Chapter 16

Wilms’ Tumor Gene (WT1) Expression and Minimal Residual Disease in Acute Myeloid Leukemia

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Abstract

The identification of minimal residual disease (MRD) has led to substantial improvements in early recognition of the recurrence of acute myeloid leukemia (AML). Flow cytometry (FC), real-time quantitative polymerase chain reaction (RQ-PCR) and fluorescence in situ hybridization are useful methods for the detection of MRD in AML patients although molecular monitoring of leukemia-specific rearranged (RUNX1-RUNX1T1 and CBFB-MYH11) or mutated genetic (NPM1, CEBPA) sequences represents the most sensitive methodology.

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Besides, more than 50% of all AML patients lack one of these specific sequences, so it is crucial to identify molecular targets applicable for the majority of patients. WT1 is overexpressed at the mRNA level in 80-90% of AML cases at diagnosis in both peripheral blood and bone marrow, and is detectable in a consistent low range in normal donors. These features have led to its adoption for MRD detection using RQ-PCR. A European LeukemiaNet Study found the magnitude of WT1 log reduction after induction chemotherapy to be an independent predictor of relapse. Other studies showed a poorer outcome in patients having WT1 levels above reference thresholds at specific time points. WT1 expression was compared with other modalities of MRD assessment, such as RQ-PCR of specific fusion genes and FC, but no differences in terms of predictive value emerged. Finally, some authors translated the use of WT1 in the clinic giving donor lymphocytes infusions to patients with increasing WT1-mRNA levels after allogeneic stem cell transplantation and obtaining an improvement of survival in this subset. Data collected on WT1 expression over the past years provided evidence for the use of this molecular marker to stratify high-risk AML patients. It can also be used as a marker for early interventional therapy, but further studies are needed to demonstrate it.

Key words: Acute myeloid leukemia; Allogeneic stem cell transplantation; Minimal residual disease; Multiparameter flow cytometry; WT1 expression

Introduction

WT1 is an important regulatory molecule involved in cell growth and development. The presence of zinc fingers in the C-terminal half of the protein confers WT1 the role of a potent transcriptional factor, including important genes for cellular growth and metabolism among the targets (1). It has been found that WT1 can either enhance or repress the expression of specific target genes, depending on the levels of WT1 expression, the isoforms, the location of the transcriptional start site, and the cell type in which the experiment was performed (2, 3). In human hematopoietic cells, WT1 appears to behave as a tumor suppressor gene as the overexpression of WT1 in early human bone marrow (BM) cells leads to growth arrest and reduced colony formation. Indeed, in normal human BM, WT1 is expressed at extremely low levels and is confined to the primitive CD34+ population of cells (4, 5). Besides, WT1 is highly overexpressed in the BM or peripheral blood (PB) of a variety of leukemias, and these evidences support the role of WT1 as an oncogene in this subset (6, 7). Increased levels of WT1 expression can be found in both acute lymphoblastic and acute myeloid leukemia (AML) although more frequently in AML (frequencies varying from 73% to 91%) (8-10). Following the discovery of overexpression of WT1, there has been growing evidence that the WT1 expression levels may have a prognostic role in AML. In 139 de novo AML, Bergmann et al. (11) observed that the probability of the 3-year overall survival (OS) was 59%
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in patients with low WT1 levels compared to 21% in patients with high levels. Similarly, Galimberti et al. (12) showed a higher probability of disease progression in AML patients presenting high WT1 levels, and recently, Nomdedeu et al. (13) also confirmed the prognostic role of high WT1 levels at diagnosis in a larger study population. However, these data are in contrast with results reported by others where WT1 levels did not correlate with the outcome (8–10, 14), thus suggesting a controversial role for WT1 expression at presentation. On the contrary, a greater agreement was found among groups that have used WT1 levels as a marker of minimal residual disease (MRD) in AML remission BMs (less than 5% of blast cells). In particular, WT1 expression has been shown to predict disease progression in AML patients treated with conventional chemotherapy (8–10, 15–17) and patients undergone allogeneic stem cell transplantation (allo-SCT) (18–22). Furthermore, when WT1 expression was compared with widely used techniques in monitoring MRD such as multiparameter flow cytometry (MFC) (23) or specific molecular targets such as fusion genes transcripts (PML-RARa, AML-ETO, and CBFb-MYH11), comparable sensitivities were found in predicting the relapse in AML. Thus, we addressed our review on the main papers that focused on the predictive role of WT1 expression as an MRD marker in AML patients, as well as results from comparison between WT1 and other methodologies in monitoring MRD.

WT1 as a minimal residual disease marker after conventional chemotherapy

Many studies have shown that the assessment of MRD may prove useful to better stratify high-risk patients and address treatment intensity in AML (Table 1). The most sensitive method for this strategy involves the detection of fusion genes derived from chromosome translocations, such as PML-RARa, AML-ETO, and CBFb-MYH11 (24, 25), and more recently gene mutations such as NPM1 (26, 27). Besides, more than 50% of AML lack known genetic lesions or clonality markers suitable for MRD monitoring. Thus, alternative markers for MRD are highly sought, and WT1 gene has been suggested as a candidate. Nondisease-specific genes should be abnormally high expressed in malignant cells when compared with normal controls to be used as an MRD marker. Cilloni et al. (8) first showed that the number of WT1 copies in 71 AML BMs and 14PB was 27,669 (ranges: 1,081–121,086) and 10,244 × 10^4 (ranges: 758–86,140) copies of Abelson gene (ABL) mRNA, respectively. Conversely, WT1 levels were extremely low in normal samples: median number of WT1 copies was 78 (range: 3–180) and 4 (1–22) × 10^4 ABL in BM and PB samples, respectively. Second, in order to assess the significance of the WT1 expression for the detection of MRD, the authors monitored WT1 levels in 10 AML patients characterized by the presence of fusion gene transcripts (CBFb-MYH11 and AML1-ETO); a good parallelism between sequential WT1 and fusion transcripts values was found: some patients who remained in complete remission (CR) (28) constantly showed WT1 values within the normal range, while patients who experienced a relapse showed a conversion to WT1 levels above the normal range in concomitance with fusion
Table 1. WT1 expression after conventional chemotherapy

<table>
<thead>
<tr>
<th>Authors</th>
<th>MRD (cutoff)</th>
<th>LOG reduction</th>
<th>Time of assessment</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weisser et al.</td>
<td>0.4%</td>
<td>≤2 Log</td>
<td>16–60 vs 61–120 vs 121–180 days after start of therapy</td>
<td>Within 61–120 and 121–180 days, levels ≤0.4%, and ≥2 log reduction were associated with improved EFS and OS</td>
</tr>
<tr>
<td>Cilloni et al.</td>
<td>250 × 10^4 ABL copies</td>
<td>≤2 Log</td>
<td>Postinduction/postconsolidation</td>
<td>WT1 transcript reduction ≥2 log after induction, and WT1 levels more than 250 × 10^4 ABL copies after consolidation predicted a significantly increased risk of relapse</td>
</tr>
<tr>
<td>Nomdedeu et al.</td>
<td>170 and 100× 10^4 ABL copies</td>
<td>-</td>
<td>Postinduction/postintensification</td>
<td>WT1 levels greater than 170 copies after induction and 100 copies after intensification identified patients with the highest probability to relapse and die</td>
</tr>
<tr>
<td>Lapillone et al.</td>
<td>50 × 10^4 ABL copies</td>
<td>-</td>
<td>Postinduction</td>
<td>WT1 &gt; 50× 10^4 ABL copies after induction is an independent prognostic risk factor of relapse and death</td>
</tr>
<tr>
<td>Rossi et al.</td>
<td>77 × 10^4 ABL copies</td>
<td>≥1.96 Log</td>
<td>Postinduction/postconsolidation</td>
<td>Only postinduction MRD ≥ 77× 10^4 ABL copies and log reduction ≤1.96 predicted a shorter DFS and OS</td>
</tr>
</tbody>
</table>

*Study performed on adults.
†Study performed on children.

transcript increasing although patients were still in CR. Indeed, the quantitative assessment of WT1 transcript allows to distinguish between normal and pathological samples, as well as increasing WT1 levels above the normal range can be prognostically significant during the follow-up of patients. Weisser et al. (16), some years later, confirmed a significant correlation between WT1 levels and fusion genes (96%, median r = 0.996) in a similar study population. The authors also showed that more than 2 log reduction of WT1 levels within 61 and 180 days from the start of chemotherapy was associated with a significantly improved OS and event-free survival (EFS). Comparable results were published by the European LeukemiaNet (ELN) study group (9). In order to standardize the WT1 assay, Cilloni et al. (9)
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first undertook a systematic evaluation of nine published and “in-house” real-time quantitative polymerase chain reaction (PCR) assays in a quality control study involving 11 ELN laboratories. Then, the selected ELN WT1 assay was applied to samples from 129 follow-up patients, and a significantly increased risk of relapse was found in patients achieving less than 2 log reduction in WT1 transcripts after induction therapy (p = 0.004). This study suggests that application of a standardized WT1 assay early during the patients’ therapy could potentially be used to refine risk stratification in AML and decisions on the role of allogeneic transplant in first morphological CR. Recently, in a large study population of AML (n = 584), Nomdedeu et al. (13) defined three different prognostic groups after induction and intensification on the basis of WT1 levels. Patients having more than 170 copies after induction and more than 100 copies after intensification showed the highest probability to relapse and the lowest to OS. On pediatric AML also, similar results were obtained when WT1 was investigated in AML. Lapillone et al. (29) observed that WT1 higher than $50 \times 10^4$ ABL copies after induction was an independent prognostic risk factor of relapse (p = 0.002) and death (p = 0.02) in pediatric AML. Published results conferred to WT1 an important role in monitoring MRD and stratifying patients with AML, similarly to results obtained by MFC (30–37). When the techniques were compared, a different role was addressed to each one on the basis of the timing of assessment and quantification of MRD or log reduction. Generally, our group and others showed that detection of MRD by WT1 expression and MFC had comparable prognostic value and technical performance described in terms of sensitivity (sens), specificity (spec), predictive value (PV), and likelihood ratio (LR) (23). Besides, when we compared log reduction with MRD measured after conventional chemotherapy by both WT1 expression and MFC, important differences between the two methodologies were found (38). Log reduction and MRD well predicted the outcome at both timing of assessment according as both methodologies, but WT1 log reduction after induction (spec 84.2%, sens 46.2%, LR+ 2.92, LR- 0.64) identified the relapse better than the MRD (spec 57.7%, sens 84.2%, LR+ 1.99, LR- 0.27) and opposite results were true after consolidation for MFC (spec 80.8%, sens 57.9%, LR+ 3.01, LR- 0.52 vs spec 73.1%, sens 63.2%, LR+ 2.35, LR- 0.50 for MRD and log reduction, respectively), thus confirming what was previously published about either WT1 or MFC singularly.

WT1 as minimal residual disease marker in allogeneic stem cell transplantation

Allo-SCT represents the only effective therapy for high-risk patients with AML in first or subsequent CR. Nevertheless, relapse remains a crucial issue in this setting, and new methods able to prevent it are needed (39). Cytogenetics and response after induction therapy were uniformly recognized as predictors of relapse, but there is a growing evidence that quantification of MRD is also a powerful, independent predictor of prognosis (Table 2). Ogawa et al. (18) studied the impact of WT1 levels after allo-SCT on the relapse and the capability to prevent it
Table 2. WT1 expression in allogeneic stem cell transplantation

<table>
<thead>
<tr>
<th>Authors</th>
<th>MRD (cutoff)</th>
<th>Time of assessment</th>
<th>Intervention MRD-based</th>
<th>Main results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ogawa et al. (18)*</td>
<td>$10^{-4}$-$10^{-2}$</td>
<td>Post-transplant</td>
<td>Immune interventions (discontinuation of immunosuppressive therapy or DLI)</td>
<td>Probability to relapse within 40 days was significantly associated with WT1 expression levels. Among high-risk patients, a significantly longer doubling time of WT1 levels in patients who underwent preemptive measures</td>
</tr>
<tr>
<td>Zhao et al. (19)*</td>
<td>0.60%</td>
<td>Pre- and post-transplant (+120 days)</td>
<td>Immune interventions (DLI, tapering of immunosuppressive therapy) when WT1 levels were $&gt;$0.60%</td>
<td>Greater than 0.60% after transplant has been shown as an independent risk factor for DFS and OS. High-risk patients who received immune interventions displayed a longer OS</td>
</tr>
<tr>
<td>Pozzi et al. (20)*</td>
<td>$100 \times 10^4$ ABL copies</td>
<td>Pre- and post-transplant</td>
<td>DLI if MRD $&gt;180 \times 10^4$ ABL copies</td>
<td>Post-transplant WT1 expression was the strongest predictor of relapse. Patients with increasing WT1 levels receiving DLI and showed an improved OS</td>
</tr>
<tr>
<td>Rossi et al. (43)*</td>
<td>$138 \times 10^4$ ABL copies</td>
<td>Pre- and post-transplant (+30 days)</td>
<td>–</td>
<td>A shorter DFS was found in patients having high levels ($\geq 138$ copies) of WT1 at day +30 from transplant. The combination of MFC and WT1 may be preferred for preemptive immune interventions</td>
</tr>
</tbody>
</table>

*Study performed on adults.

by preemptive therapeutic measures in patients with leukemias [AML, acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML)]. First, the authors showed that the probability of relapse that occurred within 40 days significantly increased according to the increase in WT1 expression levels ($100\%$ for $1.0 \times 10^{-2}$ to $5.0 \times 10^{-2}$, $44.4\%$ for $4.0 \times 10^{-3}$ to $1.0 \times 10^{-2}$, $10.2\%$ for $4.0 \times 10^{-4}$ to $4.0 \times 10^{-3}$, and $0.8\%$ for $<4.0 \times 10^{-4}$). Then, among high-risk patients, they found a significantly longer doubling time of WT1 levels in patients who underwent the discontinuation of immunosuppressive therapy or donor lymphocyte infusions (DLIs). In conclusion, they stated that WT1 was a very useful marker to predict and manage the relapse following the allo-SCT. Similar data were reported by Zhao et al. (19), who investigated the
prognostic significance of WT1 expression in a large study population (n = 138) of AL (AML, ALL) patients following allo-SCT. After measuring MRD by WT1 levels at designed time points, the authors showed that WT1 levels ≥0.60% before allo-SCT indicated higher rates of relapse post-transplant. Similarly, WT1 levels ≥0.60% at median time of +120 days from transplant was associated with lower DFS and OS. Besides, 20 patients showing high levels of WT1 expression received modified DLI, and a median of 0.22% of WT1 levels was observed after intervention. Indeed, patients showing a recurrence trend after allo-SCT, did not experience it due to interventions MRD-based. Recently, Pozzi et al. (20) also confirmed that AML patients in CR before transplant and with a median expression of WT1 >100 × 104 ABL had a higher relapse risk (53% vs 26%) and a lower 5-year survival (36% vs 62%) when compared with patients who had less than this cutoff. Similar results were obtained when the threshold of WT1 ≤100 copies was considered at 30 days after allo-SCT. Thirty-eight patients achieving a CR but exceeding 180 × 104 ABL copies post-transplant were eligible for immune intervention by DLI: 17 patients received DLI and 21 did not. The interval between MRD positivity and relapse was significantly longer in patients receiving DLI. These studies clearly defined the predictive effect of WT1 expression on relapse in AML patients who underwent allo-SCT. In particular, post-transplant WT1 expression was the strongest predictor of outcome in multivariate analysis and was found to be a useful marker to select patients for preemptive immune intervention (DLI, tapering of immunosuppressive therapy). Comparable data were reported in a smaller number of patients monitored before and after transplant (21, 22). However, discordant results on prognosis were obtained when MFC and WT1 levels were compared (40–42). In our recent paper, we investigated technical performance of MRD detected by the two techniques at different time points, before and after transplant. At day +30 post-transplant, we recommended to study MRD by either or both methods, as it had a strong predictive role. Although post-transplant WT1 measurement is a valuable and essential marker for MRD monitoring also in our series, the combination of MFC and WT1 may be preferred to a single one when further treatments should be administered to prevent the relapse. In fact, double-positive MRD after allo-SCT correlated with a higher probability to experience a recurrence, based on higher product between specificity and sensitivity (43).

Conclusions

The relapse remains the main cause of treatment failure and death in AML. Although more than 80% of patients achieves a CR after conventional chemotherapy, a significant number of them experiences a recurrence disease (44). Indeed, more stringent criteria of response than CR are needed. The monitoring of leukemia-specific gene mutation by PCR represents the gold standard method to stratify patients on the basis of the risk to relapse. Unfortunately, more than 50% of AML cases lack one of these specific genes, and new genes to detect MRD are desirable. WT1 is a transcriptional factor, which has found an important role in acute
leukemias as MRD marker. To date, all published papers have confirmed the prognostic value of WT1 levels in AML patients achieving a CR after chemotherapy or allo-SCT. Indeed, despite the controversial role of WT1 expression at the presentation of disease, WT1 levels higher than the given thresholds in AML remission BM predicted the risk of relapse and death. The main concerns grown on this technique referred to cutoff that should be used and the influence of regenerating BM on quantification of the number of WT1 copies. Although WT1 assay has been standardized by ELN, methods to determine the positive threshold of MRD differentiate from one to another study group, with values ranging from 50 to 250 × 10^4 ABL. Further, WT1 transcript values were not univocally normalized with respect to the number of ABL. On the contrary, regenerating CD34+ cells may be WT1 levels, affecting the sensitivity (45). According to the better sensitivity of 2log reduction compared to MRD and the amply demonstrated prognostic value of this cutoff after induction chemotherapy, the log reduction of copy number may overcome these pitfalls. Finally, post-transplant MRD positive by WT1 is a strong predictor of outcome, and it has been found that WT1 levels may be useful for preemptive immune intervention after transplant. Besides, the low product between sensitivity and specificity for WT1 expression suggests using another method such as MFC to detect MRD and decide for further treatments in case of double positivity.

Conflict of Interest

The authors declare no potential conflicts of interest with respect to research, authorship and/or publication of this article.

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