UNIVERSITI PUTRA MALAYSIA

CHARACTERISATION OF MYELOID, LYMPHOID AND ERYTHROID CELL LINEAGES IN MYELODYSPALSTIC SYNDROME USING FLOW CYTOMETRY

MOHADESE HASHEM BROOJERDI

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By

MOHADESE HASHEM BROOJERDI

Thesis submitted to the School of Graduate Studies of University Putra Malaysia in the fulfillment of the requirements for the Degree of Master of Science

September 2011

DEDICATION

This thesis is dedicated to

My dearest Parents, Brother and Sister

The understanding and encouragement they provided during all the these years of the study

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Chairman: **Zainina Binti Seman, PhD**

Faculty: **Faculty of Medicine and Health Sciences**

Introduction: Myelodysplastic syndromes (MDSs) are a group of disorders characterised by ineffective haematopoiesis, leading to dysplasia in one, two or all three cell lineages. Although morphological diagnosis is a traditional and the conventional method, method for the diagnosis of MDS, some patients show blood and bone marrow (BM) characteristics that make MDS diagnosis difficult. Flow cytometric immunophenotyping is an accurate and faster method for the detection of abnormalities in different cell lineages. **Objective:** To determine the expression pattern of antigens in myelomonocytic, lymphoid and erythroid lineages in non-MDS (control group) and MDS, to compare the expression patterns of antigens in myelomonocytic, lymphoid and erythroid lineages in MDS and non-MDS cases, to compare the expression patterns of

antigens in myelomonocytic, lymphoid and erythroid lineages in MDS subtypes, and to compare full blood count parameters in MDS and control group in addition between different MDS subtypes. **Methods:** 30 BM samples from newly-diagnosed MDS patients were analysed by 4-colour FACS Canto flow cytometer (Becton Dickinson, USA) to investigate the antigen expression patterns in granulocytic, monocytic, erythroid and lymphoid lineages and myeloid precursors. The results were compared with those obtained from patients with idiopathic thrombocytopaenic purpura (ITP) (30 samples) as a control. Gating on CD45/SSC (Side Scatter) plot was used for choosing the population of interest. A descriptive analysis was done for all variables studied. Student's t-test was used for statistical analysis of differences between the MDS and control groups. The one-way ANOVA was used to test of differences between mean percentages of antigens in MDS subtypes. A p-value of ≤ 0.05 was considered as statistically significant. **Result:** Between full blood count parameters, Hb, Hct, RBC count, WBC count and the ANC were significantly lower in MDS cases. The mean ranges of MCV and platelet count were higher in MDS patients. Between MDS subtypes only the difference of Hct, RBC count and Plt count was statistically significant. The mean percentages of CD33, CD13, CD11b, HLA-DR, CD10 and CD34 positive granulocytes were 91%, 84.98%, 77.20%, 14.59%, 40.34% and 34.25%, respectively, in MDS and 96.89%, 91.57%, 81.47%, 10.56%, 58.30% and 32.37%, respectively, in non-MDS cases. The mean percentage of CD71 (64.54%) was lower in the MDS subtype than non-MDS (83%). In addition, $CD235a^+$ and $CD71^+/CD235a^+$ erythroid precursors showed lower mean values of 35.96% and 6.61%, respectively, in MDS subtypes, as compared to 52.83% and 10.48%, respectively, in non-MDS cases. The mean proportions of $CD14^+$, $CD33^+$, $CD13^+$, $CD34^+$ and HLA-DR^+ monocytes were lower in MDS as compared to non-MDS with values of 65.89%, 79.92%, 74.04%, 44.43%, 36.25% and 73.36%, 86.57%, 87.74%,

45.30%, 38.86%, respectively. Investigation of antigen expression in the myeloid precursors of MDS patients showed mean proportions of: CD117 (19.89%), CD34 (59.53%), HLA-DR (57.26%), CD33 (69.24%), CD13 (60.64%) and CD11b (23.43%). In non-MDS cases the mean percentages of CD117 (11.73%), CD34 (45.67%), HLA-DR (58.90%), CD33 (74.28%), CD13 (70.16%) and CD11b (15.66%) were detected. There was no significant difference in lymphoid antigen expression among MDS and non-MDS cases. Between MDS subtypes only the expression pattern of CD71 on erythroid lineage was statistically significant. **Discussion:** Decrease of $CD10^+$ granulocytes was an important result in this study that had shown by others. Low percentage of $CD14^+$ monocytes and high percentage of $HLA\text{-}DR^+/CD11b^+$ myeloid precursors were another findings of this study that confirmed immaturity of cells in MDS cases. Erythroid lineage was found by low expression of $CD235a^+/CD71^+$, decrease of CD71 and CD235a expression. All results showed cells in MDS patients had lower maturity as compare to cells in non-MDS cases. In addition, our results support the idea that maturity of cells in RAEB subtype is lower than other MDS subtypes. **Conclusions**: In conclusion, flow cytometric immunophenotyping is useful for confirming cases in which is difficult to determine by morphology, even though the current morphological diagnostic methods are enough to diagnose straightforward MDS cases and are cheaper and more accessible.

Key words: Myelodysplastic Syndromes, Flow cytometry, Immunophenotyping;

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

KAREKTERISASI MIELOID, LIMFOID DAN ERITROID KETURUNAN SEL DALAM SINDROM MIELODISPLASIA MENGGUNAKAN SITOMETRI ALIRAN

Oleh

MOHADESE HASHEM BROOJERDI

September 2011

Pengerusi: Zainina Binti Seman, PhD

Fakulti: Fakulti Perubatan dan Sains Kesihatan

Pengenalan: Sindrom mielodisplasia (MDS) merupakan satu kumpulan penyakit yang dikarekterisasikan oleh hematopoiesis yang tidak efektif, lalu membawa kepada displasia dalam satu, dua atau ketiga-tiga keturunan sel. Walaupun diagnosis morfologikal merupakan kaedah tradisional serta Kaedah konvensional untuk diagnosis MDS, beberapa pesakit menunjukkan ciri-ciri darah dan sumsum tulang yang menyukarkan diagnosis MDS. Keadah imunofenotip aliran sitometri adalah kaedah yang tepat dan lebih cepat untuk pengesanan keluarbiasaan dalam keturunan sel berlainan. **Objektif:** Untuk menentukan corak ekspresi antigen-antigen dalam keturunan myelomonositik, limfoid dan eritrosit dalam kes-kes bukan-MDS (kumpulan kawalan) dan MDS, untuk membandingkan corak ekspresi antigen-antigen dalam keturunan myelomonositik, limfoid dan eritrosit dalam kes-kes bukan-MDS (kumpulan kawalan) dan MDS, untuk membandingkan corak ekspresi antigen-antigen dalam keturunan myelomonositik, limfoid

dan eritrosit dalam subjenis-subjenis MDS, dan untuk membandingkan parameter-parameter kiraan darah penuh MDS dan kumpulan kawalan serta antara subjenis-subjenis MDS. **Kaedah:** 30 sampel BM daripada para pesakit MDS yang baru didiagnos dianalisis oleh pengalir sitometer FACS Canto 4-warna (Becton Dickinson, USA) untuk menyiasat corak ekspresi antigen dalam keturunan-keturunan granulositik, monositik, eritrosit dan limfoid serta prekursor myeloid. Keputusan ini telah dibandingkan dengan keputusan yang telah diperolehi daripada para pesakit purpura trombositopenia idiopatik (ITP) (sebanyak 30 sampel) sebagai kawalan. Pemagaran atas plot CD45/SSC (serak tepian) telah digunakan untuk memilih populasi yang diingini. Analisis deskriptif telah dilakukan untuk kesemua pembolehubah yang dikaji. Student's t-test telah digunakan untuk analisis statistik perbezaan antara kumpulan MDS dan kawalan. satu-aliran ANOVA digunakan untuk menguji perbezaan antara peratusan antigen dalam subjenis MDS. Nilai-$p \leq 0.05$ dianggap sebagai signifikan secara statistik. Penilaian kehilangan antigenik atau aberasi telah dilakukan dengan membandingkan fluorescen purata populasi berpagar dengan kawalan. **Keputusan:** Antara parameter-parameter kiraan darah penuh, Hb, Hct, kiraan RBC, kiraan WBC dan ANC adalah lebih rendah dalam kes-kes MDS. Julat purata MCV dan kiraan platelet lebih tinggi dalam pesakit MDS. Antara subjenis-subjenis MDS, hanya perbezaan antara Hct, kiraan RBC dan kiraan Plt adalah signifikan secara statistic. Peratusan purata granulosit yang positif untuk CD33, CD13, CD11b, HLA-DR, CD10 and CD34 masing-masing adalah 91%, 84.98%, 77.20%, 14.59%, 40.34% dan 34.25% dalam kes-kes MDS, serta 96.89%, 91.57%, 81.47%, 10.56%, 58.30% dan 32.37% dalam kes-kes bukan MDS. Peratusan purata CD71 (64.54%) lebih rendah dalam subjenis MDS berbanding dengan bukan MDS (83%). Tambahan pula,precursor eritrosit $CD235a^+$ dan $CD71^+/CD235a^+$ menunjukkan nilai purata yang lebih rendah dalam subjenis MDS (masing-masing 35.96% dan 6.61%) berbanding dengan kes-

kes bukan MDS (masing-masing 52.83% and 10.48%). Kadaran purata monosit $CD14^+$, $CD33^+$, $CD13^+$, $CD34^+$ dan HLA-DR^+ lebih rendah dalam kes-kes MDS (masing-masing 65.89%, 79.92%, 74.04%,44.43% dan 35.25%) berbanding dengan kes-kes bukan MDS (masing-masing 73.36%, 86.57%, 87.74%, 45.30% dan 38.86%). Penyiasatan ekspresi antigen dalam precursor mieloid pesakit-pesakit MDS menunjukkan kadaran purata: CD117 (19.89%), CD34 (59.53%), HLA-DR (57.26%), CD33 (69.24%), CD13 (60.64%) dan CD11b (23.43%). Dalam kes-kes bukan MDS, peratusan purata CD117 (11.73%), CD34 (45.67%), HLA-DR (58.90%), CD33 (74.28%), CD13 (70.16%) dan CD11b (15.66%) telah dikesan. Tiada perbezaan signifikan dalam ekspresi antigen limfoid antara kes-kes MDS dan bukan MDS. Antara subjenis-subjenus MDS, hanya corak ekspresi CD71 pada keturunan eritrosit adalah signifikan secara statistic. **Perbincangan:** Pengurangan granulosit $CD10^+$ merupakan keputusan penting dalam kajian ini yang telah ditunjukkan oleh penyiasat lain. Peratusan monosit $CD14^+$ yang rendah dan peratusan precursor mieloid HLA-DR^+/$CD11b^+$ yang tinggi merupakan penemuan lain kajian ini yang mengesahkan ketidakmatangan sel dalam kes-kes MDS. Keturunan eritrosit telah dijumpai melalui ekspresi rendah $CD235a^+$/$CD71^+$, dan pengurangan ekspresi CD71 dan CD235a. Semua keputusan menunjukkan bahawa sel-sel dalam para pesakit MDS mempunyai kematangan yang lebih rendah berbanding dengan sel-sel dalan kes-kes bukan-MDS. Dengan itu, keputusan kami menyokong ide bahawa kematangan sel-sel dalam subjenis RAEB adalah lebih rendah berbanding subjenis-subjenus MDS lain. **Kesimpulan**: Kesimpulannya, imunofenotip aliran sitometrik berguna untuk mengesahkan kes-kes yang sukar dipastikan melalui morfologi, walaupun kaedah-kaedah diagnostik morfologi kini adalah mencukupi untuk mendiagnos kes-kes MDS jelas, lebih murah dan lebih mudah diperolehi.

Kata kunci: Sindrom Mielodisplasia, Sitometri Aliran, Kaedah Imunofenotip;

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I certify that a Thesis Examination Committee has met on 27/9/2011 to conduct the final examination of **Mohadese Hashem Broojerdi** on his thesis entitled "**Characterisation of myeloid, lymphoid and erythroid cell lineages in myelodysplastic syndrome (MDS) using flow cytometry**" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the University Putra Malaysia [P.U. (A)] 15 March 1998. The Committee recommends that the Student be awarded the Master of Haematology.

Members of the Thesis Examination Committee are as follows:

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfillment of the requirement for the degree of Master of Haematology. The members of the Supervisory Committee were as follows:

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Professor and Dean
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Date:

DECLARATION

I declare that the thesis is my original work except for quotations and citations, which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

MOHADESE HASHEM BROOJERDI

Date: 27 September 2011

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