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FLT3 internal tandem duplication and FLT3-D835 mutation in 80 AML patients categorized into cytogenetic risk groups

Wewnętrzna tandemowa duplikacja oraz mutacja D835 genu FLT3 u 80 pacjentów z AML w trzech cytogenetycznych grupach ryzyka

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Summary

Background: Acute myeloid leukemia (AML) is a clonal disorder characterized by various genetic abnormalities and variable response to treatment. About 50% of patients with AML have no cytogenetic aberrations, presenting normal karyotype, and are categorized in the intermediate risk group. In this group detection of FLT3 mutations move a patient from the intermediate to the adverse risk group.

Material/Methods: Bone marrow from 80 AML patients was cultured to obtain chromosome slides and then karyotype. Simultaneously DNA was isolated from bone marrow and PCR reaction was conducted to test the FLT3 mutation status (ITD and D835). For statistical analysis Chi squared test was used.

Results: From the group of 80 AML patients seven were classified as a favorable risk group and FLT3/ITD was found only in one of these patients (14.28%), and FLT3/D835 in another one (14.28%). Fifteen patients showed a complex karyotype with more than three aberrations or with any aberration known as a poor prognosis. Among the adverse group FLT3/ITD was detected in three patients (20%) and D835 mutation in two other patients (13.33%). Among 58 patients with normal karyotype in GTG banding FLT3/ITD occurred in six cases (10.34%) and D835 mutation in two cases (3.45%). No significant difference was found among these three risk groups regarding presence or absence of FLT3/ITD and FLT/D835.

Discussion: Molecular characterization of mutations in several genes, such as FLT3, NPM1, MLL, CEBPA, in acute myeloid leukemia, especially in normal karyotype cases, could be another factor after cytogenetic analysis to stratify AML patients into different prognostic categories.

Key words: **acute myeloid leukemia • cytogenetic • FLT3-ITD internal tandem duplication**

Streszczenie

Wstęp: Ostra białaczka szpikowa (AML – acute myeloid leukemia) jest klonalną chorobą rozrostową charakteryzującą się wieloma genetycznymi zaburzeniami i różną odpowiedzią na leczenie. Prawie 50% pacjentów z AML nie ma w kariotypie żadnych aberracji chromosomowych i są oni zaliczani do grupy pośredniego ryzyka. W grupie tej wykrycie mutacji genu FLT3 powoduje przeniesienie chorego z grupy pośredniego ryzyka do grupy złego rokowania.

Cel: Przebadano grupę 80 pacjentów z AML z ustalonym kariotypem na obecność mutacji genu FLT3: ITD (ITD – internal tandem duplication) oraz D835.

Materiał/Metody: Szpik kostny od 80 pacjentów z AML został poddany hodowli *in vitro* w celu uzyskania preparatów chromosomowych, a następnie kariotypowania. Jednocześnie izolowano DNA w celu przeprowadzenie reakcji PCR do wykrycia mutacji genu FLT3: ITD oraz D835. Do analizy statystycznej użyto testu Chi2.

Wyniki: Wśród 80 chorych z AML 7 pacjentów zaliczono do grupy korzystnego rokowania na podstawie kariotypu, a mutacja FLT3/ITD wystąpiła tylko u jednego pacjenta (14,28%), podobnie jak mutacja D835 (14,28%). Piętnastu pacjentów reprezentowało kariotyp złożony z trzech lub więcej aberracji lub aberracjami uznanymi jako źle rokujące. W grupie tej mutację FLT3/ITD wykryto u trzech pacjentów (20%), a FLT3/D835 u dwóch (13,33%). Grupa 58 pacjentów miała kariotyp prawidłowy (po zastosowaniu techniki GTG), a mutacja FLT3/ITD wystąpiła w sześciu przypadkach (10,34%) i FLT3/D835 w dwóch (3,45%). Nie stwierdzono statystycznych różnic w trzech grupach rokowniczych w zależności od występowania mutacji FLT3/ITD oraz D835.

Dyskusja/Wnioski: Pogłębienie badań molekularnych nad mutacjami w genach FLT3, a także NPM1, MLL, CEBPA w ostrej białaczce szpikowej, zwłaszcza w przypadkach gdy kariotyp jest prawidłowy może się przyczynić do ustalenia kolejnych czynników rokowniczych, po badaniach cytogenetycznych, niezbędnych w diagnostyce i leczeniu AML.

Słowa kluczowe: **ostra białaczka szpikowa • mutacja genu FLT3**

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Background

Acute myeloid leukemia (AML) is a clonal disorder characterized by various genetic abnormalities and variable response to treatment. In AML cytogenetic methods are used to stratify patients into three different risk groups: good, intermediate and poor [1]. Unfortunately about 40–50% of AML cases represent normal karyotype (NK) with intermediate prognosis [10]. Due to this fact, molecular genetic investigations are of increasing importance. The discovery of an internal tandem duplication in the FLT3 gene was a significant step in the process of understanding the genetic background of AML.

The FLT3 gene encodes the tyrosine kinase receptor critical for normal hematopoiesis. There are two types of FLT3 mutations: an internal tandem duplication (ITD) and a point mutation (D835), which both have constitutively active tyrosine kinase, thereby promoting proliferation and inhibiting apoptosis in leukemic cells [5]. Approximately 20% to 30% of AML cases carry an FLT3-ITD mutation and 8% to 12% an FLT3-D835 mutation [11]. AML patients with an FLT3-ITD mutation appear to have poorer clinical outcome, while the significance of FLT3-D835 is still not clear. Not only the presence of FLT3-ITD affects a poor prognosis but also the size of the duplicated fragment and the number of duplicated copies [8]. Surprisingly, some studies have also shown that AML patients without the FLT3-ITD mutation but with over-expression of FLT3 also had an unfavorable prognosis [9].

In our study we investigated the status of FLT3 (ITD and D835 mutations) in 80 adult AML patients categorized into cytogenetic risk groups. There is limited information in the literature about the frequency of FLT3 mutation in Polish patients (adult and pediatric) with AML.

Material and Methods

Samples of bone marrow from 80 adult AML patients were collected in the Department of Medical Diagnostics between 2006 and 2009. In order to obtain chromosome

slides a standard protocol was applied and bone marrow was cultured *in vitro* for 24 hours. Then slides were banded using a GTG technique and karyotyping was performed. For fluorescent *in situ* hybridization (FISH) with Kreatech probes t(8;21) AML/ETO, t(15;17) PML/RARa and inv16/t(16;16) CBFB slides were obtained from uncultured cells. Microscopic analysis was conducted using Zeiss Imager D1 with Metasystems software (Ikaros and Isis v 5.3.3.). Twenty GTG metaphases for karyotype and 200 interphase nuclei were analyzed for each probe in the FISH experiment.

For FLT3 analysis DNA was isolated from bone marrow cells using NucleoSpin Blood kit (Macherey-Nagel). Then polymerase chain reaction was conducted using FLT3 Mutation Assay kit (InVivoScribe Technologies).

For statistical analysis Chi squared test was used.

Results

From the group of 80 AML patients (43 men and 37 women; range 18-60 years) seven were classified as a favorable group risk with karyotypes presenting t(8;21) (cases 2 and 6), t(15;17) (case 1) and inv16 (cases 3, 4, 5 and 7). FLT3/ITD was found only in one of these patients (case 1) (14.28%), and FLT3/D835 in another one (case 2) (14.28%) (table 1). Fifteen patients showed a complex karyotype with more than three aberrations or with an aberration known as a poor prognosis (such as t(6;9) in case 77 or isolated tetrasomy 13 in case 78). Among the adverse group FLT3/ITD was detected in three patients (20%) and D835 mutation in two other patients (13.33%). The most numerous group was with intermediate prognosis according to normal karyotype. Among 58 patients FLT3/ITD occurred in six cases (10.34%) and D835 mutation in two cases (3.45%). The median age in the favorable risk group was 51 years, in the intermediate risk group 50 years and in the unfavorable risk group 44 years. The median age in all 80 AML patients was 49 years. FLT3/ITD was found in 10 patients (12.5%) and D835 mutation in 5 patients (6.25%). No significant difference was found among these three risk groups regarding presence or absence of FLT3/ITD and FLT/D835.

Discussion

For patients with *de novo* AML the best clinical approach is the classical cytogenetic analysis including FISH technique to categorize patients into specific risk groups. For AML patients with normal karyotype (NK-AML) it is particularly difficult to establish the prognosis during diagnosis. Their overall survival rate can range from 24% to 42% [8]. Further studies are needed in order to elucidate the molecular background in AML. Detection of mutations in genes such as FLT3, NPM1 or CEBPA can be valuable in providing prognostic information during diagnosis [7].

AML patients with FLT3/ITD mutation have shorter remission duration and overall survival [5]. Not only the presence of ITD affects poor prognosis, but also the size of the internal tandem duplication, which can vary from three to hundreds of nucleotides. Longer tandem duplication correlates with worse overall survival [8]. The occurrence of D835 mutation is still unclear; the data from the literature are inconsistent and further studies are required in order to establish the role of this mutation as a prognostic factor in AML [7].

Nowadays cytogenetic prognostic factors are more precise and in wide clinical use. But there is a need to improve molecular factors (the mutation status of genes such as FLT3, MLL, NPM1, CEBPA), especially in cases with normal karyotype [14]. FLT3/ITD, which is known to be a poor prognostic factor, is present in 20–30% of AML patients with different karyotype status. In the group with complex karyotype or with aberration with poor prognosis (monosomy 5 or 7) there is no difficulty in interpretation of clinical outcome. However, the group with a good cytogenetic factor such as t(15;17) with coexisting FLT3/ITD is more difficult to interpret. Patients with acute promyelocytic leukemia (APL) showed a similar frequency of FLT3/ITD and D835 mutation as the other groups within AML. A study with transgenic mice revealed that PML/RARa is necessary but not sufficient for APL to develop and additional aberrations are needed [2]. Presence or absence of FLT3/ITD in APL cases seems to have no impact on survival, but a study using quantitative techniques on FLT3/ITD ratio to wild type of FLT3 showed a significant impact on the outcome in APL. Patients with higher ITD ratios and lower PML/RARa transcript levels at diagnosis have much worse prognosis [6].

The group of AML patients with normal karyotype is the most difficult clinical problem in treatment selection. After FLT3 the most studied gene is NPM1 (nucleophosmin gene), which plays a role as a partner in many chromosomal translocations and as a nuclear chaperone has a function in genome stability, DNA duplication and transcriptional regulation [3]. The frequency of NPM1 mutations in AML patients with normal karyotype is about 50% and their presence is associated with favorable outcome (if not coexisting with FLT3/ITD) [11]. Detection of NPM1 mutations should be accessible during diagnosis of *de novo* AML, but unfortunately it is rare in Polish laboratories.

In our study the group of patients with normal karyotype was fairly numerous (72.5%) while in the literature NK patients represent about half of all AML cases [13]. The frequency of FLT3/ITD in all 80 AML patients (only 12.5%) was lower compared to other authors (in the literature about 20–30% of AML cases carry this tandem duplication). Small [12] indicates a different percentage frequency of FLT3/ITD, ranging from 15% to 34%. In our study only in the adverse group the frequency of FLT3/ITD was 20%. Within AML patients the frequency of D835 mutation was about 6.25% and this is consistent with other authors [5].

In recent years novel targeted therapies for FLT3/ITD patients have been developed. Mutated FLT3 has the activity of tyrosine kinase, and special FLT3 inhibitors have been created such as lestaurtinib (CEP-701) which in preclinical studies reduced blasts with mutations [4]. However, the best approach at the present time is to combine new drugs such as inhibitors for FLT3 mutation together with conventional chemotherapy.

Table 1. Status of FLT3 mutation and karyotype in 80 AML patients categorized to three risk groups: favorable, intermediate and adverse

Risk group	Nr	FLT3 status	Karyotype
FAVORABLE	1	ITD +, D835 –	46,XY,t(15;17)(q22;q21)[20]
	2	ITD –, D835 +	45,X,–X, t(8;21)(q22;q22) [20]
	3	–	46,XX,inv(16)(q22)[20]
	4	–	46,XX,inv(16)(q22)[8]/46,XY[12]
	5	–	46,XX,inv(16)(p13q22)[20]
	6	–	46,XY,t(8;21)(q22;q22)[2]/46,XY[18]
	7	–	46,XX,inv(16)(q22)[6]/46,XY[14]
INTERMEDIATE	8	ITD +, D835 –	NORMAL KARYOTYPE
	9	ITD +, D835 –	NORMAL KARYOTYPE
	10	ITD +, D835 –	NORMAL KARYOTYPE
	11	ITD +, D835 –	NORMAL KARYOTYPE
	12	ITD +, D835 –	NORMAL KARYOTYPE
	13	ITD +, D835 –	NORMAL KARYOTYPE
	14	ITD –, D835 +	NORMAL KARYOTYPE
	15	ITD –, D835 +	NORMAL KARYOTYPE
	16–64	–	NORMAL KARYOTYPE
ADVERSE	65	ITD +, D835 –	46,XY,del(5)(q31),add(7)(q36),inv(21)(q11.2q22.3)[20]
	66	ITD +, D835 –	46,XY,del(7)(qq22),add(9)(p24),del(12)(p12)[20]
	67	ITD –, D835 +	47,XX,+8,inv(16)(p13q22)[9]/ 46,XX,inv(16)(p13q22)[7]/ 46,XX,der(1)(1p32→1qter),der(2)(pter→2q31),der(5)(5pter→5q33: : 21q21→21qter), del(7)(q22), der(15)(15pter→15q26: : 1p32→1pter), der(15)(22q11.2→22p11.2: : 15p11.2→15qter), der(16)(2qter→2q31: : 16q22: : 16p13→16q22: : 16p13→16pter), der(21)(21pter→21q21: : ?), der(22)(?: : 22q12→22qter) [4]
	68	–	46,XX,del(7)(q11.2),t(12;14)(p11.1;q11.1),+mar [20]
	69	–	46,XX,–1,+der(1)t(1;2)(p21;q21)dup(1)(p36.3p12), del(5)(q11.1),der(7)ins(7;5)(p13;?),del(22)(q22) [20]
	70	–	45,XY, –18, der(3)(3pter→3q25:), der(6)(18qter→18q12: : 3q27→3q29: : 6p21.3→6qter), der(6)(3q25→3q27: : 6p21.3→6qter), dup(11)(p13p15) [11]/46,XY [9]
	71	–	45,XX,der(2)del(2)(p13)t(2;12;?)(?: : 12q24.1→12p12: : 2p13→2qter), del(5)(q22), del(6)(q25),del(7)(q32),der(8)t(8;12)(q24;q24.1),i(11)(q11),–12 [20]
	72	–	42~46,XY,der(4)t(4;5)(q21;?),der(?)t(?;5;4)(?;?;q27),–7, der(17)t(?;17)(?;p11.1),–7,–19,+2mar [cp15]/ 46,XY[5]
	73	–	46,XY, der(4)(4pter→4q26: : 16q22→16qter),der(15)(15pter→15q23: : 4q31.3→4qter), der(16)(16q22→16p11.2: : 17q25→17q21: : 4q26→4q31.3: : 17q21: : 15q23→15qter), der(17)(17qter→17q21: : 16p11.2→16pter) [20]
	74	ITD –, D835 +	55,XX,+X,+6,+10,+14,+14,+18,+18,+21,+21 [20]
	76	–	46,XY,del(7)(q34),t(10;12)(q15;p15)[2]/46,XY[18]
	77	–	46,XX,t(6;9)(p23;q34) [20]
	78	–	48,XY,+13,+13 [7]/46,XY[13]
	79	–	47,XY,+8[7]/48,XY,+X,+8[1]/46,XY[12]
	80	ITD+, D835–	47,XX,+8,del(11)(q21)[1]/47,XX,+8[19]

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