

The Sixth Combined Scientific Meeting of The Malaysian Society of Pathologists and The Singapore Society of Pathology was held in Port Dickson on 17-19th November 1995. Abstracts of the free paper communications follow:

Oral presentations:

1. Efficiency of use of blood for elective surgery in University Hospital, Kuala Lumpur.

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Human blood is a precious commodity. Every blood bank should aim to use this resource efficiently without compromising on the quality of patient care and blood products. With this in mind, a prospective audit of the UHKL Blood Bank was carried out over three months to determine how efficiently blood was being used in elective surgery. Every case with blood crossmatched was monitored to determine the amount transfused and the posttransfusion Hb. Overcrossmatching was noted in almost all surgical procedures and overtransfusion in 74.03% of patients transfused. These indicate inefficient utilization of blood, MLT's (medical laboratory technologist) time and funds. The transfusion index and range of units transfused were calculated for each procedure. They are useful as indicators of blood requirement and the potential severity of haemorrhage for each procedure. The rate of case postponement was 18.12%. Suggestions to improve efficiency of blood utilization include adopting the "group, screen and hold" practice for surgical procedures with large crossmatch-transfusion ratios, low transfusion indices and a small range of units transfused. A hospital blood transfusion committee can also be formed to set up guidelines for crossmatching and transfusion practices in elective cases based on the data obtained here.

2. Lesions of the central nervous systems: A Malaysian experience with 1064 consecutive cases

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A study of the incidence of central neural lesions over a period of 4 years, was conducted in two major hospitals in Malaysia. One is a University Hospital located at the north-Eastern part of the peninsular, while the other is a General Hospital located at the central region and is a national neurological referral centre. During this period, a total of 1064 (histologically proven) consecutive lesions were seen involving the brain and the spinal cord. Of these 872 cases were of neoplastic nature, of which 293 were malignant. 109 were inflammatory, 69 were congenital lesions and the rest was miscellaneous in nature. In descending order, there were 203 (23.3%) tumours of neuroglial origin, 193 (22.1%) of meningotheial origin, 90 (10.3%) of nerve and nerve sheaths origin, 70 (8.0%) of vascular origin, 68 (7.8%) from primitive and undifferentiated cells, 66 (7.6%) pituitary adenomas, 57 (6.5%) metastatic tumours, 54 (6.2%) malformative tumours, 12 (1.4%) germ cell tumours, 11 (1.3%) non-Hodgkin lymphomas and 5 (0.6%) neuronal tumours. 43 (4.9%) cases were categorised as miscellaneous tumours. Of the 108 inflammatory lesions, 55 (50.92%) cases were due to pyogenic abscesses, 16 (14.8%) were due to tuberculosis, 10 (9.25%) due to cryptococcosis while the micro-organisms were not detected or identified in the rest of the inflammatory lesions. Of the 64 congenital lesions, meningoencephalocoeles formed the majority 85.9% (55 out of 64 cases). Malaysians comprise of three major ethnic groups: the Malays, Indians and Chinese. It is noteworthy that for all the five main primary brain tumours (meningiomas, astrocytomas, schwannomas, pituitary adenomas and medulloblastomas) the total incidence among the Malays was the highest being 54.7% (293/536), followed by the Chinese, 27.6% (148/532). This was least common among the Indians, 11.7% (63/536). The incidence of all central neural lesions per 100,000 population was 1.5, while the incidence of malignant lesions was 0.5. The incidence was compared with the incidence of non-neural lesions in Malaysia.

3. Percutaneous fine needle aspiration biopsy of lung and mediastinal lesions

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In a ten-year period between 1985 and 1994, 746 percutaneous fine needle aspiration (FNA) of the lung and mediastinal lesions were carried out. About 90% of patients were between 30 and 80 years of age. Males predominate with a male:female ratio of 2.5 : 1. The majority (54%) of patients were ethnic Chinese. A diagnosis of primary lung cancer was made in 47% of patients, and metastatic lung cancer in 5%. Of the mediastinal lesions, lymphoma and thymoma constituted 28% and 26% respectively. Benign lesions were diagnosed in 19% of cases. Of these, an infectious aetiology was detectable in 25%. In 24% of lesions aspirated, the results were inconclusive. Fine needle aspiration of the lung is a useful procedure in the diagnosis of pulmonary and mediastinal lesions. In this series, a high pick-up rate of about 76% was obtained.

4. Prevalence of IgG and IgM anticardiolipin antibodies in healthy blood donors

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Circulating anticardiolipin antibodies (ACA) are frequently associated with recurrent thrombosis, frequent abortions and thrombocytopenia. Previous studies have shown that ACA is also found in normal healthy individuals. We have therefore studied the prevalence of isotypes IgG and IgM ACA in a population of 182 blood donors of whom 71.4% were Malays, 28% Chinese and 0.5% Indians. Their mean age was 31.6 years (age range 16-58 years). A commercial ELISA kit was used to quantitate ACA levels. Since the distribution patterns of ACA were not normally distributed, we used a non-parametric method of statistical analysis to determine the population prevalence. Using the definition that values falling within 95% were normal, we found that abnormal IgG ACA and IgM ACA levels were greater than 7.126 U/ml and 6.995 U/ml respectively. The prevalence of elevated IgG ACA and IgM ACA were both 4.9%. Fifteen individuals (8.2%) had either one or both elevated ACA while 2 (1.1%) were found to have both IgG and IgM ACA elevated. However, we did not find any significant association of raised ACA levels with age, sex, and race. The highest frequency of positive cases (21.7%) was observed within the younger age group (16-25 years).

5. Nucleolar organizer regions distribution in cytologic smears of breast lesions

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Fine needle aspiration (FNA) cytologic study is now an integral part of the pre-operative investigation of breast lesions and the therapeutic protocol is today often planned on the basis of cytodiagnosis. However, from time to time the cytologic picture may be equivocal or inconclusive. In recent years, the study of nucleolar organizer regions (NOR) has been explored as a potential tool for the diagnosis of malignancy as their frequency in malignant nuclei is seen to be more than in benign or reactive nuclei. With a view to applying NOR scoring as a tool in the evaluation of cytologically equivocal cases, we applied the argyrophil technique for staining NOR's (AgNOR) in FNA cytologic smears of 56 breast lesions. These included 31 benign and 25 malignant lesions. Histologic correlation was possible in 26 of these cases (17 malignant and 9 benign) and AgNOR scoring was done on paraffin sections as well. There was a significant difference between AgNOR scores in benign and malignant breast lesions in the cytologic smears ($P < 0.001$). The AgNOR scores ranged from 2.5 to 5.0 per cell in the benign lesions. In malignant lesions the range was wider i.e. from 5.8 to 17.2 per cell. None of the cases fell into the "gray" zone of overlap. One malignant lesion that was cytologically equivocal showed a mean AgNOR score of 6.08. From this preliminary study, we conclude that an AgNOR score of 5.0 and less indicates a benign lesion whereas a score above 5.0 would be in favour of a

malignant lesion. A study with a larger number of cases would be needed to verify our impression that AgNOR scoring can be useful in cytologically equivocal cases.

6. Mitochondrial myoencephalopathy, lactic acidosis and stroke-like episodes (MELAS): a report of 3 cases

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MELAS is a distinct clinical syndrome comprising mitochondrial myopathy, encephalopathy, lactic acidosis and recurrent cerebral insults resembling strokes. We report 3 adult cases (mean age = 28 yrs) of MELAS who presented at the University Hospital Kuala Lumpur with chronic, progressive neurological deficits that includes sensorineural deafness, ophthalmoplegia and dysphasia. Only one patient had muscle weakness. "Ragged red fibres", the hallmark of mitochondrial myopathy, were found in the muscle biopsies of all 3 patients. Electron microscopy showed abnormal mitochondria in the muscle tissue. A greater awareness of this condition, both from the clinical and histopathological perspective, could improve its diagnosis and enable some cases at least to benefit from new drug therapies.

7. Minimum inhibitory concentration determination of penicillin in *Streptococcus pneumoniae* - a comparison between the E-test and standard agar dilution

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Streptococcus pneumoniae is a major cause of pneumonia, meningitis, otitis media, bacteremia, and sinusitis. Penicillin has remained the antibiotic of choice for the treatment of pneumococcal infections. However, over the last two decades penicillin-resistant strains have been increasingly reported worldwide. It is therefore important to screen all clinically relevant strain of *Streptococcus pneumoniae* for penicillin susceptibility. Eighty-six strains of *Streptococcus pneumoniae* isolated from clinical specimens between January 1989 to August 1995 were included in the present study. The sensitivity to penicillin was determined by disc diffusion and dilution methods. Minimum inhibitory concentration (MIC) was determined by the standard agar dilution technique and a commercial kit (E-TEST strip). Sixteen strains (18.6%) were penicillin-resistant and seventy (81.4%) were penicillin sensitive by the disc diffusion method. MIC determination confirmed seven strains (8.14%) as penicillin-resistant by both agar dilution and E-TEST methods, with MIC ranging from 0.125 mg/L - 2 mg/L. E-TEST was found to be a simple and rapid method of MIC determination.

8. Epstein-Barr virus (EBV) in Malaysian Burkitt's lymphoma

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Aim: The association of EBV and Burkitt's Lymphoma (BL) is known to show geographical variation. More than 95% of endemic African cases are EBV associated. There is a suggestion that the association of EBV and BL is related to the socio-economic status of the population. This study aims to compare the pattern seen in Denmark and Britain, representing Western developed, industrial countries, and Malaysia, a developing country from the East.

Materials and Methods: A total of 19 cases of reconfirmed BL (16 male, 3 female) were retrieved from the files in the Department of Pathology, University of Malaya, and 39 non-AIDS related cases (26 males, 13 female) from the Danish (n=37) and British files. The immunomorphological study on

Malaysian cases were based entirely on sections from paraffin-embedded tissue. All these lesions were tested for the presence of EBV by in-situ hybridization technique for EBV early RNA (EBER), using a cocktail of oligonucleotides in all Malaysian and British cases, plasmid RNA probe and or oligonucleotide probe in the Danish cases.

Results: In the Malaysian cases (13 Chinese, 5 Malay, 1 Indian), 14 presented at or below the age of 16, and 4 of these cases (29%), all were Chinese, were EBV positive (3 small intestine, 1 maxillary sinus), and 3 out of the 5 older patients (2 Chinese, 1 Malay) were EBV positive. The overall incidence of EBV in Malaysian BL was 37%: 6/13 (45%) Chinese, 1/6 (17%) non-Chinese. In the Western series, there were 16 patients at or below the age of 16, one of whom had a lesion which was EBV positive. In the remaining 23 older patients, 1 expressed EBER positivity. The frequency of EBV positivity in the Western series was 5%.

Conclusion: The results confirm the low frequency of EBV associated BL in developed countries, even though the incidence of EBV associated BL in their AIDS-related cases are high. The frequency of EBV associated BL in Malaysia appears to fall between that of the endemic African and the industrial countries in the West, although both EBV and malaria are endemic in Malaysia, a situation similar to tropical African countries.

9. A survey to assess the impact of cytochemistry, immunophenotyping and cytogenetics on the diagnosis of acute leukaemia in the University Hospital in 1995 and their clinical implication

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A survey was performed to look at all cases of adult acute leukaemia diagnosed in the University Hospital, KL during 1995 up to the month of August. There were 15 cases of Acute Lymphoblastic Leukaemia (ALL) and 25 cases of Acute Myeloblastic Leukaemia (AML). In ALL cases the median age of patients was 26.6 yr. The median presenting white cell count was $5.4 \times 10^9/L$. 2 patients presented with high white counts of $> 30 \times 10^9/L$. 4 cases did not have any cytochemistry, immunophenotyping and cytogenetic tests performed. Only 2 patients (13%) had complete cytochemistry, cytogenetic and immunophenotyping results. In 9 patients (60%) with available immunophenotyping results, all added new information that was not provided by cytochemistry. Cytogenetic results were available in 5 patients (30%), all the results were relevant. However, when the clinical impact of these tests were assessed, in only 2 cases was the treatment strategy significantly altered by these laboratory tests. One case had Philadelphia chromosome and the other showed a myeloid antigen positive ALL with positive CD34 as well. There were 25 AML cases. The median age was 44.5 yr. The median presenting white cell count was $19.2 \times 10^9/L$. 5 cases had no cytochemistry, immunophenotyping or cytogenetic tests performed. The cytochemical reaction proved to be very helpful in subtyping AML in 6 (24%) cases, an additional 3 (12%) differed from the morphological interpretation. Immunophenotyping results were available in only 9 cases, but unlike ALL cases, in only 2 of these did the results add new information not available from other tests. Cytogenetic studies were performed on 9 cases but in only 5 of these cases (20%) were the tests successful: all five readings were clinically relevant. However, in only one case was the treatment regimen significantly altered by the laboratory tests. In conclusion, less than 10% (4/41 patients) of acute leukaemia cases had all the routine biodata as provided by cytochemistry, immunophenotyping and cytogenetic tests. Immunophenotyping is more informative in the diagnosis of ALL than AML. The total impact as far as the clinical practice is concerned is minimal at the present time.

10. The Quality Assurance Programme (QAP) for Pathology Laboratories of the Ministry of Health, Malaysia

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The Ministry of Health initiated a Quality Assurance Programme in 1985 starting with the patient care service programme. It was aimed at covering all areas of health care and was extended to cover other services contributing to health care, that is, health, laboratory, dental, engineering and pharmacy services. The QAP for Pathology Laboratories was established in 1991 and served by 4 subcommittees which represented the various pathology disciplines, that is Chemical Pathology, Medical Microbiology, Haematology & Blood Banking, Histopathology & Cytology. The programme emphasis was more on promoting quality awareness and education to propel an organisational and cultural transformation committed to improving quality. Three different indicators - analytical performance, timeliness of the service and appropriateness of services - were identified for each subcommittee and the performance of these indicators were monitored at regular intervals. Results from a few years monitoring have shown that measurement of laboratory performance is possible and these findings are being used for further improving the service.

Poster presentations:

P1. A quantitative study of mast cells in Kimura's disease

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The association and possible role of the mast cell in the pathogenesis of Kimura's disease has not been adequately studied. We quantified mast cells in tissue sections from 9 cases of Kimura's disease and compared mast cell counts with those obtained in control tissues (5 normal skins and 5 reactive lymph nodes). In addition, blood vessels were also quantified, and the distribution of mast cells in relation to blood vessels examined. Formalin-fixed, paraffin-embedded, 4 μ thick tissue sections were stained immunohistochemically for Factor VIII-related antigen which stained up blood vessels, and counterstained with toluidine blue which stained up mast cells. Mast cells and blood vessel cross sections were counted in 100 consecutive high-power fields (X1000, oil immersion). The total number of mast cells in 100 fields in Kimura's disease ranged from 541 to 1705 cells (mean = 892). Counts in normal skins and lymph nodes ranged from 102 to 584 (mean = 269) and 43 to 341 (mean = 146) respectively. Using the **Mann-Whitney** test, the higher mast cell count in Kimura's disease was found to be statistically significant ($p < 0.004$). Mast cells were not predominantly found around blood vessels. Vascularity was also found to be significantly increased in Kimura's disease. The increase of mast cells in Kimura's disease suggests that they may have an important role in the pathogenesis of Kimura's disease, and is consistent with the theory that Kimura's disease represents an aberrant immune response to unknown antigens.

P2. A case report on Acetaminophen overdose in a young child

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Acetaminophen is a safe and effective antipyretic and analgesic widely used in children. Overdosage from this medication is common and frequently missed due to similarity in the clinical presentation and laboratory findings with other conditions such as Reye's syndrome, sepsis and metabolic liver disease. We report a case of acetaminophen overdose which was subsequently confirmed by the level of acetaminophen in the serum. AW, a 22-month old Chinese boy presented in a semi-comatose state

at admission. He had been unwell 4 days prior to admission and had been given antipyretics. Clinical assessment showed that the patient was in shock. Initial investigations documented hypoglycemia and metabolic acidosis. Laboratory investigations showed significant hepatotoxicity with grossly elevated liver enzymes and prolonged prothrombin time. An initial diagnosis of Dengue shock syndrome and a differential of Reye's syndrome were made. Patient was admitted into intensive care unit but succumbed within 24 hours after admission. Post-mortem findings which included a liver biopsy showed a massive liver necrosis which was suggestive of a drug reaction and this was confirmed by acetaminophen level of 969.3 micromol/l in the serum. (Therapeutic range 66-199 micromol/l).

P3. The significance of ionized calcium measurement in patients with abnormal serum total calcium

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Ionised calcium is physiologically active calcium pool. As a clinical parameter, directly measured ionised calcium has particular important in the care of neonates, patients with sepsis or other cardiovascular instability, massively transfused patients and those undergoing cardiopulmonary bypass. This study was carried out to analyse the relationship of corrected total calcium (TCa) and ionised calcium (ICa) in 234 patients with abnormal serum calcium. 127 patients had high TCa levels and 107 had low TCa levels. TCa concentration varied from 0.66 to 3.56 mmol/L. A good correlation between measurements of TCa and ICa levels was demonstrated ($r = 0.83$, $p < 0.0001$). In patients with high TCa levels, 10 (7.87%), 81 (63.78%) and 36 (28.35%) of them had high, low and normal ICa levels respectively. In contrast, all patients with low TCa had low ICa levels ($n = 107$, 100%). Though a good correlation between the two measurements was observed, the determination of TCa did not reflect the true status of ICa levels in some of the cases. We conclude that measurement of ICa may be useful in the management of patients with calcium disorders.

P4. Primitive neuroectodermal tumour mimicking acute lymphoblastic leukaemia - a case report

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Primitive neuroectodermal tumour (PNET) is a small round cell malignancy arising from soft tissue or bone and occurring predominantly in children and adolescents. Histological differentiation of PNET from other small cell tumours can be difficult. This case is a 6-year-old boy who presented with lethargy and loss of weight of two months' duration. Two weeks prior to admission he had a left hip pain. On examination he was pale. The peripheral blood investigation on admission showed anaemia and thrombocytopenia. Bone marrow aspirate smears revealed numerous blast cells which morphologically resemble L2-lymphoblasts of the French-American-British (FAB) Classification, but cohesiveness of the cells was also observed in some areas. The peroxidase reaction was negative and the Periodic-Acid Schiff (PAS) showed block positivity similar to that of common acute lymphoblastic leukaemia. Pelvic X-ray showed a lytic lesion at the left pelvis. A tissue biopsy was taken from the site and the histological examination showed a tumour with the differential diagnosis of being an embryonal tumour or a non-Hodgkin's lymphoma. Immunohistochemistry reactions were inconclusive. Immunophenotyping of the bone marrow blasts by flow cytometry revealed that they were of non-haemopoietic origin and showed positivity for CD56, which is consistent with tumour of neuroectodermal origin (PNET). These cells also expressed BCL2 oncoprotein.

P5. Application of molecular techniques in the prenatal diagnosis of Thalassaemias

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Prenatal diagnosis is a single most effective step in the prevention of severe forms of thalassaemia. We report here the use of molecular techniques on DNA obtained from chorionic villus sample (CVS) and fetal cord blood during early pregnancy in the prenatal diagnosis of 9 cases of thalassaemias. Southern blot analysis using ³²P-labelled α -globin gene and C-gene specific probes and PCR-based techniques using allele-specific primers were performed on DNA extracted from CVS and fetal blood to establish fetal genotype in 5 cases at risk from homozygous α^0 -thalassaemias and 4 cases from homozygous β -thalassaemias respectively. Two of the α -thalassaemia cases showing deletions of all the four α -globin genes (genotype $-/-$) had their pregnancies terminated. All the four cases of β -thalassaemias showed fetal DNA to be heterozygous for a specific mutation. Two of the α -thalassaemia cases were heterozygous for α^0 (genotype: $\alpha\alpha^0/-$) and one case showed normal fetal genotype ($\alpha\alpha/\alpha\alpha$). In these cases their pregnancies were continued. In all the 9 cases DNA analysis findings performed prenatally were confirmed with DNA analysis of either the cord blood or fetal tissue obtained after birth or after termination of pregnancy respectively. These findings demonstrate the successful application of molecular techniques in the prenatal diagnosis of thalassaemias in Malaysia.

P6. Unexpected EMA and cytokeratin expression in a case of infantile acute monoblastic leukaemia

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A previously healthy eleven-month-old male Malay infant presented with fever, upper respiratory tract infection and right knee swelling. Pallor, bilateral proptosis, hepatosplenomegaly, multiple scalp swellings and a right cheek swelling were observed. Investigations revealed that he had acute monoblastic leukaemia or M5a of French-American-British Classification. Immunophenotyping by flow cytometry showed that the blast cells were positive for CD13, CD33, HLA-DR, CD11c and CD71. They were negative for CD34, CD19, CD10, CD22, CD2, CD3, CD4, CD8, CD7, CD61, NK and Glycophorin A. The monoblasts were used to test out anti-EMA and anti-cytokeratin. They were unexpectedly found to be positive. Acute monoblastic leukaemias are well known to show extramedullary infiltration and this may be their primary mode of presentation. Thus, in immunochemistry, when using EMA and cytokeratin expressions in the differential diagnosis of neoplastic diseases, it is important to consider that monoblasts may express these markers as illustrated by this case.

P7. BCL-2 oncoprotein expression in leukaemic cells

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The BCL-2 proto-oncogene encodes a mitochondrial protein that blocks programmed cell death. BCL-2 oncoproteins are found in normal tissues characterised by apoptotic cell death and also in malignancies. We studied the expression of this protein in 77 cases of leukaemias between September 1994 and August 1995, and examined its relationship with other markers of prognostic significance. We found 36% (15/42 cases) of B-precursor acute lymphoblastic leukaemia to be positive for this oncoprotein. BCL-2 expression bears no relationship to the expression of CD10 which carries a good prognosis, and myeloid associated antigens (CD13/CD33) which carries an unfavourable prognosis. BCL-2 oncoproteins were also found in 75% (618 cases) of acute T cell leukaemia and 46% (11/24 cases) of acute myeloid leukaemia. Expression of oncoproteins do not appear to be associated with a particular myeloid subtype of French-American-British Classification. In this period, we also studied

three cases of chronic lymphocytic leukaemia (CLL), an uncommon disease in Malaysia. Two were CD5 positive B-CLL and expressed the oncoprotein, whilst the other B-CLL was negative for both CD5 and BCL-2 oncoprotein. Absence of BCL-2 expression may have an unfavourable prognosis as CD5 expression in B-CLL carries a good prognosis.

PS. Malondialdehyde and conjugated diene contents in normal human skin

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Abdominal skin samples were taken from 9 adult male cadavers aged between 20 - 40 years. The samples were free from any skin diseases. The lipid peroxidation products (malondialdehyde and conjugated diene) were extracted from the samples and measured by spectrofluorophotometer. The results for malondialdehyde were: $n = 9$, mean = 6.85 nmol/g protein, SD = 2.59 nmol/g protein, and SE = 0.86 nmol/g protein. The results for conjugated diene were: $n = 9$, mean = 46.6 I.U./g protein, SD = 13.2 I.U./g protein, and SE = 4.4 I.U./g protein (I.U. = 0.D._{233nm}). These results can be used as a baseline data to study the fluctuation pattern of lipid peroxidation products (used as indicator) in determining the age of skin injury.

P9. Magnesium, copper, zinc, iron and manganese contents in normal human skin of different parts of the body

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Skin samples were taken from various parts of the body (the head, neck, chest, abdomen, forearm and leg areas) from ten adult male cadavers aged between 20-40 years. The cations from the samples were extracted using wet digestion technique (by dissolving them into nitric and perchloric acids). The Mg, Cu, Zn, Fe and Mn contents in the skin samples were measured using flameless atomic absorption spectrophotometer. The Mg contents (mean \pm 2SD) in the skin of the head, neck, chest, abdomen, forearm and leg were 61.8-82.2 $\mu\text{g/g}$, 61.4-81.2 $\mu\text{g/g}$, 55.3-69.9 $\mu\text{g/g}$, 58.7-75.7 $\mu\text{g/g}$, 101.7-143.7 $\mu\text{g/g}$ and 43.5-61.9 $\mu\text{g/g}$ respectively. The CU contents were 2.3-3.7 $\mu\text{g/g}$, 2.3-4.1 $\mu\text{g/g}$, 1.7-2.9 $\mu\text{g/g}$, 4.0-6.2 $\mu\text{g/g}$, 3.8-8.4 $\mu\text{g/g}$ and 2.1-4.1 $\mu\text{g/g}$ respectively. The Zn contents were 20.9-25.3 $\mu\text{g/g}$, 18.1-22.5 $\mu\text{g/g}$, 12.3-15.1 $\mu\text{g/g}$, 17.6-32.4 $\mu\text{g/g}$, 17.5-30.7 $\mu\text{g/g}$ and 18.6-25.6 $\mu\text{g/g}$ respectively. The Fe contents were 78.5-113.1 $\mu\text{g/g}$, 67.2-99.6 $\mu\text{g/g}$, 50.6-76.0 $\mu\text{g/g}$, 65.2-96.8 $\mu\text{g/g}$, 69.6-100.6 $\mu\text{g/g}$ and 65.1-101.9 $\mu\text{g/g}$ respectively. The Mn contents were 6.1-13.9 $\mu\text{g/g}$, 7.1-9.3 $\mu\text{g/g}$, 5.1-6.7 $\mu\text{g/g}$, 6.8-9.0 $\mu\text{g/g}$, 12.1-22.1 $\mu\text{g/g}$ and 9.8-19.0 $\mu\text{g/g}$. In general, there is no significant difference of each cation content in different parts of the body except for Mg. The Mg contents in the lower limb is much higher than other parts of the body. These data are useful in the study to estimate the age of a skin wound using skin cations contents as indicator.

P10. The impact of palm oil and its minor constituents on serum lipids, atherosclerosis and vitamin A status

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The nutritional and health attributes of palm oil has been the subject of much scientific scrutiny in recent times, following its increasing share of the global edible oil market. This presentation reviews a number of studies on the effects of consumption of (i) a diet enriched with palm oil on serum lipids (ii) tocopherol and tocotrienol supplements on the atherosclerotic process and (iii) red palm oil supplements on serum retinol levels. A palm oil enriched diet was not hypercholesterolemic in relation to other edible oils in normocholesterolemic individuals. On the other hand it altered the LDL : HDL cholesterol ratio favourably. The vitamin E in palm oil (tocopherols and tocotrienols) reduced the level of peroxidation products and also the atherosclerotic process in subjects with carotid artery stenosis. Red palm oil supplementation resulted in elevated serum retinol levels, thereby enhancing the vitamin

A status and suggesting the ready bioavailability of carotenoids from palm oil. The outcome of these studies reaffirms the versatility of palm oil as a nutritious edible oil with health promoting qualities.

P11. Aberrant expression of MHC Class II antigens in lupus nephritis

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Class I major histocompatibility complex (MHC) molecules, HLA-ABC, are ubiquitous and found in virtually all nucleated cells and platelets. In contrast, expression of MHC Class II molecules is generally restricted to cells of the immune system although expression has also been noted in the glomerular, peritubular capillary and venular endothelium of normal kidneys. Expression in the arteriolar endothelium, tubular epithelium and the glomerular mesangium of the normal kidney however remains controversial. Induction of aberrant expression of MHC Class II molecules, presumably by T-interferon, in non-MHC Class II expressing cells is also known to occur in certain immune diseases. The distribution of MHC Class II molecules in 38 cases of lupus nephritis, all satisfying the American Rheumatism Association diagnostic criteria for systemic lupus erythematosus, was immunohistochemically studied to determine the possibility of aberrant MHC Class II expression. Archival formalin fixed, paraffin-embedded renal biopsy tissue were stained with a monoclonal anti-human HLA-DR (Dako: TAL 1B5) antibody against the protein product of the HLA-D, MHC Class II, gene region, using a standard avidin-biotin complex method. Expression of HLA-DR was observed in the interstitial mononuclear cell infiltrate in all cases. In addition, HLA-DR was expressed in peritubular capillaries in all (100%) cases, veins in 97.2% of cases, arterioles in 76.5%, proximal convoluted tubules in 84.2%, distal convoluted tubules in 54.1%, collecting ducts in 16% and mesangium in 78.9%. Unlike the normal kidney, HLA-DR was also expressed in the arterial endothelium in 86.5% of cases of lupus nephritis. The anomalous expression of MHC Class II molecules in the arterial endothelium may reflect upregulation of MHC Class II expression in lupus nephritis and this may account for increased antigen presentation to CD4+ T cells leading to heightened antibody production in lupus nephritis.

P12. Cellular proliferative activity in hydatidiform moles compared with non-molar abortuses

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Cellular proliferative activity of the trophoblastic cells of complete (CHM) and partial (PHM) hydatidiform moles were compared against those of first and second trimester non-molar abortuses by studying the immunohistochemical expression of proliferating cell nuclear antigen (PCNA). Archival formalin-fixed, paraffin-embedded tissue of 25 CHM, 11 PHM and 30 abortions were stained using the standard avidin-biotin complex method with a monoclonal antibody to PCNA (DAKO : PC10). Expression of PCNA was quantitated by counting expression in 100 trophoblastic cells of each case of CHM, PHM and non-molar abortuses. PCNA was expressed predominantly by the cytotrophoblasts in all three categories. PCNA immunopositivity ranged from 47%-93% (mean = 78.7%) in CHM, 66%-96% (mean = 80.3%) in PHM and 27%-81% (mean = 80.3%) in PHM and 27%-81% (mean = 61.0%) in non-molar abortuses. Although no significant difference in PCNA expression was observed between partial and complete hydatidiform moles, the trophoblastic cells of first and second trimester abortuses showed significantly less ($p < 0.05$) proliferative activity than those of hydatidiform moles. These findings are consistent with the diagnostic morphological observation of trophoblastic proliferation in hydatidiform moles.

P13. High prevalence of autoantibodies in chronic hepatitis B carriers

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The development of autoantibodies during and following alpha-interferon (IFN) treatment has been reported. To evaluate the extent of this problem, we studied the frequency of various autoantibodies in a group of chronic hepatitis B virus (HBV) carriers who were selected for IFN therapy. All subjects were tested for anti-nuclear antibody (ANA), smooth muscle antibodies (SMA), anti-mitochondrial antibody (AMA), thyroglobulin antibody (TgAb) and thyroid microsomal antibody (Tm Ab) prior to, during and after IFN treatment. As controls, 30 apparently healthy HBV carriers comprising 16 males and 14 females were also tested for the same antibodies. Out of the 16 chronic hepatitis B patients studied, 10 (62.5%) had ANA with titers ranging from 1:40 to 1:640 prior to institution of therapy. Likewise, SMA was detected in the majority (68.75%) of patients. 6 patients (37.5%) were positive for both autoantibodies and 1 for neither. In comparison, 9 out of 30 control subjects (30%) were positive for ANA and 12 out of 30 (40%) were positive for SMA. None of the controls and patients had AMA or autoantibodies to thyroid antigens. The patients who were positive for ANA remained positive during and after IFN treatment. 5 out of 11 patients lost reactivity for SMA following IFN therapy while 1 patient developed SMA 1 year after treatment. The results of this study demonstrate a high frequency of ANA and SMA among patients with HBV related chronic hepatitis. In addition, we found a high frequency of occurrence of the same autoantibodies among "healthy" HBV carriers, albeit at a lower frequency compared to the carriers with evidence of chronic hepatitis. Contrary to reports in the literature, we did not find evidence of induction of autoimmunity during or after IFN treatment. The significance of the frequent occurrence of ANA and SMA in chronic hepatitis B is speculative.

P14. Lymphocyte predominant Hodgkin's disease in Malaysian patients

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Aim: The nature of Hodgkin and Reed-Sternberg cells has long been a subject of discussion. In nodular lymphocyte predominant Hodgkin's disease (NLPHD), the lymphocytic and histiocytic (L&H) cells have been demonstrated to be of B-cell phenotype. However, both kappa and lambda light chains are often present in the cytoplasm of these cells when demonstrated by immunocytochemistry. We aimed to use the *in-situ* hybridization (ISH) technique to demonstrate light chain restriction in the L&H cells, and also to ascertain that they are the proliferating clone of cells.

Materials and methods: A total of 14 cases of histologically reconfirmed NLPHD were retrieved from the files in the Department of Pathology, University of Malaya. Sections from paraffin-embedded material were used for immunohistochemical staining and for detection for presence of light chain immunoglobulin mRNA by ISH, using a cocktail of 15 and 11 oligonucleotide probes for kappa and lambda mRNA respectively. We also probed for the presence of histone mRNA in the L&H cells with a cocktail of oligonucleotides to histone genes H2b, H3 and H4.

Results: There were 10 males and 4 females in this series. Eight patients presented at or below 13 years of age, the youngest was 3 years old. In 13 of these cases, the lesions were nodal in presentation, with 1 presenting on the anterior aspect of the upper thigh. The L&H cells in all these cases were CD20+, with no co-expression of T-cell phenotype. In 12 of these cases, 5 showed light chain restriction (4 kappa, 1 lambda). In 7/9 cases, there were strong staining for histone mRNA in the cytoplasm of L&H cells. One case showed weak positivity.

Conclusion: Our study shows that it is possible to demonstrate light chain restriction in the L&H cells by mRNA ISH technique on archival material. The results ascertain that L&H cells are an actively

proliferating population in NLPHD, and they are of clonal B-cell origin in a proportional cases. In the Malaysian series, large majority of the patients were young.

P15. Fine needle aspiration cytology characteristics of Wilms' tumour

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The cytologic features of 19 cases (including one bilateral) of Wilms' tumour are described. All three elements identifiable histologically are usually present in the fine needle aspiration smears. Blastema was a constant finding in all 19 cases. Varying degrees of epithelial and stromal differentiation was seen. Early epithelial differentiation in the form of rosettes, glandular structures, spherical three dimensional groups of cells were present in 15 of the 19 cases. More differentiated epithelial cells were seen as elongated tubules and cell clusters. Stromal components were present in 18 cases. A myxoid matrix which stained magenta on MGG, was sometimes present amongst the stromal component. Rhabdomyoblastic differentiation was seen in one case and anaplasia was noted in another one. Tumour necrosis was present in 65% of the cases studied. Diagnostic difficulty was encountered in one case which displayed only blastema and tumour necrosis. Fine needle aspiration under ultrasound guidance to accurately localize the tumour and a posterior approach to avoid peritoneal spillage are recommended.

P16. Congenital mesoblastic nephroma: clinicopathological, immunohistochemical and flow cytometric analysis

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A clinicopathological study of nine cases of congenital mesoblastic nephroma (CMN) (3 classical, 5 cellular and 1 mixed histological types) was done. Immunohistochemical studies using six different markers (vimentin, desmin, **actin**, **S-100**, cytokeratin and EMA) were carried out on six cases to determine the possible cell of origin. Similar tumours were subjected to flow cytometric analysis to determine tumour ploidy. All six tumours were vimentin positive, indicating a mesenchymal origin. All classical types displayed a diploid cell line while aneuploidy was detected in one cellular CMN which demonstrated an aggressive behaviour, and one mixed histology CMN. Ploidy analysis may be helpful in the identification of children who may require adjuvant chemotherapy.

P17. Loss of heterozygosity analysis in nephrogenic rests and Wilms' tumours by polymerase chain reaction

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Fixed paraffin-embedded tissues from 12 cases of Wilms' tumour containing normal kidney and nephrogenic rests on the same section were studied for loss of heterozygosity (LOH) using three primers : tyrosine hydroxylase (TH), insulin-like growth factor II (P2/P3) and the putative Wilms tumour gene, WT1 (WT13/WT14). The aims were to determine the feasibility of using fixed paraffin-embedded material in molecular biological studies as well as to determine the molecular relationship between normal kidney, nephrogenic rests and Wilms' tumour. DNA was successfully extracted amplified from nine cases. The resulting pattern of each amplified DNA consisted of two bands, one maternal and one paternal. Two cases were uninformative (homozygous), and six were informative (heterozygous). Of the latter, allele loss (LOH) was detected in two cases. This study has shown that fixed paraffin-embedded archival material can be used in molecular studies and that LOH may be one of many mechanisms involved in Wilms' tumourigenesis.

P18. The role of serology in the treatment and follow up of patients with H. pylori infection*N Parasakthi, KL Goh and NW Wong**Departments of Medical Microbiology and Medicine, University of Malaya, Kuala Lumpur, Malaysia*

Noninvasive tests are an attractive alternative to biopsy diagnosis in the treatment and follow up of patients with H. pylori (HP) infection. This study was carried out to evaluate the usefulness of serial serology in determining HP eradication. Gastric biopsies and serum samples were collected from 18 patients enrolled in a treatment trial for the eradication of HP. The HP status of the biopsies were determined by a rapid urease test, tissue Gram stain and culture. Serum samples were tested for the presence of HP antibody by an ELISA method using a commercial kit, the HEL-p test (AMRAD, Australia). Biopsy and serum samples were collected pretreatment and at 14 weeks, 6 months, 1 year and 2 years posttreatment. However, at 6 months and 1 year follow up, serum samples were available for 10 and 13 patients respectively. Pretreatment biopsy and serum samples were positive for HP in all the 18 patients and these patients were HP negative by biopsy diagnosis in all the follow up samples. 2 of 18 patients (11.1%) were noted to have a 50% reduction in pretreatment titres at 4 weeks, 9 of 10 (90%) at 6 months, 12 of 13 (92.3%) at 1 year and 17 of 18 (94.4%) at 2 years. If the criteria of 50% reduction in pretreatment antibody titres were used as indicative of successful eradication, the majority of patients were correctly predicted by serology to have eradicated the bacteria from 6 months onwards.

P19. Surface membrane antigen expression in adult acute leukaemias*Chin Yuet Meng and Zubaidah Zakaria**Division of Haematology, Institute for Medical Research, Kuala Lumpur, Malaysia*

The acute leukemias are classified into acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) based on conventional morphological and cytochemical studies. AML occurs more frequently in adults than in children. Immunophenotyping together with conventional studies has enabled a more refined diagnosis of acute leukemias. The objective of this study is to detect surface membrane antigen expression using flow cytometry in adult ALL and AML. A total of 57 acute leukaemia patients (36 AML and 21 ALL), twelve years old and above were immunophenotyped from January 1994 to September 1995. 85.7% of the ALL were of B-lineage expressing CD10 and CD22 B-lymphoid antigens, while 14.3% were T-lineage ALL expressing CD3 and CD7 T-lymphoid antigens. Myeloid associated antigen was detected in 38.9% of the B-lineage ALL. A majority of the AML patients (86.1%) were positive for both CD13 and CD33 myeloid antigens. The rest of the AML patients express either CD13 or CD33 myeloid antigen. Lymphoid associated antigen was detected in 8 out of the 36 AML patients.

P20. The effects of EDTA contamination and varying storage conditions of serum total protein, albumin, calcium, magnesium, phosphate, alkaline phosphatase and alanine transaminase*AB Aishah, AR Fatimah and YN Foo**Department of Pathology, Universiti Kebangsaan Malaysia, Kuala Lumpur, Malaysia*

Appropriate collection techniques and storage conditions are part of the essential requirements in maintaining quality performance in biochemical testing. A study was carried out to investigate the effects on venous blood of 1) ethylene diamine tetraacetate (EDTA) contamination and 2) delayed serum separation at room temperature and at 4°C storage on serum total protein (TP), albumin (Alb), total bilirubin (TbI), calcium (Ca), magnesium (Mg), inorganic phosphate (PO₄), alkaline phosphatase (ALP) and alanine transaminase (ALT). The results showed that EDTA contamination reduced serum Ca, Mg and ALP significantly to 31.3, 42.9 and 67.6 per cent of the initial values in the least contaminated tube, respectively. There were undetectable levels of these parameters in the tubes with higher amounts of EDTA. Serum Ca and Mg reduced only when measured by a dye binding method but the values remained not significantly different from control when measured by atomic absorption

spectrophotometry. The serum TP, Alb, TBi, PO, and ALT were not altered significantly by EDTA. Storage of blood samples at room temperature versus refrigerator temperature (4°C) affected only serum Ca and PO₄, with a significant lowering of serum Ca after delayed separation at room temperature, but this decrease was not observed when the blood was stored at 4°C. The serum PO₄ increased with storage at room temperature, but remained stable up to 5 days of storage at 4°C. This study has contributed to the increased recognition of inadequate patients' samples due to EDTA contamination and improper storage conditions by our laboratory staff.

P21. Measurement of free thyroxine index using ABBOTT T-uptake kit

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Free thyroxine index (**FT₄I**) is an indirect measure of free thyroxine. It is measured when total thyroxine is not representative of the true thyroid status, such as in the presence of thyroid binding protein abnormalities. With the Abbott T-Uptake (T-U) kit, T-U can be mathematically converted to % T3 Uptake (T3U), from which the **FT₄I** is derived. Contrary to the traditional % T3U, which is elevated in hyperthyroid and low in hypothyroid individuals, it is observed that the transformed % T3U is usually within the normal range for both hypo and hyperthyroid patients. A retrospective study was carried out to determine whether the current reference range of % T3U and **FT₄I** were valid. Thyroid function data were retrieved from the computer from hypothyroid, (T₄ = <40 nmols/l, TSH = >20 uIU/L, n = 45), euthyroid (T₄ = 67 - 167 nmols/l, TSH = 0.3 - 5 uIU/l, n = 313) and hyperthyroid individuals (T₄ = >200 nmols/l, TSH = <0.1 uIU/l, n = 162). This study showed that the reference ranges for % T3U and **FT₄I** were 17 - 47% (current reference range : 23 - 39%) and 15 - 44 (current reference range : 18 - 54) respectively. A correlation between the **FT₄I** and free thyroxine (Abbott) showed a good correlation (r = 0.89, p < 0.01). This study illustrates the importance of establishing proper reference ranges whenever a new method is introduced in the laboratory.

P22. Acute lithium and imipramine poisoning - a case report

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Acute lithium and imipramine poisoning is very rare. Both drugs have very harmful effects and taken together in toxic doses could be fatal. Prompt adequate treatment is necessary to save the patient. We report a case which had a successful outcome. A 41-year-old Chinese woman was admitted to Kuala Lumpur Hospital in September 1994, in a coma, following self-administered toxic ingestion of lithium (28 abs of 250 mg/tab) and imipramine (28 tabs of 25 mg/tab). On physical examination, she was unconscious, blood pressure was 100/70 mmHg and pulse rate was 60/min. She suffered cardiorespiratory arrest and was resuscitated successfully. Peritoneal dialysis was instituted. Serum lithium levels taken before the dialysis measured 3.47 mmol/l. (therapeutic range : 0.6 - 1.0 mmol/l). She regained consciousness on the third day of admission. Subsequently, she developed pneumonia and respond well to chest physiotherapy and antibiotics. She was referred for psychiatric assessment and discharged relatively well 9 days after admission. This is the first case, to the best of our knowledge of acute lithium and imipramine poisoning treated by peritoneal dialysis, to be documented in Malaysia.

P23. Evan's syndrome - a case report as seen in the IMR

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We present, in a Malay male, a combination of idiopathic autoimmune hemolytic anemia with idiopathic thrombocytopenic purpura, referred to as Evan's Syndrome, associated with a

myeloproliferative disorder. He presented with bleeding gums, epistaxis, malaenic stools and conjunctival haemorrhage with blurring of vision due to haemorrhages in the fundus. On examination, he had petechiae over both temples, thighs and abdomen, haematoma left thigh and mucosal ulcers over dorsum of tongue. Liver was palpable 2 FB. Direct and Indirect Coombs Tests were positive. FBP showed a leucoerythroblastic picture with clumping of RBCs, low Hb, markedly decreased platelet count and increased reticulocyte count and ESR > 150 mm/hr. Bleeding and clotting times were prolonged. Trepine biopsy indicated a myeloproliferative disorder. Urine examination showed Albumin +++++. ANF, LE cell were negative excluding on SLE. C3, C4 levels were decreased. Initially, he was given packed cells and prednisolone intravenously, followed with oral prednisolone, tapered off to a life maintenance dose of 5 mgm.bd since the last 6 years. A splenectomy was indicated, which he refused. BMT recommended highly for such a case was not possible due to lack of funds. Patient, however, on regular follow-up, has no bleeding tendencies and leads a normal life.

P24. Fibrillary glomerulonephritis: report of the first Malaysian case

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Fibrillary glomerulonephritis is a recently recognised glomerulopathy characterised by deposition of high organised Congo red-negative fibrils extracellularly in the glomeruli. We report a case of this distinctive form of glomerulopathy to highlight the importance of considering this new entity in the differential diagnosis of glomerular hyaline deposits. A 31-year-old Malay woman presented with nephrotic syndrome of one year's duration, not associated with impairment of renal function, to the Penang General Hospital where a renal biopsy was performed to determine the nature of the renal disease. She had no clinical or serological evidence of systemic lupus erythematosus, cryoglobulinaemia, paraproteinaemia or light chain disease. Histological examination of the renal biopsy tissue revealed hyaline deposits within the mesangium with irregular thickening of the glomerular capillary basement membrane by deposition of a similar material. Congo red staining of the above deposits was negative. C3 and IgG were seen in the capillary walls and mesangium on immunofluorescence study. Ultrastructurally, non-branching fibrils of varying lengths ranging around 20 nm in width were seen predominantly in the mesangium. Segmental deposition in the subepithelial region and beneath the parietal epithelial cells of the Bowman's capsule was also evident. The histological, immunofluorescence and ultrastructural features of this case fulfills the diagnostic criteria of fibrillary glomerulonephritis, the pathogenesis of which is still poorly understood but presumed to be immunological in origin.

P25. Discriminant analysis of normal and malignant breast tissue based upon instrumental neutron activation analysis of elemental distribution

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Discriminant analysis of 6 trace element concentrations measured by instrumental neutron activation analysis in 26 paired-samples of malignant and histologically normal human breast tissues shows the technique to be a potentially valuable clinical tool for making malignant-normal classification. Justification for the use of linear discriminant analysis on skewed data from a mixture of distribution is given. The elements which have been found to be most important in distinguishing between malignant and normal tissues are Ca, Rb and Br, providing correct classification for 24 out of 26 normal samples and 22 out of 26 malignant samples.

P26. Solitary rectal ulcer

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Solitary rectal ulcer (SRU) is a relatively uncommon condition. Patients with SRU usually present with a history of constipation and rectal bleeding. Although clinically it can be mistaken for carcinoma, the histologic findings are characteristic. Recognition of the microscopic features is important to prevent patients from being diagnosed as having inflammatory bowel disease. A retrospective analysis of all the colonic biopsies seen in the Department of Pathology between 1991 to 1994 revealed 5 cases of SRU. The clinical symptoms were rectal bleeding (5 cases), constipation (4 cases) and mucus discharge (1 case). Endoscopy revealed rectal ulcer. The initial clinical diagnosis was carcinoma of the rectum in all cases. The histological features were typical of SRU in all cases. The mucosa revealed focal superficial ulceration, thickening with bifid and distorted crypts. The lamina propria showed focal obliteration by fibroblasts and smooth muscle fibres. In one case, focal colitis cystica profunda was noted.

P27. Serum typhus antibodies in Malaysian aborigines presenting with pyrexia

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Scrub, endemic and tick typhus are among the causes of febrile illnesses in Malaysia. No study have been conducted on the degree of illness caused by typhus in Malaysian aborigines. This study was conducted to determine the prevalence of typhus antibodies in Malaysian aborigines presenting with pyrexia. Serum samples from aborigine patients presenting with fever received from January 1992 until December 1994 were included in this study. Slides for indirect immunoperoxidase test were predotted in fixed order with scrub, endemic and tick typhus antigens and tests were carried out on the samples. A total of 258 serum from aborigine patients were collected over the three year period. One hundred and thirty four (52%) were from male patients and 124 (48%) were from female patients. Most of the serum samples received were single serum samples (95%). From the single serum samples sent, antibodies to scrub typhus only were seen in 110 patients. Four patients had antibodies to endemic typhus only while 11 patients have antibodies to tick typhus only. Antibodies against two more typhus antigen were detected in 55 patients. Scrub typhus appears to be the commonest form of typhus infecting aborigines followed by tick typhus and endemic typhus. Scrub typhus is a notifiable disease and should be identified as a health problem in this population.

P28. Is single test adequate for lupus anticoagulant screening?

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The most commonly used screening procedure for lupus anticoagulant (LA) are activated Partial Thromboplastin Time (APTT), Kaolin Clotting Time (KCT) and Diluted Russel Viper Venom Test (DRVVT). We conducted a study to determine the variability of results obtained in screening for LA in 42 SLE patients employing 4 methods namely APTT, KCT, DRVVT and a simplified DRVVT method known as Gradipore LA-Screen/LA-Confirm Ratio (GLAR). The cut off value, obtained from the screening of 106 normal adults, of more than 27.0 seconds for DRVVT was taken to indicate presence of LA. Weak positivity (Ratio 1.2) for GLAR was detected in 4 out of 37 normals screened. Findings amongst the SLE patients, when tests are considered individually, showed 14 positive LA cases (9 weak positive) detected by GLAR method, 4 with DRVVT and only 2 positive LA were picked up by KCT procedure. However, when all the tests were combined, 17 cases (40.5%) showed positivity for at least one test. The APTT was within normal limits for all cases even though 4 cases showed upper limit of normal values. The results from this study suggest that the use of a single test for the screening of LA is inadequate and multiple tests should be employed to increase sensitivity of detection.

P29. Detection of point mutations in α_1 AT deficiency by sequencing of ds products of polymerase chain reaction using allele specific oligonucleotides**Norsiah M Desa, G Scobie* and Zakiah Ismail***Division of Biochemistry, Institute for Medical Research, Kuala Lumpur, Malaysia and *Division of Clinical Chemistry, Queen's Medical Centre, Notts*

Genetic variants of α_1 AT deficiency by isoelectrofocussing alone does not elucidate the heterozygosity in certain situations and this may be overlooked during visual analysis of the S and Z allele bands. By using allele specific oligonucleotides, the genome fraction containing the specific mutation sites can be relatively easily amplified by the PCR technique. Sequencing of these ds PCR products by the Sanger reaction, using quick heat-cold treatment and radioactive labelling is shown to be useful and a relatively quick method to confirm the heterozygosity of the alleles. This paper describes the attempts made in distinguishing the mutated Z allele from M alleles for AAT using these techniques. Specific oligonucleotides chosen quite near to the mutated site on Axon V, namely AAT-V-I and AAT-V-11, were used as primers for the amplification of the DNA sequence to yield products of about 300 bp. Direct sequencing of this product using a third specific oligonucleotide upstream to the first primer revealed a base change G \rightarrow A on the sequencing autoradiograph for PiZZ. These techniques have been shown to be useful in genetic counselling especially for parents with affected offspring(s).

P30. Primary thrombocythaemia (PT), polycythaemia rubra vera (PRV) and chronic neutrophilic leukaemia (CNL) compared**Khalid Hassan and *Zubaidah Zakaria***Tawakal Specialist Centre and *Institute for Medical Research, Kuala Lumpur, Malaysia*

A case of PT in a 52-year-old Malay man, one of PRV in a 71-year-old Malay woman, and one of CNL in a 80-year-old Malay man, are compared clinically, haematologically and histologically. Thrombocytosis is the common feature in all these patients, and their marrows show increased thrombopoiesis as part of the myeloproliferative picture, with a fibrotic response commonly seen. The clinical presentation relates to the dominant peripheral blood abnormality, thrombocythaemia being associated with bleeding and/or thrombosis (including platelet microthrombi effects on the peripheral or cerebral circulation), erythrocytosis with hyperviscosity, and leucocytosis with leucostasis symptoms. The LAP score is high in the case of CNL, which has no Ph, chromosome, and is therefore easily differentiated from CGL. Response to antimetabolic therapy with Hydroxyurea is predictable but outcome is less certain with CNL, which has a more sinister prognosis.