Diagnostic Value of Flow Cytometry in Cases with Myelodysplasia

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Abstract: Background: Myelodysplastic syndrome (MDS) is a term used to encompass a spectrum of clonal (neoplastic) myeloid disorders The combination of obvious marrow dysplasia and clonal karyotypic abnormalities is considered diagnostic for MDS, with each technology confirming the other. However, not all patients with MDS will have this combination of findings. In this study, we evaluated the utility of flow cytometric immunophenotyping in the diagnosis of MDS. **Material and Methods:** We studied 20 patients with MDS , two of them were chronic myelomonocytic leukemia (CMML) (as diagnosed by morphologic evaluation of the initial bone marrow specimen) and compared results with those obtained in healthy controls subjects. All patients and controls were subjected to full history taking , Clinical examination, complete blood count, Bone marrow aspirate ,iron stain and immunophenotyping using a panel of antibodies CD13, 33, 34, 38, 16, 14,45 ,56 and CD11b to analyze dyspoiesis by quantifying the expression of each monoclonal antibodies (MoAb) on blasts ,granulocytes and monocytes with respect to controls. Bone marrow biopsy was done in some cases. **Results:** The results are classified according to the gate into blast, granulocytes and monocytic gates. On blast gate, we found statistically significant increase in expression and percentage of CD34 + cells , also decrease in CD 38 expression on CD34 + cells in cases of MDS in comparison to control group. Granulocytic gating revealed statistically significant increase of CD13 expression and decrease in CD56 expression in cases in comparison to control group, while the differences in expression of CD45, CD14, CD33 and CD11B were statistically insignificant. Monocytic gating revealed statistically significant decrease of CD38 expression in cases of RA and increase of CD14 & CD11b expressions in cases in comparison to control group, while the differences in expression of CD45, CD13, CD33 and CD56 were statistically insignificant. **Conclusion:** We emphasis on the role of flow cytomerty in MDS for accurate blast count and identification of abnormal myeloblasts on the basis of antigenic profiles, even in the marrow with less than 5% of myeloblasts. Also recognition of immunophenotypic dysplastic changes in mature myeloid cells and monocytes. No one single simple immunophenotypic parameter has been proved to be diagnostic of MDS.

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Keywords: MDS, Flow cytometry

1. Introduction

The myelodysplastic syndromes (MDSs) are characterized by bilineage or trilineage dysplasia. Although diagnostic criteria are well established for MDS, a significant number of patients have blood and bone marrow findings that make diagnosis and classification difficult. The diagnosis of MDS is based on a combination of clinical history, the morphological features of the peripheral blood (PB) and BM (e.g., percentages of blasts and dysplasia of cells), cytogenetic data, and ruling out other diseases.(1)

However, clonal cytogenetic abnormalities are typically found in less than 50% of these disorders, while morphologic evaluation is intrinsically subjective. Because reproducible patterns of antigen expression are identified in both normal myeloid maturation and benign/reactive settings such as marrow regeneration following injury, significant deviations from these benign maturational patterns can provide objective evidence supporting the presence of MDS or chronic myelomonocytic leukemia .(2)

A diagnostic challenge exists in low-grade MDS that lack conventional, specific diagnostic markers, ringed sideroblasts and karyotypic aberration. The diagnosis of this category (called *low-grade MDS*) largely relies on the presence of dysplasia, and therefore experienced examiners (hematologists/hematopathologists) are required to make the diagnosis. On the other hand, the dysplastic features of myeloid cells do not in themselves establish a diagnosis. Conditions other than MDS can induce dysplastic myeloid cells (e.g., deficiencies of vitamin B_{12} and folate, viral infections, ethanol or lead), and thus such conditions should be ruled out by careful history taking and physical and laboratory examinations. (3)

Flow cytometric immunophenotyping is an accurate method for quantitative and qualitative evaluation of hematopoietic cells, and several groups have used flow cytometry in the study of MDS. MDS patients have been found to have abnormal expression of several surface antigens, as indicated by either the

intensity of fluorescence or the percentage of positive cells. No one single simple immunophenotypic parameter has been proved to be diagnostic of MDS.(4).

The aim of this study is to diagnose and categorize cases of myleodysplastic syndrome by applying more accurate and objective techniques such as flow cytometry to detect abnormal maturation patterns.

2. Material and Methods

This study was conducted on twenty (20) patients diagnosed with myelodysplastic syndrome or myelodysplastic syndrome /myeloproliferative disease (MDS/MPD) at presentation at the department of Clinical Pathology, main University hospital of Alexandria and at Hematology department, Medical Research Institute, University of Alexandria. Ten (10) healthy subjects age and sex matched will be recruited as a control group whom were subjected for bone marrow aspiration for hypersplenism

Methods

All patients and controls were subjected to full history taking, clinical examination, complete blood count, Bone marrow aspirate, Prussian blue stain and immunophenotyping. Bone marrow biopsy was done for some cases.

Bone marrow sampling:

Bone marrow aspiration was done, between 2 and 3 mls were aspirated and the sample was used to prepare bone marrow films, and the remaining was used for immunophenotyping. The diagnosis and classification of myelodysplasia was primarily based on the morphologic characteristics of different lineages in PB and BM according to WHO 2008 classification. (5)

PB and BM films were stained with leishman stain, and were used for the morphological identification of various cell types. Prussian blue stain was also performed on BM films for proper classification. Morphological analysis of marrow specimens was performed by two hematologists. A total of 500 bone marrow nucleated cells per sample were assessed. In the myeloid lineage, the following abnormalities were considered: bizarre nuclear shape, hypo- or agranularity, nuclear/cytoplasmic asynchrony, and pseudo-Pelger anomaly. The evaluation of the erythroid lineage was based on the detection of megaloblastic changes, nuclear lobulation, multinuclearity, internuclear bridges and cytoplasmic granules/inclusions. . Micromegakaryocytes, small binucleated megakaryocytes, megakaryocytes with small round separated nuclei, and megathrombocytes were considered signs of megakaryocytic dysplasia.

Immunophenotyping of the cells

Analysis was performed on total nucleated bone marrow cells after erythrocytes lysis. All samples were processed and analyzed within 24 hours. Samples should be stored at room temperature until processed in the laboratory. The detailed characterization of hematopoietic cells is obtained by analyzing the expression of a given set of antigen in a cell population. Peripheral blood and / or BM cells from patients in the present study were analyzed by immunophenotyping with panel of M_OAbs. (CD 45, 14,16,38,33,13,34, 11b,56) (DAKO, Denmark)

In the present study, the direct immunofluorescence technique was employed using labeled antibodies. Immunofluorescence on the viable cells in suspension was analyzed using Becton Dickinson, FACS calibur flow cytometer equipped with cellquest software. Isotopic antibodies were used as negative control .

Gating:

We quantify the expression of each MoAb on blast, granulocytes and monocytes gates..

Statistical analysis

Data were fed to the computer using the Predictive Analytics Software **(PASW Statistics 18).** Quantitative data were described using median, minimum and maximum as well as mean and standard deviation.The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test. D'Agstino test was used if there was a conflict between the two previous tests. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used.

For normally distributed data, comparison between two independent population were done using independent t-test. For abnormally distributed data, Mann-Whitney Test (for data distribution that was significantly deviated from normal) were used to analyze two independent population**.** Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

3. Results

The present study was conducted on twenty new diagnosed patients with MDS or MDS/MPD and ten healthy subjects of matched age and sex as control. The patients were 8 (40%) females and 12 (60%) males with a mean age of 54.70±14.02 years while the control group included 5 (50 %) males and 5 (50%) females of mean age 40.67±11.00 years.

The distribution of the studied patients among the WHO subtypes was as follows; five patients were Refractory anaemia (RA) (25%), three were Refractory

anaemia with multilineage dysplasia (RAMD) (15 %), seven were Refractory anaemia with excess blasts type 1 (RAEB 1) (35 %), three were Refractory anaemia with excess blasts type 2(RAEB 2) (15 %) and two were CMML (myelodysplastic syndrome /myeloproliferative) (10 %).

Figure (1a,1b) shows dysmegakaryopoiesis in a patient with refractory anemia , Figure (2a,2b) shows abnormal localization of immature precursors in bone marrow biopsy in a patient with refractory anaemia with excess blast type 1 whereas figure (3) shows dysplastic changes in a case of CMML.

There is statistically significant higher CD 34 percentage on blast gate of cases (total), RAEB1 & RAEB2 cases in comparison to control group. (Table 1). Figure (4) shows CD34 expression in the control while figure (5) shows increased CD34 expression in a case with refractory anaemia with excess blast type 1. There's statistically significant lower CD 38 expression on CD34 + cells of cases (total), RA, RAMD & RAEB1 cases in comparison to control group.(Table 2)

There is statistically significant higher mean fluorescent intensity (MFI) of CD 13 expression on neutrophils in cases (total), RAMD, RAEB1 & RAEB2 in comparison to control group. (Figures 6,7).The difference of the mean fluorescence intensity of CD33 expression on neutrophils between cases & subgroups and the difference of the mean fluorescence intensities of CD14 & CD45 expressions on neutrophils in cases of MDS in comparison to control was statistically insignificant.The decrease of the mean fluorescence intensity of CD38 expression & increase of CD16 expression on neutrophils in cases of MDS in comparison to control was statistically insignificant. There was decrease in mean fluorescence intensity of CD 11B expression on neutrophils in cases (total) of MDS in comparison to control but was statistically insignificant. There's statistically significant lower MFI of CD 56 expression on neutrophils in cases (total) in comparison to control group (Table 4, Figures 8 and 9).

The difference of the mean fluorescence intensity of CD 45 expression on monocytes in cases of MDS in comparison to control was statistically insignificant. There is statistically significant higher MFI of CD 14 expression on monocytes of cases (total), RA, RAMD & RAEB2 in comparison to control group.(Table 5).There is statistically significant lower MFI of CD 38 expression on monocytes in RA cases in comparison to control group. There is statistically significant higher MFI of CD 16 expression on monocytes in RAEB 1 cases in comparison to control group. (Table 6)

The difference of the mean fluorescence intensity of CD 13 and CD 33 expressions on monocytes in cases of MDS in comparison to control was statistically insignificant.There is statistically significant higher MFI of CD 11B expression on monocytes of cases (total) & RAMD in comparison to control group.(Figures 10,11). The difference of the mean fluorescence intensity of CD 56 expressions on monocytes in cases of MDS in comparison to control was statistically insignificant. (Table 7)

Table -1- Comparison between control and cases group & subgroups according to CD 34 percentage

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 34 percentage							
Range	4.0 – 14.0	2.0 – 26.0	2.0 – 12.0	5.0 – 23.0	6.0 – 25.0	12.0 – 26.0	4.0 – 14.0
Mean ± SD	6.20 ± 2.90	12.15 ± 7.87	5.40 ± 4.10	11.57 ± 6.37	15.67 ± 9.50	18.67 ± 7.02	6.20 ± 2.90
Median	5.50	11.0	5.0	10.0	16.0	18.0	5.50
p	0.035*		0.453	0.053	0.047*	0.017*	0.063

p: p value for Mann Whitney test between control and other groups; *: Statistically significant at $p \leq 0.05$

Table -2- Comparison between control and cases group & subgroups according to CD 38 expression on CD 34 + cells

	Control	Cases (Total)	Cases subgroups				
			Refractory anemia	Refractory anemia with lineage dysplasia	RAEB 1	RAEB 2	MDS / MPN
CD 38 / CD 34							
Range	45.0 – 243.0	4.0 – 126.0	42.0 – 126.0	30.0 – 91.0	4.0 – 116.0	22.0 -110.0	58.0 – 94.0
Mean ± SD	138.30 ± 62.34	61.20 ± 35.08	73.40 ± 32.11	62.0 ± 30.61	42.71 ± 37.69	73.33 ± 45.80	76.0 ± 25.46
Median	131.50	61.50	66.0	65.0	28.0	88.0	76.0
p	0.001*		0.037*	0.043*	0.003*	0.091	0.133

p: p value for Mann Whitney test between control and other groups *: Statistically significant at $p \leq 0.05$

Table -3 Comparison between control and cases group & subgroups according to CD 13 and CD 33 MFI on neutrophils

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 13							
Range	97.0 – 260.0	45.0 – 1151.0	50.0 – 623.0	213.0 – 631.0	102.0 – 632.0	183.0 – 1151.0	45.0 – 299.0
Mean ± SD	175.30 ± 62.58	349.35 ± 270.40	244.0 ± 225.27	414.0 ± 209.46	319.29 ± 177.78	648.67 ± 485.04	172.0 ± 179.61
Median	159.50	277.0	205.0	398.0	278.0	612.0	172.0
p	0.048*		1.000	0.042*	0.040*	0.042*	1.000
CD 33							
Range	47.0 – 385.0	88.0 – 181.0	78.0 – 178.0	100.0 – 252.0	47.0 – 385.0	65.0 – 319.0	135.0 – 170.0
Mean ± SD	150.85 ± 94.19	125.40 ± 23.55	111.60 ± 40.99	169.0 ± 76.96	167.29 ± 128.91	158.67 ± 139.51	152.50 ± 24.75
Median	121.0	121.0	91.0	155.0	118.0	92.0	152.50
p	0.930		0.270	0.397	0.845	0.498	0.085

p: p value for Student t-test between control and other groups; *: Statistically significant at $p \leq 0.05$

Table -4-Comparison between control and cases group & subgroups according to CD 11 B and CD 56 MFI on neutrophils

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 11 B							
Range	392.0 – 3074.0	499.0 – 2160.0	392.0 – 3064.0	1088.0 – 2756.0	801.0 – 3019.0	495.0 – 3074.0	45.0 – 299.0
Mean ± SD	1524.50 ± 917.26	1045.80 ± 478.54	1286.0 ± 1075.91	1978.0 ± 839.62	1622.86 ± 897.61	1523.33±1366.54	172.0 ± 179.61
Median	1161.0	1052.50	970.0	2090.0	1218.0	1001.0	172.0
p	0.244		1.000	0.128	0.130	1.000	0.667
CD 56							
Range	26.0 – 439.0	78.0 – 311.0	26.0 – 420.0	224.0 – 274.0	133.0 – 439.0	109.0 – 352.0	135.0 – 170.0
Mean ± SD	233.40 ± 114.52	140.40 ± 83.07	217.20 ±157.63	250.0 ± 25.06	246.86 ± 125.07	261.0 ± 132.49	152.50 ± 24.75
Median	207.0	113.50	201.0	252.0	201.0	322.0	152.50
p	0.026*		0.327	0.091	0.057	0.091	0.519

p: p value for Mann Whitney test between control and other groups; *: Statistically significant at $p \leq 0.05$

Table -5-Comparison between control and cases group & subgroups according to CD 45 and CD 14 MFI on monocytes

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 45							
Range	44. – 542.0	97.0 – 811.0	114.0 – 743.0	141.0 – 407.0	97.0 – 811.0	113.0 – 360.0	111.0 – 340.0
Mean ± SD	195.90 ± 152.64	307.95 ± 230.92	362.0 ± 260.88	282.0 ± 133.72	348.29 ± 307.88	204.67 ± 135.25	225.50 ± 161.93
Median	151.0	219.50	328.0	298.0	128.0	141.0	225.50
p	0.428		0.269	0.234	0.845	1.000	1.000
CD 14							
Range	50.0 – 1079.0	129.0 – 3008.0	519.0 – 2901.0	1245.0 – 3008.0	129.0 – 2901.0	2240.0 – 3008.0	255.0 – 499.0
Mean ± SD	275.10 ± 326.57	1792.95±1186.53	2127.80 ± 953.98	2420.33±1017.87	1293.71±1329.61	2716.33 ± 415.97	377.0 ± 172.53
Median	99.50	2284.50	2329.0	3008.0	417.0	2901.0	377.0
p	0.001*		0.003*	0.011*	0.057	0.011*	0.282

p: p value for Mann Whitney test between control and other groups; *: Statistically significant at $p \leq 0.05$

Table 6: Comparison between control and cases group & subgroups according to CD 38 and CD 16 MFI on monocytes

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 38							
Range	35.0 – 250.0	4.0 – 830.0	27.0 - 106	24.0 – 830.0	4.0 – 229.0	51.0 – 369.0	111.0 – 142.0
Mean ± SD	122.20 ± 54.89	140.10 ± 182.74	69.40 ± 29.84	297.0 ± 461.64	101.43 ± 73.61	200.33 ± 159.88	126.50 ± 21.92
Median	119.50	88.50	77.0	37.0	95.0	181.0	126.50
p	0.244		0.020*	0.498	0.353	0.397	0.830
CD 16							
Range	91.0 – 1688.0	110.0 – 1989.0	142.0 – 1782.0	110.0 – 1727.0	419.0 – 1989.0	215.0 – 1396.0	150.0 – 1613.0
Mean ± SD	523.10 ± 466.49	894.50 ± 642.09	635.60 ± 667.35	1115.67 ± 877.67	1083.14 ± 541.72	673.33 ± 633.32	881.50 ± 1034.50
Median	491.50	687.0	307.0	1510.0	1148.0	409.0	881.50
p	0.113		0.713	0.236	0.032*	0.735	0.667

p: p value for Mann Whitney test between control and other groups; *: Statistically significant at $p \leq 0.05$

Table 7: Comparison between control and cases group & subgroups according to CD 11 b and CD 56 MFI on monocytes

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 11 b							
Range	104.0 – 1262.0	190.0 – 3116.0	302.0 – 1467.0	994.0 – 1704.0	190.0 – 1932.0	651.0 – 3116.0	45.0 – 299.0
Mean ± SD	495.10 ± 476.60	1069.15 ± 690.77	860.20 ± 444.78	1437.67 ± 386.79	1001.71 ± 612.80	1515.67±1387.43	172.0 ± 179.61
Median	181.50	882.50	830.0	1615.0	876.0	780.0	172.0
^{MW}p	0.016*		0.141	0.042*	0.070	0.127	0.517
CD 56							
Range	64.0 – 588.0	0.0 – 595.0	129.0 – 470.0	52.0 – 180.0	0.0 – 470.0	374.0 – 595.0	135.0 – 170.0
Mean ± SD	350.80 ± 193.81	258.65 ± 173.25	252.60 ± 131.71	107.33 ± 65.74	224.86 ± 180.80	469.67 ± 113.45	152.50 ± 24.75
Median	324.50	237.0	202.0	90.0	279.0	440.0	152.50
p	0.197		0.330	0.061	0.196	0.342	0.760

^{MW}p: *p* value for Mann Whitney test between control and other groups

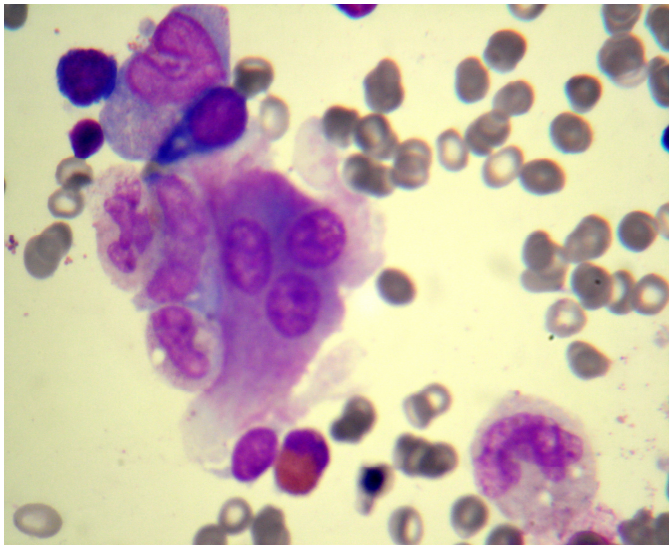

Figure (1a) :Shows dysplastic megakaryocyte in a patient with refractory anaemia

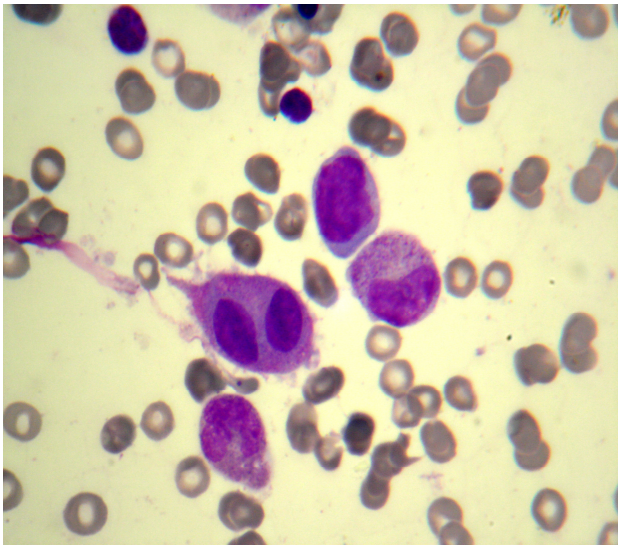

Figure (1b):Shows binucleated megakaryocyte in a patient with refractory anaemia

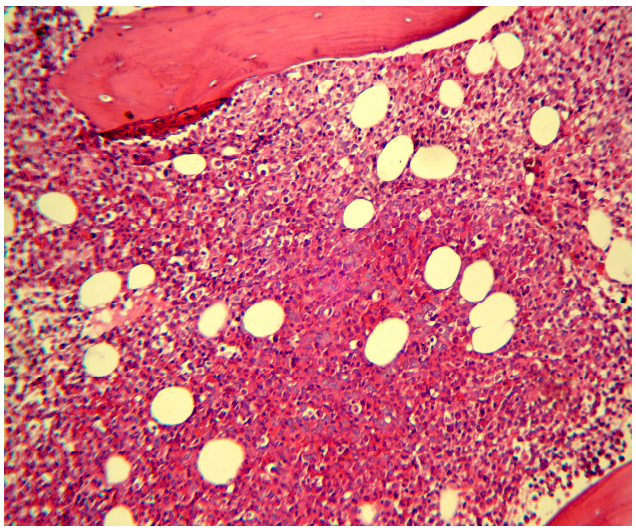

Figure (2a): Shows abnormal localization of immature precursors in a patient with refractory anaemia with excess blasts type 1 (by low power.)

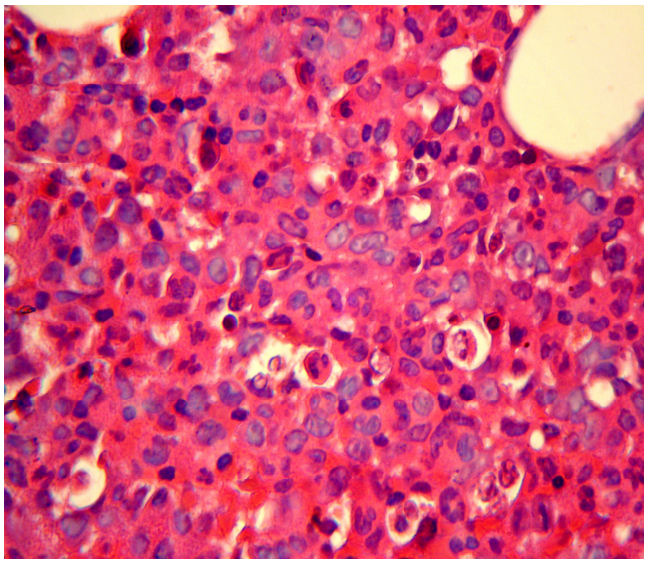

Figure (2b):Shows abnormal localization of immature precursors in a patient with refractory anaemia with excess blasts type 1(by high power)

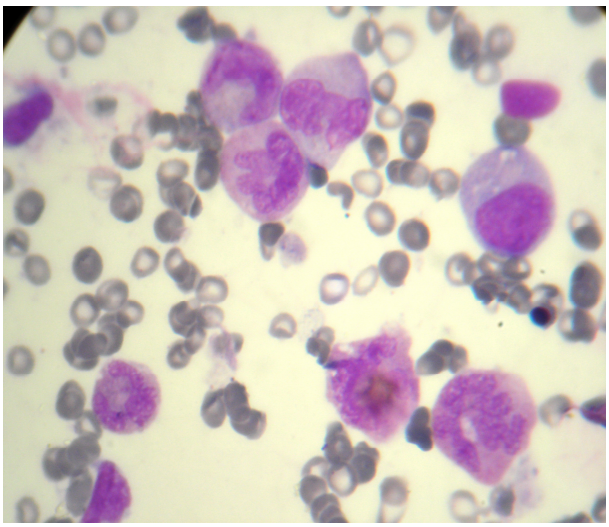

Figure (3):Shows dysplastic feature in a patient with CMML

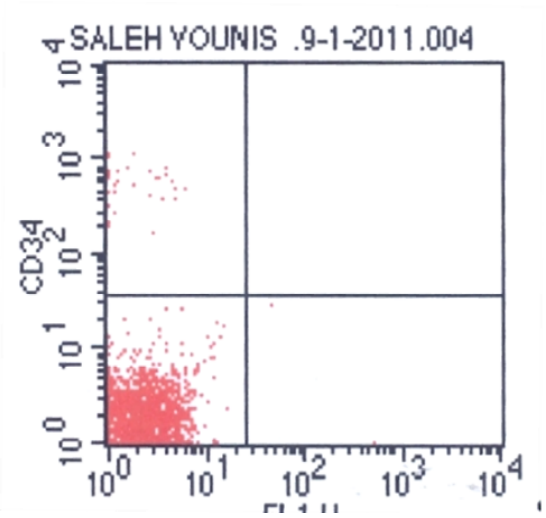

Figure 4: Normal CD 34 expression in a control

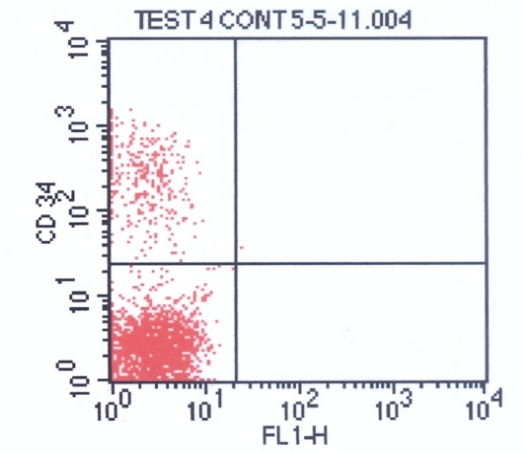

Figure 5: Higher percentages of CD 34 in a patient with refractory anaemia with excess blast type 1

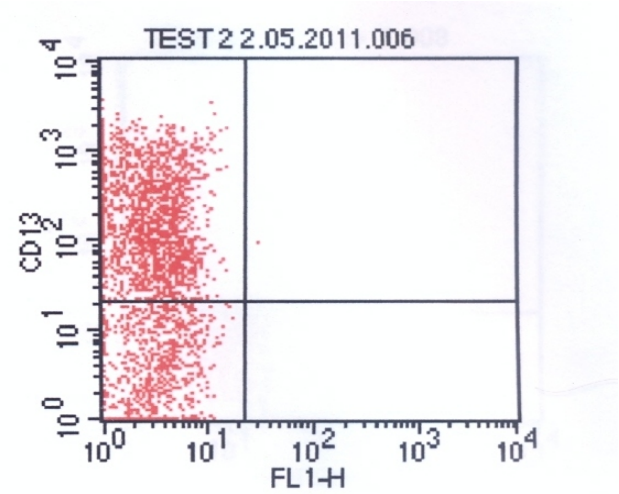

Figure 6: Higher CD 13 expression on granulocytes in a patient with refractory anemia with multilineage dysplasia

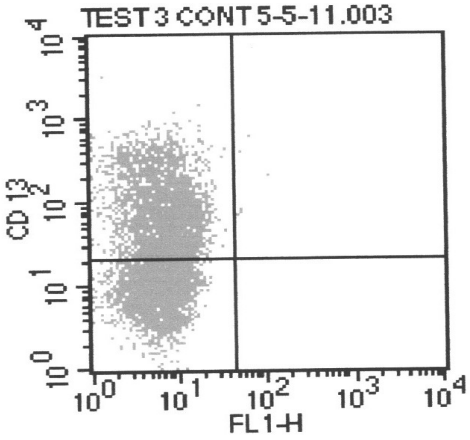

Figure 7: CD13 Expression on granulocytes in a control

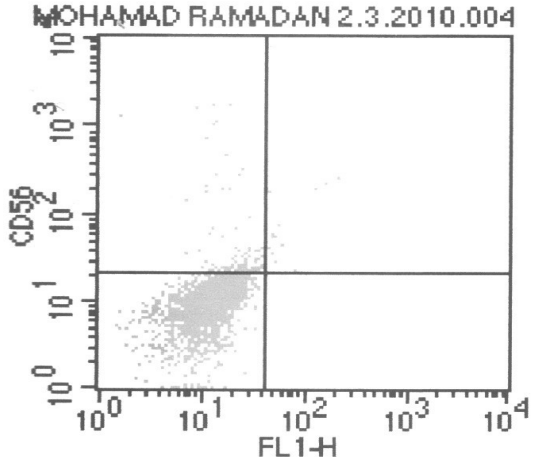

Figure (8): Low CD 56 expression on granulocytes in a case with myelodysplasisa

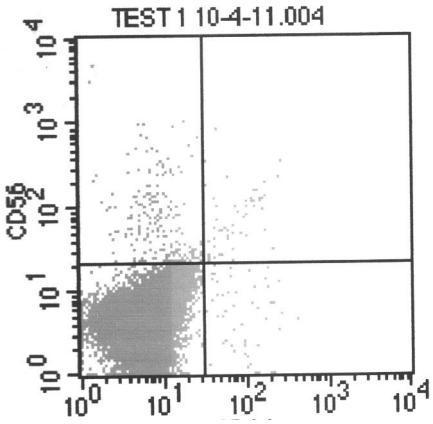

Figure (9): CD 56 expression on granulocytes in a control

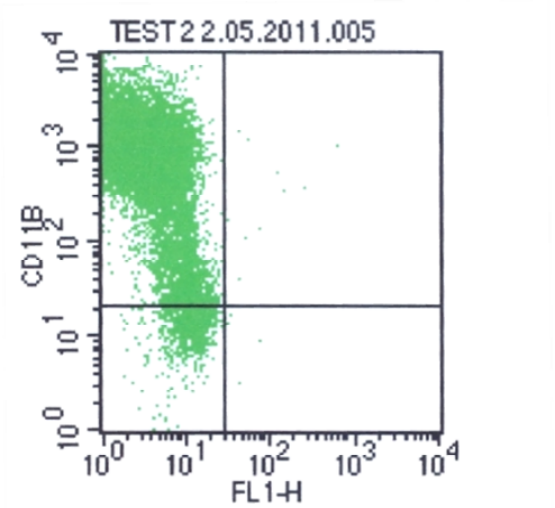

Figure 10: Higher expression of CD11B on monocytes in a case with refractory anaemia with multilineage dysplasia

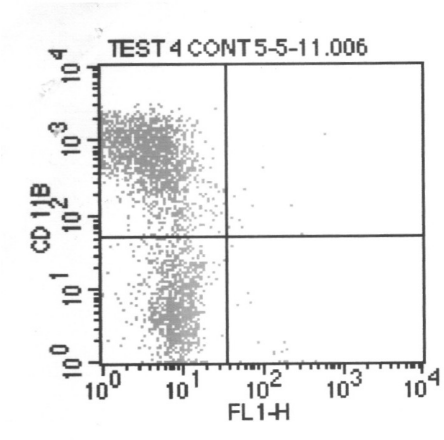

Figure (11): Expression of CD11B on monocytes in a control

4. Discussion

Myelodysplastic syndromes (MDS) are malignant disorders of haematopoietic cells. The bone marrow(BM) in MDS is composed of clonal myeloid cells showing various degrees of differentiation in each case. MDS usually exhibit cytopenia, mainly due to the early death of partially or fully differentiated myeloid cells and insufficient differentiation capacity of the progenitors to transform into mature blood cells. Manifestations caused by cytopenia and transformation to acute myeloid leukaemia(AML) due to further loss of the ability of clonal cells to differentiate are the major causes of death in MDS.(6)

The diagnosis of MDS is straightforward if clearly objective abnormalities, such as increase in blasts and/or ringed sideroblasts and/or presence of chromosomal aberration as evidence of clonal myelopoiesis are detected. A diagnostic challenge exists in low-grade MDS without ringed sideroblasts with normal karyotype For this reason laboratory scientists have investigated the use of flow cytometry (FC) to increase the sensitivity and specificity of diagnosis in such cases. (7)

Recently, many laboratories have been working to develop MDS FCM and are still struggling to determine suitable flow parameters .Flow cytometry can detect minimal aberrancies in the differentiation of myelomonocytic cell populations by changes in antigen expression in BM that are otherwise not detected by morphology.

The aberrancies in hematopoiesis that can be observed are the expression of lymphoid antigens on myeloid cells, over, under and/or loss of antigen expression on mature cells and vice versa and abnormal differentiation patterns as compared to antigen expression levels from normal hematopoietic cells.(8)

Flow cytometric role in MDS is based upon the knowledge that maturation and differentiation of hematopoietic cells is a tightly controlled process, leading to highly conserved levels of antigen expression at different stages of development. In myelodysplastic syndromes (MDS), progenitor cell formation is affected resulting in deviation from the normal level of antigen expression in the mature and immature myelo-monocytic, erythroid and megakaryocytic cell lineages. [9]

Both flow cytometric immunophenotyping and morphologic evaluation were more sensitive than cytogenetics in detecting MDS .As more antibodies useful in studying erythroid and megakaryocytic maturation are developed , the sensitivity of flow cytometric testing may increase.(1)

In this study, we evaluated the utility of flow cytometric immunophenotyping in the diagnosis of MDS. We studied 20 patients with MDS (as diagnosed by morphologic evaluation of the bone marrow specimen) and compared results with those obtained in healthy controls subjects using combination of antibodies CD13, 33, 34, 38, 16, 14,45,56 and CD11b to analyze dyspoiesis by quantifying the expression of each MoAb on granulocytes and monocytes with respect to controls. The results are classified according to the gate into blast, granulocytes and monocytic gates.

On blast gate, we found statistically significant increase in the expression and percentage of CD34+ cells and decrease in CD38 expression on CD34 + cells in MDS cases in comparison to the control group.

In between cases there was higher CD 34+ cells percentage in cases of RAEB1 & RAEB2 subtypes in comparison to other subtypes. This difference between the subtypes of MDS showed a good correlation with the number of bone marrow blast cells assessed by morphology.

In Consistence with this finding of our study; Fuchigami *et al.* [10] scored absolute numbers of CD34-positive cells using CD34 monoclonal antibody and found that the total CD34+ cells were decreased in RA patients, but increased in patients with RA with excess of blasts (RAEB) as compared to normal.

Similarly, Ogata *et al.* [11] and Matarraz *et al.* [12] focused on blast immunophenotype and found that quantifying CD34+ cells in blast compartment is useful in diagnosing patients with low grade MDS with or without karyotype abnormalities. Also increased CD34 expression was associated with a poor international prognostic scoring system, a poor cytogenetic risk factor, and a high blast cell count on bone marrow smears.

We conclude that this method allows distinguishing RA from other MDS subtypes more reliably than by morphology alone and providing early signs of progression to acute leukemia. Also CD34 expression could be significant as a prognostic marker rather than as a diagnostic marker of MDS. On blast gate, we found statistically significant decrease in CD38 expression on CD34+ cells in cases of MDS in comparison to control group.

Goardon *et al.* [13] investigated whether reduced mean fluorescence intensity (MFI) of CD38 expression on CD34+ cells could be used as a surrogate marker for abnormalities in the MDS CD34+ compartment, and whether this may provide a single simple useful flow cytometric measurement diagnostic of MDS. They found that the examined immunophenotypic parameter diagnosed low-risk MDS with 95% sensitivity and 92% specificity, and concluded that it may be of value in the routine clinical diagnosis of MDS, especially in cases with a low blast count and normal karyotype.

The present work revealed that the difference of the mean fluorescence intensity (MFI) of CD33 expression on granulocytes and monocytes in MDS cases in comparison to control were statistically insignificant while there is statistically significant

higher MFI of CD 13 expression on neutrophils in cases (total), RAMD, RAEB1 & RAEB2 in comparison to control group.

Some studies on peripheral blood neutrophils in MDS found no abnormality in CD33expression while others found an increased number of CD33-positive neutrophils, particularly in RAEB and RAEB-t. Several mechanisms may be involved in the abnormal expression of surface antigens in MDS including defective granulopoiesis, defective intracellular storage pool, abnormal membrane of cytoplasmic granules, and the effect of high levels of marrow cytokines such as tumor necrosis factor alpha and transforming growth factor-beta(5). Other studies have reported a much higher CD13 expression in high-risk groups than low-risk patients.(14) .Decreased or absent CD 33 expression in MDS has been reported by others. (15)

While Maynadié *et al.* ([16]) studied the immunophenotypic abnormalities that could be defined in MDS and the data obtained from granulocytes showed that the most discriminating markers were CD11b, CD13, CD33, CD38 and HLA-DR also the increased mean fluorescence intensities of CD38, CD13, and CD33 were associated with more advanced MDS stages (refractory anemia with excess blasts and RA with excess blasts in transformation).

The present study showed insignificant increase of MFI of CD16 expression on granulocytes and there was decrease of CD11b expression on granulocytes in the cases (total) in comparison to controls but that decrease was statistically insignificant. This might be explained by low sample size in the study.

Bowen and Davis [17] studied the pattern of CD16 and CD11b expression on maturing granulocytes in the bone marrow of patients with MDS and healthy controls. There was a highly consistent normal pattern of CD11b and CD16 expression in the granulocytic series in healthy subjects, but in MDS patients there was an increased percentage of granulocytic cells with low CD16 or both low CD16 and low CD11b.

In the present study we found decrease in CD56 expression on neutrophils in cases in comparison to control group whereas there was increase of 11b expression on monocytes in cases(total and RAMD) in comparison to control group that were not reported by others.

Also there's a statistically significant lower MFI of CD 38 expression on monocytes of RA cases in comparison to control group and higher MFI of CD 16 expression on monocytes in RAEB 1 cases in comparison to control group.

Van de Loosdrecht, *et al.* [18] **found** the following aberrancies in maturing monocytes and considered them relevant: decreased or increased proportion of monocytes as compared to lymphocytes, abnormal intensity of CD13 or CD33, an abnormal CD11b/HLA-DR pattern, abnormal intensity of CD14, CD36 or CD64, over-expression of CD56 and expression of lineage infidelity markers CD2, CD7 or CD19.

Del Canizo *et al.* [**(Error! Bookmark not defined.)**] found abnormally low CD45 expression on monocytes more frequent in MDS RAEB patients as compared to lower risk MDS patients and normal controls. This was in disagreement to the present study as the difference of mean fluorescence intensity of CD 45 expression on neutrophils and monocytes in cases of MDS in comparison to controls was statistically insignificant. This might be explained by low sample size in the study.

The results of the present study show significant increase of CD14 expression on monocytes between cases (total), RA, RAMD & RAEB2 and controls. On the other hand there was an insignificant difference of CD56 expression on monocytes between cases of MDS and normal controls.

As regarding CD56 expression which is a normal marker on natural killer cells, Lacronique-Gazaille *et al.* [20] and Xu Y *et al.* [21] used the flow cytometric analysis of monocytes to detect the phenotypic abnormalities in cases of CMML and found a significantly higher expression of CD56 and CD14 on marrow monocytes in CMML than in reactive monocytosis and normal marrow samples. Our result showed higher expression of CD 14 on monocytes in CMML compared to controls but the result was statistically insignificant as we have only two cases .

Also a study by Van de Loosdrecht [22] detect aberrancies in the myelo-monocytic lineage in cases of MDS patients in the form of over expression of CD 56 on monocytes in cases and the expression of CD56 was only scored as aberrant when its intensity exceeded that of CD56 expression as detected on monocytes in some of the normal samples by 1 log.

Our results as regarding CD56 expression in cases of CMML was insignificant ,this might be explained by the few CMML cases included in this study.

In conclusion, flow cytometry is of value in diagnosis of RA especially when the morphologic and cytogenetic evaluations are equivocal or non informative as it can detect accurate blast count and identification of abnormal myeloblasts on the basis of antigenic profiles, even in the marrow with less than 5% of myeloblasts. Also we were able to detect immunophenotypic dysplastic changes in mature myeloid cells and monocytes.

No one single simple immunophenotypic parameter has been proved to be diagnostic of MDS and because the panels needed for complete immunophenotypic analysis of all 3 lineages are extensive as well as costly, we do not recommend flow cytometric evaluation as a screening procedure for all cases of MDS.

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5. References

1-Stetler-Stevenson M, Arthur DC, Jabbour N, Xie XY, Molldrem J, Barrett AJ et al. (2001): Diagnostic utility of flow cytometric immunophenotyping in myelodysplastic syndrome. Blood; 98: 979–87.

2- Steven J. (2007): Multiparametric .Flow Cytometry in the Diagnosis of Myelodysplastic Syndromes and Related Disorders. LabMedicine; 38, 305-313

3-Kiyoyuki O,Matteo G.,Luca M,Cristina P, Norio Y, Akira M et al. (2009): Diagnostic utility of flow cytometry in low-grade myelodysplastic syndromes: a prospective validation study. haematol ;. 94 :1066-1074

4-Della Porta MG, Malcovati L, Invernizzi R, Travaglino E,Pascutto C, Maffioli M, et al. (2006): Flow cytometry evaluation of erythroid dysplasia in patients with myelodysplastic syndrome.Leukemia; 20:549-55.

5- Brunning RD, Orazi A, Germing U et al. (2008): Myelodysplastic syndroms/neoplasms, overview. In: Swerdlow SH, Canpo E, Harris NL et al, (eds). World Health Organization Classification of Tumors: Pathology and Genetics of Tumors of Haematopoietic and Lymphoid Tissues. Lyon: IARC Press;. pp. 88–93.

6- Ogata K. (2006): Myelodysplastic syndromes: recent progress in diagnosis and understanding of their pathophysiology. J. Nippon Med Sch; 73: 300–307.

7- Ogata K. (2008): Diagnostic flow cytometry for low-grade myelodysplastic syndromes. Hematological Oncology; 26: 193–8.

8- Matarraz S. (2010): Bone marrow cells from myelodysplastic syndromes show altered immunophenotypic profiles that may contribute to the diagnosis and prognostic stratification of the disease: a pilot study on a series of 56 patients. Cytometry B Clin Cytom; 78:154–68.

9- Stachurski D. (2008): Flow cytometric analysis of myelomonocytic cells by a pattern recognition approach is sensitive and specific in diagnosing myelodysplastic syndrome and related diseases: emphasis on a global evaluation and recognition of diagnostic pitfalls. Leuk Res.; 32:215–24

10- Fuchigami K, Mori H, Matsuo T, et al. (2000): Absolute number of CD34+ cells is abnormally low in refractory anemias and extremely high in RAEB and RAEB-t: novel pathologic features of myelodysplastic syndromes identified by highly sensitive flow cytometry. Leuk Res.; 24: 163–74.

11- Ogata K, Kishikawa Y, Satoh C, Tamura H, Dan K, Hayashi A. (2006): Diagnostic application of flow cytometric characteristics of CD34+ cells in low-grade myelodysplastic syndromes. Blood; 108: 1037–44.

12- Matarraz S. (2010): Bone marrow cells from myelodysplastic syndromes show altered immunophenotypic profiles that may contribute to the diagnosis and prognostic stratification of the disease: a pilot study on a series of 56 patients. Cytometry B Clin Cytom; 78:154–68.

13- Goardon N. (2009): Reduced CD38 expression on CD34+ cells as a diagnostic test in myelodysplastic syndromes. Haematologica; 94:1160–3.

14- Bonde J, Meyer K, Kjervig Broe M, Hokland M, TurleyH, Hokland P. (1996): Improved flow cytometric identification of myelopoiesis by the simultaneous labeling with CD13, CD14 and CD66 monoclonal antibodies. Br J Haematol; 92:269-79

15-Loken MR .(2008): Flow cytometry in MDS: report from working conference. Leuk Res.; 32:5-17.

16- Maynadi EM, Picard F, Husson B, et al. (2002): Immunophenotypic clustering of myelodysplastic syndromes. Blood; 100: 2349–56.

17- Bowen KL, Davis BH. (1997): Abnormal patterns of expression of CD16 (FcR-III) and CD11b (CRIII) antigens by developing neutrophils in the bone marrow of patients with myelodysplastic syndrome. Lab Hematol;3:292-8.

18- van de Loosdrecht, A. A., C. Alhan, et al. (2009): "Standardization of flow cytometry in myelodysplastic syndromes: report from the first European LeukemiaNet working conference on flow cytometry in myelodysplastic syndromes." Haematologica. 94(8): 1124-1134.

19- Del Canizo MC, Fernandez E, Lopez A, et al. (2003): Immunophenotypic analysis of myelodysplastic syndromes. Haematologica; 88: 402–7.

20- Lacronique-Gazaille C, Chaury MP, Le Guyader A, Faucher JL, Bordessoule D, Feuillard (2007): J. Haematologica; 92(6):859-60.

21- Xu Y, McKenna RW, Karandikar NJ, Pildain AJ, Kroft SH (2005): Flow cytometric analysis of monocytes as a tool for distinguishing chronic myelomonocytic leukemia from reactive monocytosis. Am J Clin Pathol;124:799-806

22- Van de Loosdrecht AA. (2008): Identification of distinct prognostic subgroups in low and intermediate-1 risk myelodysplastic syndromes by flow cytometry. Blood; 111:1067–77.

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