24th Autometry Meeting and Course

Florida, USA

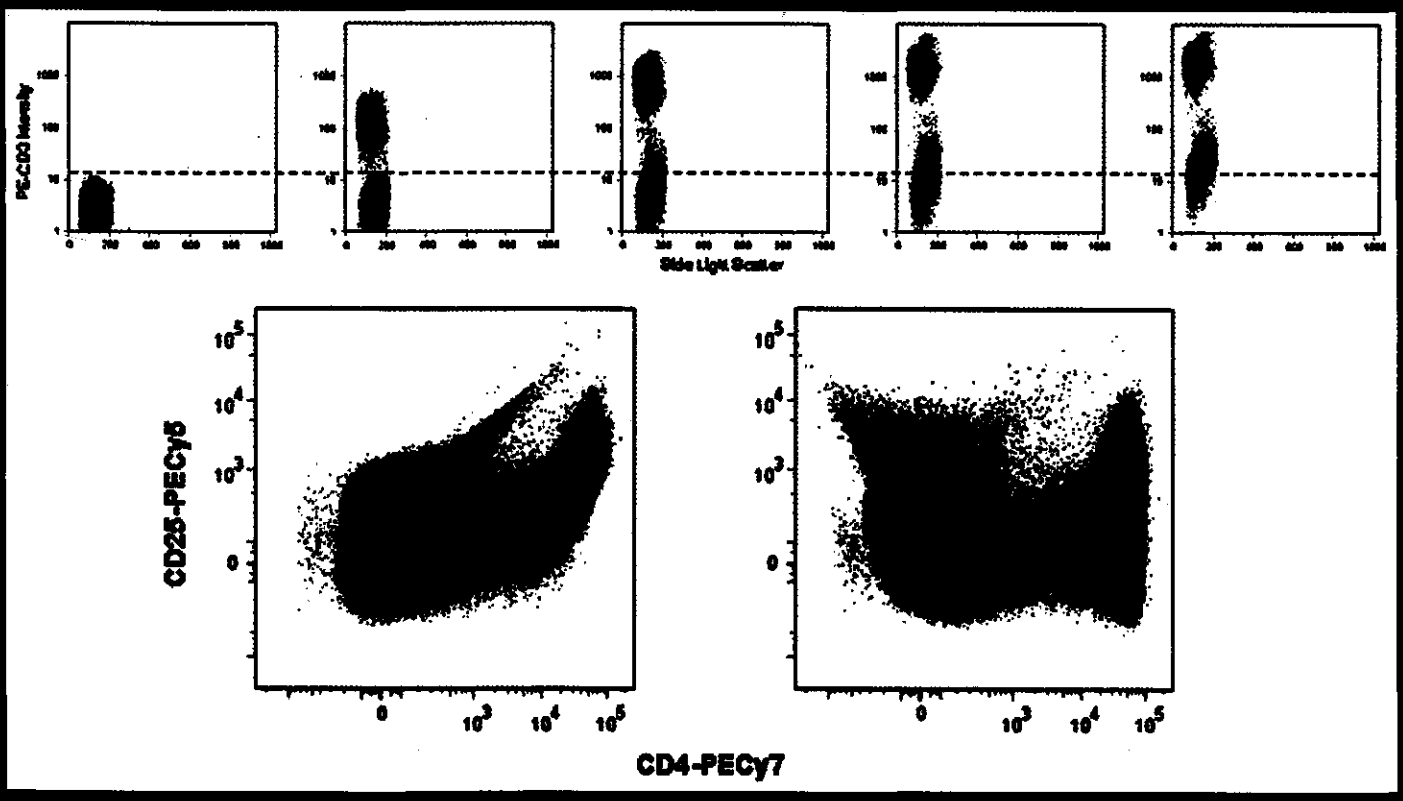
16 – 21 Oktober 2009

Prof. Madya Dr. Rosline Hassan
Jabatan Hematologi
Pusat Pengajian Sains Perubatan

THE OFFICIAL JOURNAL OF THE CLINICAL CYTOMETRY SOCIETY

PART

CLINICAL CYTOMETRY



PUBLISHED IN AFFILIATION WITH
THE EUROPEAN SOCIETY FOR CLINICAL CELL ANALYSIS

WILEY-BLACKWELL

ISSN 1552-4949

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CD123 IS USEFUL FOR IMMUNOPHENOTYPING CLASSICAL HODGKIN LYMPHOMA IN LYMPH NODES BY FLOW CYTOMETRY

Jonathan R. Fromm and Anju Thomas
University of Washington, Seattle, WA, USA

While the diagnosis of classical Hodgkin lymphoma (CHL) is still made primarily in tissue sections, CHL can now also be immunophenotyped and diagnosed by flow ctyometry (FC). In an effort to identify additional antigens on Hodgkin and Reed-Sternberg (HRS) cells that are useful for immunophenotyping this lymphoma by FC, we examined the expression of CD123 (α chain of the IL-3R) on HRS cells by FC. 372 tissue specimens (98 reactive, 274 neoplastic) were immunophenotyped by a 9-color FC tube containing CD123, to evaluate CD123 expression on reactive, non-Hodgkin lymphoma (NHL), and HRS cell populations. CD123 was expressed on the majority of HRS cell populations (59% of 59 CHL lymph nodes examined), rarely on B-cell NHL (3 of 3 hairy cell leukemias cases, 3 of 29 CLL/SLL cases, 1 of 55 large B-cell lymphoma cases, but not in the 95 other B cell NHL examined), and not on any of 16 T-cell lymphoma cases examined. CD123 expression in reactive tissues was largely limited to plasmacytoid dendritic cell populations and rare histocyte populations that can readily be distinguished from HRS populations. As CD123-positive populations are relatively rare in B- and T-NHL, CD123 is useful in immunophenotyping CHL in lymph nodes.

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A RESEARCH FLOW CYTOMETRIC BEAD ASSAY FOR THE DETECTION OF PML-RARα FUSION PROTEIN IN ACUTE MYELOID LEUKEMIA

Deborah Greenberg,[1] Eric Hsi,[2] Eric Dixon,[3] Yen Ping Liu,[4] Xiao-Yuan Liu,[1] Johanna Reneke,[1] Menilou Franzblau,[4] Rudi Varro,[4] and Charlene Bush-Donovan[4]
[1]*Kaiser Permanente TPMG Regional Laboratory, Berkeley, CA, USA*
[2]*Cleveland Clinic, Cleveland, OH, USA*
[3]*BD Technologies, Research Triangle Park, NC, USA*
[4]*BD Biosciences, San Jose, CA, USA*

Acute promyelocytic leukemia (APL or AML-M3) is one of the most lethal forms of acute myeloid leukemia when not promptly diagnosed and treated. APL is characterized by a t(15;17)(q22;q21) translocation, which fuses the *RARα* gene on chromosome 17 with the *PML* gene on chromosome 15. The fusion protein arrests the maturation of myeloid cells at the promyelocytic stage and is responsible for pathogenesis. Treatment with all-trans-retinoic acid activates the retinoid receptor RAR, which allows the promyelocytes to differentiate and mature. Currently, a diagnosis of APL is based on karyotyping, FISH, or RT-PCR, delaying molecular confirmation by at least 1 to 2 days, even when a molecular diagnostics laboratory is on site. To aid in the rapid detection of the PML-RARα fusion protein, a novel research-based flow cytometric bead assay (BD™ CBA, BD Biosciences) was developed. It employs an anti-RARα antibody capture bead and a PE-conjugated anti-PML detector. The assay detects the presence of the fusion protein within 4 to 5 hours in cell lysates from blood or bone marrow. The assay detects less than 10% of the APL cell line NB4 in a background of normal leukocytes. Preliminary study has shown 100% concordance (9/9) with FISH or cytogenetics in samples from newly diagnosed APL patients. This novel bead assay has the potential for fast and easy detection of PML-RARα fusion protein in APL samples and can easily be performed in a standard flow laboratory. Future studies are needed to demonstrate the full clinical utility of this assay.

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FLOW CYTOMETRY PROLIFERATION RATE STUDIES IN HAIRY CELL LEUKEMIA AND HAIRY CELL LEUKEMIA VARIANT USING THE DNA STAIN DRAQ5

Marlene Grönborg,[1] Robert J. Kreitman,[2] Maryalice Stetler-Stevenson,[1] and Constance Yuan[1]
[1]*Laboratory of Pathology/Department of Flow Cytometry, National Institutes of Health/National Cancer Institute, Bethesda, MD, USA*
[2]*Laboratories of Molecular Biology and Clinical Pathology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD.*

Hairy Cell Leukemia (HCL) and Hairy Cell Leukemia variant (HCLv) are two rare, chronic B-lymphoproliferative disorders. While HCL is highly treatable with the purine analogs cladribine and pentostatin, HCLv has a more rapid disease progression, shorter median survival, and is often unresponsive to standard treatments. Accurate diagnosis is necessary to identify patients who will not benefit from purine analog therapy to avoid unnecessary toxicicty. HCL and HCLv both express CD19, CD20 (bright), CD22 (bright), CD11c (bright), FMC7, CD79b, CD45 and monoclonal light chains, and are negative for CD5, CD10, CD23 and CD38. They differ in the expression of CD25, CD123 and sometimes CD103 but can still be difficult to distinguish. We retrospectively examined data from 103 HCL and 18 HCLv cases received at NIH. White blood cell (WBC) count comparisons between HCL and HCLv produced a mean of 8.1 K/uL (range: 0.3-142.0) vs. 69.8 K/uL (range: 2.0-839.2), respectively (P= 0.0018). Based on this, we hypothesized that HCLv cells would have a higher S-phase. Proliferation rate studies were conducted using the novel DNA dye DRAQ5, which enters the cell without fixation or membrane permeabilization while preserving light scatter and surface antigens. Initial data from 15 HCL and 11 HCLv specimens gave S-phase means of 0.54% (range: 0.00-2.07) and 0.31% (range: 0.00-0.99), respectively (P= 0.2597). These results do not suggest a higher S-phase for HCLv. Possibly, the higher WBC counts in HCLv cases may be due to an escape of apoptosis rather than an increased cell proliferation rate.

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EVIDENCE OF PLATELET ACTIVATION BY FLOWCYTOMETRY FOLLOWING HORMONE REPLACEMENT THERAPY IN POST MENOPAUSAL WOMEN

Rosline Hassan, Shah Reza Johan Noor, Rapiaah Mustaffa, Shabbir Ahmad Sheikh, Noor Adzha Abd Majid, and Tariq Mahmood Roshan
Universiti Sains Malaysia, Kelantan, Malaysia

Platelet activation is one of the underlying mechanisms causing thrombosis. However women are generally protected from thrombotic events before menopause. Previous study showed that there was an evidence of platelet activation in post menopausal women and it was related to the level of serum estradiol yet remain questionable. Many immunoassays by ELISA were used to measure platelet activation. Thus our aim was to study the platelet activation by flow cytometry on post menopausal women receiving hormone replacement therapy.

Methodology: Total of 48 volunteers postmenopausal women were recruited from gynaecology clinic. All women were later given HRT (Primarine or Progestigen) for 2 weeks. 10 mls of blood pre and post HRT was collected in 3.2% sodium citrate bottle. Platelet activation was measured by flow cytometric analysis using CD62P and PAC-1 FITC as monoclonal markers.

Results: CD 62P and PAC-1 FITC expression markers in post treatment with HRT show dramatically decreased compare to pre treatment level. CD62P were reduced significantly from 8.51 ± 12.56% to 3.15 ± 6.64% and PAC-1 FITC from 41.75 ± 26.85% to 20.86 ± 19.02% after two weeks treatment ($p<0.05$).

Conclusion: CD62P and PAC-1 by flow cytometry are both useful markers to measure platelet activation. Short-term treatment with Primarine or combined HRT reduced the circulating activated platelets as measured by flow cytometry which indicates a reduced risk of thrombotic event. Further study is required on its long term effect.

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B-CELL LYMPHOPROLIFERATIVE DISORDERS IN PATIENTS WITH NK-CELL LYMPHOCYTOSIS

Matthew T. Howard,[1] Nelli Bejanyan,[2] Jaroslaw P. Maciejewski,[2] and Eric D. Hsi[1]

[1]Cleveland Clinic Department of Pathology and Laboratory Medicine, Cleveland, USA

[2]Cleveland Clinic Taussig Cancer Center, Cleveland, OH, USA

B-cell dyscrasias have been described in association with T-cell Large Granular Lymphocyte Leukemia. We examined flow cytometry and clinical data in 5 patients with NK Cell large granular lymphocytosis (NK-LGL) to find abnormal B-cell populations. 5 patients with NK-LGL were studied based on available 4-color or 6-color FC data. FC data files from tubes with antibodies to CD5/CD19/CD23/CD45 and CD19/CD45/k/l/CD5/CD20in 6 color) were reviewed retrospectively. The tube containing surface immunoglobulin (sIg) was acquired with a "live-gate" on CD19+ events, collecting 20,000 events or 0.25 ml of sample. Specimens were considered positive for a B-cell dyscrasia if one of the following criterion were met: 1)B-cells with a k:l > 3:1 or k:l <0.2; 2) > 25% of B-cells were sIg negative; 3) B-cells with a specific disease phenotype were found as a subset of a sIg polytypic B-cell population, though not in sufficient quantity to cause an abnormal overall k:l ratio. 3 of 5 patients were identified as having abnormal B-cell populations. Of these, one patient had a known history of γ-Waldenstrom Macroglobulinemia, one patient was subsequently diagnosed with γ-heavy chain disease, and one patient had no other findings of a B-cell lymphoproliferative disorder. NK-LGL may be commonly associated with B-cell lymphoproliferative disorders or monoclonal B-cell lymphocytosis and maybe under recognized. These concurrent disorders may reflect dysregulated immune response in these patients.

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A METHOD FOR ABSOLUTE QUANTIFICATION OF PLASMA CELLS BY FLOW CYTOMETRY USING A LYSE NO WASH PROCEDURE

Teresa K. Kimlinger, Tim M. Halling, S. Vincent Rajkumar, and Shaji K. Kumar

Mayo Clinic, Rochester, NY, USA

Background: In myeloma the tumor cell is the clonal plasma cell (PC). An accurate method for PC quantitation is necessary to study MRD (minimal residual disease) in patients undergoing treatment and for detection of relapse. Current techniques for plasma cell quantitation include slide and molecular based assays; these can be difficult to interpret at MRD levels.

Methods: This study involved a 'Lyse no Wash' method using BD TruCount tubes with CD31PE, CD45Fitc, and CD38APC. A PE threshold allowed for separation of WBC from noncellular events. Plasma cells were identified by CD38/45 staining patterns and bead events were gated on the FL3 parameter. Samples were collected for 5 minutes or 2 million events. CD138 isolated PC were spiked into blood to determine sensitivity. A sample was run multiple times to determine the reproducibility of the assay. Samples stored on the bench were processed over multiple days to determine stability.

Results: Spiking studies were linear from 1 PC to 500 PC ($R = 0.9835$). The standard deviation was under 10% in the reproducibility studies. The standard deviation for the sample stability was <25% from day 0 to day 6 on a 0% sample.

Conclusion: This 'lyse no wash' technique requires little manipulation of the sample, reducing processing time. The results are easily interpreted and reproducible. Flow cytometry allows for the analysis of high numbers of cells required for MRD and the quantitative results obtained using this assay allow for standardized monitoring of tumor cells in patients with myeloma and other PC disorders.

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STEM CELL MARKER EXPRESSION IN CELLS FROM BODY CAVITY FLUIDS

Awtar Krishan, Deepati Sharma, Siddharth Sharma, Ronald Hamelik, Parvin Ganjei-Azar, and Mehrdad Nadji

Pathology Department, University Of Miami Miller School Of Medicine, Miami, FL, USA

Reduction of Platelet Activation Measured by Flowcytometry Following Hormone Replacement Therapy in Post Menopausal Women

Rosline H[1], Shah Reza JP[2], N Adzna AM[2], Rapiaah M[1], Abdul Aziz B[2], Shankar AS[2], W Sulany WMZ[1], Tariq M. Roshan.[1]

[1]Department of Haematology, [2]Department of Medicine, [3]Department of Obstetrics & Gynaecology, School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, Kelantan, Malaysia

ABSTRACT

Platelet activation is one of the underlying mechanisms causing thrombosis. However women are generally protected from thrombotic events before menopause. Previous study showed that there was an evidence of platelet activation in post menopausal women and it was related to the level of serum estradiol yet remain questionable. Many immunoassays by ELISA were used to measure platelet activation. Thus our aim was to study the platelet activation by flow cytometry on post menopausal women receiving hormone replacement therapy. **Methodology:** Total of 48 volunteers postmenopausal women were recruited from gynaecology clinic. All women were later given HRT (Primarine or Progestogen) for 2 weeks. 10mls of blood pre and post HRT was collected in 3.2% sodium citrate bottle. Platelet activation was measured by flow cytometric analysis using CD62P and PAC-1 FITC as monoclonal markers. **Results:** CD 62P and PAC-1 FITC expression markers in post treatment with HRT show dramatically decreased compare to pre treatment level. CD62P were reduced significantly from 7.0±5.9% to 3.05 ± 2.47% and PAC-1 FITC from 41.75 ± 26.85% to 20.88 ± 19.02% after two weeks treatment ($p<0.001$). **Conclusion:** CD62P and PAC-1 by flow cytometry are both useful markers to measure platelet activation. Short-term treatment with Primarine or combined HRT reduced the circulating activated platelets as measured by flow cytometry which indicates a reduced risk of thrombotic event. Further study is required on its long term effect

INTRODUCTION

Platelet activations play a major role causing thrombosis which resulted in cardiovascular disease (1). However women are generally protected from cardiovascular disease before menopause compared to their male counterparts. Platelet hyperactivity have been reported to be associated with many common clinical conditions including unstable angina, acute myocardial infarction, stroke and other thrombotic states. Thromboxane which is largely a product of activated platelets is increased in post menopausal women but in recent study shows that thromboxane level decrease with the commercially hormone replacement therapy. Previous study showed that there was an evidence of platelet activation in healthy post menopausal women who were not on hormone replacement therapy and it was related to the level of serum estradiol in these patients(1). Thus our aim was to study the platelet activation on post menopausal women on hormone replacement therapy.

METHODOLOGY

Total of 48 volunteers postmenopausal women were recruited from gynaecology clinic after providing written informed consent. All exclusion criteria were done during patient selection session. Critical procedures to avoid platelet activation were applied to avoid any in-vitro activation. No cuff or tourniquet was used during blood withdrawing. All women received HRT for 2 weeks on Primarine or and Progestogen. 10mls of blood pre and post HRT was collected in 3.2% sodium citrate bottle. Platelet activation was measured by standardized 3-colours analysis flow cytometric analysis using CD62 PE (P-selectin) and PAC-1 FITC as monoclonal markers.

OBJECTIVE

To determine the effect of HRT on the platelet activation markers (CD62p PE & PAC-1 FITC) by flow cytometry in healthy post-menopausal women

RESULTS

Table 1

	Mean	Std. Deviation
Age	53.8958	5.57059
BMI (kg/m²)	27.1915	4.11479
Hb	12.8313	0.94580
WBC	6.2469	1.24033
Platelet	242.46	51.679
Triglycerides	1.4488	0.70505
Cholesterol	5.9021	0.92270
Fasting Blood Sugar	4.9313	0.69470
Estradiol	57.4021	37.57840

Figure 1

Platelet Activation Markers in Pre & Post Hormone Replacement Therapy (N=48)

Platelet activation markers	Pre-tx (SD)	Post-tx (SD)	Mean diff (95%CI)	t-statistic (df)	p-value
CD62P	7.00% (5.91)	3.05% (2.47)	3.94 (2.59-5.30)	5.85 (47)	<0.001
PAC1	41.75% (26.85)	20.88% (19.02)	20.89 (14.78-26.10)	6.88 (47)	<0.001

Figure 2

Correlation Between Platelet Activation Markers & Serum Estradiol, BMI And Age. (N=48)

Platelet activation markers	S.Estradiol	BMI	Age
CD62P	-0.34*(0.02)*	0.23 (0.12)	0.13 (0.39)
PAC1	-0.29 (0.04)	0.08 (0.58)	0.12 (0.41)

* Pearson correlation coefficient
* p-value

DISCUSSION

1. Demographic data of post menopausal women are shown in Table 1
2. CD 62P PE and PAC-1 FITC expression markers in post treatment with HRT show dramatically decreased compare to pre treatment level. (Figure 1)
3. CD62P PE and PAC-1 FITC were reduced significantly from 7.0±5.91% to 3.05 ± 2.47% and 41.75 ± 26.85% to 20.88 ± 19.02% after two weeks treatment ($p<0.001$) respectively (Figure 1)
4. From previous study thromboxane levels decreased after one year treatment of HRT which indicates reduction in platelet activity. Thromboxane is a product of activated platelets which causes platelet aggregation and vasoconstriction and it is used as an index of platelet activation (2)
5. Serum estradiol was noted to be high in these patients after HRT treatment thus causing an increase in nitric oxide and PGI_2 and subsequently reduce in thromboxane. This event might contribute to the underlying pathophysiology of a decrease in the activated platelets among post menopausal women on HRT.
6. There is a significant negative fair correlation between estradiol and platelet activation markers (CD62P & PAC-1) however, there was no significant relation among BMI, age and platelet activation markers.(Figure2)
7. Our study did support the recent evidence that there is a dose-dependant relationship between estrogen and thrombogenesis.
8. The lowest effective dose of estrogen does not increase the relative risk of thrombogenesis and cardiovascular events during short period of HRT.

CONCLUSION

Short-term treatment with estradiol or combined HRT decreases the amount of circulating activated platelets as measured by flow cytometry. Thus we conclude that CD62P and PAC-1 is a useful marker for platelet activation

ACKNOWLEDGEMENT

Deepest gratitude to all project's members and person that involve in this research with the support and their kindness during learning and guiding.

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