HAEMORAL-01: A RELIABLE TTP MODEL IN BABOONS

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Thrombotic thrombocytopenic purpura (TTP) is the archetypical microangiopathy characterized by disseminated microthromboses, haemolytic anemia, and ultimately organ dysfunction. A link with deficiency of the von Willebrand factor (VWF) cleaving protease (ADAMTS13) has been demonstrated but additional genetic and/or environmental triggers are believed to be required to incite acute illness. Here we report that four days of ADAMTS13 functional inhibition is sufficient to induce TTP in the baboon (Papio ursinus), in the absence of inciting triggers, since injections with an inhibitory monoclonal antibody (mAb) consistently (n=6) induced severe thrombocytopenia (<12x109/L), schistocytic haemolytic anaemia and a rapid rise in serum lactate dehydrogenase. Immunhistochemical staining revealed the characteristic disseminated platelet-and VWF-rich thrombi in kidney, heart, brain and spleen but not lungs. Prolonged inhibition (14 days, n=1) caused myocardial ischemic damage and asplenia but not death. Control animals (n=5) receiving equal doses of a non-inhibitory anti- ADAMTS13 mAb remained unaffected. Our results provide evidence for a direct link between TTP and ADAMTS13 inhibition and for a mild disease onset without overt clinical symptoms. Furthermore, we present the first reliable animal model of this disease as an opportunity for the development and validation of novel treatment strategies.

HAEMPOST-01: OPTIMAL SPECIMEN VOLUME REQUIREMENTS FOR ROUTINE COAGULATION TESTING AT UNIVERSITAS NHLS SERVICE LABORATORY AND CLINICAL IMPLICATIONS

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Background: The aim of the study was to establish the optimal specimen fill volume necessary for accurate routine coagulation testing results in our local circumstances, be used as a guideline for the rejection of coagulation specimens in standard operating procedures and lead to a reduction in the percentage of specimens rejected with improved patient care.

Methods: Cross-sectional study design. Specimens were collected from 30 healthy volunteers and 30 patients on anticoagulation. 20ml of blood was drawn into a plastic syringe and distributed into 5 ml glass BD Vacutainer® tubes for total fill volumes (FV) of: 5 ml (100%), 4,5 ml (90%), 4,0 ml (80%), 3,5 ml (70%) and 3 ml (60%). Routine coagulation tests done included: PT, aPTT, TT and Fibrinogen levels. For patients on oral anticoagulation an INR was done with Thrombotest® reagent.

Results: For normal healthy individuals PT results were constantly accurate. APTT results prolonged, but stayed within the reference intervals. Accuracy was maintained in all tests up to 80% fill volume. The TT shortened from 100% to 60% and was concluded that citrate played a role in this finding. The fibrinogen level decreased from 100% to 60%, but did not have a clinical impact. For patients on oral anticoagulation the INR increased slightly without any clinically significant impact. Prolonged aPTT's due to anticoagulation had a more pronounced effect.

Conclusion: Specimen volume requirements form an important part of the pre-analytical sample processing and present a great potential for quality improvement once the optimal fill volume has been identified and properly applied. Our study concluded that for accurate routine coagulation testing fill volumes down to 80% can still be accepted. The clinical impact of lesser fill volumes was found to be insignificant and tests may be performed on clinician's request, but with a consciousness that accuracy may be lost.

HAEMPOST-02: D-DIMER INTER-ASSAY AGREEMENT STUDY FOR THREE COMMERCIALLY AVAILABLE D-DIMER ASSAY'S

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D-Dimers are a type of fibrin degradation product (FDP) formed during clot lysis, this makes them more useful than for FDP's in general because FDP's may be fragments broken down from fibrin or fibrinogen, unlike D-Dimers which may only arise from fibrin degradation. They are utilized as a means to exclude thromboembolic disease. This is because a negative D-Dimer result virtually rules out such a diagnosis. However a positive D-Dimer result does not always indicate thromboembolic disease as other factors such as trauma, inflammation or liver disease may also raise D-Dimer levels. It is because of this that the D-Dimer is an important test in the haemostasis laboratory. However there are various different D-Dimer measurement kits available. In this study three of the D-Dimer kits that are currently being utilized in three different laboratories have been compared to establish if the different methods yield significantly different results. For this study 45 samples were prospectively collected and then analysed using each of the three assays being assessed, namely the Innovance, Vidas and Liatest D-Dimer assays. The Innovance and Liatest are both latex based immunoturbimetric particle-enhanced immunoassays while the Vidas assay is an ELISA based method. Statistical analyses were performed to assess the comparability between assays using Bland-Altman plots, the calculation of Kappa statistics and the percentage agreement. The comparison showed the Vidas and Innovance assays to have a relatively high percentage agreement (95.4%) while the assay that showed the poorest agreement with the other methods was the Liatest D-Dimer assay which showed only 74.4% agreement with the Innovance assay and 69.8% agreement with the Vidas assay. In conclusion it appears that there is a large amount of potential inter-assay variability, and it is important for laboratories to consider this when selecting a D-Dimer assay.

HAEMORAL-02: **THROMBOELASTOGRAPHY IN BABOONS AND HUMANS**

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Introduction: Animal models have enhanced better understanding of the pathophysiology of haemostatic disorders. These models also play a pivotal role in clinical trials of new haemostatic drugs, where these drugs are first tested in animals to prove efficacy and safety before they are tried in humans. The baboon, which physiologically and immunologically, closely resembles humans, is commonly used in such trials. Several studies comparing conventional clotting times of baboons and humans have shown many similarities, however such tests do not reveal clot dynamics. In our study, we compare the clotting dynamics between the baboons and humans using thromboelastography thus justifying whether the baboons are ideal animal models.

Methodology: Blood specimens were collected from each of the 12 baboons and 13 healthy volunteers. TEG analyses were performed on the citrated specimens using TEG® analyzer. Kaolin was used as an activator. The following TEG variables were compared: reaction time (r), k-time (k), alpha angle, maximum amplitude (MA), clot lysis in 30 min (LY30).

Results: Baboons had a shorter r- and k- time and a higher alpha angle ($p=0.0017$, $p=0.0003$, $p=0061$ respectively) compared with the human volunteers. No statistical difference was noted with the MA and LY30 ($p=0.0514$, $p=0.5738$ respectively). These results demonstrate that the rate at which the clot forms in baboons is faster compared with humans. There was no difference in the clot strength and rate of clot lysis.

Conclusion: Although there were statistical differences between certain variables, these differences were not clinically relevant as the mean values of both baboons and human volunteers were within the reference interval (provided by the manufacturer). Hence baboons can be used as animal models for haemostatic studies involving thromboelastography

HAEMORAL-03: PHASE I, RANDOMIZED, DOUBLE-BLIND, PLACEBO-CONTROLLED, SINGLE-DOSE ESCALATION STUDY OF THE RFVIIA VARIANT (BAY 86-6150) IN HAEMOPHILIA A OR B WITH OR WITHOUT INHIBITORS

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Introduction: The absence of a longer-acting activated factor VII (FVIIa) is currently an unmet need in the therapeutic management of bleeds in haemophiliacs with inhibitors. BAY 86-6150, a human recombinant FVIIa (rFVIIa) variant, was developed to address this need. In preclinical animal studies, BAY 86-6150 was well tolerated and had improved pharmacokinetic and efficacy profiles, compared with the currently available rFVIIa (Novoseven®).

Objectives: To investigate safety, tolerability, pharmacodynamic/pharmacokinetic profiles, and immunogenicity of BAY 86-6150 in nonbleeding patients with haemophilia Methods: This is a randomized, double-blind, placebo-controlled, single-dose escalation study of BAY 86-6150. The study population comprised nonbleeding patients with moderate or severe haemophilia A or B with or without inhibitors aged 18 to 65 years. Sixteen patients were randomized in a 3:1 ratio to placebo (n=4) or escalating doses of BAY 86-6150 at 6.5, 20, 50, or 90 μg/kg (n=3 each). Patients were followed up for 50 days postdose. Measured endpoints were adverse events, pharmacokinetic, pharmacodynamic, and immunogenicity parameters.

Results: There was a dose response seen with the thrombin generation assay (TGA), prothrombin time (PT), and activated partial thromboplastin time (aPTT) hemostasis parameters, which was consistent across all three measurements. The TGA increased corresponding to a decrease in the PT and the aPTT in peak and duration of effect.There was no effect of BAY 86-6150 on the fibrinogen level. There was no dose response to BAY 86-6150 seen with the D-dimer, prothrombin factor 1+2 (F1+2), and the antithrombin III and thrombin-antithrombin complex (TAT) thrombogenic markers.

Conclusions: In this first-in-human study, BAY 86-6150 was not associated with any adverse events. The data safety monitoring board recommended progression to the highest proposed dose (90 μg/kg). Further safety and efficacy of this agent will be evaluated in Phase II/III studies. This research was supported by Bayer HealthCare.

HAEMPOST-03: THREEWAY COMPARISON OF METHODS OF HAEMOGLOBIN MEASUREMENT IN LEUKOCYTOSIS SAMPLES.

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Background: Haemoglobin concentration [Hb] is a commonly measured analyte often done on its own in general medicine, surgery, obstetrics and paediatrics practices at primary, secondary and tertiary healthcare levels. Accurate and fast [Hb] estimation is therefore critical for efficient healthcare delivery. Whilst analytical systems are different in different instruments, the Cyanhaemoglobin (HiCN) method is the gold standard against which these methods are calibrated. The Advia 2120 instrument recently acquired in our laboratory measures colorimetric and cellular [Hb] using the modified HiCN and Mie principles respectively. The advantage of the cellular [Hb] measurement is its measurement is independent of high white cell count(WCC) interference. The practice in our laboratory was to manually confirm all [Hb] measured in the presence of high WCC. The aim of this study was to compare the three methods of [Hb] measurement in the presence of high WCC.

Material and methods: Study population comprised all age groups in and out-patient facilities in our tertiary care centre. Selected samples left after routine diagnostic analysis that had adequate EDTA sample volume and high or normal WCC. Analysis was done by all three methods by the same technologist within 3hrs of sample collection. Results were collated on spreadsheets and analyzed using statistic comparison methods.

Results: 106 analyzed by all three methods were evaluable. The mean WCC was 164 x10^9/l (135.4-193.0). The mean [Hb](95%CI) measured by colorimetric(col), cellular(cell) and manual HiCN(cyn) methods were 11.05g/dl(10.47-11.6), 10.34g/dl(9.7-10.9) and 10.34g/dl(9.7-10.9) respectively. The bias was 0.7, 0.0 and 0.7 between col-cyn, cyn-cell and col-cell respectively. There was no difference in [Hb] measured by cellular and hiCN in presence of high WCC.

Conclusion: There is a stronger agreement between the cellular and HiCN [Hb] measurement methods. The Advia 2120 cellular method can replace the manual HiCN method in our laboratory.

HAEMORAL-04: MOLECULAR CYTOGENETICS CHARACTERISATION OF HIV RELATED DIFFUSE LARGE B-CELL LYMPHOMA WITH FEATURES OVERLAPPING BURKITT LYMPHOMA

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The 2008 WHO classification of tumours of lymphoid tissues has introduced a new category of high-grade B-cell lymphoma with overlapping features of diffuse large B-cell lymphoma (DLBCL) and Burkitt lymphoma (BL). These aggressive tumours resemble Burkitt lymphoma but have morphologic, immunophenotypic or molecular cytogenetic characteristics that differentiate them from classical BL. There is scarce data regarding the conventional and molecular cytogenetic of these lymphoma, particularly in the context of HIV infection. We present ten cases (seven females and three males) of HIV related DLBCL/BL. We reviewed the clinical, morphological and cytogenetic findings and performed fluorescence in situ hybridization (FISH) with the LSI IGH/MYC/CEP8 tricolour, the dual fusion probe LSI MYC dual colour, break apart rearrangement probe as well as the BCL6 dual colour, break apart rearrangement probe (Abott Molecular). The mean age was 30 years, nine of the 10 cases had a Burkitt like translocation involving the oncogene C-MYC. Of these, 7 patients had a complex karyotype and two patients had trisomy for chromosomes 12 and 19 respectively in addition to the t(8;14)(q24;q32). One patient had a 'double-hit' with the involvement of both C-MYC and BCL6 oncogenes on the both IGH alleles. Interestingly chromosome 1 long arm was the most frequent secondary aberration observed in the form of one or 2 chromosomal aberrations in 5 of the cases with complex karyotypes. While the dysregulation of C-MYC is likely to be responsible for the aggressive phenotype of these tumours, the secondary aberrations are thought to compound the severity of these tumours. It will be important to verify the non-random involvement of chromosome 1q in HIV related DLBCL/BL, highly suggested by these results.

HAEMPOST-04: A NOVEL, LYMPHOCYTE DIFFERENTIAL COUNT AS A COST-EFFECTIVE METHOD IN THE ASSESSMENT OF IMMUNE ACTIVATION LEVELS IN CHRONIC HIV-INFECTION. A PILOT STUDY.

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Chronic HIV-infection is associated with aberrant and ongoing activation of both the innate and adaptive immune systems. Immune activation has been identified as a stronger risk factor for disease progression than either the CD4 count or viral load tests. The current international recommendation to commence antiretroviral therapies at CD4 counts of 350 may be difficult to implement in resource-poor countries. Affordable tests for direct clinical application would be of value in the management of patients with chronic HIV-infection in resource-limited settings. A full blood count or components thereof are regularly requested in the work-up of HIV-infected patients. A blood film and morphological assessment of the lymphocytes may be a cost-effective approach to determining immune activation levels in these patients. Activated lymphocyte morphology is regularly detected on morphological review of the blood films; however, definitive numerical values have not been applied to these findings. The addition of absolute values to the different morphologic subsets of lymphocytes may have a direct correlation with more expensive immune activation levels as measured by flow cytometry. This pilot study will present the first design of a differential lymphocyte count based on the morphological assessment of peripheral blood films of 40 HIV-infected patients as reviewed by two pathologists and compared with the films from 27 matched uninfected controls. Well established markers of immune activation in HIV-infection such as CD38 on CD8 lymphocytes and other markers of activation such as CD25 and/ or CD69 levels on CD4 lymphocytes will be correlated with the lymphocyte differential count values. This will be the first study to correlate blood film morphological findings with markers of immune activation as measured by flow cytometry. The implementation of this approach may facilitate earlier access to treatment in resource-limited settings and thereby assist ultimately in delaying the onset of AIDS.

HAEMORAL-05: THE INCIDENCE OF ALPHA THALASSAEMIA IN THE SOUTH AFRICAN POPULATION WITH UNEXPLAINED MICROCYTOSIS.

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Common causes of microcytosis include iron deficiency, anaemia of chronic disorder (ACD) and thalassaemia. At the Johannesburg academic complex hospitals we found that there is no identifiable cause for microcytosis in 10 – 15% of cases, where iron studies and Hb electrophoresis are within normal limits. An assumption of alpha thalassaemia as the most likely cause is made on the premise of a high incidence of alpha thalassaemia in Africa. Alpha thalassaemia results from reduced or absent synthesis of alpha globin chains and is caused by deletions/mutations affecting either one or both α globin genes in cis, referred to as α+ or α0 thalassaemia respectively. The majority of the mutations are gene deletions but a small number of point mutations within one of the 2 α globin genes resulting in α+ thalassaemia have been described. The common alpha thalassaemia deletions can be detected using a multiplex PCR method. (Chang et al, 2000, Lin et al. 2000, Tan et al 2001).

Materials and methods: FBC samples with a low MCV were identified for further investigation. Inclusion criteria for the study were: 1) Low MCV, 2) No evidence of iron deficiency or ACD, 3) No evidence of beta thalassaemia on Hb electrophoresis. Samples were prepared for PCR and a single tube multiplex PCR technique was employed to detect six most common deletion that account for 87% of α+ and α0 thalassaemia.

Results: Alpha thalassaemia was found in 60% of the samples analysed, which confirmed our suspicion. However this still leaves 40% of microcytosis with no identifiable cause. A possible reason might be lack of identification of less common mutations that were not tested for. The study is in progress.

HAEMPOST-05: USE OF THE RETICULOCYTE HAEMOGLOBIN CONTENT (CHR) AS A SCREENING TEST FOR IRON DEFICIENCY IN THE SOUTH AFRICAN SETTING

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Although nationally representative data are limited, iron deficiency and iron deficiency anaemia have been found to be prevalent in a number of subsets of the South African population. Iron deficiency contributes to disease burden and is amenable to prevention and therapeutic measures. Automated measurement of the reticulocyte haemoglobin content (termed the cHr in reports generated by our instrument) is accepted as a useful tool in the diagnosis and treatment of iron deficiency states, and in previous studies has been compared to traditional biochemical markers of iron deficiency. A retrospective analysis was performed on 533 sets of results from patients in a variety of inpatient and outpatient settings, where both automated reticulocyte count (with associated parameters including cHr) and iron studies (ferritin, transferrin, transferrin saturation, serum iron) were available, with cHr and ferritin being the variables of interest. As per laboratory reference ranges, cHr <29 and ferritin <15 were assumed as cut-off levels. Utilising the cHr as a screening test for iron deficient states was found to have a high sensitivity (92.68%) with a specificity of 65.85% and a negative predictive value of 98.02%. This could be performed as an initial investigation with subsequent testing of iron studies where cHr <29. Employment of this strategy would have resulted in a cost saving of over R17000 in this sample of patients, a significant consideration in our resource-limited setting.

HAEMPOST-06: MALIGNANT LYMPHOMA INCIDENCE AND HIV-RELATED LYMPHOMA SUBTYPES SEEN AT TYGERBERG ACADEMIC HOSPITAL, WESTERN CAPE, SOUTH AFRICA, 2002-2009

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Background: The incidence of malignant lymphomas (ML) in the Western Cape, a province of South Africa (SA), with a population of 5 million and an estimated HIV prevalence of 15% (census report 2002) has not been previously documented. Highly active antiretroviral therapy (HAART) was introduced into the public patient sector in 2004 with 28% estimated coverage by 2007 (UNAIDS/WHO 2008). People living with HIV (PLWH) have 60-200 times increased risk of developing HIV-related lymphoma (HRL). Therefore, based on HIV prevalence, HRL would be expected to increase but is undocumented.

Materials and methods: We reviewed all patients diagnosed with ML from the Tygerberg Academic Hospital catchments area in the Western Cape of SA for years 2002-2009. In this time frame 606 cases of ML were identified of which 488 were HIV negative and 118 HIV positive. ML were sub-typed according to WHO classification (2008) based on cell or tissue morphology and molecular and immunophenotypic platforms.

Results: ML cases increased each year from 2002 to 2005 and remain elevated in both the HIV negative and positive patients through 2009. HRL increased from 5% in 2002 to 30% in 2009 with a profile of subtypes differing from the HIV negative cases. ML subtypes of HIV negative (488) and positive (118) cases are in Table 1.

Table 1. Percentages of ML subtypes by HIV status

Subtype	*%HIV-*	*%HIV+*
Burkitt lymphoma		**35**
Diffuse large B-cell lymphoma	44	33
Plasmablastic lymphoma		**16**
Follicular lymphoma	12	
Hodgkin's lymphoma	11	5

Small cell lymphoma	4	1
Mucosa-associated lymphoid tissue (MALT)	4	1
Anaplastic large cell lymphoma	3	3
Marginal zone lymphoma	3	
Cutaneous T-cell lymphoma	3	
T-cell lymphoma	2	1
Primary effusion lymphoma and Castleman's disease		**2**
Other	13	4
Total	*100*	*100*

Conclusions: ML cases increased from 2002 to 2009 including a dramatic increase in HRL, currently at 29% of all cases. This changing pattern of subtypes in PLWH presents new challenges to histopathology diagnosis as well as a clinically more therapeutically difficult patient population. Burkitt lymphoma, the most common HRL, is among emerging subtypes, along with plasmablastic lymphoma, not previously seen in this geographic region. We anticipate the continued rise in HRL cases as PLWH live longer with HAART. Emergence of more aggressive lymphoma subtypes inevitably poses a major strategic health concern in the region. We participate in the Sub-Saharan Africa Lymphoma Consortium [http://www.ssalc.org] to expand the understanding of HRL in this region of the world.

HAEM ORAL **GUIDELINES FOR MANAGEMENT OF SICKLE CELL DISEASE**

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Sickle cell disease (SCD) is a severe inherited condition due to a mutation in the beta globin gene. SCD affects approximately 72 million subjects worldwide, 55 million of whom are located in sub-Saharan Africa. The sickle gene has arisen around the world on at least 5 different occasions. Its high prevalence in Blacks and selected ethnic groups resulted from the survival advantage of the heterozygote under the selective pressure of Plasmodium Falciparum infestation, thereby compensating for the lower fitness of the homozygous state (balanced polymorphism). The prevalence of the sickle gene in South Africa is <1%, not unexpectedly so, since South Africa falls outside the malarial belt. However, lifting of travel restrictions post-1994 (first democratic election) has led to an influx of people from other parts of Africa, paralleled by an influx of the sickle gene. Hospitals are now seeing a steady increase in the number of outpatient as well as admission cases of SCD, prompting a candid re-assessment of our preparedness of managing the condition viz., resources, infrastructure and expertise. In addition, there are significant cost implications that could place our health budget under strain. There is a need to educate health care workers and patients, as well as create awareness among the public at large on the subject. Learning institutions and media are ideal platforms to achieve this objective. Management of SCD can be categorised under the following: 1) Acute crises, 2) Complications, 3) End organ damage, 4) Preventive measures. The only chance of a cure is bone marrow transplantation which has its inherent risks and limitations. Given the chronic nature of the disease, standardised management is of paramount importance. In this vein, national guidelines for management of SCD have been drafted, which will be presented in this educational session.

DISPLAY POSTERS

HAEMPOST-07 A DIAGNOSTIC DILEMMA: CASE REPORT OF A NON-HAEMOPOIETIC MALIGNANCY

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Objective: Herewith a report of a non-haemopoietic malignancy that presented with clinical features suggestive of a haemopoietic malignancy. Case presentation: The patient is a 31year old female, serologically confirmed HIV positive, with a CD4 count of 64x10^6/l. At presentation, she was not on antiretroviral therapy. She presented at Helen Joseph Hospital, complaining of one week history of per vaginal bleeding and two day history of generalized body weakness with fatigue. Physical examination revealed pallor, cervical lymphadenopathy and hepatosplenomegaly. A fine needle aspiration of a neck lymph node was suggestive of Hodgkin's lymphoma, showing moderate tumour necrosis. Full blood count and the differential count revealed pancytopenia, leuco-erythroblastic reaction and occasional primitive mononuclear cells/blasts. Bone marrow aspirate showed a markedly hypercellular marrow with approximately 88% of the nucleated cell population comprising primitive mononuclear cells. These cells appeared singly or in clumps, with nuclear moulding. Immunophenotypic analysis performed on the bone marrow aspirate revealed a population of ~10-15% intermediate sized cells which were CD45 negative. These cells did not express CD10, CD19, CD3, CD33 and CD34. The trephine biopsy showed a markedly hypercellular marrow, effaced by a neoplastic infiltrate. Focal fibrosis was also seen. An extensive panel of immunohistochemical stains performed in attempt to establish a final diagnosis, suggested an epithelial neoplasm with neuroendocrine immunophenotype. Conclusion: Although a haemopoietic neoplasm was initially suspected, the bone marrow investigations favoured a diagnosis of metastatic carcinoma, with neuroendocrine immunophenotype. A primary site of tumour origin was however not established with further immunohistochemical staining.

HAEMPOST-08 A NEW FORM OF ACQUIRED RESISTANCE TO IMATINIB MESYLATE (GLEEVEC®) IN CHRONIC MYELOID LEUKAEMIA

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Introduction: Tyrosine kinase inhibitors (TKIs) such as imatinib have proven highly effective in the treatment of chronic myeloid leukaemia (CML). However, despite the efficacy of such targeted drug therapy, some patients develop resistance to imatinib. The principal cause of acquired resistance to TKIs is the acquisition of mutations in the kinase domain of the BCR-ABL oncoprotein. These mutations are either single base substitutions or indels (insertions and/or deletions). The former impair the binding affinity of imatinib to the kinase domain thereby conferring varying degrees of drug-resistance based on the position of the mutation. In addition to this, BCR-ABL gene amplification has also been implicated in resistance development in studies involving cell lines. We report on a CML patient with resistance to imatinib displaying a combination of gene amplification and deletions in the BCR-ABL kinase domain. Method: A 40 year old Philadelphia positive CML patient was prescribed imatinib at a dose of 400 mg/day. However, after displaying a lack of response to imatinib, a mutation screen of the BCR-ABL kinase domain was performed by sequencing. Results: Sequence analysis showed the presence of the native BCR-ABL transcript and two additional BCR-ABL variants, each with different deletions, respectively. The deletion in variant 1 resulted in a truncated protein that does not contain the gatekeeper domain, catalytic domain, activation loop, and C-terminal lobe. Variant two lacked a major part of the catalytic domain, activation loop, and C-terminal lobe. No other mutations were detected. Conclusion: Based on sequence data, it appears that the deletion variants were present in equal quantity to that of the normal BCR-ABL, suggesting that this is a gene triplication as opposed to two separate duplication events in different clones. Nevertheless, this is the first report of BCR-ABL amplification combined with deletion variants that appear to be associated with drug resistance.

HAEMPOST-09 A RETROSPECTIVE ANALYSIS OF PURE RED CELL APLASIA IN HIV POSITIVE PATIENTS.

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Introduction: Pure red cell aplasia (PRCA) refers to a specific anaemia affecting erythrocyte precusors. PRCA secondary to Parvovirus infection is typically seen in immunodeficient hosts including those diagnosed with leukaemia, recent bone marrow transplantation, HIV and AIDS patients. Anaemia in general, is common in HIV patients and is often multifactoral with underlying opportunistic infections, neoplasms as well as medication e.g Antiretroviral therapy (ART) or HIV infection itself contributing. An increase in the incidence of PRCA has been noted, almost all occurs in conjunction with HIV positivity. Aim: To assess patients diagnosed with PRCA relative to their HIV status and haemoglobin (Hb) levels. The effect of Parvovirus B19 viral load on the severity of the anaemia will also be assessed. Method: A two-year retrospective analysis was performed on patients diagnosed with PRCA. Both HIV positive and negative samples were included and processed for Parvovirus PCR at CMJAH NHLS PCR Laboratory. Other investigations included: HIV viral loads, CD4 counts and percentages, Parvovirus serology and viral load, Hb levels and BMA examinations. Results: A total of 30 patients were included (22adults & 8children) with a mean Hb of 3.96 g/dl (2.3-6.6); a CD4 count (adults) of 124x10^6 (6-524) and a CD4% (paediatrics) of 7.2% (0.28-22.7). Furthermore, the mean Parvo VL was: 1.6 x10^11. Of the HIV positive group parvovirus PCR was positive in 47% patients tested, whereas serology (IgM) was positive in 18% of patients tested. Discussion: Neither parvovirus nor ART are exclusively responsible for PRCA in HIV. The incidence of PRCA remains increased in HIV even after correcting for the role of ART. Conclusion: The incidence of parvoviral-induced PRCA in HIV is increased most likely due to the immuno-deficient state and would be appropriate to do a prospective study on the progress of these patients Hb in comparison to Parvovirus viral load.

HAEMPOST-10 A SUCCESSFUL PREGNANCY IN A PATIENT WITH CHRONIC MYELOID LEUKAEMIA TREATED WITH IMATINIB

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Introduction: Imatinib was introduced as first line treatment for patients diagnosed with chronic myeloid leukaemia (CML) in 2001. Due to the dramatic improvement in outcome of these patients we are occasionally faced with women wanting to fall pregnant. This poses a challenge as Imatinib is potentially teratogenic and interruption of imatinib may result in disease progression. Females of childbearing age on imatinib are therefore advised to use effective contraception Materials and methods: We describe the clinical presentation, course and outcome of a known patient diagnosed with CML who became pregnant; and was treated with Imatinib through the course of her pregnancy Case presentation: A 15-year old female was diagnosed with Philadelphia chromosome positive chronic phase CML in 2004. She achieved haematological and cytogenetic remission but had not yet achieved a major molecular response. At age 21 in October 2009 she discovered that she was eight weeks pregnant. Alternative treatment modalities were discussed with the patient. After which the patient decided to continue on imatinib throughout the pregnancy. The pregnancy was carefully monitored and resulted in the birth of a normal healthy boy in March 2010 via a caesarian section. The patient remained in cytogenetic remission postpartum. Conclusion: This fetus was exposed to imatinib at a dose of 400mg throughout the pregnancy and born without any complications. This case illustrates that after individualized decision making with the patient, a normal pregnancy is possible in patients with chronic myeloid leukaemia despite continued imatinib therapy.

HAEMPOST-11 CASE REPORT: ACQUIRED BLEEDING TENDENCIES IN PLASMA CELL DYSCRASIAS

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Clinical History: A 49 year old female patient presented in March 2009 with a one year history of a swollen right breast. A week later her left breast became swollen and turned blue following mild trauma. Later the patient developed multiple subcutaneous nodules. She is also known with hypertension and a previous episode of heart failure. Physical Examination: Enlarged axillary and supraclavicular lymph nodes. Special Investigations: Mammograms revealed only evidence of trauma. The bleeding time was prolonged. Testing for von Willebrand disease (VWD) demonstrated borderline low results (VWF:Ag=48%, VWF:RCo=53%, CB:VWF=60%) with an abnormal multimer pattern suggestive of type 1 VWD. A lymph node biopsy showed primary amyloidosis. A bone marrow examination [July 2009] revealed Waldenstrom macroglobulinaemia (WM). Treatment: The patient was started on cladribine in October 2009 with nominal response. In March 2010 the treatment was changed to melphalan and prednisone, with addition of cyclophosphamide in May 2010. Re-evaluation: In April 2010 the acquired VWD had cleared and the bone marrow showed no signs of WM. In June 2010 the lymph nodes had shrunken. Discussion: Plasma cell dyscrasias are malignant proliferations of monoclonal plasma cell populations that secrete detectable levels of monoclonal immunoglobulins. WM is a B cell disorder characterized by bone marrow infiltration with clonal lymphoplasmacytic cells and an IgM monoclonal gammopathy. Acquired von Willebrand disease has been associated with WM and the different mechanisms described include selective adsorpsion of VWF by tumour cells and antibodies directed against functional domains of VWF. Conclusion: Patients presenting with an acquired bleeding diathesis should be investigated for a relevant underlying pathological disorder, and recovery from the bleeding tendency may suggest a favourable therapeutic response.

HAEMPOST-12 ASSESSMENT FOR THE PRESENCE OF FLT3 AND NPM1 MUTATIONS IN DE NOVO AML

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Introduction: Acute Myeloid Leukaemia (AML) is known to be a heterogeneous clonal disorder of haemopoietic progenitor cells and more recently, the molecular heterogeneity of AML has become apparent. Two of the most commonly found mutations, NPM1 (Nucleophosmin protein-1) gene mutation and the FLT3-ITD (FMS-like tyrosine kinase 3 internal tandem duplication) mutation are now routinely assessed for and used in both prognostication and treatment decisions. The primary aim was to assess for the presence of NPM1 and FLT3-ITD mutations in adult patients diagnosed with de novo AML at a single academic centre and to determine whether these mutations are as commonly found in the South African setting as elsewhere and to introduce this assay as part of routine diagnostics. In addition, previous immunophenotypic analysis and other routine parameters have been analyzed to determine any possible associations with these mutations. Methods: We have established the use of a reliable method for the detection of both NPM1 and FLT3-ITD mutations in our laboratory. This method requires genomic (g)DNA, extracted from blood or bone marrow aspirates. Two single PCR reactions are then run with final analysis by means of capillary electrophoresis using the ABI 310 Genetic Analyzer. Results: 165 patients were included from various hospitals in and around Johannesburg. Of these the NPM1 mutation rate was found to be 27%, while FLT3-ITD was present in 18% of AML patients. 34% of the patients with an NPM1 mutation were found to also harbour an FLT3-ITD mutation. This assay has been found to be highly sensitive. Conclusion: This much needed, reliable method of detection is able to further enhance genetic analysis in AML. The incidence of these mutations concurs with that found internationally. An assessment of the other parameters show interesting variations from the literature and stresses our unique setting and the need for further study.

HAEMPOST-13 THE CONSTRUCTION AND CHARACTERIZATION OF A STABLY TRANSFECTED HUMAN FIBROBLAST CELL LINE EXPRESSING BCR/ABL

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Introduction: Despite the successes of tyrosine kinase inhibitors in the treatment of Chronic Myeloid leukemia (CML), the search continues for alternative therapies that may cure or even prevent this disease. One approach is to completely characterize the malignant cell, trying to find unique cell-surface proteins that can either be used as targets for cell-specific drug delivery or immunotherapy. As part of this process, we endeavored to create a human primary cell line, stably expressing BCR/ABL, that was easy to culture and manipulate, so that tools such as phage display could be used to identify BCR/ABL-associated cell-surface markers. Key BCR/ABL functions in the cell such as its affect on proliferation, apoptosis and cytoskeletal features were then assessed to ensure that these cells exhibited characteristics observed in both BCR/ABL positive mouse fibroblast models and CML cells. **Results:** Human neonatal fibroblasts, as well as NIH3T3 cells expressing high levels of *bcr/abl* at both the mRNA and protein levels were obtained after 3 weeks of G418 (200 and 400ug/ul, respectively) selection, following transfection using Lonza's Nucleofector technology and the pDG210/ PCVT vectors. Despite altered actin arrangements and a difference in growth patterns indicating active BCR/ABL protein in these cells, the human cells were not protected from serum withdrawal or mitochondrial apoptotic stress and showed no proliferative advantage. This was in complete contrast to the mouse NIH3T3 counterparts, which exhibited these BCR/ABL specific characteristics. Conclusion: Nucleofection successfully allowed for the creation of a novel, stable BCR/ABL positive human fibroblast cell line, which has historically been difficult to achieve with standard technology. However, the characterization thus far has indicated a usually limited effect of this oncogene on the human cells, which could further indicate a novel mechanism for BCR/ABL inactivation. Phage display analysis will have to be pursued in the mouse fibroblast model.

HAEMPOST-14 CLASSICAL BCR/ABL NEGATIVE MYELOPROLIFERATIVE NEOPLASM BONE MARROW HISTOLOGY, DIAGNOSES REVISITED ACCORDING TO THE 2008 WHO CRITERIA

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Introduction and aim: The diagnosis and classification of BCR/ABL-negative myeloproliferative neoplasms (MPN) is interdisciplinary. The correct diagnosis of MPN directly impacts on prognosis and risk stratification. In the past the role of bone marrow biopsies in the diagnosis of MPN was neglected. In the revised classification of MPN in the 2008 World Health Organization (WHO), bone marrow biopsies are recognized as critical. We reviewed the histology of classical MPN bone marrow biopsies over a ten year period at Universitas Academic Laboratories according to the 2008 WHO criteria to ascertain what the impact that the new classification would have been. Methodology: We reviewed the diagnoses of 44 bone marrow biopsy specimens, according to the 2008 WHO criteria. The original haematoloxylin and eosin and reticulin stains were examined. No blinding was done and there was access to all the data available on the request form and the laboratory records. Results: Of the original 44 specimens eight (18.2%) were classified as polycythaemia vera (PV), 15(34.1%) as essential thrombocythaemia (ET), one (2.3%) as post-PV myelofibrosis and 13(29.5%) as primary myelofibrosis (PMF). Seven (15.9%) were only classified as myeloproliferative disorder. The review identified 18(40.9%) cases as PV, three (6.8%) cases as ET, 18 (40.9%) as PMF. The post-PV myelofibrosis remained unchanged, but four cases were reclassified as not being MPN. In total 30 (68.2%) of the cases were reclassified, the most being on PV and ET. Conclusion: This study confirms that the new criteria have a significant influence on the diagnosis of MPN, and in our situation might have influenced the management of thirty patients.

HAEMPOST-15 COMPOUND HETEROZYGOUS INHERITANCE OF DELTA/BETA THALASSEAMIA AND BETA THALASSEAMIA IN A PATIENT CAUSING THALASSEMIA INTERMEDIA

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Beta (β) thalasseamia is caused by a mutation/deletion of the β globin gene, leading to decreased (designated β+) or absent (designated β0) β globin chains. Since >95% of haemoglobin (Hb) is HbA, comprising of 2α and 2β chains, homozygous β0 thalassaemia causes severe anaemia that is incompatible with life in the absence of transfusion. Delta-beta (δβ) thalassaemia is caused by deletions that span the δ and β globin genes. Patients with homozygous δβ thalassaemia present with a mild anaemia that is exacerbated during periods of stress. Here we describe an 87-day infant of Indian descent who presented with anaemia (Hb 8.1 g/dl). Haemobinopathy screening formed part of the investigation process and a diagnosis of compound heterozygous δβ and β thalassaemia (δβ/β thal) was suspected. The suspicion was confirmed after testing of both parents where the mother and father had heterozygous δβ and β thalassaemia respectively. In δβ thalassaemia the expression of γ chains, and therefore HbF, is increased. This reduces the α:non-α imbalance and consequently lessens the severity of anaemia. Clinically these patients often behave as thalassaemia intermedia, i.e. anaemia that is well compensated and not requiring regular transfusions. However, ad hoc transfusions may be necessary during periods of stress e.g. infection. Interestingly, the subject's older sibling has also been diagnosed with compound heterozygous δβ/ β thalassaemia in 2004 who, at the age of 4 months, presented in similar fashion. Even though there might not be transfusion dependency, such chronic haemolytic states can still lead to long term complications such as iron overload and organ dysfunction. δβ Thalassaemia is described in many ethnic groups including Indians and Chinese and is caused by a variety of mutations. This family study also highlights the importance of screening of family members.

HAEMPOST-16 DEATH SIGNALS AS MARKERS OF IMMUNE SYSTEM ACTIVATION IN CHRONIC HIV-INFECTION. A PILOT STUDY.

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Disease progression in HIV-infection is driven by immune activation and inflammation and it is now established that the degree to which the immune system is activated has greater prognostic value than either the CD4 count or viral load tests. Novel markers that correlate with levels of immune activation would therefore add to the prognostic value of the CD4 count and / viral load tests. The binding of Fas ligand (FasL / CD178) to its death receptor Fas (CD95) on the surface of cells triggers the activation of a cascade of enzymes that systematically dismantle the cell by the physiological death process known as apoptosis. This mechanism is a significant cause of death of CD4 T cells in chronic HIV-infection and particularly of the uninfected or 'bystander' CD4 T cells. Repeated exposure of CD4 T cells to antigen induces the up-regulated expression of Fas ligand and results in a form of apoptosis termed activation-induced cell death (AICD). Levels of Fas and Fas ligand may therefore correlate with levels of activation of the immune system in chronic HIV-infection. This pilot study will compare the levels of Fas and Fas ligand on CD4 and CD8 T lymphocytes from 40 HIV-infected patients with those from 27 un-infected controls. In addition, these markers will be correlated with well-described markers of immune activation such as CD38 on CD8 T lymphocytes and CD25 or CD69 on CD4 lymphocytes. Fas and/ FasL may form the basis of a panel of tests that add prognostic value to the CD4 count by correlating with markers of immune activation and thereby impact on treatment algorithms in the chronic stage of the disease.

HAEMPOST-17 DEGRADATION OF ANTI-HLA ANTIBODIES, DESPITE STORAGE AT -70°C, COMPROMISES THE UTILITY OF STORED SERUM FOR FLOW CYTOMETRIC CROSSMATCHES IN RENAL TRANSPLANTATION

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Introduction: Flow cytometric crossmatch is a sensitive assay that is used at our centre to identify antibodies in recipient serum that may react against the cells of the cadaver kidney donor. A positive crossmatch due to HLA specific antibodies is a contraindication to transplantation and predicts hyperacute rejection. Cadaver crossmatches are urgent and need to be processed and reported as soon as possible. Major setbacks are the inconvenience of sick patients commuting from far-away places and the time spent travelling to our centre. To prevent delays that may compromise the quality of donor kidneys and increase the quality of patient care, recipient serum is stored prior to crossmatching. Aims/Rationale: We evaluated the effect of length of serum storage at -70°C on flow crossmatch results. Methods: A known positive crossmatch and negative crossmatch were run with fresh serum. Serum was stored at -70°C monthly for six months. Results: The negative crossmatch gave negative results for up to six months. The positive crossmatch gave positive results only up to the three-month time point, becoming negative thereafter. Conclusions: These data suggest that recipient serum should only be stored at -70°C for a maximum of three months before use in a flow cytometry crossmatch. This is also likely to apply to other assays relying on antibody integrity.

HAEMPOST-18 DIAGNOSIS OF CRYPTOCOCCOSIS IN HIV PATIENTS ON TREPHINE BIOPSIES- HELEN JOSEPH HOSPITAL EXPERIENCE

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Diagnosis of cryptococcosis in HIV patients on trephine biopsies – Helen Joseph Hospital (HJH) experience. Background: The genus Cryptococcus comprises approximately 37 species, however Cryptococcus neoformans (CN) is the commonest pathogen. Disseminated cryptococcosis is a life-threatening disease and is common in immunocompromised individuals particularly AIDS patients. The majority of patients present with cryptococcal meningitis and a few may have cytopenia(s) when there is bone marrow (BM) involvement. We describe four cases in AIDS patients presenting with BM involvement at HJH over the last 2 years (May 2008 to March 2010). Cases presentation Case 1: 27 year old female with a CD4 count of 27, on HAART. Presented with symptoms and signs of meningitis, hepatosplenomegaly, anaemia and thrombocytopenia. CN was isolated on cerebro-spinal fluid (CSF). Case 2: 23 year old male with a CD4 count of 7, HAART naive. Presented with headache and persistent pancytopenia. No hepatosplenomegaly. CN was isolated on CSF and blood culture. Case 3: 42 year old female with a CD4 count of 12, HAART naïve. Presented with symptoms and signs of meningitis, hepatosplenomegaly, anaemia and thrombocytopenia. CN was isolated on CSF and blood culture. Case 4: 32 year old female with a CD4 count of 31, HAART naïve. Presented with menorrhagia, hepatosplenomegaly, anaemia and thrombocytopenia. CN was isolated on CSF and blood culture. Trephine biopsies revealed intracytoplasmic yeast-like organisms in macrophages which stained positive with Periodic acid schiff (PAS), Grocott's and mucicarmine stains confirming disseminated cryptococcosis. Conclusion: These cases although few, illustrate the diagnostic value of trephine biopsies in the diagnosis of disseminated cryptococcosis. BM involvement should be considered in HIV patients with low CD4 counts, hepatosplenomegaly and cytopenia(s).

HAEMPOST-19 **COMPARISON OF MULTIPLE METHODS FOR DIAGNOSIS OF MYCOBACTERIAL INFECTION IN BONE MARROW SAMPLES OF HIV INFECTED PATIENTS.**

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Background: Diagnosis of Tb infection remains a challenge particularly in the setting of HIV. The gold standard for TB diagnosis is culture. Sputum microscopy and trephine are importnat adjunct diagnostic methods. Nucleic acid amplification assays for TB diagnosis have recently become commercially available. Comparison of the various methods of TB diagnosis is important to inform public health policy and surveillance strategies. This study compared the diagnostic usefulness of TB culture, trephine biopsy granulomata, ZN stain and TB polymerase chain reaction (PCR) analyses in establishing the diagnosis of TB infection in an HIV infected patient population. Method: Trephines from clinically suspected TB with HIV+ patients were identified and examined for granulomata stained with Ziehl-Neelson(ZN) stain. Corresponding blood/bone marrow culture results were also reviewed. Unstained bone marrow aspirate samples were subjected DNA real-time PCR analyses using Roche TB PCR assay. Test results were correlated with the corresponding CD4 counts. RESULTS: Of the 60 evaluable patients, 51% were females and the mean age was 35.9yr (range 21-55yrs). The mean CD4 count was 67.7x106/l (range 0 to848x106/l). Fifty seven percent had positive culture results whilst 55%, 40% and 87% were positive for ZN stain, TB PCR and granulomata respectively. Only two mycobacterium species were identified comprising 61% Mycobacterium tuberculosis and 38% Mycobacterium avium intracellulare. With culture gold standard, the sensitivities/specificities were 97%/23% for granulomata, 65%/58% for ZN staining and 50%73% for PCR analysis. Ninety-seven percent of granulomata were positive with ZN stain. Sixty percent of of culture negative patients had granulomata whilst 18% had positive ZN stain. Only one patient was positive with ZN stain with no granulomata.
Conclusion: Granulomata in HIV infected patients, appear to have a high diagnostic yield whilst PCR has the lowest yield but highest specificity. These results should be confirmed in a prospective case controlled study.

HAEMPOST-20 **DIAGNOSTIC UTILITY OF A D-DIMER TEST IN A PRIVATE HOSPITAL EMERGENCY UNIT IN JOHANNESBURG.**

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Background: The D-Dimers test has a high negative predictive value used primarily to confirm or exclude possible thromboembolic disease. In emergency units(EUs) practice, this test is often done not only for suspected PE but also to rule out atypical pulmonary emboli(PE). Diagnostic utility of this test has been evaluated in many public hospital EUs studies but not in our private hospital EUs. Patients presenting in public and private EUs often differ and therefore we hypothesized that the utility of the D-Dimer test in these two settings may be different. Results of this study will inform private hospital EU best practice. Objective: To evaluate the usefulness of the D-Dimer test at the private Morningside MediClinic(MMC) EU in Johannesburg. METHODS After approval by the Wits Ethics Committee, clinical audit of records was done at the MMC EU from 1 March to 1 June 2009. All patients who had a D-Dimer test done in the EU diagnostic workup were included. Extracted data included demographics, diagnoses and confirmatory tests done. Continuous and categorical variables of data collected were summarized using Stastistica 9.0 package. Results: In study period, 189 of 2948(5%) patients seen at MMC EU had D-Dimers measured. Their mean age was 57yrs(38.0 – 84.0) and 51% were males. Postive D-Dimers were present in 40(21%) of patients comprising PE(5)(100%), chest infection(5)(63%), AMI(2)(33%), arrythmia(2)(33%), hypertension(2)(25%), chest pain(6)(14%), anxiety(3)(23%), headache(1)(14%), (syncope(1)(14%) and others(5)(7%). The mean Wells score in PE was 3.6(3.0-4.5.) indicating medium probability of PE. All other diagnostic groups had low probability Wells score. Conclusion: The D-Dimer is done in only a fifth of patients seen at the private MMC EU and positive in less than half of cases. The test yield is highest in PE and has high negative predictive value in more than half of non-PE diagnoses except for chest infection.

HAEMPOST-21 EVALUATION OF THE CLINICAL VALUE OF MANUAL PERIPHERAL BLOOD SMEAR OF UNFLAGGED FULL BLOOD COUNTS.

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Introduction: An examination of the blood smears requested by clinicians is usually a response to perceived clinical features or an abnormality shown in a full blood count. A shortage of experienced morphologists at the CH Baragwanath Hospital and an increased workload has obligated us to consider other means to identify haematological abnormalities whilst providing quality service. Most manufacturers of automated haematology analyzers claim that instrumentation flags could be used as a preliminary screen to identify samples that require manual review. We therefore evaluated the significance of smear findings and the clinical value of comments made by morphologist to unflagged automated differential counts. Aim This study aims to determine whether manual review of peripheral blood smears is necessary in instances where the Sysmex XE 5000 instrument does not show any flags. Methods 200 samples were used in this study. All samples were taken from routine full blood count and differential request from patients at CHB hospital. Samples where no abnormalities were flagged by the instrument were evaluated manually by an experienced morphologist. Results In 98% of the unflagged results, no significant abnormalities were detected by the morphologists. For the remainder 2%, additional findings by the morphologists included occasional elliptocytes/ ovalocytes, occasional teardrops, mild rouleaux formation and some pencil cells. These additional findings/ comments did not seem to add any clinical value. Conclusion: With respect to samples where the instrument had no flags, the automated full blood count and differential results can reliably be reported without manual review of the peripheral blood smear. This should reduce the workload and improve turnaround time.

HAEMPOST-22 EVALUATION OF THE HYDRASYS AGAROSE HYDROGEL SYSTEM FOR HAEMOGLOBIN ANALYSIS.

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Background: The haemoglobinopathy diagnostic standard is to confirm all abnormal results of a haemoglobin screening test by a confirmatory test. In our laboratory, abnormal haemoglobins identified by HPLC screening tests (D10, Birad, USA) are confimred by electrophoretic analysis (Beckman Coulter Paragon agarose gel electrophoresis system(BCP). The BCP was abruptly discontinued by the manufacturer and we replace it with the Sebia Hydrasys Agarose Hydragel system (SHAH) (Sebia, UK). The SHAH is a semi-automated system with improved throughput, analytical efficiency capable of both acid and alkaline pH analyses with savings on labour and cost. The aim of this study was to evaluate the diagnotic performance of the SHAH. Material and methods: As the BCP system was discontinued, we could not do a direct comparison between two systems. We therefore compared the qualitative diagnostic assessments made using the BCP system with the same samples prospectively analyzed with the SHAH. Our laboratory is tertiary care centre in which a number of patients would require repeat testing to monitor therapeutic intervention. Repeat samples from these patients were prospective analyzed with the SHAH system and results compared to the previous analysis done with the BCP system. Comparison was from 1 December 2009 to 31 May 2010 and included assessment of the presence or absence of Hb variants and clinical interpretation of results. Results: In the 6 month study period, 48 abnormal screen samples required confirmation of which 18 analyzed with the both systems were available for comparison. There was concordance between the two systems in identifying the HbS, HBE and HbD variants. Clinical interpretation of the results was identical between the two methods. Conclusion: In this limited qualitative evaluation of the SHAH, there was 100% agreement between it and the BCP. Further comparison including analysis of other Hb variants is ongoing to complete this evaluation.

HAEMPOST-23 HAEMATOLOGICAL FINDINGS IN SOUTH AFRICAN HIV EXPOSED UNINFECTED INFANTS

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Introduction: Haematological parameters of HIV exposed uninfected (HEU) infants are affected by many factors, of which maternal antiretroviral therapy and prophylaxis have been identified as major contributors to anaemia and neutropaenia during the first three months of life. Haemoglobin, neutrophil, lymphocyte, and CD4 counts are documented to be significantly lower at 0-2 months in HEU infants exposed to antiretroviral therapy. At 6-24 months haemoglobin levels and neutrophil counts normalize, whereas differences in platelet, lymphocyte, CD4 and CD8 counts can persist. Data from sub-Saharan Africa also show that HIV-infected infants at 4-6 weeks have lower haemoglobin levels than HIV-uninfected counterparts. Automated blood count analysis was used in most of the studies. Methods: We compared the haematological findings in South African HEU infants with unexposed (UE) infants at 2, 6, 12 week, and 6 month intervals. The automated leucocyte differential count includes large unstained cells (LUC). The LUC reflect activated lymphocytes, paediatric lymphocytes, peroxidase negative blasts, plasma cells and hairy cells. A manual differential count corrects the LUC of the automated counter; a statistically significant difference between LUC in the HEU and UE populations may indicate exposure to infection. 25 HEU and 25 UE infants automated full blood counts were analyzed and compared at 2, 6, 12 week, and 6 months of follow up. Manual differential and automated differential counts were performed at these intervals for both study populations. Results: Preliminary results for the 2, 6, 12 week, and 6 month follow up confirmed an excess of anaemia in the HEU group which corrected at age 6 weeks. LUC reflecting predominantly activated lymphocytes were observed in infants of both populations, with a statistically significant increase of LUC in the HEU. A possible correlation between an excess of LUC and episodes of infections or failure to thrive will be explored.

HAEMPOST- 24 **HAEMOPHAGOCYTOSIS**

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Haemophagocytosis describes the pathological finding of activated macrophages, engulfing erythrocytes, leukocytes, platelets and their precursors in the bone marrow and other tissues. It is an important finding in patients with haemophagocytic lymphohistiocytosis. Haemophagocytic lymphohistiocytosis is characterized by fever, pancytopenia, splenomegaly. It is associated with a variety of viral, bacterial fungal and parasitic infections as well as collagen-vascular diseases and malignancies such as T-Cell lymphomas. This poster illustrates the striking haemophagocytosis in some patients with Human Immune Deficiency Virus (HIV). The most prominent lab findings were pancytopenia (Anemia, Thrombocytopenia and Neutropenia). Chemistry findings included elevated Lactate Dehydrogenase (LDH) and hyperbilirubinaemia. Activated macrophages engulfing erythrocytes, leukocytes, platelets and cellular fragments on blood film and bone marrow were identified from the poster.

HAEMPOST-25 IMATINIB RESISTANT CHRONIC MYELOID LEUKAEMIA: A CASE FOR HYDROXYUREA

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Introduction: Tyrosine Kinase Inhibitors (TKI) such as Imatinib (Glivec) is currently the drug of first choice in the management of Chronic Myeloid Leukaemia (CML). When a patient acquires resistance to Imatinib it is recommended to try other 2nd or 3rd generation TKIs such as Dasatnib and Nilotinib etc. Unfortunately these newer TKIs are not available in our center. We report a case of Imatinib-resistant CML who has responded very well to the re-introduction of Hydroxyurea in the management of her disease. Case Report: MN is a 56 years old Trankeian woman, one of our longest surviving cases of CML who was diagnosed in 1998 and started on treatment with Busulphan. She is also a known Epileptic (due to Neurocysticercosis) on anti-eplilectics. Her disease was initially well controlled but over the years her counts expectedly began to rise such that in March 2006 the WBC count was 133 x 109/l. Fortunately Imatinib became available at our center (courtesy Max Foundation of USA) and she qualified as her disease was Philadelphia chromosome positive. Within 6 weeks of therapy at 400mg daily the WBC had dropped to normal values (5.29 x 109/l in May 2006). However she remained in remission for less than 18 months as we noted a gradual rise in the counts to 40 x 109/l. A repeat FISH for the bcr/abl oncogene confirmed 100% positivity in October 2007. We feared she was going into the 'accelerated phase' of the disease and in the absence of the newer TKIs started her on Hydroxyurea at 1G po daily. We are happy to report that she has been well controlled for almost 3 years.

HAEMPOST- 26 **PLATELET DYSFUNCTION AS A SIDE EFFECT OF VALPROIC ACID: AN UNDERDIAGNOSED PHENOMENON**

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Introduction: Sodium valproate is commonly used as an anticonvulsant. It has been shown to inhibit the secondary phase of platelet aggregation. This can be reflected in increased bleeding times and haemorrhage Methods: We describe a case of a 56-year-old male with a meningioma. He was preoperatively screened for a bleeding tendency after he gave a history of bleeding during a previous operation. A prolonged bleeding time of 15 minutes lead to further investigations. A detailed medication history, full blood count, platelet function tests, PFA-100 screening test, Von Willebrand screening tests, and thromboelastography was done. We learned that he was taking valproic acid. All the screening tests were abnormal except for the full blood count and Von Willebrand tests. Results: Valproic acid was stopped for two weeks and the bleeding time and platelet function tests repeated. The bleeding time normalised and the platelet function tests were all normal. Conclusion: This case emphasises the role of a proper medication history in the workup of a patient with a bleeding diathesis.

HAEMPOST-27 **POLYCYTHAEMIA VERA IN A KNOWN HAEMOPHILIAC**

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Rosemary Khanyile. Nelson Mandela Academic Hospital, Mthatha .

Introduction: Polycythaemia is a disorder characterized by an abnormal increase in the number of circulating red blood cells. There are many causes of secondary polycythaemia. Polycythaemia Vera (PV) is a clonal disorder of unknown aetiology and the diagnosis can only be made when all other causes of polycythaemia have been ruled out. A significant complication of polycythaemia is thrombosis for which Aspirin is recommended as a prophylaxis. Haemophilia A is an inherited bleeding disorder that afflicts about 1 in 10 thousand live male births. It is due to a deficiency of coagulation Factor VIII. Sufferers are prone to excessive bleeding following even the mildest of traumas and in severe cases bleeding especially into joints may be spontaneous. Haemophiliacs are advised to avoid all aspirin containing drugs. We report on a known Haemophilia A person who was incidentally found to have Polycythaemia. Case Report KM is a 46 years old severe haemophiliac with Factor VIII level of <1% whose bleeding episodes are managed with Factor VIII concentrate. It is interesting to note that he has 2 other male siblings with the same affliction. It was during a routine check for Factor VIII inhibitors, that blood was drawn for other baseline studies including FBC. He had normal platelet and white cell counts, but the red cell indices were: Red Cell Count 7.2 x 1012/l (N= 4.50-5.50) Haemoglobin 19.2 g/dl (N= 13.0-17.0) Haematocrit 0.584 l/l (N= 0.400-0.500) We have investigated and ruled out all common causes of Polycythaemia and came to the conclusion that it is a PV. Thus far he remains asymptomatic, but with a gradually rising Haemoglobin concentration there may come the time to consider placing him on anti-platelet drug as a prophylaxis for DVT. Aspirin is the cheapest one available but is contraindicated in haemophiliacs.

HAEMPOST-28 **RARE CASE OF DONOR CELL LEUKEMIA**

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Donor cell leukemia (DCL) is the occurrence of leukemia in donor cells after allogeneic SCT (stem cell transplantation) with a healthy donor. Acute leukemia of donor origin is a rare event after allogenic BMT (bone marrow transplant). On occasion one may encounter a new leukemia or myelodysplastic syndrome of host origin, presumebly derived from a host stemcell that survived the myeloblative therapy. Rarely a leukemia of donor origin developes after BMT. A 51 year old male patient was diagnosed with T-ALL . The patient underwent allogenic BMT from a HLA matched sister aged 60 years, approximately one year after the diagnosis of T-ALL was made. 7 Years later a bone marrow biopsy performed for persistent neutropenia showed features consistent with AMML (Acute Myelo Monocytic Leukemia). Conventional cytogenetic analysis yielded 20 metaphases that were analysed from the BM culture of which were all female donor cells 46XX.FISH analysis identified a female sex chromosome complement (XX) in 98% of 514 cells examined, consistent with a leukemia of donor origin. In conclusion a AMML DCL was diagnosed. A major problem in the analysis of DCL is that demonstration of donor cell origin of leukemic relapse after allogeneic transplantation is difficult as follows: 1. Artefacts and limitations of the methods to establish the chimeric status. 2. The genetic lability which is intrinsic to the leukemic transformation. 3. Some of the proposed mechanisms leading to donor cell transformation. Various laboratory tests are currently available to characterize the chimeric status of haemopoietic compartments of a transplanted patient. The essential purpose of each of these tests is to recognize genetic material specific to the host and the donor.

HAEMPOST-29 THE EFFECT OF CURCUMIN ON TELOMERE LENGTH AND CASPASE ACTIVITY IN THE U937 ACUTE MYELOID LEUKAEMIA CELL LINE

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Introduction: Apoptosis is a controlled cell death mechanism of eradicating irreversibly damaged or unwanted cells from the body. Activation of this process is achieved by the shortening of telomeres at chromosomal endpoints to a critical length. Apoptosis is then induced by either caspase-dependent or -independent pathways. Highly activated telomerase, a reverse transcriptase may prevent shortening of telomeres to the critical length needed to activate apoptosis. This may result in uncontrolled proliferation of cancerous cells and lead to disorders like acute myeloid leukaemia. Curcumin, commonly known as turmeric, has been reported to have anti-tumour and apoptotic potential. It may influence telomerase activity and cause apoptosis via a caspase dependent pathway. Aim: To determine the effect of Curcumin on telomere lengths and caspase 3 and -4 activity in U937 acute myeloid leukaemia cells Material and methods: The U937 cell line was cultured and treated with different Curcumin concentrations for 24- and 48 hours respectively. The relative telomere lengths were determined using Flow FISH. This assay was performed to determine how Curcumin influences telomerase. ELISA's on caspase 3 - and caspase 4 activity, involved in the endoplasmic reticulum apoptotic pathway were performed. This was done to determine whether Curcumin influence the endoplasmic reticulum apoptotic pathway. Results: An increase in telomere lengths were observed with increasing Curcumin concentrations at 24 and 48 hour treatment periods. At a concentration of 10μM for the 24 hour treatment period, the telomeres were shortened. Caspase 3 - and caspase 4 activity mostly increased with increasing Curcumin concentrations. Conclusion: Curcumin might influence telomerase and cause telomere shortening, however an alternative way of telomere elongation have to be considered in that case. Curcumin does influence the endoplasmic reticulum pathway of apoptosis as illustrated by increasing caspase 3 - and caspase 4 activity and decreasing cell viability with increasing Curcumin concentrations.

HAEMPOST-30 THE EFFECTS OF BUDGETARY CONSTRAINTS ON COAGULATION TESTING AT THE UNIVERSITAS HOSPITAL NHLS SERVICE LABORATORY.

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Introduction: Budgetary restrictions were in force from 1 December 2008 up to March 2010 at Universitas Academic Hospital. The effects of these restrictions on the number of specimens received for coagulation testing as well as on the proportion of coagulation specimens requiring direct human involvement were investigated. Methods: All specimens received for coagulation testing from 1 December 2008 to 30 September 2009 were audited. Specimens inspected by technologists and found to be subject to suboptimal pre-analytical influences and those requiring additional sample preparation, were recorded in a logbook and the two periods (with and without budgetary constraints) compared. Results: For the 121 day period with budgetary constraints 3198 specimens (median=27/day) were received and 459 (14.4%) required human involvement. For the 183 day period without constraints 5416 (median=29/day) were received and 632 (11.7%) required human involvement. Data for most of the different categories requiring human involvement remained relatively stable. Conclusion: Budgetary constraints had only a minor impact on the number of specimens received for coagulation testing and the proportion requiring direct human involvement. Although a lower number of specimens for lupus anticoagulant testing (essentially an elective investigation) were expected for the period with budgetary constraints, this was not the case. It would appear that budgetary constraints do not deter clinicians from requesting lupus anticoagulant testing. Furthermore, clinicians at Universitas Academic Hospital do not generally order unnecessary coagulation testing, since the number of specimens received for testing did not differ greatly during the two periods.

HAEMPOST-31 THE VALIDATION OF THE MANUAL PLATELET COUNT USING A PERIPHERAL SMEAR STAINED BY WRIGHTS OR GIEMSA STAINS

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Introduction A manual platelet count using the chamber method is time consuming, requires high expertise and is expensive. An alternative method which reduces all of the above would be of great assistance to the laboratory for the verification of platelet counts that are produced by the instrument and requires to be verified. Aim To determine the accuracy of the manual platelet count using a peripheral smear stained by the Giemsa or Wrights stain. Study design A three ways comparison of results obtained from the instrument, from the chamber and also manual platelet count peripheral smear method. Results Greater than 80% of the counts performed thus far compare with that of the instrument and the chamber method. Conclusion The use of the manual platelet count using the peripheral smear compares well with that of the instrument and the chamber hence it is acceptable to use this method where the platelet count is questionable. This will reduce time spend on the preparation of reagents and the staff personnel that would have been required to perform a manual platelet count using the chamber method.

HAEMPOST-32 POST-TRANSPLANT IMMUNE RECONSTITUTION MIMICKING RELAPSE OF HEPATOSPLENIC T-CELL LYMPHOMA

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We present the case of a 35-year old female with mild mental retardation who was diagnosed with Hepatosplenic T-Cell Lymphoma with a γδ-phenotype. Following remission induction with chemotherapy, the patient successfully underwent allogeneic stem cell transplantation from a matched sibling male donor. The post-transplant period was complicated by Pneumocystis Jirovecii pneumonia, but no significant graft versus host disease. Seven months post-transplant, the patient developed pancytopenia and constitutional symptoms. Clinical and radiological examinations were unremarkable, but a bone marrow biopsy was markedly abnormal, with increased numbers of T-lymphocytes and morphological features suggestive of relapse and granulomatous inflammation. Flow cytometry for T-cell receptors showed an aß: γδ ratio of 60%:40%. Fluorescent activated cell sorting of T-lymphocytes, followed by Fluorescent in situ hybridisation (FISH) for X/Y chromosome discrimination proved that the vast majority of T-lymphocytes, including γδ-cells, were of donor rather than patient origin. The cytopenias subsequently recovered spontaneously and the patient remains clinically well and clear of disease. Although T-cell immune reconstitution after allogeneic stem cell transplantation has been well documented, little published data exists on the nature and extent of γδ-cell reconstitution, and the function and relative importance of these cells in the post-transplant setting remain unclear.

HAEMPOST-33 TOTAL LABORATORY AUTOMATION: CHALLENGES AND SOLUTIONS IN AN ACADEMIC TERTIARY LABORATORY

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Introduction: Total laboratory automation (TLA) is an analytical laboratory solution combining pre-analytical, analytical and post-analytical functions into a single system. Advantages of the TLA system are analytical efficiency, reduced turnaround time (TAT), improved throughput, improved safety and quality. In South Africa, there is critical shortage of technical staff and TLA is a plausible solution to this challenge. For these reasons, the NHLS implemented TLA solution at the Charlotte Maxeke Johannesburg Academic Hospital (CMJAH) in September 2009. This process has been characterized by challenges at all levels of operation. The aim of this presentation is to share our experience with implementation of the TLA solution. Materials and Methods We reviewed documented evidence of challenges since implementation of TLA and mapped these to the solutions implemented. The information sources were minutes of the regular TLA project meetings and discipline specific project champions we spoke to. Identified challenges were grouped into categories and presented as quantitative data. Results Thirty four major challenges were identified and these were grouped into 5 categories as in figure 1 below. The majority of challenges were hardware failures. Vendor and workflow issues were the most challenging to resolve. The majority of outstanding issues relate to personnel and software. Category Problem (nos) Resolution Achieved (%), Resolution Pending (%) .1) Personnel 42575. 2) Workflow 812.550. 3) Hardware 104030. 4) Software 4075. 5)Vendor 7057 Figure 1: Problems and Resolutions encountered during the implementation of Total Laboratory Automation Discussion This study has enabled the identification and categorization of the major challenges encountered during the implementation of TLA while highlighting specific problems that have proven more difficult to resolve. The results will enable other laboratories embarking on the TLA project to plan appropriately in order to minimize unexpected problems.

HAEMPOST-34 TRANSFORMATION OF CHRONIC MYELOID LEUKAEMIA (CML) TO AN ACUTE LYMPHOBLASTIC LEUKAEMIA (ALL) ASSOCIATED WITH ACQUISITION OF A RARELY DESCRIBED TRANSLOCATION(5;17).

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CML is a clonal stem cell disorder, characterized by proliferation of granulopoietic cells in all stages of maturation and a prominence of basophils in the peripheral blood. Untreated it is a progressive disease which usually transforms to an acute leukaemic process within 3 to 5 years; most commonly an Acute Myeloid Leukaemia (AML). However, 20-30% of cases transform to an ALL. Clonal evolution / transformation is associated with acquisition of chromosomal abnormalities in addition to the Philadelphia Chromosome (Ph) [such as a double Ph chromosome, trisomy 8 and isochromosome (17q)]. In this case study we present a 45 year old female patient with known CML who transformed to an ALL with acquisition of a rarely described mutation t(5;17) in conjunction with a complex karyotype. At presentation analysis showed: 46,XX, t(9;22)(q34;q11),add(17)(q25) in all metaphases. FISH analysis confirmed that 87% of cells carried a BCR/ABL fusion gene, with a double Philadelphia Chromosome noted in a small population of cells. The patient was started on Gleevec therapy and a full haematological remission was achieved within 2 months. The patient remained in haematological remission for a further 5 months, at which stage she was noted to have 54% blasts in the blood. Flow cytometry confirmed transformation to a Precursor B-Cell ALL. Cytogenetics revealed: 46,XX,t(5;17)(q13;q25), t(9;22)(q34;q11),-der(9)t(9;22),t(12;?)(q10;?),del(13)(q13),-20,+der(22)t(9;22),+mar [17]. Mutational analysis of the ABL oncogene showed the presence of a T315I mutation which typically confers resistance to Gleevec and second-line drug therapies. Translocation (5;17) is an extremely rare cytogenetic finding. To the best of our knowledge, t(5;17)(q13;q25) has only previously been described once in the setting of a haematological disease. Interestingly the previous description was also in a patient with CML and associated with a complex karotype. Translocation (5;17)(q13;q21) has been described three times previously: once in AML, once in T-ALL and once in a Biphenotypic Leukaemia.

HAEMPOST-35 TRENDS IN CONGENITAL BLEEDING DISORDERS IN THE FREE STATE, NORTHERN CAPE & KZN USING THE SA WEB-BASED HAEMOPHILIA REGISTRY: 2010 UPDATE

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In South Africa there are three sources of information about patients with congenital bleeding disorders: annual Haemophilia Treatment Centre (HTC) reports, a stand-alone Access-based database, and the web-based registry. Since its inception in 2008 the web-based registry has slowly been implemented. It is the simplest method of capturing basic data on persons with congenital bleeding disorders. It is being used in the Haemophilia Treatment Centres (HTC) in the Northern Cape, Free State and KwaZulu-Natal, with slow progress. Both the Free State Adult Centre outreach to the Northern Cape and the paediatric Kimberley HTC, have been active. In the Northern Cape the number of active patients increased from 25 to 32, five 'lost' patients could be re-identified, but number of deceased patients identified on family trees increased by 47.1%. The KZN Paediatric HTC has added 20 new patients to the registry, with the data of 46 patients being sanitised. No new deceased patients were identified. Among the new patients two had inhibitors. Issues that have been identified include: lack of man-power to update the Registry, the need for more simple computers at nursing stations, the need to request routine follow-up blood tests, and to obtain these results. Two of these centres have each lost a full-time staff members. The stringent ethics approach taken by the web-based registry committee has also slowed down its roll-out. It is not cost-effective for every small laboratory to be able to determine factor VIII or IX. The transport of specimens for coagulation tests is challenging, as they have to be maintained at -20°C. In the Northern Cape this problem is being addressed. The supply of coagulation factors can be intermittent at this stage, thus it is vital to have very basic accurate statistics to lobby and plan. Our three centres hope to lead the way.

HAEMPOST-36 TWO AFRICAN ACUTE LYMPHOBLASTIC LEUKAEMIA CASE REPORTS USING SINGLE-NUCLEOTIDE POLYMORPHISM MICROARRAYS

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Single nucleotide polymorphism (SNP) array technology offers potential for cytogenetic analysis of haematological malignancies. Array based technology can be used to explore haematological malignancies (and other malignancies) with an unprecedented degree of resolution. Two paediatric case-reports are described of pre-cursor B-Cell/common paediatric acute lymphoblastic leukaemia (ALL) which have been analysed using Affymetrix SNP 6.0 Genome-Wide chips. Both patients were admitted to the same academic centre, were diagnosed with conventional techniques and treated with the BFM-95 protocol. Case 1, a 9 year old male, with t(9:10) and an otherwise normal karyotype, after one month of treatment did not go into adequate remission. Array technology identified homozygous deletion of IKZF1 and TARP and heterozygous loss EBF1. These genomic deletions drive resistance to treatment and have been correlated with prognosis in ALL; however, these have not been identified in an African population and the presence of these deletions in a t(9:10) translocation is novel. Case 2, is a 4 year old female (12:21 positive) with a complex karyotype consisting of at least three distinct clones as identified by karyotyping. The case presents an interesting case for array diagnostics due to the multiple-clones present. Genetic abnormalities from all three clones were identified with refined chromosomal breakpoints. An amplified region of Chr 8 was also identified that was previously unreported. A highly complex karyotype with multiple sub-populations may successfully be interrogated using array based technology. IKZF1 and EBF1 mutations in an African patient failing treatment with t(9:10) is reported.

HAEMPOST-37 WHERE THERE IS ONE, THERE MAY BE MORE. A CASE PRESENTATION

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The occurrence of multiple myeloma (MM) and chronic lymphocytic leukaemia (CLL) in the same patient is rare with only isolated case reports having been published. Our patient is a 49yr old retroviral positive female who presented with a history of an enlarging right sided chest wall mass over a 2-3 month period. A plasmacytoma was diagnosed on histology. In the work-up for multiple myeloma, no serum monoclonal peak could be demonstrated with electrophoresis. A free light chain assay, however, confirmed the presence of increased serum lambda light chains. Urine fixation showed a lambda band. Other biochemistry tests for total protein, albumin, calcium, urea and creatinine were within normal limits. Peripheral blood report showed a mild anaemia with a lymphocytosis of 9.8x109/l. Morphology revealed pleomorphic lymphocytes but no circulating plasma cells. The immunophenotype of the peripheral blood lymphocytes was consistent with B-cell chronic lymphocytic leukaemia with 98% of B-cells displaying lambda light chain restriction. The core biopsy showed ±20% B-lymphocytes and ±30% plasma cells with lambda light chain restriction. Fluorescence in site hybridization (FISH) analysis of the blood and bone marrow slides showed 49% positivity and 60% positivity for del13q14.3 respectively. The patient was therefore diagnosed with both multiple myeloma and chronic lymphocytic leukaemia. Conclusion: Multiple myeloma and chronic lymphocytic leukaemia are distinct neoplasms that are thought to arise from different stages of B-cell maturation. The concomitant appearance of both these entities in the same patient is rare and to our knowledge this phenomenon has not yet been described in a retroviral positive patient. The controversy regarding the clonal origins(s) of the malignant cells remains a source of debate in the literature. The presence of both CLL and MM is associated with an unfavourable myeloma disease course with early progression.

HAEMPOST-38 DERIVATIVE(Y) TY;1)(Q12;Q12) FOUND IN AN ALL PATIENT IN CONJUNCTION WITH A DER(19)T(1;19)(Q23;P13)- A CASE STUDY

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Acute lymphoblastic leukemia (ALL) is a malignancy of lymphoblasts, which proliferate in the marrow and replace normal haemopoietic elements. It is the most common leukaemia found in childhood. Cytogenetic translocations associated with specific molecular genetic abnormalities in ALL are: cryptic t(12;21), t(1;19)(q23;p13), t(9;22)(q34;q11), t(4;11)(q21;q23), t(8;14)(q24;q32) and t(11;14)(p13;q11). Our case study shows a der(Y)t(Y;1)(q12;q12) found in an ALL patient in conjunction with a der(19) t(1;19) (q23;p13). We present a 9 year old male patient, diagnosed to be a Precursor B-Cell ALL. Cytogenetic analysis and Fluorescent in situ Hybridization (FISH) studies were performed. Chromosomal analysis on the bone marrow sample revealed the following karyotype: 46,XY,der(19)t(1;19)(q23;p13)[7]/46,X,idem,der(Y)t(Y;1)(q12;q12)[14]/ 46,XY [1]. FISH studies were performed on bone marrow smears using Vysis probes. Interphase cells revealed a small subclone with a partial/complete trisomy 8. The results for FISH analysis for t(12;21), t(9;22) and the rearrangement of the MLL gene were negative. The patient presented in this report had a diagnosis of a de novo precursor B-cell ALL with a more mature B-cell immunophenotype. The der(Y)t(Y;1)(q12;q12) was found as an additional aberration to a der(19)t(1;19)(q23;p13) in a subclone of cells. It is therefore a secondary event resulting in partial tetrasomy for 1q23qter and trisomy for 1q12q22. To the best of our knowledge a der(Y)t(Y;1)(q12;q12) has been described once previously in the setting of ALL in an infant. Interestingly in both infants, this aberration was secondary to t(1;19).