The 22nd Scientific Meeting of the Malaysian Society of Pathologists and 8th Combined Meeting of the Malaysian Society of Pathologists and the Singapore Society of Pathology was held in the Lake Pedu Resort, Kedah from 12th to 15th December 1997. Abstracts of the free paper communications follow:

Oral presentations:

1. Inducible DNA repair in the fission yeast *Schizzosaccharomyces Pombe*

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Inducibility of DNA repair in prokaryotes has been proven over 20 years ago in ***Escherichia Coli.*** This inducible repair is also known as SOS repair. SOS repair in E. ***Coli*** is repressed in undamaged wild-type cells but is induced in response to UV radiation and other agents that damage DNA or interrupt its replication. An analogous system is yet to be proven to exist in eukaryotic cells, although there are some evidence shown in experiments with ***Saccharomyces cereviseae.*** In this study, we investigated the presence of inducible repair in the fission yeast ***Schizzosaccharomyces pombe.*** Wild type ***Schizzosaccharomyces pombe*** cells were subjected to split dose protocols with UV radiation and their survival following the second dose of UV radiation and their survival following the second dose of UV radiation were monitored. The cells were exposed to 70 J/m.sq. UV radiation to induce the repair system and left to recover on complete growth media. At various times, cells were given a high dose of 300 J/m sq. UV radiation and colonies formed after 7 days of incubation were scored. The percentage of survival is compared to that of uninduced cells (cells given only the 300 J/m.sq dose).

Results obtained showed that the percentage of survival of logarithmic phase cells was 3 times that of uninduced cells. Maximum increase in survival was observed after a recovery period of 3 hours. On the other hand stationary phase cells failed to show similar increase in survival when induced. This is probably because the stationary phase cells are not active and also ATP needed for repair synthesis is lacking from depleted nutrients.

2. Monoclonal gammopathies: A review of cases seen at the University of Malaya Teaching Hospital over a 7 year period.

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A retrospective review of cases with diagnosis of monoclonal gammopathy seen over a 7 year period (January 1991 to September 1997) was carried out. During this period, specimens including sera and urine were a received from a total of 1809 patients in the Immunology Laboratory, Department of Pathology, for electrophoretic analysis. Out of these 1809 analysis, 143 cases were found to have evidence of monoclonal g-globulins based on protein electrophoresis and immunofixation studies. These included 126 cases (88.1%) with "single gammopathies", 6 cases (4.2%) with "double gammopathies" and 11 cases (7.7%) with light chain disease. The distribution of the imrnunoglobulin isotypes of the 126 cases with "single gammopathies" were as follows: IgG - 901126 (71.4%), IgA - 221126 (17.5%), IgM - 12/126 (9.5%) and IgD - 21126 (1.6%). There were no cases with IgE isotype. The proportion of monoclonal proteins with kappa to that with lambda light chain was about 2:1 which is consistent with the normal 2:1 ratio of kappa to lambda in the serum. Of the 6 cases with "double gammopathies", 1 had M-proteins that differ in the light chain class indicating the biclonal origin of the proteins. It was not possible to ascertain whether the remaining cases were of monoclonal or biclonal origin without resorting to analysis of the idiotype of these M-proteins.

Demographic information was available for 130 subjects. The mean age of the patients was 63.7 years (range : 32 - 84); there were almost equal number of males (n=66) to females (n=64). The distribution by ethnic group was : 61 (46.9%) Chinese, 33 (25.4%) Malays and 33 (25.4%) Indians. The most common clinical presentations were general malaise with loss of weight and appetite followed by back pain with or without associated pathological fractures.

3. Effect of tocotrienols on human breast cancer cells

Kalanithi Nesaretnam and Philippa Darbre

Antiproliferativeeffects of tocotrienols, the major vitamin E component in palm oil, were investigated on the growth of estrogen-responsive MCF7 human breast cancer cells and compared with effects of a-tocopherols (αT). The tocotrienol-rich fraction (TRF) of palm oil inhibited growth of these cells in the absence of estradiol at concentrations as low as 0.5 µg/ml and complete suppression of growth was achieved at 8 µg/ml. Growth inhibition was found also in the presence of estradiol. Separation of the TRF into individual tocotrienols revealed that all fractions could inhibit cell growth. However, the γ and 6- fractions were the most inhibitory, resulting in complete suppression of growth at concentrations of 6 µg/ml in the absence of estradiol. By contrast, αT had no inhibitory effect on MCF7 cell growth. These results confirm studies using other sublines of human breast cancer cells and demonstrate that tocotrienols can exert direct inhibitory effects on the growth of breast cancer cells. By comparison to studies using ER-MDA-MB-231 human breast cancer cells, these results suggest that ER+ cells are more sensitive to tocotrienol-mediated growth inhibition than ER- cells. In searching for the mechanism of inhibition, studies of the effects of TRF on estrogen-regulated pS2 gene expression showed that tocotrienols do not act via an estrogen receptor-mediated pathway and must, therefore, act differently from estrogen antagonists. Furthermore, tocotrienols did not increase levels of growth-inhibitory insulin-like growth factor binding protein (IGFBPs) implying also a different mechanism from that proposed for retinoic acid inhibition of breast cancer cell growth. This could assume a clinical importance in potential growth suppression of breast cancer cell otherwise resistant to antiestrogen and retinoic acid growth inhibition.

4. An Immunohistochemical Study of Laminin Expression in Fibroadenoma, Fibrocystic Disease, In-situ and Invasive Ductal Carcinoma of Breast

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Objective: The basement membrane (BM), of which laminin is a major glycoprotein component, is an important barrier to tumour cells which must be breched before metastatic spread can occur. We have attempted to compare the pattern of laminin expression in a range of benign and malignant breast lesions to better understand the process of tumour progression. ***Method:*** 162 breast samples comprising 18 fibroadenomas, 22 fibrocystic disease, 96 invasive ductal carcinoma and 26 carcinomas with intraductal components were evaluated for laminin expression by a standard immunoperoxidase method on formalin-fixed, paraffin-embedded histological sections using a commercial antibody against laminin. The pattern of laminin expression was charted as follows: Type I: >70% of .BM complete/continuous; Type II: >70% of BM moderately disrupted; Type III: >70% of BM completely disrupted. ***Results:*** The Type I pattern was observed in all cases of fibroadenoma and fibrocystic diseases, and in 73% of intraductal carcinoma components. Various patterns of BM disruption were observed in invasive ductal carcinoma. Severity of BM disruption were correlated with histological grade of the carcinomas ($p < 0.001$) and histologically detectable vascular invasion ($p < 0.01$). Small size tumours, those without lymphatic invasion and lymph node negative tumours showed more complete patterns of laminin expression. ***Concluswns:*** These findings suggest that tumour cells with high histological grade posses an enhanced capacity to disrupt the basement membrane, an important step in the metastatic process. The detection of BM disruption by immunohistochemical staining for laminin

is technically easy and may be usefully applied for the differentiation of "in-situ" and microinvasive carcinoma.

5. Fluorescent *in situ* hybridization (FISH) as a tool for ploidy analysis of formalin-fixed, paraffin-embedded tissues.

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Ploidy status and specific chromosomal abberations are useful information in the diagnosis and prognosis of premalignant and neoplastic lesions, particularly when histological features are ambiguous. The DNA ***in situ*** hybridization technique permits assessment of the chromosomal make-up of targetted cells or cell populations while retaining cellular morphology and tissue architecture. The employment of fluorescein-labelled probes in the hybridization process has allowed the detection of positive hybrids directly using ultraviolet microscopy. **Objective:** To develop the FISH technique for ploidy status determination of hydatidiform moles and optimize it for our local histopathology laboratory. **Method:** Formalin-fixed, paraffin-embedded tissue blocks of two cases each of histological-proven complete hydatidiform mole (CHM) and partial hydatidiform mole (PHM) were retrieved from the archives of the Department of Pathology, University of Malaya. Sequential 4 mm thick histological sections were cut. Digoxigenin-labelled DNA probes specific for the centromeric region of human chromosomes 12 and 18 (Boehringer-Mannheim, Germany) were hybridized separately with the tissue sections. Subsequently, positive hybrids were amplified with the final incorporation of fluorescein labels. Under the UV microscope, 100 distinct interphase nuclei were scored for the number for fluorescent dots within each nucleus. CHMs were found to be diploid and the PHMs were triploid. **Conclusion:** The FISH technique is an easy and rapid method of determining the ploidy status for formalin-fixed, paraffin-embedded tissues. The technical details and limitations of the FISH technique and the possible implementation of the technique in the routine histopathology laboratory and in other cytogenetic studies will be discussed.

6. Accurate cord blood stem cell enumeration using Procount ™ kits

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Bone marrow (BM) cells have been, in the past, the only source of stem cells for autologous or allogeneic transplantation. Recently, cord blood and peripheral stem cells (PSC) have been increasingly used. Preliminary results have indicated that the frequency and severity of acute and chronic Graft Versus Host Disease (HVHD) following unrelated cord blood transplantation is less than that observed following BM or mobilised PSC transplantation with the same degree of histocompatibility. With this in mind and the easy availability of this usually discarded material, we embarked on a prospective study from September to November 1997 to enumerate stem cells on at least 30 samples of cord blood using Procount ™ kit.

This is a more accurate method of stem cell enumeration utilizing CD34 reagent which is able to bind specifically to cell surface antigens and at the same time able to stain the DNA and RNA of all nucleated cells. CD34 positive cells in the sample can thus be determined more accurately compared with the, earlier method which utilises CD34 and 45. Preliminary results of stem cell enumerations using Procount Kit show a total of 36 cells/ul, 8 cells/ul and 588 cells/ul for cord blood, peripheral blood and bone marrow samples respectively. Comparisons will be made with the former method utilising CD34 and CD45.

7. Cervical cancer, tumour virus and anti-tumour virus?

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The association between Human Papillomavirus (HPV) infection and cervical cancer is well established. The HPV, which encodes several onco-proteins is present in a large proportion of cervi-

cal cancers. Recently, the Adeno-Associated Virus (AAV) was reported to be also present in the cervix. To date the AAV has not been associated to any cancers or diseases. Nevertheless, its presence in the cervix prompted us to question if these two viruses (HPV and AAV) interact in the cell, and the consequences of such an interaction.

Our *in vitro* experiments show that the AAV-encoded replication protein *(Rep)* forms a tight complex with the E2 protein of HPV. Further experiments showed that this interaction also occurs *in vivo.* More surprisingly, E2 protein alone can increase the expression of *Rep;* which usually occurs only when AAV co-infects a cell with a helper virus (e.g. adenovirus). We also show that the HPV can provide help to AAV for its replication. As *Rep* is extremely toxic, the consequence of the help given by HPV to AAV is the death of the host.

Hence, it appears that the AAV might act as an anti-tumour virus which when triggered by a helper-virus (e.g. HPV), expresses a protein which kills the doubly-infected host cell. This would explain old sero-epidemiological studies which show that the prevalence of AAV-infection is high in healthy individuals, but low in cervical cancer patients.

8. Characterization of plasmids mediating ESBL in multi-resistant *Klebsiella pneumoniae*

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Strains of *Klebsiella pneumoniae* which produce extended-spectrum beta-lactamases (ESBL) have become important nosocomial pathogens in oncology and intensive care units. Selection of these strains is thought to be secondary to the use of third-generation cephalosporins. ESBL production in these strains is often plasmid-mediated. Twenty-eight strains of *K. pneumoniae* isolated from blood cultures of patients from University Hospital, Kuala Lumpur were identified as ESBL producers by their resistance to ceftazidime in disc-diffusion susceptibility tests and by a double-disc synergy test. Their plasmid profiles were analysed and it was observed that these strains carried a group of common plasmids ranging from 60kb to 150kb in molecular size.

Conjugation studies were carried out using *E.Coli* 562.1 strain as recipient and selection of transconjugants were done on agar plates containing ceftazidime and nalidixic acid antibiotics. Plasmids from eight strains were successfully transferred to the recipient strain. All the eight transconjugants selected for ceftazidime resistance harboured a single plasmid ranging from 60kb to 100kb in molecular size. Five of the transconjugants carried a plasmid of 60kb each. All these transconjugants were then characterized by digesting them with Eco R1 restriction enzyme. All eight transconjugants showed different restriction profiles suggesting that these ESBL producing *K.pneumoniae* strains were not clonal in origin.

9. *Chlamydia pneumoniae (TWAR)* and coronary heart disease

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Chlamydia pneumoniae, a respiratory pathogen, has been implicated in the pathogenesis of coronary heart disease. The organism has been detected in atheromatous plaques and coronary endothelium by culture, DNA detection, immunbperoxidase staining and electron microscopy. It has been postulated that acute myocardial infarction (AMI) may be triggered off by an exacerbation of chronic chlamydial infection. In this study, patients with AMI were examined for laboratory evidence of C. *pneumoniae* infection.

Thirty male patients admitted to the TAR hospital in Klang, Selangor with AMI were studied. Blood was withdrawn for microimmunofluorescence (MIF) serology, serum polymerase chain reaction (PCR) and immunoblotting. Throat swabs were collected for chlamydial culture and PCR. The control group consisted of 158 asymptomatic patients with no evidence of respiratory tract infections and coronary heart disease.

Preliminary results showed that 56% of the AMI cases had raised antibody titres (>1:32), of which 23% had high *C.pneumoniae* IgG titres ≥ 1:512 indicating possible acute infection and 33% had titres of (1:32-256). In the control group, only 19% had IgG titre >1:32 against *C.pneumoniae*. This higher prevalence of IgG titres among AMI patients support the fact that acute myocardial infarction may be the results of an acute exacerbation of chronic TWAR infection. By imrnunoblotting it was shown that 5 out of 7 patients with the IgG titre ≥ 1:512 had antibodies against two chlamydial antigens with molecular weight of 46kda and 56kda by immunoblotting. These antigens were specific to *C.pneumoniae* and the 56kda protein was known to be heat shock protein. The serum PCR, with and without prior DNA extraction had a sensitivity of 1-10 elementary bodies per specimen but none of the patients were serum PCR positive. Of the throat swabs examined, none were positive by culture or PCR. The patients are being followed-up for changes in chlamydial antibodies titres.

10. Chlamydial upper genital tract infection diagnosed by DNA amplification tests

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Chlamydia trachomatis is a major cause of pelvic inflammatory disease (PID) which occurs as a result of ascending infection from the uterine cervix. The laboratory diagnosis of chlamydial PID is usually indirect from the result of cervical cultures or serology. In this study, an attempt was made to detect the organism in endometrial tissue by DNA amplification tests.

Fifty-four women presenting with symptoms of acute PID were studied. From each patient, two endocervical swabs were collected for chlamydial enzyme immunoassay (EIA), polymerase chain reaction (PCR) and ligase chain reaction (LCR). Endometrial tissue obtained with a Pipelle was homogenized and examined for chlamydia by PCR and LCR and the results were correlated with chlamydial antibody titres obtained by microirnmunofluorescence(MIF) serology.

C. trachomatis infection was diagnosed in 17 (31.5%) of the patients. The number of positive tests for cervical swabs were 4 by EIA, 5 by PCR and 6 by LCR. There was 98.1% concordance between cervical swab EIA and PCR and 100% between PCR and LCR. Of 54 endometrial samples 10 were positive by PCR and 17 by LCR. Chlamydial IgG titres > 1:64 were found in 5 patients, 1 of whom also had raised IgM titres. These preliminary results showed that *C. trachomatis* is a significant cause of acute PID in Malaysian women. The examination of endometrial tissue by DNA amplification tests appears to be superior to the testing of endocervical swabs for women with upper genital tract infection.

11. Comparison of two molecular typing systems based on sequence specific primer directed amplification for the determination of Hepatitis C virus genotypes

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A variety of systems for genotyping the hepatitis C virus (HCV) have been developed to address the issue of the association between genotypes and pathogenecity, infectivity and response to immunomodulatory therapy. Among the available systems, typing based on the use of type specific primers in *in-vitro* amplification (PCR) is the most widely used due to its relative simplicity. The approach was originally deschbed by Okamoto *et al* in 1992; in this assay system, 4 distinct genotypes (1a, 1b, 2a, 2b) could be distinguished. In a following paper in 1993, the assay was extended to include additional primers specific for a fifth genotye (3a). Using this system, we are able to classify about 70% of our isolates; of the remaining cases, ~9% are of mixed genotypes and 21% are unclassified. Recent reports pointed out that Okamoto's system of typing (i) may be inadequate with respect to typing specificity and (ii) is unable to detect the more newly identified genotypes (3b, 4, 5, 6). Apaper published in 1997 by Ohno *et al* reported the capability to type genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a & 6a with improved specificity. In the present study, the typing system described by Ohno *et al* was used to analyse samples in parallel with Okamoto's system with the objective of

determining if the former is indeed an improvement over the latter as a routine assay for typing of HCV isolates. A total of 79 samples were analysed. Overall, in this series of samples, we were able to type ~73% of the cases using Okamoto's system and only ~66% of cases using Ohno's system. The concordance rate was about 62%. In view of the results obtained, further evaluation studies will need to be carried out and isolates with discrepant results subjected to sequence analysis.

12. Sputum liquefaction with dithiothreitol - a more effective technique for lung cancer detection

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Objective: To compare the efficacy of sputum cytology using dithiothreitol (DTT) as a mucolytic agent with that of conventional "pick and smear" (PS) method for lung cancer detection. **Method:** Fifty sputum specimens were examined from 50 patients, of which 35 were clinically suspected to have lung cancer. 25 were routine samples and another 25 were obtained postbronchoscopically. Smears were first prepared using PS method to act as controls. The remainder sputum were subjected to mucus liquefaction using 0.3% DTT in 70% ethanol and subsequently smeared. Both types of smears were screened for malignant cells. The overall appearance of cell distribution, cell morphology and background clarity were subjectively assessed. **Results:** From the 35 clinically suspected lung cancer cases, only one sputum was positive for malignant cells in the PS method (2.9%), whereas 5 positive cases were detected by DTT-liquefaction technique (14.3%) including the case positive in PS method. Thus the rate of lung cancer detection had been increased by 11.4% using the latter method. DTT-treated smears exhibited higher concentration of evenly distributed cells. The morphology of both normal and malignant cells were well preserved and definitive typing of the latter was possible. The background was generally clear of mucus and RBCs. **Conclusion:** Sputum liquefaction with DTT is a simple and effective method for lung cancer detection. It is also inexpensive. For these reasons we propose this technique over the conventional pick and smear method for routine sputum cytology.

Poster presentations:

P1. Frequency of Hepatitis B virus variants containing a TAG stop codon in the precore region in chronic carriers of the virus

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Hepatitis B virus (HBV) replication in the absence of the Hbe antigenaemia is attributed to the presence of a mutation in the precore region of the viral genome resulting in the introduction of a TAG stop codon. This event tends to occur during spontaneous or interferon (IFN) induced HBeAg seroconversion. The clinical relevance of these observations is based on reports of associated rapid progression of liver disease in chronic hepatitis B patients who are infected by the mutant virus. In addition, infection with the wild type virus appear to show a better response to interferon therapy compared to that by the mutant strain. Therefore, a rapid and reliable method for detecting the mutant HBV may be useful for identifying chronic hepatitis patients with poor prognosis and in predicting antiviral response in HBeAg negative subjects. In the present study, sera from a total of 66 chronic HBV carriers including 23 HBeAg positive and 43 HBeAg negative cases were used. HBV DNA was subjected to amplification by the polymerase chain reaction (PCR) using primers derived from the precore region spanning nucleotides (nt) 1730 and 2458. PCR product were then subjected to stringent hybridisation using 3 oligonucleotide probes specific for (i) the wild type virus, (ii) the mutant virus harbourng a point mutation at nt 1896 and (iii) the mutant virus harbouring 2 point mutations at nt 1896 & nt 1899. Nineteen out of 23 (82.6%) HBeAg positive samples carry only the wild type virus (MO) and 4 carry both the wild type and the mutant virus containing the TAG stop codon (M1). In contrast, only 17/43 (39.5%) of HBeAg negative samples carry the wild type virus

exclusively. Of the remaining samples, 4 (9.3%) carry M1, 3 (7.0%) carry the virus with mutations both at nt 1896 and nt 1899 (M2) and 19 (44.2%) carry both MO and M1 viruses. Our data indicate frequent occurrence of point mutations at the precore region in chronic HBV carriers who are seronegative for the HBeAg.

P2. Role of cyclic-AMP dependent protein kinase (PKA) in the regulation of DNA repair

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A major obstacle to the success of cancer chemotherapy is the emergence of drug resistance. Recent studies with cAMP-dependent protein kinase (PKA) mutants have suggested that the increase in resistance may be due to an increase in recognition and protein binding to the damaged DNA in these cells. This finding suggests that PKA may be involved in the regulation of DNA repair and the increase in resistance in these mutants is due to an increase in the repair activity.

In this study we used an ***in-vitro*** DNA repair assay to investigate the role of PKA in DNA repair. We evaluated the effects of two pharmacological agents that modulate PKA activity : 8-Bromo-cAMP and H-89, and the effect of immuno-depletionof the RIa subunit of the PKA. When cell-free extracts prepared from Schizosaccharomycespombe were pretreated with 5mM 8-Bromo-CAMP(an activator of PKA), repair activity was reduced to near background level. Repair activity was increased by nearly 60% when cell-free extracts were pretreated with H-89 (an inhibitor of PKA). These results suggest that PKA modulators which regulate the activity of PKA does affect the level of DNA repair activity. In immuno-depletionstudies, cell-free extracts pretreated with antibody to the RIa subunit of PKA showed inhibition of repair activity. Our ***in-vitro*** studies, therefore, support the earlier ***in-vivo*** studies and provide further evidence that PKA does play a role in the regulation of DNA repair. Our results suggest that PKA modulators may be clinically useful pharmacologic tools for combating the emergence of drug resistance during chemotherapy.

P3. a,-antitrypsin variant: frequency of detection among patients referred for protein electrophoretic analysis in the University of Malaya Teaching Hospital

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a,-Antitrypsin (AAT) is a glycoprotein which forms the major component of the discrete band at the a,-region on protein electrophoresis. It is the most important protease inhibitor (Pi) in serum and is responsible for >90% of the trypsin inhibiting capacity of serum. The most significant clinical finding on examination of the a,-electrophoretic band is a decrease or absence of the band, reflecting AAT deficiency. Genetic polymorphism of AAT results in phenotypic variations that can be detected on protein electrophoresis. Although there are over 40 alleles described, the M allele (PiM) is the most common, accounting for about 90% of most populations. Next in frequency is the S allele (PiS). The Z allele, which is the slowest moving on electrophoresis is uncommon and is associated with severe deficiency. The most common genetic variant that is evident on protein electrophoresis is PiMS which gives rise to 2 faintly staining a,-bands. Subjects heterozygous for the deficiency phenotypes PiMZ, PiMS, generally do not suffer frdm clinical effects; PiSZ, however, may have clinically significant deficiency.

High resolution electrophoresis (HRE) provides an excellent means to screen for AAT deficiency; however, detailed analysis of the genetic variants of this protein require more sophisticated methods. We report the results of screening a total of 1995 sera, spanning an 8 year period (1990-1997) to determine the frequency of detection of AAT deficiency and AAT variants. A total of 19 cases with AAT variant were found, all of which gave double a,-Antitrypsin bands on HRE. Review of the case records of these 19 patients did not reveal clinical evidence of AAT deficiency. From the results of this retrospective review, it appears that AAT deficiency is rare in our community and that the genetic variants encountered are not associated with significant clinical problems.

P4. Evaluation of methods for detection of anti-double stranded DNA antibody in human serum

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Systemic Lupus Erythematosis (SLE) is a classic multi-systemic auto-immune disease with characteristic auto-antibodies including anti-nuclear; anti-double-stranded & single-stranded DNA. Anti-double-stranded DNA (anti-dsDNA) is more specific. Its level correlates with the clinical manifestation, relapse and response to treatment. Hence it is an excellent indicator for monitoring disease progression. The method currently in use is qualitative immunofluorescent technique (IFT). This is very specific due to the use of dsDNA in the kinetoplast of *Crithidia lucilae* as substrate. In this study, ELISA and Amersham kits used for the measurement of dsDNA are evaluated and compared with IFT. 49 healthy volunteers and 119 patients with autoimmune disease were analysed to determine the reference range, precision, clinical specificity and sensitivity as well as correlation between these methods. The results obtained show that ELISA has a higher reference value than Amersham. IFT is the most sensitive and specific test but being a qualitative method is only useful as a screening test. Amersham kit is more specific in detecting SLE but is not very sensitive thus is good for discriminating between SLE and non-SLE. ELISA is sensitive but not specific. The coefficient of correlation between ELISA and Amersham is 0.84. The discrepancy is due to ELISA detecting both low and high avidity anti-dsDNA whereas Amersham detects only high avidity antibody. The high false-positive results with ELISA is attributed to the binding of IgM to the pre-coating used. ELISA is adequate for screening for SLE from normal individuals but inadequate for differentiating from other autoimmune diseases.

P5. Flow cytometric analysis of intracellular myeloperoxidase with myeloperoxidase monoclonal antibody - a preliminary report

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Morphology and cytochemistry are fundamental to the diagnosis and classification of acute leukaemias. Immunophenotyping of leukaemic cells with monoclonal antibodies against surface marker molecules complement the morphologic and cytochemical methods of diagnosis leading to a more precise classification of acute leukaemias.

Myeloperoxidase (MPO) is localised in azurophlic granules of myeloid cells. Cytochemical demonstration of MPO activity is classically used in the diagnosis of acute leukaemias, where in the FAB classification presence of at least 3% of MPO containing blasts with conventional light microscopy is diagnostic of acute myeloblastic leukaemia (AML). However, in poorly differentiated AML (MO) MPO activity can only be demonstrated at the ultrastructural level requiring laborious techniques. Negative MPO activity by light microscopy makes diagnosis difficult for such cases. An alternative method of MPO detection that has been described is by using monoclonal antibodies against MPO. This intracellular MPO activity detection is by flowcytometry.

Here we report the results of intracellular MPO detection in patients with acute leukaemia using monoclonal anti-MP0 antibody by double fluorescence flowcytometry (using BD-FACScan). These cases were also subjected to conventional cytochemical MPO analysis.

10 cases of acute lymphoblastic leukaemia (ALL) and 7 cases of AML were analysed. All ALL cases and 3 cases of acute monoblastic leukaemia (M5) showed negative reaction to anti-MP0 antibody while 2 cases of AML(M2) and 2 cases of AML(M4) were positive. In this study, we found no difference in the sensitivity and specificity of MPO-activity detection between the standard cytochemical MPO staining and imrnunochemical method.

P6. The spectrum of p-thalassaemia mutations in the Chinese in Malaysia - A comparison with β-mutations in the Chinese in Asia

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The spectrum of p-thalassaemia mutations in the Chinese in Malaysia was studied. Allele specific priming was used to determine the mutations in p-carriers at -28, Codon 15, Codon 17, Codon 19, Codon 26 (HbE), IVSI#1, IVSI#5, Codon 41-42 and IVSII #654. Five p-mutations (Codon 41-42, IVSII #654, -28, Codon 17 and Hb E) were responsible for 80% of the β-thalassaemias. Three p-mutations at Codon 41-42, IVSII #654 and -28 accounted for 76% of p-thalassaemias in the Chinese in Malaysia. Hb E which is one of the most common p-mutations present in the Malays was detected in 1.5% of the Chinese studied.

Comparison of the mutations responsible for p-thalassaemia in the Chinese in Asia indicated that the p-mutations were similar but the frequency of the mutations differed in different regions. In Singapore, two mutations at Codon 41-42 and IVSII #654 caused 80% of the p-thalassaemias, while the same two mutations caused only 65% of the p-thalassaemias in South China. Hb E was not detected in the Singaporean Chinese but was present in 4% of Chinese in South China.

W. Laboratory investigation of deep vein thrombosis

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Numerous factors have been identified to constitute a risk to thrombosis. Clinical diagnosis of deep vein thrombosis (DVT) is highly non-specific due to the fact that the signs and symptoms are non-diagnostic. Patients with relatively minor symptoms or none at all may have extensive DVT with or without pulmonary embolism. We carried out a study to identify the presence of known risk factors such as Protein C (PC), Protein S (PS), Antithrombin III (ATIII) and or Activated PC resistance as predictive markers for risk of thrombosis in DVT patients referred to Hospital Kuala Lumpur. Normal range for the various parameters was established from blood donors. The following results were obtained for the normal ranges; ATIII (95.26 +/- 31.34 U/dl), PC (112.05 +/- 32.66 U/dl), PS (93.23 +/ - 45.98 U/dl) and APC ratio (2.26 +/- 0.46). Laboratory investigation on available DVT samples showed deficient levels of either PC, PS or ATIII in some of the patients. APC ratio, a measure of APC resistance, revealed abnormality in 2 patients and the molecular analysis of factor V mutation in the R506Q was not detected in both patients as indicated by the presence of fragments 157bp, 93bp and 37bp in the PCR digest. More cases need to be looked at in order to establish the local incidence for the deficiency of the various proteins. Identification of thrombotic tendency in patients may lead to more aggressive management and screening of asymptomatic family members for similar defects may result in the prevention of future thrombotic events.

P8. *In-vitro* susceptibility of 28 strains of extended-spectrum beta-lactamase producing strains of Klebsiella pneumoniae

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Strains of ***Klebsiella pneumoniae*** which produce extended-spectrum beta-lactamases (ESBL) have become important nosocomial pathogens in intensive care and haematology units where there is an increased use of antibiotics especially broad-spectrum cephalosporins. Treatment of invasive infections with these organisms is often with carbapenems or fluoroquinolones. This study was undertaken to evaluate the ***in-vitro*** susceptibility of multiresistant ***K.pneumoniae***.

28 strains of ***K. pneumoniae*** isolated from blood cultures of patients from University Hospital, Kuala Lumpur were identified as ESBL producers by their resistance to ceftazidime in disco-diffusion

susceptibility tests and by a double-disc synergy test. Minimum inhibitory concentrations (MIC) of the strains to 11 antimicrobial agents were determined by an agar dilution method. The MIC_{90} (mg/l) of the strains to the various antimicrobial agents were as follows:- ceftazidime (>256), cefoperazone (>256), cefotaxime (128), ceftriaxone (256), cefoxitin (32), cefipime (32), piperacillin (>256), imipenem (4), aztreonam (>256), gentamicin (>256) and amikacin (64). All strains were confirmed to be resistant to ceftazidime by the agar dilution method. Imipenem had the highest degree of *in-vitro* activity with 100% of the strains being fully susceptible at a breakpoint of ≤ 4 mg1 followed by cefipime with 79% of the strains being fully susceptible using a breakpoint of ≤ 8 mg/1.

P9. *In-vitro* susceptibility of *34* strains of *Streptococcus pneumoniue*

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Until recent years penicillin was the drug of choice in the treatment of pneumococcal disease. With the emergence of strains with reduced susceptibility to penicillin, the choice of drugs for the treatment of severe pneumococcal disease especially meningitis is limited. In this study we evaluated the *in-vitro* susceptibility of *S.pneumoniae* to 6 antimicrobial agents.

The minimum inhibitory concentration (WC) of 34 strains of *S.pneumoniae* isolated in the Department of Medical Microbiology, University of Malaya, to the various antimicrobials were determined by the E-test method. NCCLS breakpoints were used to interprete the results. The antimicrobialsevaluated were penicillin, amoxicillin-clavulanic acid, cefaclor, cefuroxime, ceftriaxone and azithromycin.

28 isolates were from the respiratory tract and 6 were from blood. The MIC_{90} ug/ml (% strains fully susceptible) in decreasing order of activity were as follows: amoxicillin-clavulanicacid 0.5/0.25 (94), ceftriaxone 1.0 (88), cefuroxime 2.0 (85), penicillin 0.5 (79), azithromycin 256 (74) and cefaclor 8.0 (53). The most active drug tested was amoxicilin-clavulanic acid with all but two strains fully susceptible at a breakpoint of ≤0.5/0.25 ug/ml.

Seven strains (21%) had reduced susceptibility to penicillin; of these, four (12%) had intermediate resistance (MIC 0.1 - 1.0 ug/ml) and three (9%) high-level resistance (MIC ≥2.0 ug/ml). All these strains were from respiratory specimens. Six of the strains with reduced susceptibility to penicillin also showed reduced susceptibility of azithromycin. Only 25 (74%) of the strains tested were fully susceptible to azithromycin at a breakpoint of ≤ 0.5ug/ml. Strains with high-level resistance to penicillin were also resistant to ceftriaxone (MICs≥2ug/ml).

P10. Cathepsin D expression in fibroadenoma, fibrocystic disease, in-situ and invasive ductal carcinoma of breast

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Objective: In the metastatic process, proteolytic enzymes play an important role in mediating the passage of cancer cells through the basement membrane and extracellular matrix. Biochemical analyses of tumour cytosol have suggested that high levels of cathepsin D relate to poor outcome in breast cancer patients. We have attempted to compare cathepsin D expression in a range of benign and malignant breast lesions to investigate its role in breast cancer progression. ***Method:*** 162 breast samples comprising 18 fibroadenomas, 22 fibrocystic disease, 96 invasive ductal carcinoma and 26 lesions with intraductal carcinoma components were evaluated for cathepsin D expression by a standard immunoperoxidase method on formalin-fixed, paraffin-embedded histological sections using a commercial antibody against cathepsin D. Cathepsin D expressions by both epithelial/tumour and stromal cells were semiquantitatively assessed and given a histoscore that combined staining intensity with the estimated population of stained cells. This was correlated with histomorphology, vascular and lymphatic invasion and lymph node status of the case. ***Results:*** 61.5% of invasive ductal

carcinoma showed cathepsin D expression in stromal cells whereas 47.9% showed cathepsin D positivity in cancer cells. H-scores in both stromal and tumour cells showed a positive correlation with histological grade ($p.<0.01$), but was not correlated with patient's age, tumour size, and histological evidence of vascular and lymphatic invasion. Only 1.8% of intraductal carcinoma expressed cathepsin D and this was limited to neoplastic cells. There was no cathepsin D expression by fibrocystic lesions and fibroadenomas, but a weak to moderate positivity was observed within myoepithelial cells in mammary ducts. ***Conclusions:*** These findings may have identified a mechanism whereby tumours with high histological grade mediate invasion into tissue. Stromal cells appear to have a role in abetting tumour progression and the means of their recruitment deserve further study.

P11. Molecular and cytogenetic investigation of the DCC gene in colorectal cancer

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We are investigating the status of the tumour suppressor gene, DCC (Deleted in Colorectal Carcinoma) in several colonic cell lines and colorectal cancers by cytogenetic and molecular approaches. Cytogenetic analysis showed 2 copies of chromosome 18 in 3 carcinoma cell lines but chromosome rearrangement and loss involving chromosome 18 was seen in a transformed adenoma line and confirmed by FISH with chromosome paints and a-satellite probes. FISH with 7 YAC contig probes from the DCC locus (18q21) showed that 2 copies of the DCC gene were present but evidence for DNA rearrangement was also seen. Using the PCR-SSCP technique with 3 microsatellite markers on 18q (D18S61), including the DCC gene (DCC1 & DCC2), intragenic DCC deletion was found in the transformed cell line. We have extended our observations to resected sporadic colorectal carcinomas in 20 patients. Interphase FISH on fixed normal and tumour smears with DCC YAC probes detected less LOH of 18q compared to the PCR based procedures. The D18S61 marker was able to detect LOH of 18q in 2/20 cases (10%) and replication error (RER) in an additional 3/20 cases (15%) by the PCR-SSCP method. Using another marker, DCC-M2 which detects an Mspl polymorphic site within the DCC gene, LOH of 18q was found in 1/20 cases (5%) by the RFLP analysis. These molecular changes may be involved in the progression of adenoma to carcinoma. Correlation between these changes with the pathological stage of the tumours will provide information on the use of chromosome 18q loss as a prognostic marker in patients with non-metastatic colorectal cancer.

P12. Molecular characterization of β-thalassaemia in the Malays in Malaysia - Evaluation of the ARMS in prenatal diagnosis.

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Molecular characterization of the β-thalassaemia genes in the Malays in Malaysia was carried out using the Amplification Refractory Mutation System (ARMS). Beta-mutations at 9 sites along the P-gene were studied - Codon 17, -28, Codon 15, Codon 19, Codon 26 (Hb E), IVSI#1, IVS#5, Codon 41-42 and IVSII #654. Seven β-mutations (Hb E, IVSI #5, IVSI #1, Codon 41-42, IVSII #654, Codon 17 and Codon 19) were responsible for 75% of p-thalassaemias. The remaining 25% of p-thalassaemias were caused by rare β-mutations. Direct genomic sequencing will be carried out to detect the rare p-mutations. Three p-mutations common to the Chinese race (Codon 41-42, IVSII #654 and Codon 17) were also found in the Malays.

Using the ARMS, prenatal diagnosis results can be obtained in 5 days and diagnosis can be carried out with less than 50 mg of chorionic villi. The cost of prenatal diagnosis is low as radioisotopes are not required and the enzyme Taq polymerase is used at 1:5 dilution. We conclude that the ARMS .provides a rapid, sensitive and inexpensive prenatal diagnosis technique for P-thalassaemia in the Malays in Malaysia.

P13. Malignant tumours of the skin - a histopathological study

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A review of all surgical biopsies received by the Department of Pathology between 1986 and 1996 revealed 171 cases of malignant tumours of the skin, constituting 0.2% of all cases. The racial distribution of patients with skin cancers was 115 (67%) Chinese, 30 (18%) Malay, 22 (13%) Indian and 4 (2%) others. Compared with hospital attendances during the period of study, there appears to be a higher prevalence of malignant cutaneous tumours among the Chinese ($p<0.0001$). There were 103 males with male to female ratio of 1.5. Metastatic tumours constituted 25% of the cases.

The commonest primary tumour was basal cell carcinoma (46 cases) of the face. Squamous cell carcinoma (SCC) formed the second most common cancer (41 cases) and in 53% (22 cases), the tumour occurred in the extremities. A history of radiation dermatitis, and exposure to chemical carcinogen was obtained in 2 cases. The SCC was well differentiated in most cases including 5 cases of verrucous carcinoma. There were 13 cases of nodular malignant melanoma with 10 cases (77%), originating in the legs. Primary cutaneous lymphoma constituted 13.2% (17 cases) and 94% (16 cases) was of T cell type. Metastatic adenocarcinoma formed 40% of secondary tumours.

This study shows that majority of the malignant tumours of the skin are primary tumours and the common ones are basal cell carcinoma and squamous cell carcinoma.

P14. M3 variant - a frequently misdiagnosed entity

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The diagnosis of Acute Promyelocytic Leukaemia (APL;M3) is rather straight forward due to its distinctive morphology. The diagnosis can even be made on PBF in the presence of abnormal hypergranular promyelocytes. However, the hypogranular variant (M3V) poses diagnostic difficulty and to inexperienced eyes, it may be mistaken for M2 or M5. Wrong diagnosis is detrimental to the patients as coagulopathy, a clinical presentation, may be aggravated by chemotherapy and results in early death. Death may be prevented by early diagnosis, timely starting of alltransretinoic acid (ATRA) and appropriate transfusion support.

M3 is a distinct type of AML which represents 5 - 15% of all AML. It is not only distinct in its mode of treatment (i.e. ATRA) but also in its morphologic, cytochemical, immunophenotypic markers and cytogenetic findings.

We studied retrospectively cases referred/diagnosed at our centre from July 1995 to September 1997. An attempt to correlate the morphology, cytochemistry, immunophenotyping and cytogenetic findings of the two subtypes of APL was done. The morphologic/cytochemical differences between M3, M3V, M2 and M5 are also highlighted.

P15. Malignant gastrointestinal stromal tumour - a case report

NR Nik Mustapha, MZM Kamal and Minn Lwin

Gastrointestinal stromal tumours (GIST) constitute a group of mesenchymal tumours arising from the wall of the gastrointestinal tract. Both the cellular differentiation and prognostic importance of various pathologic factors of GIST have been controversial. We recently reported a case of malignant GIST in a 44 year old Malay lady who presented with 2 episodes of haematemesis. A tumour was detected by oesophagogastroduodenoscopy, located mainly in the gastric antrum. Partial gastrectomy was performed. Grossly the tumour measured (75 x 50 x25) mm and vaguely lobulaetd with 3 small mucosal ulcerations. Microscopically it was composed of fairly uniformly small cells in a solid sheets and trabeculae with dense intervening fibrous stroma. They exhibited round to oval vesicular nuclei and minimal cytoplasm. Mitotic figures were between 9 to 20 per 50 h.p.f. The tumour irregularly

infiltrated the surrounding muscle coat. The mucosa was unremarkable except for the pressure ulcerations. Morphologically we could not rule out lymphoma and neuroendocrine tumour with certainty.. Special stains and immunohistochemistry had however failed to demonstrate its differentiation. Unfortunately we do not have electron microscopy for ultrastructural studies. Based on its size, mitotic index and the poor cellular differentiation, a final diagnosis of malignant GIST of uncertain differentiation was made. A review of the literature had shown that most GIST exhibit smooth muscle differentiation but only a few are true leiomyomas. A smaller fraction have neural or mixed smooth muscle-neural differentiation. Undifferentiated GIST form the smallest group. The histogenesis is probably from an indeterminate or pluripotent mesenchymal cell.

P16. A case of active ulcerative colitis masquering as a neoplasm

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A 37-year-old Indian lady was admitted in December 1995 when she presented with her second episode of diarrhoea. She was then at 20 weeks of gestation. Two years ago, she had a history of spontaneous abortion after an untreated bout of prolonged diarrhoea. The diagnosis of active ulcerative colitis was made after an extensive investigation. The patient responded symptomatically with medication. A year later, she presented with a tender umbilical mass, which on colonoscopy appeared to be malignant. Total colectomy and ileorectal anastamosis was immediately performed. Macroscopically, there was a collection of giant pseudopolyps forming sea-weed like fronds and occupying 13 cm of the transverse colon. This case describes the occurrences of localized giant pseudopolyposis, an interesting but unusual manifestation of ulcerative colitis.

P17. Glioblastoma multiforme of the cerebellum in a child - a case report and review of Literature

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Glioblastoma Multiforme is rare in children, those reported are those of the supratentorium. Cerebellar glioblastoma has not been documented.

A 7 year old boy presented with occipital headache, vomiting and papilloedema. CT Scan and MRI showed a tumour arising from the posterior fossa causing obstructive hydrocephalus of the 4th, 3rd and lateral ventricles, suggestive of medulloblastoma or ependymoma. Temporary relief of hydrocephalus by insertion of Rickham's catheter was done. At craniotomy a posterior fossa tumour arising from the vermis and obstructing the 4th ventricle was seen; the brainstem was not involved. Histological examination showed a hypercellular tumour exhibiting mild pleomorphism with an increased mitotic index. Peripheral palisades of tumour cells around necrotic areas are seen. Endothelial cells proliferation forming glomeruloid tufts are easily identified. These features are consistent with Glioblastoma Multiforme.

In conclusion although the most likely cause of space occupying lesion in the posterior fossa in a child is a medulloblastoma, a rare possibility of Cerebellar Glioblastoma should be considered.

P18. p53 protein expression in squamous and glandular malignancies of the cervix

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20 squamous and 20 adenocarcinoma of the cervix were immunohistochemically studied for the expression of p53 protein using a monoclonal antibody (DO7; DAKO). 17 (85%) squamous carcinoma and 11 (55%) adenocarcinoma showed nuclear p53 staining. Of these, 4 (20%) squamous and 3 (15%) adenocarcinoma exhibited immunopositivity in more than 75% of the neoplastic nuclei.

In comparison, 16 (80%) of 20 normal cervices removed during hysterectomies performed for various non-malignant conditions of the uterine corpus showed no p53 expression. 4 showed positivity in the occasional (<10%) normal ectocervical or endocervical cell nuclei. Although it would appear that p53 protein expression is increased in both squamous and glandular malignancies of the cervix compared to the normal controls, it is generally believed that only immunopositivity occurring in the "majority" of cellular nuclei is indicative of p53 tumour suppressor gene mutation. Hence, accepting >75% of neoplastic cellular nuclei to equate "majority", only 20% squamous and 15% adenocarcinomas of the cervix appear to have undergone p53 tumour suppressor gene mutation. This suggests that p53 mutation is not a frequent event in either squamous or glandular malignancies of the cervix. The finding of increased p53 protein expression in cervical carcinoma, although to levels not indicative of mutation, is consistent with the known observation that the majority of cervical cancers are HPV-related. This results in HPV E6/E7 proteins inactivating and possibly stabilising the inactivated wild-type p53 proteins to irnrnunohistochemically detectable levels.

P19. Expression of oestrogen receptor, progesterone receptor, p53 and c-erbB-2 proteins in invasive breast carcinoma

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Objective: This study correlates the expression of oestrogen receptor (ER), progesterone receptor (PR), c-erbB-2 and p53 proteins with histomorphological parameters in invasive ductal carcinoma of the breast, in an attempt to better understand the biological behavior of the tumour. **Method:** 51 cases of invasive ductal carcinoma of the breast treated at the University Hospital, Kuala Lumpur were randomly selected for this study. The tumours were evaluated for the above mentioned markers by the standard immunoperoxidase method on formalin-fixed, paraffin-embedded tissue sections using commercially available antibodies. For each parameter a score that combined the staining intensity and the estimated population of tumour cells stained was determined. This was then correlated with the various histomorphological features: histological grade, vascular invasion and lymph node metastasis. Results: There was good correlation between ER and PR expression. ER expression had an inverse correlation with histological grade, p53 and c-erbB-2 expression. 88% of the tumours expressed c-erbB-2 oncoprotein, but there was no relation with histological grade. However, c-erbB-2 expression was associated with a significant incidence of vascular invasion and lymph node metastasis. **Conclusion:** The association of higher grade tumours with p53 expression and loss of ER and PR expression indicate a change in the biological behavior towards less controlled cell growth. The inverse correlation between ER and c-erbB-2 expression has raised the possibility of a subset of cancer resistant to hormonal therapy. The association between c-erbB-2 expression and vascular and lymph node invasian suggests a role for this oncoprotein in tumour progression, that warrants further study.

P20. Clinical presentation of aggressive non-Hodgkin's lymphoma (NHL) seen at University Hospital between 1995-1997

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One hundred and twelve patients with aggressive NHL were studied. In 26 (14.4%) patients, the disease is confined mainly to the lymph nodes. In 69 (62.2%) patients, the lymphoma had extended from the lymph nodes to extranodal sites (secondary extranodal disease). In 16 patients, the disease arose primarily from extranodal sites (primary extranodal disease). The commonest site of primary extranodal disease is the gastrointestinal tract. The commonest extranodal sites for the secondary extranodal disease is the liver (42%) followed closely by the spleen (39.1%) and the bone marrow (30.5%). The survival for patients with nodal disease only and those with primary extranodal disease are fairly compatible, and the median survival is not reach by 2 years. But the group with secondary extranodal disease which probably represent more advanced disease, the median survival is only 38

weeks with only 15% of the patients still alive at 2 years (p = 0.005). The survey suggests the current treatment is ineffective for patients with secondary extranodal disease. Further improvement is required in this area.

P21. Toxic epidermo-necrolysis from cyanide or a post-mortem appearance mimicing burns? • a case report

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A case of a chronic alcoholic, who was found dead in the afternoon, on the terrace, after consuming some 'moonshine' liquor in the morning, is presented. His skin at the time of discovery, showed a blackened charred-like appearance, with slippage, prompting an impression at arrival at the A & E, as a case of foul play, both to the admitting Medical Officer, as well as the investigating police officer. Toxicological analysis of his body tissues and blood showed significant levels of alcohol, along with cyanide. This raised the question of whether these cutaneous blisters were not "toxic epidermo-necrolysis" as a result of cyanide. However, on histopathological examination, the skin tissues did not suggest any blister formation of the superficial layers, and there was no evidence of vital response in the tissues in the form of cellular infiltration. It was thought to be an accelerated postmortem phenomenon, of very short duration, coupled with the effect of prolonged surface contact (i.e. of near about 3 hours) of those parts with a hot surface from the sun baked terrace with surface temperatures above 51°C, with the summer in Delhi at its peak.

P22. CA 72-4 compared with CEA and CA 19-9 as a marker of some gastrointestinal malignancies

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The CA 72-4 assay measures in blood the tumour-associated glycoprotein TAG 72 which is expressed in gastrointestinal malignancies. CA 72-4 was determined by RIA while CEA and CA 19-9 were measured on the Abbott Imx analyser. This study had 65 cases of confirmed gastrointestinal cancer. The controls consisted of 16 cases with benign gastrointestinal diseases which served as diseased controls, and healthy adults. The cut-off values used were: CA 72-4, 4.0 U/ml; CEA, 5.0 ng/ml; and CA 19-9, 37 U/ml. For colorectal, gastric, and oesophageal carcinomas, CA 72-4 gave sensitivities of 58.3, 28 and 20 percent respectively, while CEA gave 83.3, 35 and 14.3 percent,; the sensitivities of CA 19-9 for these malignancies were 39.1, 18.2 and 23.1 percent. Marker expression in serum was highest in colorectal carcinoma and generally low in gastric and oesophageal carcinomas. Among the three cancers studied, CA 72-4 had the best sensitivity (58.3%) for colorectal carcinoma. When the combination of all 3 markers was considered, the best improvement was seen in oesophageal carcinoma when the sensitivity improved to 53.8%. Although the specificity of CA 72-4 for carcinomas was very high, it did not appear to be a useful a marker for gastrointestinal malignancies in view of its poor sensitivity.

P23. Laboratory findings of ornithine transcabamylase (OTC) deficiency in urea cycle enzyme defect

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The urea cycle, through a series of biochemical reactions incorporates unused nitrogen into urea and prevents the accumulation of toxic nitrogenous compounds. Deficiencies of ornithine transcabamylase

(OTC) are characterised by the signs and symptoms induced by the accumulation of the precursors of urea. We report a case of OTCD in a female patient who was admitted during neonatal period presenting with history of sudden onset of convulsions, vomiting and refusal of feeds. Laboratory findings revealed metabolic acidosis, hyperammonemia (626 umol/L) and a blood urea of 2.4 mmol/L. Serum electrolytes and random blood sugar was within the reference range. Liver function tests showed elevated levels of AST (2695 IU/L) and ALT (2156 IU/L). Amino acids by HVPE showed a normal pattern. A sample of urine was analysed by GC/MS and the results showed the presence of lactic acid, pyruvic acid, B-Hydroxybutyric acid, 2-ketoglutaric acid and adipic acid. A very high level of orotic acid and urucil was also detected. HPLC findings of plasma amino acid showed the presence of elevated omithine and very low level of citrulline.

We conclude that hyperammonemia and elevated levels of liver enzymes could be the presentation of urea cycle enzymes defects. The confirmation of the defective enzyme, Omithine Transcabamylase was made based on the presence of low levels of citrulline and an elevated level of orotic acid. The condition is mostly due to an X linked disorder. Antenatal diagnosis is available by a number of methods such as analysis of cultured fibroblasts and DNA analysis and this could help in early detection and prevention.

P24. A potential role of newer biochemical bone markers in patients with multiple myeloma

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Enhanced bone resorption is a characteristic finding in multiple myeloma (MM) but assessment of the newer biochemical bone markers has been poorly studied. We studied 17 MM patients. [10 males, (3 untreated, 5 in remission, 2 responding); 7 females (3 in remission, 4 responding)] and 15 controls. Serum bone-specific alkaline phosphatase (BSALP), osteocalcin and procollagen type 1 C-terminal peptide (PICP) were measured for bone formation while serum tartrate resistant acid phosphatase (TRAP), urinary deoxypyridinoline (Dpyr) and calcium (Ca) for resorption, the later two were expressed as a ratio to urinary creatinine (Cr) excretion. There were significantly: (1) higher (Dpyr/Cr) :PICP ratio (mean ± SEM = 0.100 ± 0.013 vs 0.064 ± 0.004, $p<0.05$) in male MM patients than in controls, (2) higher Dpyr/Cr excretion (10.48 ± 1.15 vs 5.48 ± 0.043, $p<0.001$), (Dpyr/Cr): BSALP ratio (0.783 ± 0.051 vs 0.359 ± 0.028, $p<0.001$) and (Dpyr/Cr): PICP ratio (0.147 ± 0.016 vs 0.064 ± 0.004, $p<0.001$) in untreated male MM subgroup than in control subjects, (3) higher (Dpyr/Cr): BSALP ratio (0.783 ± 0.051 vs 0.338 ± 0.114, $p<0.05$) in untreated than responding male MM subgroup, (4) higher (Dpyr/Cr): PICP ratio (0.147 ± 0.016 vs 0.077 ± 0.013, $p<0.05$) in untreated than those in remission male MM subgroup. In conclusion, (a) Dpyr is a sensitive marker in assessing bone resorption in MM patients, (b) (Dpyr/Cr): BSALP and (Dpyr/Cr): PICP ratios are even more sensitive in distinguishing untreated MM patients from the other MM subgroups and control subjects. The use of a combination of these markers may have potential role in monitoring bone disease progression and response to treatment in MM.