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

TAKAHIRO YAMAUCHI, YASUFUMI MATSUDA, MIHOKO TAKAI, TOSHIKI TASAKI, NAOKO HOSONO, EIJU NEGORO, SATOSHI IKEGAYA, KAZUTAKA TAKAGI, SHINJI KISHI, AKIRA YOSHIDA, YOSHIMASA URASAKI and TAKANORI UEDA

Department of Hematology and Oncology, Faculty of Medical Sciences, University of Fukui, Matsuoka, Eiheiji, Fukui, Japan

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.*

Pancytopenia is a descriptive term referring to a reduction in all three blood cell lineages, erythrocytes, leukocytes, and platelets, which can result from diverse mechanisms (1, 2). Pancytopenia with bone marrow dysfunction can be caused by deficiency in vitamin B12 or folate, aplastic anemia (AA), myelodysplastic syndrome (MDS), aleukemic leukemia, paroxysmal nocturnal syndrome, or infiltration of malignant cells. Pancytopenia can also be associated with a decrease in hematopoietic cell production in the bone marrow (BM) secondary to medication (1-3). Possible non-hematological causes include collagen disease, hypothyroidism, systemic infection including tuberculosis, and hypersplenism as well as chronic liver dysfunction and Banti syndrome. Therefore, it is important to achieve a correct diagnosis before treating patients with pancytopenia.

Among the diseases that can cause pancytopenia, it is critical to rule out hematological malignancies, especially acute myeloid leukemia (AML) and MDS. An examination of the BM is essential for obtaining these diagnoses; however, an appropriate tumor marker suggesting the underlying AML and MDS may help diagnose such patients before examining the BM. Wilms' tumor-1 (*WT1*) was first identified as a tumor suppressor gene associated with the etiology of Wilms' tumor (4, 5). Because different deletions and point mutations of this gene have been described in this tumor type, the product of the mutated *WT1* gene is thought to be involved in carcinogenesis. *WT1* is also highly expressed in AML. High levels of *WT1* gene expression in AML were reported to be associated with lower remission rates and reduced overall and disease-free survival (6-10). With respect to MDS, not only does the percentage of cells expressing *WT1* increase as MDS progresses, but also so does the expression level (6-8). Therefore, it is suggested that elevated *WT1* expression levels may be a helpful marker for diagnosing AML and MDS in patients with reduced blood counts.

In the present study, patients who were presented with reduced blood cell counts and underwent the measurement of *WT1* mRNA expression in peripheral blood (PB) were retrospectively analyzed. The PB *WT1* transcript levels were examined for any association with the final diagnosis.

Correspondence to: Takahiro Yamauchi, Department of Hematology and Oncology, Faculty of Medical Sciences, University of Fukui, 23 Shimoaizuki, Matsuoka, Eiheiji Fukui, 910-1193, Japan. Tel: +81 776613111, Fax: +81 776618109, e-mail: tyamauch@u-fukui.ac.jp

Key Words: WT-1, pancytopenia, acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), anaplastic anemia (AA), Wilms' tumor.

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. Cytopenia 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 $p \leq 0.05$ were considered statistically significant.

Results

Patients. The study population consisted of a total of 47 patients, who were presented with pancytopenia or bicytopenia 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 cytopenia were AA, drug-induced cytopenia, 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 cytopenia, 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 *WT-1* levels in PB and the BM ($R^2=0.61$, $p=0.0043$) (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.

Table I. *Patients' characteristics*.

No.	Age (years) gender	Dx	WBC (n/μl)	Neutro-phil (n/μl)	Hb (g/dl)	Plt (n/μl)
1	55 F	Drug induced	3200	2220	6.6	9.3
2	74 M	AA	1000	820	6.5	3.7
3	65 F	AA	1900	304	10.7	3
4	77 M	MDS (RA)	4900	2993	8	5.2
5	80 M	AML (M2)	1800	516	9.5	6
6	73 F	vit B_{12} deficiency	2500	1500	10.8	27.2
7	71 M	MDS (RAEB-II)	1900	589	7.2	9.5
8	68 F	MDS (RA)	2400	1279	12.3	12.7
9	82 F	AA	2400	813	8.3	0.6
10	79 F	drug-induced	3100	1943	8	11.4
11	78 M	AML (M6)	2200	1298	6.8	3.1
12	73 M	AA	1000	150	6.5	1.8
13	67 F	AML (M0)	1100	420	7	5.7
14	63 M	MDS (RAEB-II)	1500	225	6.6	0.8
15	78 M	MDS (RAEB-II)	1200	264	7.7	6.7
16	81 M	MDS (RA)	2400	1200	10.4	4.9
17	82 F	MDS (RAEB-I)	2300	715	6.5	16.9
18	73 M	AML (M2)	1700	187	8.2	3
19	80 M	MDS (RAEB-I)	2500	875	10.5	6.4
20	57 M	MDS RAEB-II	2400	624	6.1	1
21	47 F	Drug induced	1300	403	5.8	6
22	58 M	MDS (RAEB-I)	2200	198	6.2	0.15
23	68 M	MDS (RAEB-II)	3000	1051	6.4	2.3
24	68 M	LC	1500	553	10.2	6.7
25	67 M	MDS (RA)	4300	1333	6.9	4.8
26	82 M	DIC	3200	1756	6.6	4.1
27	82 M	MDS (RA)	2700	1836	10.5	4.2
28	77 F	AML (M2)	3900	2145	6.7	15
29	80 F	Drug induced	1700	986	9.9	4
30	67 F	Drug induced	2500	890	10.8	9.6
31	84 F	Drug induced	2600	1999	8.3	13.6
32	73 M	AML (M2)	2000	560	11.8	13.7
33	64 F	MDS (RA)	1800	280	10.2	20.5
34	83 F	AA	900	144	5.5	12.4
35	89 M	MDS (RAEB-I)	1900	494	7.1	47.6
36	88 F	AA	700	56	5.1	0.1
37	74 M	LC	1600	977	8.6	3.3
38	39 F	MDS (RA)	2400	1012	8.2	2.4
39	24 F	SLE	1400	1023	9.4	4.3
40	81 M	AML (M2)	700	308	8.9	8.2
41	73 F	MDS (RAEB-II)	1700	708	12.8	16.6
42	82 F	MDS (RA)	4000	2360	6.9	2
43	78 F	viral infection	1900	1178	8.9	11.7
44	88 F	drug-induced	2500	1582	8.9	8.4
45	75 M	AML (M0)	500	110	5	4.5
46	70 M	MDS (RAEB-II)	2300	713	5.8	25.6
47	70 M	MDS (RAEB-II)	3300	825	6.2	2.7

AML, Acute myeloid leukemia; M0, M2, M6, French-American-British classification of leukemia; MDS, myelodysplastic syndrome; RA, refractory anemia; RAEB-I, II, refractory anemia with excess of blasts-I, II; AA, aplastic anemia; LC, liver cirrhosis; DIC, disseminated intravascular coagulation; SLE, systemic lupus erythematosus. WBC, white blood cells; Hb, hemoglobin; Plt, platelets. The blood counts were those at the initial presentation.

Table II. *The enrolled patients categorized by the final diagnosis*.

Diagnosis	Number	Comments
AML	8	1, M6; 5, M2; 2,M0
MDS	20	8, RA; 4, RAEB-I; 8, RAEB-II
Aplastic anemia	6	
Drug-induced cytopenia	7	candesartan, allopurinol, carbamazepine, amlodipine, quetiapine, risedronate, milnacipran, nicergoline
Non-hematological diseases	6	3, LC; 1, DIC; 1, SLE 1, systemic infection

AML, Acute myeloid leukemia; M0, M2, M6, French-American-British classification of leukemia; MDS, myelodysplastic syndrome; RA, refractory anemia; RAEB-I, II, refractory anemia with excess of blasts-I, II; LC, liver cirrhosis; DIC, disseminated intravascular coagulation; SLE, systemic lupus erythematosus.

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).

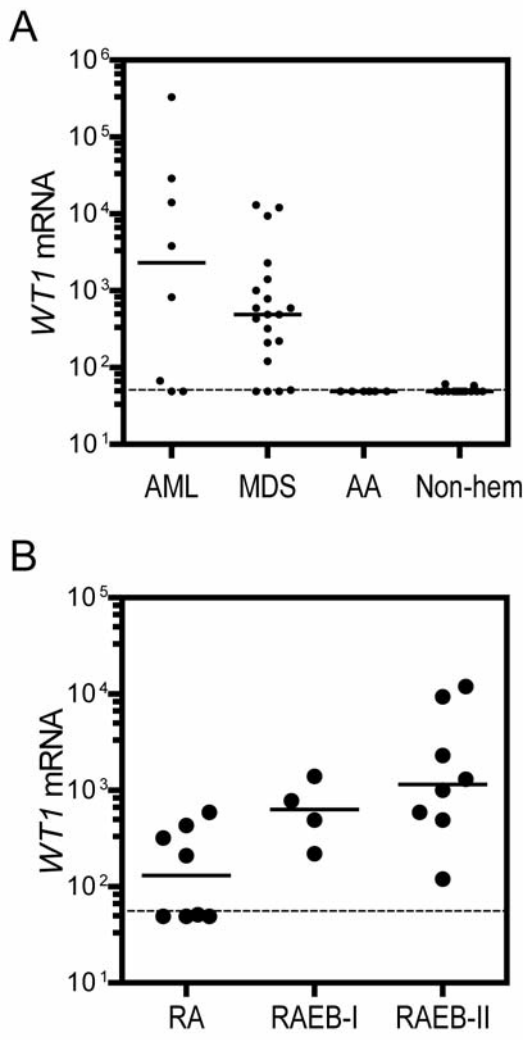

Figure 1. *Peripheral blood Wilms' tumor-1 (WT-1) mRNA levels in patients with cytopenia. A: The WT1 mRNA levels (copies/μg RNA) in the peripheral blood were plotted with respect to the diagnosis in 47 patients. B: WT1 mRNA levels in peripheral blood in myelodysplastic syndrome (MDS). WT1 mRNA levels (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.*

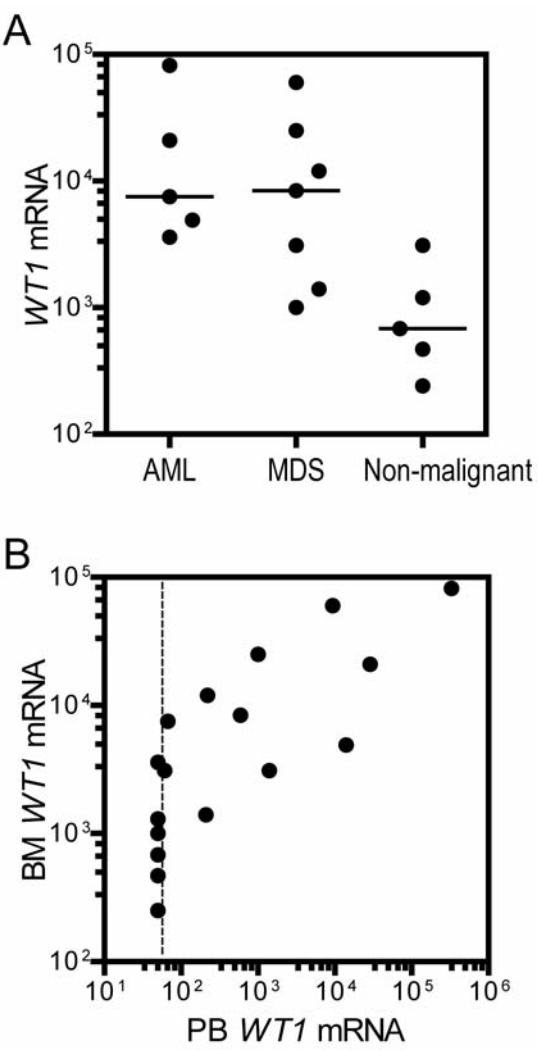

Figure 2. *A: Bone marrow Wilms' tumor-1 (WT-1) mRNA levels. WT1 mRNA levels (copies/μg RNA) in bone marrow were plotted with respect to the diagnosis in 17 patients. B: The relationship between WT1 transcripts in peripheral blood and the bone marrow in 17 paired samples from patients. The dotted line 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.*

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

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, *WT1* transcript levels were elevated in PB and BM in MDS, which might be attributable to MDS clonal cells rather than to 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.

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