Osmangazi Tıp Dergisi
Osmangazi Journal of Medicine 2022;

Research Article / Araştırma Makalesi

Flow Cytometric Analysis of Chronic Phase Chronic Myeloid Leukemia Patients

Kronik Faz Kronik Miyeloid Lösemi Hastalarının Akış Sitometrik Analizi

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Abstract

Bone marrow aspirate for morphology and cytogenetics and qualitative reverse transcriptase polimerase chain reaction on peripheral blood cells is mandatory for the diagnosis of chronic myeloid leukemia (CML). Bone marrow biopsy and fluorescence in situ hybridisation may be necessary in selected patients. Performing other tests and diagnostic procedures depends on characteristics of the individual patient. Although flow cytometry is an essential tool in the diagnosis and monitoring of many hematological malignancies, it has a limited role in CML. In this study, we evaluated the CD45 side scatter results of our CML patients at diagnosis and during follow up. Totally 56 CML patients (22 female and 34 male) in chronic phase treated with imatinib were included. Patients were also evaluated after 8 (3-19) months follow up. Complete blood cound parameters and CD45/SSC results of the patients at diagnosis and follow up were evaluated retrospectively. The Wilcoxon T test was used to compare the means between the two groups. $p<0.05$ was considered statistically significant. All of them had decreased leukocyte and platelet counts. There was no difference in hemoglobin value. Comparison of CD45/SSC results at diagnosis and follow up revealed a decrease in granulocyte and blast percentages, and an increase in lymphocyte, monocyte, normoblast percentages. Complete blood counts and CD45/SSC results were not different when we divided patients into 2 groups according to being MMR positive or MMR negative. Survival of MMR positive and negative patients were also found similar. According to the results of our study, we were unable to suggest using CD45/SSC as a routine diagnostic and/or follow up tool. However, there were limitations of our study such as the limited number of patients, the variance between the time of MMR evaluation and the evaluation at only one time point. The results may change in larger studies with serial CD45/SSC analysis and with different tyrosine kinase inhibitors.

Keywords: chronic myeloid leukemia, CD45/SSC, imatinib.

Özet

Morfoloji ve sitogenetik için kemik iliği aspiratı ve periferik kan hücrelerinde kalitatif revers transkriptaz polimeraz zincir reaksiyonu, kronik miyeloid lösemi (KML) tanısı için zorunludur. Seçilmiş hastalarda kemik iliği biyopsisi ve floresan in situ hibridizasyon gerekli olabilir. Diğer testlerin ve teşhis prosedürlerinin uygulanması, bireysel hastanın özelliklerine bağlıdır. Akış sitometrisi birçok hematolojik malignitenin tanısında ve izlenmesinde önemli bir araç olmasına rağmen, KML'de sınırlı bir rolü vardır. Bu çalışmada KML hastalarımızın tanı anında ve takip sırasındaki CD45 dağılım sonuçlarını değerlendirdik. İmatinib ile tedavi edilen kronik fazdaki toplam 56 KML hastası (22 kadın ve 34 erkek) çalışmaya dahil edildi. Hastalar ortalama 8 (3-19) aylık takipliydi. Hastaların tanı ve takipteki tam kan sayımı parametreleri ile CD45/SSC sonuçları retrospektif olarak değerlendirildi. İki grup arasındaki ortalamaları karşılaştırmak için Wilcoxon T testi kullanıldı. $p<0.05$ değeri istatistiksel olarak anlamlı kabul edildi. Hepsinde lökosit ve trombosit sayıları azalmıştı. Hemoglobin değerinde farklılık görülmedi. Tanı ve takipteki CD45/SSC sonuçlarının karşılaştırılmasında granülosit ve blast yüzdelerinde azalma, lenfosit, monosit, normoblast yüzdelerinde artış saptandı. Hastaları MMR pozitif veya MMR negatif olarak 2 gruba ayırdığımızda tam kan sayımı ve CD45/SSC sonuçları farklı değildi. MMR pozitif ve negatif hastaların sağkalımı da benzer bulundu. Çalışmamızın sonuçlarına göre, CD45/SSC'nin rutin tanı ve/veya takip aracı olarak kullanılmasını öneremedik. Ancak çalışmamızın sınırlı hasta sayısı, MMR değerlendirme zamanı ile sadece bir zaman noktasındaki değerlendirme gibi kısıtlılıkları vardı. Seri CD45/SSC analizi ve farklı tirozin kinaz inhibitörleri ile yapılan daha büyük çalışmalarda sonuçlar değişebilir.

Anahtar Kelimeler:kronik miyeloid lösemi, CD45/SSC, imatinib.

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Received 06.08.2021 Accepted 24.08.2021 Online published 27.08.2021

Davutoglu NO, Gunduz E, Yaman F, Arslan S, Cilingir O, Isik S, Durak Aras B, FlowCytometric Analysis of ChronicPhaseChronicMyeloidLeukemiaPatients,Osmangazi Journal of Medicine, 2022;44(2): 193- 198 Doi: 10.20515/otd.978914

1. Introduction

When chronic myeloid leukemia (CML) is diagnosed, a bone marrow aspirate is required for morphology to distinguish chronic phase (CP) from accelerated phase (AP) or blastic phase (BP) and for cytogenetics. A core biopsy may be done to evaluate the degree of fibrosis and may identify blasts not evident in the aspirate (1-3). A qualitative reverse transcriptase polimerase chain reaction (PCR) on peripheral blood cells is mandatory to identify the type of Breakpoint Cluster Region-Abelson1 (BCR-ABL1) transcripts. If a molecular assay demonstrates BCR-ABL1, but the Philadelphia (Ph) chromosome cannot be identified by cytogenetics, Fluorescence in situ Hybridisation (FISH) is required. The diagnostic work-up is completed by physical examination, a standard biochemical profile including hepatitis B serology, cholesterol, lipase, and hemoglobin A1c values and an electrocardiogram. Performing other tests and diagnostic procedures depends on characteristics of the individual patient (4).

Several prognostic systems (Sokal, Euro, EUTOS, ELTS) including peripheral blood blasts have been used to estimate the survival risk at baseline (5-8). Blasts are usually defined morphologically.

Blood cell counts and differential cell counts are required every 2 weeks until a complete hematologic response is achieved , quantitative PCR on blood cells must be performed at least every 3 months. FISH monitoring may be needed in patients with atypical transcripts (4).

Although flow cytometry is an essential tool in the diagnosis and monitoring of many hematological malignancies, it has a limited role in CML especially in CP. In this study, we evaluated the flow cytometry (CD45 side scatter (SSC)) results of our CML patients at diagnosis and during follow up and aimed to find a role for flow cytometry in CML.

2. Methods

The study group comprised 56 CML patients (22 female and 34 male) in CP treated with imatinib. Age at diagnosis was 52 (24-82) years. Patients were also evaluated after 8 (3-19) months follow up. Treatment responses were categorized according to European Leukemia Net criteria (4). Informed consent was obtained from all patients. No additional bone marrow biopsy was needed and all tests were performed during routine diagnostic and monitoring procedures. Ethics committee approval was obtained from the Non-interventional Clinical Studies Ethics Committee of Eskişehir Osmangazi University Faculty of Medicine with the number 2020-55 on 30.12.2020.

BCR-ABL expression was quantitated using real-time quantitative reverse-transcriptase PCR according to the Europe against Cancer Protocol using ABL as a control gene. The BCR-ABL/ABL ratio was expressed as a percentage and normalized to the international scale using a correction factor established during external standardization.

Flow cytometry was performed on 50 microliter bone marrow aspirate in EDTA on Becton Dickinson flow cytometer using 20 microliter CD45FITC/CD14PE Becton Dickinson monoclonal antibodies. Further processing was done using whole blood lyse method. A total count of 1x104 cells per tube was obtained and analyzed on CD45/SSC gate. The SSC versus CD45 plots were evaluated for CD45 expression, side scatter expression, normoblasts, granulocytes, lymphocytes, monocytes and the blasts if present.. The Wilcoxon T test was used to compare the means between the two groups. $p<0.05$ was considered statistically significant.

3. Results

Fifty (89.3%) patients were still alive at the time of data collection. Causes of death were pneumonia (n=2), heart failure (n=1), colon perforation (n=1) and unknown in 2 patients. Sokal risk score was low in 26 (46.4%), intermediate in 22 (39.3%) and high in 8 (14.3%) patients. Bone marrow biopsy revealed fibrosis in 23 (41.1%) and dysplasia in 19 (33.9%) patients. Bone marrow cytogenetics was evaluable in 20 (35.7%) patients and revealed 46,XX,t(9;22)(q34;q11) in 16 patients, 46,XY,t(9;22)(q34;q11) in 2

patients, 46,XY,t(1;9;22) in 1 patient and 46,XY,t(9;22;10) in 1 patient. Hematologic parameters at diagnosis and follow up are listed in Table 1.

Table 1. Hematologic parameters at diagnosis and follow up

Parameter	At diagnosis				At follow up				
	Min	Max	Mean	Standard deviation	Min	Max	Mean	Standard deviation	p
Hemoglobin (g/dl)	7	16	11.53	2.06	8.1	20.9	12.26	2.01	0.089
White blood cell (/mm^3)	3400	600000	82810.71	108511.04	1500	96000	8816.07	14254.78	<0.001
Absolute neutrophil(/mm^3)	1100	580000	73324.11	102233.83	600	47000	4317.86	6055.72	<0.001
Absolute lymphocyte(/mm^3)	1800	55000	5551.10	7731.77	800	4600	1736.78	723.04	<0.001
Absolute monocyte (/mm^3)	0	30300	2213.21	4074.12	0	3300	455	477.68	<0.001
Absolute eosinophil (/mm^3)	0	26500	1585.89	3732.14	0	900	158.21	156.33	<0.001
Absolute basophil(/mm^3)	0	8700	1211.07	2122.80	0	2400	80.18	321.68	<0.001
Platelet (/mm^3)	34000	2023000	485928.57	351508.02	14000	1243000	236339.29	161803.17	<0.001

Major molecular response (MMR) was obtained in 25 (44.6%) patients. Control bone marrow biopsies were obtained in only 26 (46.4%) patients. Fibrosis was observed in 11 (42.3%) and dysplasia was observed in 3 (11.5%) patients. CD45 SSC results at diagnosis and follow up are shown in Table 2.

Table 2. CD45 SSC results at diagnosis and follow up

Parameter (%) (n=56)	At diagnosis				At follow up				
	Min	Max	Mean	Standard deviation	Min	Max	Mean	Standard deviation	p
Granulocyte	31.6	92	78.20	12.91	19.7	74.9	58.09	11.57	<0.001
Lymphocyte	0	32.2	3.82	50.04	6	42.1	17.36	7.92	<0.001
Monocyte	0	6.1	1.36	1.21	1.6	9.6	3.53	1.33	<0.001
Normoblast	0	47	8.68	9.60	4	39	12.4	6.66	<0.001
Blast	0	9.6	1.56	2.44	0	3.7	0.18	0.67	<0.001

When we divided patients into 2 groups as MMR positivite and negative, we found no difference between MMR positivity and Sokal risk groups at diagnosis (p=0.378).

MMR positivity was not different between the patients with fibrosis (p=0.33) and dysplasia (p=0.087) in the bone marrow biopsy at diagnosis.

Complete blood count parameters and CD45 SSC results at diagnosis and follow up in MMR positive and negative patients are shown in Table 3 and 4.

Survival of MMR positive and negative patients was not found different (101.43∓8.42 months vs 109.36∓7.44 months) (Figure 1).

Table 3. Hematologic parameters of MMR positive and negative patients

Parameter	MMR positive (n=25)				MMR negative (n=31)				
	Min	Max	Mean	Standard deviation	Min	Max	Mean	Standard deviation	p
Hemoglobin (g/dl)	8.5	16.5	12.1	1.61	8.1	20.9	12.3	2.30	0.96
White blood cell (/mm^3)	2400	96000	10556	18859.41	1500	55000	7412.9	9134.13	0.81
Absolute neutrophil(/mm^3)	1300	8700	3492	1751.89	600	47000	4983.87	7984.32	0.54
Absolute lymphocyte(/mm^3)	800	4600	1751.6	808.48	800	3900	1724.83	659.68	0.99
Absolute monocyte (/mm^3)	100	3300	506.4	608.28	100	1750	420	336.81	0.45
Absolute eosinophil (/mm^3)	0	900	186.4	194.65	0	400	138	112.77	0.55
Absolute basophil(/mm^3)	0	100	41.6	43.27	0	2400	111.29	431.24	0.181
Platelet (/mm^3)	14000	338000	205120	73341.84	67000	1243000	261516.12	205491.42	0.63

Table 4. CD45 SSC results of MMR positive and negative patients

Parameter (%)	MMR positive (n=25)				MMR negative (n=31)				
	Min	Max	Mean	Standard deviation	Min	Max	Mean	Standard deviation	p
Granulocyte	26	71	59.06	10.45	19.7	74.9	57.3	12.50	0.77
Lymphocyte	8	42.1	16.36	6.97	6	35.4	18.1	8.63	0.42
Monocyte	2.1	9.6	3.6	1.61	1.6	6.4	3.46	1.07	0.66
Normoblast	4	19.8	11.4	4.35	4	39	13.2	8.03	0.77
Blast	0	1	0.04	0.2	0	3.7	0.28	0.86	0.27

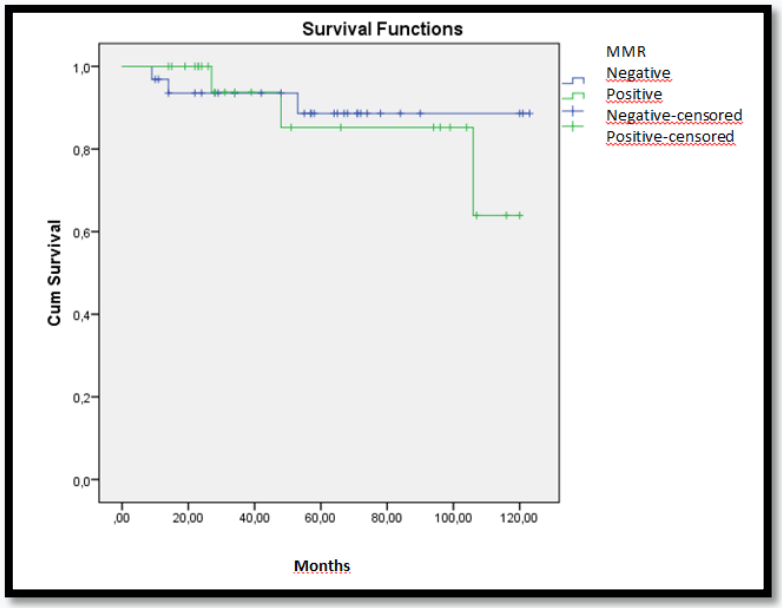

Figure 1. Survival of MMR positive and negative patients

4. Discussion

Flow cytometric analysis data in CML patients is scarce. Dybko et al (9) evaluated 54 CML patients and indicated that CD34+CD117+ cells must be eliminated through an apoptotic pathway to achieve optimal treatment response in CML patients.

Shima et al (10) demonstrated that granulocye-macrophage progenitor (GMP) predominance in CML progenitors at diagnosis and sustained expression of cMpl in bone marrow progenitors at 3 months may be predictive of poor outcome in children with chronic phase CML treated with imatinib.

Sun et al (11) demonstrated that flow cytometric analysis of phosphotyrosine levels is a reliable and convenient adjuvant technique for diagnosis of BCR-ABL positive leukemias and shows promise for serial evaluation of patients undergoing treatment.

In another study (12), the results of flow cytometry analysis showed that in patients with the acquisition of resistance to imatinib the number of CD34+ cells increases in the bone marrow and peripheral blood.

Oka et al (13) showed that decrease in CD19 positive B lymphoid cells in the blast region is associated with a poor response to imatinib. Cell compositions in the blast region in some patients were similar to those in myelodisplastic syndrome and healthy volunteers and they concluded that cell compositions in the blast region of bone marrow may be directly related to the proliferation of CML progenitors, leading to response to imatinib.

The flow cytometry with a CD45/SSC gate separates immature cells including blasts from other cells in bone marrow and is superior to flow cytometry with a forward scatter/side-scatter gate to identify small populations of the immature cells in bone marrow (14,15).

Janssen et al (16) found that in 24 of 40 newly diagnosed CML patients residual normal CD34+CD38- stem cells could be identified by lower CD34 and CD45 expression, lower forward/sideward light scatter and by differences of lineage marker expression (CD7, CD11b and CD56) and of CD90. Patients with residual non-leukemic stem cells had lower clinical risk scores (Sokal, Euro), lower hematological toxicity of imatinib and better molecular responses to imatinib than patients without.

In this study we only used CD45/SSC gate in CP CML patients because it is widely used for

determining leukemia phenotypes as well as detecting minimal residual disease in leukemia as a routine practice in flow cytometry laboratories at hospitals. Therefore, physicians can easily order the analysis of bone marrow in CML using flow cytometry with a CD45/SSC gate. Moreover,flow cytometry results are obtained within one or two days and data can be easily re-analysed if needed.

Firstly we compared the complete blood count parameters of patients at diagnosis and follow up. All white blood cell and platelet counts were decreased as expected and hemoglobin was not different. Comparison of CD45/SSC results at diagnosis and follow up revealed a decrease in granulocyte and blast percentages, and an increase in lymphocyte, monocyte, normoblast percentages. However complete blood counts and CD45/SSC results were not different when we divided patients into 2 groups according to being MMR positive or MMR negative. Survival of MMR positive and negative patients were also found similar.

In conclusion, our results were not enough to suggest using CD45/SSC as a routine diagnostic and/or follow up tool. The reasons for this result can be the limited number of patients, the variance between the time of MMR evaluation and the evaluation at only one time point. The results may change in a larger study with more CML patients, serial CD45/SSC analysis and with different tyrosine kinase inhibitors.

Acknowledgement

We would like to thank to Associate Professor Cengiz Bal from Eskisehir Osmangazi University School of Medicine Department of Statistics for the statistical analysis.

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