Disease status in patients with chronic myeloid leukemia is better characterized by BCR-ABL/BCR transcript ratio than by BCR-ABL transcript level, which may suggest a role of normal BCR gene in the disease pathogenesis*

J. MORAVCOVÁ, J. REGNER, D. MOUČKOVÁ, K. FIŠER, V. ZMEKOVÁ, R. MALÁČOVÁ, K. MICHALOVÁ, H. KLAMOVÁ

Institute of Hematology and Blood Transfusion, e-mail: jana.moravcova@uhkt.cz, 128 20 Prague, Czech Republic

Received August 23, 2004

Monitoring of BCR-ABL transcript level is widely used in chronic myeloid leukemia (CML) to follow up response to therapy. In this study we compare abilities of two quantitative RT-PCR assays to characterize the disease status in CML patients: RT-PCR quantifying the BCR-ABL transcript concentration and RT-PCR determining the BCR-ABL/BCR transcript ratio (R). We demonstrate that in non-responders only R, but not BCR-ABL, unambiguously characterizes the state of disease. Moreover, R values >1 found in all poor responders indicate lower BCR expression compared to BCR-ABL in these patients. Our results demonstrate the importance of BCR-ABL/BCR transcript ratio for the disease status and the disease prognosis characterization and suggest a possible role of the normal BCR gene expression in CML pathogenesis.

Key words: CML, BCR-ABL, BCR-ABL/BCR, quantitative RT-PCR, disease status, disease prognosis

Chronic myeloid leukemia (CML) is characterized by the reciprocal translocation t(9;22) resulting in a shortened chromosome 22, known as the Ph chromosome. At molecular level, proto-oncogene ABL is juxtaposed to the BCR gene and under its regulatory sequences. The product of normal gene ABL, the ABL protein with nonreceptor tyrosine kinase activity, is localized predominantly in the cell nucleus and is involved in the regulation of the cell cycle and response to genotoxic stress [21, 23]; the role of normal BCR protein bearing serine-threonine kinase activity is still not well known. The fusion BCR-ABL gene codes BCR-ABL oncoprotein endowed with constitutively elevated tyrosine kinase activity relocated to cytoplasm. BCR-ABL protein was proved to play a crucial role in CML pathogenesis [5].

The intensity of BCR-ABL expression correlates with the disease status and the disease prognosis, being high and increasing in poor response to treatment and poor prognosis [8, 11]. The most sensitive method for monitoring of BCR-ABL expression is quantitative RT-PCR (Q-RT-PCR). It is used for early detection of disease relapse in patients after stem cell transplantation (SCT) [4, 14] and generally for characterization of treatment efficacy in CML patients. Q-RT-PCR methods commonly used at present are: 1) real-time RT-PCR [3, 6, 7, 9, 12] and 2) more rarely, competitive quantitative RT-PCR [4,16]. The competitive method is more labor intensive, however, more sensitive [9]. Numerous Q-RT-PCR assays have been published; their results might, depending e.g. on choice of internal control-gene, differ less or more. Therefore, at present BCR-ABL Q-RT-PCR monitoring, as a very important tool of assessment of response to therapy, is standardized to be worldwide comparable [2, 7].

In this study we evaluate two Q-RT-PCR assays in which results of long-term monitoring of BCR-ABL transcript level showed some differences in their abilities of characterizing the disease status in patients with CML. The aim of the study was 1) to compare values of both methods in different responses to treatment and, 2) to compare kinetics of both assay values during treatment in regard to disease status and disease prognosis characterization.

*This study was supported by the grant NC/7550-3 from the Internal Grant Agency of Ministry of Health of the Czech Republic.

Patients and methods

Patients and sample processing. Forty-eight not-transplanted CML patients (25 men and 23 women; median age of 54 years; range 22–71) longitudinally investigated during treatment (median period of follow-up 42.8 months; range 14.3–112.4) and 9 CML patients in blast crisis were enrolled in this study. During the follow-up the patients were treated with hydroxyurea (HU), busulfan (BS), interferon-α (IFN), cytosine arabinoside (Ara-C) or combinations of drugs or recently with imatinib mesylate (STI571, Glivec); blast crises were treated with combined intensive chemotherapy according to standard treatment protocols. Patient's samples were obtained with the consent of the Ethics Committee of the Institute of Hematology and Blood Transfusion, Prague. Peripheral blood samples (PB) for molecular analyses were collected at 2–3 month intervals, where possible. Total leukocytes were isolated from 20 ml of PB in citrate anticoagulants by the method of hypotonic red cell lysis within 36 hours after collection and lysed in a guanidinium-thiocyanate (GTC) buffer in concentration 10^7 leukocytes per ml. Total RNA was extracted as mentioned previously [16]. cDNA synthesis was performed using random hexamer primers and SuperScript II reverse transcriptase (Invitrogen, Carlsbad, CA, USA).

Molecular monitoring

The BCR-ABL transcript concentrations in peripheral blood samples were determined by a simple competitive Q-RT-PCR as described earlier together with the assay validation [16]. The assay used natural competitors from cell lines K562 and BV173 added to samples prior to RNA isolation, which eliminated the problem with quantification of sample cDNA concentration and with checking for the RNA quality and quantity and reverse transcription efficiency and in consequence it obviated the necessity of housekeeping control-gene usage. The level of BCR-ABL transcripts was given by BCR-ABL transcript concentration per 10^6 leukocytes. In this study the BCR-ABL transcript concentration was expressed in percentage. 100 % BCR-ABL positivity was defined as the BCR-ABL transcript concentration per 10^6 leukocytes, detected in non-treated patients at the time of CML diagnosis, with 100 % of Ph-positive metaphases and the BCR-ABL/BCR transcript ratios equal to one [16]. This definition enables easy comparison of RT-PCR results with cytogenetic examinations. The BCR-ABL transcript concentration was calculated after densitometric analysis of electrophoretically separated bands (UVP, Cambridge, UK) by means of linear regression analysis.

The BCR-ABL/BCR transcript ratios in peripheral blood samples were determined by a duplex RT-PCR as described previously [17]. Primer design ensured the same amplification efficiency of both BCR-ABL and BCR templates, which enabled the precise BCR-ABL/BCR ratio determination. Evaluation was performed by capillary electrophoresis on ABI PRISM 310 System (Applied Biosystems, Foster City, CA, USA). Functional dependence of values determined by both methods enabled to indirectly estimate the BCR transcript level.

Cytogenetic analysis. For standard G-banding technique bone marrow cells were cultivated 24 hours in RPMI medium with 10 % fetal calf serum without stimulation. Mitoses were harvested after hypotonic treatment with 0.075 M KCl, and slides were prepared using conventional techniques. Karyotypes were prepared according to ISCN (1995) nomenclature. Only results derived from at least 20 metaphases were considered. Interphase fluorescence in situ hybridization (i-FISH) analyses using the extra signal LSI bcr-abl ES dual color translocation probe set from VYSIS were performed according to the recommendation of manufacturers. To identify the presence and localization of BCR-ABL fusion gene 200 interphase nuclei were screened. The cut-off level of FISH analyses was 2.5 %.

Response criteria. Cytogenetic response was defined as follows: complete remission (CCR): 0 % Ph+ metaphases; partial response (pCR): 1–34 % Ph+ metaphases; minor response (mCR): 35–94 % Ph+ metaphases. Hematological response (HR) was defined by WBC $<10x10^9$/l, platelets $<450x10^9$/l, presence of no immature forms in peripheral blood and disappearance of splenomegaly, without cytogenetic response. Patients who lost HR or did not achieve HR or any improvement of hematological and clinical status for more than two months, i.e. patients resistant to treatment, were considered as non-responders (NR). Blast crisis (BC) was defined by more than 30 % of blasts in peripheral blood or bone marrow.

Statistical analysis. Comparison of RT-PCR values in different response groups was made using Wilcoxon singed-rank test and by Analysis of variants for hierarchical model. To eliminate influence of different number of individual patient measurements, only 3 values of each patient (median, the second highest and the second lowest value) were included in Wilcoxon test, and a weighted least squares regression weighted by 1/N (N, number of measurements/patient) was used in the Analysis of variants. Comparison of the two RT-PCR methods to characterize the disease status was performed using McNemar paired value test. Only values measured within stable responses lasting six months or longer were included in statistical analyses. The points of equivalence in competitive Q-RT-PCR were calculated using linear regression.

Results

RT-PCR values in different response groups. A total of 401 peripheral blood samples of patients with CML during treatment were tested in pairs by both Q-RT-PCRs determining: a) BCR-ABL transcript concentration [16] and b) BCR-ABL/BCR ratio (R) [17]. The Q-RT-PCR results were compared with cytogenetic, hematologic and clinical data and the Q-RT-PCR values were divided into different re-

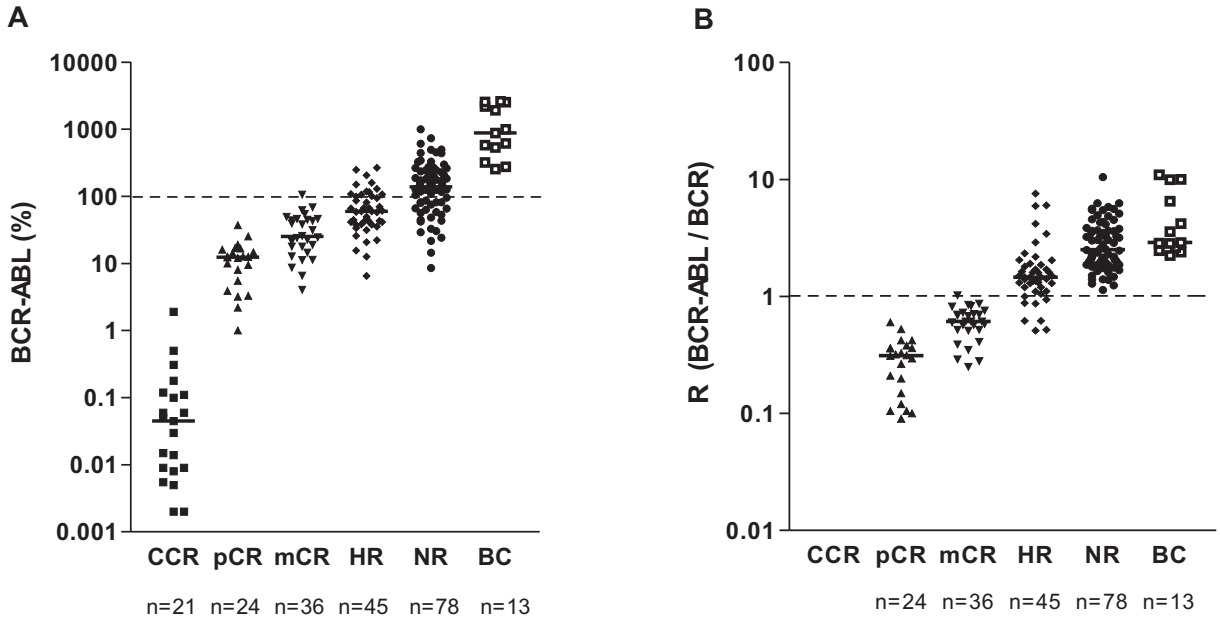

Figure 1. BCR-ABL transcript levels and BCR-ABL/BCR ratios (R) in different response groups (Wilcoxon singed-rank test).
(A) BCR-ABL transcript levels, (B) BCR-ABL/BCR transcript ratios (R). CCR, complete cytogenetic remission; pCR, partial cytogenetic response; mCR, minor cytogenetic response; HR, hematological response; NR, non-responders; BC, blast crisis. Definitions of response criteria see in Patients and methods. The median values of both RT-PCR assays in each response group were significantly different, p<0.0001. The difference between R for non-responders and blast crisis was characterized by p=0.0003. R values were not determinable in CCR; due to very low BCR-ABL transcript concentrations only BCR peak was detected.

sponse groups according to criteria given in Patients and methods (Fig. 1).

In patients with cytogenetic (CG) responses, BCR-ABL transcript levels were <100 % and R<1, which corresponded to Ph+ cell reduction. The lowest BCR-ABL transcript values were found in patients in complete cytogenetic remission (CCR), however, PCR negativity was not achieved in patients enrolled in this study. In CCR, BCR-ABL median was 0.045 % (range 0.002–1.9). R values in CCR were mostly indeterminable; due to very low BCR-ABL transcript concentrations only BCR peak was detected. With decreasing quality of CG response the values of both assays increased, however, the values of 100 % for BCR-ABL and 1 for R were not significantly exceeded in any of CG responders. The values of BCR-ABL <100 % and R<1 were found to be typical for patients with CG response. In partial response (pCR) BCR-ABL median was 12.4 % (range 0.99–37.0), R median was 0.31 (range 0.09–0.61); in minor response (mCR) BCR-ABL median was 25.2 % (range 4.1–103.6), R median was 0.61 (range 0.25–1.02).

Only with the loss of CG response we could find BCR-ABL >100 % and R>1 (Fig. 1). In hematologic responses (HR) BCR-ABL median was 60.0 % (range 6.5–265.5), R median was 1.46 (range 0.51–7.59); in non-responders (NR) BCR-ABL median was 138.8 % (range 8.4–1001.4), R median was 2.52 (range 1.14–10.50). The highest values were measured in blast crisis with BCR-ABL median 883.2 % (range 257.4–2606.1) and R median 2.87 (range 2.25–10.70). The median values of both methods statistically significantly correlated with responses to treatment (both Wilcoxon test and Analysis of variants: p<0.0001).

Poor correlation of BCR-ABL transcript levels with disease status in some of non-responders. R values clearly differentiated between groups of good responders with CG response (CCR, pCR, mCR) and group of non-responders, being <1 in CG responders and >1 in non-responders. On the contrary, BCR-ABL values did not show such unambiguous difference. In CG responders BCR-ABL values were always <100 % but in non-responders we found values ranging from 8 % to 100 %. Thus a part of the BCR-ABL values in patients without response to treatment fell in the area typical for CG responders. After focusing on individual patients, we found that the group of non-responders, with regard to BCR-ABL level, was in fact composed of three subgroups: 1) patients with the BCR-ABL transcript levels permanently >100 % (15/26), 2) patients with a great fluctuation of BCR-ABL levels, with values both >100 % and <100 % (5/26) and 3) patients with transcript levels permanently <100 % (6/26), however, in some of them with BCR-ABL increase in the terminal stage of disease. The low BCR-ABL transcript levels were not caused by the type of therapy; the therapy did not differ between the three groups. Non-responders of all groups were treated with hydroxyurea, busulfan, interferon-α, cyto-

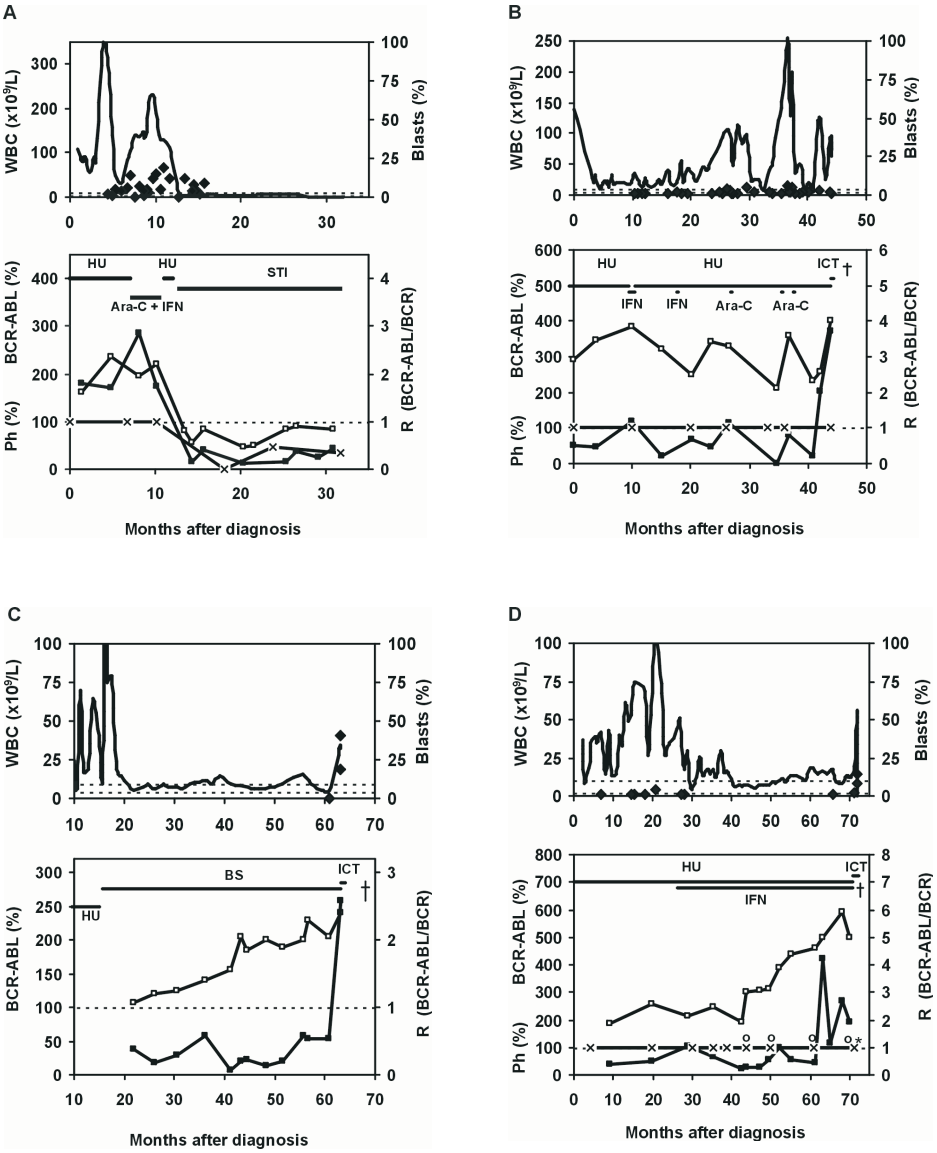

Figure 2. Kinetics of BCR-ABL transcript levels and BCR-ABL/BCR transcript ratios (R) in CML patients during treatment.

Values of both Q-RT-PCR assays in four illustrative patients were compared with white blood cell (WBC) count, percentage of peripheral blood blasts and with cytogenetic data. Upper panels: WBC counts (lines) and percentage of blasts (diamonds), normal WBC values (4–10x10^9/l; dotted lines). Lower panels: percentage of Ph+ metaphases (lines with crosses), percentage of BCR-ABL transcripts (lines with full squares) and R (BCR-ABL/BCR transcript ratios) (lines with empty squares). Horizontal lines represent type of treatment: HU, hydroxyurea; BS, busulfan; IFN, interferon-α; Ara-C, cytosine arabinoside; STI, imatinib mesylate; ICT, intensive chemotherapy. (A) Good correlation of both BCR-ABL transcript levels and R with response to treatment. During poor response to therapy BCR-ABL transcript values were >100 % and R>1. BCR-ABL<100 % and R<1 were detected only after the patient had achieved cytogenetic response (here to imatinib therapy). (B, C, D) Good correlation only of R but not BCR-ABL transcript levels with the response to treatment. BCR-ABL transcript concentrations were usually low in these patients although response to therapy was poor. Only in the terminal stage of the disease BCR-ABL level increased. However, in all these patients R values were always >1 and further increased in patients C and D. In all patients B,C and D the disease progressed and the patients died. (B) Patient B was treated with HU due to his intolerance to IFN. (C) Patient C was treated with BS because of relatively high age (72 years) at the time of diagnosis and poor response to HU. In this patient, cytogenetic analysis at the time of diagnosis showed 100 % of Ph+ metaphases; further analyses were not available because of patient's disagreement with this examination, therefore this patient's data are not included in statistic analyses. Although the patient achieved HR, R values were permanently high and increasing. (D) Patient D exhibited BCR-ABL transcript values <100 % even after the appearance of an additional Ph chromosome (empty circles). R values were stably >1 and further increased after the second Ph chromosome appearance. Interestingly, the additional Ph chromosome and increase of R were found shortly after WBC count normalization, 10 months before hematologic relapse. Asterisk denotes appearance of chromosome-8 trisomy. At the time of treatment of patients B, C and D imatinib was not available (years 1995–2001).

sine arabinoside or their combinations. Only one patient in the second group was treated with imatinib. Where samples were available, the low BCR-ABL values in the third group had been already detected at the time of diagnosis. The third group was fully beyond any assumption and low values of BCR-ABL transcript levels could be misleading. Although the BCR-ABL transcript levels were low, the patients did not achieve cytogenetic response, hematologic response was exceptional and all but one, successfully treated with transplantation, died due to disease progression 37–72 months after diagnosis. In other two groups only 3 patients (3/20) died of disease related complications during follow-up (29, 81 and 89 months after diagnosis).

The BCR-ABL/BCR transcript ratio statistically significantly better characterized the disease status than the BCR-ABL transcript concentration itself (McNemar test: $p<0.0001$)

Kinetics of BCR-ABL levels and BCR-ABL/BCR ratios during treatment. In all patients of our study group the kinetics of BCR-ABL transcript levels and R values were followed up during treatment. The median period of follow-up was 42.8 months (range 14.3–112.4 months). In most cases the kinetics of both assay values well characterized the course of disease (Fig. 2A). Increase of values always indicated bad prognosis. However, in some non-responders or patients with poor outcome only R but not BCR-ABL values correlated with the response to therapy (Fig. 2B–D). Although the response was poor and the disease progressed later, BCR-ABL levels were permanently low, except the terminal stage of disease in some of them. On the contrary, R values were always >1, in patients C a D with increasing trend (Fig. 2 C, D). In our patients, R>1 always indicated CML not-responding to treatment, R permanently >2 or increasing signalized disease progression later confirmed by worsening of patient's status.

Discussion

Two quantitative RT-PCR assays for BCR-ABL, in which preliminary results showed some discrepancies in BCR-ABL monitoring, were tested in regard to their ability of determining the disease status and prognosis in CML patients. The first method was a simple competitive Q-RT-PCR determining the BCR-ABL transcript concentration [16]. One of the advantages of this method was a very wide detection scope ranging from 0.001 % to unlimited values of BCR-ABL overexpression. The preciseness and reproducibility of the method was verified [16]. Figure 1A shows statistically significant differences of measured values in different response groups. This Q-RT-PCR has been used in clinical practice at the Institute of Hematology and Blood Transfusion, Prague since 1994 and 10 years' practice confirmed the great importance of sensitive monitoring of BCR-ABL transcript levels for early detection of relapses after SCT [18, 19] and for monitoring responses to treatment in general. BCR-ABL overexpression (BCR-ABL >100 %) was highly predictive for the loss of hematological response in patients treated with imatinib mesylate. The increase of BCR-ABL above 100 % preceded the hematological relapse 4–16 weeks [20].

We have compared this competitive method with real-time RT-PCR using TaqMan chemistry as designed in the Protocol of Europe Against Cancer Program [2, 7]. We have found close correlation between competitive method and real-time RT-PCR with B2M (beta-2-microglobulin) as the control gene. However, using total ABL control gene, concordance was found only when low BCR-ABL levels were present; higher BCR-ABL values were underestimated and differences increased with the rising number of the BCR-ABL transcripts, overexpression could not be detected. In our opinion, the total ABL transcript is not convenient to be used as an internal standard for BCR-ABL monitoring. The relation BCR-ABL/(ABL+BCR-ABL), i.e. BCR-ABL to total ABL, does not express linear dependence of real and measured BCR-ABL values. As the upper limit of this expression is 1 (100 %), higher BCR-ABL transcript levels are underestimated and BCR-ABL overexpression cannot be detected. Although the advantage of Q-RT-PCR is mainly in its high sensitivity to monitor patients in CCR, also the advantage of precise monitoring of high BCR-ABL levels in 100 % Ph positive patients and early detection of BCR-ABL overexpression should not be omitted.

The second method used in this study was duplex RT-PCR determining BCR-ABL to normal BCR transcript ratio [17]. The preciseness of the BCR-ABL/BCR ratio determination was ensured by the primer design that enabled the same amplification efficiency of both templates throughout PCR. This method was not sensitive enough to be used for early detection of relapses in post-SCT patients but it was found to be very important for monitoring patients without CCR. The advantages of this method were small laboriousness similar to that of qualitative RT-PCR and high preciseness.

We found that BCR-ABL/BCR transcript ratio (R) better characterized the disease status and disease prognosis than BCR-ABL transcript concentration. Although the statistical analysis confirmed that values measured by both methods statistically significantly correlated with response to therapy (Wilcoxon test and Analysis of Variants, both: $p<0.0001$), it was only R that really unambiguously denoted all poor responders. Permanently high (R>1) or further increasing R values characterized poor response and bad prognosis, while permanently low (R<1) or decreasing R values characterized good response and good prognosis. We did not find any exception. On the contrary, BCR-ABL transcript levels did not unambiguously distinguish between cytogenetic responders and patient resistant to therapy. BCR-ABL values <100 % were found to be typical for good responders with cytogenetic response, however, values <100 % were also detected in some non-responders. These atypically low levels of BCR-ABL transcripts in patients without response to ther-

apy may indicate that the disease became BCR-ABL independent. This possibility has been under study.

However, at the same time our results showing R>1 in non-responders and R<1 in cytogenetic responders indicate that in non-responders expression of BCR is always lower, while in good response higher than that of BCR-ABL. R<1 in good responders corresponded with low leukemic burden in these patients. R>1 in poor responders with 100 % Ph positivity could be caused by 1) BCR-ABL gene amplification or overexpression, 2) blocking of BCR expression or 3) both. Results showing increase in R, while stable trend was found in BCR-ABL level (Fig. 2 C,D), suggest different regulation of BCR-ABL and BCR genes expression, although both genes are controlled by an identical promoter. The results indicate that in poor responders expression of BCR is always lower than that of BCR-ABL and can further decrease in disease development. It suggests that the disease status may depend on the relation of BCR-ABL and BCR expression. Although speculative, this may indicate that not only BCR-ABL but also normal BCR gene may play a role in CML and the role of BCR is antagonistic to that of BCR-ABL. This fact has never been described in patients yet, however, the ability of normal BCR protein to inhibit the tyrosine kinase activity of BCR-ABL oncoprotein has been reported and studied in detail [1, 10, 15]. Increased expression of BCR gene, measured at protein level, in cell cultures [22] as well as in a mouse model [13] interfered with oncogenic effect of BCR-ABL. To test this hypothesis in human CML, comprehensive experiments on patients' samples should be done.

In conclusion, this study shows that different results may be obtained by different approaches to BCR-ABL transcript monitoring. In this study we report that in poor responders only BCR-ABL/BCR transcript ratios but not BCR-ABL transcript levels unambiguously characterize the disease status. It shows not only the importance of BCR-ABL/BCR transcript ratio in CML monitoring but may also suggest a role of normal BCR as BCR-ABL antagonist in human CML. To verify this suggestion future experiments on patients' samples should investigate the relation between the two proteins in patients' cells and study the mechanism of regulation of the two gene expression in CML pathogenesis.

We would like to thank MSc. Z. ROTH, PhD. for his help with statistical analysis.

References

[1] ARLINGHAUS RB. Bcr: a negative regulator of the Bcr-Abl oncoprotein in leukemia. Oncogene 2002; 21: 8560–8567.

[2] BEILLARD E, PALLISGAARD N, VAN DER VELDEN VH, BI W, DEE R et al. Evaluation of candidate control genes for diagnosis and residual disease detection in leukemic patients using 'real-time' quantitative reverse-transcriptase polymerase chain reaction (RQ-PCR) – A Europe Against Cancer Program. Leukemia 2003; 17: 2474–2486.

[3] BRANFORD S, HUGHES TP, RUDZKI Z. Monitoring chronic myeloid leukaemia therapy by real-time quantitative PCR in blood is a reliable alternative to bone marrow cytogenetics. Br J Haematol 1999; 107: 587–599.

[4] CROSS NCP, FENG L, CHASE A, BUNGEY J, HUGHES TP et al. Competitive polymerase chain reaction to estimate the number of BCR-ABL transcripts in chronic myeloid leukemia patients after bone marrow transplantation. Blood 1993; 82: 1929–1936.

[5] DALEY GQ, VAN ETTEN RA, BALTIMORE D. Induction of chronic myeloid leukemia by the P210 bcr/abl gene of the Philadelphia chromosome. Science 1990; 247: 824–830.

[6] EMIG M, SAUSSELE S, WITTOR H, WEISSER A, REITER A et al. Accurate and rapid analysis of residual disease in patients with CML using specific fluorescent hybridization probes for real-time quantitative RT-PCR. Leukemia 1999; 13: 1825–1832.

[7] GABERT J, BEILLARD E, VAN DER VELDEN VH, BI W, GRIMWADE D et al. Standardization and quality control studies of 'real-time' quantitative reverse transcriptase polymerase chain reaction of fusion gene transcripts for residual disease detection in leukemia – a Europe Against Cancer program. Leukemia 2003; 17: 2318–2357.

[8] GAIGER A, HENN T, HÖRTH E, GEISSLER K, MITTERBAUER G et al. Increase of BCR-ABL chimeric mRNA expression in tumor cells of patients with chronic myeloid leukemia precedes disease progression. Blood 1995; 86: 2371–2378.

[9] GUO JQ, LIN H, KANTARJIAN H, TALPAZ M, CHAMPLIN R et al. Comparison of competitive-nested PCR and real-time PCR in detecting BCR-ABL fusion transcripts in chronic myeloid leukemia patients. Leukemia 2002; 16: 2447–2453.

[10] HAWK N, LIU J, SUN T, WANG Y, WU Y et al. Inhibition of the Bcr-Abl oncoprotein by Bcr requires phosphoserine 354. Blood 2001; 98b: 167.

[11] HOCHHAUS A, LIN F, REITER A, SKLADNY H, MASON PJ et al. Quantification of residual disease in chronic myelogenous leukemia patients on interferon- therapy by competitive polymerase chain reaction. Blood 1996; 87: 1549–1555.

[12] JONES CD, YEUNG C, ZEHNDER JL. Comprehensive validation of real-time quantitative bcr-abl assay for clinical laboratory use. Am J Clin Pathol 2003; 20: 42–48.

[13] LIN F, MONACO G, SUN T, LIU J, LIN H et al. BCR gene expression blocks Bcr-Abl induced pathogenicity in a mouse model. Oncogene 2001; 20: 1873–1881.

[14] LION T, HENN T, GAIGER A, KALHS P, GADNER H. Early detection of relapse after bone marrow transplantation in patients with chronic myelogenous leukemia. Lancet 1993; 341: 275–276.

[15] LIU J, WU Y, ARLINGHAUS RB. Sequences within the first exon of BCR inhibit the activated tyrosine kinases of c-Abl and the Bcr-Abl oncoprotein. Cancer Res 1996; 56: 5120–5124.

[16] MORAVCOVÁ J, LUKÁŠOVÁ M, STARÝ J, HAŠKOVEC C. Simple competitive two-step RT-PCR assay to monitor minimal residual disease in CML patients after bone marrow transplantation. Leukemia 1998; 12: 1303–1312.

[17] MORAVCOVÁ J, MUCHOVÁ S, KALBÁČOVÁ R, HAŠKOVEC C. Single-tube quantitative RT-PCR for monitoring of the response to IFN treatment in CML patients. Leukemia 1998; 12: 1326–1327.

[18] MORAVCOVÁ J, MUCHOVÁ S, LUKÁŠOVÁ M, KLAMOVÁ H, BŘEZINOVÁ J et al. Late molecular relapse in two CML patients in molecular remission after donor lymphocyte transfusion for post-BMT relapse. Bone Marrow Transplant 1999; 23: 857–859.

[19] MORAVCOVÁ J, NÁDVORNÍKOVÁ S, LUKÁŠOVÁ M, KLAMOVÁ H. Polymerase chain reaction analyses should be used as a basis for clinical decision making in patients with chronic myelogenous leukemia. Blood 1999; 94: 3609–3611.

[20] MORAVCOVÁ J, ZMEKOVÁ V, KLAMOVÁ H, VOGLOVÁ J, FABER E et al. Differences and similarities in kinetics of BCR-ABL transcript levels in CML patients treated with imatinib mesylate for chronic or accelerated disease phase. Leukemia Res 2004; 28: 415–419.

[21] SAWYERS CL, MC LAUGHLIN J, GOGA A, HAVLIK M, WITTE O. The nuclear tyrosine kinase c-Abl negatively regulates cell growth. Cell 1994; 77: 121–131.

[22] WU Y, MA G, LU D, LIN F, XU HJ et al. Bcr: a negative regulator of the Bcr-Abl oncoprotein. Oncogene 1999; 18: 4416–4424.

[23] YUAN ZM, SHIOYA H, ISHIKO T, SUN X, GU J et al. p73 is regulated by tyrosine kinase c-Abl in the apoptotic response to DNA damage. Nature 1999; 399: 814–817.