

## THE SECOND COMBINED MALAYSIA-SINGAPORE PATHOLOGY CONFERENCE

The Second Combined Scientific Meeting of the Malaysian Society of Pathologists and the Singapore Society of Pathology was held at the Awana Golf & Country Club, Genting Highlands, Malaysia on 28-29 November 1991. Abstracts of the scientific communications follow:

### ORAL PRESENTATIONS:

#### 1. PATHOLOGICAL CHANGES IN SAMBA DEERS NATURALLY INFECTED WITH TUBERCULOSIS: A PRELIMINARY REPORT

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Tuberculosis is an important disease in both animals and man. Although the disease has been occasionally reported in cattle, it has never been reported in deers in this country. This report presents some of the pathological lesions in lungs and lymph nodes of deers infected with tuberculosis.

28 deers suspected of having tuberculosis were killed at the Shah Alam abattoir. Lung and lymph node samples were taken for bacterial isolation. Other tissues, including the lung and lymph nodes, were fixed in 10% buffered neutral formal saline, embedded in paraffin wax and stained with routine haematoxylin and eosin. Special stains were also performed on selected lung and lymph node tissues.

11 deers had lesions in the lungs, thoracic cavity and retropharyngeal lymph nodes and consisted of multiple circumscribed abscesses. Microabscesses were also detected in the lymph nodes. Histologically, typical *Mycobacterium tuberculosis* with giant cells together with a secondary bacterial exudative pneumonia were present in the lungs. *Mycobacterium bovis* was isolated from these abscesses, many of which were calcified.

The gross changes and histological lesions in deers are similar to those reported in other species of animals. The actual source of the infection cannot be accurately determined.

#### 2. ACUTE HYBRID LEUKAEMIA DIAGNOSED BY TWO COLOUR FLOW CYTOMETRY

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Immunophenotypic studies using immunofluorescent flow cytometry were performed on the blast cells of 50 patients with acute leukaemia using a panel of eight monoclonal antibodies. Six patients had blast cells which expressed markers for both lymphoid and myeloid differentiation, therefore defined as acute hybrid leukaemias. Peripheral blood counts were variable, however, bone marrow infiltration were extensive (blasts >75% in all). None of these patients achieved remission yet. The data suggested that acute hybrid leukaemia was not rare and represented a subset of acute leukaemias identifiable by immunophenotyping but eluded the French-American-British (FAB) classification. The two colour flow cytometry proved to be a useful tool for diagnosis and classification of acute leukaemia.

#### 3. FLOW CYTOMETRIC ANALYSIS (FCM) OF NUCLEAR DNA CONTENT IN NEUROBLASTOMA

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Neuroblastoma (NBL) is one of the commonest malignancy in childhood and is usually associated with a poor prognosis despite recent advances in diagnosis and treatment. Well known prognostic factors are age, stage of disease, resectability and histological grading. In recent

years, N-myc oncogene and DNA content have been found to correlate well with prognosis.

In this study, we analysed the nuclear DNA content of NBL cells obtained from deparaffinised formalin-fixed tissues. There were 12 patients, age ranging from 12 months to 7 years. There was 1 stage 1, 2 stage 2, 6 stage 3 and 3 stage 4 diseases. 7 patients were found to have the diploid mode and 5 were aneuploid. These findings were correlated with the Shimada's histological classification and survival of the patients. All 7 patients with diploid DNA content were found to have a bad prognosis and the 5 patients with aneuploid DNA content had a good prognosis.

FCM analysis of nuclear DNA content is possible on formalin-fixed tissues and the results obtained from NBL cells correlate well with prognosis.

#### 4. LEUCONOSTOC SPECIES - ITS SIGNIFICANCE IN' BLOOD CULTURES

*ML Ling*

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*Leuconostoc* species, a gram-positive coccal bacterium that is classically moderately susceptible to ampicillin and penicillin but resistant to vancomycin is a potential pathogen in the immunocompromised host.

28 blood isolates of *Leuconostoc* species were collected in the year 1989-1990 by the Diagnostic Bacteriology section of the Department of Pathology, Singapore General Hospital. Case records of 19 of these patients were traced and reviewed. 5 of these patients were not treated for the infection but recovered uneventfully. In 8 of them, other organisms were isolated together with *Leuconostoc* species. This poses a question of the clinical significance of a *Leuconostoc* species isolated from a blood culture specimen.

#### 5. NON-AMYLOID FIBRILLARY GLOMERULONEPHRITIS: A CLINICOPATHOLOGICAL STUDY

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This study details the clinicopathological features of 23 cases of non-amyloid fibrillary

glomerulonephritis (FGN) from the files of the Brigham and Women's Hospital between 1979 and 1990.

Renal biopsies were prepared for light, immunofluorescence and electron microscopy according to conventional methods. Additional sections were stained with Congo red. Fibril thickness was determined ultrastructurally by comparison with a 28,800 line per inch grating. Clinical data was obtained from request forms, referral notes and followup inquiries.

The ages of FGN patients ranged from 18 to 81 years with a mean of 50 years. The male:female ratio was 1:2. The majority of patients presented with haematuria (44%), proteinuria (44%), nephrotic syndrome (35%) or hypertension (30%), often with renal insufficiency. Histology showed a diffuse mesangioproliferative (39%) or mesangiocapillary (26%) pattern and occasional focal proliferative, focal sclerosis or crescentic features. The classical morphological findings were: glomerular capillary thickening and wrinkling (90%), mesangial proliferation (80%), mesangial and capillary PAS-positive, Congo red-negative globules (70%), and hypertensive vasculopathy (>50%). Immunofluorescence examination typically revealed irregular, coarse linear positivity for polyclonal IgG. Ultrastructurally, diagnostic fibrillary-tactoid deposits (10-20 nm width) were present in the glomerular basement membrane and mesangium.

FGN is a recently described primary glomerulopathy of uncertain aetiology, commonly confused with amyloid glomerulopathy. It is hoped that with increasing awareness, a clearer understanding of its pathogenesis, natural history and prognosis will emerge.

#### 6. CONGENITAL MESOBLASTIC NEPHROMA: A CLINICOPATHOLOGICAL STUDY

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In a 10 year period of 1982-1991, 9 patients with congenital mesoblastic nephroma (CMN) were seen at Universiti Kebangsaan Malaysia, Kuala Lumpur. They were all less than 6 months old ranging from 2 days to 5 months of age. There were 5 males and 4 females. All presented with an abdominal mass. 2 patients had associated macroscopic haematuria and another 2 were hypertensive. There were 5 involving the right

kidney and 4, the left kidney. A diagnosis of renal tumour was made on ultrasonography in all patients and the diagnosis of CMN was made on fine needle aspiration in 6 patients. Total nephrectomy was performed on all 9 patients. Histologically, there were 5 patients with a cellular type and 4 patients with an ordinary type of CMN. All patients are alive and well on follow-up.

## 7. ENDOMETRIAL CHANGES IN ECTOPIC PREGNANCY

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In the absence of chorionic villi, trophoblastic tissue or foetal parts, it is difficult to determine, by examining the curettings, if a pregnancy is intrauterine or ectopic.

We aim to identify endometrial changes that would raise our index of suspicion to the presence of an ectopic pregnancy.

Twenty-six cases of confirmed ectopic pregnancies with curettage specimens over a one year period in Kandang Kerbau Hospital were analysed. As controls curettings from twelve unwanted pregnancies and twenty-two missed abortions were randomly selected to form the group of intrauterine pregnancies.

We looked for the Arias-Stella change, noting its degree and proportion of glands affected; presence of decidua; dilated muscular spiral arterioles; in addition to other histological features.

Our findings showed an Arias-Stella change in the endometrium of 69% of the ectopic pregnancies, with over a third (39%) demonstrating a florid pattern. This compares with the intrauterine pregnancies where 59% were positive for the Arias-Stella phenomenon and only 20% showed florid changes. Decidua was found in all cases of intrauterine pregnancies, but in only 62% of the ectopics. Spiral arterioles occurred in both groups, but significant dilatation was seen only in the intrauterine cases (71%) ( $P < 0.05$ ).

Although a florid Arias-Stella reaction and absence of decidua in a curettage specimen from a pregnant patient may indicate that an ectopic pregnancy must be excluded, the most useful parameter is the absence of dilated spiral arterioles.

## 8. LIPID PROFILES OF THE POPULATION OF KELANTAN MALAYSIA

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(Abstract not available at time of printing)

## 9. RUPTURE OF STOMACH AND DIAPHRAGM - SPONTANEOUS OR TRAUMATIC?

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A nine month old baby was found dead in her cot by her "single parent" mum in the morning, when she went to give the baby's feed. The previous evening, the mother, having fed the baby tucked her in bed before going out for the evening. To keep the room warm (as it was winter) she switched on the single strip fire heater although the central heating system was on. As the child was crying she accepts that she punched on the baby's abdomen twice. At the post-mortem examination there were no external injuries. Internally the only positive findings were of the ruptured left leaf of diaphragm and fundus of stomach. Was it a traumatic or spontaneous rupture? What is your verdict?

**POSTER PRESENTATIONS:****P1. PREVENTION OF RESORPTION BY CEMENTOID AND ENAMEL EPITHELIUM: A FALLACY***Hashim b Yaacob**Dental Faculty, University Malaya, Kuala Lumpur*

Many authorities believe, though without any scientific proof, that external resorption of teeth is brought about as a result of the loss of cementoid around the roots or the reduced enamel epithelium of the crowns. To determine the validity of such claims, three *Macaca mulatta* were used to study whether the removal of such tissues would lead to external resorption. Selected areas of cementum from the anterior teeth were scraped away to expose root dentine after a mucoperiosteal flap was raised. The teeth were examined microscopically 12 months later. The crowns of unerupted molars of the monkeys were surgically exposed and the reduced enamel epithelium over the crown were scraped away to expose the coronal enamel to the surrounding connective tissues. The wounds were closed and the molars were microscopically examined when they erupted. The findings were exactly contra to the popular belief above, as the loss of the said tissues did not result in resorption. Instead, the cementum were replaced by repair tissues of variable structures. The crowns, on eruptions were normal. Assuming that these findings are also true in humans, then any dental procedures that involve the unavoidable removal of cementum or the reduced enamel epithelium may be carried out safely. It is concluded that in *Macaca mulatta*, the belief that the loss of cementoid or the reduced enamel epithelium results in tooth resorption is now rejected.

**P2. METASTATIC FOLLICULAR CARCINOMA OF THE THYROID PRESENTING AS PULSATING MASSES - A REPORT OF TWO CASES DIAGNOSED BY FINE NEEDLE ASPIRATION CYTOLOGY (FNAC)***Sharifah NA, Zaini Marsan, Jalil J\*, F Meah†**Department of Pathology and Surgery\*,  
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Case No.1: A 74 years old Indian lady presented with 1 year history of a pulsating mass

in the scalp measuring 3x3 cm. She also had a thyroid swelling of more than 10 years' duration.

Case No.2: A 62 years old Malay lady presented with a large diffuse thyroid swelling of more than 10 years and a pulsating sternal mass measuring 3x2 cm which was of 3 months duration.

Both patients were referred for fine needle aspiration cytology (FNAC) to plan their management. FNAC of the enlarged thyroid gland, pulsating scalp and sternal masses yielded cellular material which were heavily bloodstained. Neoplastic cells appeared to have a follicular pattern with scanty colloid. A diagnosis of metastatic follicular carcinomas of the thyroid was suggested. Total thyroidectomy specimens confirmed the FNAC diagnoses.

**P3. FINE NEEDLE ASPIRATION CYTOLOGY OF A CASE OF LANGERHANS CELL HISTIOCYTOSIS - A CASE REPORT***Sharifah NA, Megat Radzi, Suraya Hussain\*, Janie Lau\*\***Department of Pathology, Universiti  
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A 2 year old female Malay child presented with generalised papular rashes over the scalp, trunk and abdomen of 3 months duration with bilateral cervical lymphadenopathy and hepatosplenomegaly. She also developed spiking fever in the ward. She was referred to the Cytopathology Unit, UKM for fine needle aspiration of the enlarged cervical lymph nodes. Fine needle aspiration cytology of the cervical lymph node yielded cellular material composed of a monotonous population of cells with abundant cytoplasm and peripherally placed clefted nuclei. Immunohistochemical studies showed S100 positivity. Electron microscopic analysis of the aspirated material showed presence of Birbeck granules, confirming the diagnosis of Langerhans cell histiocytosis.

#### **P4. HANDLING OF FINE NEEDLE ASPIRATION SPECIMENS**

*Kent Mancer.*

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Ability to make a diagnosis on FNA specimens will be limited if the fine needle aspirate is entirely used to produce only smears.

We utilize several additional techniques depending upon the type of case.

A trolley is brought to the aspiration site, equipped with a microscope, fixatives and stains.

The specimen is expressed directly from the bore of the needle to a glass slide, smeared with a second slide to produce two identical smears, one of which is immediately fixed in 95% ethanol, and the other air dried. One of these is stained and examined immediately. An adequate specimen is defined as allowing a good working diagnosis after examination of the smear. If inadequate, the procedure is repeated.

If the examination indicates an unusual diagnostic problem, additional aspirates should be done to obtain material for cell block sections, which may be used for electron microscopy, special stains, and immunohistochemistry. If a lymphoma is being considered additional air dried slides may be made for immunohistochemistry.

The syringe is rinsed with balanced salt solution to obtain cells or fragments of tissue that have entered the barrel of the syringe. This can be used for cytospin or filter preparations.

Grossly bloody specimens should be allowed to clot, are placed in formalin and histologic sections are made. Fragments of tissue trapped in the clot may allow histological diagnosis. In general, large amounts of blood indicate the need for repeat aspiration.

In our experience there have been cases where a specific diagnosis was only possible on one of the following: air dried smear, fixed smear, washings, sections of the cell block, immunohistochemistry, and electron microscopy of cell block sections.

Optimum handling is dependent on making correct management decisions at microscopic examination of the specimen at the aspiration procedure.

#### **P5. MASSIVE OESOPHAGOSTOMIASIS IN MAN: THE FIRST REPORTED CASE IN MALAYSIA**

*Norain Karim, C.K.Ow Yang*

A case of oesophagostomiasis, producing pseudotumours of large intestine in 8 years old Malay boy from Grik, Perak presenting with features of acute peritonitis probably due to ruptured appendix is described. Oesophagostomum commonly invades apes, monkeys and herbivora but is rare in man. To date, 70 human infestation were reported in the literature, mainly from Africa and a few from South America and Indonesia. This case appears to be the first reported in Malaysia. The pathology, parasitology and the difficulties in differential diagnosis are discussed, especially with reference to the need for increased awareness of the disease in the country.

#### **P6. PRIMARY SKIN MALIGNANCIES IN HOSPITAL BESAR, KUALA TERENGGANU FROM JANUARY 1990 TO JUNE 1991**

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A retrospective study of primary skin malignancies in Hospital Besar Kuala Terengganu from January 1990 to June 1991 was done to show the prevalence and types of malignant neoplasm. A total of 217 primary neoplasms from various organs were seen during this 18 months period. 21 malignant neoplasms were seen in the skin giving an incidence rate of 9.7%. The types of malignant neoplasms were squamous cell carcinoma (9 cases), basal cell carcinoma (9 cases), malignant melanoma (1 case) and verrucous carcinoma (2 cases). 6 of the 9 cases of squamous cell carcinoma were located in the lower limbs and the presenting age ranged from 47 to 73 years. 8 of the 9 cases of basal cell carcinoma were located in the head region and the presenting age ranged from 27 to 80 years. Malays formed the predominant racial group in all these cases which correlated with the hospital admissions.

## W. MALIGNANT MELANOMA - A RETROSPECTIVE STUDY OF 14 CASES

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Fourteen cases of malignant melanoma were diagnosed in the Pathology Department, UKM between the year 1985 and 1990. The distribution among races were as follows:

Malays: 8, Chinese: 5, Indians: 1. 43% were aged 60 years or above and none was below 20 years. 8 out of 14 cases were located in the trunk. Seven cases had metastasis on presentation and one presented with metastasis 2 years after excision of primary lesion.

## P8. THE EFFECTS OF EDIBLE OILS ON THE ULTRASTRUCTURE OF COMMON CAROTID ARTERY OF THE RAT

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It has been shown that monkeys fed a high cholesterol diet of egg yolk, butter and supplemented with cholesterol show thickening of the intimal layer and development of arteriosclerosis. Coconut oil and ghee have also been reported to cause thickening of the intimal layer. Since palm oil is widely used in Malaysia, it would be appropriate to make a comparative study of its effects with that of the other edible oils on ultrastructure of the artery.

12 rats aged 3 months were divided into 5 groups, the control group was fed with rat chow only, the other 4 groups were fed rat chow containing either 20% w/w of palm oil, soya bean oil, coconut oil or ghee for a period of nine months. The common carotid artery of the rats were then prepared for electron microscopic study.

There was an obvious thickening of the intimal layers of the ghee and coconut oil fed rats, compared to the control, palm oil and soya bean fed rats. No change was noted in their medial layer.

The effect of palm oil on the intimal layer of

the common carotid artery resembles more closely the effect of soya bean oil rather than coconut oil or ghee. This observation at the subcellular level is in agreement with its effect on lipid profile which resembles soya bean oil rather than coconut oil.

## P9. PREVENTION OF LIPID PEROXIDATION-INDUCED ERYTHROCYTE MEMBRANE-DAMAGE BY ALPHA-TOCOPHEROL

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Erythrocyte membrane-damage following lipid peroxidation (LPX) could be demonstrated as the result of an autocatalytic activation of free radicals leading to hemolysis. Alpha-tocopherol is an essential component within the erythrocyte membrane which prevents lipid peroxidation. Therefore procedures resulting in an increase membrane alpha-tocopherol (a powerful free radical scavenger) will prevent the lysis of the membrane following LPX. Erythrocyte malonaldehyde (an intermediate product of LPX) formation was examined. Following alpha tocopherol supplementation erythrocyte malonaldehyde content was found to be significantly decreased ( $P < 0.05$ ) from  $16.1 \pm 6.54$  nmol/g protein ( $N=28$ ) to  $10.32 \pm 1.85$  nmol/g protein (decreased by 36%). This finding suggested that increased free radical induced LPX could be prevented partly by maintaining an adequate level of vitamin E in erythrocyte membrane.

## P10. AN ASSESSMENT OF ANTIOXIDANT STATUS OF BLOOD FROM THALASSAEMIC PATIENTS

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Anemia is a feature in thalassaemia intermedia and homozygous beta thalassaemia patients. The cause of anemia is multifactorial which include ineffective erythropoiesis, hypersplenism and folic acid deficiency. Another possible predisposing factor may be an increased erythrocyte membrane fragility (as shown by decreased red cell survival) due to generation of

free radicals. This study assessed the plasma antioxidant status and the level of lipid peroxides in stressed erythrocyte from thalassaemic patients. Lipid peroxides induce erythrocyte hemolysis in vivo and in vitro. An adequate amount of antioxidants such as caeruloplasmin and transferrin are required to cope with this stress. Blood samples from 12 healthy donors and 28 thalassaemia patients were examined for the caeruloplasmin and transferrin content, total antioxidant activity (AOA), and erythrocyte susceptibility to lipid peroxidation induced by hydrogen peroxide. The latter was monitored by malonaldehyde content in erythrocytes. The findings showed significant positive correlation between (1) plasma caeruloplasmin content and erythrocyte malonaldehyde ( $r=+0.88$ ,  $p<0.01$ ) and (2) plasma total antioxidant activity and erythrocyte malonaldehyde ( $r=0.81$ ,  $p<0.01$ ). However no significant correlation was found between transferrin and malonaldehyde content.

#### **P11. FAMILY STUDY OF ALPHA-THALASSAEMIA BY GENE ANALYSIS**

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The varied clinical severity in the alpha-thalassaemia syndromes depend on the number of alpha globin genes deleted. The diagnosis of the two most severe forms i.e. Barts Hydrops foetalis (genotype  $--/a$ ) and Hb-H disease (genotype  $--/--$ ) can easily be made by routine haematological studies. However, the identification of the heterozygous carriers can only be made with certainty by doing DNA analysis. We performed DNA analysis in a family study of a 42 year old Chinese patient with Hb-H disease. DNA was extracted from peripheral blood of propositus, his wife and four siblings. To determine genotype, gene mapping studies were carried out by using restriction enzymes Bam HI and Bgl II and  $p^{32}$ -labelled alpha and zeta gene specific DNA probes. The findings confirmed the three alpha globin genes deletion ( $---/\alpha$ ) of Hb-H disease in propositus. One sibling whose red cells indices were low showed loss of two genes (genotype  $--/\alpha\alpha$ ) and the other three siblings, although haematologically normal showed a single gene

deletion (genotype  $-\alpha/\alpha\alpha$ ). This study illustrates the usefulness of DNA analysis in the diagnosis of alpha thalassaemias, especially in the identification of heterozygous carriers.

#### **P12. A FREEZE-DRIED METHOD FOR PREPARATION OF G6PD REAGENT TUBES**

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Mixed reagents for the Glucose-6-phosphate dehydrogenase deficiency (G6PD) fluorescent screening test were freeze-dried in plastic tubes. The reagents were then reconstituted with distilled water and the test was performed in the standard way. Testing with the freeze-dried mixed reagents gave consistent positive reaction to 12 normal blood samples and negative reaction to 9 G6PD deficient blood samples. This will enable a laboratory with freeze-drying facilities to prepare reagent tubes in bulk. As these tubes can be kept at  $4^{\circ}\text{C}$  and do not require to be stored at  $-20^{\circ}\text{C}$ , a major laboratory can prepare these tubes and supply small laboratories for screening purposes. Stability studies had been performed on the reagents. The shelf-life at room temperature was 1 week whereas at  $4^{\circ}\text{C}$  was 4 months. We conclude that freeze-drying mixed reagents for screening G6PD is a viable method of preparation and of commercial potential.

#### **P13. SERUM DEOXYTHYMIDINE KINASE GIVES PROGNOSTIC INFORMATION IN ACUTE LEUKAEMIAS**

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A recently developed deoxythymidine kinase assay, utilizing [2-C14] thymidine as substrate, and capable of detecting normal levels of serum deoxythymidine kinase (sdTK), was used in an investigation of sera from 21 untreated patients with acute leukaemias (12 cases of acute lymphoblastic leukaemia [ALL] and 9 cases of acute myeloblastic leukaemia [AML]). The results showed a significant correlation between sdTK level and disease status, i.e. the more ad-

vanced the disease, the higher the s-dTK values. Greater than 100-fold increases in s-dTK levels were found in some patients compared to those reported for healthy individuals. A high pretreatment level of s-dTK correlated with a poor prognosis for the patient. In ALL, the mean pretreatment s-dTK level was 126.5 U/L, whereas in AML the mean level was 72.6 U/L. Longitudinal studies of s-dTK levels in 14 acute leukaemia patients showed that s-dTK increases when the disease is active, decreases during successful therapy, and finally increases during relapse.

#### **P14. GENE REARRANGEMENT IN THE DIAGNOSIS OF A CASE OF ACUTE LEUKAEMIA MIMICKING SYSTEMIC LUPUS ERYTHEMATOSUS (SLE) - A CASE REPORT**

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A 16 year-old Chinese patient was referred to UKM Haematology Unit as a case of aplastic anaemia. Full blood picture done here revealed about 10% atypical lymphoid cells and trephine biopsy showed that the bone marrow was fibrotic and in part normal. In view of the manifestations including photosensitive skin rashes, multiple joint pains and erythema nodosum, a diagnosis of collagen-vascular disease was made. He responded clinically to prednisolone. LE cells and ANF (antihnuclear factor) were found to be negative. DNA analysis was carried out to look for immunoglobulin and TCR (T-cell receptor) genes rearrangements. The results showed a rearrangement in the immunoglobulin J-H gene indicating a clonal disease of B-cell leukaemia/lymphoma.

#### **P15. A PRELIMINARY STUDY OF RICKETTSIAL DISEASES IN TERENGGANU FROM JANUARY 1990 TO JUNE 1991**

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Rickettsial diseases are endemic within the Asia-Pacific region. Scrub typhus is a common cause of febrile illness throughout Malaysia. Taylor and Kelly have shown that *R. tsutsugamushi* (scrub typhus) specific antibody prevalence rates vary within Peninsular Malaysia

from 6% in urban areas to 69% in rural areas.

This study showed the number of active cases detected by the Serology Unit, Makmal Kajipenyakit, Hospital Besar Kuala Terengganu during the study period. Specimens were obtained from patients in the four district hospitals in Terengganu. The method used for diagnosis was the Immunoperoxidase test as recommended by IMR (Institute of Medical Research Kuala Lumpur).

Results showed a total of 221 cases of Rickettsial diseases with 165 cases (74.7%) of scrub typhus, 22 cases (9.9%) of tick typhus, and 34 cases (15.4%) of endemic typhus.

Therefore the prevalence rate of scrub typhus in Terengganu is higher than that quoted by Taylor and Kelly for the rural areas. This study also showed an increasing number of cases towards the end of 1990 with the maximum in the month of October.

#### **P16. SQUAMOUS CELL CARCINOMA RELATED ANTIGEN: ITS USE AS A TUMOUR MARKER IN CERVICAL CARCINOMA**

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Squamous cell carcinoma related antigen (SCC-Ag) is a glycoprotein which is being increasingly recognised as a marker for various squamous cell carcinomas. In January 1989, we embarked on a prospective multivariate study, using the Abbott SCC-RIA diagnostic kit, to determine the importance of measuring serum SCC-Ag levels in our patients with cervical carcinoma. As part of this study, we analysed the association between serum SCC-Ag level and tumour bulk. Twenty-three cases of cervical carcinoma with stage 0 to 2a disease, who had undergone curative surgery, and had post-operative serum specimens available for analysis were studied. There were 4 stage 2a, large cell non-keratinising carcinoma (LCNK). Eight LCNK, 1 adenosquamous carcinoma (AS), 1 small cell carcinoma (SC) and 2 adenocarcinoma (AD) respectively were in stage 1b. Two microinvasive carcinoma (MI) and 5 carcinoma-in-situ (CIS) were also included. Preoperative serum SCC-Ag levels were raised above the arbitrarily selected cut-off value of 2 ng/ml in 4 LCNK, 2 of which were in stage 2a and 2 in stage 1b; 1 AS (stage 1b); and 1 CIS. After surgical

removal, SCC-Ag levels dropped below 2 ng/ml in all these cases whereas those with pre-operative serum SCCAg levels below the cut-off value remained so post-operatively. Hence it appears that serum SCC-Ag elevation correlates with the amount of tumour tissue present, perhaps better with actual tumour bulk than clinical stage. However, it is noteworthy that among other factors, the degree of squamous differentiation, as described in an earlier report, also plays a role in determining the final serum SCC-Ag value attained.

#### **P17. AN IMMUNOCYTOCHEMICAL STUDY OF PLASMA CELL PROLIFERATIVE LESIONS**

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Monoclonal plasma cell proliferative diseases such as multiple myeloma and plasmacytoma can involve extramedullary sites at the time of first presentation, or subsequently in the course of the disease. It is not uncommon that both clinically unsuspected cases and cases with presentation at unusual sites can mimic primary or metastatic carcinomas, neuroendocrine or neuroectodermal tumours and lymphomas. We studied 10 cases of previously confirmed monoclonal plasma cell proliferative lesions, retrieved from the files in the Department of Pathology, University of Malaya. We aimed to study the morphology and immunohistochemical properties of these lesions. Serial 4 $\mu$  thick paraffin sections were examined. They were stained with H&E, Unna-Pappenheim technique for nucleic acid, and a panel of common antibodies using standard immunoperoxidase technique.

Most of the tumours resembled plasma cells of varying degrees of maturity and were stained positive with Unna-Pappenheim technique. However, a number of these lesions bear minimal resemblance to plasma cell and formed distinct packets, simulating carcinomas.

Immunohistochemical staining showed that these tumours demonstrated light chain restriction: 7(70%)  $\kappa$  and 2(20%)  $\lambda$ . The remaining 1 case was not stainable with all the antibodies in the panel. Majority (80%) of these tumour cells had accompanying IgG heavy chain in the cytoplasm, and 1 case had IgA. Immunohistochemical staining for  $\kappa$  and  $\lambda$  light chain is helpful in differentiating plasma cells of monoclonal from poly-clonal origin. Seven (70%) of these tumours

showed membrane positivity with antibody to epithelial membrane antigen (EMA). This study enhances our awareness that both normal and neoplastic plasma cells can be EMA positive, and cautions us from misinterpreting these lesions as carcinomas.

#### **P18. INTENSIFICATION OF THE END-PRODUCT OF THE DIAMINOBENZIDINE (DAB) REACTION . IN PEROXIDASE HISTOCHEMISTRY**

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Peroxidase histochemistry has been widely used in medical research to study tissue disease. Lately, several techniques have been introduced in an attempt to intensify the end product of DAB to demonstrate the activity of peroxidase. In this paper we present 4 cases on which this technique was applied.

3  $\mu$  tissue sections were subjected to the usual immunoperoxidase technique. Slides were then washed in deionised water and incubated for 10-30 minutes with silver solution (modified from Methenamine Impregnation method) at 60°C. Positive DAB reactions which normally show up as light brown deposits in the cells appear intensified in all 4 cases.

This modified method is simple and the results more satisfactory.

#### **P19. ALPHAFETOPROTEIN AND T-CELL HELPER/SUPPRESSOR RATIO IN HBSAG-POSITIVE INDIVIDUALS**

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It is well recognised that alphafetoprotein concentrations may be elevated in subjects yielding a positive test for hepatitis B surface antigen (HBsAg) and that chronic infection with hepatitis B virus (HBV) has a strong association with the development of primary hepatocellular carcinoma (HCC). It has also been reported that there is a decrease in helper to suppressor T-cell ratio (HIS) in HBV-induced liver diseases. This preliminary study was undertaken to determine

whether there is any correlation between alphafetoprotein and peripheral T-cell subsets in HBsAg-positive individuals. There was no significant difference between the proportions of helper to suppressor T-cells in the peripheral blood of normal (n=22) and HBsAg-positive individuals (n=14), the H/S ratio being  $0.9 \pm 0.32\%$  of lymphocytes for the normal and  $0.75 \pm 0.64\%$  of lymphocytes for the HBsAg-positive individuals. The mean alphafetoprotein for the HBsAg-positive individuals is four times (12.13 IU/ml) that of the normal (3.75 IU/ml). However, no significant correlation was observed between AFP and H/S ratio ( $r=0.17$ ).