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ORIGINAL ARTICLE

WT1 and TP53 as valuable diagnostic biomarkers for relapse after hematopoietic stem cell transplantation in acute myeloid leukemia

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Abstract

Background Relapse following hematopoietic stem cell transplantation (HSCT) occurs relatively frequently and is a significant risk factor for mortality in patients with acute myeloid leukemia (AML). Early diagnosis is, therefore, of utmost importance and can provide valuable guidance for appropriate and timely intervention. Here, the diagnostic value of two molecular markers, Wilms tumor 1 (*WT1*) and tumor suppressor protein p53 (*TP53*), were studied.

Methods and results Twenty AML patients undergoing HSCT participated in this investigation. Some had relapsed following HSCT, while others were in remission. Peripheral blood (PB) and bone marrow (BM) samples were collected following relapse and remission. *WT1* and *TP53* messenger RNA (mRNA) expression was evaluated using reverse transcription-quantitative polymerase chain reaction (RT–qPCR). The diagnostic value of genes was evaluated by utilizing receiver-operating characteristic (ROC) curve analysis. ROC analysis showed *WT1* and *TP53* as diagnostic markers for relapse after HSCT in AML patients. The mRNA expression level of *WT1* was elevated in individuals who experienced relapse compared to those in a state of remission (p value < 0.01). Conversely, the expression level of *TP53* mRNA was lower in individuals who had relapsed compared to those in remission (p value < 0.01).

Conclusions *WT1* and *TP53* possess the potential to serve as invaluable biomarkers in the identification of molecular relapse after HSCT in patients with AML. Further studies for a definitive conclusion are recommended.

Highlights

- In acute myeloid leukemia disease relapse is a major contributor to mortality.
- After transplantation, up to 50% of acute myeloid leukemia patients may relapse.
- Making early detection of molecular relapse essential for effective treatment.
- *WT1* and *TP53* emerged as diagnostic biomarkers for early detecting molecular relapse.

Keywords Acute myeloid leukemia · Hematopoietic stem cell transplantation · Relapse · *WT1* · *TP53* · Biomarkers

Introduction

Acute myeloid leukemia (AML) is a malignancy that impacts hematopoietic stem cells, resulting in abnormal proliferation of immature myeloid cells with abnormal differentiation and forming a clone of cells with a myeloid lineage [1]. In addition to conventional chemotherapy, allogeneic stem cell transplantation (allo-SCT) serves as a fundamental treatment modality for AML patients who meet the necessary criteria. Regardless of its predictive characteristics, allo-SCT possesses the highest potential as a post remission therapy for enduring survival of intermediate- or high-risk diseases and as a salvage therapy for relapsed or resistant disease [2]. Self-renewing leukemic stem cells

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maintain the malignant clones in leukemia, which are rare and quiescent, making them highly resistant to cytotoxic chemotherapy. This resistance can contribute to relapse and disease progression [3].

In patients with AML, disease relapse and related leukemia-associated complications are major contributors to mortality. The relapse of AML is associated with a notable increase in molecular complexity, as numerous novel subclones and mutations arise that lead to increased resistance to cytotoxic chemotherapy [4]. Up to 50% of AML patients may relapse after transplantation; thus, early molecular relapse diagnosis is crucial for successful treatment. Waiting for hematological relapse, which has a poor prognosis, is less effective than starting treatment at the molecular relapse stage with a modest disease burden [5]. Inactivation of the tumor suppressor protein p53 (*TP53*) is a strong promoter of AML and Loss or alteration of *TP53* is a significant predictor of poor outcomes in AML. However, *TP53* mutations are commonly found in AML cases with increased genomic instability. This suggests that additional factors may contribute to the development and progression of AML, highlighting the complex relationship between *TP53* mutations and AML [6].

In AML, the overexpression of Wilms tumor 1 (*WT1*) functions as an oncogene, unlike its expected role in the BM. The effect of *WT1* depends on its interactions with multiple protein partners, such as p53 and Fms-related receptor tyrosine kinase 3 (*FLT3*). However, it is unclear what triggers the overexpression of *WT1* in AML or whether it occurs early or late in disease onset. Furthermore, it is still unclear how a pro-apoptotic factor such as *WT1* transforms into an oncogene. The conflicting results from multiple studies continue to be a source of discussion about the prognostic significance of *WT1* overexpression in AML [7]. *WT1* expression levels increase during relapse and decrease in the bone marrow after HSCT, making the *WT1* transcript assay highly sensitive for detecting relapse [8].

This study aimed to investigate the diagnostic potential of *WT1* and *TP53* gene expression as markers for relapse in AML patients who have undergone HSCT using real-time PCR.

Table 1 Primers for the expression of mRNAs

mRNAs	Sequences
WT1 forward primer	ACGCCCCTTCATGTGTGCTTA
WT1 reverse primer	GCTGGTCTGAACGAGAAAACCT
TP53 forward primer	GCCCCTCCTCAGCATCTTATC
TP53 reverse primer	GTACAGTCAGAGCCAACCTCA
ABL forward primer	ACACTCTAAGCATAACTAAAGG
ABL reverse primer	TGAAAAGC GATGTAGTTGCTTGGGACCCA

Materials and methods

Patients and sample collection

Twenty AML patients who underwent HSCT at Taleghani Hospital transplant center in Tehran were included in the study. All protocols and sampling were performed following the completion of informed consent forms by patients and obtaining confirmation from the ethics committee of Shahid Beheshti University of Medical Sciences (Ethics code No: IR.SBMU.RETECH.REC.1401.360). Peripheral blood (PB) and bone marrow (BM) samples were collected from patients with relapsed AML after HSCT and individuals in remission after HSCT at Taleghani Hospital in Tehran.

mRNA extraction and expression analysis

Using reverse transcription-quantitative polymerase chain reaction (RT–qPCR), the relative expression levels of *WT1* and *TP53* messenger RNAs (mRNAs) in PB and BM samples were determined. PB and BM samples were subjected to total RNA extraction using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) in accordance with the manufacturer's recommendations. Then, agarose gel electrophoresis was used to assess RNA integrity, and the purity of intact RNA was assessed by NanoDrop. According to the manufacturer's instructions, cDNA synthesis was performed utilizing the ExcelRT™ Reverse Transcriptase kit developed by SMOBio, a company based in South Korea. A Real-time PCR reaction was conducted in a total volume of 13 µl for the expression analysis step. The composition of the reaction mixture consisted of 6.5 µl of 2× Master Mix (Real Q Plus Master Mix, Denmark), 0.5 µl of each primer, 1 µl of synthesized cDNA, and 4.5 µl of nuclease-free water. The Applied Biosystems StepOnePlus™ Real-time PCR System and Thermal Cycler (USA) was used for this purpose. The reactions were performed under the following conditions: an initial denaturation at 95 °C for 15 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60–62 °C for 15 s, extension at 72 °C for 15 s, and a final step of 10 min for the melting curve analysis. The *ABL* geometric mean cycle threshold (CT) was utilized to calculate the relative expression level, represented as $2^{-\Delta CT}$. The sequences of the housekeeping gene and mRNA primers are provided in Table 1.

Statistical analysis

The data analysis was performed using two software programs: SPSS version 25 and GraphPad Prism version 9. The Shapiro–Wilk test was used to confirm the normality of the WT1 and TP53 variable distributions. Additionally, a t test

was utilized to assess whether there was any discernible difference in the expression of *WT1* and *TP53* between AML patients who had relapsed following HSCT and those who were in remission. Furthermore, Pearson's test was used to measure the linear correlation between *WT1* and *TP53* expression. The diagnostic value of *WT1* and *TP53* in the diagnosis of relapse was analyzed by receiver operating characteristic (ROC) analysis. A p value less than 0.05 was considered statistically significant.

Results

Patient data

The study included 20 AML patients who underwent HSCT. The average age of the individuals in the study was 51.3 ± 20.5 years, and half of them were of the female gender. Also, the median and age range were 47 and 25–64, respectively. The demographic information was recorded and is presented in Table 2, out of the patients who underwent HSCT, seven experienced relapse, while 13 achieved remission.

mRNA expression

Compared to individuals who experienced remission following HSCT, AML patients who relapsed after HSCT exhibited significantly higher levels of *WT1* expression (p value< 0.01). *TP53* expression was significantly lower in AML patients who relapsed after HSCT than in the individuals in remission after HSCT (p value < 0.01) (Fig. 1). The analysis of PB samples revealed that individuals who experienced relapse had significantly higher expression levels of *WT1* compared to those in remission (p value < 0.05) (Fig. 2A). The analysis further revealed that among individuals who relapsed, those in the age group of 47< exhibited higher expression levels of *WT1* compared to those who achieved remission (p value < 0.05) (Fig. 2B).

The analysis of BM samples revealed that individuals who experienced relapse had significantly lower expression levels of *TP53* compared to those who achieved remission (p value < 0.05) (Fig. 2C). Furthermore, when considering the age factor, it was observed that among individuals who relapsed, those in the age group of 47 < had lower expression levels of *TP53* compared to those who remained in remission (p value < 0.01) (Fig. 2D). Blast percent was significantly higher in the relapse group than in the remission group at diagnosis (p value < 0.05).

Table 2 Participant variables (N = 20)

Variables at the diagnosis time	Number/Frequency (%)	Number/Frequency (%)
Features	Relapse (7)	Remission (13)
Age (year)		
Age < 47	5 (50%)	5 (50%)
Age > 47	2 (20%)	8 (80%)
Gender		
Male	3 (30%)	7 (70%)
Female	4 (40%)	6 (60%)
Sample type		
BM	4 (28.57%)	10 (71.43%)
PB	3 (50%)	3 (50%)
FAB classification		
AML M3	1 (50%)	1 (50%)
AML non-M3	6 (33.33%)	12 (66.66%)

BM: Bone marrow, **PB**: Peripheral blood, **FAB**: French American British classification systems, **AML**: Acute myeloid leukemia

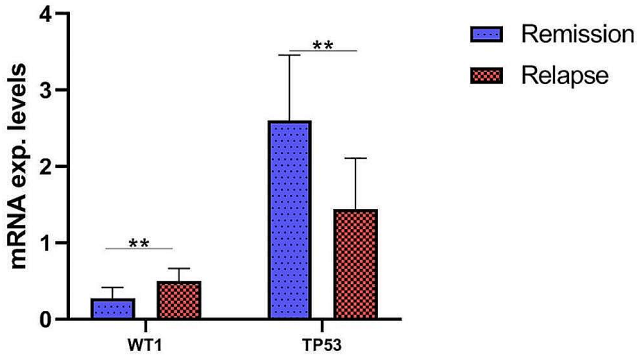

Fig. 1 Relative expression of *WT1* and *TP53* in individuals who had relapsed after HSCT and those who had remission after HSCT. NS stands for not significant, $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***

ROC analysis results

In our study, we conducted a ROC analysis to assess the diagnostic value of *WT1* and *TP53* mRNAs in the detection of relapse. The analysis of ROC curves demonstrated that both *WT1* and *TP53* mRNAs serve as convenient diagnostic markers for relapse after HSCT in AML patients (Fig. 3A and B) (p value: 0.008, 0.008). *WT1* had a sensitivity of 0.85 and a specificity of 0.76, and *TP53* had a sensitivity of 1 and a specificity of 0.61 for diagnosing relapse after HSCT in AML patients.

Discussion

Recent studies have demonstrated that AML patients who relapse post-HSCT have a poor prognosis. However, it has been increasingly recognized that early intervention at the molecular relapse stage, when the disease burden is minimal, yields more favorable outcomes than waiting for

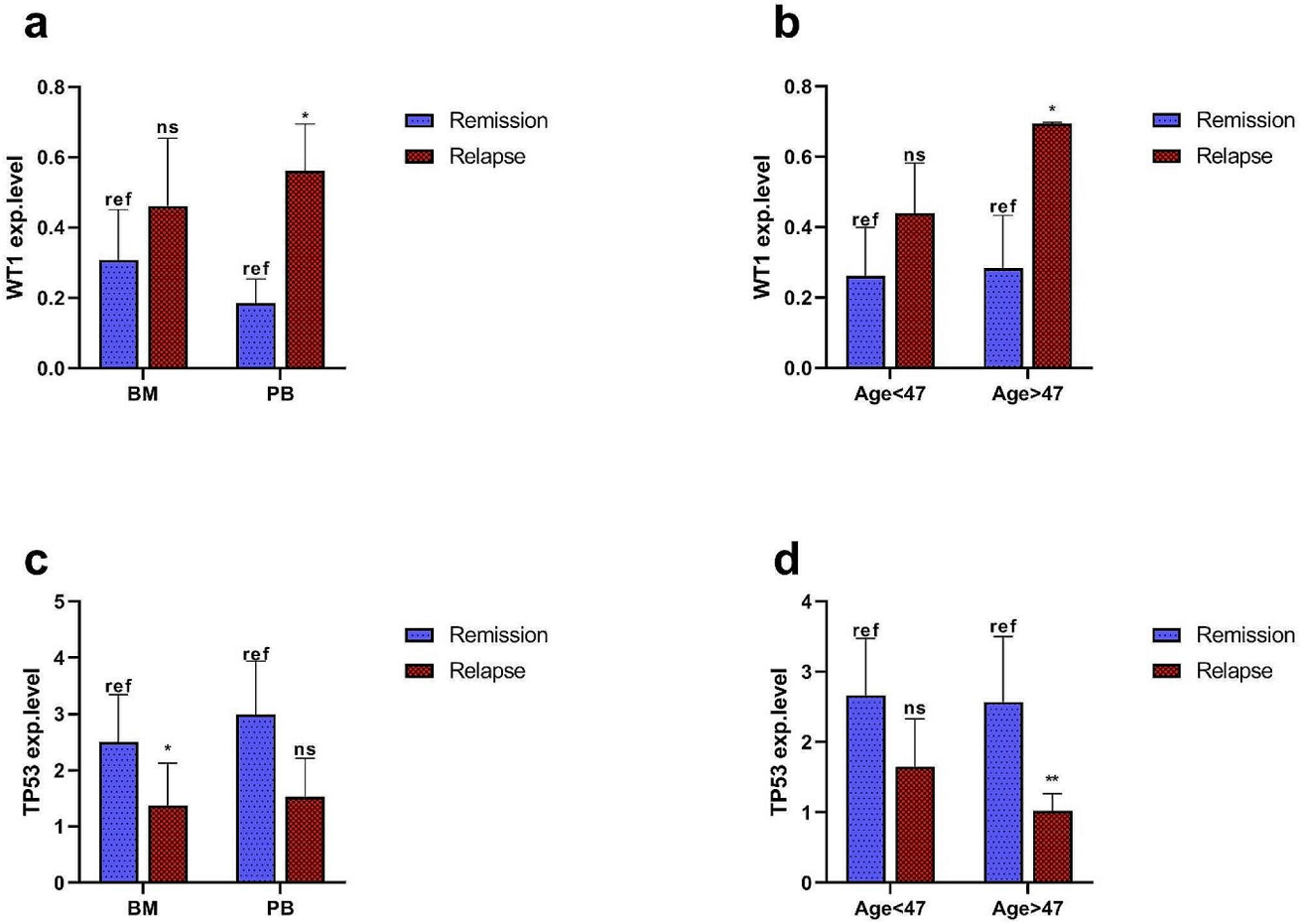

Fig. 2 Relative expression of *WT1* and *TP53* in individuals who had relapsed after HSCT and those who had remission after HSCT. **a** Expression of *WT1* in BM and PB samples. **b** Expression of *WT1* in the age < 47 and age > 47 groups. **c** Expression of *TP53* in BM and PB samples. **d** Expression of *TP53* in the age < 47 and age > 47 groups. NS stands for not significant, $P < 0.05$ *, $P < 0.01$ **, $P < 0.001$ ***

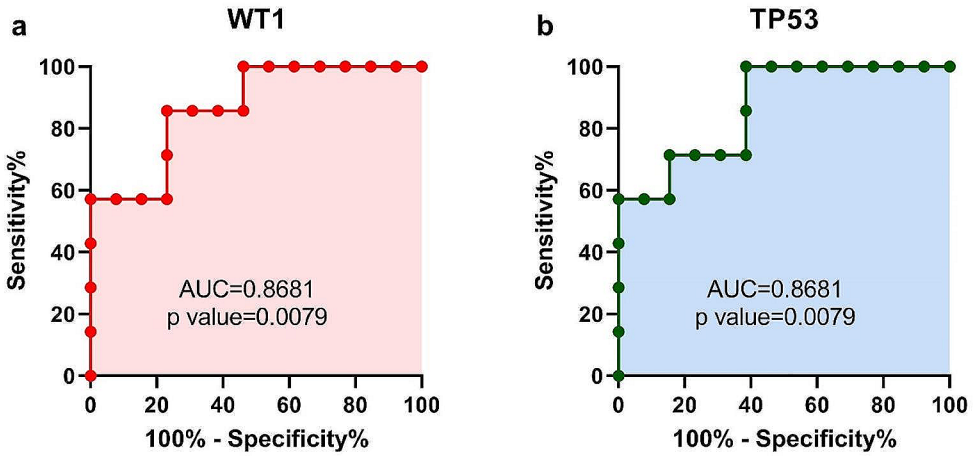

Fig. 3 Receiver-operating characteristic (ROC) analysis. **a** ROC curve indicated the powerful diagnostic value of *WT1* for relapse. **b** ROC curve analysis indicated the powerful diagnostic value of *TP53* for relapse

hematological relapse. Therefore, early detection of imminent relapse at the molecular level is crucial for successful therapeutic intervention [9–11]. The goal of our study was to examine the potential diagnostic use of *WT1* and *TP53* gene expression as potential recurrence biomarkers in AML patients who had received HSCT. Real-time PCR and ROC curve analysis were used for the evaluation. The *WT1* gene can function as both an oncogene and a tumor suppressor in the development of AML. The tumor suppressive protein p53 plays a crucial role in regulating *WT1* activity by

physically interacting with it and facilitating its recruitment to the promoter region of *WT1* target genes, which modulates their expression. However, the disruption of p53 and *WT1* interaction caused by the p53 mutation (p53R248Q) in AML cases can result in the loss of *WT1* target gene modulation. Moreover, wild-type p53 is necessary for the antiproliferative activity of *WT1* in AML cells, while *WT1* promotes AML cell proliferation in the absence of p53 or in the presence of mutated p53. In general, *WT1* inhibits AML cell proliferation via a p53-dependent mechanism [12].

According to our findings, patients with relapsed AML had higher levels of *WT1* mRNA expression than those in remission. Consistent with our findings, studies on *WT1* as a marker for measurable residual disease (MRD) have demonstrated that *WT1* expression is generally low in the bone marrow of healthy people but rises in AML patients during diagnosis. After successful treatment for AML, it has been observed that the expression of *WT1* decreases but may rise again before clinical relapse [13–20].

In our results, AML patients who had relapsed after HSCT had lower expression of *TP53* mRNA compared to the individuals in remission. We did not find any studies that investigated the expression of *TP53* mRNA in AML patients who relapsed after HSCT. Nevertheless, a study by Mattsson K et al. revealed upregulation of p53 protein in patients who relapsed compared to relapse-free individuals at 3–6 months post-HSCT, which is in contradiction with our data [21]. It's difficult to explain. It is anticipated that the expression of *TP53* mRNA will be lower in AML patients who relapsed after HSCT than in those who were in remission because *TP53* is a tumor suppressor and works in conjunction with *WT1* to inhibit proliferation. The *WT1* assay is very useful for predicting and managing relapse following allogeneic stem cell transplantation, regardless of the presence of chimeric gene markers [22]. *WT1* transcript levels are a more sensitive diagnostic relapse test than the morphologic readout approach utilized in the clinic [23]. *WT1* expression post-transplant appears to be a reliable marker of MRD, which is the most important predictor of relapse and survival in AML patients undergoing allogeneic HSCT [24]. In patients with AML undergoing allo-SCT, monitoring of MRD^{WT1} can be conducted in more than 80% of cases. According to the European LeukemiaNet (ELN) criteria, *WT1* overexpression in PB is a highly accurate predictor of post-transplant relapse. Consequently, it is advised to quantify *WT1* expression in PB after allo-SCT to identify patients at high risk of poor outcomes. Patients classified as being at very poor risk may benefit from early immunosuppressive drug withdrawal, preemptive donor lymphocyte injection, and/or chemotherapy [25]. These published findings suggest that *WT1* can be utilized to detect relapse in AML patients after HSCT at an early stage. Consequently, morphological relapse can be prevented by performing appropriate therapeutic interventions.

So, we decided to evaluate the expression of *WT1* and *TP53* mRNAs in AML patients who relapsed after HSCT compared to those in remission to study their probable diagnostic potential for relapse after HSCT. On the basis of ROC curve analysis, we determined that *WT1* and *TP53* expression levels have an excellent capacity for discriminating between relapsed and remission patients. Thus, *WT1* and *TP53* mRNAs proved to be valuable markers for the diagnosis of relapse in AML patients following HSCT.

We had some limitations in conducting the present study, such as its small sample size. It is of great importance to acknowledge that our sampling procedure was conducted during the COVID-19 pandemic when the frequency of patients visiting medical centers reached its lowest point. This issue had a significant impact on our sampling.

Conclusions

In conclusion, *WT1* and *TP53* emerged as valuable diagnostic biomarkers for potential early detection of molecular relapse following HSCT in patients with AML. Utilizing these markers can facilitate the timely implementation of appropriate therapeutic interventions to prevent hematological relapse.

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Author contributions AA collected the samples, performed the experiments, performed the statistical analyses, and wrote the first version of the manuscript. FF, JG and MM drafted the manuscript and analyzed the data. MM conceived the study, designed and coordinated it, and finalized the manuscript. All authors gave final approval for publication.

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Data availability The data have been incorporated into the manuscript and properly documented and archived under the supervision of the corresponding author.

Declarations

Ethics approval All experimental procedures and protocols were conducted strictly with the ethical guidelines and standards set forth by the institutional and/or national research committee. The present study obtained ethical approval from the ethics committee of Shahid Beheshti University of Medical Sciences, with the assigned ethics code number IR.SBMU.RETECH.REC.1401.360.

Consent to participate Before participating in the study, all patients provided informed consent by completing the requisite consent form.

Consent to publish Not applicable.

Competing interests The authors declare no competing interests.

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