Prognostic value of quantitative analysis of WT1 gene transcripts in adult acute lymphoblastic leukemia

Acute Lymphoblastic Leukemia

Prognostic value of quantitative analysis of *WT1* gene transcripts in adult acute lymphoblastic leukemia

We quantified Wilm's tumor gene (*WT1*) using a real time quantitative polymerase chain reaction in 20 adult patients with acute lymphoblastic leukemia at presentation. A *WT1* level greater than 906 (median value for the whole series) was a significant predictor of a poor disease-free and overall survival in uni- and multivariate analyses.

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Wilms' tumor gene (*WT1*) is a tumor suppressor gene involved in regulation of cell growth and differentiation. *WT1* transcripts and nuclear protein have been described in the majority of human acute leukemias.[1] The level of *WT1* expression is associated with the presence, persistence or reappearance of leukemic hematopoiesis,[2,3] and has been suggested to represent a potential prognostic factor. However, while a significant association was shown between *WT1* and prognosis in acute myeloid leukemia and acute lymphoblastic leukemia (ALL),[2,4,5] no correlation was found in other series.[6,7] We analyzed *WT1* expression by a real-time quantitative polymerase chain reaction (RQ-PCR) in 20 adult patients with ALL at diagnosis to investigate whether the level of *WT1* expression was associated with clinico-pathologic features and prognosis of the disease.

All adult patients with newly diagnosed ALL admitted to the Division of Hematology, S.Giovanni Hospital, Turin, Italy in 2001 and 2002, were included in the study. There were 12 females and 8 males; their mean age was 36.6 years (range, 16 to 65). Fifteen had B-ALL and five had T-ALL. In all, five patients had a normal karyotype, six had the t(9:22) translocation [Ph positive] and one had miscellaneous cytogenetic abnormalities. Using a reverse transcription-polymerase chain reaction (RT-PCR) technique,[8] eight cases showed a *BCR/ABL* gene rearrangement. All patients were treated according to the multicenter GIMEMA (*Gruppo Italiano Malattie Ematologiche dell'Adulto*) ALL 0496 protocol.[9] Follow-up data were analyzed as of August 31, 2004.

Total cellular RNA was extracted from bone marrow mononuclear cells. cDNA was prepared by Reverse transcription following the standardized BIOMED-1 protocol.[8] RQ-PCR was carried out on the i-Cycler iQ Real PCR Detection System (BioRad Laboratories, Hercules, CA, USA) using Taqman fluorescent probes.[3] All samples were processed in triplicate. Serial dilutions of a plasmid construct containing the sequence targets were used to obtain a calibration curve for the quantitative assessment of *WT1* and *ABL* (Figure 1). *WT1* values were normalized to the number of *ABL* transcripts and expressed as copy numbers of *WT1* for every 10^4 copies of *ABL*.

The mean number of *WT1* copies/10^4 *ABL* copies for the whole series was 7824 (median, 906; SD, 19786; range, 3.6 to 86766). A *high* level of *WT1* expression, defined as >906 copies/10^4 *ABL* copies was found in all five cases of T-ALL but in only five out of the 15 cases of B-ALL (p=0.01); no association was found with sex, age, white cell count, cytogenetics or *BCR/ABL* status.

Seventeen of the 20 patients achieved complete remission. Using the median *WT1* value (906 copies/10^4 *ABL* copies) as a cut-off, the 3-year disease-free survival rates were 47% for the whole series, 89% for patients with a *WT1* level ≤906 and 0% for those with a higher level (p=0.01) (Figure 2A). No other parameter was associated with the duration of disease-free survival. At the time of analysis nine patients (45%) had died of their disease and 11 (55%) were alive (censored). The mean follow-up for censored patients was 19.5 months (median, 18.2; range, 3 to 44). Three-year overall survival rates were 42% for the whole series, 91% for patients with a *WT1* level ≤906 and 0% for those with a higher level (p=0.005) (Figure 2B). Overall survival was also shorter for patients with a white cell count > 50×10^9/L (p=0.02) and for those with T-ALL (p=0.04), but was not related to sex, age, cytogenetics or *BCR/ABL* status. At multivariate analysis, WT1 level (χ^2, 7; p=0.008; risk ratio, 9.3) and white cell count (χ^2, 3.7; p=0.05; risk ratio, 3.95) retained independent prognostic significance.

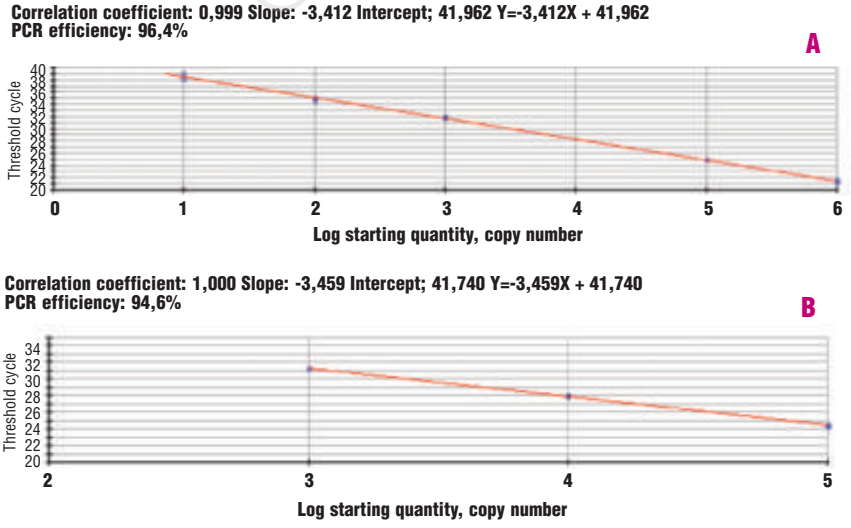

Figure 1. Representative standard curves for *WT1* (A) and *ABL* (B) using real-time quantitative polymerase chain reaction analysis.

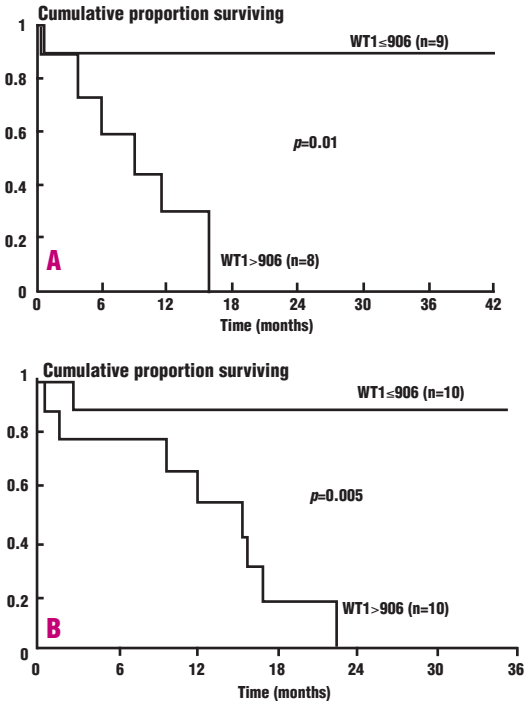

Figure 2. Actuarial probability of disease-free survival (A) and overall survival (B) for adult patients with acute lymphoblastic leukemia categorized according to the median *WT1* level.

Our results indicate that the *WT1* gene is expressed in all cases of adult ALL, contrary to reports showing *WT1* expression in only 44 to 86% of ALL.[1,10] This result may depend on the higher sensitivity and specificity of the RQ-PCR. Indeed, qualitative or semi-quantitative techniques for detecting *WT1* transcript do not take into account the variation resulting from sample handling and quality of RNA and cDNA, contrary to the double quantification of both *WT1* and *ABL* transcripts.

Secondly, we have clearly demonstrated the high prognostic value of the amount of *WT1* expressed in adult ALL at presentation: patients with high *WT1* expression had shorter disease-free survival and overall survival. Furthermore, *WT1* expression was the most significant independent prognostic factor in multivariate analysis. Our results agree with studies showing that high *WT1* expression is associated with a poor prognosis in acute myeloid leukemia and ALL,[2,4,5] but contrast with the results of other studies[6,7] that did, however, only investigate cases of acute myeloid leukemia using qualitative or semi-quantitative RT-PCR assays. To our knowledge, no study has been performed on a homogeneously treated series of adult ALL patients using a quantitative approach. We believe that the level of *WT1* expression, as assessed by RQ-PCR, can be regarded as a risk parameter in adult ALL.

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