Wilms’ Tumor-1 Transcript in Peripheral Blood Helps Diagnose Acute Myeloid Leukemia and Myelodysplastic Syndrome in Patients with Pancytopenia

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Abstract. Background/Aim: Pancytopenia is caused by acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), aplastic anemia (AA), or by non-hematological diseases. Because Wilms’ tumor-1 (WT1) is overexpressed in patients with AML and MDS, its expression level may be helpful for diagnosing these hematological malignancies. Patients and Methods: We retrospectively investigated the WT1 transcripts in peripheral blood (PB) from 47 patients with decreased blood cell counts. Results: The final diagnoses included AML, MDS, AA, drug poisoning, and non-hematological diseases. PB WT1 mRNA was overexpressed in AML and MDS, whereas the patients with other diseases mostly tested negatively for the transcript. The patients with MDS with higher marrow blast counts had higher PB WT1 mRNA levels. The sensitivity of the PB WT1 transcript in detecting AML and MDS was 78%, and the specificity was 90%. Conclusion: The WT1 mRNA level in PB may help diagnose AML and MDS in patients with pancytopenia.

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Patients and Methods

Patients. Patients who were presented with decreases in at least two blood cell lineages in the PB between January 2008 and December 2012 were included in this study. Cytopения was defined as a white blood cell count <4,000/µl, hemoglobin (Hb) <12 g/dl in males or 11 g/dl in females, and platelets <100,000/µl. The patients who met these criteria were referred to our department for further evaluation. The patients then underwent blood tests, including the measurement of the PB WT1 transcript level, and BM aspiration in certain cases. Among these patients, those for whom a final diagnosis was achieved were retrospectively analyzed. Patients who exhibited an infiltration of leukemia cells into the PB were excluded. This retrospective study was approved by the Ethics Committee of the University of Fukui Hospital.

Determination of the final diagnosis. To confirm the diagnosis, BM samples were aspirated and evaluated using standard techniques, including hemograms, cell surface markers, and karyotypes in certain cases. The diagnosis of leukemia and MDS was made based on standard cytological and histochemical examination of BM smears according to the French–American–British criteria and the World Health Organization criteria (11, 12).

Measurement of WT1 mRNA levels. The PB samples were evaluated for WT1 mRNA levels using real-time reverse transcriptase-polymerase chain reaction (RT-PCR). The primers were prepared by BML (Tokyo, Japan) (13). WT1 mRNA in BM was also evaluated in certain cases.

Statistical analyses. All statistical analyses were performed using the Microsoft Excel 2007 software (Microsoft, Redmond, WA, USA). All graphs, linear regression lines, and curves were generated using the GraphPad Prism software (version 5.0) (GraphPad Software, Inc. San Diego, CA, USA). Values of p≤0.05 were considered statistically significant.

Results

Patients. The study population consisted of a total of 47 patients, who were presented with pancytopenia or bcytopenia and for whom there was a final diagnosis. The median age was 74 years (range=24-89 years), with 25 males and 22 females. Table I shows the characteristics for each patient, including the final diagnosis and PB counts on presentation. Sixty-six percent (31/47) of the patients were presented with pancytopenia (Table I). Table II summarizes the patient groups categorized according to the final diagnosis. Nearly 60% (28/47) of the patients were confirmed to have a final diagnosis of AML or MDS (Table II). Apart from these myeloid malignancies, the diseases that presented with cytopenenia were AA, drug-induced cytopenian, and other systemic diseases, such as liver cirrhosis, systemic lupus erythematosus, and infections. There were 13% (6/47) AA cases, the frequency of which was close to the one (16%) reported by a Swedish study (3). Importantly, the patients examined in this study exhibited no blasts in the PB, which might otherwise be strongly suggestive of underlying hematological malignancies. Thus, the final diagnoses of the patients with cytopenia varied, suggesting that not all the patients had hematological diseases.

Determination of the WT1 mRNA levels in PB. The PB samples were evaluated for WT1 mRNA levels using real-time RT-PCR. Overall, the values varied widely among the patients. The data were plotted according to the four patient groups, namely, AML, MDS, AA, and non-hematological (drug-induced, liver cirrhosis, systemic lupus erythematosus, disseminated intravascular coagulation, and systemic infection). The WT1 mRNA was overexpressed in PB in the AML group and the MDS group (Figure 1A). Overall, 78% (22/28) of the patients with myeloid malignancies (AML, MDS) had elevated levels of WT1 transcripts in PB (for a sensitivity of 78%), whereas 90% (17/19) patients in the AA and non-hematological groups tested negatively for the WT1 transcript in PB (indicating a specificity of 90%) (Figure 1A). The increase in WT1 transcript levels in PB was not significant between the AML group and the MDS group (p=0.39, two-tailed Mann-Whitney test). Several studies demonstrated an elevation of WT1 mRNA levels in PB in patients with MDS (14-16). In this study, WT1 transcript levels in PB were apparently elevated in proportion to the increase in the BM blast count (Figure 1B), although WT1 transcripts were not found in half of the patients with refractory anemia (RA). WT1 levels in PB of the patients with refractory anemia with excess of blasts I (RAEB-I) were significantly higher than those of the patients with RA (p=0.049, two-tailed Mann-Whitney test) (Figure 1B), as were WT1 levels of the patients with RAEB-II (p=0.006, two-tailed Mann-Whitney test), which were slightly but insignificantly higher than those of patients with RAEB-I (p=0.39, two-tailed Mann-Whitney test) (Figure 1B). Thus, elevated WT1 transcript levels in PB would be suggestive of underlying AML or MDS.

Determination of WT1 mRNA levels in BM. Seventeen patients were also examined for WT1 transcripts in BM. The WT1 levels were significantly higher in the AML group and the MDS group than in the non-malignant group (four patients with AA, two with drug-induced cytopenian, one with disseminated intravascular coagulation) (Figure 2A) (AML versus non-malignant, p=0.008, two-tailed Mann-Whitney test; MDS versus non-malignant, p=0.028, two-tailed Mann-Whitney test). Using 11 samples with elevated WT1 transcript levels in PB, there was a close correlation between the WT1 levels in PB and the BM (R²=0.61, p=0.0043) (Figure 2B). Conversely, among the samples that tested negatively for WT1 transcripts in PB, the WT1 levels in BM were not predictive (Figure 2B). The results thus suggest that WT1 mRNA in PB, but not in BM, might be a preferred marker for diagnosing myeloid malignancies.

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Discussion

Pancytopenia can result from diverse mechanisms (2). It was previously reported that many serious conditions, including AA (16%), were found in 100 patients with pancytopenia in the Swedish portion of an international study of AA conducted between 1983 and 1986 (3). In that report, nearly one-third of the patients were not found to have any obvious explanation at the time of discovery of their pancytopenia, suggesting difficulties in diagnosing conditions with decreased blood counts. Nassem et al. evaluated the etiological and clinicohematological profile in children with bicytopenia and pancytopenia (17). They aspirated the BM from 990 children for different indications. Out of these, 571 (57.7%) had either pancytopenia (17.7%) or bicytopenia (40%). The most common etiology was acute leukemia (66.9%) in bicytopenic children and AA (33.8%) in pancytopenic children (17). In our study, the major causes of cytopenia among the patients who had a diagnosis were AML, MDS, and AA. A limitation of the present study was that we only recruited patients who underwent WT1 transcript measurement and received a confirmed final diagnosis. Nevertheless, our study indicates that hematological malignancies may underlie a cytopenic condition without blast infiltration in the PB.

The present retrospective study was conducted to assess correlation of WT1 transcript levels in PB with a diagnosis of AML or MDS in patients presenting pancytopenia, or at least bicytopenia. It was previously reported that the expression of WT1 was demonstrated in 75% of acute leukemias and blast crises of chronic myeloid leukemia (10). Monitoring WT1 mRNA in the PB and the BM has been previously shown to be useful for estimating minimal residual disease and predicting relapse of leukemia (4-6).
Moreover, the degree of WT1 expression was highly correlated with the type of MDS, and a significant correlation was found between WT1 expression levels, blast cell percentage, and the presence of cytogenetic abnormalities (16). The present study showed that the sensitivity for WT1 overexpression in PB in AML and MDS was 78% (22/28) and the specificity was 90% (17/19) (Table I, Figure 1A). Two out of eight patients with AML and four out of twenty patients with MDS tested negatively for the WT1 transcript in PB. In the study of the patients with AML, not all of the patients exhibited overexpression of WT1 (10). With respect to MDS, it was reported that there was no significant difference in the WT1 mRNA levels between AA and RA, suggesting that WT1 might not be a good marker to discriminate AA from RA (18). However, other tumor markers, including carcinoembryonic antigen, CA19-9, and

Figure 1. Peripheral blood Wilms’ tumor-1 (WT-1) mRNA levels in patients with cytopenia. A: The WT1 mRNA levels (copies/μg RNA) in the peripheral blood were plotted with respect to the diagnosis in 47 patients. B: WT1 mRNA levels in peripheral blood in myelodysplastic syndrome (MDS). WT1 mRNA levels (copies/μg RNA) were plotted with respect to the diagnosis in 20 patients with MDS. The dotted line represents levels in controls. AML, Acute myeloid leukemia; AA, aplastic anemia; non-hem, non-hematological diseases; RA, refractory anemia; RAEB-I, refractory anemia with excess of blasts-I; RAEB-II, refractory anemia with excess of blasts-II. The horizontal bar in each group indicates the mean value.

Figure 2. A: Bone marrow Wilms’ tumor-1 (WT-1) mRNA levels. WT1 mRNA levels (copies/μg RNA) in bone marrow were plotted with respect to the diagnosis in 17 patients. B: The relationship between WT1 transcripts in peripheral blood and the bone marrow in 17 paired samples from patients. The dotted line represents the level of controls. AML, Acute myeloid leukemia; MDS, myelodysplastic syndrome; non-malignant, the disease group including aplastic anemia, drug-induced cytopenia, and non-hematological diseases. The horizontal bar in each group indicates the mean value.
CA125, are not always elevated in the corresponding cancer patients (19). Therefore, the absence of WT1 in a small percentage of patients with AML or MDS may not negate the usefulness of this specific marker in the clinic. The present results thus suggest that the WT1 transcript in PB may be helpful for diagnosing myeloid malignancies in patients with reduced peripheral blood counts.

WT1 transcripts were found in BM in non-hematological diseases, although the level was low compared with the levels found in AML and MDS (Figure 2A). WT1 mRNA expression is observed in normal hematopoietic CD34+ cells, but decreases during differentiation, and becomes undetectable in mature cells (20). The expression in the patients with non-hematological diseases would reflect the background of the normal CD34+ cells of the BM (13, 14). Conversely, hematological diseases would reflect the background of the mature cells (20). The expression in the patients with non-decreases during differentiation, and becomes undetectable in residual normal cells (13, 21). In this regard, the use of WT1 transcript in PB might be preferable for screening myeloid malignancies, compared with WT1 transcript in BM.

In conclusion, pancytopenia occurs in a wide variety of systemic diseases including myeloid malignancies. WT1 transcript in PB, as a sensitive and specific marker, may help to properly diagnose AML and MDS in patients who present with lowered blood counts.

References


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