

# Abstracts of the First Joint Meeting of the Singapore Society of Pathology and the Malaysian Society of Pathologists.

The first combined meeting of the Singapore Society of Pathology and the Malaysian Society of Pathologists was held at the National University Hospital, **Singapore** on 5–7 September 1990. Abstracts of the scientific communications follow:

## ORAL PRESENTATIONS:

### 1. COLORECTAL CARCINOMA IN ADULTS UNDER THE AGE OF 40 YEARS – THE UNIVERSITY HOSPITAL EXPERIENCE.

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2. Former Assoc. Professor, Department of Surgery, University Hospital, Kuala Lumpur, Malaysia.

A total of 430 cases of colorectal carcinomas were **diagnosed** in the University Hospital, Kuala Lumpur over a 14-year period. 37 cases (8.6%) were under the age of 40 years. These cases were reviewed and aspects of the clinical presentation, operative **findings**, pathological features and response to treatment were analysed.

The disease was predominant in Chinese (**p<0.01**) who formed 70% of cases. The mean age was 32.6 years and the **male:female** ratio was 1.3:1. 82% of cases presented in Duke's stage B, C or with distant metastases. The mean 5-year survival time was 30 months (sample size = 24). Polyps were present in 27% of cases. Only 5% of the tumours were well differentiated.

The results showed that **poorly** differentiated tumours infiltrated in a diffuse manner (**100%**), produced abundant **mucin** (89%), elicited a poor lymphocytic response (80%) and were associated with a poor prognosis.

### 2. RENAL TUMOURS OF CHILDHOOD

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Renal tumours occurring in children at **UKM**, Kuala Lumpur from 1980 to 1990 were reviewed. 43 cases were available for study, of which 38 were malignant.

Wilms' tumours accounted for 33 cases, 87% of the malignancies. Of these 4 (12%) were the anaplastic type.

There were 2 cases of rhabdoid sarcoma and 3 cases of clear cell sarcoma. These two types of sarcomas, along with the anaplastic type of Wilms' tumour comprised the 'unfavourable histology' group of renal tumours of childhood. 'Unfavourable histology' tumours have a poor prognosis, which can be improved with more intensive radiotherapy and chemotherapy regimens.

These 'unfavourable histology' tumours and benign congenital mesoblastic nephroma have a higher incidence in this series than that in the US. The Western incidence of anaplastic Wilms' tumour is **7.2%**, clear cell sarcoma 2.9% and rhabdoid sarcoma 1.3% of all childhood renal malignancies, versus **10.5%**, 7.9% and 5.3% respectively in this series.

There were 5 cases of congenital mesoblastic nephroma, of which 3 were the cellular variant. This was 11.6% of all renal tumours in this series, compared with a 5.4% incidence in the West. CMN is treated by nephrectomy and seldom requires any additional treatment.

The recognition of these entities as distinct from Wilms' tumour **is important** as there are significant differences in management.

### 3. LECTIN HISTOCHEMISTRY OF OVARIAN MUCINOUS CYSTADENOMAS

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The lectin histochemistry of ovarian **mucinous** cystadenoma was studied using a panel of lectins comprising Triticum vulgare, Lotus tetragonolobus, Arachis hypogaea, Bandeiraea simplicifolia I, Concanavalin A and Dolichus biflorus. **All** of the 23 cases examined in this study stained extensively with Triticum **vulgare**. Of all the lectins, **Dolichos** biflorus was the least reactive. Concanavalin A stained mainly the perinuclear zone while the other lectins stained frequently the cytoplasm, glycocalyx and extracellular luminal mucin. This pattern of lectin reactivity

resembles endocervical more than intestinal epithelium and suggests that the ovarian **mucinous** cystadenomas are of Mullerian origin. The lack of difference in lectin reactivity between cystadenomas with and without **goblet/Paneth** cells supports a common histogenetic origin in both groups.

#### 4. OESTROGEN RECEPTOR RELATED PROTEIN IN BREAST CARCINOMA: AN IMMUNOHISTOCHEMICAL AND BIOCHEMICAL STUDY

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Breast carcinoma removed by excision or mastectomy from 31 unselected patients at the University Hospital, Kuala Lumpur were studied for oestrogen receptor (ER) status by both immunoenzymic and immunohistochemical methods. Tumour tissue was received fresh and sampled for the 2 methods of assay accordingly.

Fresh tissue was snap-frozen and stored at  $-70^{\circ}\text{C}$  prior to enzyme immunoassay (EIA) for ER cytosol protein (Abbott). Tumours with ER values of  $>15$  fmol/mg cytosol protein were considered ER positive. Immunohistochemistry for ER status was performed on **formalin-fixed paraffin-embedded** material using the **avidin biotin complex** method. Monoclonal antibodies against cytoplasmic ER related protein was obtained commercially (Amersham). A positive ER reaction was recognised by the presence of dark brown granular staining in the cytoplasm of tumour cells. The nucleus was not stained. The corresponding H & E sections of the tumours were reviewed for classification and grading of the lesions.

**24** tumours (77%) were found to be ER related protein positive by immunohistochemistry while 16 (52%) were positive by EIA. There was acceptable comparability between both assays. Well differentiated carcinomas were more often ER positive than poorly differentiated tumours. No relationship was demonstrated between the histological types of breast carcinoma and ER status. Patients older than 60 years had a higher percentage of ER positive cancers. No

correlation **was** shown between ER status and prevalence of lymph node metastasis or tumour size.

Immunohistochemical assessment of ER related protein status appears to be a good alternative to EIA assay as it is fast, easy to perform, requires little tissue and allows retrospective studies to be carried out.

#### 5. REFLUX NEPHROPATHY AND CHRONIC PYELONEPHRITIS.

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**Reflux** nephropathy is a common cause of renal damage and renal failure. The terms 'chronic atrophic pyelonephritis' and '**reflux** nephropathy' are used interchangeably. However, **reflux** per se may cause renal damage. To determine whether lesions caused by **reflux** can be distinguished histologically from those due to pyelonephritis, we reviewed **54** nephrectomy and partial nephrectomy specimens from 30 males and 24 females (age range 1 month to 17 years) divided by clinical and radiological criteria into **2** groups: **reflux** nephropathy and chronic pyelonephritis. 35 patients had vesico-ureteric **reflux** and **12** had chronic pyelonephritis with urinary tract obstruction; in addition, 7 kidneys showed histological evidence of renal dysplasia and were not considered further.

Refluxing kidneys showed distinctive histological features. The lesions were sharply demarcated from the surrounding renal cortex. There were few nephrons and the residual glomeruli were all small and sclerosed. Unlike chronic pyelonephritis, inflammation was minimal and scar tissue absent. The arteries were thickened and tortuous but the veins were dilated and thin-walled. These **reflux** lesions were often associated with changes of chronic pyelonephritis. We conclude that intra-renal **reflux** produces a histologically distinctive lesion and predisposes to pyelonephritis.

## 6. **PYELONEPHRITIS: THE SIGNIFICANCE OF RENAL RENIN AND THE VASCULAR CHANGES IN THE HUMAN KIDNEY.**

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Hypertension complicates chronic pyelonephritis. Since arterial narrowing is common in the damaged kidney, activation of the **renin-angiotensin** system due to renal ischaemia has been suggested as a pathogenetic mechanism. We used an antiserum to human renin and immunoperoxidase technique to study the anatomy of **renin-containing** cells (RCCs) in 18 kidneys removed for **pyelonephritis**. We independently assessed the degree of arterial narrowing and correlated these variables with the clinical **findings**. There was histological evidence of hyperplasia and RCCs in 5 of the 6 hypertensive patients and 7 of the 12 non-hypertensive cases. There was no difference in the apparent number or distribution of RCCs between the hypertensive and non-hypertensive cases. Also, the **degree** of arterial narrowing did not correlate with either the hyperplasia of RCCs nor with the **blood** pressure of the patients. Our results do not support the hypothesis that narrowing of the intrarenal arteries is important in the pathogenesis of hypertension in pyelonephritis. These raise the possibility that the arterial narrowing may be the consequence rather than the cause of the renal scarring in pyelonephritis. In our cases the renal veins were more severely damaged than the arteries and their lumina were often obliterated by organised thrombus. We suggest that such widespread obliteration of the renal venous tree could impair blood flow and contribute to the tissue damage in the pyelonephritic kidney.

## 7. **FINE NEEDLE ASPIRATION CYTOLOGY: A NEW CONCEPT IN DIAGNOSIS IN PAEDIATRIC PRACTICE**

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Fine needle aspiration cytology (FNAC) is widely used as a diagnostic tool in adults.

However, there has been very little experience with this technique in children. At the Universiti Kebangsaan Malaysia, FNAC has been increasingly used in the under-16 population, resulting in the saving of operating time and patient days in hospital.

In the past 18 months, we carried out 127 FNA on 114 patients. The types of conditions included solid tumours of the head and neck, thorax and abdomen, benign and malignant lymphadenopathy and benign and malignant lesions of soft tissue and bone. A definite cytological diagnosis was made in 124 aspirates (98% pick-up rate). There were no false positive or false negative results when benign versus malignant lesions were compared. Histological correlation was available in 53 patients and was **accurate** in 46 cases (87% specificity rate).

The technique is rapid, accurate, cost-effective, labour saving, atraumatic and requires no anaesthesia. It is well tolerated by infants and older children. Based on this experience, we would like to advocate the use of FNAC as a simple diagnostic procedure in paediatric practice.

## 8. **THE ASSAY OF VARIOUS MUTATIONS IN THE BETA-GLOBIN GENE OF BETA-THALASSAEMIA PATIENTS IN MALAYSIA.**

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Oligonucleotide probes were synthetically prepared using a DNA synthesizer such that these probes were homologous in structure to known mutations associated with the **beta**-thalassaemia phenotypes. Beta-globin genes from beta-thalassaemia patients were amplified several fold by the polymerase chain reaction (PCR) and analysed in a dot-blot hybridization technique. Various mutations were probed for their occurrence in the beta-thalassaemia patients. We found 14 different mutant genes associated with our beta-thalassaemia patients. 7 different mutations occurred in the **Chinese** patients, 3 in Indians, 2 in Malays. These mutations have been previously **characterized** and occur worldwide. 2 other mutations occurred in Malay patients: one of these was

a novel mutation not previously described before and the other previously described for an American black. This particular mutation occurred spontaneously and was not related to the American black.

9. **PNEUMOCYSTIS CARINII IN PATIENTS WITH AIDS – A STUDY OF 2 CASES AND REVIEW OF DIAGNOSTIC METHODS AND HISTOLOGICAL FEATURES.**

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*Pneumocystis carinii* is an important cause of opportunistic infections in immunodeficient hosts, and usually manifests clinically as a diffuse interstitial pneumonia.

We present 2 patients with AIDS who developed *Pneumocystis carinii* pneumonia.

Both cases were diagnosed via a bronchoalveolar lavage and a transbronchial lung biopsy.

With the number of AIDS cases on the increase, there will be a parallel rise in the frequency of opportunistic infections diagnosed in these patients. *Pneumocystis carinii* pneumonia accounts for over half of such infections.

The diagnosis of *Pneumocystis carinii* can be made by a variety of techniques, with the current trends favouring the more rapid and less invasive methods. These methods as well as the different histological patterns will be discussed.

10. **IMMUNOPHENOTYPIC CHARACTERIZATION AS AN ADJUNCT TO THE SUBCLASSIFICATION OF ACUTE NON-LYMPHOCYTIC LEUKAEMIA.**

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The FAB Classification of acute non-lymphocytic leukaemias (ANLL) has been shown to have some clinical use in the prognosis of patient survival. ANLL with

monocytoid features (M4, MS) fares worse than those which are predominantly myeloblastic (M1, M2). Classification is, however, sometimes difficult if non-specific esterase activity is not strongly expressed. Monoclonal antibody characterisation of myeloid differentiation antigens may be of some use as a supplementary aid. We immunophenotyped the blast cells from 25 cases of ANLL for Ia, CD14 (Mo2, My4), CD13 (My7) and CD 33 (My9) expression using the APAAP method. A combination of CD 14 and CD 33 used together had a high correlation with ANLL M1 and M2 morphological subtypes of the FAB Classification. We conclude that the use of surface marker analysis is a useful adjunct to the diagnostic means presently available.

11. **POLYACRYLAMIDE GEL ELECTROPHORESIS OF PSEUDOMONAS PSEUDOMALLEI TOTAL PROTEIN AS A POTENTIAL METHOD FOR RAPID PRESUMPTIVE DIAGNOSIS OF MELIOIDOSIS**

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Melioidosis is endemic in South-east Asia. The septicaemic form of the disease is frequently fatal. Early diagnosis and treatment are essential for a better prognosis.

Present biochemical methods of identifying the causative agent, *Pseudomonas pseudomallei*, take 24 to 48 hours.

We analysed several isolates of *Ps. pseudomallei* and representative strains of *Ps. cepacia*, *Ps. aeruginosa*, *Ps. maltophilia*, *Ps. putrefaciens*, *Ps. stutzeri*, *Ps. fluorescens*, *Ps. paucimobilis*, *Ps. acidovorans*, *Ps. alcaligenes* and *E. coli* by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE). Although the total protein profiles were complex, distinctive patterns of adequate resolution were obtained which allowed *Ps. pseudomallei* to be readily differentiated from other species.

All *Ps. pseudomallei* isolates tested so far possessed almost identical profiles. The *Ps. cepacia* profiles appeared similar to that of *Ps. pseudomallei* but specific differences in band pattern at several foci, permitted the two related species to be distinguished.

Using a mini-gel format (8 x 10 cm), the complete procedure took less than four hours, from harvesting colonies from a plate to the Coomassie Brilliant Blue-stained gel. This method is currently being assessed as a routine diagnostic technique for the identification of plate-cultured *Ps. pseudomallei* organisms. More cultures of *Ps. pseudomallei* are also being tested to determine the extent of strain variation, if any, among local isolates.

## 12. ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES IN SYSTEMIC LUPUS ERYTHEMATOSUS

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Antineutrophil cytoplasmic antibodies (ANCA) are well established as serological markers for idiopathic crescentic glomerulonephritis and systemic necrotising vasculitis, in particular, Wegener's granulomatosis and microscopic polyarteritis nodosa. Its association with systemic lupus erythematosus (SLE) is less well studied.

We examined ANCA in 77 serum samples from 68 patients with SLE by the indirect immunofluorescence technique using alcohol fixed neutrophil as substrate. Almost half (38/77) of the sera were positive for ANCA. Titres ranged from 1:20 to 1:320. Two patterns of immunofluorescence were seen: speckled cytoplasmic and diffuse cytoplasmic patterns. In the 4 patients with 2 serum samples each taken at a different time, 2 patients demonstrated a different pattern each time.

We conclude that ANCA may be present in patients with SLE and as two patterns of immunofluorescence were demonstrated, it is likely that more than one type of ANCA are present in SLE.

## 13. DOT-BLOT HYBRIDIZATION ASSAY FOR THE DETECTION OF THE HEPATITIS B VIRUS DNA IN SERUM: FACTORS DETERMINING ITS SENSITIVITY AND SPECIFICITY.

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A systemic study was carried out to determine the optimal assay conditions for

the detection of Hepatitis B virus (HBV) DNA in serum. The assay was based on filter hybridization (dot-blot) and the probe used was a purified HBV-DNA which was labelled by standard nick-translation using <sup>32</sup>P dCTP or dATP. Methods for pretreatment of serum samples, blot treatment and prehybridization conditions were found to affect the sensitivity of the assay. The optimum procedure was found to be the indirect serum blot assay. In this assay, the viral particles were first isolated from serum, lysed and their DNA extracted prior to blotting. Although the process time was longer compared to the direct serum spot method, this method was preferred because the results obtained were more reliable. Sensitivity studies showed that labelling with dCTP was more efficient than with dATP. This can be explained partly by the higher proportion of dGTP in the HBV genome. Probes labelled to a specific activity of 1 x 10<sup>7</sup> cpm/ug were found to give a sensitivity of 1 picogram. The specificity of the assay was ensured firstly by using a highly purified probe. Non-specific binding (NSB) of probe to the hybridization paper was minimised by overnight prehybridization and thorough washing of the papers post-hybridization. Negative serum controls were introduced in each experiment to check for NSB. Hybridization of the probe to the vector minus the HBV insert was also negative.

Due to the long processing time of the assay, various modifications to shorten the process time were looked at. Factors that limited the speed of the assay included 1) centrifugation time, 2) proteinase K treatment, 3) prehybridization time and 4) autoradiography time. A modification of the original method that shortened the process time from 11 to 4 days will also be presented.

## 14. HEPATITIS B VIRUS DNA IN SERUM: ITS RELATIONSHIP WITH THE HBe/ ANTI-HBe STATUS

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Parameters used for measuring Hepatitis B virus (HBV) replication include serum level of Hepatitis B e antigen (HBeAg), HBV DNA polymerase (HBV DNAP) activity and HBV DNA concentration. A positive correlation between serum HBeAg and HBV DNA, and

a negative correlation between anti-HBe and HBV DNA is widely reported. However, HBV DNA has also been reported in anti-HBe positive sera. The association between HBV DNA and anti-HBe is described mainly in the Mediterranean and Oriental populations. It has been suggested that this atypical profile may be a reflection of genetic **and/or** unknown environmental factors influencing an early clearance of the HBeAg disparate from that of HBV DNA from **the** serum.

We studied the HBV DNA status in 92 cases of HBeAg positive and in 114 cases of **anti-HBe** positive, asymptomatic, HBV carriers. Our data confirmed published reports on the high frequency of HBV DNA positivity among HBeAg positive carriers (86/92, 93%). Of the 86 cases who were HBV DNA positive, 55 (64%) had high levels of circulating viral genome ( $> 100$  pg/ml). 2 out of the 6 cases who were HBeAg positive but HBV DNA negative showed evidence of sero-conversion.

10 out of 114 (8.8%) **anti-HBe** positive carriers showed the presence of HBV DNA in their sera. All but one of these 13 cases had low levels of viral DNA. The last case had a serum level of 300 pg/ml of viral genome. Serum transaminases were looked at in these subjects 5 out of the 10 carriers (50%) had raised levels of transaminases. In comparison, 9 out of 104 **anti-HBe/HBV** DNA negative carriers (8.7%) had elevated enzymes.

The coexistence of HBV DNA and **anti-HBe** may be due to one of several reasons. A discrepancy between the presence of serum HBV DNA and HBeAg has been noted during seroconversion. Additionally, the virus may persist beyond clearance of the HBeAg in a minority of cases. It is also possible that reactivation of viral replication in anti-HBe positive carriers may occur. The possible **reason(s)** for the atypical profile in our study subjects will be discussed.

#### 15. **IMMUNOHISTOCHEMICAL LOCALIZATION OF THROMBOSPONDIN IN BREAST CANCER**

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Thrombospondin (TSP) is a 420 kD cytoadhesive protein present in the alpha granules of platelets. It plays an important

haemostatic role in platelet-platelet interaction. **In vitro** experiments using cultured malignant cells suggest that it may help to mediate malignant cell attachment to connective tissue matrix. Furthermore, it may be important in cancer invasiveness and metastasis. Using an APAAP immunohistochemical technique, we studied 29 fresh specimens of confirmed breast carcinomas and found **TSP** to be present in the desmoplastic stroma or at the basement membrane associated with the malignant ductal epithelium. Tumour cells abutting these tissues revealed cytoplasmic staining for TSP. Stronger TSP staining was found in the poorly differentiated tumours. The findings suggest an important role for TSP in the tumour biology of **invasiveness** and metastasis.

(This work is generously supported by the National University of Singapore Research Fund for Biological Sciences RP890364 and RP890365.)

#### 16. **MULTIPLE MYELOMA PRESENTED WITH SEVERE SENSORY AND MOTOR NEUROPATHY IN A 51-YEAR OLD MAN – A CASE REPORT**

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A 51-year old Malay teacher from Tumpat, Kelantan was admitted to the General Hospital, Kuala Lumpur for resection of an abdominal aortic aneurysm. For the last 5 months before admission, he had progressive sensory and motor peripheral neuropathy for which he was referred to the neurologist who confirmed the diagnosis peripheral neuropathy by nerve conduction studies and electromyographic examinations. Investigations showed that the patient had **IgA** heavy chain/lambda light chain multiple myeloma by serum immunoelectrophoresis, later confirmed by bone marrow examination. Skeletal survey appeared normal. There was no clinical evidence of amyloidosis. The symptoms improved subjectively after a course of prednisolone and melphalan. The clinical presentations, laboratory results and pathophysiological features of the peripheral neuropathy in multiple myeloma are presented.

**ABSTRACTS OF POSTER  
PRESENTATIONS:**

**P1. IN-HOUSE MICROCOMPUTER  
SOFTWARE PACKAGE FOR MANAGE-  
MENT OF HISTOPATHOLOGY  
REPORTS.**

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**Histopathology** reports are currently stored in files and they are arranged in numerical order according to the laboratory number. This gives rise to difficulty in retrieval of data as one has to sift through many files to locate the report. This is particularly trying when an enquiry is made through the telephone. It is also almost impossible to determine whether a patient has any previous reports.

An in-house programme written in dBASE IV, a database management software with programming facilities, was designed, developed and tested between April and May 1990. The programme contained a friendly user interface with a pull-down menu system where a user can choose the database, update records, enquire and print information. There are also house-keeping utilities for maintaining the databases. The programme is suitable for single users as well as multiple users accessing the same database simultaneously in a local area network environment. After implementation, the programme not only fulfilled the needs mentioned above, but also facilitated research in medical practice.

**P2. A dBASE III SYSTEM FOR  
MANAGING 35 MM PROJECTION  
SLIDES**

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We describe the design of a 35 mm projection slide database and the use of a menu-driven dBASE III-plus programme to manage such slides. Low-cost, easily available, industry-standard computer hardware and software were used.

In the database, each record, which represented a projection slide, had fields for the slide number, case number, slide category, SNOMED codes, and a description of the slide in natural language. These fields

seemed adequate for **slide** definition. The menu-driven programme, written in dBASE language, was used to access and manage this database. Its functions include the abilities to add, delete, edit and back-up records, and to search for desired slides. Although slides may be searched for in various fields, we found that searches using natural language alone to be both comprehensive and efficient, provided a standard format of description was adhered to and data entries were scrutinized carefully for errors.

A complete inventory of our slides to detect omission or under-representation in any subject matter is possible, thus enabling us to build up a more comprehensive slide collection. With a collection of more than 10,000 slides, we found this system to be far superior to the card filing system.

**P3. PRELIMINARY REPORT OF A RENAL  
HISTOPATHOLOGY SERVICE FOR  
OUTSIDE CENTRES.**

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From June 1988, the author undertook a project to provide a diagnostic histopathology service for renal biopsies from the following centres in Malaysia: Penang General Hospital, Ipoh General Hospital, Johor Baru General Hospital and Sarawak General Hospital. From the first 3 centres, biopsies were received by courier service in 2 portions: 1 in 10% buffered **formalin** and the other embedded in OKT compound, wrapped in aluminium foil and kept in a vacuum flask containing dry ice. **Biopsies** from Sarawak were sent in 10% buffered **formalin** via normal parcel post.

14.5% of biopsies were inadequate for interpretation. Immunofluorescence (IF) examination was successful on OKT-embedded material in 85% of adequate biopsies (excluding Sarawak materials). Biopsies from Sarawak were assessed using an immunoperoxidase (IP) method with satisfactory results. 48% of patients were Chinese, 43% Malay, 5% Indian and 4% others. The histopathological patterns encountered were minimal change glomerulonephritis (GN) 30.6%, focal glomerulosclerosis 8.7%, proliferative GN 13.7%, end-stage disease 3.3% and others 5.5%. These findings did not differ much from those encountered in University Hospital patients based on the in-house service.

This project showed that it is possible to establish a viable diagnostic histopathology

service for renal biopsies from outside centres. Occasional problems encountered were related to freezing artifacts, delay in transport of specimens causing failure of IF and difficulties in the interpretation of IP-stained sections.

**P4. THE PATTERN AND IMMUNOCYTOCHEMICAL PROFILE OF HODGKIN'S DISEASE IN THE UNIVERSITY HOSPITAL, KUALA LUMPUR.**

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From 1983 to 1987, 200 cases of histologically confirmed malignant lymphomas were seen in the Department of Pathology, University Hospital. 161 (80.5%) were non-Hodgkin's lymphoma (NHL) and 39 (19.5%) Hodgkin's disease (HD). The ratio of NHL:HD was 4:1. The ages of these patients ranged from 3 to 77 years.

The ages of our patients with HD ranged from 3 to 73 years. We noticed a bimodal age pattern with peaks at 5-9 years and young adult age-groups. There were 26 male and 13 female patients. The male:female ratio was 2:1. The commonest morphological subtype of HD was nodular sclerosing HD (18), closely followed by mixed-cellularity HD (13). There were 4 each of lymphocyte depleted HD and lymphocyte predominant HD.

4-µm-thick sections of available paraffin-embedded tissue of HD (19) were cut for immunocytochemical staining. We used the conventional PAP method when staining with polyclonal antibodies, and the ABC method for monoclonal antibodies. The panel of common antibodies used for this study included UCHL-1, L26, kappa and lambda light chains, IgM, BerH2 (CD 30) and Leu-M1 (CD 15). In 13 cases (68.4%), we noted Golgi and/or membrane positivity in the Reed-Sternberg cells and Hodgkin's cells when using antibody Leu-M1. Only 4 cases of HD stained positively with BerH2. All the 3 cases of lymphocyte-predominant HD showed negative reaction with Leu-M1 and BerH2.

**P5. CD5 POSITIVE B-CELLS IN PERIPHERAL BLOOD AND ENLARGED LYMPH NODES IN RHEUMATOID ARTHRITIS - A CASE REPORT**

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A 68-year-old mason with 20-year history of seropositive rheumatoid arthritis presented with bilateral cervical lymphadenopathy. Immunofluorescence flow cytometric and APAAP immunostaining analysis of the peripheral lymphocytes showed an increase in CD5 and CD19 positive B-cells which expressed both kappa and lambda light-chains. CD5 antigen expression in B-cells is known to be associated with autoimmunity which is a recognised feature in rheumatoid arthritis. Lymphadenopathy can be an extra-articular manifestation in rheumatoid arthritis. The enlarged lymph nodes in this patient showed diffuse lymphoid hyperplasia of the T-dependent paracortical zones resulting in marked compression of the cortical lymphoid follicles. The lymphocytes in the paracortical areas showed an increase in CD5 positive B-cells expressing both light chains. Both peripheral blood and lymph node thus revealed a reactive process to the rheumatoid disease.

**P6. DISSEMINATED HISTOPLASMOSIS: REPORT OF A CASE SIMULATING MILIARY TUBERCULOSIS.**

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A 35-year old Chinese man who was known to have insulin-dependent diabetes mellitus was admitted for fever and weight loss. The clinical features and radiological findings

were suggestive of miliary tuberculosis. During his hospital stay, he fell to his death from his ward at the twelfth floor. At autopsy, the appearance of the lungs was indistinguishable from miliary tuberculosis. The adrenal glands were grossly enlarged and large areas of necrosis resembling caseous necrosis were noted. Histology revealed necrotizing granulomas in the adrenal glands, lungs, spleen, kidneys and thyroid gland associated with the presence of *Histoplasma capsulatum* organisms. The liver contained many tiny granulomatous lesions but yeast organisms could not be demonstrated. Disseminated histoplasmosis as seen in this patient is often not suspected in non-endemic areas. The organisms are usually not easily seen in routine histological sections and the lesions may be passed off as tuberculous in aetiology. There is a need for greater awareness of this disease in order to help lower the high mortality associated with it.

**W. CYTOPHAGIC HISTIOCYTIC PANNICULITIS: A UNIVERSITY HOSPITAL EXPERIENCE**

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Cytophagic histiocytic panniculitis (CHP) is a syndrome recently described by Winkelmann *et al.* in 1980 and Crotty *et al.* in 1981. It is characterised by recurrent febrile episodes with accompanying subcutaneous nodules distributed over the extremities, face, neck or trunk, a chronic clinical course and systemic involvement. The subcutaneous nodules are typified by lobular panniculitis with infiltration by cytologically benign histiocytes exhibiting marked cytophagia, giving rise to 'bean-bag' cells. Systemic involvement includes infiltration by similar histiocytes in extracutaneous sites, particularly the lymph nodes, liver, spleen and bone marrow. This condition (CHP) was initially believed to run a variable but ultimately fatal course, with death resulting from coagulopathy or sepsis. Recently, the syndrome has been demonstrated to precede the development of non-Hodgkin's lymphoma by several years, both of the T-cell (Aronson *et al.*) and B-cell (Petrers and Winkelmann) types. It is now believed to have a benign variant, based on clinical and pathological criteria. This report describes 2 cases of CHP seen in the University Hospital, Kuala Lumpur, in the past 9 months.

The typical clinical and pathological features seen in these 2 cases are illustrated, with a discussion on clinical management.

**P8. USEFULNESS OF S-100 PROTEIN IMMUNOREACTIVITY IN SOME BENIGN AND MALIGNANT LESIONS OF THE BREAST.**

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S-100 protein was first isolated by Moore in 1965. It was initially thought to be a protein specific to the central nervous system. Later it was also found in a variety of normal and neoplastic tissues in man, including the myoepithelial cells in the terminal duct lobular units of the breast. Because of its ubiquitous nature, there have been conflicting opinions regarding its usefulness in the practice of surgical pathology of the breast. The present study attempts to determine the usefulness of S-100 protein in breast pathology. 20 cases each of histologically characteristic fibrocystic change (FC), ductular carcinoma *in situ* (DCIS) and infiltrating ductal carcinomas (IDC) were studied using antibody to S-100 protein by immunoperoxidase (PAP) method. The degrees of reactivity and the patterns of staining in these categories were compared. The results showed that there are significant differences in the degrees of staining between FC and DCIS ( $p=0.003$ ) and between FC and IDC ( $p=0.00001$ ). It is concluded that positive staining by S-100 protein is still useful in differentiating benign ductal proliferations of the breast from malignant lesions, based on the degree and pattern of staining, especially in small needle biopsy specimens.

**P9. PAPILLARY-CYSTIC NEOPLASM OF PANCREAS – A CASE REPORT**

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A 36-year old Chinese female presented with a history of haematemesis. A mass was felt in the left hypochondrium. She was subsequently discharged and readmitted for passing tarry stools. Ultrasound of the abdomen

showed a mass present around the tail of the pancreas. She underwent laparotomy. The gross specimen consisted of a yellowish firm nodular mass, measuring 15 x 11 x 9 cm containing cystic spaces. Histology showed that the mass was composed of solid cellular and papillary areas, cholesterol granulomas, necrosis and haemorrhage. A second look laparotomy was performed. However, she died soon after diagnosis with extensive metastases.

This was an unusual presentation for this tumour which is considered to be of low grade malignancy.

#### **P10. CLEAR CELL SARCOMA OF KIDNEY: A UNIVERSITY HOSPITAL EXPERIENCE**

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5 cases of clear cell sarcoma of kidney (CCSK) were seen in the Department of Pathology, University Hospital, Kuala Lumpur over a 22-year period from 1968 to 1989. 4 of the patients were males. Ethnically, 4 patients were Malays and 1 Chinese. The patients' ages ranged from 8 months to 3 years. CCSK was correctly diagnosed in 2, while 3 other cases were diagnosed as 'blastemal-predominant' Wilms' tumours at initial presentation. Lesions clinically and radiologically compatible with tumour metastases developed in the thoracic vertebra; orbit, shoulder and lung; and liver of 3 patients respectively. Prognosis was generally poor. However, a prolonged survival of 8 years and 3 months in one patient, despite omission of Adriamycin (doxorubicin) from the chemotherapeutic regime was seen. All the cases of CCSK could not be differentiated from Wilms' tumour clinically, biochemically, radiologically or macroscopically. We therefore emphasise the importance of 1) careful histology in the diagnosis of CCSK and 2) differentiating this entity from Wilms' tumour if the final outcome of CCSK patients is to be improved by incorporation of Adriamycin to the chemotherapeutic protocol.

#### **P11. A MIXED MEDULLARY AND FOLLICULAR CARCINOMA OF THYROID: A CASE REPORT**

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A mixed medullary and follicular carcinoma is an exceedingly rare malignant tumour of the thyroid. While medullary carcinoma is the least common of all thyroid carcinomas, accounting for only five to ten percent of the total, follicular carcinoma ranks as the second commonest thyroid carcinoma. Histogenetically, medullary carcinoma arises from the parafollicular or C cell, a cell of neuroendocrine origin, whilst follicular carcinoma is a malignant neoplasm of the follicular epithelium. As early as 1985, Uribe *et al.* had suggested the possibility of the co-existence of a papillary/follicular carcinoma with medullary carcinoma, based on the presence of thyroglobulin in a significant proportion (35%) of medullary carcinoma in his series of 20 cases. In this report, we describe the occurrence of such a mixed tumour in a 49-year old Malay woman. The tumour consisted predominantly of medullary carcinoma, with a definite follicular component. The relevant clinical and pathological features in this patient are discussed.

#### **P12. BILATERAL GONADBLASTOMA IN A 46XY INDIVIDUAL - A CASE REPORT**

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This is to report a case of bilateral gonadoblastoma occurring in an 18-year old Chinese 'female' who presented with primary amenorrhoea. Examination showed a phenotypic female. The external genitalia revealed scanty pubic hair with poorly developed labia majora and minora. Exploratory laparotomy showed a vestigial uterus, fallopian tubes and streak gonads.

Histological examination of the gonads showed a mixed tumour comprising primitive germ cells and sex cord cells arranged in characteristic nests containing **hyaline** material. The patient is alive and well 4 years later.

Gonadoblastoma is a rare tumour and the literature is briefly reviewed

### **P13. MALIGNANT TRANSFORMATION OF GIANT CELL TUMOUR**

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A case of malignant transformation of a giant cell tumour is presented in a 46-year old Malay man. He was initially diagnosed to have a giant cell tumour fifteen years ago, which originated in the iliac crest. Primary treatment was effected; no radiotherapy was given then.

The patient now presented with recurrence of the tumour for which an **excision/debulking** was done. The tumour was classified histologically as a fibrosarcoma.

It is a moot point whether **this** 'recurrence' may in actual fact be a second primary.

Malignant transformation in a giant cell tumour is a well recognized entity which most often results from previous irradiation. A review of literature revealed only 15 cases of malignant transformation in previously diagnosed benign giant cell tumours without radiotherapy.

### **P14. SQUAMOUS CELL CARCINOMA RELATED ANTIGEN: A MARKER FOR CARCINOMA OF THE UTERINE CERVIX**

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**Squamous** cell carcinoma related antigen (SCC-RAg) is a sub-fraction of tumour-associated antigen (TA-4), a glycoprotein purified from squamous cell carcinoma tissue of the uterine cervix. First described by Kato

*et al.* in 1977, this marker has been shown by various workers to be of prognostic value and predictive of recurrences in carcinomas of the uterine cervix, head and neck and oesophagus. Over the 18-month **period** from January 1989 to June 1990, we assayed the pre-therapy serum levels of **SCC-RAg** of **35** newly **diagnosed** cases of cervical epithelial malignancies. Of these, 18 were large cell non-keratinising carcinoma (LCNK), 4 keratinising squamous cell carcinoma (KS), 3 adenosquamous carcinoma (AS), 7 adenocarcinoma (AD) and 3 carcinoma *in situ* (CIS). Using a cut-off value of 2 **ng/ml**, all the cases of **KS (100%)**, 6 cases of **LCNK (33%)**, 1 case of **AS (33%)** and 1 case of **CIS (33%)** were positive for **SCC-RAg**. All cases of adenocarcinomas showed serum levels below 2 **ng/ml**. The highest serum level of 78 **ng/ml** was found in a case of **LCNK**. The preliminary results of our on-going study indicate that **SCC-RAg** is a useful serological marker of epithelial malignancies of squamous origin of the uterine cervix,

### **P15. HYPOCALCAEMIA IN THE CRITICALLY ILL PATIENT**

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Hypocalcaemia is often a recognised laboratory finding in many patients admitted to the intensive care unit. This poster deals with the plasma total and ionised calcium levels in twenty critically ill patients at various time intervals. The results were correlated with plasma albumin, electrolytes and blood pH. None of the patients had any primary parathyroid disease. Many of the patients had reduced levels of total and ionised plasma calcium. However, the usual complications of hypocalcaemia were not manifested because of continuous monitoring and therapy. There is a **disturbance** of calcium homeostasis in the critically ill and the various factors contributing to it are discussed.

**P16. SOME PERFORMANCE CHARACTERISTICS OF SPOTCHEM - 4410**

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The immediacy of results and convenience afforded by physician office analysers (POA) has led to their increasing popularity. However, tests are performed singly on strips, slides, or cartridges. "Spotchem" (Kyoto Daiichi, Japan) is a new POA capable of running a panel of tests on a single strip using serum or plasma except for haemoglobin which uses whole blood. "Spotchem" is available in 2 models - SP 1510 (electrolytes only) and SP4410 (non-electrolytes). For the non-electrolyte chemistries the result is available within 2-4

**P17. PREVALENCE OF THALASSAEMIA/ HAEMOGLOBINOPATHIES IN A MALAY COMMUNITY**

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Thalassaemias and haemoglobinopathies are known to be common among the Malays in Malaysia. Recently we undertook a cross-sectional study of the prevalence of this problem in a Malay community in Tanjung Karang, Selangor. An exhibition on health matters was organised in the location and visitors who wished to have their blood tested were included in the study. The intention was explained to all the participants. The laboratory studies carried out included a full blood count with a 5-parameter Coulter

ANALYTE	REGRESSION EQUATION	N		R
GLUCOSE	Y = 1.16X - 0.36	83	0.9890	0.9945
AST	Y = 0.74X + 7.2	100	0.9879	0.9939
ALP	Y = 1.05X + 6.63	101	0.9586	0.9791
ALBUMIN	Y = 0.97X + 2.95	101	0.9059	0.9518
CHOLESTEROL	Y = 0.94X + 0.5	30	0.8498	0.9218
UREA	Y = 0.81X + 1.7	98	0.8006	0.8948
LDH	Y = 0.68X - 0.05	116	0.7588	0.8710
GGT	Y = 0.82X + 28.7	100	0.7563	0.8697
CREATININE	Y = 0.85X + 25.6	45	0.7444	0.8628
TRIGLYCERIDES	Y = 0.85 + 0.344	43	0.7561	0.8695
ALT	Y = 0.71X + 20.7	100	0.7395	0.8599
CK	Y = 0.61X + 186.4	98	0.6209	0.7879
TBILIRUBIN	Y = 16.4X - 138.2	53	0.5777	0.7600
URIC ACID	Y = 0.89X + 66.6	42	0.5646	0.7514
CALCIUM	Y = 0.17X + 1.88	55	0.0084	0.0917

min (single-strip) and 8 min (multi-strip). The basic principle is colorimetric reflectance spectrophotometry.

As a preliminary evaluation, we have chosen to assess the single test strip for each chemistry against the Ektachem 700 using fresh serum samples rather than the multi-test strip. The correlation coefficients (R) ranged from 0.75 to 0.99 for most of the chemistries evaluated (see table below). However, the performance of the "Spotchem" calcium test strip was particularly poor towards the extreme ends of the reference range (2.10 - 2.60 mmol/l) [r = 0.091.

Counter, a reticulocyte stain, a blood smear, isopropanol precipitation test, haemoglobin electrophoresis with cellulose acetate, haemoglobin A2 and haemoglobin F quantitation. A total of 111 visitors were studied.

We found that 23% of the participants had some form of thalassaemia/haemoglobinopathy trait. There were 4 cases of alpha-thalassaemia trait, 11 cases of beta-thalassaemia trait. 12 cases of delta-beta-thalassaemia trait, 5 cases of beta-thalassaemia trait with high A2 values (8% and 12.6%) and 3 cases of possible beta-thalassaemia trait with raised A2 values but normal red

cell indices.

In conclusion, thalassaemia/haemoglobinopathy is prevalent in the Malay community studied and not all cases fit into the classical description of the heterozygotes. Since no family studies were carried out, one may speculate that it could be due to the interaction of the various heterozygotes or these are variants that need further elucidation by DNA analysis.

#### **P18. PATTERNS OF ANTIMICROBIAL RESISTANCE IN THE NATIONAL UNIVERSITY HOSPITAL, SINGAPORE IN 1989**

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In serious infections antimicrobial therapy should be started before the culture results are available. Hence, clinicians should be constantly aware of the pattern of organisms affecting the particular patient populations they are concerned with, and their antimicrobial sensitivities, for prompt institution of therapy. The patterns have varied in different periods of time.

In this paper, the patterns of antimicrobial resistance of most frequently isolated strains in NUH during 1989 were analysed; the urinary organisms were compared with organisms isolated from other sites.

The sensitivity patterns of organisms such as *Staphylococcus aureus*, *Pseudomonas* and *Enterobacteriaceae* are analysed and illustrated in graphic forms for quick reference.

#### **P19. LIPOPROTEIN (A) LEVELS IN CHINESE AND INDIAN PATIENTS UNDERGOING ELECTIVE CORONARY ANGIOGRAPHY**

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Coronary heart disease (CHD) ranks as one of the top two killers in Singapore with a distinct preponderance of Indians being affected. Recently, lipoprotein (a) [Lp (a)] has been shown to be a strong independent marker for CHD. We have investigated the association of Lp (a) with CHD. Serum Lp (a) from 195 Chinese and 90 Indians males admitted for elective coronary angiography were determined using the Macra Lp (a) [Terumo Medical Corporation] enzyme immunoassay kit. The ages of the subjects recruited into the study ranged from 33–78 years (Chinese) and 29–71 years (Indians). They were classified as having zero-, one-, two-, or triple-vessel disease according to the angiographic extent of coronary stenosis.

Both groups showed a highly skewed distribution in Lp (a) levels. Mean levels ( $\bar{x}$ ) were higher in the Indians than the Chinese ( $\bar{x}$  = 19.4 and 16.3 mg/dl respectively). The Chinese and Indian populations were subdivided into two groups, namely, the diseased and control (non-stenosed) groups. Comparisons were also made between the controls and the severely diseased patients (triple-vessel). The Lp (a) levels of these subjects were ranked (10th to 90th percentile) and are shown below.

There was a distinct elevation in Lp (a) in subjects with CHD compared to those without CHD. Among subjects with CHD, the Indians had much higher Lp (a) levels than the Chinese. This may account in part for the higher frequency of CHD among the local Indian population.

Population percentile	Zero-vessel disease	Diseased vessels	Triple-vessel disease
Chinese (n)	<b>48</b>	147	50
10 th	1.0	1.7	2.0
25 th	2.0	4.4	5.1
50 th	7.3	13.0	13.0
75 th	<b>16.3</b>	24.1	31.8
90 th	<b>31.6</b>	42.8	48.3
Indians (n)	13	77	31
10 th	1.0	1.9	1.5
25 th	1.5	5.3	4.3
50 th	2.9	16.8	12.9
75 th	12.8	30.0	30.0
90 th	26.5	51.4	49.2