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Wilms tumor 1 gene expression in acute myeloid leukemia: prognostic significance and usefulness in minimal residual disease monitoring—a case–control study

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Abstract

Background: Minimal residual disease (MRD), which is characterized as leukemic cells at a level below morphologic detection, has been connected to the risk of relapse in acute myeloid leukemia. In 80–90% of acute myeloid leukemia (AML) patients, the Wilms tumor (*WT1*) gene is overexpressed at the mRNA level. In our prospective study, a total of 55 patients were enrolled in the study. Group I involved 40 AML patients and group II involved 15 patients healthy controls. *WT1* gene expression was quantified using quantitative real-time PCR on bone marrow samples from AML patients at initial diagnosis and at day 28 after induction chemotherapy, and compared to 15 healthy controls in group II. Follow up of patients for prognosis evaluation was assessed. IBM SPSS software was used to capture and analyses the data.

Results: At diagnosis, the mean *WT1* transcript value in AML patients was substantially higher than the expression observed in control patient's Bone marrow. There was no statistically relevant relationship between the onset of relapse and *WT1* expression. Patients with *WT1* overexpression at diagnosis had a shorter overall survival than patients with negative *WT1* expression.

Conclusions: Wilms tumor 1 gene expression was found to be significantly higher in AML patients than control cases, overall, our results confirmed the prognostic significance of *WT1* overexpression in AML patients. Our findings support the application of MRD in AML patients based on WT1 overexpression.

Keywords: Acute myeloid leukemia, Wilms tumour 1, Minimal residual disease

Background

Acute myeloid leukaemia (AML) is a disease with a wide range of genetic anomalies, as well as immunophenotypes, and clinical outcomes. AML is currently the most common type of acute leukaemia in adults [1]. After achieving morphologically specified complete remission (CR) with induction chemotherapy, more than half of adult patients with AML relapse [2]. Traditionally, cytomorphology has been used to determine post-treatment remission, with relapse being identified as 5% blasts in the BM that are not due to other causes. Microscopic evaluation of BM or PB morphology is based on the analysis of a limited number of cells (200–500 cells), and its accuracy is influenced by sample quality and pathologist expertise [3].

Post-chemotherapy perseverance of minimal residual disease (MRD), which is characterized as leukemic cells at a level underneath morphologic detection, has been linked to the risk of relapse [4].

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In the treatment of patients with acute lymphoblastic leukemia, acute promyelocytic leukemia (APL), and chronic myeloid leukemia, MRD monitoring has become standard practice. The existence of MRD is a solid, autonomous prognostic marker of amplified risk of relapse and shorter survival in patients with AML compared to patients with a negative MRD, according to mounting evidence [5].

Flow cytometry and molecular techniques for detecting remaining disease are more sensitive than morphologic assessment, and there is growing agreement that MRD should be renamed "measurable residual disease," since the existence of any disease identified by these methodologies after treatment is linked to an inferior prognosis, and identification of residual disease even in morphologic remission is associated with a poorer prognosis [6]. MRD monitoring is rapidly becoming the most successful method and technique for determining prognosis and therapeutic strategy for AML patients, and it is now widely tracked using qRT-PCR, as evidenced by numerous studies from various laboratories. Over the last few years, real-time PCR has been implemented, allowing for a remarkable degree of sensitivity in diagnosis and the ability of leukemia to be detected [7].

Detecting fusion genes derived from chromosomal translocations, such as PML-RARA, AML-ETO1, and CBFb-MYH11, and more recently gene mutations, such as NPM1, is currently the most sensitive tool for this strategy [8, 9]. Unfortunately, more than half of all AML patients do not have the genetic lesions that can be monitored for MRD. As a result, alternative MRD markers are in high demand, and Wilm's tumor is one of them [10].

In BM samples from 80 to 90% of AML patients at diagnosis, the *WT1* gene is overexpressed at the mRNA level, and it is detectable in a stable low range in normal donors [11]. It can be thought of as a universal molecular marker of malignant hematopoiesis, and several studies have suggested that quantifying *WT1* expression level as a molecular marker for MRD monitoring is useful. Moreover, it's been proposed that its level of expression can have prognostic consequences for AML patients' remission rate and overall survival [12]. While treatment outcomes of AML have improved steadily over the last decades in younger and adults, limited changes have been observed in survival. We investigated the function of WT1 gene expression in AML patients' prognosis and its utility as an MRD marker after induction chemotherapy.

Materials and Methods

A total of 55 patients were recruited from the hematology unit of Alexandria main university hospital for the research. Group I: *WT1* expression was tested in bone marrow samples of 40 adult patients with newly diagnosed de novo AML at diagnosis and at day 28 after induction chemotherapy. Group II: included 15 (age and sex-matched) healthy controls with no prior history of hematological malignancy. The revised French-American-British classification was used to diagnose and classify AML established on morphologic, immunophenotypic, and cytochemical parameters [3]. From all participants an informed consent was taken in this study.

Laboratory investigations

- Complete blood image and morphological analysis in the laboratory.
- Aspiration of bone marrow
- Cytogenetic study
- Assays for fluorescence in situ hybridization (FISH).
- Immunophenotyping by flowcytometry. The Mo Abs mentioned below were used Table 1.
- RT-PCR quantification of a bone marrow sample for *WT1* gene expression assay

A Blood QI Aamp RNA blood mini kit was used to isolate RNA (Qiagen, Germany). Thermocycler (Applied Biosystems, USA) was used for reverse transcription, and the samples were held at -800 until PCR amplification. Finally, using Ipsogen WT1 Profile-Quant, real-time quantitative detection was performed on Stratagene (PCR MX 3000P, USA) (QIAGEN, Germany). The WT1 gene level was determined and expressed as a ratio to the ABL gene (endogenous control) found in the human body.

Table 1 The monoclonal antibodies used for immunophenotyping by flowcytometry

	FITC	PE	PERCP	PECY7	APC	APC-H7
Primary panel, tube 1	CD7	CD14	CD34	HLA-DR	CD13	CD45
Primary panel, tube 2	CD2	CD10		CD33	CD19	
Monocytic markers	CD11c	CD11b		CD64	CD4	CD45
Additional myeloid markers	MPO	CD117				

Treatment protocol

Induction chemotherapy for AML patients consisted of 3 days of anthracycline within 7 days of cytarabine ("3 + 7" protocol) [13]. From day one to day three, patients were given 45 mg/m^2 Anthracyline (Daunorubicin) intravenously. From days 1 to 7, they were given Cytarabine (cytosine arabinoside) 100 mg/m^2 via continuous infusion [14].

Assessment and consolidation after remission with four additional cycles of (HiDAC); high-dose Cytosine Arabinoside or (HAM regimen); high-dose Cytosine Arabinoside with Mitoxantrone.

Response criteria for AML patients after induction chemotherapy: [13]

Complete remission (CR) was defined as morphologically normal bone marrow with less than 5% lasts, neutrophil count more than 1.5 × 109/l, and platelet count more than 100 × 109/l.

Complete remission with incomplete hematologic recovery (CRi) Partial remission.

Primary induction failure (PIF), Relapse is characterized as disease recurrence after complete remission (CR) with more than 5% leukemic blasts in BMA or new extra medullary leukemia. Early relapse occurs within 6 months of CR1, while late relapse occurs after a period of more than 6 months [15]. The overall survival (OS) of a trial's patients is determined. It is calculated from the time of enrollment in a clinical trial or diagnosis to the time of death [16].

Statistical analysis of data

IBM SPSS software was used to capture and analyses the data. Quantitative data were defined using range (minimum and maximum), mean, standard deviation, median, and interquartile range, while qualitative data were described using numbers and percentages (IQR). The significance of the gained results was determined at a 5% level of significance.

Chi-square test, Fisher's exact correlation, Mann Whitney test, Student t-test, Spearman coefficient, Receiver operating characteristic curve (ROC), were some of the tests used.

Results

Demographic data

AML patients were divided into 23 females (57.5%) and 17 males (42.5%). The participants' ages ranged from 30 to 50 years old, with a mean of 38.40 6.62 years. The FAB classification of AML patients is shown in Fig. 1. The majority of them were FAB-M5 (42.5%). AML patients' cytogenetic risk stratification. The majority of the people were at a medium risk (55%) Table 2.

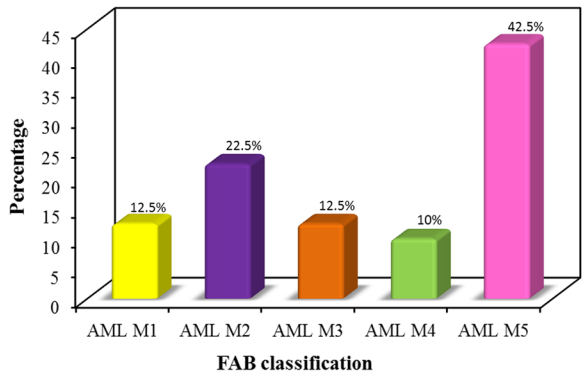

Fig. 1 Distribution of the AML patients according to FAB classification ($n = 40$)

WT1 Expression in AML patients and controls

The mean value of *WT1* transcript in AML patients at presentation was 11,109.3 ± 133,387.3 × 104/ABL, which was significantly greater than expression found in control patients BM. (median value of 69 × 104/ABL) Table 3. To determine a cut-off value for WT1 positivity, a receiver operating characteristic (ROC) curve analysis was created, based on the NCN of *WT1* gene expression of both AML patients and controls Fig. 2. According to the Youden index, a cut-off value of 1059 was used, which was 87.5% adaptive, had an AUC of 0.95, 100% accuracy, a negative predictive value (NPV) of 75%, a positive predictive value (PPV) of 100%, and was significant statistically (p value 0.001) Table 4.

WT1 Expression at diagnosis

WT1 expression levels in the BM of 40 adults with newly diagnosed AML were tested using real-time quantitative polymerase chain reaction (RQ-PCR). According to the cut-off value for *WT*1 positivity, 35 AML patients (87.5%) showed *WT1* overexpression at diagnosis and 5 patients (12.5%) were *WT*1-negative. *WT1* expression at diagnosis did not correlate with age, sex, hemoglobin level, leukocyte count, or peripheral blood & bone marrow last percentage at diagnosis. Also, no correlation was found between *WT*1 expression at diagnosis and AML FAB subtype & cytogenetic findings.

WT1 Expression after induction treatment

WT1-positive AML patients were followed up on post-induction chemotherapy by real-time PCR for their level of *WT1* expression and morphological assessment by BM analysis in a trial to see whether *WT1* expression could

Table 2 Cytogenetic findings in AML patients and their distribution according to risk stratification ($n=29$)

Risk group according to cytogenetics	Cytogenetics	Number	Percentage (%)
High risk group ($n=3$)	Unfavorable cytogenetics		
	inv.3	1	3.5
	t (6;9)	2	6.9
Intermediate risk group ($n=22$)	Intermediate cytogenetics		
	Cytogenetic abnormality not classified as adverse or favorable	8	27.5
	Normal karyotype	14	48.3
Low risk group ($n=4$)	Favorable cytogenetics		
	t (8; 21)	2	6.9
	t (15; 17)	2	6.9
Total		29	100

Table 3 Comparison between the AML patients and controls regarding WT1 expression

NCN of WT1 gene expression	AML patients ($n=40$)	Controls ($n=15$)
Min.–Max	33.0–74,741.0	5.0–1059.0
Mean ± SD	11,109.3 ± 13,387.3	262.13 ± 355.6
Median	7294.0	69.0
IQR	3084.5–14,272.0	35.0–390.0
Significance	$p<0.001$	

p: *p* value for Mann Whitney test for comparing Group I and group II

Statistically significant at $p \leq 0.05$

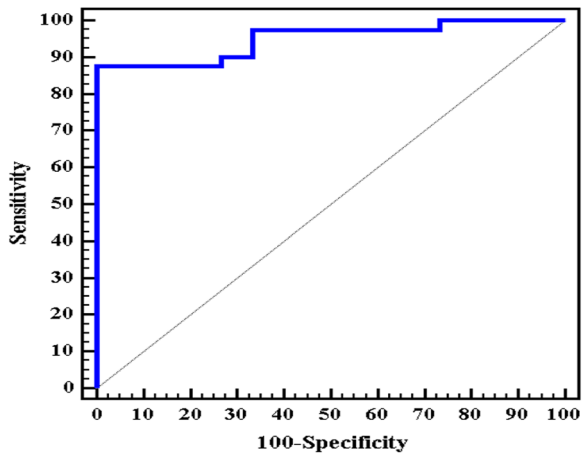

Fig. 2 ROC curve generated for level of WT1 expression among AML patients and controls

be utilized as a marker for follow-up and MRD assessment. A statistical significance was found between *WT1* expression post-induction & hematological response on BM examination, thus, indicating a higher sensitivity of *WT1* assessment over the morphological examination of the BM in the follow-up of AML patients to estimate the response to chemotherapy Table 5.

WT1 expression and long term outcome of AML

AML patients were followed up on for 15 months after diagnosis to see whether *WT1* expression levels had any impact on their overall survival. The OS was calculated using the Kaplan–Meier survival study Fig. 3. The potential difference between *WT1* positive and WT1 negative was analyzed by the log-rank test. Although the *WT1*-positive patients had a shorter OS than those with negative *WT1*, the disparity was not statistically significant. (Average of 10.14 vs. 13 months) Tables 6 and 7.

We then used Cox regression analysis between OS and *WT1* expression at diagnosis among the *WT1*-positive AML patients. *WT1* expression seems to affect statistically significant OS (*P* value < 0.038). As a result, it appears that patients with *WT1* overexpression at diagnosis have a shorter OS than those who do not have *WT1* positivity.

WT1 expression and relapse prediction of AML

Among AML patients 44.8% experienced early relapse and 55.2% late relapse. There was no significant difference among the time of occurrence of relapse and *WT1* expression Table 8.

Discussion

In AML, relapse is still the leading cause of treatment failure and death. Despite the fact that more than 80% of patients receive a CR after traditional chemotherapy, a large proportion of them develop recurrent disease [17]. Indeed, more stringent response requirements than CR are needed. The gold standard approach for stratifying patients based on the likelihood of relapse is to detect leukemia-specific gene mutations using PCR. Unfortunately, more than half of all AML cases lack one of these

Table 4 Characteristics of the WT1 cutoff for WT1 positivity

	AUC	*P*	95% CI	Cut off	Sensitivity	Specificity	PPV	NPV
WT1 cut-off	0.950	< 0.001*	0.897–1.003	> 1059#	87.50	100.0	100.0	75.0

AUC: Area under a curve, *p* value: probability value

CI: Confidence intervals

NPV: Negative predictive value, PPV: positive predictive value

* Statistically significant at $p \leq 0.05$

Cut off was choose according to Youden index

Table 5 Relation between hematological response and WT1 expression post-induction chemotherapy ($n = 35$)

	Treatment response				χ^2	FE *p*
	Complete remission ($n = 31$)		Remission failure ($n = 4$)			
	No	%	No	%		
WT1 expression post-induction						
WT1-Positive ($n = 14$)	10	71.4	4	28.6	6.774*	0.019*
WT1-Negative ($n = 21$)	21	100	0	0.0		
Total	31		4			

χ^2 Chi square test, FE: Fisher Exact

p: *p* value for association between different categories

* Statistically significant at $p \leq 0.05$

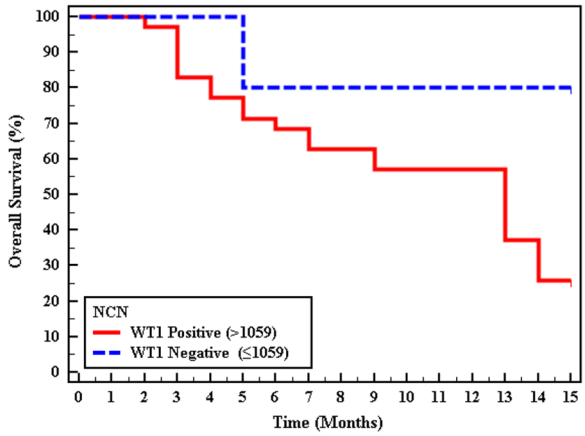

Fig. 3 Kaplan–Meier survival curve for overall survival with WT1 expression ($n = 40$)

unique genes, so new MRD-detecting genes are required. *WT1* is a transcriptional factor that has been identified as an MRD marker in acute leukemia [11].

The aim of this study was to look into the prognostic value of *WT1* gene overexpression in AML patients, as well as its utility as an MRD marker after treatment. At a statistically significant amount, the *WT1* level in AML patients was significantly higher than in control cases in our cohort. At the time of diagnosis, 87.5% of AML patients had an overexpression of the *WT1* transcript. This finding was similar to that revealed by other studies where *WT1* overexpression was reported to be approximately between 70 and 90% of AML patients [18–21].

Regarding our AML patients in this study, *WT1* expression was not affected by the patient's hematological profile such as platelet count, hemoglobin level, or WBC count. This was also in agreement with Østergaard et al. who conducted a study on BM samples from 133 newly diagnosed AML patients and compared them with those in healthy volunteers and found no statistical significance between *WT1* expression and hemoglobin level, WBC, or platelet count [18, 23]. No significant association was found between a higher PB and BM blasts percentage and *WT1* overexpression which came in congruence with Assem et al. and Ibrahim et al. who also had similar findings in their studies [23, 24]. However, Lane et al. conducted a study on 58 de novo AML patients and found, by using multivariate Cox regression analysis, that elevated *WT1* levels were significantly associated with higher PB and BM blast percentage [25]. The high *WT1* expression is hypothesized to originate from CD34-positive cells, thus it seems that the discrepancies might be due to differences in sample size.

In the current research, we discovered that AML patients who were *WT1*-positive at diagnosis had a shorter OS than those who were *WT1*-negative. Similarly, Bergmann et al., who conducted a study on 139 de

Table 6 Relation between overall survival (OS) and WT1 expression at diagnosis ($n=40$)

WT1 level of expression	Overall survival (OS)				χ^2	^{FE}p
	< 15 months ($n=27$)		≥ 15 months ($n=13$)			
	No	%	No	%		
WT1-Positive ($n=35$)	26	74.3	9	25.7	5.877*	0.031*
WT1-Negative ($n=5$)	1	20.0	4	80.0		

χ^2: Chi square test, FE: Fisher Exact

p: *p* value for association between different categories

* Statistically significant at $p \leq 0.05$

Table 7 Kaplan–Meier survival curve for overall survival with WT1 expression ($n=40$)

	Mean	Median	% End of study	Log rank	
				χ^2	*p*
WT1 level at diagnosis					
WT1-Positive	10.14	13.0	25.7	3.623	0.057
WT1-Negative	13.0	–	80.0		

Table 8 Relation between patient outcome and WT1 expression in relapsed AML patients ($n=29$)

WT1 expression	Patient outcome		*U*	*P*
	Early relapse ($n=13$)	Late relapse ($n=16$)		
Min.–Max	127.0–74,741.0	33.0–29,543.0	99.0	0.846
Mean ± SD	14,847.6 ± 20,102.4	10,998.5 ± 9572.4		
Median	7015.0	10,667.0		

U: Mann Whitney test

p: *p* value for association between different categories

novo AML patients reported that Patients with low *WT1* levels had a 59% chance of 3-year overall survival (OS), while patients with high levels had a 21% chance [27]. Galimberti et al. also discovered that AML patients with elevated *WT1* levels have a higher risk of disease progression [26]. In a larger sample population, Nomdedéu et al. reported the prognostic function of high *WT1* levels at diagnosis [27]. In contrast, Noronha et al. conducted a study on 155 AML patients and found no correlation between OS and *WT1* expression [28]. Similarly, Ibrahim et al. followed up 50 AML patients over 20 months and found no significant impact of WT1 on OS [22]. The difference in sample size and the ability to follow up AML patients over a longer period might confer an explanation to these discrepancies.

In our cohort, all *WT1*-positive patients that turned negative post-induction have achieved CR on BM examination, a finding that reflects concordance between the *WT1* status and morphological response to chemotherapy. However, some patients who were considered to have achieved CR on BM examination remained *WT1*-positive post-induction. Given that *WT1* is reported to be expressed on CD34 positive blast cells may reflect a higher sensitivity of *WT1* monitoring over the morphological examination of the BM in the evaluation of treatment response and detection of residual disease after induction chemotherapy. Candoni et al. found that 24% of his AML patients who were in CR were still WT1-positive [29]. In a study of 197 AML patients, Liu et al. discovered that low and high *WT1* expression is correlated with clinical remission and relapse, respectively [30]. A strong correlation between *WT1* expression and BM morphological remission was also found in other studies [31–33].

Conclusions

WILMS tumor 1 gene expression was found to be significantly higher in AML patients than control cases, overall, our results confirmed the prognostic significance of *WT1* overexpression in AML patients. Our findings support the application of MRD in AML patients based on WT1 overexpression.

Abbreviations

ABL: Abelson; AML: Acute myeloid leukemia; APL: Acute promyelocytic leukemia; BM: Bone marrow; BMA: Bone marrow aspiration; CD34: Cluster of differentiation 34; CR: Complete remission; CRi: Complete remission with incomplete hematologic recovery; FAB: French American British; FISH: Assays for fluorescence in situ hybridization; HiDAC: High-dose cytosine arabinoside; HAM: High-dose cytosine arabinoside with mitoxantrone; MRD: Minimal residual disease; NPM1: Nucleophosmin 1; NPV: Negative predictive value; OS: Overall survival; PB: Peripheral blood; PIF: Primary induction failure; PPV: Positive predictive value; QRT-PCR: Quantitative real-time polymerase chain reaction; ROC: Receiver operating characteristic; WT1: Wilms tumor 1.

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Author contributions

NF designed the research, HD carried out the molecular genetics studies, EN recruited the patients with management and follow up and NE processed the samples and performed the statistical analysis. All authors shared in writing

the manuscript, read and approved the final version of this manuscript. All authors read and approved the final manuscript.

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Availability of data and materials
Data and materials are available upon request.

Declarations

Ethics approval and consent to participate
The authors give thanks to all the study participants. Written consent to inclusion was obtained from all participants and they were informed of the study. The study was conducted after being authorized by the Medical Ethics Committee of Alexandria Faculty of Medicine. Reference Number Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors confirm that they have no competing interests.

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