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ABSTRACTS

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CLINICAL CYTOMETRY SOCIETY

Future Meetings

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BONE MARROW PLASMA CELL LABELLING INDEX BY FLOW CYTOMETRY IN MYELOMA

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Assessment of the proliferative capacity of plasma cells (labelling index) is an important independent prognostic indicator in myeloma. A high labelling index (LI) is associated with bad prognosis or worsening of disease. Conversely, a low index corresponds to a good prognosis. Traditionally, the LI has been determined by a microscope immunofluorescent assay. In recent years, a modified method utilising flow cytometry has been developed. We studied the use of flow cytometry to determine LI in 30 patients with newly diagnosed or relapsed myeloma. Using a combination of CD38 PE and CD138 FITC, and propidium iodide to intercalate double stranded DNA, the DNA content of S phase cells (cycling cells) was calculated using Multicycle software (Phoenix Flow Systems, USA). A cut-off point of 4% was adopted as threshold for significant increase in the LI. Of the 30 patients, 23 (77%) had LI of greater than 4% while 7 (23%) had LI less than 4%. Adoption of flow cytometric method over the traditional manual slide method to determine LI should be encouraged and further studied. It is more objective and much easier to perform as the counting procedure is automated. Studies have found it to correlate well with slide based technique.

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INCREASED CD8+CD57+ CMV TETRAMERS IN HIV AND CMV SEROPOSITIVE INDIVIDUALS

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To evaluate MHC class I and II immune responses by the use of CMV tetramers with CD8+ T cells and IFN- γ production in CD4+ T cells after stimulation with viral lysate in HLA A2 and HLA B7 positive healthy CMV+ donors and individuals seropositive for HIV and CMV.

Whole blood was collected from 7 healthy donors and 8 HIV-1 + individuals (average absolute CD4+ T cell counts 875.77, SD \pm 446.61). Standard 4-color flow cytometry techniques were utilized, a total of 20,000 CD8+ T cells were acquired for tetramers and the percentage of tetramer+CD57+ cells obtained; for cytokine intracellular assays 50,000 CD4+ T cells was collected and the percentage obtained reflected co-expression of IFN- γ and CD69. Membrane marker acquisition of 40,000 cells was acquired.

HIV+ individuals showed significantly increased CD8+CD38+ expression and tetramers (Mann-Whitney U test, $p=0.001$; 0.002) in comparison to healthy subjects. HIV+ individuals demonstrated significantly reduced Absolute CD4+ T cell counts (Mann-Whitney U test, $p=0.004$)

and CD4+/CD8+ ratio (Mann-Whitney U test, $p=0.001$) in comparison to controls. However, assessment of intracellular IFN- γ in these subjects revealed no significant differences (Mann-Whitney U test, $p>0.05$) compared to controls.

This study suggests the existence of homeostatic mechanisms in HIV disease that selectively preserve memory CD8+ T cell populations reactive towards ubiquitous pathogens. The preservation of reactive CMV responses may be at the expense of T cell memory to infrequently encountered infectious agents.

3

FLOW CYTOMETRIC ANALYSIS OF NUCLEATED RED BLOOD CELLS IN HUMAN PLACENTAS

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Umbilical cord nucleated red blood cells (NRBCs) have been implicated as both markers and predictors of chronic fetal hypoxia and adverse neonatal outcome. It has been shown that placentas with an unusually high proportion of NRBCs are associated with a high incidence of fetal distress and perinatal asphyxia. In an attempt to identify a method for antenatal detection of elevated fetal NRBC counts, we sought to evaluate the correlation between placental and umbilical cord NRBC counts by flow cytometry. We collected 80 placentas and their matched umbilical cord blood samples immediately following delivery. In-vitro fine needle aspiration (FNA) biopsies were used to obtain placental tissue samples. In order to identify NRBCs and WBCs in both umbilical cord and placental samples by flow cytometry, cells were labeled with CD45-PerCp and CD71-FITC. Nucleated RBC counts were expressed as: (#NRBC events)*100/ (#WBC events). Spearman correlation was used for statistical analysis.

The mean (\pm SD) gestational age, birth weight and placental weight were 38.6 \pm 1.7 weeks, 3266 \pm 482 GM and 579 \pm 107 GM, respectively. Flow cytometric analysis showed a significant correlation between umbilical cord blood NRBC counts and placental fine needle aspirations NRBC counts (Spearman Correlation; $R=0.74$, $p<0.0001$). The inter observer variability ($N=20$) of flow cytometric NRBC counts was 7.6%.

Umbilical-placental NRBC counts:

	Umbilical cord NRBC counts	Placental NRBC counts	Spearman Correlation
Median	11.3	8.6	
Range	0-275	0.3-92	$R=0.74$, $p<0.0001$

This study demonstrates that placental NRBC counts highly correlate with umbilical cord NRBC counts and strongly suggests that antenatal evaluation of fetal NRBCs can be achieved by placental FNA biopsy.

4

FAILURE TO RECOVER CIRCULATING EBV-SPECIFIC CD8⁺ T CELLS MAY PREDISPOSE FOR HIGH EBV LOAD AND EBV⁺ LYMPHOPROLIFERATIONS IN ALLOGENEIC, PARTIALLY T-DEPLETED STEM CELL TRANSPLANTATION

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The clinical success of adoptive cellular (pre-emptive) immunotherapy of EBV⁺ lymphoproliferative disease (LPD) using EBV-specific cytotoxic T lymphocytes (CTL) indicates the pivotal role of these CTL in controlling this virus in allogeneic stem cell transplant (SCT) recipients. Here, we studied the recovery of EBV-specific CD8⁺ T cells in recipients of partially T-cell-depleted SCT using a panel of 11 class I HLA tetramers presenting peptides derived from 8 latent and 3 lytic EBV proteins. The median time to EBV reactivation was 65 days post SCT. In 24 patients EBV-specific CD8⁺ T cells were quantified prior to EBV reactivation. In 6 of 9 patients with low viral load (<1,000 gc/ml), EBV-specific CD8⁺ T cells were detectable (>0.5 T cells/ μ l), whilst this was the case in only 2 of 15 patients with high-level EBV load ($p = 0.02$). Six of these 15 patients were pre-emptively treated with CD20 mAb and did not progress to LPD. The remaining 9 patients did not receive pre-emptive CD20 mAb; 6 of them progressed to LPD. In 5 of these 6 patients, EBV-specific CD8⁺ T cells were undetectable, whilst all 3 patients not developing LPD had recovered these cells ($p = 0.048$). These observations indicate the clinical utility to monitor the reconstitution of EBV-specific T cells post SCT using tetramer technology.

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AUTOMATED VIABLE CD34 COUNTS USING StemONE™ ON THE COULTER XL

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The accurate enumeration of CD34⁺ cells by flow cytometry (FCM) represents a critical step in evaluating products in patients undergoing autologous or allogeneic hematopoietic stem/progenitor transplantation. The enumeration of CD34⁺ cells is a challenge to the clinical FCM laboratory, as they usually represent 0.1-1% nucleated cells. Processing of samples including overnight storage and cryopreservation can complicate the analysis. Recently a CD34⁺ enumeration system, consisting of reagents and automated software (StemONE, Beckman Coulter) has become available. We compared StemONE to our manual gating strategy using the single platform ISHAGE method on the following samples: mobilized peripheral blood (mPB, n=45), apheresis (Aph, n=59), cord blood (CB, n=16) and post cryopreserved apheresis

(cAph, n=12). All samples were stained in duplicate using StemKIT, which contains CD45FITC/CD34PE and the viability dye 7-AAD. After staining, samples were lysed with ammonium chloride and StemCOUNT™ fluorospheres added immediately before acquisition on a Coulter XL flow cytometer. Analysis on ranks showed no significant difference between manual and automated analysis ($p > 0.05$). Correlation coefficients (R^2) were 0.85 (mPB), 0.91 (Aph), 0.92 (CB) and 0.98 (cAph). The data presented here shows it is possible to automate the process of CD34 analysis in a wide range of sample types using a standardized reagent kit and the viability dye 7-AAD, with StemONE automated software. Coupled with automated sample preparation and instrument setup, fully automated analysis of this complex testing is possible, requiring only that the operator review histograms after sample preparation and analysis has been performed.

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VARIABILITY OF ABSOLUTE CD4 AND CD8 COUNTS: EFFECT OF GATING STRATEGY AND STAINING PROTOCOL

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The aim of this study was to assess the impact of gating strategy (CD45/side-scatter versus CD3/side-scatter) and staining protocol (three- versus four-color staining) on the variability of absolute CD4 and CD8 counts. Peripheral blood specimens from 67 patients were analyzed using the whole blood lysis method, a single platform FACSCalibur instrument (Becton Dickinson), and MultiSET V1.0.1 software. Each specimen was analyzed in three ways: (1) CD45 gating and three-color staining (CD3-FITC/CD4-PE/CD45-PerCP and CD3-FITC/CD8-PE/CD45-PerCP); (2) CD45 gating and four-color staining (CD3-FITC/CD8-PE/CD4-APC/CD45-PerCP); and (3) CD3 gating and three-color staining (CD4-FITC/CD8-PE/CD3-PerCP). All samples were run and analyzed by the same operator. Absolute CD4 counts ranged from 2 to 1082 cells/ μ l, and absolute CD8 counts from 34 to 2422 cells/ μ l. Statistical analysis, performed with a one-way ANOVA procedure showed no significant variability among absolute CD4 ($p=0.91$) or CD8 ($p=0.97$) counts. The gating strategies and staining protocols used in this study did not significantly affect absolute CD4 and CD8 counts.

7

ACUTE MYELOID LEUKEMIA BEARING THE FLT3 INTERNAL TANDEM DUPLICATION HAS A PROMINENT IMMATURE BLAST POPULATION WITH A UNIQUE IMMUNOPHENOTYPE.

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In an effort to identify myeloblast subpopulations which may represent the ³leukemic stem cell² in cases of acute myeloid leukemia (AML), we have evaluated the expression of CD34, CD38, CD45, CD90, CD110 (c-Mpl), CD117 (c-Kit),

CD123 (IL3-R_α), CD133, and CD135 (Flt3) in 100 cases of AML, including FAB types M0 to M7. In parallel, DNA PCR to look for the internal tandem duplication (ITD) of the Flt3 receptor tyrosine kinase gene, which has been associated with an adverse prognosis in a number of studies of AML, was performed on all cases. The molecular and immunophenotypic data were correlated with basic clinical features of the cases, including age, gender, peripheral leukocyte count, and cytogenetic findings. Our findings suggest that patients whose AML bears the Flt3 ITD are significantly more likely to present with leukocytosis, normal cytogenetics, and monocytic differentiation, compared to patients whose AML lacks the ITD. Strikingly, Flt3 ITD-positive AML demonstrates a higher level of expression of CD123 regardless of FAB subtype, in comparison to patients whose AML lacks the ITD. In addition, a significant subset of cases contains a prominent ³immature² blast population expressing CD34 with little or no CD38 and showing complete loss of CD133 on all blasts. We hypothesize that this less-differentiated blast population harbors the leukemic stem cell for Flt3 ITD-positive cases of AML, and makes possible the immunophenotypic identification of cases harboring the Flt3 internal tandem duplication.

8

Minimal Residual Disease Detection in Hairy Cell Leukemia: Comparison of Flow Cytometric Immunophenotyping and Clonal Analysis Employing Polymerase Chain Reaction for the Heavy Chain Gene

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86 consecutive specimens from 24 patients treated for hairy cell Leukemia (HCL) were studied to determine the sensitivity of flow cytometry (FC) and PCR in minimal residual disease detection. A panel of antibodies that included CD19, CD20, CD22, CD103, CD25, CD11c, CD10, CD5, CD23, FMC-7 and both monoclonal and polyclonal light chain reagents was used in all cases. PCR was performed on each sample using JH alpha and VH framework consensus primers. Concurrent CBC and differential counts were available on 76 specimens. Results were analyzed using repeated measures analysis. FC was more sensitive detecting HCL in 48/86 specimens (56%) compared to detection of clonal B cell populations in 23/86 (28%) of PCR specimens. FC and PCR were both more sensitive than morphology. A positive PCR result was associated with higher tumor cell numbers as determined by FC ($p=0.0017$). We determined cutoff values for the number of tumor cells at which PCR is consistently positive. 6.8 tumor cells/uL would yield positive PCR results in 90% of samples. Flow cytometry was adequate in all 86 cases (100%) regardless of white blood cell count(WBC) while PCR was adequate in 74 (86%) cases. FC was found to be superior to PCR in detecting minimal residual HCL. It is more

sensitive, more specific and allows for calculation of tumor cell number.

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CORRELATION OF ABSOLUTE CD11b+/CD28- CD8+ T CELL COUNTS AND PROGNOSIS IN EARLY-STAGE B-CLL

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Early-stage (Rai 0, 1) B-Cell Chronic Lymphocytic Leukemia [B-CLL] is marked by a wide variability among patients in the rate of disease progression: Some may not ever progress or require treatment, while others will progress rapidly and die despite best available therapy. Several B cell variables have been proposed as prognostic markers for B-CLL (e.g. Ig V gene mutation, CD38 expression, soluble CD23, beta-2 MG), however our group has previously reported novel clinical data on early-stage cases, representing the first indication that a T cell variable may enable prediction of disease prognosis for patients with B-CLL.

This study, which used multicolor immunocytometry and new cytometry software, explored whether the presence of expanded subsets of CD11b+/CD28- putative 'immune suppressor' lymphocytes among CD8+ T cells heralds a more rapidly-progressive form of B-CLL in the early stages of disease. The median clinical follow-up of our updated data is 49 mo. after experimental cytometry and 60 mo. after diagnosis.

The 13 cases in our pilot study contained two groups, based on progression of their disease: Among 6 cases which continue to have non-progressive disease, the mean absolute count of CD11b+/CD28- CD8+ T cells was 161 (range 91-338; SD = 89), while the corresponding absolute counts among 7 cases with progressive disease was 507 (range 180-932; SD = 251). Alternatively, these cases could be divided into two groups based on absolute CD11b+/CD28- CD8+ T cell counts: 8 cases had high counts (>2 SD above the mean for normal healthy controls), and 5 cases had normal counts. Although the age, sex, Rai stage, and absolute lymphocyte counts were not significantly different in the two groups, there was a statistically significant difference in progression-free survival between the two groups: 7 of the 8 cases with high CD11b+/CD28- CD8+ T cell counts (180 or greater) had disease progression, while 5 of 5 with normal counts remain progression-free and asymptomatic ($p = .01$). These findings highlight the need for further study of the prognostic and therapeutic implications of abnormal CD8+ T cell subsets in B-CLL.

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COMPARISON OF A MIXTURE VERSUS A SINGLE ANTI-HLA-B27 ANTIBODY REAGENT IN FLOW CYTOMETRIC HLA-B27 TYPING

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HLA-B27 (B27) typing is used in the diagnosis of ankylosing spondylitis and other arthropathies. The single mono-

clonal anti-B27 antibody (Ab) based test often gives equivocal results, which require confirmation. We compared Com-B27 (a mixture of anti-B27Abs) from IQ Products to FD705 (single anti-B27 Ab) from One Lambda, with 60 (20 B27⁺ and 40 B27⁻) patients. Blood was incubated with anti-CD3 and anti-B27 Abs for 15 min. Binding of anti-B27 Ab(s) on CD3⁺ stained T cells was quantified after lysis of red blood cells. HLA status was determined by the standard microcytotoxicity test. The fluorescence intensity of FD705 binding to B27⁺ and B27⁻ cells are 107 ± 25 and 24 ± 25 mean channels (MC, mean \pm 2SD), respectively. The positive and negative range overlapped by 31 MC. In contrast, the binding of Com-B27 to B27⁺ and B27⁻ cells is 119 ± 6 and 35 ± 19 MC, respectively. B27 positive and negative ranges did not overlap. Com-B27 has a significantly higher cross-reaction to HLA-B7 antigen than FD705 (55 ± 16 vs. 16 ± 17 MC, Mean \pm 1SD, n=7, $P < 0.001$) but less than the low range of B27⁺ samples. FD705 has higher cross-reaction to HLA-B44 antigen than Com-B27 (51 ± 31 vs. 28 ± 15 MC, n=10, $P > 0.05$) and one false negative test to a B27⁺ patient whose HLA status was confirmed by family study. The lower variability of Com-B27 binding to B27 antigen than FD705 may reduce the number of costly confirmation test by microcytotoxicity. In addition, Com-B27 may increase the sensitivity of the flow cytometric B27 typing.

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A QUALITY CONTROL TOOL FOR SINGLE-PLATFORM ABSOLUTE COUNTING TECHNOLOGY: USING BEAD FLOW RATE AS PIPETTING PRECISION VERIFYER.

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The single-platform technology (SPT) is now the preferred method for enumeration of absolute CD4 T-cells. Both accuracy and precision of the absolute counts are dependent on the quality of pipetting of blood and the calibrator beads (one exception, TruCOUNT TM tubes, BDBiosciences). This study was designed to use the bead calibrator flow rate, dependant on pipetting, as a built-in internal quality control tool.

Method: Twelve specimens from HIV positive individuals were stained with the four-color monoclonal antibody tetraCHROME TM (CD45 FITC/ CD4 RD1/CD8 ECD/ CD3PC5) using the ImmunoPrep lysing agent from Beckman Coulter. Flow-CountTM beads, the calibrators, were added to each preparation prior to analysis. The preparations were analyzed on an Epics XL (Beckman Coulter). Total CD4 T-cells, bead count and time were monitored as follows: (1) One standard preparation with 100 μ L of Flow-Count beads was analyzed 5 consecutive times to establish the bead flow rate variation inherent to the instrument. (2) A sample with approximately 200 CD4 T-cells/ μ L was prepared with five different amount of beads (50 μ L, 60 μ L 70 μ L, 80 μ L, 90 μ L) and analyzed parallel with 11 normally prepared specimens (100 μ L beads) to

establish the effect of pipetting error on the bead flow rate. The median bead flow rate, SD's and CV's were calculated for all specimens.

(1) The instrument bead flow rate precision was 5 %CV at 100 μ L. (2) The bead flow rate variation with five different bead preparations gave CV's of 12 %, 10 %, 8 %, 6 % and 5% respectively for 50, 60, 70, 80, and 90 μ L.

Using 2 standard deviation limit to define outlier values, preparations with 70, 60 and 50 μ L of beads were identified as outliers. In this study, it was demonstrated that bead flow rate can be used to identify significant pipetting errors.

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EVALUATION OF TRANSFIX®, A COMMERCIAL WHOLE BLOOD STABILIZING REAGENT. THIS PRODUCT REDUCES HIV REPLICATION

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TransFix is a reagent designed by UK NEQAS to stabilize whole blood specimens for leucocyte Immunophenotyping. TransFix stabilized whole blood preparations were assessed as candidate product for external quality assessment programs (QAP). Samples treated with TransFix retained morphology and cell surface expression over time. Shipping and handling HIV+ samples involves inherent elevated cost and a risk of accidental exposure to Human Immunodeficiency Virus-1 (HIV). Does TransFix have a capacity to reduce infectivity of HIV positive blood preparations and thus minimize the risk of exposure to HIV?

To study the effect of TransFix on reduction of HIV replication.

CEM 13D cells (15x10⁶cells) were infected with various dilutions of subtype B HIV-1(10⁻¹,10⁻⁵,10⁻¹⁰). The cells were incubated until cytopathic effect (CPE) was observed (~ three days). Cultures were then treated with diluted TransFix (1/10) and incubated for 7 days. Cell-free supernatants were harvested every 24 hours and tested by p24 AG assay (Beckman Coulter).

At 10⁻⁵ HIV dilution, the results obtained within 24 hours of treatment with TransFix showed a 1-log reduction in HIV replication as measured by HIV p24 Ag production.

The results indicate that TransFix has the ability to cause significant reduction of HIV replication.

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TARGETED IMMUNOTHERAPY FOR ACUTE LYMPHOBLASTIC LEUKEMIA

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Significant advances have been made in the treatment of acute lymphoblastic leukemia (ALL) over the past twenty years. However, patients who fail standard treatment protocols do not usually have a successful outcome, even with allogeneic bone marrow transplantation (BMT) (1) and cellular immunotherapy (2), largely because immunotherapy protocols currently in use are not specific for ALL. Although leukemia-reactive T cells have been isolated and leukemia-associated antigens have been identified, the isolation of ALL-specific T cells that offer the potential of widespread therapeutic use has been generally unsuccessful.

The presence of increased $\gamma\delta$ T cells in relapse-free survivors of BMT was first noticed in our laboratory (3). Eight of ten (80%) patients who developed a spontaneous increase in $\gamma\delta$ T cells during the first year following BMT remain alive and free of disease for up to seven years, as compared to a disease-free survival (DFS) probability of 31% patients with a normal number of $\gamma\delta$ T cells ($p = 0.009$). No other factor was found to be independently associated with improved DFS in these patients (4), and the two patients who relapsed did so only after the $\gamma\delta$ T cell count returned to the normal range. Further studies have shown that enrichment of the marrow graft with $\gamma\delta$ T cells contributed to the later development of increased $\gamma\delta$ T cells (5). The $\gamma\delta$ T cells from these patients are predominately V δ 1+ "normally" recovering patients and healthy donors show predominant expression of V δ 2.

We also have generated $\gamma\delta$ T cells *in vitro* identical those seen in the patients described above. The cultures expand rapidly in the presence of primary ALL and are almost exclusively V δ 1+ cells that express activation antigens CD25, HLA-DR, and CD69. These cells are also cytotoxic to primary ALL, lymphoid cell lines, and to K562 cells but are not cytotoxic to myeloid cell lines or third party AML. This effect is not seen with AML or biphenotypic acute leukemia suggesting that it may be restricted to lymphoid leukemias (5). The *in vitro* allogeneic effect of $\gamma\delta$ T cells is minimal (5-6) and they do not initiate graft-versus-host disease (7-10). Finally, recent work from our laboratory shows that activation of these leukemia-specific $\gamma\delta$ T cells occurs through the T cell receptor, although the costimulatory pathway remains unclear at this time. Killing of ALL is likely via perforin and granzyme. These data strongly suggest a distinct role for $\gamma\delta$ T cells against ALL independent of an allogeneic effect, and the direct recognition of a protein surface antigen on ALL by the $\gamma\delta$ T cell. Isolation and purification of this antigen may subsequently lead to production of mAbs directed against the antigen which can then be developed as specific pharmaceutical therapy for ALL. This treatment may have less systemic

toxicity than conventional chemotherapy and would likely be more widely applicable than cellular therapy.

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A CASE OF TWO SYNCHRONOUS LYMPHOID NEOPLASMS: ONE WITH FEATURES OF CLL, AND THE OTHER WITH FEATURES OF B-CELL LYMPHOMA

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A case of two synchronous B-lymphoid neoplasms is presented, detected by three colour flow cytometry in a bone marrow sample from a 79 year old woman suspected of having chronic lymphocytic leukaemia (CLL). A population comprising 53% of the lymphocytes co-expressed CD5/CD19, dim CD20 and CD23/sIg (dim) with lambda light chain restriction. The findings in this population were diagnostic of CLL. A second population comprising 22% of the lymphocytes expressed CD19, CD20 (moderate), CD22, CD79b and sIg (moderate) with kappa light chain restriction, but was negative for CD5. Both populations were negative for CD10, CD11c, CD25, CD103 and FMC7. Morphologically the populations could not be distinguished in either the aspirate or trephine.

Expression of sIg and lambda/CD19 light chain restriction in the CLL population was masked by the kappa/CD19 clonal expression in the second population. It was only when kappa and lambda antibodies were coupled with CD23 that the lambda light chain restriction was demonstrated.

The clinical behaviour of the two clones cannot be predicted, and follow-up of the patient is proceeding.

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DISTRIBUTION OF 90 BI- AND TRICLONAL LEUKEMIAS AND LYMPHOMAS WITH FLOW CYTOMETRIC ANALYSES.

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The coexistence of more than one abnormal lineage is well known to cytogenetics and is increasingly apparent in FCM studies with the use of expanded antibody repertoires and increased numbers of analytic parameters. The purpose of this study is to review the incidence and composition of multiclonal detection, and whether this holds informative consequences for analysis. 90 patients with two or three abnormal populations were evaluated. When age was known, nearly all were 70s or 80s. The majority of cases were detected at initial presentations, including three triclinal samples. 100% of samples included at least one mature B cell type. CD5+ B cells were most common (51 patients, 59%). 56 patients had two B cell clones, including - double CLL/SLL types: 18 (21%); B with immature B-ALL: 2; B with T: 4; B with NK: 1; B with AML: 14; B with MDS: 12. 3 triple clones were: 1) B,K+/cyL+ plasma cells/ MDS; 2) CLL K+/CLL L+/B, CD5-,K+; and 3) B,10+,K+/B,10+,L+/B,10+,Ig-. AML coincidence with CD5- B cell clones was significantly greater than with CD5+ B cell clones ($p=0.006$); this association was not seen with MDS. Detection of clonal K and L light chains, either simultaneously within one sample or for two sites, or at sequential collection times is not unexpected. Small initial panel size may fail to identify a clonal population of significance. Panel reduction for follow up of previous diagnoses in some patient groups can miss the emergence of a second population.

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A FLOW CYTOMETRIC ASSAY FOR THE QUANTITATION OF ACTIVATION MARKERS CD11a, CD11b, CD62L, CD64, CD66b, and HLA-DR

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A flow cytometric assay to assess inflammation in vivo and cell responses ex vivo in peripheral blood neutrophils and monocytes was validated. After lysing the red cells in heparinized whole blood the expression of leukocyte function antigen-1 (LFA-1, CD11a), Mac-1 (CD11b), L-selectin (CD62L), IgG Fc receptor I (CD64), CD66b and HLA-DR were evaluated. Each assay included anti-CD45-PerCP and anti-CD14-APC for leukocyte discrimination in addition to FITC- and PE-conjugated antibodies for respective activation markers. QuantiBRITE PE-conjugated calibration beads were used to quantify PE-fluorescence intensity for CD11b, CD64 and HLA-DR expressed as antibodies bound per cell (ABC). FITC-conjugated antibodies CD11a, CD62L and CD66b data were expressed in mean fluorescence intensity units. As an in vitro model of inflammation, heparinized whole blood was stimulated ex vivo with 10 ug/mL of lipopolysaccharide. CD62 was lost on the activated neutrophils, whereas, CD64, CD66b and CD11b were upregulated. On activated monocytes, CD62L was down modulated, while CD66b, CD11b, CD11a and HLA-DR increased. Precision data were generated for both unstimulated whole blood and LPS-stimulated whole blood. Intra-assay coefficients of variation (CVs) were typically less than 6%. Inter-assay CVs of antigen expression were typically less than 8%. Samples were unstable when held at 4 °C or RT for 3 h prior to processing. However, the markers are stable on cells frozen after the lyse step. This six-biomarker assay may be useful in clinical trials for anti-inflammatory, immunosuppressive, and anti-infective compounds.