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DOI: 10.4103/ijh.ijh_49_22

Relation between FMS-like tyrosine kinase 3 factor and hematological parameter in acute lymphoblastic leukemia patients by flow cytometry

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Abstract:

BACKGROUND: Acute lymphoblastic leukemia (ALL) is a heterogeneous disorder that is caused by the clonal expansion of immature lymphoid cells with a high rate among children more than adults. FMS-like tyrosine kinase 3 (FLT3) is a cellular receptor belongs to the Class III receptor tyrosine kinase family. The main expression of FLT3 on bone marrow (BM) cells especially $CD34^{+}$ hematopoietic stem cells, early progenitor cells, dendritic progenitor cells, and other cells of organs (brain, placenta, and testis). Activation of FLT3 results in increased cell proliferation, decreased cell apoptosis, and inhibition of differentiation of cells. This study aims to detect the expression of the FLT3 cluster of differentiation antigen 135 (CD135) in childhood B-ALL patients. Moreover, to correlate this expression with hematological parameters include a complete blood count and BM examination findings and clinical parameters.

PATIENTS, MATERIALS AND METHODS: This study was conducted on 30 newly diagnosed pediatric ALL patients. Diagnosis of the disease was based on the blood film, BM examination findings, cytochemistry, and flowcytometry of peripheral blood (PB) and/or BM sample, 1 ml of PB and/or BM sample was collected in EDTA tubes for flowcytometry for detection of CD135.

RESULTS: This study found that male patients were more than females with a male-to-female ratio (1.14:1) and a median age of 5 years. Most of the patients had a positive expression of the FLT3 receptor and according to NCI risk groups, 60% of patients fall in the standard risk and 40% in the high-risk group. There was a significant correlation between the level of FLT3 (CD135) and age but no significant correlation with hemoglobin, white blood count, platelets, and peripheral or BM blast percentage.

CONCLUSION: In this study, the patients with positive FLT3 blast cells (which is a bad prognostic factor) were associated with good prognostic factors. This proves that FLT3 is an independent prognostic factor.

Keywords:

Childhood acute lymphoblastic leukemia, FMS-like tyrosine kinase 3, receptor tyrosine kinase

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Submission: 18-09-2022
Revised: 29-10-2022
Accepted: 31-10-2022
Published: 06-12-2022

Introduction

Childhood acute lymphoblastic leukemia (ALL) is caused by recurrent clonal chromosomal abnormalities (numerical and structural changes) in ~80% of ALL. Approximately 30% of childhood ALL patients do not have cytogenetic abnormalities, these abnormalities lead to an increase in the proliferation and differentiation of the clonal population of lymphoid cells.[1-4]

Cluster of differentiation antigen 135 (CD135) is also known as FMS-like tyrosine kinase 3 (FLT3), which is a Class III receptors

How to cite this article: Al-Ali ZS, Mohammed B. Relation between FMS-like tyrosine kinase 3 factor and hematological parameter in acute lymphoblastic leukemia patients by flow cytometry. Iraqi J Hematol 2022;11:175-81.

tyrosine kinase. When this receptor binds to the FLT3 ligand (FLT3 L), leads to the formation of a complex, in which two FLT3 molecules are bridged by one (homodimeric) FLT3 which brings the two intracellular domains in proximity to each other, resulting in initial transphosphorylation of each kinase domain, leading to phosphorylation, and activation of signal transduction molecules that transfer the signal through the cell.[5,6] The FLT3 (CD135) is about 160-KDa, Type I transmembrane glycoprotein. FLT3 is expressed in many human tissues included marrow stromal cells, $CD34^+$ stem cells, progenitor cells, human brain, placenta, and testis.[6]

Constitutive activation of the FLT3 receptor can happen in leukemia cells by two mechanisms:

1. Overexpression of FLT3 ligand (wild type FLT3), which lead to constitutive activation through autocrine, paracrine, or intracrine signaling.[7] Wild type is widely expressed in hematopoietic malignancies, including ALL with mixed lineage leukemia rearrangement and hyperdiploidy[8]
2. Mutations of the FLT3 receptor, which lead to ligand-independent autophosphorylation and activation of the FLT3 receptor. In childhood, leukemia FLT3 receptor mutations are associated with a poor prognosis.[9,10] There are two mutations: internal tandem duplications (ITDs) and kinase domain mutations. The FLT3 mutations are common in both subtypes (B and T) of childhood ALL.[11,12]

FMS-like tyrosine kinase 3 internal tandem duplication mutation

It is one of the most common mutations and leads to transcription and translation of FLT3 receptor with an elongated juxtamembrane domain. This mutated receptor can dimerize, phosphorylate, and activate the kinase domains without the need to bind the FLT3 ligand, which is called ligand-independent constitutive activation of FLT3. This may be as a result to eliminating an auto-inhibitory function of the wild-type receptor, which is involved without ligand binding and there is no dimerization and kinase domain activation.[13]

Point mutations in FMS-like tyrosine kinase 3

They are the second most common type of FLT3 mutations (less common than ITD mutation) involving point mutations in the activation loop of FLT3, lead to constitutive activation of FLT3 which is inhibited by FLT3 inhibitor.[14,15]

The most common activating point mutation is the sub-situation of tyrosine for aspartic acid at position 835 within the activation loop of the kinase domain. Point mutations at other positions, such as 836 or 841, all are associated with FLT3 L-independent activation.[15,16]

FLT3 has a serious role in the survival, proliferation, and differentiation of cells. FLT3 is expressed in acute myeloid leukemia in about 89%. Furthermore, its high expression is found in other hematologic malignancies such as B-cell precursor ALL (94%), a fraction of T-cell ALL (32%), and chronic myelogenous leukemia in lymphoid blast crisis.[17-19]

Patients, Materials and Methods

This cross-sectional study was done on 30 newly diagnosed ALL male and female pediatric patients with median age of 5 years old from Central Teaching Hospital of Pediatrics and Welfare Children Teaching Hospital.

The diagnosis depends on complete blood count (by Sysmex), morphology, cytochemistry of peripheral blood (PB) and/or bone marrow (BM) sample examined by hematopathologist in the laboratory of hospital, and on flow cytometry study which was done at private laboratory or flowcytometry Department in Medical City in Baghdad.

From each patient after admission, about 1 ml of PB and/or 1 ml of BM sample were collected in EDTA tubes for flow cytometry analysis (the marker CD135 was used in this study).

After the diagnosis of ALL have done by the flow cytometry Department in the Medical City of Baghdad using four-color BD Biosciences fluorescence activated cell sorting (BD FACS) for detection of CD19, CD79a, CD22, and cytoplasmic immunoglobulin. About 1 ml of PB sample and/or BM sample from each patient were transferred to private laboratory in the cool box within 6–8 h after collection for detection of surface markers CD19 and CD135 using four-color (Partec CyFlow® Cube 6, Germany). Gating of the cells was done depending on CD19/ Side scatter (SSC) gate.

Positive expression of FLT3 is considered when >20% of lymphoblast cells expressed CD135 (expression of most membrane antigens is positive if >20% of gated events are positive)[20] [Figure 1].

Ethical consideration

This study was approved by the review ethical committee of Iraqi council for medical specialization. Written informed consent was obtained from parents of children who enrolled in the study.

Statistical analysis

Statistical analysis was carried out using Statistical Package for Social Sciences (SPSS) version 23, Released 2015. (IBM SPSS Statistics for Windows, Version 23.0. Armonk, NY: IBM Corp). Data were presented in

the form of numbers and percentages (qualitative) and in the form of mean ± standard deviation (SD) and median (quantitative). The association between categorical variables was analyzed by Fisher's exact tests. $P < 0.05$ is considered statistically significant.

Results

This study included 30 pediatric patients newly diagnosed ALL included 16 males (53.3%) and 14 females (46.7%) with a median age of 5 years at the time of diagnosis, range from 0.17 to 13, and interquartile range (IQR) is 5.5.

The patient in this study divided into two groups according to National cancer institute (NCI) risk groups into high risk (HR) which is included age <1 or >10 years or WBC count >50,000/μL 40% (12 patients) and standard risk (SR) which is included age 1-10 years and WBC count <50,000/μL 60% (18 patients) , as shown in [Figure 2].

The study involved comparison between high NCI risk groups (HR) and SR groups in age and laboratory parameters showed significant statistically differences between HR and SR only in white blood count ($P = 0.009$), while there were no statistically significant differences founded between male and female regarding age distribution and laboratory parameters, as shown in Table 1.

The patients in this study are divided into two groups (group 1 with positive expression FLT3 (CD135) which are 27 patients and group 2 of 3 patients with negative expression), patients with positive expression associated with many favorable prognostic factors as shown in Table 2.

Table 1: Comparison between NCI risks groups (high risk and standard risk) in regard to age and hematological parameters

Parameter	Mean±SD	HR (*n*=12)	SR (*n*=18)	*P*
Age (years)	Mean±SD	7.74±5.22	4.75±2.12	0.158
	Median	9.0	4.0	
WBC (×10³/μL)	Mean±SD	39.77±23.74	17.67±16.49	0.009
	Median	44.38	10.8	
Hb (g/dL)	Mean±SD	6.46±1.01	7.39±1.77	0.158
	Median	6.59	7.31	
PLts (×10³/μL)	Mean±SD	66.06±49.97	71.71±82.86	0.662
	Median	41.4	44.75	
Blast PB (%)	Mean±SD	70.92±24.5	53.78±28.96	0.146
	Median	83.0	58.5	
Blast BM (%)	Mean±SD	90.25±7.47	85.28±18.13	0.983
	Median	92.5	92.0	
CD135 (%)	Mean±SD	51.91±21.63	43.01±20.75	0.267
	Median	53.63	39.13	

SD=Standard deviation, HR=High risk, SR=Standard risk, WBC=White blood cell, Hb=Hemoglobin, PLts=Platelets, PB=Peripheral blood, BM=Bone marrow, CD135=Cluster of differentiation antigen 135, IQR=interquartile range, NCI=National Cancer Institute

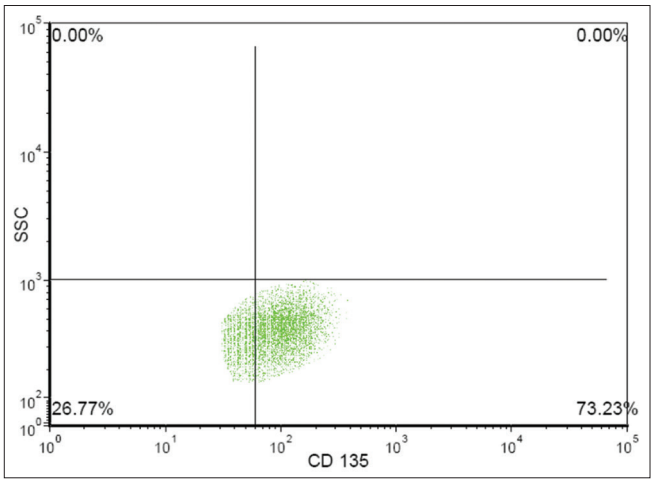

Figure 1: Flowcytometric dot plot shows positive CD135. CD135 = Cluster of differentiation antigen 135

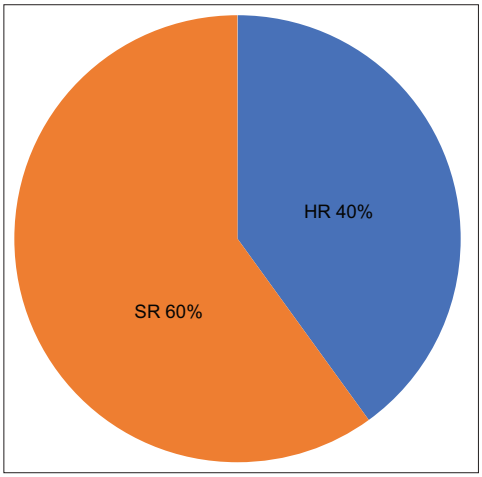

Figure 2: Classification of patients according to NCI risk groups. N (HR)=12; N (SR)=18 NCI=National cancer institute

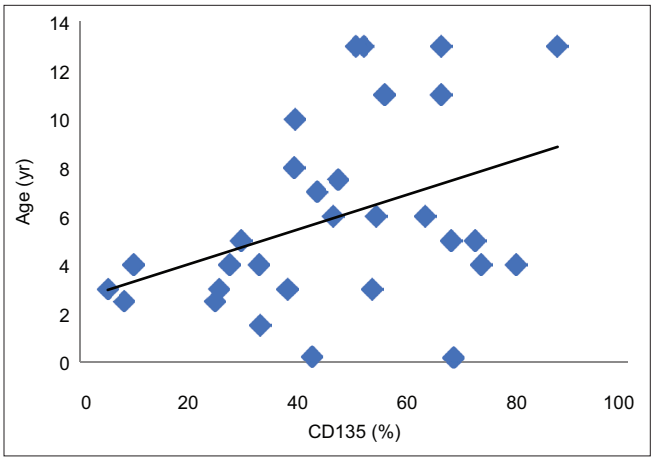

Figure 3: Correlation between age and CD135%. $r = 0.390$; $P = 0.033$

Table 2: Description of FMS-like tyrosine kinase 3 expression with many parameters

Parameters	FLT3-positive patients (*n*=27)	FLT3-negative patients (*n*=3)
Age (years)		
<1	2	0
1–9	18	3
≥10	7	0
Sex		
Male	13	3
Female	14	0
WBC (×10³/μL)		
<50	22	2
≥50	5	1
Hb (g/dL)		
<8	18	3
8–10	7	0
>10	2	0
PLts (×10³/μL)		
<50	16	1
50–100	5	2
>100	6	0
Blast PB (%)		
<90	24	2
≥90	3	1
Blast BM (%)		
<90	8	0
≥90	19	3
LAP		
Present	13	1
Absent	14	2
HSM		
Present	21	3
Absent	6	0
Fever		
Present	20	2
Absent	7	1
Bone pain		
Present	6	1
Absent	21	2
FAB classification		
L1	14	3
L2	13	0
NCI risk groups		
HR	11	1
SR	16	2

HR=High risk, SR=Standard risk, WBC=White blood cell, Hb=Hemoglobin, PLts=Platelets, PB=Peripheral blood, BM=Bone marrow, FLT3=FMS-like tyrosine kinase 3, LAP=Lymphadenopathy, HSM=Hepatosplenomegally, FAB=French-American-British, NCI=National Cancer Institute

There was a significant positive correlation between CD135 and age ($P = 0.033$, $r = 0.390$), as shown in Figure 3. However, there was no significant correlation between CD135 and other parameters, as shown in Figures 3-8 and Table 3.

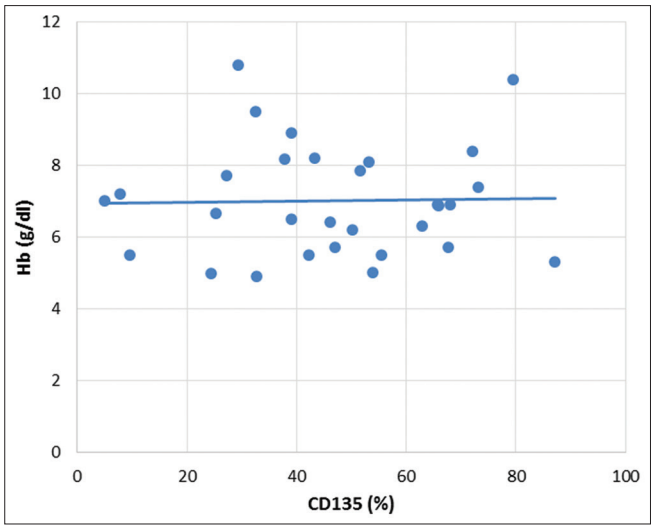

Figure 4: Correlation between hemoglobin and CD135%. $r = 0.021$, $P = 0.91$

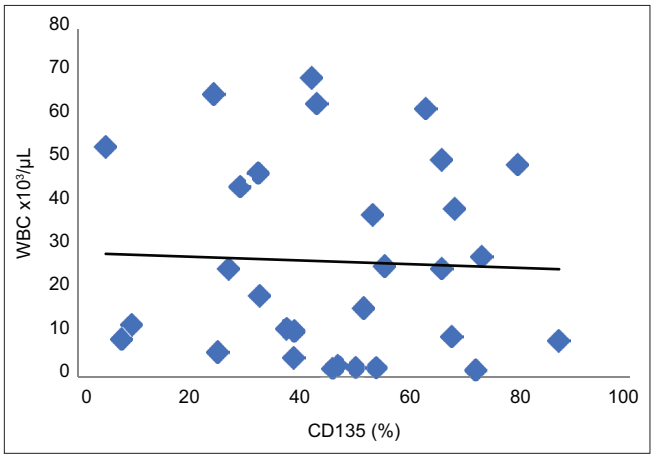

Figure 5: Correlation between white blood cell count and CD135%. $r = -0.041$, $P = 0.828$

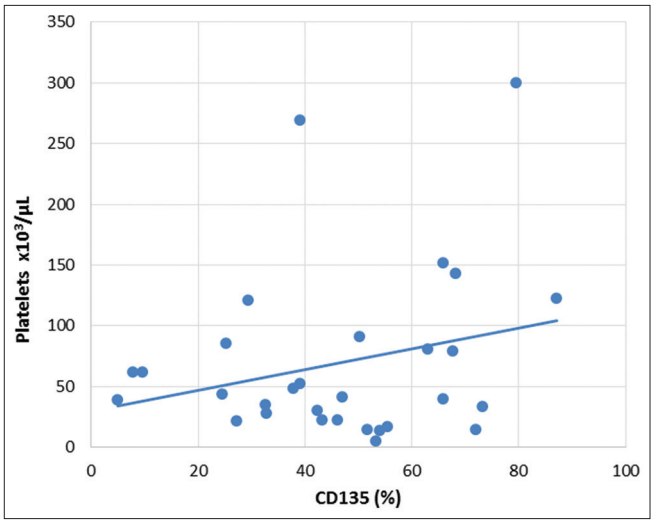

Figure 6: Correlation between platelets count and CD135%. $r = 0.257$, $P = 0.170$

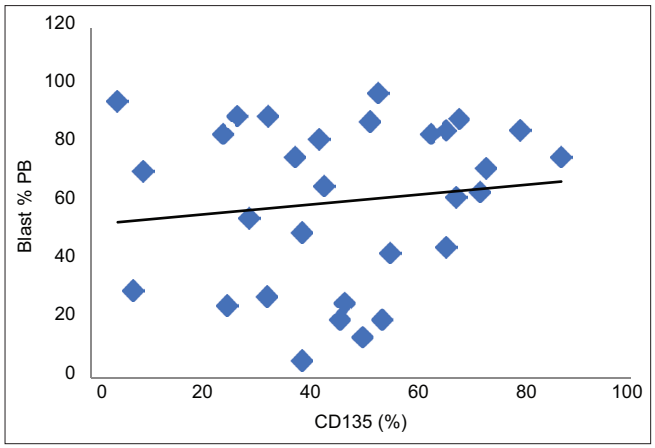

Figure 7: Correlation between blast % in peripheral blood and CD135%. $r = 0.128$, $P = 0.501$

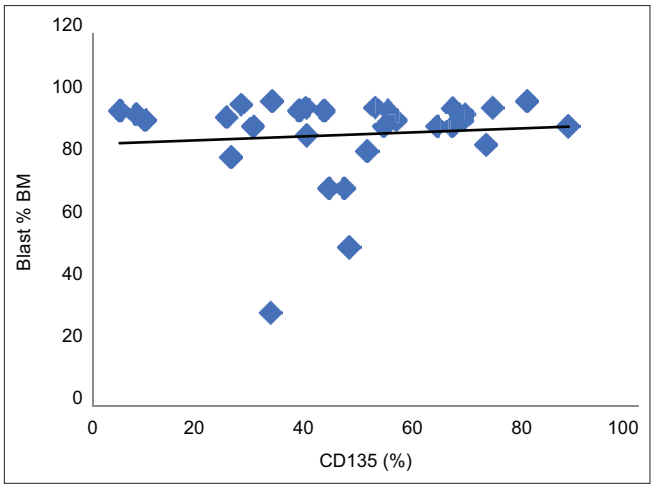

Figure 8: Correlation between blast % in bone marrow and CD135. $r = 0.092$, $P = 0.627$. CD135 = Cluster of differentiation antigen 135

Table 3: Correlation of cluster of differentiation antigen 135 with hematological parameters

Parameter	CD135 (%)	
	r	*P*
Age (years)	0.390	0.033
WBC (×10³/μL)	−0.041	0.828
Hb (g/dL)	0.021	0.911
PLts (×10³/μL)	0.257	0.170
Peripheral blast (%)	0.128	0.501
BM blast (%)	0.092	0.627

WBC=White blood cell, Hb=Hemoglobin, BM=Bone marrow, CD135=Cluster of differentiation antigen 135, PLts=Platelets

Discussion

FLT3 or CD135 has an important role in the pathogenesis of leukemia[19] and it may be consider independent prognostic factor.[21,22] The median (IQR) expression of CD135 in this study is 46.57% (34.06) with a wide range of about 5.01–87.13. The patients with positive expression of CD135 are (27) 90% of total child patients (total patients in this study are 30 pediatric patients) while patients with negative expression of CD135 are only (3) 10% of total patients, this observation is in line with the study[6] but incompatible with these studies,[22,23] the small size of patients may be the cause.

There are 18 patients with positive expression CD135 with a good prognostic age range (1–9) years and only 9 of them with poor prognostic age (<1 year or ≥10 years), these findings were also founded in the study.[23] It means that the positive expression of CD135 is associate with favorable age range. The median age (IQR) of patients in this study is 5 years (5.5) and range from (0.17-13) years which are also compatible in other studies.[23-26] but differed from another with higher (7yr) or lower median age (4yr).[27-30] These differences because age reproduces the different underlying biology of ALL which differs between populations.[31]

These patients with positive expression of CD135 (27) consist of 14 females and 13 males. This finding is opposite to the results of other study.[23] Gender is considered as one of the prognostic factors of childhood leukemia. In the present research 16 males (53.3%) and 14 females (46.7%) with male to female ratio about 1.14:1 (males were predominant) were founded, these findings are compatible to other studies.[23,32,33] Male is one of unfavorable factor for detection of outcome of childhood leukemia and more likely to have other unfavorable prognostic factors such as (T- cell subtype, high WBC count and age of 10 or more).[34,35]

According to hematological parameters, 21 patients in this study with positive CD135 have WBC count (<50,000/μL) and only 6 patients of them have WBC count (≥50,000/μL) compatible to study.[23] This means that the favorable feature of WBC count is predominant in our patients as founded in other studies.[36-38] The median Hb level and platelets count of total patients are (6.89g/dL) and (42.3x 10^3/μL) respectively. Anemia and thrombocytopenia in childhood ALL result mainly from bone marrow suppression by infiltration of blasts but there is no significant relation between hemoglobin level and platelets with remission rate of childhood ALL.[39-41] In this study no statistically significant differences between males and female in clinical and hematological parameters.

Most of patients with positive CD135 expression are associate with hepatosplenomegaly and fever as mentioned in Table 2.

It is found that significant positive correlation between age and CD135%, this finding is like study.[23] However,

no significant correlation between CD135% and the percentage of blasts in BM, opposite to others[32] which have a significant positive correlation between CD135% and blast % in BM, also there is no significant correlation between CD135% and Hb, PLts, and WBC count or blast % in PB. In this study, 16 patients with positive expression CD135 are with standard NCI risk (SR) and 11 patients of them are with high NCI risk (HR). Majority of patients in the study are with standard NCI risk group (SR) this compatible with other studies[24,26,37,42], there is statistically significant difference between high risk (HR) and standard risk (SR) regarding to total WBC count (*P* value 0.009) but no any statistically significant difference between HR and SR regarding to age, gender, clinical parameters and other laboratory parameters with no available studies like our findings, as shown in [Table 1].

It is noticed that there is a relation between positive expression of CD135 and good prognostic factors in ALL (age between 1 and 9 years, female, WBC count <50,000/μL, and standard NCI risk group); despite the role of CD135 in the pathogenesis of ALL, this mean that the FLT3 (CD135) may be act as an independent prognostic factor.

The limitation in this study is related to the small number of patients and the assessment of FLT3 (CD135) expression is not studied in relation to response to therapy, this limitation is because of time and financial restrictions that associate with repeating the assessment of marker in large number of patients and at different phases of treatment.

Conclusion

The research suggested that ALL cases with positive CD135 expression on blast cells are more common than ALL cases with negative expression. There is a significant positive correlation between CD135% and the age of patients but no significant correlation between CD135% and WBC count, PB, and BM blast percentage. Interestingly enough, the patients with positive expression of FLT3 are associated with favorable prognostic factors as age (1–9) years, gender (female), WBC count (<50,000/μL), and lastly, NCI risk group (SR) due to the FLT3 may be act as an independent prognostic factor.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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