ORIGINAL ARTICLE

Using Quantitative Assessment of the Wilms Tumor 1 Gene Expression in Residual Disease Monitoring in Patients with Acute Myeloid Leukemia

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ABSTRACT

Background. Despite considerable effort, a suitable specific molecular marker for monitoring minimal residual disease (MRD) has not been found for the majority of acute myeloid leukemia (AML) patients. The results of some studies suggest the Wilms tumor gene (WT1) as a possible molecular marker of MRD.

Methods and Results. We measured the expression of WT1 at diagnosis and during treatment of acute myeloid leukaemia (AML) patients. The expression of WT1 was measured by the quantitative real-time RT-PCR in peripheral leukocytes from 56 AML at diagnosis and 7 patients with AML transformed from myelodysplastic syndromes (MDS). The WT1 expression was significantly elevated (up to 3 orders of magnitude) in peripheral blood samples (PB) of AML patients at diagnosis compared to PB samples of healthy donors ($P<0.0001$). The level of WT1 expression depends particularly on FAB AML subtype, with the highest being found in AML patients with subtypes M4, M1, M3 and AML transformed from MDS. Conversely, AML patients with M2 and with the presence of AML1/ET0 at presentation showed a significantly lower expression of the WT1 gene compared to the remaining AML patients at presentation ($P = 0,005$). Further, sequence samples of 12 AML patients under long-term surveillance were tested for the WT1 expression in parallel with the expression of specific MRD markers - fusion genes: AML1/ETO, PML/RAR and CBFB/MYH11. The levels of WT1 gene expression and the above specific fusion genes significantly correlated. Moreover, 14 patients without the specific MRD marker were tested for the WT1 expression. The results show that hematological relapses were associated with the rise of expression of the specific fusion genes and with the WT1 gene expression. As for the rise of WT1 expression above the level seen in leukocytes from peripheral blood and/or bone marrow of healthy donors, in four patients under long-term surveillance the "molecular relapse" predicted ongoing hematological relapses as early as 2 months in advance.

Conclusions. Our results, in accordance with some of the previously published ones, show that WT1 expression seems to be a suitable marker of minimal residual disease in AML patients.

Key words: leukemia, AML, WT1, MRD, PML/RARa, AML1/ETO, CBFB/MYH11, real-time PCR

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Detection of a specific molecular marker plays an important role in the diagnosis of acute leukemias. Monitoring levels of these specific molecular markers may be useful in screening the so-called minimal residual disease (MRD), and detecting residual leukemic cells under the limit of morphological detection may help to determine an oncoming relapse. Minimal residual disease may be best detected using a sensitive reverse transcriptase polymerase chain reaction (RT-PCR), mostly using quantitative PT-PCR (RQ-RT-PCR). Detection of MRD in acute myeloid leukemia surveillance may be performed using specific markers, which include mostly translocated fusion genes t(8,21) – fusion gene AML1/ETO, t(15,17) – fusion gene PML/RAR, inv16 and t(16,16) – fusion gene CBFB/MYH11 and some others. These specific markers may be found in approximately 40 percent of patients with acute myeloid leukemia (AML), while for the rest of the patients with AML this marker is missing. Quantitative evaluation of the Wilms' tumor gene expression (WT1), which is increasingly expressed in patients with acute myeloid leukemia in diagnosis, is being tested for MRD screening.

The Wilms tumor gene encoding a transcription factor with a Motif of a Zinc finger was first identified and localised as a tumor-suppressor gene on the chromosome 11, 11p13, in 1990 [1]. Inactivation of both alleles of the WT1 gene plays a fundamental role in the pathogenesis of a kidney tumor – Wilms tumor. WT1 is usually expressed during embryogenesis and plays a key role in the development of the urogenital tract, spleen and normal hematopoietic progenitor cells [2] and [3]. Gene products of the WT1 serve as a transcription factor regulating expression of a line of genes, including those regulating cellular proliferation, differentiation and apoptosis [4]. An increased level of the WT1 is found in a number of solid tumors, including breast cancer, ovaries, colon, melanoma. Increased expression of the WT1 was found in cells of patients with leukemia [5]. The WT1 gene is expressed in bone marrow stem cells; however, it is not expressed in mature blood cells. When a decreased level of the WT1 was found during differentiation of leukemic cells lines HL60 and K562 [6], it was supposed that the WT1 gene may play an important role in leukogenesis. Particular subtypes of acute myeloid leukemias (based on the FAB classification) express different levels of the WT1 gene. The highest expression was

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found in less differentiated subtypes of AML M1-3 and lower in subtypes M4 and M5 (7), (8) and (9). Others (10), however, did not find a correlation between the WT1 gene expression and particular FAB subtypes, except for a significantly lower expression in AML M5. WT1 expression in patients with ALL is also increased, but one degree less than in acute myeloid leukemias (11, 12). Although a significantly increased expression of the WT1 gene is limited to leukemic blasts, its monitoring can be used for minimal residual disease surveillance detected best by quantitative RT-PCR (RQ-RT-PCR). In case of acute myeloid leukemias, it is possible to detect the WT1 gene using the above mentioned RQ-RT-PCR on a high level of sensitivity (detection of a single leukemic cell between 10^5 – 10^6 normal peripheral blood cells). In another work (7), the authors systematically started to survey the level of MRD using a WT1 as a marker and concluded that it may be useful for the MRD monitoring. Even though some (13) confirmed the usefulness of the WT1 as a reliable marker of MRD, the majority of other studies, especially those using a highly sensitive, reliable and standardized method – PCR in real time (12, 8) and (11), confirmed the results published in (7). From the clinical point of view, it is important to note that the methods of RQ-PCR detection of the WT1 transcript make it possible to predict an oncoming relapse of a disease 3 months in advance (12, 11). A level of the WT1 gene expression in patients with AML is of a prognostic value in diagnosis: patients with a high level of the WT1 gene expression have a shorter overall survival and shorter disease-free survival (10), (7). A crucial fact is that there is a wider dynamic interval of the WT1 transcript level in peripheral blood cells compared with bone marrow cells, taking into account the fact that peripheral blood taking is less stressful for the patient (11, 14). Further, there is increased WT1 expression when chronic myeloid leukemia transforms to the blastic phase (11, 15).

Purpose of the study

Evaluation of some preliminary results on a group of patients with AML with clearly defined molecular markers: fusion genes AML1/ETO, CBFB/MYH11 and PML/RARalpha.

METHODS AND PATIENTS

We studied 57 patients with AML and 26 patients during treatment (median of surveillance = 11.51 months). Patients surveyed during treatment were divided based on the FAB subtype: 6 AML M1, 9 AML M2 (4 of them were carriers of a fusion gene AML1/ETO), 7 AML M3 with a fusion gene PML/RAR a 4 AML M4 (3 of them carrying a fusion gene CBFB/MYH11). The source material included leukocytes drawn from patients at the time of diagnosis and during the course of treatment. The elements were taken mostly from peripheral blood or, in retrospectively evaluated samples, from bone marrow. Leukocytes were prepared using standard methods of leukocyte isolation, selective osmotic lysis. A standard amount of leukocytes (10 million) were lysed in an ITG solution (16) kept at – 20 °C. Overall RNA was isolated in a lysate solution and was reversely transcribed into a cDNA using a method described previously (17). Regions of cDNA with tracked genes were amplified using quantitative polymerase chain reaction with specific starting oligonucleotides (primers) and a specific fluorescent-marked TaqMan probe for cDNA gene WT1 based on (11) using Hot Start polymerase FasStart (Roche, Mannheim, Germany). Quantification was performed on a thermal cycler RotorGene 2000 or 3000 (Corbett Research, Sydney, Australia) enabling the use of PCR in real time. For quantification purposes a dilutant series of a standard with an incorporated PCR gene WT1 with a known concentration was evaluated. This standard was prepared using cloning of a purified PCR product from a cDNA line K562 into a plasmid pCR®2.1-TOPO® using a method TOPO TA cloning (Invitrogen Corporation, Carlsbad, USA). After cloning the plasmid was isolated, cleaned, its concentration was determined and a dilutant series was prepared in a TE buffer, pH 8, with added Herring Sperm DNA with a resulting concentration of 100ng/l (Promega, Madison, USA). In parallel, a reference ABL gene was quantified, amplified using a RQ-RT-PCR with a specific TaqMan probe as described in (18). A standard for the ABL gene was prepared using the same method of cloning, isolation and purification. The results of quantification were expressed as relative values, comparing the WT1 values to ABL, which we obtained using a formula: $2^{(Ct\ ABL - Ct\ WT1)}$, Ct standing for the amplification cycle where the fluorescence of the sample reached a threshold equal to all amplified samples during the run. Fusion genes PML/RARα, CBFB/MYH11 and AML1/ETO were quantified based on (24, 25) and (26).

Statistical evaluation of the results, i.e. medians and results of quantification differences and correlations were counted using GraphPad Prism with a Mann-Whitney linear regression.

RESULTS

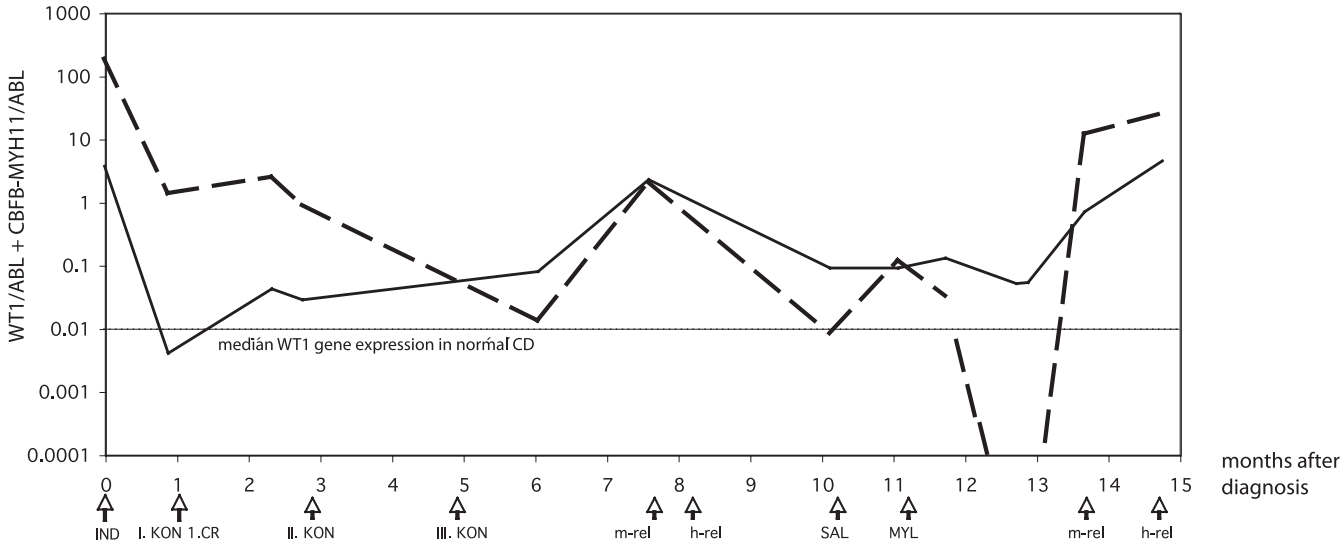
Patients at diagnosis

The WT1 gene expression in the peripheral blood of patients with AML at diagnosis was significantly increased by 3 orders (median expression of WT1 = 1.211, number = 57) compared with expression in peripheral blood of 26 healthy donors (median expression of WT1 = 0.002, P<0.0001). After dividing the patients with acute myeloid leukemia based on the FAB classification, we found the highest expression in 14 patients with AML M1 (median expression of WT1 = 2.112) and in 14 patients with AML M4 (median expression of WT1 = 2.84) and lowest in 4 patients with AML M5 (median expression of WT1 = 0.577) and in 19 patients with AML M2 (median expression of WT1 = 0.276). Patients with AML M3 at the time of diagnosis had a relatively high WT1 expression (median expression of WT1 = 1.199). The highest expression of WT1 at the time of diagnosis was found in a patient with AML M4 (WT1 = 24) and the lowest expression of the WT1 gene was found in a patient with AML M5 (WT1 = 0.0004). After selecting a group of patients with AML M2 and with the presence of a fusion gene AML1/ETO, we found that this group showed a significantly lower expression of the WT1 gene at the time of diagnosis than in other patients with AML (P = 0.005). We also found a high expression of the WT1 gene in 8 patients with MDS transformed in AML (median WT1 gene expression = 2.595). In 6 patients with ALL at the time of diagnosis we found increased WT1 expression (median expression of the WT1 gene = 0.088).

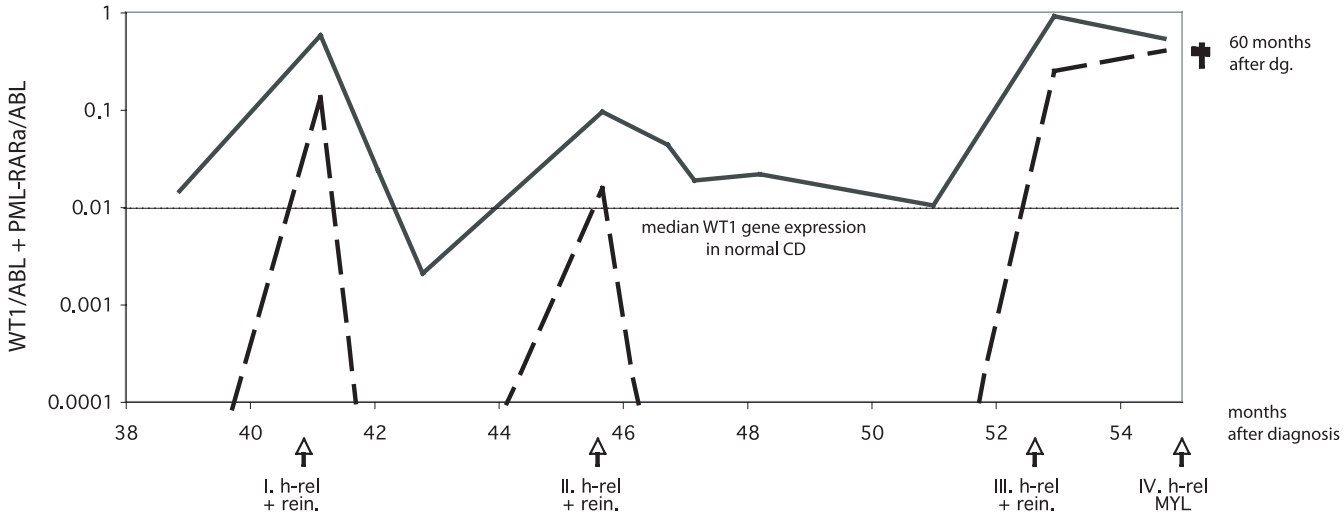
MRD surveillance

In 102 retrospectively evaluated samples, mostly from bone marrow, we performed correlation between the WT1 gene expression during the course of the disease in 12 patients with AML and with a presence of a specific MRD marker (fusion gene). We found a highly significant correlation between the course of the WT1 gene expression and expression of other specific fusion gene markers (r^2 = 0.63 – 0.99). Considering that gene expression in bone marrow of 31 healthy donors (median expression of WT1 = 0.01) was significantly increased compared to the WT1 expression in peripheral blood in 26 healthy donors (median expression of WT1 = 0.002, P<0.0001), it is more convenient to monitor WT1 expression in leukocytes from peripheral blood.

In most patients successful therapy led to a decrease of WT1 expression by 3 orders reaching the values of normal donors and

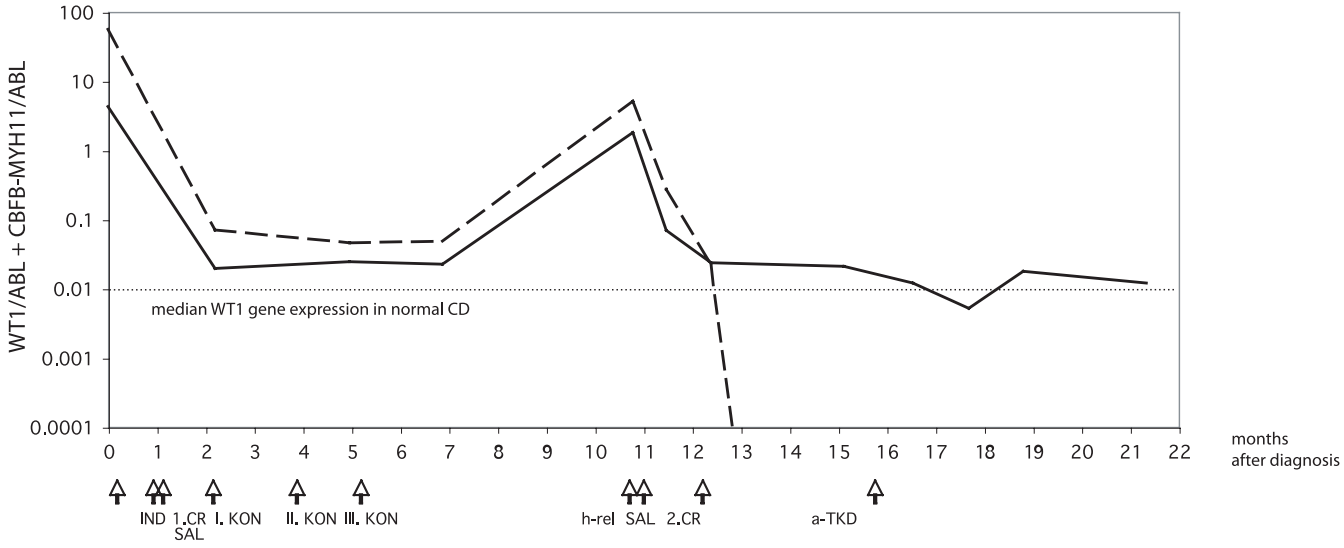


Graph 1: WT1 gene expression(———) and CBFB/MYH11 (- - - - - - -) in CD in a patient with AML M4Eo
The therapy is marked with an arrow – induction therapy: "ind" (protocol"3+7") and 3 consolidation therapies: "con" (protocol HiDAC), complete remission: "1.CR", molecular relapse: "m-rel.", hematological relapse: "h-rel", therapy SALVAGE: "SAL" (protocol FLAG-IDA), therapy using Mylotarg: "MYL".

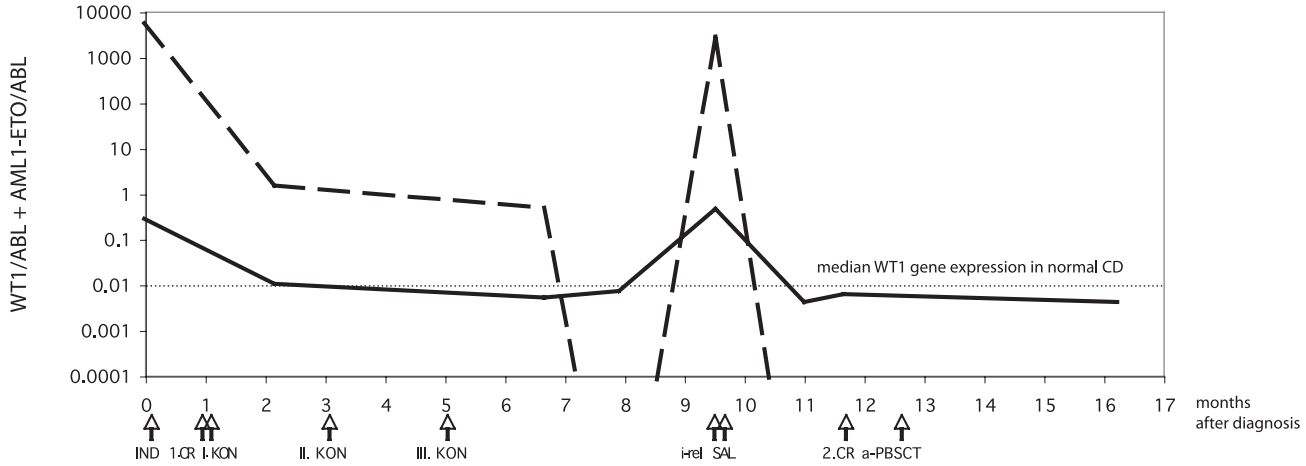


Graph 2. Graph of the WT1 gene expression (———) and PML/RARα (- - - - - - -) in bone marrow in a patient with APL
Hematological relapse: "h-rel", 1 and 2 reinduction: "rein" (protocol IDA + ATRA), 3 reinductions (protocol AIDA) and 4 relapses – 2 therapies using Mylotarg: "MYL".

complete remission; however, in some it later led to a relapse of the disease. Concurrently, the relapse was accompanied with an increased expression of the WT1 gene and in some patients this preceded the hematological relapse. For example, in the case of one patient with AML M4Eo, the WT1 expression started to rise about 2 months before the hematological relapse. This was followed by therapeutical remission of the disease accompanied by a decrease of the WT1 gene expression down to values one order higher compared to the median WT1 expression in normal bone marrow (see Graph. 1). Further on, the patient experienced another relapse accompanied by increased WT1 expression by one order (molecular relapse) preceding the hematological relapse by 32 days. The WT1 gene expression significantly correlated with CBFB/MYH11 gene expression ($r^2 = 0.63$, $P = 0.019$). Another patient (see Graph. 2) repeatedly experienced (3 times) major increase of the WT1 gene expression in parallel with increased expression of the specific marker for MRD – fusion gene PML/RARα followed by a relapse of the disease. Expression of both markers of MRD significantly correlated ($r^2 = 0.67$, $P = 0.011$). There was an analogous situation in another patient (see Graph. 3) where the remission was accompanied with a decrease in the WT1 gene expression in bone marrow samples by three orders together with a decrease of the CBFB/MYH11 gene expression by three orders. After approximately 10 months of remission the patient had another relapse accompanied by a rapid increase of both markers of MRD. After successfully overcoming the relapse and reaching complete remission (CR), the WT1 gene expression and the fusion gene CBFB/MYH11 expression both decreased to values equal to those found in normal bone marrow, and the patient reached a second complete remission. In this patient too, expression of both markers



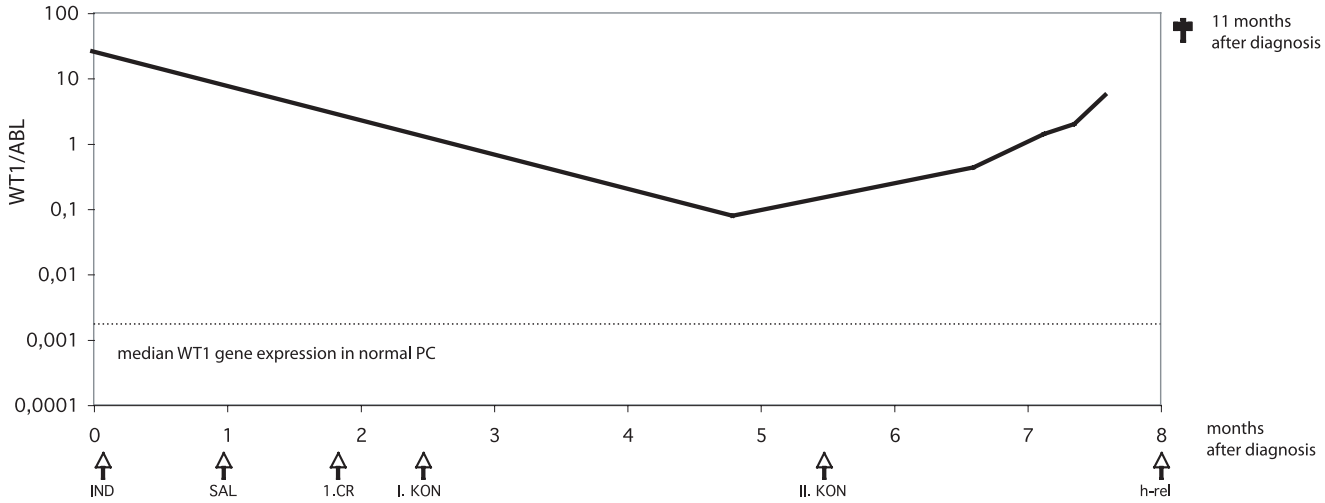
Graph 3. Graph of the WT1 gene expression (———) and CBFB/MYH11 (- - - - - - -) in bone marrow in a patient with AML M4Eo.
Induction therapy: "ind" (protocol AIE), therapy Salvage: "SAL" (protocol HAM), complete remission: "1.CR" 3 consolidation therapies: "con" (protocol HAV), hematological relapse: "h-rel" – treated with reinduction: "rein" (protocol FLAG), 2 complete remissions: "2.CR", allogenic bone marrow transplantation "a-BMT".



Graph 4. Graph of the WT1 gene expression (———) and AML1/ETO (- - - - - - -) in bone marrow in a patient with AML M2
Induction therapy: "ind" (protocol"3+7") a 3 consolidation therapies: "con" (protocol HiDAC), 1 complete remission: "1.CR", "incipient" relapse: "i-rel", therapy Salvage: "SAL" (protocol FLAG-IDA), 2 complete remissions: "2.CR", allogenic peripheral stem cell transformation: "a-PBSCT".

of MRD showed high correlation ($r^2 = 0.90$, $P < 0.0001$). As mentioned above, in patients with AML M2 and a presence of the AML1/ETO gene we observed lower values of the WT1 gene expression at the time of diagnosis; therefore at the time of remission there is a relatively lower decrease of this marker compared to more pronounced decrease of the AML1/ETO gene expression. At the time of a relapse there is a higher increase in the AML1/ETO gene expression compared with the WT1 gene (see Graph. 4). From this point of view, we can conclude that there is a higher dynamic interval of the AML1/ETO gene expression compared with the WT1 gene. An opposite tendency, i.e. higher dynamic interval of the WT1 gene expression compared with the PML/RARα gene, can be observed in patients with AML M3.

In the patients mentioned above, as well as some others, we were able to monitor the levels of the WT1 gene expression and levels of expression of both specific MRD markers – fusion genes. In most patients with AML (about 60 percent) these markers are not, however, present. Monitoring of the WT1 gene expression could replace them. In another 14 patients without the presence of a specific marker we monitored the MRD only by detection of the WT1 gene expression in peripheral blood compared with morphological clinical features. Similar to a group of patients with AML with a specific marker of MRD, we found such individuals who, following successful therapy, reached complete remission (CR) accompanied with a decrease of the WT1 gene expression down to values measured in peripheral blood leukocytes of healthy donors. Aside from these patients, we also included a group of patients who did not reach CR at all (patients resistant to treatment) or they reached complete remission and relapsed. In this group too, we found a correlation between the WT1 gene expression and morphological-clinical features. A typical example is a patient with AML M4 subtype with a very high WT1 gene expression at the time of diagnosis (WT1 = 24)



Graph 5. Graph of the WT1 gene expression (———) in peripheral blood in a patient with AML M4.
Induction therapy: "ind" (protocol "3+7"), therapy Salvage: "SAL" (protocol FLAG-IDA), 1 complete remission: "1.CR", 1 and 2 consolidation therapies: "con" (protocol HiDAC). Hematological relapse: "h-rel" therapy: (protocol HAM).

Tab. 1. Correlations of the WT1 gene expression with fusion gene

Patient	number of retrospective samples	specific marker	level of significance	r^2
patient 1	8	AML1/ETO	P<0,0001	0,98
patient. 2	7	AML1/ETO	P=0,0003	0,85
patient 3	6	AML1/ETO	P<0,0001	0,99
patient 4	5	AML1/ETO	P=0,0002	0,99
patient 5	5	AML1/ETO	nekoreluje	0,46
patient 6	6	PML/RARa	P<0,0001	0,998
patient 7	12	PML/RARa	P=0,011	0,67
patient 8	6	PML/RARa	P=0,0004	0,968
patient 9	13	CBFB/MYH11	P=0,019	0,63
patient 10	12	CBFB/MYH11	P<0,0001	0,901
patient 11	5	CBFB/MYH11	P=0,0001	0,99
patient 12	8	CBFB/MYH11	P<0,0001	0,99

where we observed a decrease of the WT1 gene expression on a value of 0.075, that is a decrease by 3 orders, however, not reaching the values of healthy individuals (note: the patient was refractory to induction therapy). This patient experienced a relapse of the disease preceded by an increase of the WT1 gene expression anticipating hematological relapse by 46 days (see Graph. 5).

We can summarize that in patients under surveillance we found a strong correlation between the WT1 gene expression and specific markers, fusion genes AML1/ETO, CBFB/MYH11 and PML/RARα and correlation of an increase of the WT1 gene expression before a hematological relapse. These results confirm the usefulness of monitoring the WT1 gene expression as a marker of minimal residual disease especially in those patients without a presence of specific markers.

DISCUSSION

Results of the WT1 gene expression in patients with AML show that the highest values were found in patients with a subtype M4 and lowest in patients with subtypes M2 and M5. In patients with AML M2 we also found the widest interval of values of the WT1 gene expression. In patients with AML M2 the median of the WT1 gene expression is relatively low compared with patients with subtypes AML M1 and M3, which is in contradiction with results published in (11) and (8). In these studies they found that the value of the WT1 gene expression in patients with AML M2 is relatively high. Patients with AML M2 and a presence of the AML1/ETO fusion gene show a significantly lower WT1 gene expression at the time of diagnosis compared with other patients with AML. This is in agreement with other published studies. From this point of view we can also explain the relatively lower median of the WT1 gene expression in all patients with AML M2 at diagnosis. Furthermore, 2 patients with AML M2 (without a presence of a fusion gene AML1/ETO) had a very low WT1 gene expression at the time of diagnosis, and the course of their disease was unfavorable, in contrast to results published in (7) and (8). One of these patients was most probably a patient with myeloproliferation with a presence of the t(5;12) translocation who transformed into an AML. Searching for correlation between the WT1 gene expression at the time of diagnosis and prognosis was not the goal of the study.

In accordance with published data (11, 9) and (8), we found

a very high correlation between the high WT1 gene expression at the time of diagnosis in patients with AML M1, AML M3 and in patients with AML transformed from MDS. Although the number of patients with the AML M5 subtype was very small (4 patients), median WT1 gene expression showed a relatively low value and a wide interval of values, similar to the results published in another study (8).

Even though on a limited number of patients with ALL, we detected, in accordance with results published in (8) and 11), a significantly lower WT1 gene expression in 6 patients with ALL at the time of diagnosis compared with the WT1 gene expression in patients with AML at diagnosis. Monitoring the WT1 gene expression in peripheral blood and in bone marrow showed that the WT1 gene expression in bone marrow is significantly higher than in peripheral blood. The difference in the WT1 gene expression at the time of diagnosis and during remission is higher in peripheral blood samples than in bone marrow. This observation is in accordance with results published in other studies (14). Also, blood taking from peripheral blood is less stressful for the patient than bone marrow biopsy. It is again another reason why monitoring the WT1 gene expression from peripheral blood as a marker of the MRD is more useful.

Our results of the MRD monitoring using the WT1 gene expression showed highly significant correlation of the WT1 gene expression from bone marrow or peripheral blood with specific markers of the MRD – fusion genes AML1/ETO, CBFB/MYH11 and PML/RARα in all studied patients at the time of diagnosis and during the course of the disease. This is not in agreement with results published in study (13); however, it is in accordance with results published in (20) and (12). In these studies they also found a highly significant correlation of the WT1 gene expression with the above mentioned specific markers of MRD. In four patients in long-term surveillance we found that a molecular relapse (i.e. increase of the WT1 gene by one order over the median found in healthy donors) preceded the hematological relapse by almost 2 months five times.

In case of acute myeloid leukemias in children, it is important for prognosis to know the expression of the appropriate MRD marker 5 weeks after diagnosis (21). But in adults suffering acute leukemia it is not possible to prove correlation between the state of MRD after the first and second induction therapy and further prognosis of the disease (22). On the contrary, there was correlation found between prognosis factors, such as DFS (disease free survival) and OS (overall survival) and the WT1 gene expression after the induction and second consolidation therapy (23). The authors established a significantly better DFS and OS in patients with the WT1 gene expression values lower than the determined limiting expression equal to 10^3 copies of the WT1/10^5 copies of the ABL gene. With regard to a common relapse in AML, it may be useful to find such a marker that would indicate a relapse early enough. Achieving the above mentioned limiting WT1 gene expression may provide a useful marker. However, the low number of patients in our study does not allow us to state a reliable conclusion, even though the results of MRD surveillance in patients with leukemia using quantitative monitoring of the WT1 gene expression show a good means of prediction value in case of a relapse.

List of used abbreviations

CIA – Czech Institute of Accreditation
EMDIS – European Marrow Donor Information System
GvHR – Graft versus Host
HLA – Human Leukocyte Antigens
HR – high-resolution – (detection on the level of alleles)
HSC – Hematopoietic Stem Cells
HSCT – Hematopoietic Stem Cells Transplantation
HvGR – Host versus Graft Reaction
IMGT – International Histocompatibility Working Group
LR – low-resolution - (detection on the level of allelic groups)
MUD – Marrow Unrelated Donor
PCR – SSP – Polymerase Chain Reaction- Sequence Specific Primers
SBT – Sequencing-Based Typing

LITERATURE

1. **Call, K.M, Glaser, T., Ito, C.Y., Buckler, A.J., Pelletier, J., Haber, D.A., Rose, E.A., Kral, A., Yeger, H., Lewis, W.H., Jones, C., and Housman, D.E.** Isolation and Characterization of A Zinc Finger Polypeptide Gene at the Human Chromosome-11 Wilms Tumor Locus. Cell, 1990, 60, pp. 509-520.
2. **Fraizer, G.C., Patmasiriwat, P., Zhang, X. H., and Saunders, G.F.** Expression of the Tumor-Suppressor Gene Wt1 in Both Human and Mouse Bone-Marrow. Blood, 1995, 86, pp. 4704-4706.
3. **Hosen, N, Sonoda, Y., Oji, Y., Kimura, T., Minamiguchi, H., Tamaki, H., Kawakami, M., Asada, M., Kanato, K., Motomura, M., Murakami, M., Fujioka, T., Masuda, T., Kim, E.H., Tsuboi, A., Oka, Y., Soma, T., Ogawa, H., and Sugiyama, H.** Very low frequencies of human normal CD34(+) haematopoietic progenitor cells express the Wilms' tumour gene WT1 at levels similar to those in leukaemia cells. British Journal of Haematology, 2002, 116, pp. 409-420.
4. **Algar, E.** A review of the Wilms' tumor 1 gene (WT1) and its role in hematopoiesis and leukemia. Journal of Hematotherapy & Stem Cell Research, 2002, 11, pp. 589-599.
5. **Miwa, H, Beran, M., and Saunders, G.F.** Expression of the Wilms-Tumor Gene (Wt1) in Human Leukemias. Leukemia, 1992; 6, pp. 405-409.
6. **Phelan, S.A, Lindberg, C., and Call, K.M.** Wilms-Tumor Gene, Wt1, Messenger-Rna Is Down-Regulated During Induction of Erythroid and Megakaryocytic Differentiation of K562 Cells. Cell Growth & Differentiation, 1994, 5, pp. 677-686.
7. **Inoue, K., Sugiyama, H., Ogawa, H., Nakagawa, M., Yamagami, T., Miwa, H., Kita, K., Hiraoka, A., Masaoka, T., Nasu, K., Kyo, T., Dohy, H., Nakauchi, H., Ishidate, T., Akiyama, T., and Kishimoto, T.** Wt1 As A New Prognostic Factor and A New Marker for the Detection of Minimal Residual Disease in Acute-Leukemia. Blood, 1994, 84, pp. 3071-3079.
8. **Trka, J., Kalinova, M., Hrusak, O., Zuna, J., Krejci, O., Madzo, J., Sedlacek, P., Vavra, V., Michalova, K., Jarosova, M., and Stary, J.** Real-time quantitative PCR detection of WT1 gene expression in children with AML: prognostic significance, correlation with disease status and residual disease detection by flow cytometry. Leukemia, 2002; 16, pp. 1381-1389.
9. **Patmasiriwat, P., Fraizer, G., Kantarjian, H., and Saunders, G.F.** WT1 and GATA1 expression in myelodysplastic syndrome and acute leukemia. Leukemia, 1999, 13, pp. 891-900.
10. **Bergmann, L, Miething, C., Maurer, U., Brieger, J., Karakas, T., Weidmann, E., and Hoelzer, D.** High levels of Wilms' tumor gene (wt1) MRDA in acute myeloid leukemias are associated with a worse long-term outcome. Blood, 1997, 90, pp. 1217-1225.
11. **Kreuzer, K.A, Saborowski, A., Lupberger, J., Appelt, C., Na, I.K., le Coutre, P., and Schmidt, C.A.** Fluorescent 5'-exonuclease assay for the absolute quantification of Wilms' tumour gene (WT1) MRDA: implications for monitoring human leukaemias. Br. J. Haematol., 2001, 114, pp. 313-318.
12. **Cilloni, D, Gottardi, E., De Micheli, D., Serra, A., Volpe, G., Messa, F., Rege-Cambrin, G., Guerrasio, A., Divona, M., Lo Coco, F., and Saglio, G.** Quantitative assessment of WT1 expression by real time quantitative PCR may be a useful tool for monitoring minimal residual disease in acute leukemia patients. Leukemia, 2002, 16, pp. 2115-2121.
13. **Elmaagacli, A.H., Beelen, D.W., Trenschel, R., and Schaefer, U.W.** The detection of wt-1 transcripts is not associated with an increased leukemic relapse rate in patients with acute leukemia after allogeneic bone marrow or peripheral blood stem cell transplantation. Bone Marrow Transplantation, 2000, 25, pp. 91-96.
14. **Inoue, K., Ogawa, H., Yamagami, T., Soma, T., Tani, Y., Tatekawa, T., Oji, Y., Tamaki, H., Kyo, T., Dohy, H., Hiraoka, A.,**

Masaoka, T., Kishimoto, T., and Sugiyama, H. Long-term follow-up of minimal residual disease in leukemia patients by monitoring WT1 (Wilms tumor gene) expression levels. Blood, 1996; 88, pp. 2267-2278.

15. **Menssen, H.D, Renkl, H.J., Rodeck, U., Maurer, J., Notter, M., Schwartz, S., Reinhardt, R., and Thiel, E.** Presence of Wilms' tumor gene (wt1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias. Leukemia, 1995; 9, pp. 1060-1067.
16. **Chomczynski, P. and Sacchi, N.** Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal.Biochem., 1987, 162, pp. 156-159.
17. **Haskovec, C., Ponzetto, C., Polak, J., Maritano, D., Zemanová, Z., Serra, A., Michalova, K., Klamová, H., Cermák, J., and Saglio, G.** P230 BCR/ABL protein may be associated with an acute leukaemia phenotype. Br.J.Haematol., 1998, 103, pp. 1104-1108.
18. **Visani, G., Buonamici, S., Malagola, M., Isidori, A., Piccaluga, P. P., Martinelli, G., Ottaviani, E., Grafone, T., Baccarani, M., and Tura, S.** Pulsed ATRA as single therapy restores long-term remission in PML-RAR alpha-positive acute promyelocytic leukemia patients: real time quantification of minimal residual disease. A pilot study. Leukemia, 2001, 15, pp. 1696-1700.
19. **Ostergaard, M., Olesen, L.H., Hasle, H., Kjeldsen, E., and Hokland, P.** WT1 gene expression: an excellent tool for monitoring minimal residual disease in 70% of acute myeloid leukaemia patients - results from a single-centre study. British Journal of Haematology, 2004, 125, pp. 590-600.
20. **Ogawa, H., Tamaki, H., Ikegame, K., Soma, T., Kawakami, M., Tsuboi, A., Kim, E. IH., Hosen, N., Murakami, M., Fujioka, T., Masuda, T., Taniguchi, Y., Nishida, S., Oji, Y., Oka, Y., and Sugiyama, H.** The usefulness of monitoring WT1 gene transcripts for the prediction and management of relapse following allogeneic stem cell transplantation in acute type leukemia. Blood, 2003, 101, pp. 1698-1704.
21. **Brisco, M.J., Condon, J., Hughes, E., Neoh, S.H., Sykes, P.J., Seshadri, R., Toogood, I., Waters, K., Tauro, G., Ekert, H., and Morley, A.A.** Outcome Prediction in Childhood Acute Lymphoblastic--Leukemia by Molecular Quantification of Residual Disease at the End of Induction. Lancet, 1994, 343, pp. 196-200.
22. **Schnittger, S., Weisser, M., Schoch, C., Hiddemann, W., Haferlach, T., and Kern, W.** New score predicting for prognosis in PML-RARA(+), AML1-ETO+, or CBFB-MYH11(+) acute myeloid leukemia based on quantification of fusion transcripts. Blood, 2003, 102, pp. 2746-2755.
23. **Garg, M., Moore, H., Tobal, K., and Yin, J. A. L.** Prognostic significance of quantitative analysis of WT1 gene transcripts by competitive reverse transcription polymerase chain reaction in acute leukaemia. British Journal of Haematology, 2003, 123, pp. 49-59.
24. **Schwarz, J, Markova, J., Pekova, S. et al.** A single administration of gemtuzumab ozogamicin for molecular relapse of acute promyelocytic leukemia. Hematology Journal. 2004,5, pp. 279-280.
25. **Marcucci, G., Caligiuri, M.A., Dohner, H. et al.** Quantification of CBF beta/MYH11 fusion transcript by Real Time RT-PCR in patients with INV(16) acute myeloid leukemia. Leukemia. 2001,15, pp. 1072-1080.
25. **Wattjes, M.P., Krauter, J., Nagel, S. et al.** Comparison of nested competitive RT-PCR and real-time RT-PCR for the detection and quantification of AML1/MTG8 fusion transcripts in t(8;21) positive acute myelogenous leukemia. Leukemia. 2000,14, pp. 329-335.

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