Prognostic value of WT-1 gene combined with recurrent cytogenetic gene in acute myeloid leukemia

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Research Article

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Abstract

**Background:** Wilms tumor gene 1 (WT-1 gene) is overexpressed in most patients with acute myeloid leukemia (AML) and provides the evidence for an indicator of minimal residual disease (MRD) monitoring, but further studies of the combined prognostic value of WT-1 gene are needed to be illustrated due to its relatively low specificity. The aim of the study is to explore the prognostic value of WT-1 gene combined with recurrent cytogenetic gene in AML.

**Methods:** We dynamically examined the transcript expression of WT-1 gene in the bone marrow samples of adult patients with AML, and then validated the prognostic value of WT-1 gene with or without recurrent cytogenetic gene.

**Results:** In AML, the transcript expression of WT-1 gene was closely related to leukemic tumor burden and could act as an accurate indicator of molecular MRD detection. Most patients with low level expression of WT-1 gene after induction and consolidation therapy were significantly associated with favorable relapse free survival (RFS) and overall survival (OS), but there were still 16.7% patients were relapsed and died of primary disease. However, when analyzing of WT-1 gene combined with recurrent cytogenetic gene, none of the patients with low level expression of WT-1 gene and negative of recurrent cytogenetic gene were relapsed and died in the median follow-up time of 19 months (range: 3-94months).

**Conclusion:** WT-1 gene combined with recurrent cytogenetic gene is a more accurate indicator of MRD monitoring and prognosis evaluation in AML.

1 Background

Acute myeloid leukemia (AML) is a clinical and molecular cytogenetic heterogeneous malignant clonal disease originating from myeloid stem progenitor cells [1]. Unraveling the heterogeneity of AML allows improved prognostic and predictive abilities and leads to the development of selected therapies for AML subsets. Efforts have been made to find the reliable predictive biomarkers to evaluate clinical efficacy and identify prognostic value in AML.

The identification of minimal residual disease (MRD) has led to substantial improvements in understanding the depth of remission and recognition of no hematological recurrence of AML [2]. Multiparameter flow cytometry (MFC), polymerase chain reaction (PCR), fluorescence in situ hybridization (FISH) and next-generation sequencing (NGS) are frequently-used methods for the MRD detection in AML with specific immunophenotype or recurrent cytogenetic gene [3]. However, there is no absolute specificity in the immunophenotype of leukemia blasts, and more than half of cases lack recurrent cytogenetic gene, so it is urgent to identify other molecular targets applicable for the majority patients of AML.

Wilms tumor gene 1 (WT-1 gene) encoding transcription regulatory factors and is highly expressed in a certain tissue or a specific developmental stage of hematopoietic cells. Especially, WT-1 gene is overexpressed at the transcription level in 80–90% patients with AML, which usually provides the
evidence for an indicator of MRD monitoring and early interventional therapy, but further studies of the combined prognostic value of WT-1 gene are needed to be demonstrated due to its relatively low specificity [4, 5]. The purpose of the present study is to explore the prognostic value of WT-1 gene combined with recurrent cytogenetic gene quantitative detection in AML.

2 Materials And Methods

2.1 Patients and samples

This study included adult patients with AML composed of 15 newly diagnosed (ND) cases, 343 complete remission (CR) cases, 61 refractory cases and 38 relapsed cases (Table 1), in the Second Hospital of Anhui Medical University between 2012 and 2021. All patients were characterized their own diagnostic stratification and received standard induction chemotherapy followed by either chemotherapeutic consolidation therapy or allogeneic hematopoietic stem cell transplantation (allo-HSCT) in accordance with Chinese guidelines for diagnosis and treatment of adult AML [6, 7]. CR was defined as meeting all the criteria of the National Comprehensive Cancer Network (NCCN). Relapse free survival (RFS) was defined as the duration from the day of CR to the day of relapse. Overall survival (OS) was defined as the time from date of diagnosis to the date of death. The bone marrow samples derived from all patients were collected and processed within 6 hours.

| Disease status and number of AML patients with the measurement of WT-1 gene |  
|---|---|---|
| ND | N = 15 |  
| CR | N = 343 |  
| With WT-1 gene ± recurrent cytogenetic gene and follow-up data | N = 77 |  
| Risk stratification | Low risk 19 |  
| | Medium risk 31 |  
| | High risk 27 |  
| Curative effect | CCR 50 |  
| | Relapse 27 |  
| With only WT-1 gene | N = 266 |  
| Refractory | N = 61 |  
| Relapse | N = 38 |  

ND: newly diagnosed; CR: complete remission; CCR: continuous complete remission.

2.2 Measurement of MFC-MRD
Cells derived from bone marrow samples of AML patients were stratified, washed and incubated with monoclonal antibodies, then analyzed by MFC. MFC-MRD were identified by two hematopoietic stem progenitor cells markers, at least two leukemia-associated immunophenotype markers identified at diagnosis, and other lymphoid markers. The proportion above 0.01% was defined as the positive for MFC-MRD. Isotype-matched controls were included in experiments and were used to define the cutoff point for positive/negative staining.

### 2.3 Measurement of WT-1 gene

Total RNA was extracted from bone marrow samples were drawn from AML patients using TRIzol and then reverse-transcribed using a Transcript RT Kit. Quantitative real-time PCR was performed on the ABI 7500 System using SYBR Green PCR Master Mix. All primers were synthesized by Sangon. The relative WT-1 gene mRNA expression level was calculated using the $2^{-\Delta\Delta CT}$ method.

### 2.4 Measurement of recurrent cytogenetic gene

RNA and DNA was extracted from bone marrow samples of AML patients. Quantitative real-time PCR was performed on the ABI 7500 System using SYBR Green PCR Master Mix. The RNA samples were evaluated for recurrent cytogenetic genes including BCR/ABL P210 or P190, PML/RARα, RUNX1-RUNX1T1 and CBFβ-MYH11. The DNA samples were evaluated for recurrent cytogenetic genes including NPM1 and CEBPA.

### 2.5 Statistical analysis

Categorical variables were presented as sample size/percentage and compared using the Chi-squared test. Continuous variable with normal distribution was compared by Student’s t test. Kaplan-Meier approach was performed to estimate time-to-event analysis, and the log-rank test was used to evaluate between-group differences in survival curves. All statistical analyses were performed by SPSS 23.0, and a two-tailed p value < 0.05 was considered significant statistical analysis.

### 3 Results

#### 3.1 Transcript expression of WT-1 gene in AML

In the present study, it was found that bone marrow WT-1 mRNA relative expression levels of AML were increased in ND patients (0.3300 ± 0.0552, N = 15) and refractory patients (0.1966 ± 0.0340, N = 61), decreased in CR patients (0.0068 ± 0.0010, N = 343), but increased again in relapse patients (0.1361 ± 0.0242, N = 38). The difference in ND, refractory and relapse patients were all statistically significant compared with CR patients (all p < 0.0001) (Fig. 1A). It was indicated that WT-1 gene transcript expression was closely related to leukemic tumor burden and could act as an accurate indicator for molecular MRD detection in AML.

After strict screening, 77 AML cases in CR status with dynamic WT-1 gene expression results with or without dynamic recurrent cytogenetic gene results and follow-up data were selected to explore the
significance of WT-1 gene expression and its relationship with recurrent cytogenetic gene. Although morphological CR was achieved in these 77 cases, WT-1 gene transcript expression was still significantly higher in medium risk group (0.0229 ± 0.0074, N = 31) and high risk group (0.0151 ± 0.0067, N = 27) compared with low risk group (0.0060 ± 0.0020, N = 19). The difference between medium/high risk group and low risk group was statistically significant (Fig. 1B). It was indicated that the remission depth of AML patients with morphological CR after induction and consolidation therapy was still not enough, especially for the medium and high risk cases.

3.2 Prognostic value of WT-1 gene in AML

To explore the prognostic value of WT-1 gene in AML, the positive and negative WT-1 gene expression level of was calculated as 0.0022 based on the synchronous detection of the same sample MFC-MRD positive or not (AUC = 0.809 (0.760–0.859)) in the total 343 cases with CR status. Then, the changes of WT-1 gene transcript expression level in the above 77 cases were continuously monitored dynamically, the median follow-up time was 19 months (range: 3-94months). The WT-1 gene high level group was defined as once more than 0.0022, and the WT-1 gene low level group was defined as always less than 0.0022. Finally, follow-up and survival analysis were conducted for these two groups, and results showed that the RFS and OS of the WT-1 gene high level group were significantly shorter than the WT-1 gene low level group, there was statistical difference between the two groups (Fig. 2A and 2B). It was noteworthy that 82.4% cases of WT-1 gene low level group were associated with favorable long-term prognosis, but there were still 17.6% cases of that were relapsed and died of leukemia (Fig. 2A and 2B). It was suggested that WT-1 gene transcript expression level was an accurate indicator of MRD monitoring and prognosis evaluation in AML, but the specificity of WT-1 gene still needs to be further improved.

3.3 Prognostic value of WT-1 gene combined with recurrent cytogenetic gene in AML

Of the above 77 cases with CR status, 41 cases were only characterized with WT-1 gene, but 36 cases were simultaneously characterized with WT-1 gene and recurrent cytogenetic gene. Excluding the influence of WT-1 gene, survival data of 36 AML patients for recurrent cytogenetic gene were also analyzed and showed that recurrent cytogenetic gene expression level had prognostic value. The RFS and OS in the recurrent cytogenetic gene positive group were significantly shorter than the recurrent cytogenetic gene negative group, there was statistical difference between the two groups (Fig. 3A and 3B). It was suggested that recurrent cytogenetic gene expression level also was an accurate indicator of MRD monitoring and prognosis evaluation in AML. However, the prognostic value of WT-1 gene combined with recurrent cytogenetic gene is still unclear.

To explore the prognostic value of WT-1 gene combined with recurrent cytogenetic gene in AML, the above 36 cases with WT-1 gene data and recurrent cytogenetic gene data were divided into 3 groups based on gene expression profiles: double positive group (WT-1 gene high level and recurrent cytogenetic gene positive), single positive group (WT-1 gene high level or recurrent cytogenetic gene positive) and
double negative group (WT-1 gene low level and recurrent cytogenetic gene negative). The survival data showed that the double negative patients showed the best performance in both RFS and OS, and none of them had achieved recurrence or death in the median follow-up time of 19 months (range: 3-94 months) (Fig. 4A and 4B). Double-positive patients had the worst performance in both RFS and OS, and also had the most recurrences and deaths (Fig. 4A and 4B). While the single-positive patients with WT-1 gene high level or recurrent cytogenetic gene positive were in the middle level in both RFS and OS (Fig. 4A and 4B). It was suggested that WT-1 gene combined with recurrent cytogenetic gene is a more accurate indicator of MRD monitoring and prognosis evaluation in AML, comparing to the prognostic value of either singer WT-1 gene or singer recurrent cytogenetic gene.

4 Discussion

AML is a kind of malignant clonal disease and most cases cannot be cured, because conventional chemotherapy is difficult to completely remove the residual leukemia blasts. The major limitation in the treatment of AML is the accurate and sensitive detection of MRD. The purpose of MRD detection is to make individual treatment decisions such that those patients who require more aggressive approaches are treated promptly and to avoid toxic and expensive treatments for those patients who do not require them.

The expression of WT-1 gene mRNA is increased in the early stage of hematopoietic cell differentiation, especially in myeloid cells [4, 5, 8]. WT-1 gene transcript expression level can be used as a biomarker for diagnosis, monitoring of MRD and predicting relapse for molecular remission in AML, especially in normal karyotype AML patients [9–13]. It also has potential of being a predictive molecular biomarker for the treatment of leukemic cases after allo-HSCT [14–16]. WT-1 responds to oncogenic signaling and is part of a signaling-responsive transcription factor hub that controls leukemia blast cells growth [17]. WT-1 facilitates the self-renewal of leukemia-initiating cells through the upregulation of anti-apoptotic gene [18]. Higher expression of WT-1 gene with lower expression of CD58 or deletion of TP53 gene are biomarkers for risk stratification of AML [19, 20]. WT-1 gene specific expression on blast cells and its interaction with cytotoxic T cell has been explored for its potential usage WT-1 gene-based immunotherapy [14, 20–23]. However, due to the relatively low specificity of WT-1 gene in leukemia blasts, further studies of the combined prognostic value of WT-1 gene are needed to be discussed.

The mutational landscape of AML has revised diagnostic, prognostic and therapeutic schemata over the past decade. Recurrent cytogenetic genes have functional consequences beyond typical oncogene-driven growth and loss of tumor suppressor function [24]. They are commonly used as MRD monitoring indicators for AML, which can guide efficacy evaluation, protocol adjustment and prognosis assessment [3]. However, their application scope is limited, because more than 50% patients do not have such favorite characteristic genes in AML. The lack of effective monitoring indicators in clinical practice often leads to the untimely and unreasonable judgment of clinical efficacy and adjustment of treatment plan, and finally resulting a poor survival for most AML patients without recurrent cytogenetic genes.
In the present study, the transcript expression level and clinical prognostic value of WT-1 gene in AML were firstly evaluated. Compared with CR patients, we found that WT-1 gene transcript level was highly expressed in untreated, refractory and relapsed AML patients, even in CR patients of the medium risk and the high risk. Then, the cut off value of WT-1 gene positive and negative in our center was calculated as 0.0022 according to MFC-MRD results. So, we redefined the WT-1 gene transcript expression of AML patients as high level group and low level group, and found that the RFS and OS of WT-1 gene low level group were significantly longer than WT-1 gene high level group, but there were still 17.6% cases of that were relapsed and died of leukemia. Meanwhile, we also found that the RFS and OS of the recurrent cytogenetic gene negative group were significantly longer than those of the recurrent cytogenetic gene positive group. It was indicated that WT-1 gene and recurrent cytogenetic gene both were the accurate indicators of MRD monitoring and prognosis evaluation for the patients with AML. But the combined prognostic value of WT-1 gene and recurrent cytogenetic gene is still unknown.

Therefore, compared with the prognostic value of singer type of WT-1 gene or recurrent cytogenetic gene, we found that the WT-1 gene high level and recurrent cytogenetic gene positive group had a significant survival disadvantage both in RFS and OS. But the WT-1 gene low level and recurrent cytogenetic gene negative group had a significant survival advantage both in RFS and OS, and none of them were relapsed and died in the median follow-up time of 19 months, that was to say, the prognostic predictive value of the combing indicator for long-term remission reached 100%. It was indicated that WT-1 gene combined with recurrent cytogenetic gene could more accurately monitor the depth of disease remission and judge the long-term prognosis of the patients with AML.

5 Conclusion

The transcript expression of WT-1 gene is closely related to leukemic tumor burden and can act as an indicator of molecular MRD detection and prognosis evaluation, but its specificity still needs to be further improved. Our findings support that WT-1 gene combined with recurrent cytogenetic gene analysis is a more accurate indicator of MRD monitoring and prognosis evaluation in AML.

Declarations

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Author contributions ZM Zhai and QS Tao conceived the study and designed experimental procedures. Q Zhang and QS Tao collected data, analyzed data and wrote the draft. QS Tao, Q Zhang, HT Yan, LL Liu, XY Ren, M Zhou, SD Xiong and HP Wang performed the data. QS Tao revised and edited the draft. All authors reviewed and approved the final version.

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**Data availability** The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

**Code availability** Not applicable.

**Declarations**

**Ethical approval** This study was approved by the Institutional Review Board Institutional of the Second Hospital of Anhui Medical University (2011991) in accordance with the IRB's guidelines and regulations.

**Conflict of interests** The authors declare that they have no competing interests.

**References**


**Figures**

**Figure 1**

Transcript expression of WT-1 gene in AML. WT-1 mRNA relative expression in AML patients with different disease status (A) and different risk stratification of CR status (B).
Figure 2

The prognostic value of WT-1 gene in AML. The WT-1 gene high level group had a significant survival disadvantage compared with the WT-1 low level group in RFS (A) and OS (B).

Figure 3

The prognostic value of recurrent cytogenetic gene in AML. The recurrent cytogenetic gene positive group had a significant survival disadvantage compared with the recurrent cytogenetic gene negative group in RFS (A) and OS (B).
The prognostic value of WT-1 gene combined with recurrent cytogenetic gene in AML. The double positive group of WT-1 gene high level and recurrent cytogenetic gene positive had a significant survival disadvantage in RFS (A) and OS (B). The double negative group of WT-1 gene low level and recurrent cytogenetic gene negative had a significant survival advantage in RFS (A) and OS (B). The single positive group of WT-1 gene high level or recurrent cytogenetic gene positive was in the middle level in RFS (A) and OS (B).