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ABOUT COVER

Peer Reviewer of *World Journal of Experimental Medicine*, Wu Duan, MD, PhD, Assistant Professor, Attending Doctor, Department of Endocrinology, Qilu Hospital of Shandong University, Jinan 250012, Shandong Province, China. duanwu@qiluhospital.com

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ORIGINAL ARTICLE

Retrospective Cohort Study

Prevalence of *RUNX1* gene alterations in *de novo* adult acute myeloid leukemia

Hoda M Abd El-Ghany, Mona S El Ashry, Mona S Abdellateif, Ahmed Rabea, Nada Sultan, Omnia Y Abd El Dayem

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Hoda M Abd El-Ghany, Omnia Y Abd El Dayem, Department of Clinical Pathology, Faculty of Medicine, Cairo University, Cairo 11976, Al Qāhirah, Egypt

Mona S El Ashry, Nada Sultan, Department of Clinical Pathology, National Cancer Institute, Cairo University, Cairo 11976, Al Qāhirah, Egypt

Mona S Abdellateif, Department of Cancer Biology, National Cancer Institute, Cairo University, Cairo 11976, Al Qāhirah, Egypt

Ahmed Rabea, Department of Medical Oncology, National Cancer Institute, Cairo University, Cairo 11976, Al Qāhirah, Egypt

Corresponding author: Mona S Abdellateif, MD, PhD, Professor, Department of Cancer Biology, National Cancer Institute, Cairo University, 1 Fom Elkhaligue, Cairo 11976, Al Qāhirah, Egypt. mona.sayed@nci.cu.edu.eg

Abstract

BACKGROUND

Acute myeloid leukemia (AML) is a complicated disease with uncontrolled hematopoietic precursor proliferation induced by various genetic alterations. Runt-related transcription factor-1 (*RUNX1*) is commonly disrupted by chromosomal translocations in hematological malignancies.

AIM

To characterize *RUNX1* gene rearrangements and copy number variations in newly diagnosed adult AML patients, with an emphasis on the impact of clinical and laboratory features on the outcome.

METHODS

Fluorescence in situ hybridization was used to test *RUNX1* gene alterations in 77 newly diagnosed adult AML cases. *NPM1*, *FLT3/ITD*, *FLT3/TKD*, and *KIT* mutations were tested by PCR. Prognostic clinical and laboratory findings were studied in relation to *RUNX1* alterations.

RESULTS

RUNX1 abnormalities were detected by fluorescence in situ hybridization in 41.6% of patients: 20.8% had translocations, 22.1% had amplification, and 5.2%

had deletion. Translocations prevailed in AML-M2 (P = 0.019) with a positive expression of myeloperoxidase (P = 0.031), whereas deletions dominated in M4 and M5 subtypes (P = 0.008) with a positive association with CD64 expression (P = 0.05). The modal chromosomal number was higher in cases having amplifications (P = 0.007) and lower in those with deletions (P = 0.008). *RUNX1* abnormalities were associated with complex karyotypes (P < 0.001) and were mutually exclusive of *NPM1* mutations. After 44 months of follow-up, *RUNX1* abnormalities affected neither patients' response to treatment nor overall survival.

CONCLUSION

RUNX1 abnormalities were mutually exclusive of *NPM1* mutations. *RUNX1* abnormalities affected neither patients' response to treatment nor overall survival.

Key Words: Acute myeloid leukemia; Deletion; Disease-free survival; Fluorescence *in-situ* hybridization; Karyotyping; *RUNX1*

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Core Tip: In the current study, we characterized the runt-related transcription factor-1 (*RUNX1*) gene rearrangements and copy number variations in patients with newly diagnosed adult acute myeloid leukemia with an emphasis on the impact of clinical and laboratory features on the outcome. *RUNX1* abnormalities were mutually exclusive of *NPM1* mutations. *RUNX1* abnormalities affected neither patients' response to treatment nor overall survival.

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INTRODUCTION

Acute myeloid leukemia (AML) is a heterogeneous hematologic cancer characterized by the clonal growth of myeloid blasts in the blood, bone marrow (BM), and/or other tissues. It is the most prevalent type of acute leukemia in adults[1]. Over the past few decades, the discovery of recurrent structurally balanced and unbalanced chromosomal abnormalities has significantly influenced the clinical management of patients with AML. These chromosomal abnormalities are the most significant prognostic markers where they can define specific clinicopathologic entities of the disease. The current recommendations of the European Leukemia Network for genetic testing in AML are primarily focused on risk stratification in order to identify an effective therapeutic strategy[2].

Runt-related transcription factor 1 (RUNX1) is the founding member of the mammalian core-binding transcription factor family, which also includes RUNX2, RUNX3, and their non-DNA-binding cofactor CBF[3]. The significance of the transcription factor *RUNX1* in the t (8; 21) translocation in AML attracted initial interest. Since its discovery, RUNX1 has been found to play significant roles not only in leukemia but also in the development of the normal hematopoietic system [3]. Additionally, *RUNX1* has been associated with epithelial tissue development and carcinogenesis[4]. The *RUNX1* gene occupies approximately 261 kb on the long arm of chromosome 21. It controls the expression of genes involved in hematopoietic differentiation, ribosome synthesis, cell cycle regulation, p53, and transforming growth factor signaling pathways *via* interacting with various proteins through its domains[5].

Four forms of acquired *RUNX1* genetic abnormalities have been identified in AML: (1) Translocations involving *RUNX1* that result in fusion genes; (2) Molecular mutations; (3) *RUNX1* amplifications; and (4) Partial or total deletions of the *RUNX1* gene. It has been observed that *RUNX1* deletions and gains were most common in patients with unfavorable cytogenetics and that the prognosis differed dramatically, being best for individuals with *RUNX1* translocations and worst for those with deletions[6].

There have been reports of the *RUNX1* gene fusing with over 40 partner genes encoding structurally diverse proteins. Some RUNX1-fusions are frequent in AML, and their partner genes are known to be implicated in recurrent translocations, including *RUNX1 RUNX1T1*/t (8; 21) (q22; q22), t (3; 21) (q26.2; q22), t (1; 21) (p36; q22), and t (16; 21) (q24; q22). These translocations have been investigated extensively, and their potential prognostic influence is uncertain. Others have been documented in only a small number of trials, and their potential prognostic influence is uncertain[7].

Genetic testing of patients with newly diagnosed AML with *RUNX1* fluorescence in situ hybridization (FISH) probe provides the opportunity to identify more instances with minor rearrangements or new partner genes of the *RUNX1* locus. This will improve our understanding of the prognosis for these instances and may ultimately aid in the establishment of a very successful therapeutic treatment plan[6] that offers tailored therapy options for a large number of patients and future opportunities to prevent the development of AML[8].

This study aimed to determine the prevalence of *RUNX1* gene changes in patients with newly diagnosed AML and their influence on clinical outcomes. Various prognostic markers and other clinical and laboratory results were examined in relation to the expression of *RUNX1* genetic variations.

MATERIALS AND METHODS

Patients

Among the 263 adult patients who were diagnosed with AML between January 2018 and July 2019, 77 adult patients with *de novo* AML were included in this study. All patients were presented to the Outpatient Clinic of the Medical Oncology Department of the National Cancer Institute in Cairo, Egypt. Patients with acute promyelocytic leukemia were omitted from the study since their therapy and prognosis differ significantly from other patients with AML. Additionally, those with a history of hematologic disorders were excluded. The diagnosis of AML was based on morphological assessment of peripheral blood (PB) and BM smears, cytochemistry, immunophenotyping by flow cytometry, conventional cytogenetics, and molecular studies according to French-American-British (FAB) and World Health Organization parameters[9].

All patients underwent standard induction chemotherapy with the 3 + 7 treatment protocol (doxorubicin as a 3-day brief infusion and cytarabine 100 mg/m^2 as a 7-day continuous infusion). Depending on their risk assessment, patients who achieved complete remission (CR) were offered consolidation with high-dose cytarabine and human leukocyte antigen matching, followed by allogeneic BM transplantation. Refractory cases were given a high-dose cytarabine-based regimen for re-induction.

Clinical endpoints

Response to induction therapy was evaluated between days 14 and 28 post-induction. Response was categorized as CR, partial remission, stable disease, relapsed disease, or refractory disease. CR was defined as BM blasts 5%, absence of blasts with Auer rods, lack of extramedullary illness, neutrophil count > 1.0 × 10^9/L, platelet count > 100 × 10^9/L, and independence from red cell transfusions[10]. Patients who attained CR were divided into two groups termed normal recovery or delayed recovery based on whether they achieved CR before or after day 35, respectively[11]. Treatment failures were due to either disease resistance or relapse. The resistant disease was defined as the inability to achieve CR after completion of the initial treatment, with evidence of residual leukemia by PB and/or BM examination. Relapse was defined as BM blasts ≥ 5%, recurrence of blasts in PB, or the development of extramedullary illness.

Disease-free survival (DFS) was only defined for patients who attained a CR. It was calculated from the date of CR until the date of relapse or death, regardless of cause, censoring patients who were still alive at the time of the last follow-up. Overall survival (OS) was estimated from the date of protocol inclusion to the date of death or last follow-up/ measured from the date of diagnosis to the date of death or last follow-up.

Cytogenetic analysis

Pretreatment diagnostic conventional karyotyping was applied to BM samples employing G-banded metaphase cells from unstimulated 24-h cultures following the standard techniques. Using the IKAROS imaging system, at least 20 metaphases were analyzed in the majority of cases (Metasystems, Altlussheim, Germany). Through using International System for Human Cytogenetic Nomenclature, the karyotypes were interpreted (ISCN 2016)[12]. FISH was performed according to the manufacturer's instructions using locus-specific probes XL *RUNX1* dual-color Break-Apart probe (MetaSystems) to detect *RUNX1* rearrangements, deletions, and amplifications, which together represented total *RUNX1* abnormalities.

A minimum of 10 metaphases and 200 interphase nuclei were studied using a fluorescent microscope (AxioImager.Z1 mot; Carl Zeiss Ltd., Hertshire, United Kingdom) with the proper filter settings. The ISIS imaging system was utilized for image capture and processing (Metasystems).

Molecular detection of fusion gene transcripts and mutational analysis

Total RNA was extracted from BM or PB samples using Qiagen RNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. RNA was reverse transcribed using a high-capacity complementary DNA reverse transcription Kit (Applied Biosystems, Waltham, MA, United States) for the identification of fusion transcripts t (9; 22) (q34; q11), t (8; 21) (q22; q22), and inv (16) (p13q22) in accordance with the BIOMED-1 guidelines[13]. Mutation analysis of four other significant molecular marker genes, *NPM1*, *FLT3/ITD*, *FLT3/TKD*, and *KIT*, was carried out using genomic DNA-PCR as directed by the manufacturer.

Immunophenotype analysis

In all cases, blast cells in bone marrow aspiration samples were immunophenotyped using an EPICS XL Coulter Flow Cytometer (Beckman Coulter, Hialeah, FL, United States). A large panel of myeloid markers [myeloperoxidase (MPO), CD13, CD33, CD117, CD14, and CD15], lymphoid markers (CD10, CD19, CD22, CD79a, CD20, Cyto IgM, Kappa and Lambda for B lymphoid series, and CD3, CD2, 4, 8, 7, and 5 for T lymphoid series), and the stem cell marker CD34, as well as CD56 and human leukocyte antigen-DR, were used to confirm the diagnosis of AML.

Statistical analysis

Using version 22 of the statistical software program SPSS, data were analyzed (IBM, Armonk, NY, United States). According to the relevant normality test, quantitative data were described as mean ± standard deviation or median and interquartile ranges or as numbers and percentages for qualitative data. The relationship between *RUNX-1* anomaly and patient clinical characteristics was evaluated using the χ^2 test and/or Fisher exact test, where applicable. Numerical variables from two groups were compared using the Mann-Whitney test. The Kaplan-Meier test was utilized for survival analysis, whilst the log-rank test was utilized to compare survival curves. All tests were run with an alpha level of 0.05 and a confidence interval of 95%.

Compliance with ethical standards

Every patient gave a written informed consent. The study was conducted in accordance with the Helsinki Declaration of 2011 and was approved by the internal review board of the National Cancer Institute and the Faculty of Medicine Research Ethics Committee at Cairo University (Code: MS-38-2020).

RESULTS

Patient characteristics

The median age of the 77 Egyptian patients with *de novo* AML was 42 (range of 18 to 82) years. Males represented 61% (47/77) of patients. Thirty-eight patients (49.4%) were AML-M2, while 22 patients (28.6%) were AML-M4. Based on genetic findings, the patients with AML were classified according to the European LeukemiaNet genetic risk classification into 18 patients (23.4%) with low risk, 42 patients (54.5%) with intermediate risk, and 17 patients (22.1%) with high-risk stratification. By molecular screening, recurrent translocations were identified in 15/77 cases (19.5%); 7 cases (9.1%) with t (8; 21) (q22; q22), 6 cases (7.8%) with inv (16) (p13q22), and 2 cases (2.6%) with t (9; 22) (q34; q11.2). Forty cases (51.9%) achieved CR, while 36 cases (46.8%) died before day 28. The detailed demographic, clinical, and laboratory characteristics of our patients are illustrated in Table 1.

Cytogenetics analysis

Excluding 8 patients who failed to show mitosis, 22 out of 69 patients (31.9%) had normal karyotyping, while 9/69 patients (13.0%) had a complex karyotype including 4 cases with recurrent translocations. The median of the modal chromosome number (MCN) was 45.8 (range: 32-53); 10 patients (14.5%) had hypodiploidy, while 12 cases (17.4%) had a hyperdiploid karyotype (MCN > 46) including 4 cases with concurrent recurrent translocations, 2 cases with inv (16) (p13q22), 1 case with t (8; 21) (q22; q22), and 1 case with t (9; 22) (q34; q11.2).

RUNX1 aberrations in de novo AML

By FISH, *RUNX1* gene abnormalities were found in 32/77 patients (41.6%): 16 patients (20.8%) showed *RUNX1* rearrangements; 17 patients (22.1%) had *RUNX1* amplifications; and 4 patients (5.2%) had *RUNX1* deletion encompassing the whole *RUNX1* gene (Figure 1A).

Out of 16 cases with *RUNX1* translocations, 7 cases (43.8%) had t (8; 21) (q22; q22), 2 cases (12.5%) had t (1; 21) (Figure 1B-D), 1 case (6.3%) had t (16; 21), and 6 cases (37.5%) had unidentified partner chromosome. One or more copies of chromosome 21 in a hyperdiploid karyotype were gained in 4/17 cases (23.5%), while 10/ 17 cases (58.8%) showed *RUNX1* duplications including 4 cases with concurrent *RUNX1* translocations and 1 case with isochromosome 21. Three cases (17.6%) failed to show mitosis. Therefore, the differentiation could not be done.

Two out of four cases (50%) had -21; 1 case with a hypodiploid karyotype and another case with concurrent inv16 in a complex karyotype. Deletion of *RUNX1* was found in 2/4 cases (50%) in a diploid karyotype including 1 case with concurrent *RUNX1* translocations. The clinical characteristics of patients with and without different *RUNX1* aberrations at diagnosis are presented in Table 1.

Correlation of RUNX1 aberrations with clinical features and hematological findings

Although no statistically significant correlation between *RUNX1* abnormalities and hepatomegaly, lymphadenopathy, or splenomegaly was found (P = 0.434, 0.808, and 0.404, respectively), patients with *RUNX1* amplification presented with splenomegaly (P = 0.009) and 52.9% of them had lymphadenopathy (P = 0.076).

Hypercellular BM was more frequent in patients with *RUNX1* abnormalities (68.8%) and translocations (62.5%) than normocellular and hypocellular marrow (P = 0.042 and 0.09, respectively).

In *RUNX1* translocation positive cases, AML-M2 (43.8%) was the most frequent FAB subtype, and M0 and M7 tended to be more frequent among them than in *RUNX1* translocations negative cases (75% *vs* 25% and 100% *vs* 0%, respectively; P = 0.019). In addition, *RUNX1* translocations were positively associated with MPO expression (P = 0.031). Regarding *RUNX1* deletion, all cases were of the myeloid with monocytic phenotype (FAB-M4 and M5) (P = 0.008) and were positively associated with the expression of CD64 (P = 0.050). Otherwise, there was no significant difference between *RUNX1* positive and negative cases in different clinical characteristics. The comparison of clinical characteristics of patients with and without *RUNX1* gene alterations is shown in (Table 2 and Table 3).

Association of RUNX1 aberrations with cytogenetic and molecular abnormalities

There was a highly statistically significant relation between *RUNX1* copy number variations and MCN where positive cases to *RUNX1* amplification tended to have higher MCN, while *RUNX1* deletion cases tended to have lower MCN (P = 0.007 and 0.008, respectively). Cases positive to *RUNX1* abnormalities, translocations, and amplifications tended to have complex karyotypes compared to *RUNX1*-negative cases (P = 0.000, 0.001, and 0.001, respectively). There was no statistically significant correlation between all types of *RUNX1* abnormalities and other different cytogenetic abnormalities (as -2, -3, -7, -11, -13, -22, +8, +13, +17), inv16, and t (9; 22) (P = 0.623, 0.670, and 0.806, respectively).

To investigate the interaction of gene mutations in the pathogenesis of adult AML, screening of mutational status of four other genes was performed. Among the 32 patients with *RUNX1* abnormalities, 4 cases showed additional molecular abnormalities including 2 cases with *RUNX1* deletion, of which one had an *FLT3-ITD* mutation and the other case had concomitant *FLT3/TKD* and *NPM1* mutations, 1 case with t (8; 21) and *c-KIT* mutation, and 1 case with *RUNX1* ampli-

Table 1 Clinical features of the assessed patients with acute myeloid leukemia

Parameter	Frequency	Percent	Median (IQR)
Sex			
Male	47	61	
Female	30	39	
Age in years			42 (18-82)
< 50	52	67.5	
≥ 50	25	32.5	
TLC as × 10^9/L			20 (1-377)
Hb in g/dL			8.2 ± 2.39
Platelets as × 10^9/L			32 (1-658)
PB blast as %			53 (0-63)
BM blast as %			69 (20-97)
MCN			45.8 ± 2.62
< 46	10	14.5	
46	47	68.1	
> 46	12	17.4	
BM cellularity			
Hypocellular	3	3.9	
Normocellular	11	14.3	
Hypercellular	63	81.8	
Hepatomegaly			
Absent	57	74	
Present	20	26	
Splenomegaly			
Absent	60	77.9	
Present	17	22.1	
Lymphadenopathy			
Absent	52	67.5	
Present	25	32.5	
FAB classification			
M0	4	5.2	
M1	10	13	
M2	38	49.4	
M4	22	28.6	
M5a	2	2.6	
M7	1	1.3	
t (8; 21)			
Absent	70	90.9	
Present	7	9.1	
inv16			
Absent	71	92.2	
Present	6	7.8	

t (9; 22)			
Absent	75	97.4	
Present	2	2.6	
Genetic risk			
High	17	22.1	
Intermediate	42	54.5	
Low	18	23.4	
FLT3-ITD			
Wild	69	89.6	
Mutant	8	10.4	
FLT3-TKD			
Wild	75	97.4	
Mutant	2	2.6	
C-KIT			
Wild	76	98.7	
Mutant	1	1.3	
NPM			
Wild	63	81.8	
Mutant	14	18.2	
BM blast on day 15			0.03 (0-1)
BM cellularity on day 15			
Hypocellular	41	68.3	
Normocellular	11	18.3	
Hypercellular	8	13.3	
BM blast on day 28			0.03 (0-1)
BM cellularity on day 28			
Hypocellular	6	14.6	
Normocellular	21	51.2	
Hypercellular	14	34.1	
CR			
Negative	37	48.1	
Positive	40	51.9	
Delayed CR			
Negative	70	90.9	
Positive	7	9.1	
Resistance			
Negative	67	87	
Positive	10	13	
Relapse			
Negative	63	81.8	
Positive	14	18.2	
Death			
Negative	20	26	

Positive	57	74
Early death		
Negative	41	53.2
Positive	36	46.8

BM: Bone marrow; CR: Complete remission; FAB: French-American-British; Hb: Hemoglobin; IQR: Interquartile range; MCN: Modal chromosomal number; PB: Peripheral blood; TLC: Total leucocytic count.

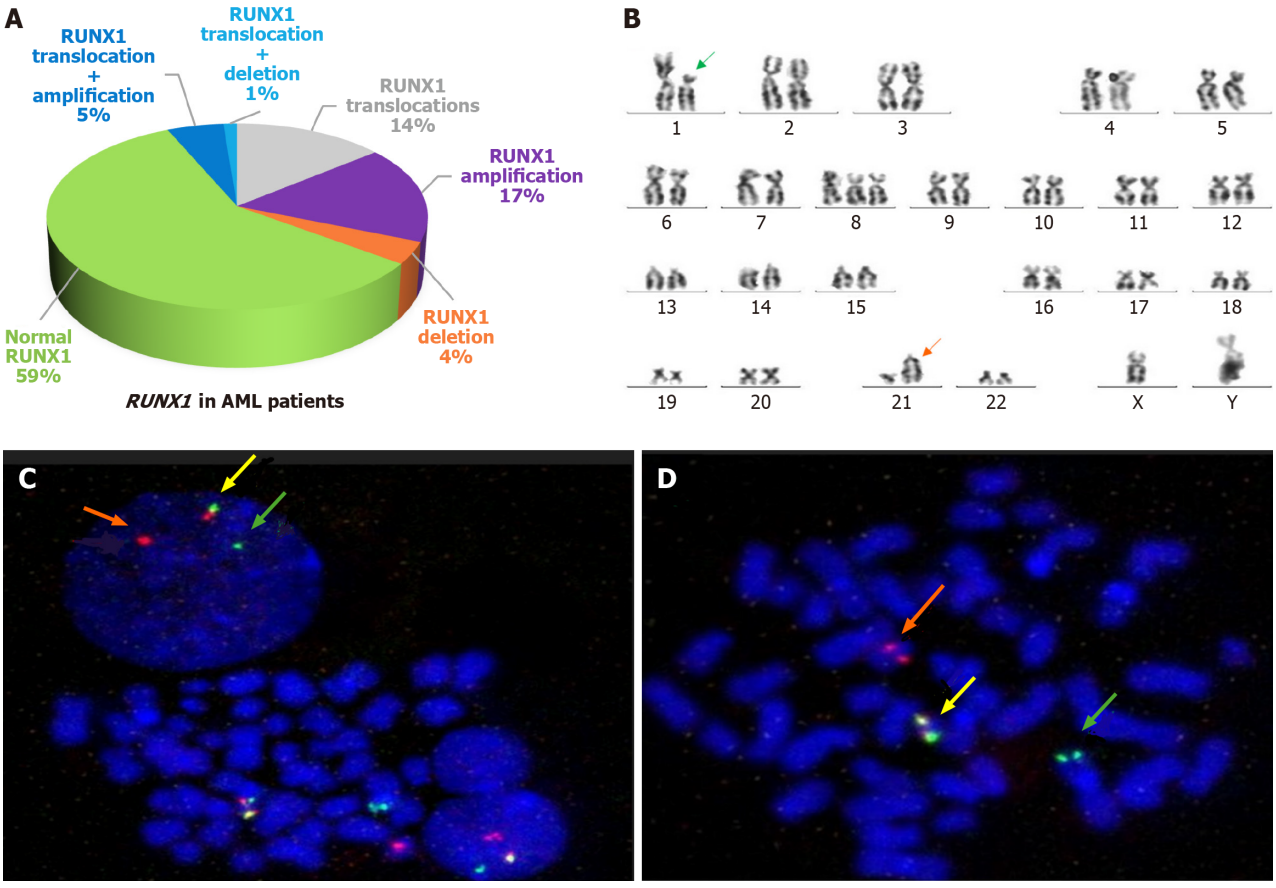

Figure 1 Runt-related transcription factor-1 in patients with acute myeloid leukemia. A: Runt-related transcription factor-1 (*RUNX1*) gene alterations in acute myeloid leukemia (AML) cases; B: G-banded karyotype of a case of t (1; 21). 48, XY, +X, t (1; 21) (p36; q22), +8; C: Interphase fluorescence in situ hybridization using *RUNX1* break apart probe showing a split of *RUNX1* signal; D: Metaphase fluorescence in situ hybridization using a *RUNX1* break apart probe showing a split of the *RUNX1* signal. The magnification factor is × 63.

fication with *NPM1* mutation.

FLT3-ITD mutations tended to be more prevalent in *RUNX1*-negative cases compared to positive cases. Out of 8 patients with AML with *FLT3-ITD* mutations, 7 patients (87.5%) were negative for *RUNX1* abnormalities, while only 1 patient was positive for *RUNX1* abnormalities. However, this relation was statistically insignificant (P = 0.078). Similarly, *NPM1* mutations were rarely seen in *RUNX1* abnormalities and translocations (P = 0.034 and 0.034, respectively). Otherwise, there were no significant differences between *RUNX1* positive and negative cases regarding other cytogenetic and molecular abnormalities, as shown in Table 2 and Table 3 (P > 0.05).

RUNX1 translocations, amplifications, and deletions were more frequent in the intermediate risk group (43.8%, 64.7%, and 50.0%) and the high-risk group (31.3%, 29.4%, and 50.0%) than in the low risk group (25.0%, 5.9%, and 0%), but the relationship was not statistically significant (P = 0.542, 0.150, and 0.288, respectively).

Impact of RUNX1 aberrations on response to treatment and clinical outcome

The response to treatment at day 28 of starting chemotherapy revealed that 36 of 77 (46.8%) cases died before day 28, and 33 cases (42.9%) achieved CR at day 15, 7 cases (9.1%) achieved delayed CR, 10 cases (13%) were resistant to treatment, and eventually 57 cases (74%) died.

Fourteen cases (18.2%) relapsed after achieving CR, 9/14 cases (64.2%) were positive to *RUNX1* abnormalities. One patient had t (9; 22) (q34; q11.2), and another patient had inv(16). The detailed karyotype and analysis of the outcome of

Table 2 Association between all runt-related transcription factor-1 abnormalities and runt-related transcription factor-1 translocations with the clinicopathological features of the patients with acute myeloid leukemia

Parameter		*RUNX1* abnormalities		*P* value	*RUNX1* translocation		*P* value
		Negative	Positive		Negative	Positive	
Age in years, median (IQR)		43.0 (20-69)	35.0 (18-78)	0.472	42.5 (20-78)	30.5 (18-70)	0.156
TLC as × 10^9/L, median (IQR)		44.7 (1-377)	20.8 (2-191)	0.620	34.8 (1-377)	20.1 (2-107)	0.187
Hb in g/dL, mean ± SD		8.38 ± 2.30	7.93 ± 2.50	0.421	8.15 ± 2.20	8.37 ± 3.10	0.748
Platelets as × 10^9/L, median (IQR)		24 (12-266)	30 (13-185)	0.549	26 (12-226)	27 (13-185)	0.390
PB blast as %, median (IQR)		60.0 (0-99)	54.0 (5-92)	0.426	53.0 (0-99)	68.5 (33-92)	0.950
BM blast as %, median (IQR)		66 (30-97)	62 (20-90)	0.598	66 (20-97)	70 (36-90)	0.711
MCN, mean ± SD		45.90 ± 0.67	45.80 ± 4.10	0.852	46.00 ± 2.50	45.30 ± 3.10	0.362
Sex, *n* (%)	Male	28 (62.2)	19 (59.4)	0.817	40 (65.6)	7 (43.8)	0.151
	Female	17 (37.8)	13 (40.6)		21 (34.4)	9 (56.3)	
BM cellularity, *n* (%)	Hypocellular	1 (2.2	2 (6.3)	0.042	3 (4.9)	0 (0.0)	0.009
	Normocellular	3 (6.7)	8 (25.0)		5 (8.2)	6 (37.5)	
	Hypercellular	41 (91.1)	22 (68.8)		53 (86.9)	10 (62.5)	
Hepatomegaly, *n* (%)	Absent	35 (77.8)	22 (68.8)	0.434	45 (73.8)	12 (75.0)	0.920
	Present	10 (22.2)	10 (31.3)		16 (26.2)	4 (25.0)	
Splenomegaly, *n* (%)	Absent	37 (82.2)	23 (71.9)	0.404	47 (77.0)	13 (81.3)	0.718
	Present	8 (17.8)	9 (28.1)		14 (23.0)	3 (18.8)	
Lymphadenopathy, *n* (%)	Absent	31 (68.9)	21 (65.6)	0.808	41 (67.2)	11 (68.8)	0.907
	Present	14 (31.1)	11 (34.4)		20 (32.8)	5 (31.3)	
MPO, *n* (%)	Negative	3 (6.7)	5 (15.6)	0.265	4 (6.6)	4 (25.0)	0.031
	Positive	42 (93.3)	27 (84.4)		57 (93.4)	12 (75.0)	
CD34, *n* (%)	Negative	26 (57.8)	16 (50.0)	0.643	35 (57.4)	7 (43.8)	0.330
	Positive	19 (42.2)	16 (50.0)		26 (42.6)	9 (56.3)	
CD64, *n* (%)	Negative	35 (77.8)	22 (68.8)	0.434	45 (73.8)	12 (75.0)	0.920
	Positive	10 (22.2)	10 (31.3)		16 (26.2)	4 (25.0)	
CD14, *n* (%)	Negative	39 (86.7)	29 (90.6)	0.728	53 (86.9)	15 (93.8)	0.447
	Positive	6 (13.3)	3 (9.4)		8 (13.1)	1 (6.3)	
FAB, *n* (%)	M0	1 (2.2)	3 (9.4)	0.241	1 (1.6)	3 (18.8)	0.019
	M1	5 (11.1)	5 (15.6)		9 (14.8)	1 (6.3)	
	M2	27 (60.0)	11 (34.4)		31 (50.8)	7 (43.8)	
	M4	11 (24.4)	11 (34.4)		19 (31.1)	3 (18.8)	
	M5	1 (2.2)	1 (3.1)		1 (1.6)	1 (6.3)	
	M7	0 (0.0)	1 (3.1)		0 (0.0)	1 (6.3)	
Complex, *n* (%)	Negative	40 (100.0)	20 (69.0)	< 0.001	50 (94.3)	10 (62.5)	0.001
	Positive	0 (0.0)	9 (31.0)		3 (5.7)	6 (37.5)	
t (8; 21), *n* (%)	Negative	45 (100.0)	25 (78.1)	0.001	61 (100.0)	9 (56.3)	< 0.001
	Positive	0 (0.0)	7 (21.9)		0 (0.0)	7 (43.8)	
inv16, *n* (%)	Negative	41 (91.1)	30 (93.8)	0.670	55 (90.2)	16 (100.0)	0.191
	Positive	4 (8.9)	2 (6.3)		6 (9.8)	0 (0.0)	
t (9; 22), *n* (%)	Negative	44 (97.8)	31 (96.9)	0.806	59 (96.7)	16 (100.0)	0.463

	Positive	1 (2.2)	1 (3.1)		2 (3.3)	0 (0.0)	
Cytogenetic risk, *n* (%)	High	8 (17.8)	9 (28.1)	0.310	12 (19.7)	5 (31.3)	0.542
	Intermediate	24 (53.3)	18 (56.3)		35 (57.4)	7 (43.8)	
	Low	13 (28.9)	5 (15.6)		14 (23.0)	4 (25.0)	
FLT3-ITD, *n* (%)	Wildtype	38 (84.4)	31 (96.9)	0.078	53 (86.9)	16 (100.0)	0.126
	Mutant	7 (15.6)	1 (3.1)		8 (13.1)	0 (0.0)	
C-KIT, *n* (%)	Wildtype	45 (100.0)	31 (96.9)	0.416	61 (100.0)	15 (93.8)	0.208
	Mutant	0 (0.0)	1 (3.1)		0 (0.0)	1 (6.3)	
NPM, *n* (%)	Wildtype	33 (73.3)	30 (93.8)	0.034	47 (77.0)	16 (100.0)	0.034
	Mutant	12 (26.7)	2 (6.3)		14 (23.0)	0 (0.0)	
BM blast on day 28, *n* (%)		0.04 (0-1)	0.02 (0-1)	0.082	0.03 (0-1)	0.04 (0-1)	0.789
BM cellularity on day 28, *n* (%)	Hypocellular	4 (19.0)	2 (10.0)	0.507	5 (16.7)	1 (6.3)	0.831
	Normocellular	9 (42.9)	12 (60.0)		15 (50.0)	6 (54.5)	
	Hypercellular	8 (38.1)	6 (30.0)		10 (33.3)	4 (36.4)	
CR, *n* (%)	Negative	23 (51.1)	14 (43.8)	0.644	30 (49.2)	7 (43.8)	0.699
	Positive	22 (48.9)	18 (56.3)		31 (50.8)	9 (56.3)	
Delayed CR, *n* (%)	Negative	43 (95.6)	27 (84.4)	0.093	57 (93.4)	13 (81.3)	0.131
	Positive	2 (4.4)	5 (15.6)		4 (6.6)	3 (18.8)	
Relapse, *n* (%)	Negative	40 (88.9)	23 (71.9)	0.075	52 (85.2)	11 (68.8)	0.128
	Positive	5 (11.1)	9 (28.1)		9 (14.8)	5 (31.3)	
Death, *n* (%)	Negative	9 (20.0)	11 (34.4)	0.192	15 (24.6)	5 (31.3)	0.589
	Positive	36 (80.0)	21 (65.6)		46 (75.4)	11 (68.8)	
Early death, *n* (%)	Negative	21 (46.7)	20 (62.5)	0.247	30 (49.2)	11 (68.8)	0.163
	Positive	24 (53.3)	12 (37.5)		31 (50.8)	5 (31.3)	

BM: Bone marrow; CR: Complete remission; FAB: French-American-British; Hb: Hemoglobin; IQR: Interquartile range; PB: Peripheral blood; MCN: Modal chromosomal number; MPO: Myeloperoxidase; *RUNX1*: Runt-related transcription factor-1; SD: Standard deviation; TLC: Total leucocytic count.

those patients are summarized in Supplementary Table 1.

Although positive cases to *RUNX1* abnormalities tended to have delayed CR and relapse compared to negative cases (71.4% *vs* 28.6% $P = 0.093$ and 64.3% *vs* 35.7% $P = 0.075$, respectively), the relationship was not statistically significant. The statistical analysis showed the absence of a relationship between the achievement of CR and *RUNX1* abnormalities ($P = 0.644$), *RUNX1* translocation ($P = 0.699$), *RUNX1* amplification ($P = 0.926$), and *RUNX1* deletion ($P = 0.616$), as shown in Table 2 and Table 3.

After a follow-up period of 44.2 months, the present study showed that there was no significant difference between positive and negative *RUNX1* aberration cases regarding the OS (Figure 2). Patients with *RUNX1* deletion had significantly poorer DFS than those without *RUNX1* deletion (mean: 3.03 months *vs* 27.20 months, respectively; $P < 0.001$). No other significant differences were observed between any other type of *RUNX1* alterations and negative cases regarding DFS (Figure 3).

DISCUSSION

The role of *RUNX1* mutations in cytogenetically normal AML had been identified. However, the prognostic impact of *RUNX1* translocations other than t (8; 21) (q22; q22), *RUNX1* deletions, and amplifications are still unknown. As a result, addressing such interactions is critical for further risk classification and eventually the development of a successful therapeutic plan.

In this study, FISH was used to screen for *RUNX1* gene alterations in 77 newly diagnosed adult patients with *de novo* AML, and the results were compared to clinical characteristics and prognosis. *RUNX1* abnormalities were divided into four categories: (1) *RUNX1* translocations; (2) *RUNX1* amplifications; (3) *RUNX1* deletions; and (4) *RUNX1* abnormalities, which encompass all three types.

Table 3 Association between runt-related transcription factor-1 amplifications and runt-related transcription factor-1 deletion with clinicopathological features of acute myeloid leukemia patients

Parameter		*RUNX1* amplification		*P* value	*RUNX1* deletion		*P* value
		Negative	Positive		Negative	Positive	
Age in years, median (IQR)		42.0 (18-70)	36.0 (20-78)	0.694	41.5 (18-78)	21.5 (21-22)	0.175
TLC as × 10^9/L, median (IQR)		44.7 (1-377)	19.2 (2-91)	0.873	29.1 (1-377)	105.3 (19-191)	0.630
Hb in g/dL, mean ± SD		8.33 ± 2.50	7.70 ± 1.70	0.359	8.20 ± 2.40	8.10 ± 1.50	0.950
Platelets as × 10^9/L, median (IQR)		24 (12-226)	45 (21-185)	0.484	26 (12-226)	24 (18-30)	0.663
PB blast (%), median (IQR)		60 (0-99)	38 (5-72)	0.515	59 (0-99)	47 (40-54)	0.232
BM blast (%), median (IQR)		66 (30-97)	60 (20-90)	0.35	65.5 (20-97)	74.5 (71-78)	0.613
MCN, mean ± SD		45.4 ± 2.5	47.5 ± 2.4	0.007	46 ± 2.1	42.5 ± 7	0.008
Sex, *n* (%)	Male	34 (56.7)	13 (76.5)	0.168	45 (61.6)	2 (50.0)	0.641
	Female	26 (43.3)	4 (23.5)		28 (38.4)	2 (50.0)	
BM cellularity, *n* (%)	Hypocellular	1 (1.7)	2 (11.8)	0.137	3 (4.1)	0 (0.0)	0.626
	Normocellular	8 (13.3)	3 (17.6)		11 (15.1)	0 (0.0)	
	Hypercellular	51 (85.0)	12 (70.6)		59 (80.8)	4 (100.0)	
Hepatomegaly, *n* (%)	Absent	47 (78.3)	10 (58.8)	0.125	53 (72.6)	4 (100.0)	0.568
	Present	13 (21.7)	7 (41.2)		20 (27.4)	0 (0.0)	
Splenomegaly, *n* (%)	Absent	51 (85.0)	9 (52.9)	0.009	56 (76.7)	4 (100.0)	0.57
	Present	9 (15.0)	8 (47.1)		17 (23.3)	0 (0.0)	
Lymphadenopathy, *n* (%)	Absent	44 (73.3)	8 (47.1)	0.076	49 (67.1)	3 (75.0)	0.743
	Present	16 (26.7)	9 (52.9)		24 (32.9)	1 (25.0)	
MPO, *n* (%)	Negative	6 (10.0)	2 (11.8)	0.833	8 (11.0)	0 (0.0)	1
	Positive	54 (90.0)	15 (88.2)		65 (89.0)	4 (100.0)	
CD34, *n* (%)	Negative	34 (56.7)	8 (47.1)	0.584	38 (52.1)	4 (100.0)	0.121
	Positive	26 (43.3)	9 (52.9)		35 (47.9)	0 (0.0)	
CD64, *n* (%)	Negative	45 (75.0)	12 (70.6)	0.758	56 (76.7)	1 (25.0)	0.052
	Positive	15 (25.0)	5 (29.4)		17 (23.3)	3 (75.0)	
CD14, *n* (%)	Negative	51 (85.0)	17 (100.0)	0.194	66 (90.4)	2 (50.0)	0.065
	Positive	9 (15.0)	0 (0)		7 (9.6)	2 (50.0)	
FAB, *n* (%)	M0	2 (3.3)	2 (11.8)	0.368	4 (5.5)	0 (0.0)	0.014
	M1	6 (10.0)	4 (23.5)		10 (13.7)	0 (0.0)	
	M2	32 (53.3)	6 (35.3)		38 (52.1)	0 (0.0)	
	M4	17 (28.3)	5 (29.4)		19 (26.0)	3 (75.0)	
	M5	2 (3.3)	0 (0)		1 (1.4)	1 (25.0)	
	M7	1 (1.7)	0 (0)		1 (1.4)	0 (0.0)	
Complex, *n* (%)	Negative	52 (94.5)	8 (47.1)	0.001	57 (87.7)	3 (75.0)	0.436
	Positive	3 (5.5)	6 (35.3)		8 (12.3)	1 (25.0)	
t (8; 21), *n* (%)	Negative	54 (90.0)	16 (94.1)	0.602	66 (90.4)	4 (100.0)	0.516
	Positive	6 (10.0)	1 (5.9)		7 (9.6)	0 (0.0)	
inv16, *n* (%)	Negative	55 (91.7)	16 (94.1)	0.739	68 (93.2)	3 (75.0)	0.282
	Positive	5 (8.3)	1 (5.9)		5 (6.8)	1 (25.0)	
t (9; 22), *n* (%)	Negative	59 (98.3)	16 (94.1)	0.395	71 (97.3)	4 (100.0)	0.737

	Positive	1 (1.7)	1 (5.9)		2 (2.7)	0 (0.0)	
Cytogenetic risk, *n* (%)	High	12 (20.0)	5 (29.4)	0.15	15 (20.5)	2 (50.0)	0.288
	Intermediate	31 (51.7)	11 (64.7)		40 (54.8)	2 (50.0)	
	Low	17 (28.3)	1 (5.9)		18 (24.7)	0 (0.0)	
FLT3-ITD, *n* (%)	Wildtype	52 (86.7)	17 (100.0)	0.188	66 (90.4)	3 (75.0)	0.361
	Mutant	8 (13.3)	0 (0)		7 (9.6)	1 (25.0)	
C-KIT, *n* (%)	Wildtype	59 (98.3)	17 (100.0)	0.592	72 (98.6)	4 (100.0)	0.814
	Mutant	1 (1.7)	0 (0)		1 (1.4)	0 (0.0)	
NPM, *n* (%)	Wildtype	47 (78.3)	16 (94.1)	0.173	60 (82.2)	3 (75.0)	0.717
	Mutant	13 (21.7)	1 (5.9)		13 (17.8)	1 (25.0)	
BM cellularity on day 28, *n* (%)	Hypocellular	6 (20.0)	0 (0)	0.152	5 (12.8)	1 (25.0)	0.22
	Normocellular	13 (43.3)	8 (47.1)		21 (53.8)	0 (0.0)	
	Hypercellular	11 (36.7)	3 (25.0)		13 (33.3)	1 (25.0)	
CR, *n* (%)	Negative	29 (48.3)	8 (47.1)	0.926	36 (49.3)	1 (25.0)	0.616
	Positive	31 (51.7)	9 (52.9)		37 (50.7)	3 (75.0)	
Delayed CR, *n* (%)	Negative	56 (93.30	14	0.177	66 (90.4)	4 (100.0)	0.516
	Positive	4 (6.7)	3 (25.0)		7 (9.6)	0 (0.0)	
Relapse, *n* (%)	Negative	50 (83.3)	13	0.496	61 (83.6)	2 (50.0)	0.149
	Positive	10 (16.7)	4		12 (16.4)	2 (50.0)	
Death, *n* (%)	Negative	13 (21.7)	7	0.125	19 (26.0)	1 (25.0)	0.964
	Positive	47 (78.3)	10		54 (74.0)	3 (75.0)	
Early death, *n* (%)	Negative	30 (50.0)	11	0.41	39 (53.4)	2 (50.0)	0.894
	Positive	30 (50.0)	6		34 (46.6)	2 (50.0)	

BM: Bone marrow; CR: Complete remission; FAB: French-American-British; Hb: Hemoglobin; IQR: Interquartile range; MCN: Modal chromosomal number; PB: Peripheral blood; MPO: Myeloperoxidase; *RUNX1*: Runt-related transcription factor-1; SD: Standard deviation; TLC: Total leucocytic count.

In agreement with Haferlach *et al*[6], total *RUNX1* abnormalities were detected in 41.6% of cases of which *RUNX1* amplification was the most common alteration (22.1%) followed by *RUNX1* translocations (20.8%) then RUNX1 deletion (5.2%). Baldus *et al*[14] investigated 12 patients with AML with complicated karyotypes and chromosome 21 anomalies and showed that amplification of two chromosome 21 areas was frequently seen in AML with complex karyotypes using comparative genomic hybridization, supporting the notion that gain of chromosome 21 material appears to be a nonrandom event implicated in AML. Baldus *et al*[14] reasoned that this might be related to the function of a specific gene or set of genes.

The clinical and genetic characteristics of patients with and without *RUNX1* alterations were compared. In agreement with Yamato *et al*[15], there were no statistically significant differences in any form of *RUNX1* abnormalities with respect to age, sex, total leucocytic count, hemoglobin level, platelet count, PB count, or BM blast cell counts at presentation. Tang *et al*[16] discovered that male patients had a higher rate of *RUNX1* alterations than female patients, while Haferlach *et al*[17] reported that patients with *RUNX1* deletion were considerably older than those with two *RUNX1* copies and had a lower WBC count. On the other hand, Said *et al*[18] used reverse transcription-quantitative PCR to explore the role of *RUNX1* gene expression in Egyptian patients with AML and found that male patients had significantly higher *RUNX1* expression. This discrepancy can be attributed to the difference in the technique used, a difference in the sample size, and variation in inclusion criteria between the two studies, in spite of conducting both studies on the same race.

The current data showed that splenomegaly is common in patients with *RUNX1* amplification, and the majority of them had lymphadenopathy. Hypercellular marrow was also more frequent than normocellular and hypocellular marrow in *RUNX1*-abnormalities and translocations. No published research had associated *RUNX1*-abnormalities with hepatomegaly, splenomegaly, lymphadenopathy, or BM cellularity at the time of diagnosis, to our knowledge.

Of interest, 43.8% of patients with *RUNX1* translocation were FAB AML-M2 and were associated with MPO expression. Also, M0 and M7 were more prevalent in *RUNX1* translocation positive cases than in negative instances. All patients with *RUNX1* deletion had a myeloid with monocytic phenotype (FAB-M4 and M5) and were favorably related with CD64 expression. These results matched those of Haferlach *et al*[6], who found that 45.2% of *RUNX1* translocation cases were FAB type M1 and M2. However, in contrast to our findings, they found that in cases of *RUNX1* deletion, M0 was the most common AML subtype. This discrepancy can be attributed to the racial and sample size variations. While

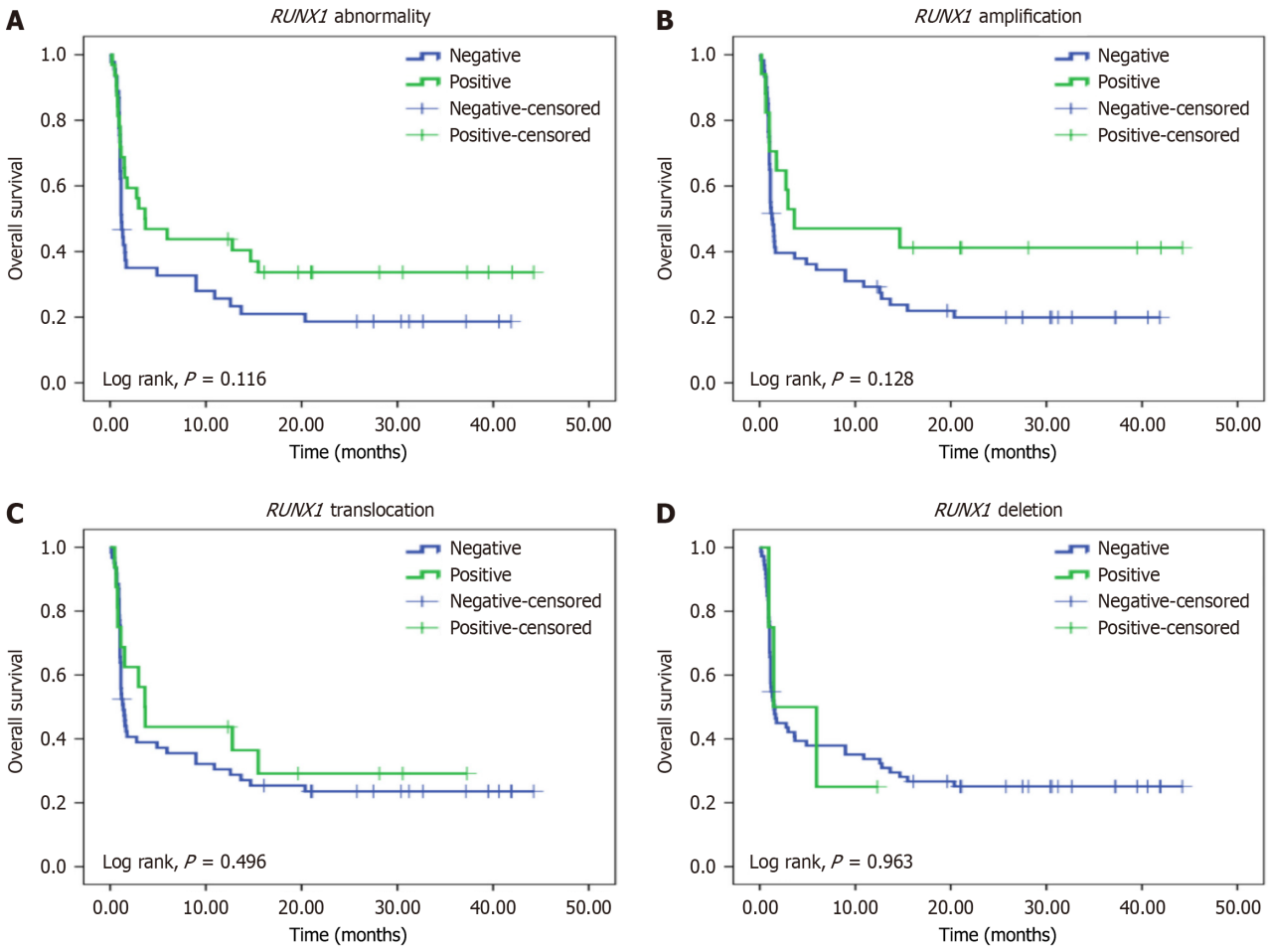

Figure 2 Kaplan-Meier survival curves. A: Runt-related transcription factor-1 (*RUNX1*) abnormality; B: *RUNX1* amplification; C: *RUNX1* translocation; D: *RUNX1* deletion on overall survival rates in acute myeloid leukemia patients.

Said *et al*[18] found that out of 42% of patients with AML classified as M2, translocation t (8; 21) was found in only 6.6% of the cases. The researchers also discovered no link between FAB subtypes and *RUNX1* expression.

In keeping with earlier studies, translocations, amplifications, and deletions of *RUNX1* were more common in the intermediate and high-risk groups, although not statistically significant.

In addition, as previously reported[17,19,20], positive cases of *RUNX1* amplification have a higher MCN, whereas *RUNX1* deletion cases have a lower MCN. This could be explained by the fact that chromosomal gain is nonrandom in patients with AML with hyperdiploid karyotypes, and chromosome 21 is one of the most frequently gained chromosomes in these circumstances.

The present data showed that *RUNX1* abnormalities, translocations, and amplifications are associated with more complex karyotypes than *RUNX1*-negative instances, which supports prior research[6,14,17]. It was concluded that chromosome 21 amplifications are common in people with complicated karyotypes.

In terms of other molecular mutations, 8 cases tested positive for the *FLT3-ITD* mutation. *RUNX1* translocations and amplifications were all negative, but *RUNX1* deletion was positive in 1 patient (12.5%). These relationships, however, did not exhibit statistical significance. According to Said *et al*[18], patients with higher *RUNX1* expression were more likely to have *FLT3-ITD* mutation than patients with lower *RUNX1* expression. This suggested that *RUNX1* could operate as an oncogene that causes leukemogenesis and as a surrogate marker for other mutations, particularly *FLT3-ITD*, when expressed at high levels.

Furthermore, *NPM1* mutations were mutually exclusive of *RUNX1* abnormalities and *RUNX1* translocations. This suggests that *RUNX1* mutation shares a similar genetic pathway role with *NPM1* mutations in leukemia development. This was supported by the study of Zuo *et al*[21], who stated that *NPM1* mutant interacts with *PU.1/CEB-PA/RUNX1* transcription factor complexes to block myeloid differentiation. Additionally, *NPM1* mutations were found at a lower frequency in *RUNX1* copy number variation-positive patients than in negative cases, but the difference was not statistically significant. These *RUNX1* mutations have genetic characteristics that are similar to those previously described in patients with AML[6,15,17].

There was no statistically significant association between any form of *RUNX1* modification and the achievement of CR or OS in terms of clinical outcome. Although the median DFS differed significantly amongst the three types of *RUNX1* changes (9.5, 25.7, and 1.5 months, respectively), *RUNX1* amplifications had the best prognosis. Only those with *RUNX1* deletion exhibited a considerably worse outcome than cases without the mutation. Consistently, previous research has

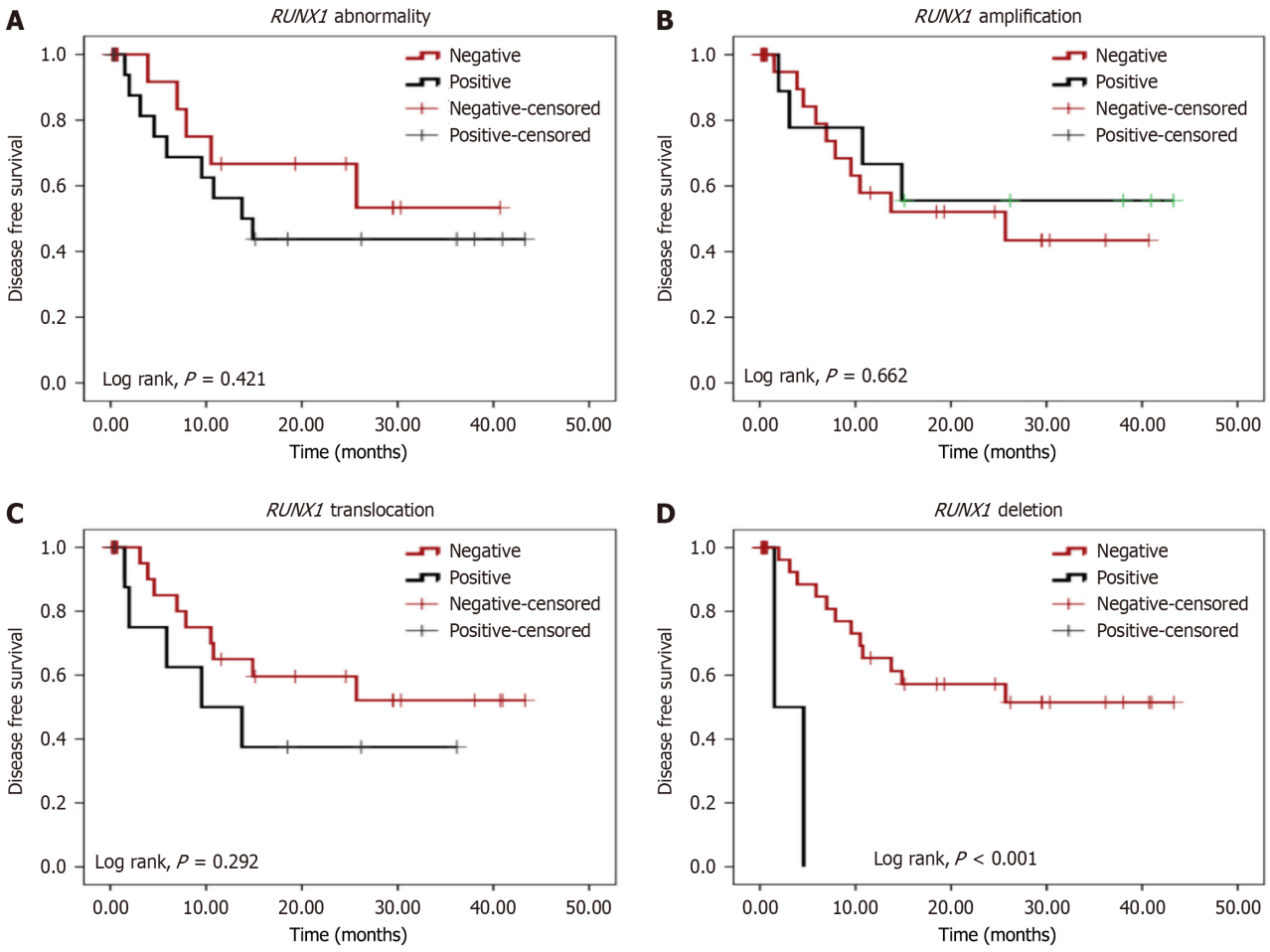

Figure 3 Kaplan-Meier survival curves. A: Runt-related transcription factor-1 (*RUNX1*) abnormality; B: *RUNX1* amplification; C: *RUNX1* translocation; D: *RUNX1* deletion on disease-free survival of patients with adult acute myeloid leukemia.

found that OS varies dramatically between the different forms of *RUNX1* alterations, with *RUNX1* deletions having the worst outcome[6].

On the contrary, other studies[6,22] found that the outcome differed considerably across *RUNX1* alterations and was better in individuals with *RUNX1* translocations. Discrepancies in the results could be related to the fact that most research looked at *RUNX1* mutations in combination with cytogenetic abnormalities, whereas just a few looked at the impact of *RUNX1* translocations, amplifications, and deletions in AML cases from different risk groups. Furthermore, it is thought that the different sorts of techniques used in the studies are a key source of heterogeneity. Said *et al*[18] found no significant impact of *RUNX1* expression on OS or DFS rates, but Chen *et al*[23] found that *RUNX1* mutation was linked to a lower risk-free survival rate.

Nine out of seventeen (53%) *RUNX1* amplification positive cases had *RUNX1* duplications. Four cases (29.4%) had *RUNX1* translocations at the same time, and five cases (29.4%) obtained one or more copies of chromosome 21, four of which were in a hyperdiploid karyotype. All of these are favorable prognostic markers that may help patients with positive *RUNX1* amplification live longer. This could explain the disparity in clinical outcomes between our data and that of others.

Furthermore, when inv16 and t (9; 22) cases with favorable prognosis were excluded from statistical analysis, the mean DFS in positive *RUNX1* deletion cases was 3.033 months compared to 27.231 months in negative *RUNX1* deletion cases. This suggests that *RUNX1* deletion has the worst prognosis, even if other strong prognostic markers like inv16 and t (9; 22) are present.

CONCLUSION

Our data presented a pilot study for *RUNX1* gene alterations in a cohort of patients with *de novo* AML. *RUNX1* abnormalities were detected in 41.6% of patients. *RUNX1* translocations occurred predominantly in FAB M2, M0, and M7 while *RUNX1* deletions were of myeloid with monocytic phenotype (FAB-M4 and M5). Cases positive for *RUNX1* abnormalities, translocations, and amplifications tended to have complex karyotypes. *RUNX1* abnormalities were mutually exclusive of *NPM1* mutations. *RUNX1* deletion was an independent adverse parameter for DFS. Further trials with larger numbers of *RUNX1* abnormal cases are warranted to further highlight the prognostic features and the

predictive significance of this abnormality.

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FOOTNOTES

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Country of origin: Egypt

ORCID number: Mona S Abdellateif 0000-0002-5510-4435.

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