

**The 10<sup>th</sup> Annual Scientific Meeting of the College of Pathologists, Academy of Medicine Malaysia was held at Meritus Pelangi Beach Resort and Spa, Langkawi, Kedah from 18-19 June 2011. Abstracts of poster presentations follow:**

**POSTER PRESENTATIONS**

**P1. Rapid detection of aneuploides in amniotic fluid by multiplex ligation-dependent probe amplification**

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**Introduction:** Diagnosis of chromosomal abnormalities in high risk pregnant women is important for rapid reassurance of those with normal results and for early management of those with abnormalities. Multiplex ligation-dependent probe amplification (MLPA), a relatively new technique that allows quantification of multiple DNA sequences at different loci in a single reaction has been reported to be useful in detecting DNA copy number. In this study we evaluated the MLPA screening kit (SALSA MLPA P095) and determined its usefulness in the prenatal diagnosis of common aneuploides. **Method:** We performed the MLPA reactions on DNA extracted from amniotic fluid of 25 pregnant women referred to the O&G Department, Hospital UKM for prenatal screening of aneuploides. The kit contains 40 specific oligonucleotide probes for 8 loci on each of the chromosomes 13, 18, 21 and X and 4 loci on chromosome Y. Amplified PCR products were analysed using capillary electrophoresis and Coffalyser software version 7 and the average relative signal probes were determined for each chromosome. All samples were sent to a referral laboratory for parallel QF-PCR and karyotyping analysis. **Results:** Seven samples were not analysable because of poor DNA concentration. Out of the 18 samples analysed, 3 cases showed relative signal probes consistent with trisomy 18 and one case with trisomy 13. All the results of the 18 samples were in concordance with cytogenetic analysis and QF-PCR results. **Conclusion:** We find the MLPA a sensitive and specific method for detection of aneuploides. It is comparable to QF-PCR, rapid, simple, and reliable for the prenatal diagnosis of common numerical chromosomal disorders and has an added advantage of being cost effective.

**P2. The use of automated immature reticulocyte fraction in predicting bone marrow recovery in patient with acute leukaemia**

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**Introduction:** Automated immature reticulocyte fraction (IRF) is becoming an important parameter in monitoring patients with many conditions including anaemia, stem cell regeneration after bone marrow transplant and chemotherapy, erythropoietin therapy and follow up of patients with acute leukaemia post chemotherapy. It is on the way to replace absolute neutrophil count (ANC) because the latter, although easily measured and inexpensive, may be affected by infections and other conditions that cause marrow stress. In this study, we established the benefits of automated IRF

measurement using automated haematology cells analyzer over ANC in predicting bone marrow recovery post induction chemotherapy in patient with acute leukemias. **Method:** Peripheral blood samples from 22 patients with newly diagnosed acute leukaemia were analyzed in this study. The blood specimens were collected from day zero after chemotherapy and every 3 days till patients recovered haematologically. All blood samples were measured for ANC and IRF using automated haematology analyzer (Beckman-Coulter LH750). Day of recovery was selected base on day with sudden increase in the ANC and IRF value. Percentage of early, late or equal recovery using both measurements were calculated. Recovery day for ANC and IFR were compared using paired t-test. **Results:** This study showed that there was statistically significant difference in days of recovery between ANC and IRF (17.18 and 14.05 days respectively),  $P = 0.005$ . The percentage of patients showing early recovery was higher in the IRF than ANC (63.6% versus 22.7%). **Conclusion:** This study has established the superiority of IRF compared to ANC in predicting bone marrow recovery in patient with acute leukaemia post-induction chemotherapy. IRF is also a better parameter since it is not affected by infection, easily measured and inexpensive.

### **P3. Prostate cancer in Malaysian men, a 1-year retrospective study of patients in Hospital Kuala Lumpur**

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**Introduction:** Prostate cancer is the 4<sup>th</sup> highest cause of cancer mortality in Malaysian men. Screening for prostate cancer is by Prostate Specific Antigen (PSA) serology in combination with direct rectal examination (DRE). When PSA level is increased along with a positive DRE, the patient is first closely followed-up. However, if both are increasing, a tissue sample will be taken for histopathology examination. **Methodology:** A retrospective study was done all prostate adenomas in HKL in 2009. Demographic data were obtained from the patients' records and the histopathology slides were received in which demographic data was obtained from records and slides reviewed of all prostate adenocarcinoma cases diagnosed in Hospital Kuala Lumpur in 2009. Data were analysed using SPSS software version 15.0. The association between selected clinic-pathological findings with three major ethnic groups (Malay, Chinese and Indian) and age groups (40-50, 51-60, 61-70, 71-80 and 81-90years) were tested using Anova and Chi Square. **Results:** There were 148 cases with a mean age of 68 years. Most cases were from TRUS biopsies (75.7%) followed by prostatic chips and prostatectomy specimens (12.2% each). The incidence was highest in the Malays (48%) followed by the Chinese (40.5%) and Indians (11.5%). The Indians presented at an older age group (> 60 years). The PSA level were available in 129 cases and levels ranged from 1.86 to 10,000.00ng/ml. The Gleason score ranged from 6 to 10. There was a correlation between ethnicity with PSA and Gleason score with the highest in the Indian community. A positive correlation was noted between PSA and Gleason score. Inflammation was observed seen in 107 cases (72.3%) but no there was correlation with PSA or Gleason score. **Conclusion:** This series of prostate carcinoma cases in Hospital Kuala Lumpur showed a positive correlation with the selected histological features and ethnicity and between PSA and Gleason score. This was in concordance with similar literature reviewed. There was no significant correlation with inflammation.

**P4. Designing the right laboratory transplantation service policy: The Malaysian experience**

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The 'National Organ, Tissue and Cell Transplantation Policy' was formulated in 2007 involving more than fifty (50) healthcare planners and policy maker, clinical experts from various institution and related NGO's in the health industry. Pathologists were also member of the team. There were numerous meeting and discussion and it stretches 2-3 years before the final policy were agreed. Numerous documents and international experiences were reviewed before it was formulated. Pathology has it's own section that focuses on pathology policy. It was agreed that there will be no specific policy for each pathology discipline. The policy statements relating to pathology saw the need that it shall assist future planning, organization and staffing, leading as well as controlling, monitoring aspects of transplantation pathology services. The words and statements was selected so that it can be used as a tool to strengthen existing and future needs. In achieving these, there were numerous challenges. Firstly, transplantation involves multi disciplines and can numerous organs. Each has it's own requirement and priority that need to be considered to ensure adoptability and feasibility. As in the general policy, it shall be being patient/person focused. Besides that, there a need to formulate the right policy to support potential donor and recipient rights and needs. The following key policy areas were finally agreed and include (i.) the need to establish a National Pathology Committee for Transplantation Programme, (ii.) laboratory services that shall be established in parallel with other clinical specialties (iii.) The establishment of National Reference Laboratory for tissue typing and. (iv.) the need of establishhing specialised services in identified centres (v.) mandatory participation in Quality Assurance Programs (vi.) that labs supporting transplantation should be accredited. (vii.) A local network to store clinical and laboratory data of all donors and recipients to ensure good monitoring. (viii.) A shared database of all recipients and donors shall be established.

\*The author is also a contributor of MOH 'National Organ, Tissue and Cell Transplantation Policy' 2007

**P5. Pathology governance and leadership: Integrating public and private sector service delivery**

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Governance describes how an organization control it's action. It lets us know the common mechanisms that an organization uses to ensure agreed processes and policies are followed. It is to ensure responsibility and accountability in a large, widespread organizational structure. Governance by itself, may not be able to detail out what's monitored and recorded or takes steps to ensure compliance with agreed policies, and provides for corrective action in cases where the rules have been unobserved or overlooked. Management is about using resources at hand to achieve organisation goals. These include establishing the right planning for usage of resources, organizing and placing the appropriate individual to do the right job and placing effective leader to lead and oversees objectives are realized. In addition, monitoring and controlling mechanisms are set in place to organization ensure objectives are met. Areas or platforms for integration of pathology services include having an excellent and seamless network of pathology consultancy services, patient's information sharing (person's lifetime records, including pathology results and reports), integrated training approaches where both public and private sector experts work together toward greater capacity building for pathology services. In addition, there is integrating strategy to ensure wider coverage, greater accessibility and more affordability to wider range of the poor and unfortunate population. A social based industry such as health, requires professional empowerment practices. Thus, there is a need for strong governance towards pathology services and related groups. On the other hand, it is moved by quality driven

processes and targeted clinical outcomes and therefore a need for an equally guarded management approaches. However, pathology service is being delivered by both the public and private sectors; the common goals are the same; that is to assist in individual as well as population health but the specific goals may differ. For example, the public sector tends not to be profit driven. As such, the management approaches by both parties will not be fully similar. Therefore, there's a need by both sectors to agree to achieve common areas/platforms goals. Specific aspects of governance and management approaches must incorporate/address all agreed aspects describe above. This is to ensure pathology service integration materialize. In conclusion, relevant aspects of governance must be addressed and pathology leaders by both sector must be visionary to ensure integration of pathology services.

#### **P6. Pure erythroid leukaemia in a four year old child: A diagnostic dilemma**

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Erythroleukaemia (acute myeloid leukaemia, French-American-British category M6) is predominantly a disease of adults and comprises approximately 5%-6% of acute myeloid leukaemia and about 3% of acute erythroid leukaemia cases. It can occur at any age, including childhood. Pure erythroid leukaemia is frequently associated with complex cytogenetic abnormalities. This is a case of pure erythroid leukaemia in a 4-year-old girl with no evidence of molecular or cytogenetic abnormality. The patient initially presented to the hospital with a history of fever, runny nose, sore throat and was unwell for 2 weeks. Upon examination, she was found to have hepatosplenomegaly with cervical lymphadenopathy. Full blood count showed severe anaemia, leucocytosis and thrombocytopenia. Peripheral blood film showed bicytopenia, reticulocytosis and presence of blasts. The first bone marrow aspiration showed erythroid hyperplasia with trilineage dysplasia and excess of blasts consistent with myelodysplastic syndrome-refractory anaemia with excess of blasts (RAEB1), however erythroleukaemia (AML 6a) cannot be excluded due to haemodiluted marrow sampling. The second bone marrow aspiration showed marked erythroid hyperplasia (90%) with 6% myeloblasts of non-erythroid cells. Considering the clinical presentation and other laboratory findings, the diagnosis of pure erythroid leukaemia was made. She was started on induction chemotherapy, however succumbed to death one month after the diagnosis was made.

#### **P7. Co-inheritance of compound heterozygous Hb constant spring and single gene deletion alpha thalassaemia (3.7 kb type) with heterozygous delta beta thalassaemia: A case report**

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Hb Constant Spring (Hb CS) mutation and single gene deletions are common underlying genetic abnormalities for alpha thalassaemias. Co-inheritance of deletional and non-deletional Hb Constant Spring may result in various thalassaemia syndromes. Concomitant co-inheritance with beta and delta gene abnormalities would result in improved clinical phenotype. We report here a 33-year-old male patient who was admitted with dengue haemorrhagic fever, with a background history of Grave's disease, incidentally noted to have mild hypochromic microcytic anaemia. Physical examination revealed no thalassaemic features or hepatosplenomegaly. His full blood count results were Hb 12.2 g/dL, MCV 63 fl, MCH 20.5 pg, MCHC 32.6 g/dl, RDW 18.3 %, platelet 25 x 10<sup>9</sup>/L and WBC 4.7 X 10<sup>9</sup>/L. Iron status were normal ( Iron 14.7 umol/L; TIBC 51 umol/L; ferritin 286.27 ug/L). Peripheral blood film showed hypochromic microcytic red cells with occasional pencil cells seen. Quantitation of Hb using capillary electrophoresis (Sebia) revealed raised HbF (9.8 %), normal Hb

A2 level (2.8 %) and Hb A of 86.4 %. H inclusions were negative. Kleihauer test was positive with heterocellular distribution of Hb F among the red cells. DNA analysis for alpha globin gene deletion showed a single  $\alpha^{3.7}$  deletion and DNA analysis for Hb Constant Spring mutation showed positivity for the mutant allele. These findings were suggestive of co-inheritance of compound heterozygous Hb CS and 3.7kb alpha gene deletion with a concomitant heterozygous delta beta thalassaemia. Co-inheritance of Hb CS and  $\alpha^+$ -thalassaemia (3.7 kb type) is expected to result at the very least in a clinical phenotype similar that of two alpha genes deletion. However we demonstrate here phenotypic modification of alpha thalassaemia presumptively as a result of co-inheritance with beta delta chain abnormality as suggested by the high Hb F level.

#### **P8. Lymphoepithelial carcinoma of the parotid gland: A case report**

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**Introduction:** The first mention of the term lymphoepithelial carcinoma was in 1962. Today, the synonyms of this entity are lymphoepithelioma like carcinoma, malignant lymphoepithelial lesion, undifferentiated carcinoma with lymphoid stroma and carcinoma ex-lymphoepithelial lesion. Lymphoepithelial carcinoma is an unusual squamous cell neoplasms which is associated with a prominent lymphocyte-rich stroma. Lymphoepithelial carcinoma accounts for approximately 0.4% of malignant salivary gland tumors. Believed to arise de novo, this carcinoma has a mysteriously strong racial prevalence, 75% of affected people being Eskimos / Mongolian and is strongly associated with EBV infection. Of the occurrence, 80% are in the parotid gland. **Method:** A case of lymphoepithelial carcinoma occurring in the right parotid salivary gland is presented. Histologic features of this rare malignant epithelial tumor is described in detail. **Results:** The main histological features of an undifferentiated carcinoma within a prominent background of mature lymphocytes and plasma cells infiltrates are observed. **Conclusion:** A final diagnosis of primary lymphoepithelial carcinoma of the parotid gland was given after thorough examination of the nasopharyngeal region which include random biopsies, to rule out metastatic nasopharyngeal carcinoma.

#### **P9.**

**(WITHDRAWN)**

#### **P10. Immunohistochemical analysis of APC expression in chronic gastritis and gastric cancer**

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**Introduction:** Gastric cancer is the 7<sup>th</sup> most common cancer in Malaysia. It is usually diagnosed at advanced stage and thus causing high mortality. Therefore, there is a need to find a diagnostic marker at its early stage. Adenomatous polyposis coli (APC) is classified as tumor suppressor gene. There are studies which showed that inactivation of APC plays a role in the development of some gastric cancers, particularly very well differentiated adenocarcinomas and signet-ring cell carcinomas but yet there is no study on the APC expression in chronic gastritis, a precursor lesion of gastric cancer. **Aim:** In this study, APC was studied by using immunohistochemistry to determine whether alteration in the expression levels of APC occurs prior to the development of gastric cancer and can be detected in chronic gastritis. It will also disclose whether these alterations may reliably predict

early stage of gastric cancer. **Methodology:** Immunohistochemical staining of APC was performed on 54 chronic gastritis, 51 *Helicobacter pylori*-associated chronic gastritis (HPCG) and 34 gastric cancer samples. Slides were scored using semi-quantitative scoring system. Wilcoxon Signed-Rank Test and Mann Whitney U Test were used for statistical analysis. **Results:** The Mann-Whitney U test showed that there is significant difference ( $P < 0.05$ ) in the APC expressions between chronic gastritis and gastric cancer (higher percentage of positivity in gastric cancer). For Wilcoxon signed-rank test, there are significant differences in APC immunoreactivity in chronic gastritis and gastric cancer with their normal adjacent mucosa ( $P < 0.05$ ). **Conclusions:** Increased expression of APC was found in the samples of chronic gastritis and gastric cancer. Further study need to be carried out to find the association of APC with gastric cancer and its precursor lesions.

#### **P11. Unique expression of CD10, CD34, CD13 and CD38 in adult precursor B-acute lymphoblastic leukaemia (Pre B-ALL) with BCR/ABL gene rearrangement**

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Pre B-ALL has been reported to be associated with translocation (9; 22) or BCR/ABL fusion gene with resistance to conventional chemotherapy and poor prognosis. Although cytogenetic and PCR analysis are standard methods for BCR/ABL gene detection, immunophenotyping has been reported to be useful in the leukaemia patients. Co-expression of CD10, CD13 and CD33 or a homogenous expression of CD10 and CD34, but low and relatively heterogenous expression of CD13 and CD38 have been reported in majority of BCR/ABL positive pre-B ALL cases (MD Taberero et al, 2001). The aim of the study is to determine the association of CD10, CD34, CD13, and CD38 in adult pre-B ALL patients with BCR/ABL gene locally. Bone marrow or peripheral blood samples from newly diagnosed adult pre-B ALL in Hospital Kuala Lumpur and UKM Medical Centre were collected in EDTA or ACD tubes with consent. Immunophenotyping with CD10, CD34, CD13, and CD38 were performed. Routine cytogenetic analysis and RT-PCR analysis for BCR/ABL fusion gene were also performed. Thirty-one cases of adult Pre B-ALL were analyzed from June 2009 to September 2010. Nine cases had to be excluded as molecular results were unavailable. Our immunophenotyping results showed that ten cases of BCR/ABL positive Pre B-ALL had homogenous expression for CD34 and CD10, and heterogenous expression for CD13 and CD38 as previously reported (MD Taberero et al, 2001). BCR/ABL fusion gene by RT-PCR analysis were positive in all these cases. There were two cases of BCR/ABL negative Pre B-ALL in this study however, that had showed similar phenotypic expression (homogenous expression for CD34 and CD10, heterogenous expression for CD13 and CD38). In conclusion, immunophenotypic markers has the potential to predict the presence of BCR/ABL fusion gene among adult Pre B-ALL cases, however, more number of cases must be studied to correlate these findings.

**P12. Angiogenic properties of Wharton's jelly stem cells versus human umbilical vein endothelial cells**Hayati AR<sup>1,2</sup>, Nur Fariha MM<sup>3</sup>, Fatimah S<sup>3</sup>, Tan GC<sup>3</sup>, Tan AE<sup>4</sup>, Chua KH<sup>5</sup>

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**Introduction:** Stem cells-based therapy for angiogenesis has emerged as a new option to treat infarcted heart tissues. Having some advantages such as ease of isolation and abundantly available, Wharton's jelly stem cells (WJSCs) can be an appropriate choice. This study aimed to compare the angiogenic and endogenic properties of WJSCs and Human umbilical vein endothelial cells (HUVECs). **Method:** WJSCs and HUVECs from human umbilical cord were isolated and cultured until passage 3 in equal volume mix of Ham's F12 medium and Dulbecco's Modified Eagle Medium, supplemented with 10%FBS, 1% Glutamax, 1% Vitamin C and 1% Antibiotic antimycotic. Total RNA was extracted from cultured cells at passage 3. Quantitative real time PCR was performed to appraise the differential mRNA expression pattern of the following angiogenic and endogenic genes; VEGF, bFGF, HGF, PECAM1, eNOS and angiopoietin-1. Primer 3 software was used to design the primers and the RT-PCR reaction was performed with 100ng of total RNA, 5uM of each primer and SYBR Green as indicator in Bio-Rad iCycler instrument. The reaction kinetic of each primer set and protocol was verified with melting profile and further confirmed with agarose gel electrophoresis. Expression level of each gene was then normalized to GAPDH. **Results:** The cultured WJSCs showed significant higher expression of angiogenic genes; VEGF, bFGF and HGF compared to HUVECs. However, the endogenic-associated genes; PECAM-1, eNOS and angiopoietin-1 were expressed at very low level in WJSCs compare to HUVECs. These results proposed that WJSCs have a great angiogenic potential and may promote angiogenesis by the secretion of crucial angiogenic growth factors but they were unlikely to become endothelial cell without further cytokine induction. **Conclusion:** These findings suggested that Wharton's jelly is a potential stem cell source for angiogenic therapeutic purpose in ischemic heart disease.

**P13. Two serological screening tests for detection of HbsAg**

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**Introduction:** Hepatitis B is highly infectious disease caused by Hepatitis B virus (HBV), which affects two billion people worldwide and more than 350 million runs a chronic course and is one of the most important causes of infectious morbidity and mortality worldwide. The present study was designed to compare the specificity and sensitivity of two serological screening tests (ie. RPHA and ICT) with that of ELISA which is a gold standard in detection of HBsAg and to compare the performance characteristics of each test. **Methodology:** Non-intervention comparative study was conducted to 200-HBsAg positive samples, among which 150 were asymptomatic blood donors and 50 were in-patients of Defense Services General Hospital, Myanmar. **Results:** Out of 200 samples, 166 were HBsAg positive and 34 were negative by ELISA, 159 were positive and 41 were negative by ICT and 167 were positive and 33 were negative by RPHA. As ELISA was standardized, out of 166 HBsAg positive samples, 156 were positive and 10 negative by ICT and 159 were positive and 7 negative by RPHA, revealing the sensitivity of 93.97% and 95.78% respectively. Out of 34 HBsAg negative samples detected by ELISA, 31 were negative and 3 were positive by ICT and 26 negative and 8 positive for RPHA with specificity of 91.17% and 76.47% respectively. **Conclusion:** Comparison of the performance characteristic showed RPHA required moderate level of training

and skilled staff, need instruments, relatively very cheap and with high sensitivity. It could be useful in large screening purposes whereas ICT only low level of training needed with few steps of procedures, although highly sensitive, cost per test is rather expensive, it could be useful in private sectors where turnover of the patient is small as rapid means of detecting HBsAg.

#### **P14. Evaluation of saliva as a source of DNA for human leukocyte antigen (HLA) – A pilot study**

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**Introduction:** Self-collection of saliva is a user friendly way to collect DNA for genetic studies. The aim of this study was to evaluate the quality and quantity of DNA using the Oragene saliva collection method and to determine its suitability for HLA typing. **Methods:** Fourteen individuals consented to deposit 2 ml of saliva into an Oragene saliva collection tube. DNA was extracted using a standardized protocol provided by DNAGenotek Oragene. The concentration of DNA extracts was measured. HLA typing was carried out using Polymerase Chain Reaction-Sequence Specific Primer (PCR-SSP) and PCR using sequence-specific oligonucleotides (PCR-SSO). HLA typing was carried out on five DNA specimens extracted from saliva and compared with DNA extracted from blood. **Results:** The mean DNA yield from saliva was 147 ng/ $\mu$ l and ranged from 9.4 ng/ $\mu$ l to 580.6 ng/ $\mu$ l. Eight HLA-A, thirteen HLA-B and eight HLA-DR alleles were identified. The results of five HLA typing from saliva DNA was in agreement with findings from the routine blood processing. **Conclusion:** We have demonstrated that saliva samples provide sufficient DNA for HLA typing by PCR-SSP and PCR-SSO methods with results comparable to DNA from blood.

#### **P15. Comparison of CD34 cell dose and T cell dose between bone marrow and peripheral blood haemopoietic stem cell harvest in paediatric patients in HKL**

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**Introduction:** Haemopoietic stem cell transplant (HSCT) begins with the use of bone marrow harvest as the source of haemopoietic stem cell (HSC). The discovery of circulating HSC in peripheral blood for several days after chemotherapy and growth factors has allowed peripheral blood HSCT. It has been noted that peripheral blood HSCT is associated with faster engraftment and increase chronic graft versus host disease when compare with bone marrow HSCT. The aim of this study is to assess the difference in CD34 cell dose and T cell dose in the bone marrow and peripheral blood allogeneic harvest in our centre. **Methods:** Retrospective study of all bone marrow (BM) and peripheral blood (PB) HSC harvest from January 2009 till December 2010. These HSC has been analysed for CD34 cell dose and T cell dose. **Results:** A total of 44 BM and PB allogeneic HSC have been received by HKL BMT lab for the 2 year period. Out of these 44 HSC harvest, 25 are from BM and 19 are from PB. Mean CD34 cell dose for BM HSC harvest is  $13.68 \times 10^6$ /kg and for PB HSC harvest is  $10.65 \times 10^6$ /kg. Mean T-cell dose for BM HSC harvest is  $16.18 \times 10^6$ /kg and for PB HSC harvest is  $70.17 \times 10^6$ /kg. **Conclusions:** T-cell dose is significantly higher in PB HSC harvest when compared to BM HSC. However there is no significant difference in the CD34 dose between PB and BM HSC harvest. These findings correlate with the increase in chronic GVHD among recipient of allogeneic PB HSC.

**P16. Sclerosing angiomatoid nodular transformation of the spleen**

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**Introduction:** Sclerosing angiomatoid nodular transformation (SANT) of the spleen is a rare, benign, proliferative lesion affecting the spleen. The lesion is a nonneoplastic, nodular vascular proliferation of the red pulp with prominent sclerosis. We report a case of a 47-year-old lady who had chronic epigastric pain and did not respond to conservative treatment. Ultrasound and CT abdomen shows splenomegaly with multiple splenic lesions. **Methods:** Splenectomy was performed in January 2011. On gross examination, the spleen is lobulated. Cut sections show multiple well circumscribed nodules which have bosselated contour with mixed whitish and brownish cut surface as well as stellate shaped whitish stroma at the periphery. The following markers were used in the immunohistochemical evaluation: CD34, CD31, CD8 and ALK. **Results:** Sections from the spleen show multinodular lesions with angiomatoid appearance. The nodules are composed of slitlike, round and irregular shaped vascular spaces lined by plump endothelial cells interspersed by spindle cells. Some of the nodules particularly the smaller ones are surrounded by concentric rings of collagen fibers. Numerous red blood cells are present and scattered inflammatory cells. Immunohistochemical stains for CD31, CD34 and CD8 demonstrated an admixture of blood vessels recapitulating those in the normal splenic red pulp. The splenic lesion was interpreted as Sclerosing angiomatoid nodular transformation (Multinodular haemangioma). **Conclusion:** Our case show characteristic gross, histological and immunophenotypical features similar to the cases previously described in the literature. Histologically, Sclerosing angiomatoid nodular transformation comprises of proliferation of vessel. Therefore, the histological differential diagnosis includes haemangioma, hamartoma, haemangioendothelioma, littoral cell angioma, inflammatory psedotumour and desmoplastic response to metastatic carcinoma. The pathogenesis and aetiology of SANT need to be ascertained to establish appropriate patient's management.

**P17. Clinicopathologic features of colorectal cancer - Hospital Sultanah Bahiyah's three and a half years experience**

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**Introduction:** Colorectal cancer is one of the commonest cancers in Malaysia. Managing it is a team process. High-quality reporting of resected specimens is very important, partly to ensure accurate staging and appropriate subsequent treatment. **Methods:** Clinicopathologic information was obtained from pathology reports of 215 colorectal cancer patients in Hospital Sultanah Bahiyah (October 2007 to March 2011). **Results:** Most patients were in 50-79 age group (77%) and only 15% were less than 50 years old. Male to female ratio was 1.4:1. Colonic and rectal tumours constituted 59% and 41%, respectively. Collectively, left sided tumours were more common, (71.1%). Majority (88.1%) was adenocarcinoma of usual-type and 85% of these were moderately-differentiated. Most (71.7%) showed infiltrative tumour edge (a negative prognostic factor). Tumour-infiltrating lymphocytes were observed in 1%. Significant mucinous component (not mucinous tumours) were noted in 15%. Only 10.3% of tumours were confined to bowel wall (pT1N0 or pT2N0); thus, the majority had spread beyond muscularis propria and/or had metastasised. Extramural venous invasion (predictor of liver metastasis) was seen in 20.8%. Polyps were reported in 29.4% of specimens. Circumferential margin involvement was seen in 11.4% of rectal tumours. Resection end margin involvement was seen in 3.5% of total cases. **Conclusion:** The majority of our colorectal cancer patients were older, with male:female ratio of 1.4:1. Tumours were mostly left sided and being adenocarcinoma of usual-type. A significant number showed histologic features representing negative prognostic

factors. A small number of tumours showed MSI-positive morphology. Most patients had growths beyond muscularis propria and/or metastatic disease, suggestive of late presentation. Achievements in resection margin clearance were good.

### **P18. Actinomycoses masquerading as an abdominal intramuscular tumour**

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**Introduction:** Actinomycosis is a chronic abscess-forming disease predominantly caused by *Actinomyces israelii*. Long-term use of an IUCD is known as a risk factor for pelvic actinomycosis. However the involvement of the abdominal wall is a rare clinical entity. We report an unusual case of abdominal actinomycosis presenting as abdominal wall intramuscular mass. **Case:** A 41-year-old lady para 3 presented with 3 months history of right iliac fossa pain associated with an infiltrative abdominal wall mass. There was also associated loss of weight and appetite. Her white cell count was raised but there was no history of fever. She was on IUCD contraception for the past 3 years and her menses were otherwise regular. The mass was then excised in view of clinical and radiological suspicion of malignancy. The histopathological findings of the mass showed presence of multiple abscesses containing *Actinomyces* colonies within the muscular wall and adjacent tissue, surrounded by chronic inflammation and tissue fibrosis. She had her IUCD removed and was started with antibiotic therapy with serial CT scan surveillance. **Conclusion:** This case illustrates that actinomycoses can mimic the presentation of a malignancy only to be confirmed histologically as an infection. Recognition of the various ways of presentation for *Actinomyces* is essential for appropriate management of the patients.

### **P19. Liver allograft biopsies: Experience in Hospital Selayang**

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**Introduction:** Liver biopsies are important to document any allograft rejection in liver transplantation. As one of the main centres providing liver transplant services, we are fortunate to experience dealing with liver allograft biopsies. Here, we briefly describe our experience over a 7-year period. **Materials and Methods:** All reports of liver allograft biopsies performed from January 2004 to December 2010 in Hospital Selayang along with the relevant clinicopathological data were retrieved from the Department of Pathology archives using Cerner Pathnet Application. **Results:** There were 29 biopsies done from 18 patients aged between 19 months to 47 years old. Eleven of them were from males and 7 were female patients. There were 10 Malays, 5 were Chinese and 3 were Indians. Biliary atresia, Tyrosinaemia, Alagille's syndrome and fulminant liver failure were some of the earlier indications or aetiologies for the liver transplantation. Most of them showed abnormal liver enzymes prior to allograft biopsy. The biopsies were evaluated using Banff scheme for liver allograft rejection. Ten (34.5%) of the cases showed acute rejection, 2 of the cases was indeterminate and another 2 cases showed chronic rejection. Fourteen (48.3%) of the cases showed no evidence of rejection. Drug induced injury, chronic biliary outflow obstruction, vanishing bile duct syndrome and chronic hepatitis were among the histopathological changes found in the allograft biopsies of these 14 cases. **Conclusion:** Liver biopsy is useful to evaluate graft dysfunction and to confirm clinically suspected rejection. Adequate interpretation is dependent on adequacy of sampling, knowledge of differential diagnoses, clinical aspects of transplant hepatology and also close interaction with the clinical team.

**Keywords:** liver allograft biopsy, liver transplant, rejection

**P20. Common chromosomal translocation in children with acute leukemia: A descriptive study**

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The study aims to show the epidemiology and the common chromosomal translocation in childhood acute leukemia cases in Institute Paediatric, Hospital Kuala Lumpur. A total of 186 bone marrow samples were collected from children with newly diagnosed acute leukaemia from January 2008 to December 2010. Their demographic features including age, race, gender and immunophenotyping were recorded. Multiplex reverse-transcriptase polymerase chain reaction (RT-PCR) was performed by using the *HemaVision* protocols for detection of 28 common translocation. The incidence of acute leukaemia was higher in boys (56.5%) than in girls (43.5%). Among the three common races, Malay (69.9%) was the most affected followed by Chinese (21.0%) and Indian (5.4%), while other races represent 3.8%. We found that acute lymphoblastic leukemia (ALL) was the most common subtype (59.1%) with a *peak* incidence at 1 to 5 years of age (44.1%). The Philadelphia chromosome t(9;22) was present in most of the cases followed by t(12;21) that accounted for 11.3% and 8.6% respectively. Our study suggests that recurring chromosomal translocations can be detected in a substantial number of cases of childhood acute leukaemia. A comprehensive subgrouping of childhood acute leukaemia by molecular technique is very useful not only for a diagnostic purpose, but also for treatment strategies and prediction of prognosis.

**P21. RCL2 is a potential formalin substitute for tissue fixation in routine pathological specimens**Noraidah Masir<sup>1</sup>, Mahdieh Ghoddoosi<sup>1</sup>, Suhada Mansor<sup>1</sup>, Faridah Abd.Rahman<sup>1</sup>, Chandramaya S. Florence<sup>1</sup>, Nor Azlin M. Ismail<sup>2</sup>, Nani Md Latar<sup>3</sup>

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**Aim:** RCL2 is a new alcohol-based, non-toxic fixative that does not cause protein cross-linking. However its use and suitability for routine histopathological examination has not been widely investigated. In this report, we describe its use as a fixative in a variety of tissue types. We also studied the suitability of RCL2-fixed tissues for various investigations including immunohistochemistry and molecular techniques. **Materials and Methods:** Forty-nine tissue samples from twenty-four benign fresh surgical specimens were fixed in three types of fixatives i.e., formalin, RCL2 diluted in 100% ethanol (RCL2-100) and RCL2 diluted in 95% ethanol (RCL2-95). The fixed tissues were assessed for suitability for microtomy, quality of histomorphology, histochemical staining, immunohistochemistry, *in situ* hybridization analysis (FISH and SISH) and quality of extracted genomic DNA. All the procedures performed on RCL2-fixed tissues followed the same protocol as that performed on formalin-fixed tissues without any adaptation. **Results:** Our result showed that RCL2-fixed paraffin-embedded (RCLPE) tissues were suitable for microtomy. In addition, the quality of tissue architecture and the results of histochemistry, histochemical staining, immunohistochemistry, SISH and FISH from RCLPE were comparable to formalin-fixed paraffin-embedded (FFPE) tissues. The concentration of extracted genomic DNA was considerably higher in RCLPE than in FFPE tissues. Using RCL2 diluted in 95% ethanol did not affect the results of fixation. **Conclusions:** This study found that RCL2 is a potential formalin-substitute fixative suitable for use in routine histopathological examination without the need for major changes in the technical procedures.

## P22. Distributions of HLA-A, -B & -DRB1 alleles among the Malay population in Malaysian Marrow Donor Registry (MMDR)

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**Introduction:** Human leukocyte antigen (HLA) genes are the most polymorphic loci in the human genome. These genes can serve as highly informative genetic markers not only in the clinical field but also in population studies. As allele frequencies differ considerably among various human populations, knowledge of the allele distributions allows inferences to be made about population relationships, histories and selective forces influencing HLA diversity. **Methods:** In the present study, a total of 12,262 healthy Malay volunteers recruited for the Malaysian Marrow Donor Registry (MMDR), were typed for HLA-A, -B and -DRB1 alleles by the polymerase chain reaction-sequence specific primers (PCR-SSP) method. **Results:** The number of known alleles identified was 19 for HLA-A, 37 for HLA-B and 14 for HLA-DRB1. The highest frequencies of HLA-A alleles were A\*24 (n=8931, freq.=0.3641), A\*11 (n=4734, freq.=0.1930), A\*02 (n=3894, freq.=0.1588) and A\*33 (n=3108, freq.=0.1267). For HLA-B alleles, the highest frequencies were B\*15 (n=6388, freq.=0.2605), B\*35 (n=2615, freq.=0.1066) and B\*18 (n=2377, freq.=0.0969) and for HLA-DRB1, the frequencies were DRB1\*12 (n=6606, freq.=0.2694), DRB1\*15 (n=6465, freq.=0.2636) and DRB1\*07 (n=2404, freq.=0.0980). **Conclusion:** The data obtained might be useful for future HLA and disease association studies apart from its importance in determining the possibility of finding matched unrelated donors in transplantation medicine.

## P23. HLA-A, -B and -DR alleles frequencies in Chinese population: A Malaysian Marrow Donor Registry experience

Norhazlin M, Dhaliwal JS, Masita A, Salawati M, Norfarhana KF, Ripen AM, Farid B, Noormalin A, Faizal B, Ainur YY, Maiselamah L, Lee YY, Shahnaz M.

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**Introduction:** The Malaysian Marrow Donor Registry (MMDR) was established in December 2000 and currently has more than 17,000 registered potential donors. We analyzed the frequency of HLA-A, HLA-B and HLA-DR in the Chinese individuals who are registered with the MMDR. **Methods:** One thousand four hundred and eighteen Chinese registered with the Malaysian Marrow Donor Registry between January 2001 and December 2010 were typed for HLA-A, HLA-B and HLA-DR using Polymerase Chain Reaction-Sequence Specific Primer (PCR-SSP) and Polymerase Chain Reaction-Sequence Oligonucleotides (PCR-SSO). **Results:** The observed alleles corresponded to 16 HLA-A, 31 HLA-B and 13 HLA-DR antigens. Out of 1418 of the donors, the gene frequency of a few alleles were predominant for each locus, such as A11 (0.33), A2 (0.29), A24 (0.16), B60 (0.16), B15 (0.13), B46 (0.13), DR4 (0.15), DR12 (0.15) and DR15 (0.15). The frequency of the most common HLA alleles was similar to those found in the Southern ethnicities in Mainland China. **Conclusion:** This data is important in estimating the likelihood of obtaining appropriately matched donors for stem cells or bone marrow transplants and for the study of HLA associated diseases in the population.

**P24. Detection of polyomavirus/BK virus associated nephropathy in the urine of a renal transplant patient**Noriah O<sup>1</sup>, Shobana MD<sup>3</sup>, Simon P<sup>1</sup>, Nik Hasimah NY<sup>2</sup>, Noor Laili MM<sup>1</sup><sup>1</sup>Department of Pathology, Selayang Hospital; <sup>2</sup>Department of Pathology, Hospital Kuala Lumpur; <sup>3</sup>Department of Pathology, Hospital Tengku Ampuan Rahimah, Klang

**Introduction:** Polyomavirus /BK virus associated nephropathy in renal transplant recipients is a rare but important cause of renal transplant dysfunction, occurring in only 3% to 4% of patients. In this case, a 39 year old Orang Asli woman developed acute borderline rejection secondary to polyomavirus / BK virus infection. **Case description:** A 36 year old Orang Asli woman with a medical history of end stage renal disease on regular haemodialysis, hypertension and hyperparathyroidism had a cadaveric renal transplant in Selayang Hospital in May 2009. Post transplant, she was well up till September 2009 when it was noted that her creatinine levels were increasing. A renal biopsy done in October 2009, showed borderline rejection which was C4D negative and SV40 negative with no viral inclusions. However the urine screening sample sent at that time was found to be positive for Decoy cells. Another renal biopsy done in December 2009 showed BK nephropathy (SV40 positive). Urine PCR for BK virus which was sent to Australia was positive (more than 1 million copies detected). The patient was then treated accordingly and by April 2011, the urine was negative for Decoy cells. Subsequent biopsies showed no evidence of BK nephropathy or rejection and serial serum creatinine levels showed a downward trend. **Discussion:** This case illustrates the importance of urine as a screening tool for detection of Polyomavirus / BK virus infection in renal transplant recipients. Even before the renal biopsy showed evidence of viral infection, the urine already demonstrated the presence of Decoy cells which goes to show how a cheap and simple investigation can be used to detect a condition that can be potentially fatal.

**P25. Renal allograft biopsies in Hospital Selayang: An overview**Noriah O<sup>1</sup>, Shahawiah AW<sup>1</sup>, Nur Syahrina R<sup>2</sup>, Nik Hasimah NY<sup>3</sup>, Noor Laili MM<sup>1</sup><sup>1</sup>Department of Pathology, Hospital Selayang; <sup>2</sup>Faculty of Medicine and Health Science, Universiti Sains Islam Malaysia; <sup>3</sup>Department of Pathology, Hospital Kuala Lumpur

**Introduction:** The most common indication for renal allograft biopsy is impaired renal allograft function and acute renal allograft dysfunction. The biopsy may also be part of post transplant renal biopsy protocols. Graft failure may be due to many causes, acute rejection being the most common. A retrospective study on renal allograft biopsies in Hospital Selayang was done to get an overview of the pattern of post transplant renal biopsy cases in the hospital. **Materials and Methods:** All histopathological reports of renal biopsies that were performed from January 2006 to March 2011 in Hospital Selayang along with the relevant clinicopathological data were retrieved from Department of Pathology archives using Cerner Pathnet Application. **Results:** Altogether there were 112 renal biopsies and these were obtained from 77 patients. The age range was 10 years to 68 years old, from 46 males and 31 females. Forty two percent of the cases (47/112) showed acute rejection changes either cellular, humoral, borderline or mixed categories. Acute cellular rejection was seen in 25/47 (53.2%) cases with most of them having tubulointerstitial rejection, Banff type 1. There were 19/47 (40.4%) cases showing borderline changes and 12/47 (25.5%) showing humoral rejection. Acute tubular necrosis was seen in 23/112 (20.5%) cases. Other changes seen are chronic allograft nephropathy, calcineurin inhibitor toxicity, pyelonephritis as well as infection with Polyoma virus and Cytomegalovirus. **Conclusion:** These findings showed that rejection changes are the commonest entity emphasising the value of renal biopsy in post transplant patients. Appropriate management can be instituted effectively if early changes of rejection can be detected.

**P26. Immunohistochemical study of P16<sup>INK4A</sup> and survivin expressions in cervical squamous neoplasm**

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**Introduction:** Cervical cancer is the second most common cancer affecting Malaysian women. And despite pap smear screening, many women are still diagnosed in the advanced stage of cervical cancer. This could partly be due to failure of detection of its precursor lesions. This study aims to determine the expression of p16<sup>INK4A</sup> and survivin as possible predictive biomarkers in cervical squamous neoplasm. **Methods:** This is a retrospective study on 201 cases of cervical neoplasms comprising of 129 cervical intraepithelial neoplasia (CIN) and 72 squamous cell carcinomas (SCC). All samples were evaluated by two independent observers using p16<sup>INK4A</sup> and survivin monoclonal antibodies. The p16<sup>INK4A</sup> expression was graded as negative, focal and diffuse positivity. The intensity for survivin expression was graded as weak, moderate and intense. **Results:** It is seen that p16<sup>INK4A</sup> expression in CIN 1, CIN 2 and CIN 3 were 25.4%, 42.9% and 95.9% respectively. The majority of SCC (98.6%) showed p16<sup>INK4A</sup> expression. Survivin expressions in CIN 1, CIN 2, CIN 3 and SCC were 56.7%, 33.4%, 87.5% and 98.6%. There was a linear relationship between increasing grade of CIN and p16<sup>INK4A</sup> expressions. **Conclusion:** Our study showed that p16<sup>INK4A</sup> expressions correlate well with the increasing grade of CIN. Although survivin does not correlate well with the increasing grade of CIN, it could be useful in differentiating CIN 3 from SCC.

**P27. A case of acute megakaryoblastic leukaemia with translocation t(1;22) in a non-down syndrome child**

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The t(1;22)(p13;q13) is an uncommon subtype of AML comprising <1% of all cases. It exclusively occurs in non-down syndrome infant and has female predominance. Majority presents with marked organomegaly, anaemia, thrombocytopenia and moderately elevated white cells. Presence of this translocation carries a poor prognosis. We described this rare form of leukaemia in a 9-month-old girl who presented with bruises, massive hepatosplenomegaly and multiple cervical and inguinal lymphadenopathy. The blood film showed severe anaemia with ovalostomatocytosis, thrombocytopenia and mild increase in white cells. The bone marrow aspirate showed numerous blasts showing high nuclear-cytoplasmic ratio and agranular cytoplasm with cytoplasmic blebs. Peroxidase staining was negative. The immunophenotyping showed blasts expressing CD117, CD 13, CD33 and CD 61 which confirmed the diagnosis of acute megakaryoblastic leukaemia. Interestingly, the cytogenetic finding of translocation (1;22) which is most common in acute megakaryoblastic leukaemia in infants without Down syndrome was found in this case. She received the AML trial 15 ADE protocol chemotherapy regime and as a consequences developed severe neutropenic sepsis and respiratory distress requiring ventilatory support, multiple antibiotics and granulocyte colony stimulating factor (G-CSF). However, after recovery and following discharge, she was not brought in for further follow-up.

**P28. Mesenchymal stem cell delivery system via different bore size needles**\*Nurul Ain N MY<sup>1</sup>, \*Mamidi MK<sup>2</sup>, Nathan KR<sup>2</sup>, Thrichelvam ST<sup>2</sup>, Zubaidah Z<sup>1</sup> and Das AK<sup>2</sup>*<sup>1</sup>Hematology Unit, Cancer Research Centre, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia; <sup>2</sup>Stempeutics Research Malaysia, Technology Park Malaysia, 57000, Kuala Lumpur, Malaysia*

Numerous preclinical and clinical studies are investigating the regenerative capacity of stem cells following their injection into a target organ. However, clinicians favor the use of smallest bore needles for delivery of stem cells during therapy, particularly when working with vascular organs like heart, liver and spleen. Limited data exist on the factors critical for the survival of the implanted cells following their injection via a different needle sizes. Human mesenchymal stem cells (hMSCs) were isolated, expanded and characterized for their mesenchymal cell status. These cells were ejected using 1ml syringe via a variety of needle sizes (20, 24, 25, 26 & 27G). Thereafter, cells were cultured and characterized for their morphology, attachment, viability, phenotypic expression and proliferative abilities. Following manipulation with different bore needles, morphological observations revealed abnormal cell growth and poor attachment to the tissue culture dishes with needle sizes >26G. Further, viability and phenotypic expression of hMSCs was observed to be inversely proportional to the bore size of the needles. These detrimental effects were found to be increased when the cells were left in the syringe chamber for extended period of time. However, upon increasing the needle bore diameter, a significant reduction in these effects was observed. This study highlights the use of wider bore size needles facilitates the improvement in viable cell density; which is crucial for cell delivery in cell therapy applications. Further, determination of narrow bore size needles where cells remains stable could be extremely important for clinical settings.

**P29. Acute myeloid leukaemia presenting with epigastric pain – A case report**

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The acute myeloid leukemia (AML) is a heterogeneous group of diseases with various morphologic features, cytogenetic and molecular genetic abnormalities and clinical manifestations. Although AML is usually considered a disease confined primarily to the bone marrow and peripheral blood, certain subtypes are associated with the presence of extramedullary infiltration, either at diagnosis, or during the course of the disease. Yet extensive acute myeloid leukemic infiltration of the gastrointestinal tract has rarely been reported. We report a case of extramedullary acute myeloid leukemic infiltration of gastrointestinal tract that presented to the gastroenterology specialist centre with two months history of abdominal pain and loss of weight. A diagnosis of AML was subsequently made by a gastrointestinal biopsy and bone marrow aspiration based on morphological and immunophenotypic features.

**P30. Identification of protein markers in embryonic stem cells**Baharuddin PJN<sup>1</sup>, Loring JF<sup>2</sup>, Schwartz PH<sup>3</sup>, Zakaria Z<sup>1</sup>*<sup>1</sup>Haematology Unit, Cancer Research Centre, Institute for Medical Research, Kuala Lumpur, <sup>2</sup>Children's Hospital of Orange County, CA, <sup>3</sup>Centre for Regenerative Medicine, The Scripps Research institute, CA*

The BG01 hESC line was established and characterized in 2001 and is listed on the NIH Stem Cell Registry. We are using the BG01 line to elucidate a characterization scheme for hESC lines for the following properties; (1) stability (karyotype) and (2) maintenance of the undifferentiated

state (antibody staining). *In vitro* propagation of BG01 hES cells were conducted in culture dishes containing DMEM/F12, supplemented with FBS, knockout serum replacement, L-glutamine, nonessential amino acids, b-mercaptoethanol, penicillin/streptomycin, and bFGF at 37°C, 5% CO<sub>2</sub>. Colony formation was visible within 2-3 days. The karyotype analysis was performed using a standard G-banding technique. Immuno cytochemical staining for the following specific embryonic markers was done: NANOG, Brachyury, OCT3/4, GATA4, SOX2, SMA, MAP2, Nestin, SSEA1, SSEA4, Tra-1-81 and Troponin. Positively stained cells were visualized using an epifluorescence microscope. **The visual assessment** of the embryoid bodies display the appearances and growth patterns typical for embryonic stem cells. Its karyotype verifies the stability of the cells after long-term growth in culture. BG01 cells express the protein markers of undifferentiated hESC: NANOG, OCT3/4, SSEA4, and Tra-1-81, and negative for early differentiation markers: Brachyury, GATA4, SOX2, SSEA1, SMA, MAP2, Nestin and Troponin. BG01 is stable at the level of resolution provided by G-banding and the undifferentiated state of the cells exhibited by immunostaining.

### **P31. Development of a gas chromatography-mass spectrophotometry (GCMS) method for the quantitative analysis of amphetamine type stimulants in human urine**

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**Introduction:** The abuse of designer drugs is becoming a serious global problem, with Amphetamine-type stimulants (ATS) being the most prevalent in many countries. In Malaysia, the abuse of ATS drugs has been increasing dramatically since the late 1990s. A method for the quantitative analysis of ATS drugs-amphetamine, methamphetamine, 3,4-methylenedioxy-N-amphetamine (MDA) and 3,4-methylenedioxy-N-methylamphetamine (MDMA) in human urine was developed using gas chromatography-mass spectrometry (GCMS). **Method:** Liquid liquid extraction step was carried out for sample clean up. Derivatization was done using ethyl chloroformate. To reduce cost, mexiletine was used as the internal standard to replace deuterated standards which are normally used. **Results:** The linearity for all ATS drugs was in the range of 0 to 50000 ng/mL with mean coefficients of determination ( $r^2 = 0.988$ ). The intra and inter day precisions were within 1 % to 8 % and 4 % to 11 %, respectively. The accuracies at different concentrations (500ng/mL – 4000 ng/mL) were within  $\pm 20\%$ . The Limit of Detection (LODs) and Limit of Quantification (LOQs) for each analyte were lower than 15 ng/mL and 45.62 ng/mL, respectively. The Retention Time (RTs) for ATS drugs were detected between 5.4 to 7.4 minutes. **Conclusion:** The method which utilizes only one internal standard reduces cost and has the sensitivity and measurement range suitable for the quantitative analysis of drugs in human urine.

### **P32. Performance evaluation of volume, conductivity and scatter (VCS) technology in Beckman Coulter Unicel DXH 800 at Hospital Kuala Lumpur**

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**Introduction:** Highly accurate automated WBC, PLT and NRBC counts, especially in the presence of interference, has a large impact in the operational efficiency of the hematology laboratory. Inaccurate automated enumeration of any parameter involves the traditional labour-intensive process of manually inspecting and assessing peripheral blood film. Therefore, technology that refine automated cell counting with minimal manual inspection requirement has the potential to significantly improve the turnaround time. The UniCel DxH 800 uses the technology known as Volume, Conductivity and Scatter (VCS) to determine its parameters. To enhance the VCS, DxH 800 comes with new innovative Data Analysis Techniques not seen in previous models of Coulter

Hematology analysers. **Methods:** Precision, linearity, carry over, correlation and flagging verification were evaluated. Correlation was made by comparing the analyser with existing analysers. The flagging verification was done by performing slides review. **Results:** All parameters are within manufacturer's specification for both inter and intra-run precision. DxH 800 was well correlated with two existing haematology analysers ( $r^2 > 0.98$  for WBC, RBC, HB and PLT). Correlation coefficient,  $r^2$  for both absolute counts and percentage of reticulocytes were  $> 0.90$  while for red blood cell size factor (rsf), its  $r^2$  was  $> 0.7$ . Carry over and linearity were also within acceptable limit for WBC, RBC, HB and PLT. The efficiency rate of flagging was 71%. **Conclusion:** From the present evaluation, VCS technology was technically proven in producing accurate and reliable results comparable to existing haematology analyzers. However, further verification for the flagging system with bigger sample size is warranted.

### **P33. HLA-A, -B and -DR alleles and frequencies among Indians in Malaysian Marrow Donor Registry**

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**Introduction:** The first bone marrow donor registry in Malaysia was established in December 2000, in a collaboration between the Ministry of Health (MOH) and the National Cancer Council (MAKNA). This is a registry of unrelated donors who are willing to donate their bone marrow to a patient in need. This study was carried out to determine the gene frequencies among Indians in Malaysian Marrow Donor Registry. **Method:** One thousand and fifty-one Indian donors registered between February 2001 and December 2010 were included in this study. HLA typing was carried out by polymerase chain reaction sequence specific primer (PCR-SSP) and sequence specific oligonucleotides (PCR-SSO). **Result:** Sixteen HLA-A, twenty-eight HLA-B and thirteen HLA-DR alleles were detected. The most common HLA-A alleles and their gene frequencies for Indians were HLA-A24 (0.21), HLA-A02 (0.18) and HLA-A01 (0.15). The most common HLA-B alleles and their gene frequencies were HLA-B35 (0.11), HLA-B61 (0.11) and HLA-B51 (0.09). For HLA-DR, the most common alleles and gene frequencies were HLA-DR15 (0.22), HLA-DR07 (0.16) and HLA-DR04 (0.12). **Conclusion:** Our data may provide useful information when searching for HLA matched unrelated donors among Indians.

### **P34. A case series of unusual presentation of idiopathic hypereosinophilic syndrome**

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Idiopathic hypereosinophilic syndrome (HES) is a rare disorder with significant morbidity and mortality. It is characterized by persistent and marked primary eosinophilia with end-organ dysfunction. Tissues infiltrated by the eosinophils and with the release of the damaging proteins by the eosinophilic granules are responsible for the tissues and organ system damage. We report two cases of idiopathic hypereosinophilic syndrome and highlight the unusual presentations and challenges posed by this rare disease. *Case 1:* A 21-year-old girl, with fever associated with nausea, diarrhoea and significant weight loss. She was pale with palpable multiple bilateral cervical lymphadenopathy, bilateral upper limb swelling and hepatomegaly. Her FBP reported anaemia (8.5g/dl) and leucocytosis ( $61.8 \times 10^9/l$ ) predominantly with neutrophilia (47.3%) and eosinophilia (42.7%). Platelet count was normal ( $233 \times 10^9/L$ ). Bone marrow aspirate were normocellular with no

excess of blast but with an increase in the eosinophils and its precursors. Nevertheless, the patient had succumbed to multiple thrombotic complications despite treatment with anticoagulations and cytoreductive agents. *Case 2:* A 20-year-old girl was admitted with fever associated with facial oedema and rashes. Clinically she had hepatomegaly. The FBP showed leucocytosis ( $20 \times 10^9/L$ ) with neutrophilia (51%) and eosinophilia (36%). Platelet count was  $300 \times 10^9/L$ . She failed antibiotic treatment and developed pleural effusion with gross ascites. Pleural tap exhibited increase in the number of eosinophils present. Bone marrow aspirate reported eosinophilia with a background of normocellular marrow. There was no excess of blast. Treatment with steroids was started. However, patient deteriorated with the further development of thrombotic thrombocytopenic purpura. In both cases, the patients were treated as idiopathic HES after secondary causes were excluded. Both were negative for BCR-ABL gene. However, the FIP1L1-PDGFR fusion genes were not excluded due to unavailability. Currently, there is no universally effective therapy for HES and there is no cure. The primary goal for the management of HES is to prevent the tissue damage that can be caused by the hyper eosinophilia. Thus, early diagnosis and intervention are therefore essential in preventing disease progression and end-organ damage.

### **P35. Evaluation of blood/liquid sample total RNA rapid extraction kit**

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**Introduction:** Molecular diagnosis of Hemato-oncology has become an important diagnostic tool nowadays. This advanced method is very helpful to investigate, screen and able to precisely confirm a very wide range of hematological disorders, such as leukemia. Use of precious bone marrow samples increases the importance of reliable method to obtain optimum quantity and quality of RNA. **Methods:** Two commercially available RNA extraction kit were used to extract 20 whole blood samples. *Blood/Liquid Sample Total RNA Rapid Extraction kit* from BioTeke Corporation was used according to manufacturer's protocol and QIAamp RNA blood mini kit (QIAGEN), used together with *On Column DNase* treatment according to manufacturer's procedure and with certain modification to reduce the processing time. **Results:** Spectrophotometer reading showed BioTeke kits extracted better RNA yield and purity without undergone DNase treatment compare to QIAGEN kit. No DNA contamination was observed in agarose gel electrophoresis. **Conclusions:** Evaluation of BioTeke *Blood/Liquid Sample Total RNA Rapid Extraction kit* showed that it is very cost effective, used less consumables, shorter processing time and the RNA yield and purity were better compared to QIAamp RNA blood mini kit (without DNase treatment). Furthermore, this kit only requires 250 $\mu$ L of whole blood compare to latter which suggested 500 $\mu$ L to 1500 $\mu$ L of whole blood.

### **P36. Orbital leiomyosarcoma in a survivor of hereditary retinoblastoma: A case report**

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**Introduction:** There is predisposition to development of non ocular tumours in 'heritable' bilateral cases of retinoblastoma. **Materials and Method:** We describe, a 18-year-old girl with a mass over the left lower eyelid. Physical examination, radiological investigations and histological examination on biopsy and resection of the mass were performed. **Result:** A 18-year-old girl presented with history of progressively enlarging painless mass over the left infraorbital area for the past 6 months. The mass later became painful. No associated nasal symptoms. The patient had a history of bilateral retinoblastoma in which enucleation were performed for both eyes at age of 3 and 4 years old and followed by subsequent radiotherapy and chemotherapy. The magnetic resonance imaging

and tomography scan of the mass showed a heterogenous enhancing lesion which was suggestive of local recurrence of left orbital retinoblastoma with extension into maxillary sinus. The patient underwent biopsy and later excision of the mass. The histological examinations of the mass showed a leiomyosarcoma, which was positive for smooth muscle actin and h-caldesmon. **Conclusion:** Soft tissue sarcoma can present in the long term survivors of hereditary retinoblastoma with or without radiation, in which osteosarcoma is the commonest. We report a case of leiomyosarcoma in a survivor of hereditary retinoblastoma following post radiation therapy.

**P37. Right heel malignant melanoma of soft part: A case report**

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**Introduction:** Malignant melanoma of soft part also known as clear cell sarcoma is a rare tumour affecting young adult. **Material and Method:** We present, a 23 year old lady with a mass over the right heel. Physical examination, radiological investigations and histological examination on biopsy and resection of the mass were performed. **Result:** A 23-year-old lady presented with a progressively enlarging mass over her right heel, which later became painful for the past 1 year. She also had palpable lymph nodes at her right groin area. There was a non progressive warty lesion over the same area for the past 7 years and it was excised in a private clinic. Physical examination showed a mass over right heel with ulceration. The radiological examinations show a mass with features suggestive of myxoid lipomatosis over the right heel with suspicious lung nodule on the right lower lobe. The patient underwent biopsy and excision of the mass and part of her right calcaneal bone followed by fine needle aspiration of the right inguinal lymph node. The histological examinations of the mass showed spindle shaped and epithelioid cells arranged in fascicles separated by fibrocollagenous septae, which are positive for Vimentin, S100 and HMB45. Similar cells are seen in lymph node aspiration smears. **Conclusion:** Clear cell sarcoma is a rare tumour and has similarities both in morphology and immunohistochemistry with cutaneous malignant melanoma. Clinicopathological correlation is deemed necessary to aid in diagnosis in a centre where molecular diagnosis is not available.

**P38. Henoch-schonlein purpura- a case report in elderly diabetic patient**

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**Introduction:** Henoch-Schonlein purpura (HSP) is a systemic IgA-related vasculitis affecting small vessels. It is commonly seen in children with smaller frequency in adult over the age of 20 years. The disease is characterized by four cardinal clinical features which include purpuric rash, abdominal pain, arthralgia, and renal involvement. **Method:** We describe a case of an elderly female who is a known case of diabetic nephropathy and newly diagnosed to have HSP, presenting with cutaneous and gastrointestinal manifestations. **Results:** The skin biopsy showed typical leucocytoclastic vasculitis with IgA deposition in postcapillary venules, and the gastrointestinal biopsy showed similar leucocytoclastic vasculitis with evidence of ulcer. Our patient developed hypertension with acute renal failure characterized by proteinuria and increasing trends of serum creatinine and urea. **Discussion:** The pathophysiology of HSP is related to antigen-antibody complex, mostly IgA form, as a result of bacteria and viral infection, vaccination, drugs and autoimmune mechanism. Renal involvement is a common and significant finding, as it can in rare cases progress to end-stage renal disease. The urinalysis usually shows mild proteinuria with active sediment and red cell casts. Most

patients have relatively mild disease, with a normal or slightly elevated creatinine. However, severe involvement may occur, with nephrotic syndrome, hypertension, and acute renal failure. **Conclusion:** Henoch-Schönlein purpura is a systemic vasculitis disorder with a prominent cutaneous component. It is not always benign and can be associated with serious complications such as renal failure as well as gastrointestinal events.

**Keywords:** Henoch Schonlein Purpura, Leucocytoclastic vasculitis

### **P39. Chromosomal aberrations in adult acute lymphoblastic leukemia using high-density single nucleotide polymorphism arrays**

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The majority of cases of acute lymphoblastic leukaemia (ALL) demonstrate an abnormal karyotype, either in chromosome number or structural changes. These changes are detected in only half of ALL patients in the first banding studies. This study aimed to identify recurrent and/or novel chromosomal aberrations in adult ALL. Single Nucleotide Polymorphism (SNP) arrays was employed to map the DNA copy number changes on a series of 35 adult ALL specimens. The most frequent copy number gains were on chromosome regions 8p23.1: 7297328-7752131 (46%), 15q11.2:20585976-22588019 (40%) and 14q11.2:19689305-20395115 (34%), whereas copy number losses were most frequently seen on chromosomes 11p11.2: 50573214-50769305 (54%), 9q12: 66722409-67669320 (51%), Xp22.33: 152664-2666341 (46%), 2p11.2 (Loss chr2: 89124136 – 91789700 (43%), 4q13.2: 69374929-69489323 (43%), 7q11.21: 61728395-61836567 (40%), 12q12: 37966421-38393648 (40%), 8p11.23: 39230171-39385182 (34%), 17q21.32: 44401055-44752288 (34%) and 3q26.1: 162512645-162629570 (31%). Gain of 8p23.1: 7297328-7752131 and loss of 11p11.2: 50573214-50769305 (54%) were the most frequently found aberrations in this study, suggesting that these chromosomal regions are likely to contain potential genes that may contribute to adult ALL. The cytogenetic and molecular mechanisms underlying these chromosome changes deserve further investigations.