aganglionosis. The four commonest presenting features were abdominal distention (96%), constipation (62%), vomiting (55%) and delayed passage of meconium (54.5%). 160 patients had definite pull-through surgery. The commonest post-operative complications were enterocolitis (30 patients), wound infection (8 cases) and wound dehiscence (6 cases). On follow at a mean of 14.2 (SD 10.5) months, 92% had normal bowel movement. Enterocolitis occurred in 68 patients (30 post operative and 38 preoperative patient) of which 14 had septicemia including 2 toxic megacolon and one perforation and enterocolitis was responsible for all four deaths occurring in this series. *Conclusion:* The presentation and outcome of HD in Malaysia does not appear to have any major differences with the western literature although enterocolitis is more common. This might reflect a later presentation.

## 6. Isolation and identification of mesenchymal cells from the bone marrow

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Mesenchymal stem cells are pluripotent progenitor cells located in bone marrow that have the capacity of differentiating into bone, cartilage, tendon, fat, muscle, and early progenitors of neural cells under certain conditions. It has also been shown that they support haematopoiesis in culture. We have successfully isolated mesenchymal cells in a few samples of bone marrow aspirates from normal and leukaemic patients by using DMEM supplemented with 10% of FBS. They could be distinguished from other cells by their tendency to adhere to tissue culture plastic. The adherent time was about three to seven days. Microscopically, the cultured cells showed morphology resembling fibroblast and they divided actively. Early passage cultures were heterogenous and contained four morphologically distinct cell types; long spindle-shaped cells, star-shaped cells, petal-shaped cells and large flattened granular cells with vacuoles. Identification of mesenchymal cells was carried out by immunocytochemical analysis and immunophenotyping by flow cytometry. Identification of these cells is vital as they have properties that appear to make them ideal candidates for studying differentiation and make them suitable for cytotherapy and gene therapy.

## 7. Determination of Hepatitis B virus genotype based on restriction analysis of the pre-S region

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The hepatitis B virus (HBV) is classically divided into 4 major subtypes based on antigenic variations in the hepatitis B surface antigen (HbsAg). This variability is dependent on the antigenic determinant, "a" which is common to all subtypes, and the "d/y" and "w/r" pairs, which are mutually exclusive. More recently, the HBV has been classed into 7 genotypes (A-G) on the basis of full-length genomic variation. This inter-genomic variability, which is relatively pronounced in the pre-S region of the virus, is the basis for a system for routine genotyping of the HBV (Magnus Lindh et.al., 1998). The approach is dependent on the different restriction patterns obtained following enzymatic digestion of polymerase chain reaction (PCR) amplified pre-S fragments. Using 2 different restriction enzymes, *Sau3A1* and *Ava 2*, 6 genotypes (A-F) can be differentiated. We used this method to analyse 47 randomly selected HBsAg positive samples to determine the genotypes of our isolates.

The samples included 22 from chronic HBV carriers followed-up regularly in the Hepatitis Clinic, University of Malaya Medical Centre (UMMC), and 25 from HBV carriers managed by physicians at various private medical centres. Overall, we were able to determine the genotype of 27/47 (57.4%) cases. The distribution of genotypes for samples from the UMMC were 11/22 (50.0%) genotype B (RFLP pattern B4), 2/22 (9.1%) genotype C (RFLP Patterns C2 & C4). The genotypes in the remaining 9 cases could not be identified by this method. In comparison, the results of samples referred to us from other medical centers are 6/25 (24%) genotype B (pattern B4) and 8/25 (32.0%) C (pattern C7). The genotype could not be determined in 11 cases. The restriction patterns shown by the "indeterminate" samples can be due to either the presence of mixed genotypes or new genotypes/sub-genotypes not previously described. To resolve this, it is necessary to type the samples using a different region of the HBV genome or by sequence analysis. The differential distribution of the genotypes in the 2 different groups of samples is interesting, but we are unable to draw conclusions on this observation until more detailed evaluation of the patient groups.

## 8. Immunophenotypic characterization of acute lymphoblastic leukemia for purpose of minimal residual disease monitoring

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Identification of residual blasts by multiparameter flow cytometry has been proven to be an effective tool for predicting impending relapses before clinical or haematological manifestations, and for establishing different risk categories in patients with acute leukemia. In this retrospective study, we aimed to determine the proportion of acute lymphoblastic leukemias that would have been amenable to investigation of minimal residual disease, using surface immunophenotyping alone. Seventy-five cases of acute lymphoblastic leukemia (FAB L1 and L2) with complete surface immunophenotyping results were identified from laboratory records. Co expression of CD10, CD19 and CD34 was observed in 56.0% of the cases. Of the remaining cases, 5.1% were CD13+, CD19+ and CD34+ while another 1.3% was CD13+, CD19+ and CD10+. Using these three combinations, a total of 62.4% of the cases could be investigated for minimal residual disease. When restricted to childhood acute lymphoblastic leukemia alone, 66.7% of the cases were CD10+, CD19+ and CD34+. In the remainder of the childhood cases, CD13+, CD19+, CD34+ cases and CD13+, CD19+, CD10+ cases occurred in 1.7% each. The proportion of cases expressing CD13 was significantly less among childhood acute lymphoblastic leukemia as compared to adult acute lymphoblastic leukemia. Only 8.8% of childhood leukemia expressed CD13 as compared to 23.5% in the adult population. We

conclude that minimal residual disease detection using surface immunophenotyping is feasible in the majority of patients with acute lymphoblastic leukemia with better yields observed among childhood lymphoblastic leukemia. Myeloid expression in acute lymphoblastic leukemia appears more frequently in the adult population.

## 9. Telomere length analyses in cervical, breast and colonic cancers

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It has been reported that telomeres in normal human cells shorten at a rate of 50-200bp with each normal somatic cell division due to the end replication problem. This progressive shortening serves as a mitotic clock that limits division in somatic cells to between 50-70 times before cellular senescence. As tumour cells are immortal, it could be assumed that telomere lengths would differ from those of normal cells. A study was carried out at the Department of Pathology, University of Malaya Medical Centre to study the telomere lengths of tumours in the uterine cervix, breast and colon compared with normal corresponding control tissues. 20 histologically-confirmed cervical carcinomas, 24 infiltrating breast carcinomas, 27 colonic adenocarcinomas were analysed for telomere lengths using the TeloTAGGG Telomere Length Assay kit (Boehringer Mannheim). In addition, 6 normal cervixes, 16 normal breast and 16 normal colonic tissues were also analysed. The mean telomere lengths were 1.9 and 2.6 kbp respectively for cervical carcinomas and normal cervixes. For breast, the mean telomere lengths were 1.0 and 3.1 kbp for carcinomas and normals respectively. In colonic samples, the mean telomere lengths were 0.9 and 1.7 kbp in adenocarcinomas and normal colonic tissues. Telomere lengths in cervical, breast and colonic carcinomas appeared consistently shorter than that in the normal controls. Although it would be thought that the expression of telomerase in neoplasia, which counters natural telomeric shortening, would cause telomere lengths to be generally longer in cancers compared with normal tissues, the process appears otherwise. One possibility for this could be that the high cell turnover of cancers still leads to a concomitant telomeric shortening despite telomerase activation. Also it would appear that a minimum of 0.9 kbp telomere length is sufficient for cell survival and immortalisation.

### Poster presentations:

#### P1. The role of insulin-like growth factor I (IGF-I) in neonatal outcome

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Insulin-like growth factor I (IGF-I) plays an important role in the control of fetal growth and development (*Styne*). By using the Elisa method, we measured serum levels of IGF-I in 50 diabetic mothers and 50 control mothers at two periods of gestation (28 weeks and 36 weeks). We also evaluated and compared the serum IGF-I level in both groups in the two periods of gestation (28 weeks and 36 weeks) and correlated them with the presence of septal hypertrophic cardiomyopathy. Serum IGF-I Levels in maternal blood during delivery (36 weeks gestation) were significantly higher than the level at 28 weeks of gestation in both groups. IGF-I levels were low (IGF-I level <300 ng/ml) in very low birth weight infants (birth weigh <1500g). Among the 50 cases of diabetic mothers, there were 2 cases (4%) of neonatal septal hypertrophic cardiomyopathy. Both mother had high IGF-I level (IGF-I Level >400 ng/ml) during delivery. However, control mothers had IGF-I level of 302 +/- 15 ng/ml during delivery. In conclusion, serum IGF-I concentration may explain the variation in physical growth and development during neonatal life, or a predictor of neonatal outcome.

**P2. Porphyria cutanea tarda in a patient with chronic myeloid leukaemia**

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Porphyrias are a heterogenous group of metabolic disorders characterized by excessive accumulation and excretion of porphyrin or porphyrin precursors. Porphyria cutanea tarda (PCT) is the most common of the porphyrias and is characterized by skin lesions and liver disease. Clinical manifestations include blistering and erosions on light-exposed skin with scarring, skin fragility and hypertrichosis. Sporadic PCT (type 1), which usually presents during adulthood, is the most common type and often occurs in conjunction with other conditions such as iron overload. We describe a 55-year-old lady who was diagnosed to have chronic myeloid leukaemia in 1987 and being treated with s.c. interferon, allopurinol and hydroxyurea. In year 2000, she started to develop rashes on her hands and face with areas of hyper- and hypopigmentation. The rashes were ulcer like bullous lesions associated with skin peeling, pain and pruritus, effecting mainly the light exposed areas. Initial biopsy performed on one of the lesions was described as subepidermal bulla consistent with PCT. Patient's urine was also noted to be red in colour. Urine test confirmed the presence of urine porphria and porphobilinogen. Liver function test showed evidence of liver impairment with low total protein and albumin, and elevated liver enzymes. Serum ferritin was also markedly raised (4588 ug/l). A final diagnosis of PCT was made based on clinical presentation, laboratory findings and the initial skin biopsy. Patient was started on choloquine and s.c erythropoietin, and was advised on high carbohydrate and beta carotene diet.

**P3. CD34+ cell yields in healthy donors after granulocyte-colony stimulating factors (G-CSF) mobilisation.**

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In the recent years, peripheral blood stem cell transplantation has been accepted as an alternative to bone marrow for allografting. The optimal time for G-CSF mediated peripheral blood stem cell mobilisation in normal healthy donors had been documented to peak on day 5 after administration of G-CSF. We analyzed 30 samples from 15 healthy donors (9 males, 6 females) for allogeneic stem cell transplantation. The median age was 23 years (range, 9 – 44 years). The dosage of G-CSF given to these donors was 10mg/kg/day for 5 days. The samples were taken from the day 5 and day 6 of the harvested peripheral blood stem cell products to measure for CD34+ cell counts by flow cytometer, using the lysed, non-wash Procount method (Becton Dickinson).

In our series of donors, the median total harvested stem cell yield collected on day 5 was  $1.36 \times 10^8$  CD34+ cells (range,  $0.02 - 4.4 \times 10^8$ ) and the median stem cell yields per kg of the recipient body weight was  $2.36 \times 10^6$  (range,  $0.05 - 7.39 \times 10^6/\text{kg}$ ). The target cell dose of  $\geq 2 \times 10^6$  CD34 + cell per kg recipient body weight was achieved with one procedure in 9 donors (60%) and in 12 donors (80%) with two procedures. We conclude that in our normal healthy donors, G-CSF of 10 mg/kg/day for 5 days with single leukapheresis on the following day is only an effective regimen for peripheral blood stem cell mobilisation in 60% of donors. Alternate regimen needs to be explored to reduce leukapheresis to a single procedure.

**P4. Does optimal cutting temperature (OCT) compound deter amplification of beta-globin gene?**

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Optimal cutting temperature (OCT) compound is commonly used to embed fresh, frozen tissue before sectioning. It has been suggested that OCT affects the quality of DNA. A study was conducted at the Department of Pathology, University of Malaya Medical Centre to compare the amplification of a 268 bp segment of the ubiquitous human beta-globin gene using DNA extracts from OCT-embedded and non-OCT-embedded fresh, frozen tissue. Paired samples from 50 normal cervixes were snap frozen with and without prior embedding in OCT on receipt. Two 10 mm sections from the OCT and non-OCT embedded tissues respectively were subjected to 0.06 mg/ml proteinase K digestion at 48°C for 5 days. Following phenol-chloroform-isoamyl alcohol extraction, the DNA was quantitated and 0.25 mg from each specimen was subjected to amplification of a 268 bp ubiquitous human beta-globin gene segment. 38 (76%) OCT-embedded and 44 (88%) non-OCT-embedded fresh, frozen tissue demonstrated beta-globin gene amplification ( $p>0.05$ ). Although there is no statistical difference between OCT-embedding and non-OCT-embedding in this study, we caution that there appears to be a mild decrease in DNA quality with OCT use and would still suggest processing of fresh frozen tissue without OCT embedding, if permissible, in tissues considered for molecular diagnostic work.

**P5. Signet ring cells in the bone marrow**

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Gastric carcinoma accounts for 95% of gastric tumour and accounts for 3% death from cancer. It occurs primarily after age 50. Surgery is the only curative treatment but radiation and chemotherapy can be beneficial. The tumour may metastasise to Virchow's node, ovary, liver or peritoneum and rarely to the bone marrow. We describe a 79-year-old Chinese lady who had a history of poorly differentiated adenocarcinoma of the stomach and had undergone total gastrectomy with standard Roux-en-Y esophagojejunostomy about 10 years ago. She was completely asymptomatic following the surgery until recently when she presented with generalized body ache and had to walk with support. Blood investigations revealed markedly increased ALP and ESR. Full blood picture showed normochromic normocytic anemia with leukoerythroblastic change. Skeletal survey reported osteopenic bones with multiple lucencies, which suggested metastatic disease or myeloma. Bone marrow aspiration and trephine biopsies showed malignant cell infiltration with signet ring appearance.

**P6. The physician ordering pattern for the tumour marker CA-125 in the University Malaya Medical Centre.***PAVAI S. YAP SF and CH NG**Department of Pathology, University Malaya Medical Centre.*

CA-125 is a glycoprotein that is produced by epithelial ovarian tumours and tissues of the mullerian duct origin. It is elevated in a significant proportion of patients with ovarian carcinomas. It is useful for differentiating benign from malignant ovarian masses, for determining prognosis and monitoring response of patients to chemotherapy, and for detecting residual tumour recurrence. It is not only elevated in non-ovarian malignancies such as pancreatic, GI, lung and breast but also in a range of benign conditions. We reviewed the test requests received from various doctors from our Medical Centre (UMMC) over a period of 6 months (July to December 2001) to assess the reasons for ordering CA-125. Out of a total of 502 requests that were analysed, 318 (63.6%) originated from the Gynaecology Department. The reason for 254 of these 318 requests (79.9%) was to assist in the diagnosis and management of patients with gynaecological malignancies. In the remaining 20.1% of cases, the indications for requesting were benign gynaecological conditions such as ovarian cyst, fibroid uterus, etc. In 114 cases (22.8% of the total requests), CA -125 was requested for ruling out various forms of non-gynaecological malignancies. Eighteen additional requests were for the purpose of management of patients with colon, breast, lung carcinoma and lymphoma. In the remaining 52 cases (10.4%), the clinical diagnosis indicated included various benign conditions like pneumonia, pleural effusion, renal failure and SLE. Based on this review, we estimate that about half of the requests received do not have clear indications. The clinical utility of the test results for these cases are limited at best. The review is part of an ongoing activity to evaluate the efficiency of utilization of laboratory tests. The test results highlight the need for continued monitoring and communication with the ordering physician to optimize the utilization of the limited resources of the laboratory.

**P7. HPV prevalence in normal cervixes in a Malaysian population***PL CHEAH, B NESAMALAR, MH NG and LM LOOI**Department of Pathology, University of Malaya Medical Centre, Kuala Lumpur*

The human papillomavirus (HPV) is aetiologically-linked to cervical carcinoma. It is therefore important to know the prevalence of HPV in normal cervixes to perceive the immensity of the problem. This led to a study at the Department of Pathology, University of Malaya Medical Centre which assessed prevalence of HPV in normal cervixes obtained from hysterectomies performed for benign conditions in the female genital tract apart from those occurring in the uterine cervix. 63 normal cervixes with prior beta-globin gene amplification were admitted to the study. Of these, surgery was carried out because of leiomyoma in 30, endometriosis 7, utero-vaginal prolapse 9, ovarian cystadenoma 5, adenomyosis 3, benign ovarian teratoma 2, endometrial polyp 1, dysfunctional uterine bleeding 1, placental praevia 1, uterine laceration 1, ovarian follicular cyst 1, fimbrial cyst 1 and pelvic inflammatory disease 1. The patients' ages ranged between 30-83 years (mean = 49 years) and ethnically there were 30 Chinese, 16 Indians, 14 Malays and 3 of other minority groups. HPV PCR was carried out using consensus primers MY09/MY11 to the highly conserved L1 ORF of a wide range of HPV types. Only a case of utero-vaginal prolapse in an 83-year-old Chinese female showed presence of HPV. Further amplifications using type specific primers to HPV 6, 11, 16 and 18 were negative. It appears that HPV prevalence in the normal cervixes of this set of Malaysian patients is low ( $1/63 = 1.6\%$ ). Nevertheless, it has to be mentioned that the mean age of the cases was 49 years. Whether it is possible that the high average age has somehow selected HPV infected cases out of this cohort as most HPV infections would have presented in some manner by this age is a question that needs to be addressed.

**PS. Generation of dendritic cells from monocytes and acute myeloid leukemia cells**

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Dendritic cells (DC) are efficient and potent antigen-presenting cells. Pilot clinical trials indicated that dendritic cells loaded with tumor antigen could induce tumor-specific immune responses in various cancers. Owing to the extensively low numbers of dendritic cells in the blood circulation, a variety of sources have been used to generate dendritic cells including monocytes, CD34+ stem cells and even with AML cells which could induce anti-AML immune response in mice. We demonstrate here the optimized method to generate human dendritic cells in vitro from normal peripheral blood monocytes and from AML cells. Monocytes or AML cells were cultured in RPMI 1640 media supplemented with human serum and cytokines. The cultured dendritic cells were evaluated for their morphology by phase contrast microscope and MGG staining. Viability of cells were determined by trypan blue dye exclusion. Percentage of yield and immunophenotype were carried out by flow cytometry. As a result, the generated dendritic cells shown typical DC morphology. Immunophenotyping of these cells shown that they were CD14 -/low, CD1a+ , CD83+/-, CD80+, and HLA-DR + indicating that they were dendritic cells.

**P9. Primary malignant lymphoma of the prostate- a case report**

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Malignant lymphoma of the prostate is rare. There are less than 100 cases reported in the literature. We report of a case of primary Non-Hodgkin's lymphoma of the prostate. A 73-year-old Chinese presented with acute urinary retention following a six-month history of nocturia. His past history included hypertension, ischemic heart disease and left urinary tract surgery. Clinical examination revealed an enlarged prostate gland. Preoperative investigations showed an Hb of 128 gm/L, total white blood cell count of  $7.0 \times 10^9/l$ , ( N=52%, L=38%, E=2.5%, M=6.6% and B=1%). The platelet count was normal. The renal profile was normal. The prostate specific antigen was 5.22 ng/ml. The chest X-ray was unremarkable. Transurethral resection of the prostate was done. The left lobe of the prostate looked suspicious of malignancy at surgery. *Pathology:* Prostatic tissue weighed 25 grams. Histology showed benign prostatic hyperplasia. There was a monotonous small cell lymphocytic infiltrate in the prostatic parenchyma as well as blood vessel walls. These cells were positive for CD20 and negative for CD3. Following the diagnosis of small cell lymphocytic lymphoma, a CT scan of the abdomen was performed. There was no hepatosplenomegaly or enlargement of the retroperitoneal lymph nodes. No post-operative chemotherapy was given. The patient is symptom-free over a year following surgery.

**P10. Pulmonary artery sarcoma in a teenager – a case report**

*Sabariah Mohd Noor, Khairul Azman Ibrahim and Norraha Abd Rahman*

*Pathology Department, Hospital Ipoh.*

Pulmonary artery sarcoma is an uncommon tumour. The diagnosis is often missed during life. It is more commonly reported in the older age group. We report a case of pulmonary artery sarcoma diagnosed at post-mortem in a 16-year-old boy who was apparently healthy. This paper highlights the autopsy findings, the rarity of this histological type of tumour in this age group and describe the relevant clinical and pathological features of this tumour.

**P11. AML M4EO with pleural infiltration: a case report**

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Acute myelomonocytic leukaemia is an acute myeloid leukaemia with both granulocytic and monocytic differentiation. The M4 with eosinophilia subtype, is characterized by increased bone marrow eosinophils. M4Eo accounts for about 30% of M4 AMLs and appears to be equally distributed between children and adults. Most AML M4Eo have abnormalities of chromosome 16, with inv(16) offering the best prognosis.

We describe a 25-year-old Chinese man who presented to a private hospital for fever with productive cough associated with loss of appetite. On examination he was febrile and pale. Both lungs had bilateral coarse crepitations. The full blood count revealed high white cells count with low haemoglobin and platelet count. Full blood picture showed 89% of total white cells counts were blast cells. Chest X-ray showed bilateral pneumonic changes. He was referred to Hospital University Kebangsaan Malaysia for further management. Bone marrow examinations showed a mixture of myeloblasts and monoblasts with eosinophilia. Immunophenotyping showed expression of HLA-DR, CD 34, CD 33, CD 11c and intracellular MPO. Cytogenetics study showed absence of normal karyotype from eleven cells studied. There were 2 clones of cytogenetics abnormality detected: 45, X, -19, der(11), der(17), +mar(13) and 44, X, -16, -19, der(11), der(17), +mar(13). Patient suddenly developed shortness of breath at day 4 of admission. His lower right lung had reduced air entry with dullness to percussion. Repeat chest X-ray showed worsening of pneumonia with right pleural effusion. Pleural tap was done and cytology revealed numerous blast cells. The patient was commenced on induction chemotherapy with cytosine arabinoside dan daunorubicine. Leukaemic cells persisted 2 weeks post chemotherapy.

**P12. Orbital tuberculosis – a case report**

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In recent years, we have seen a worldwide increase in the number of cases of tuberculosis (TB). Several factors have been cited to explain this, namely HIV infection, high rate of immigration from countries with a high incidence of TB, inadequate funding for TB control, emergence of multi-drug resistance of TB. We report a case of "orbital tuberculosis" which was diagnosed by fine needle aspiration cytology. The patient is a young man who presented with multiple cervical lymphadenopathy. He was diagnosed as "suppurative tuberculous lymphadenitis" when acid-fast bacilli were found on Ziehl-Nelson's staining of pus aspirated from the lymph node. He was started on standard anti-TB treatment. While still on treatment, he developed proptosis of his right eye and a CT scan showed an intraorbital lesion eroding the bone. Fine needle aspiration of the mass through an intraorbital, extraocular approach showed "orbital TB" with many acid-fast bacilli seen in the aspirate. Streptomycin was added to his anti-TB regime with clinical response.

**P13. Resistance to extended-spectrum  $\beta$ -lactams mediated by AmpC  $\beta$ -lactamase in *Klebsiella pneumoniae***

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**AIM:** Resistance to the extended-spectrum  $\beta$ -lactams in organisms such as Enterobacter is by hyperproduction of their chromosomal AmpC P-lactamase providing resistance to both oxyimino- and 7-a-methoxy-cephalosporins and monobactams. Resistance in bacterial species that lack an inducible AmpC enzyme, such as *Klebsiella pneumoniae* and *Escherichia coli* was mediated by plasmids encoding extended-spectrum  $\beta$ -lactamases (ESBLs) and such resistance excluded the 7- $\alpha$ -methoxy-cephalosporins and was inhibited by  $\beta$ -lactamase inhibitors. With continuing use of 7- $\alpha$ -methoxy-cephalosporins and the use of  $\beta$ -lactamase inhibitor combinations, plasmids encoding class C P-lactamases appeared and have now been described worldwide, most frequently in *K.pneumoniae*. It is important for laboratories to be able to detect such novel mechanisms of resistance and in this report we describe the detection of AmpC  $\beta$ -lactamases in 2 isolates of *K.pneumoniae* derived from clinical samples of 2 patients in the University of Malaya Medical Centre. **METHODS:** Resistance to the extended-spectrum  $\beta$ -lactams was determined by a disc diffusion method and phenotypic confirmation of ESBL production was by the inhibitor-potentiated disc method. Production of AmpC P-lactamase was suspected when the isolate was resistant to ceftazidime and ceftoxitin. IEF, PCR amplification and DNA sequencing was carried out to characterize the P-lactamase. Plasmid analysis was done using the method described by Kado and Liu. **RESULTS AND CONCLUSIONS:** The strains were resistant to both oxyimino-cephalosporins and 7-a-methoxy-cephalosporins and tests for ESBL production were negative. The organisms produced P-lactamases with a pI value of 8.0 that corresponded to that of AmpC. DNA sequencing of the 396bp PCR product obtained using AmpC primers showed a 99% homology with the AmpC gene of *Enterobacter cloacae* P99 with accession number X07274. Both strains carried a single large plasmid of approximately 63kb size. While such strains remain infrequent in our setting, it is of interest to note that in a strain with decreased outer membrane permeability, such enzymes can provide resistance to carbapenems as well.

**P14. Formalin-fixed, paraffin-embedded tissues are compatible with molecular diagnostics***PL CHEAH, B NESAMALAR, MH NG, CW WANG and LM LOOI**Department of Pathology, University of Malaya Medical Centre, Kuala Lumpur*

Although it is acknowledged that extraction of nucleic acid is most efficient from fresh tissues, most tissues available in the routine histopathology laboratory are formalin-fixed and paraffin-embedded. Therefore the introduction of molecular diagnostics into a routine histopathology service is very much dependent on the possibility of applying molecular techniques on routinely formalin-fixed, paraffin-embedded tissues. A study was conducted at the Department of Pathology, University of Malaya Medical Centre to assess this. Paired samples from 50 normal cervixes were respectively (1) snap frozen and (2) formalin-fixed on receipt. The formalin-fixed tissues were processed and subsequently paraffin-embedded in a similar manner as for all other tissues received for the day in the laboratory. Two 10 mm sections from the fresh, frozen and formalin-fixed, paraffin-embedded tissues respectively were subjected to 0.06 mg/ml proteinase K digestion at 48°C for 5 days. Following phenol-chloroform-isoamyl alcohol extraction, the DNA was quantitated and 0.25 mg from each specimen was subjected to amplification of a 268 bp ubiquitous human beta-globin gene segment. 40 (80%) formalin-fixed, paraffin-embedded and 44 (88%) of fresh, frozen tissue demonstrated beta-globin gene amplification ( $p > 0.05$ ). Although the fresh frozen tissues showed a slightly higher rate of amplification for the beta-globin gene, it appears that formalin-fixed, paraffin-embedded tissues are compatible with molecular techniques.

**P15. Apolipoprotein E genotypes and their influence on cholesterol levels in Malaysian population.***WT SEET, JAMA TAN and SY TAN**Department of Medicine, Faculty of Medicine, University of Malaya, Kuala Lumpur*

Apolipoproteins are the protein components of plasma lipoproteins. They are responsible for carrying triglycerides, phospholipids, cholesterol and cholesteryl esters between organs. Apolipoprotein E (ApoE) is a polypeptide consisting of 299 amino acids. It is encoded by a polymorphic gene located on chromosome 19. In normal population, individuals carrying the  $\epsilon 2$  alleles were observed to have lower plasma cholesterol concentration compared to individuals with the E3/E3 genotype. On the other hand, individuals carrying the c4 allele were found to have higher plasma cholesterol compared to those with homozygous  $\epsilon 3$  alleles. Therefore, it can be generalised that the c4 allele causes higher plasma cholesterol concentration whereas the  $\epsilon 2$  allele has a protective effect against it. This study is designed to investigate the correlation between the ApoE gene and cholesterol levels in population groups in Malaysia. One thousand subjects were recruited from Klang Valley. EDTA and plain blood were collected from each subject for apoE genotyping and cholesterol level measurement respectively. DNA was extracted from the EDTA blood using Proteinase K – SDS digestion and purified by phenol-chloroform extraction. The apoE gene was amplified by PCR reaction and genotyped by the restriction enzyme digestion method. Statistical analysis revealed that there is a significant difference in cholesterol level among the six genotypes ( $p = 0.0001$ ). Individuals with the E2/E2 genotype have the lowest mean total cholesterol level ( $3.645 \pm 0.787$ ) whereas individuals with the E4/E4 genotype have the highest mean total cholesterol level ( $5.522 \pm 0.976$ ). The results obtained in this study are consistent with other studies performed by other researchers around the world. Therefore, there is convincing evidence that individuals carrying the c2 allele tend to have lower plasma cholesterol and individuals carrying the  $\epsilon 4$  allele tend to have elevated plasma cholesterol.

**P16. A comparison of telomere lengths in breast tissues ranging from normal to fibroadenomas and carcinomas***MH NG, LM LOOI and PL CHEAH**Department of Pathology, Faculty of Medicine, University of Malaya*

Telomeres, which cap ends of chromosomes, are known to shorten with each cell replication till a critical limit, following which the cell undergoes senescence. At the same time, it is known that tumour cells express telomerase, an enzyme which synthesizes telomeres and prevents this lethal shortening. Hence it would appear that telomere lengths in neoplasia would be a result of the balance of these 2 processes. A study was conducted at the Department of Pathology, University of Malaya Medical Centre to compare the telomere lengths in a continuum of normal to benign and malignant tumours in breast. 16 normal breast tissues, 14 fibroadenomas and 24 infiltrating breast carcinomas were analysed for telomere lengths using the TeloTAGGG Telomere Length Assay Kit (Boehringer Mannheim). The mean telomere lengths were 3.1, 1.9, 1.0 kbp for normal breast tissues, fibroadenomas and infiltrating breast carcinomas respectively. Benign fibroadenomas appear to have longer mean telomere lengths compared with invasive breast carcinomas but shorter lengths compared with normal non-neoplastic tissue. These findings support the hypothesis that telomere lengths are a balance between natural end-replication shortening and telomerase-directed lengthening in tumours. It also appears that shorter telomere lengths in tumours may be a marker of tumour aggression.

**P17. Preharvesting peripheral blood CD34+ cells quantification predicts stem cell yields in autologous stem cell transplant patients.***CF LEONG, K.SIVAGENGEI, NH HAMIDAH and SK CHEONG**Clinical Haematology & Stem Cell Transplantation Services, MAKNA-HUKM Cancer Institute, Cheras, Kuala Lumpur.*

Peripheral blood CD34+ count quantification has been used to predict the peripheral blood stem cell yield in the harvested leukapheresis products. We analyzed 17 samples from 9 patients (6 males and 3 females) undergoing autologous stem cell transplantation. They suffered from Non-Hodgkin's lymphoma and relapsed or resistant acute myeloid leukemia. The median age was 33 years (range, 14 – 54 years) and the median body weight was 53kg (range, 29 – 70kg). The samples were taken on the harvesting days just before the leukapheresis as well as from the harvested peripheral blood stem cell products. CD 34+ cell counts were measured by flow cytometer using the lysed, non-wash Procount method (Becton Dickinson). In our patients, the preharvested peripheral blood CD34+ cell counts correlated significantly with the harvested stem cell yield in terms of CD34 x10<sup>6</sup>/kg patient's body weight (R value of 0.742 p<0.05) as well as the total CD34+ cells/L (R value of 0.713, p<0.05). Our data had shown that in order to get the harvested stem cell yield of at least 2x10<sup>6</sup>/L, the preharvested peripheral blood CD34+ count must be at least 29/ $\mu$ l. Even though our patient numbers and leukapheresis procedure were low, our results agreed with the previous reports that the quantification of peripheral blood CD34+ count is a reliable and useful guide for predicting the peripheral blood stem cell yield.