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Range Determination of Antigen Expression in Myeloid, Erythroid and Lymphoid Cell Lineages among Patients with Myelodysplastic Syndrome

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Abstract

Background: Myelodysplastic syndrome is a mixed clonal disorder of bone marrow progenitor cells. Understanding the pattern of the different lineage-specific, immature, and mature markers in myelodysplastic syndrome will help in setting-up the frame of reference to diagnose.

Patients and Methods: We compared 60 bone marrow samples from 30 newly-diagnosed patients with myelodysplastic syndrome and 30 patients with idiopathic thrombocytopenic purpura as the control to perform a quantitative analysis of the antigen expression patterns in granulocytic, monocytic, erythroid and lymphoid lineages and myeloid precursors.

Results: Quantitative analysis of CD markers, showed that 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 myelodysplastic syndrome and 96.89%, 91.57%, 81.47%, 10.56%, 58.30% and 32.37%, respectively in idiopathic thrombocytopenic purpura. Flow cytometric analysis of erythroid lineage showed the mean percentage of CD71 in myelodysplastic syndrome and idiopathic thrombocytopenic purpura cases to be 64.54% and 83%, respectively. Investigation of antigen expression in the myeloid precursors of myelodysplastic syndrome patients showed the mean proportions of: 19.89%, 59.53%, 57.26%, 69.24%, 60.64% and 23.43% for CD117, CD34, HLA-DR, CD33, CD13 and CD11b, respectively. Also, idiopathic thrombocytopenic purpura cases showed the mean percentages of 11.73%, 45.67%, 58.90%, 74.28%, 70.16% and 15.66% for CD117, CD34, HLA-DR, CD33, CD13 and CD11b, respectively.

Conclusion: There is no doubt that providing the reference values for an antigen expression pattern among myelodysplastic syndrome cases enhances the utility of flow cytometric analysis interpretation among these patients.

Keywords: Myelodysplastic syndromes, flow cytometry, immunophenotyping, antigen expression.

Introduction

Myelodysplastic Syndrome (MDS) is a heterogeneous cluster of diseases characterized by ineffective haematopoiesis, which leads to the peripheral cytopenia of one, two or all three (myeloid, erythroid and megakaryocytic) lineages [1,2]. The clinical presentation of constant cytopenia supported with a morphological examination of the bone marrow (BM) is the conventional tool in the diagnosis of MDS. Because of certain limitations, the morphological findings in MDS patients are not always trustworthy [3]. Analysis of the cell lineages and expression pattern of antigens can provide a characteristic model of the disease. These findings lead to the more accurate diagnosis of this disorder as they also support the morphological diagnosis[4]. Furthermore, immunophenotypic analysis is easier and faster as compared to conventional MDS diagnostic methods [5].

Patients and Methods

Thirty patients with newly diagnosed MDS were examined in this study. Samples from patients with MDS were collected from February 2009 to November 2010 at Hospital Kuala Lumpur (HKL).

Table 1: Demographics of the MDS and control groups.

Patient Group	
MDS	30
Gender	20 Males and 10 Females
Median age	52 years old
Race	9 Malays and 21 Chinese
Control Group	
ITP	30
Gender	13 Males and 18 Females
Median age	40 years old
Race	12 Malays and 18 Chinese

This study was approved by the Faculty of Medicine and Health Sciences and performed at University Putra Malaysia (UPM). Information about the MDS and control groups is summarized in Table 1. The pattern of antibody we used in this study which is listed in Table 2 was based on van Lochem et al. suggestion [6]. The technique that was used for labeling the cells was according to Li et al. study [7]. More details have been described in our previous study [8]. For all variables in this study a descriptive analysis was performed. Differences between groups were tested by student t-test. For all statistical tests, statistical significance was defined by a p value of 0.05 or less [9]. Antigenic difference assessment was performed by comparing the mean of the gated population fluorescence with that of the control.

Results

Flow cytometric immunophenotyping

Granulocytic lineage

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, among MDS and 96.89%, 91.57%, 81.47%, 10.56%, 58.30% and 32.37%, respectively, among non-MDS patients. Table 3 shows the percentages of different antigens in granulocytic lineage in MDS and non-MDS patients.

Erythroid lineage in MDS and non-MDS

Flow cytometric analysis of erythroid lineage showed the mean percentage of CD71 in MDS and non-MDS cases to be 64.54% and 83%, respectively. In addition, CD235a-positive and CD71/CD235a-positive erythroid precursors showed mean percentages of 35.96% and 6.61%, respectively, in MDS cases, as compared to 52.83% and 10.48%, respectively, in non-MDS cases. Table 4 shows the percentages of different antigens in erythroid lineage in MDS and non-MDS cases.

Monocytic lineage in MDS and non-MDS

In this study the mean proportions of CD14, CD33, CD13, CD34 and HLA-DR were 65.89%, 79.92%, 74.04%, 44.43%, 36.25% in MDS and 74.36%, 86.57%, 87.74%, 45.30%, 38.86% in non-MDS cases. Table 5 shows the percentages of different antigens on monocytic lineage in MDS and non-MDS.

Myeloid precursors in MDS and non-MDS

Investigation of antigen expression in myeloid precursors of MDS patients showed the mean

Table 2: The monoclonal antibody-panel used in this study

CD19-FITC / CD20-PE / CD45-PerCP-Cy5.5 / CD10-APC
CD71-FITC / CD235a-PE / CD45-PerCP-Cy5.5 / CD117-AP
CD14-FITC / CD33-PE / CD45-PerCP-Cy5.5 / CD34-APC
HLA-DR-FITC / CD13-PE / CD45-PerCP-Cy5.5 / CD11bAPC

Table 3: Percentages of different antigens in granulocytic lineage in MDS and non-MDS patients.

Variable	Group	Mean percentage	*SD*	*P* values
CD13	MDS	84.98	10.77	0.006
	non-MDS	91.57	6.49	
CD34	MDS	34.25	6.76	0.218
	non-MDS	32.37	4.81	
CD10	MDS	40.34	16.19	0.000
	non-MDS	58.30	5.98	
HLA-DR	MDS	14.59	7.73	0.029
	non-MDS	10.56	3.66	
CD33	MDS	91	22.21	0.005
	non-MDS	96.89	10.18	
CD11b	MDS	77.20	17.45	0.210
	non-MDS	81.47	5.99	

Table 4: Percentages of different antigens on erythroid lineage in MDS and non-MDS patients.

Variable	Group	Mean percentage	*SD*	*P* values
CD71	MDS	64.54	18.68	**0.000**
	non-MDS	83	7.05	
CD235a	MDS	35.96	13.03	**0.000**
	non-MDS	52.83	7.98	
CD71/CD235a-positive	MDS	6.61	3.75	**0.000**
	non-MDS	10.48	3.23	

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. Table 6 shows the percentages of different antigens in myeloid precursors in MDS and non-MDS cases.

Lymphoid lineage in MDS and non-MDS cases

The mean ranges for CD19/CD10-positive, CD19/CD20-positive, CD20/CD10-positive and CD19 were 4.14%, 12.20%, 3.13%, and 14.69%, respectively, in MDS and 3.19%, 13.93%, 3.08%, and 15.57%, respectively, in non-MDS cases. Table 7 shows the percentages of different antigens in lymphoid lineage in MDS and non-MDS cases.

Table 5: Percentages of different antigens on monocytic lineage in MDS and non-MDS cases.

Variable	Group	Mean percentage	*SD*	*P* values
CD34	MDS	44.43	11.53	0.755
	non-MDS	45.30	9.69	
HLA-DR	MDS	36.25	11.72	0.376
	non-MDS	38.86	10.65	
CD19	MDS	31.64	10.12	0.350
	non-MDS	31.29	9.04	
HLA-DR/CD11b-positive	MDS	32.49	8.00	0.024
	non-MDS	28.47	5.04	
CD14	MDS	65.89	21.53	0.337
	non-MDS	74.36	9.65	
CD33	MDS	79.92	16.80	0.099
	non-MDS	86.57	8.62	
CD14/CD34-positive	MDS	35.98	15.86	**0.037**
	non-MDS	29.21	6.48	
CD13	MDS	74.04	23.90	**0.005**
	non-MDS	87.74	5.90	

Discussion

MDS is one of the common BM disorders among elderly population. Incidence of MDS in general population is about 3.5–4 per 100,000 people per year [10]. Analyzing the pattern of different CD markers in MDSs will help to set the frame of reference for identification of MDS 4. These ranges provide a basis for comparing the results from various institutes. In addition, it helps to combine such results on patients from several institutes, and will provide the methodology and equipment that are matching at all sites. Describing a range for antigen expression can also be useful in evaluating each individual patient. With a panel of thirteen monoclonal antibodies, including monoclonal antibodies against CD45, CD71, CD235a, CD117, HLA-DR, CD13, CD11b, CD14, CD33, CD34, CD19, CD20, and CD10 quantitative flow cytometric analysis of various cell lineage specific antigens were achieved in this study. We examined antigen expression pattern of erythroid, granulocytic, monocytic, lymphoid lineages and myeloid precursors in thirty patients with newly-diagnosed MDS. The results were compared with the BM samples of patients affected by disorders with no BM involvement (ITP) [9].

The gold standard for the MDS diagnosis is based on morphology but sometimes morphological diagnosis may not be enough [11,12]. Different studies have indicated that neoplastic cells in MDS demonstrate decreased or increased expression of some CD markers. They also have shown the maturational asynchrony in expression of different antigens in MDS. In fact, abnormal expression patterns of different antigens have been reported

Table 6: Percentages of different antigens in myeloid precursors in MDS and non-MDS patients.

Variable	Group	Mean percentage	*SD*	*P* values
CD33	MDS	69.24	22.21	0.263
	non-MDS	74.28	10.18	
CD34	MDS	59.53	19.33	**0.002**
	non-MDS	45.67	12.06	
CD13	MDS	60.64	20.35	**0.031**
	non-MDS	70.16	11.78	
HLA-DR	MDS	54.26	2033	0.355
	non-MDS	58.90	18.16	
HLA-DR/CD11b-positive	MDS	22.40	13.65	**0.000**
	non-MDS	9.22	5.48	
CD117	MDS	19.89	9.70	**0.000**
	non-MDS	11.73	6.05	
CD11b	MDS	23.43	14.27	**0.012**
	non-MDS	15.66	8.02	

several times in MDS cases [5, 12-16]. But to the best of our knowledge there is no report about the mean percentage of different CD markers on various cell lineages in MDS patients. Previous studies have just mentioned the decreasing, increasing and abnormal expression of different CD markers on different cell lineages. For instance, low mean percentage of CD13 and CD33 on granulocytes and monocytes has been reported before [5,8,12,13]. In addition, a lower mean percentage of CD10+ on granulocytes has been found in MDS patients [5,12,15,16]. Wells et al. [15], showed the maintenance of CD34 expression on mature granulocytes. In a series of 115 MDS cases, they reported retention of CD34 expression on differentiating granulocytes [15]. Wang et al.[17], studied immunophenotypic characteristics of BM samples from 48 MDS patients. They found the abnormal expression of CD13/CD11b on granulocytes of MDS patients but the difference between MDS and non-MDS cases was not statistically significant [17]. Other studies have shown decreased CD14 expression on monocytes of MDS patients [12,15]. Wells et al. [15] , found a variety of myeloid and monocytic abnormal antigenic patterns in MDS, atypical maintenance of HLA-DR in subpopulations of maturing myeloid cells, aberrant development in a subpopulation of monocytes that decrease CD11b presentation on maturing granulocyte and monocytes [15]. Antigen expression pattern of erythroid precursors has been investigated by the researchers as well. Based on previous findings flow cytometric analysis of erythroid progenitors from MDS patients have indicated decreased CD71 and CD235a/CD71 presentation compared to those from normal BM [16, 18]. Xu et al.[19] analyzed the Immunophenotypic features of erythroid progenitors to evaluate their diagnostic application in MDS. They showed low expression of CD71 in erythroid precursors of MDS patients. Chopra et al.[20], investigated the BM aspirates of 57 suspected MDS and 31 normal controls by five-

Table 7: Percentages of different antigens in lymphoid lineage in MDS and non-MDS patients.

Variable	Group	Mean percentage	SD	*P* values
CD19	MDS	14.69	5.34	0.510
	non-MDS	15.57	4.86	
CD19/CD10-positive	MDS	4.14	3.85	0.530
	non-MDS	3.19	5.70	
CD19/CD20-positive	MDS	12.20	2.26	0.094
	non-MDS	13.93	2.05	
CD20/CD10-positive	MDS	3.13	1.97	0.925
	non-MDS	3.08	2.09	

color flow cytometry. They showed significantly lower expression of CD71 on CD235a of erythroid precursors and in MDS cases [20].

Also, several studies have reported the phenotypic changes occurring in the myeloid precursors of MDS patients. Arroyo et al. [21, 22], and [23] reported an increase in myeloid precursors CD34 and CD38 expression in MDS. Ogata et al. [23], also reported finding an increase in myeloid precursors CD11b expression[23]. Samuel et al.[24], also showed, reduced or increased CD33 expression on myeloid precursors. CD117 is another antigen that is expressed on hematopoietic progenitors and immature myeloid cells. CD117 has been shown to be normal or increased in myeloid precursor cells in MDS patients [24]. Kussick et al.[14], showed increased CD34, HLA-DR, CD11b+, CD117, and CD11b expression on myeloid precursors in MDS when compared to non-MDS cases. On the other hand, decrease of HLA-DR, CD33 and CD13 on myeloid precursors has been reported in MDS cases compared to non-MDS cases[14]. Ogata et al .[23], also indicated the rise of CD11b expression on myeloid precursors. Previous studies have indicated enhancement of CD38 expression on myeloid precursors in MDS cases [21, 22,23]. Although, our results support the previous studies the aim of this study was showing the mean percentage of each CD markers included in our panel. There is no doubt, that having the reference values for an antigen expression pattern of various cell lineages in MDS will enhance the utility of flow cytometric analysis. In addition, using reference values will improve the accuracy of flow cytometric analysis by mixing variation due to race, gender, and age. In the absence of previously published estimates, we now report establishment of reference values for antigen expression patterns in various cell lineages of MDS cases. In conclusion, this study has determined the range of antigen expression on myeloid, erythroid and lymphoid cell lineages in MDS patients that may be useful in interpretation of laboratory and clinical findings. In addition, our result can be used as a reference for antigen expression patterns for the diagnosis of MDS, and also help to distinguish MDS from other diseases. Although this study was successfully completed some limitations were observed. First of all, the number of MDS cases was very low in Malaysia. Secondly, no normal individuals volunteered to give BM sample for the study so in this study we used the ITP cases as the control. We think these cases were suitable enough to be as a control because they did not show any BM involvement.

Conclusion

There is no doubt that providing the reference values for an antigen expression pattern among myelodysplastic syndrome cases enhances the utility of flow cytometric analysis interpretation among these patients.

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