

Peripheral FLT-3 ligand levels as a pathobiological parameter during the clinical course of acute myeloid leukemia

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Background/aim: FLT-3 ligand is a growth factor affecting the hematopoietic lineage. The aim of this study was to evaluate the variability of peripheral FLT-3 ligand during the clinical course of acute myeloid leukemia (AML) patients.

Materials and methods: Twenty-four patients were enrolled in this study in order to assess alterations in the circulating levels of FLT-3 ligand during the clinical course of AML.

Results: We studied the association in the diagnostic period between the FLT-3 ligand and peripheral blood cells together with serum electrolytes. FLT-3 ligand levels (pg/mL) during the aplastic period due to remission induction and consolidation were higher than the levels at initial diagnosis. On the other hand, the diagnostic and remission induction values of leukocytes and FLT-3 ligand showed an inverse association. These results indicate to us that higher white cell counts are associated with lower FLT-3 ligand levels. We also found a reversed association between FLT-3 ligand and serum lactate dehydrogenase level. However, there was no association between FLT-3 ligand and other serum electrolyte levels. We also found higher FLT-3 ligand levels in male patients.

Conclusion: Our study demonstrates the inverse proliferative action of FLT-3 ligand on the early myeloid lineage. In addition, this study showed us that FLT-3 receptor inhibition during chemotherapy-induced aplasia causes a compensative ligand overexpression.

Key words: Acute myeloid leukemia, FLT-3 ligand

1. Introduction

Acute myeloid leukemia (AML) is a process of increased myeloid cells in the bone marrow with maturation arrest. The progress of this situation leads to hematopoietic insufficiency (granulocytopenia, thrombocytopenia, or anemia), with or without leukocytosis (1). The progression of the leukemic blast cells is regulated by exogenous and endogenous human growth factors (2,3).

The FLT-3 ligand plays a role in complex cytokine interactions in the process of proliferation and differentiation of various hematologic periods while it is a router in early cell interactions (4–6). The FLT-3 receptor is a transmembrane protein that reaches its biologically active form by proteolytic lysis. It was suggested that with its proliferative activity, the FLT-3 ligand also has an antitumor effect (7). The FLT-3 ligand is an earlier releaser of the progenitor cells from the stem cell pool according to GM-CSF and G-CSF actions, which occur later. Additionally, a study on mouse models showed that the FLT-3 ligand increases the blood amount of CD34 cells.

These features gave rise to the idea that recombinant FLT-3 ligand could be used in bone marrow transplantation to evoke cell mobilization. Similarly, some authors theorized that the FLT-3 ligand could be used in many pathological diseases such as Fanconi and aplastic anemia that originated from stem cell defects (8).

FLT-3 ligand binding to the tyrosine kinase receptor system and the juxtamembranous region with the activation of the kinase domain mutation of most AML and acute lymphoblastic leukemia (ALL) patients was identified. Initial studies showed some benefit of FLT-3 inhibition in high-risk AML patients (9).

The aim of this study was to evaluate the variability of peripheral FLT-3 ligand during the clinical course of AML patients. In this context, the change of some critical parameters in the clinical course of AML such as white cell, platelet, and electrolyte levels and infection, age, and sex were analyzed according to the differentiation of the FLT-3 ligand. While FLT-3 receptor mutations developed in patients with AML are known to be associated with

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poor prognosis in younger patients, the effect of outside ligand stimulation is not fully understood.

Due to its critical role in the pathogenesis of AML cell proliferation, understanding the critical role of the FLT-3 ligand serves the objective of the development of targeted therapeutic approaches.

2. Materials and methods

This study was conducted with 24 AML patients who were diagnosed in the Hacettepe University Hematology Department between 2002 and 2003. Informed consent from all patients and ethical permission (Hacettepe University Ethical Committee) were obtained. The inclusion criteria were:

- 1- A new diagnosis of AML or a myeloid transformation of chronic myeloid leukemia.
- 2- Patient provision of informed consent.

Exclusion criteria were:

- 1- Patients with renal failure (creatinine value over 1.5 mg/dL).
- 2- Patients with abnormality in liver function tests (alanine transaminase higher than 1.5 times the normal value).
- 3- Patients who did not provide informed consent.

Plasma FLT-3 ligand analysis was performed with the sandwich enzyme immunoassay technique (Quantikine, R&D Systems; Minneapolis, MN, USA; Catalog Number: 308-DFK00). All blood samples were collected in the morning hours to avoid possible diurnal variation, although there is no standard literature about the diurnal variation of the Flt-3 ligand. Remission-induction therapy was administered to AML patients as cytarabine at 200 mg mm⁻² day⁻¹ (7 days) + idarubicin at 13 mg mm⁻² day⁻¹ (3 days) and consolidation treatment was performed with cytarabine at 3 g m⁻² every 12 h (days 1, 3, and 5). Only nine of the patients' FLT-3 ligand evaluations during the treatment periods could be completed. Blood samples were collected during 5 periods: 1- first diagnosis, 2- aplastic term (at the time of lowest platelet count) of idarubicin + cytarabine chemotherapy, 3- before consolidation treatment (during normal cell count), 4- cytopenic period of the consolidation treatment, and 5- after the consolidation treatment.

Statistical analysis was performed with SPSS 10. Because of the wide range of measurements and the low number of patients, nonparametric tests were preferred in our analysis. Friedman analysis was used to compare the FLT-3 ligand values for the same group in different time periods. Analysis of different groups was performed with the Wilcoxon test. Spearman analysis was used to test FLT-3 ligand variation and white cell, platelet, and hemoglobin change. The Mann-Whitney U test was used to measure the differences of sex and body temperature variation.

3. Results

There were 24 newly diagnosed AML patients in our study. Diagnostic FLT-3 ligand, white cell, and platelet levels were measured for all patients. Serum lactate dehydrogenase (LDH) and electrolyte levels were also measured at diagnosis in the study group. Demographic characteristics and the AML subtypes of patients are summarized in Table 1. Only nine of the patients' FLT-3 ligand evaluations during the treatment periods could be completed.

At the time of diagnosis, the median FLT-3 ligand value was 139 pg/mL (minimum: 6.88 pg/mL; maximum: 1419 pg/mL; interquartile range (IQR): 41.94). The median white cell value was 12 × 10³/µL (minimum: 3 × 10³/µL; maximum: 211.6 × 10³/µL; IQR: 41,750). Median platelet count was 40.5 × 10³/µL (minimum: 11 × 10³/µL; maximum: 455 × 10³/µL; IQR 62,750). FLT-3 ligand values at the time of diagnosis are shown in Figure 1.

The median FLT-3 ligand value during remission-induction treatment during aplasia was 877 pg/mL (minimum: 9.18 pg/mL; maximum: 2211 pg/mL). The median platelet and white cell counts were respectively 14 × 10³/µL (minimum: 5 × 10³/µL; maximum: 25 × 10³/µL) and 0.7 × 10³/µL (minimum: 0.2 × 10³/µL; maximum: 1.8 × 10³/µL) in the same time period.

After the remission-induction period, median FLT-3 ligand value was 61.9 pg/mL (minimum: 35.3 pg/mL; maximum: 127 pg/mL). Median platelet and white cell counts were respectively 277 × 10³/µL and 7.1 × 10³/µL.

Table 1. The demographic specifications of AML patients.

	Study group
Age (years)	
Mean ± SD	49.92 ± 3.63
Median	50.5
Distribution range	19-90
Sex	
Male	12
Female	12
AML type (FAB)	
AML-M1	1
AML-M2	14
AML-M4	4
AML-M5	1
CML blastic transformation	3
Biphenotypic	1

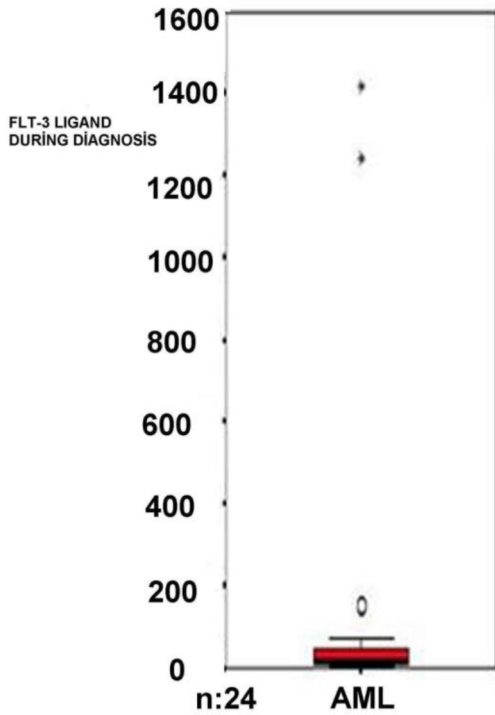


Figure 1. FLT-3 ligand value at the time of diagnosis.

The median FLT-3 ligand value during consolidation thrombocytopenia was 1812 pg/mL (minimum: 234.8 pg/mL; maximum: 2244 pg/mL). The median white cell count was $1.1 \times 10^3/\mu\text{L}$ (minimum: $0.3 \times 10^3/\mu\text{L}$; maximum: $3 \times 10^3/\mu\text{L}$) with $15 \times 10^3/\mu\text{L}$ median platelet count. The median FLT-3 value after consolidation was 68.5 pg/mL (minimum: 43.89; maximum: 231.1 pg/mL). The median value of white cell count was $4.6 \times 10^3/\mu\text{L}$ (minimum: $3.1 \times 10^3/\mu\text{L}$; maximum: $17.7 \times 10^3/\mu\text{L}$). Median platelet value was $200 \times 10^3/\mu\text{L}$ during the same treatment period. The median value trends of white cells, platelet count, and FLT-3 ligand during chemotherapy periods of AML patients are shown in Table 2.

There was no relation between age and diagnostic value of the FLT-3 ligand ($P = 0.862$). The diagnostic FLT-

3 ligand value of male subjects was significantly higher than that of the females ($P = 0.009$) (median FLT-3 for males: 16.25 pg/mL; for females: 8.75 pg/mL). During diagnosis, 12 patients had a coincidental infection while the others had no sign of infection. There was no statistical relationship between infection and diagnostic FLT-3 value. There was also no relation between fever and diagnostic FLT-3 value ($P = 0.788$).

There was a reverse relationship between white cell count and diagnostic FLT-3 ligand value ($P = 0.014$). When we analyzed the values of the 9 AML patients who could be followed during all treatment periods, there was a negative correlation between white cell and FLT-3 value ($P = 0.024$; $r = -0.736$). There was also a relationship between diagnostic FLT-3 and platelet count ($P = 0.035$). When we compared the diagnostic FLT-3 with the other treatment periods' measurements there was a significant increase in consolidation and remission-induction values ($P = 0.008$) (Figure 2).

The analysis of electrolytes such as Na, K, P, and Ca showed no relation with diagnostic FLT-3 ligand measurements. On the other hand, there was a negative relation between FLT-3 ligand and LDH values ($P = 0.03$).

4. Discussion

This study, which measured the level of FLT-3 ligand during different clinical courses of AML, also analyzed the relationship of diagnostic ligand grade with age, sex, electrolytes, and some critical blood parameters. There was a reverse relationship between diagnostic and remission-induction white cell count and FLT-3 ligand measurements. The same situation was valid for diagnostic LDH measurement. There was an elevation of FLT-3 ligand level in the remission and consolidation phases according to the diagnostic measurement. Another statistically significant point was higher diagnostic ligand levels in male subjects.

Considering the clonal characteristics of AML cells, the FLT-3 receptor and its ligand activity seem to be closely associated with prognosis and serial quantities of leukemic cells. The FLT-3 receptor activating mutations

Table 2. The median value trends of white cells, platelet count, and FLT-3 ligand during chemotherapy periods of AML patients.

	FLT-3 ligand (median)	Platelets (median)	White cell count (median)
At diagnosis (n = 24)	139 pg/mL	$40.5 \times 10^3/\mu\text{L}$	$12 \times 10^3/\mu\text{L}$
Aplasia (n = 9)	877 pg/mL	$14 \times 10^3/\mu\text{L}$	$0.7 \times 10^3/\mu\text{L}$
Before consolidation (n = 9)	61.9 pg/mL	$277 \times 10^3/\mu\text{L}$	$7.1 \times 10^3/\mu\text{L}$
Aplasia of consolidation (n = 9)	1812 pg/mL	$15 \times 10^3/\mu\text{L}$	$1.5 \times 10^3/\mu\text{L}$
After consolidation (n = 9)	68.5 pg/mL	$200,000 \times 10^3/\mu\text{L}$	$4.6 \times 10^3/\mu\text{L}$

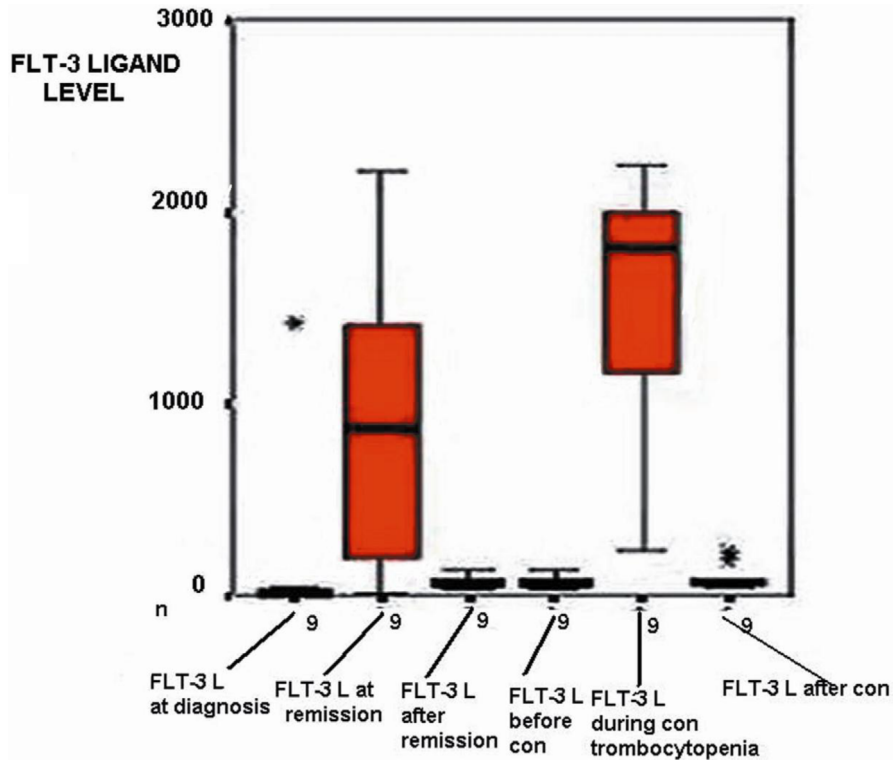


Figure 2. The FLT-3 ligand variation of 9 patients during the treatment period. con: Consolidation; n: number.

in AML could be related with white cell number in that disease. The level of the ligand used in this study is in the opposite direction of the activation of the receptor, which is associated with low white blood cell count. These results are statistically significant, especially in the early stages of treatment. It is possible that after reaching a stable serum level activity, FLT-3 ligand concentration could be compensatively elevated to overcome the depressed FLT-3 receptor function. FLT-3 ligand elevation could be used as an opposing factor of white cell proliferative activity in early-stage AML patients.

An interesting point of our study is the elevated blood concentration of FLT-3 ligand in the pancytopenic periods of the treatment phases. This could be the result of the stem cell activity to increase blood cell amount. This is in support of the FLT-3 ligand usage in stem cell transplantation.

The FLT-3 ligand has the potential to stimulate both the unmutated and the mutated FLT-3 receptor cells. This stimulation is carried out with a Lyn phosphorylation mechanism. AML cells are also stimulated by FLT-3 ligand with similar mechanisms. The same proliferative responses through phosphorylation of Lyn are observed in wild-type and mutated FLT-3 receptors (10). Our study is not divided according to the patients' receptor mutation

status. In general, the overall FLT-3 ligand levels of AML patients were analyzed during the course of the study.

Sato et al. (11) examined in vivo FLT-3 ligand inhibition in AML patients treated with chemotherapy followed by the FLT-3 inhibitor lestaurtinib. They also tried to explore the effect of FLT-3 ligand concentration on the response of therapy. After intensive chemotherapy, FLT-3 ligand levels increased to a mean of 488 pg/mL on day 15 of induction cure for newly diagnosed patients, whereas they rose to a mean of 1148 pg/mL in the relapsed patients. The FLT-3 ligand level rose progressively in the followed courses. They concluded that FLT-3 ligand elevation could be an obstacle to FLT-3 inhibition. This dramatic increase in the successive courses supported our FLT-3 ligand rise during periods of aplasia (11).

Levis et al. (12) performed a randomized trial for FLT-3 mutant AML in first relapse. Patients received chemotherapy alone or followed by 80 mg of the FLT-3 inhibitor lestaurtinib twice a day. There was no difference between control and lestaurtinib treatments according to overall survival. In the lestaurtinib arm, FLT-3 inhibition was excessively correlated with remission rate, but target inhibition on day 15 was achieved in only 58% of patients receiving lestaurtinib. They also observed a rise of the FLT-3 ligand throughout the course of therapy. At first

the measured FLT-3 ligand was less than 20 pg/mL at baseline. During the aplasia period, there were markedly elevated FLT-3 ligand levels in all patients. The activity of lestaurtinib was blunted in this aplasia course. They also supported, in accordance with our study, the elevation of FLT-3 ligand in the following treatment periods of AML (12).

LDH activity in AML is increased as a parameter correlated with the disease severity and appears to decrease with AML activity (13). FLT-3 ligand and serum LDH levels are in an inverse relationship in our study. In contrast to high FLT-3 ligand levels during aplasia, a decreased LDH level was observed in the same time period. According to

these results the FLT-3 ligand could be a relevant marker of AML activity inversely proportional to LDH level.

In conclusion, it may be mentioned that the decrease of the white cell count during the treatment course of AML resulted in a reciprocal FLT-3 ligand overexpression. There was no relation between FLT-3 ligand level and red cell count together with platelet count. The diagnostic ligand level was higher in male patients. There was no relationship between age and diagnostic ligand level. An interesting point of our study was the inverse relation between LDH and ligand level. Our study supports the hypothesis that FLT-3 receptor inhibition during chemotherapy-induced aplasia causes a compensative ligand overexpression.

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