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Polymerase Chain Reaction Analyses Should Be Used as a Basis for Clinical Decision Making in Patients With Chronic Myelogenous Leukemia

To the Editor:

The report of Faderl et al[1] deals with the question of whether polymerase chain reaction (PCR) analysis, used to detect minimal residual disease in patients with chronic myelogenous leukemia (CML), should be used in clinical decisions. The authors' view on the ability of PCR to predict relapse of the disease is very critical. However, all of their considerations, and consequent conclusion, are based only on qualitative PCR and some static results of quantitative (Q)-PCR. They do not take into account the sequential analyses of residual disease by Q-PCR and they quite ignore the advantages of Q-PCR for follow-up of the dynamics of CML, which have been confirmed by many investigators.[2-9] It was already shown several years ago that qualitative PCR confirming the presence of the BCR-ABL transcripts in patients after bone marrow transplantation (BMT) can only identify patients belonging to a group with elevated risk of relapse, but it is only of a limited predictive value for the disease evolution in individuals of this group. Only the Q-PCR, by determining the amount of BCR-ABL transcripts at regular time intervals, can find an increasing level of BCR-ABL transcripts indicating activity of the malignant clone and thus predict the impending clinical relapse.[2-5]

This is also our experience. At our institute, the Q-PCR was introduced in 1994[9]; since then, it has been used for routine monitoring of the BCR-ABL level in patients with CML after BMT. The results of the monitoring have been used as a basis for making clinical decisions. The assay permits absolute quantification with a sensitivity of 10^{-5} and was proved to be precise and reliable by comparing its results with the mathematical model of competitive PCR.[9] Before BMT, each patient is tested by multiplex qualitative reverse transcriptase-PCR (RT-PCR),[10] which is capable of identifying any of the BCR-ABL transcripts to exclude some atypical rearrangement and to prevent false-negative results in post-BMT monitoring; this is the answer to the anxiety of Faderl et al[1] about false-negative results in diagnosis and monitoring of residual disease in CML. Patients after BMT are followed-up by qualitative PCR at regular time intervals suggested by the group of European Investigators on CML (EICML group),[5] ie, at 2- to 3-month intervals in the first year after BMT, when 2-step PCR results are negative, and at 6-month intervals during further years of PCR negativity. In patients with persistent post-BMT or reappearing PCR positivity, quantitative PCR analyses are performed at 1-month or shorter intervals. In our own experience, this time schedule is necessary to enable an early detection of imminent disease relapse. Molecular (PCR) relapse is defined dynamically as a 10-fold increase of PCR positivity[5] without any signs of cytogenetic relapse. Therapeutic intervention, to be most efficient, should start at the molecular relapse. If regular sequential analyses by qualitative and quantitative PCR are performed, comparison of the 2 methods can be performed. The group of patients with permanent BCR-ABL positivity found by qualitative PCR assay can be further divided into 3 groups by Q-PCR: groups with increasing, stable, or decreasing level of BCR-ABL transcripts. These quantitative results clearly show different evolution of the disease in individual positive patients; therefore, different clinical decisions should be made on the basis of these results.

From our experience, we do not suppose that a definite level of BCR-ABL transcripts, a threshold as the investigations mention, can be found ''above which a patient is likely to relapse or, conversely, below which remission is sustained.'' A patient with a level of BCR-ABL transcripts below such a definite threshold cannot be said to be in remission if the amount of the BCR-ABL transcripts is permanently increasing. On the other hand, a patient with a relatively high but stable level of BCR-ABL positivity can be in a stable state without relapse for several years, although the danger of relapse is very high in this case. However, we assume that a definite level of BCR-ABL transcripts should be given as the threshold that marks the last appeal to start therapy before cytogenetic or even hematological relapse is diagnosed.

We cannot agree with the investigators' apprehension of injuring the patients due to decisions made on PCR assay ''given the significant morbidity and mortality associated with aggressive therapeutic interventions aimed at molecular disease eradication.'' The aim of Q-PCR is quite the opposite—not to force clinicians to eradicate leukemic clone under detectable level of PCR at any cost, but to show the increasing proliferative activity of the malignant clone still in the time of full clinical and cytogenetic remission when malignant clone is not too numerous and when nonaggressive therapy (low doses of DLI) is shown to be very efficient. The intention of Q-PCR monitoring is to prevent clinical relapse when treatment efficacy is low despite very aggressive therapeutic intervention.

The findings of BCR-ABL transcripts in leukocytes from healthy adults cannot decrease the validity and importance of Q-PCR tests for predicting disease relapses after BMT. In the study of Bose et al,[11] the overall sensitivity of Q-PCR assay used in healthy individuals is approximately 40 times higher than that used in practice; the finding of this normal positivity is, thus, highly improbable. Moreover, such a positivity would appear as a low incidental positivity, having no predictive value for the disease relapse. On the other hand, long-term highly sensitive sequential Q-PCR analyses showing a permanently increasing amount of BCR-ABL transcripts in healthy individuals should separate those predisposed to the outbreak of the disease. However, this cannot be used in practice.

As to technical pitfalls of the PCR, such as sample contaminations, etc, we do not believe that they can influence the results of the minimal residual disease monitoring at present. We hope that every responsible

laboratory worker is aware of all of the dangers resulting from high PCR sensitivity and tries to minimize them by using stringent precautions. Many recommendations in this respect have been published (eg, Cross[12]). Negative and positive controls are included to show false-positive results due to contaminations and false-negative results due to lower sensitivity of PCR, lower efficiency of reverse transcription, and RNA isolation or bad quality of samples. The check on competitor stability is also performed in every quantitative test in our laboratory.

In conclusion, on the basis of our results and reports of others, in contrast to Faderl et al,[1] we consider PCR assays, quantitative PCR together with qualitative PCR, to be a very important tool in monitoring residual disease and predicting disease relapse. They should therefore be taken into account when making clinical decisions.

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Response

The letter by Moravcová et al demonstrates how important it is that recommendations for clinical decision making based on laboratory assays are well-founded in carefully performed clinical studies and not in feelings, beliefs, assumptions, and anecdotal clinical experiences.

We agree that any meaningful follow-up of patients with minimal residual disease requires serial measurements at various time points. In addition, detection of the mere presence or absence of residual disease, such as obtained with qualitative assays, is certainly less informative than being able to quantify leukemic fusion transcripts. However, quantification of minimal residual disease markers, as desirable and important it may be, has been a major problem with PCR methods.[1,2] Furthermore, quantitative PCR techniques are not immune to drawbacks and pitfalls that are encountered with qualitative assays and that are widely accepted in the research community.[3-5] New techniques, such as real-time PCR, are exciting and produce interesting data.[6] Whether the inherent assay-related difficulties with PCR can thereby be overcome, and whether and how the results of these studies can be used in clinical decision making, has to be proven first, however. The dogma-like certainty of Moravcová et al about the infallibility of multiplex PCR is premature and requires approval by other investigators.[7] Furthermore, their statement that ''therapeutic intervention, to be most efficient, should start at the molecular relapse'' is not supported by the current literature. No randomized clinical study in patients with CML has proven that therapeutic intervention based on PCR testing and at the time of molecular (and not cytogenetic) relapse is beneficial. Rather than our ''anxiety,'' therefore, we have been expressing our concern, shared by many other investigators in the field, that such information is still lacking and that further studies are warranted to clarify these issues.[5]

Moravcová et al ''suppose'' that no definite threshold of leukemic fusion transcripts for relapse exists, but ''assume'' that a ''definite level of BCR-ABL transcript should be given'' as ''the last appeal'' to start therapy. This statement is contradictory in itself and erratic enough to make one feel uncomfortable to use it as a treatment guideline. Threshold levels of transcripts to predict relapse have been proposed by some investigators.[8-10] However, in some of these cases, the sensitivity of the threshold was so low that it approached the sensitivity of cytogenetic or even morphologic relapse.

We think that for quantitative PCR to become a clinically reliable test, it must prove to have acceptable sensitivity, specificity, and positive and negative predictive values. After the data are generated to validate the test results, randomized clinical studies should be performed to demonstrate whether it is of advantage to the patients to be treated in molecular rather than cytogenetic relapse. Until that is the case, we want

to caution the practicing clinician against blindly relying on PCR test results for clinical decision making and suggest that such studies be performed in a supervised investigational setting only.

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von Willebrand Factor Proteolysis in Thrombotic Thrombocytopenic Purpura

To the Editor:

von Willebrand factor (vWF)-cleaving protease has been shown to be deficient in patients with thrombotic thrombocytopenic purpura (TTP).[1,2] The recent article by van der Plas et al[3] confirms the observation that vWF-cleaving protease is severely decreased or missing in plasma of patients with classic TTP, whereas normal protease activities were found in patients with bone marrow transplantation (BMT)-associated TTP. The investigators found no significant difference between the 2 groups regarding the levels of cellular fibronectin. However, as shown in Table 1 of their report, there was a very significant difference in vWF:Ag and vWF:Rcof between classic TTP and BMT-associated TTP, even though the number of patients in each group was quite limited (5 and 8 patients, respectively). Thus, the median values of vWF:Ag were 88% in classic TTP and 292% in BMT-associated TTP. The respective median values of vWF:Rcof were 67% and 161%. The Mann-Whitney rank sum test resulted in *P* values of .010 (vWF:Ag) and .011 (vWF:Rcof) for the difference between classic and BMT group. These results suggest that, in contrast to the classic group, there was massive release of vWF from the endothelial cells in the BMT group, possibly due to graft-versus-host disease and/or cyclosporin treatment.[4,5] It is conceivable that the excessive mobilization of abnormally adhesive very large multimeric forms in the BMT patients leads to aggregation of the circulating platelets and to thrombotic microangiopathy, even in the presence of vWF-cleaving protease.[6]

Thrombotic microangiopathy (TMA), which is associated with BMT, is sometimes labeled as TTP and sometimes as hemolytic-uremic syndrome (HUS). Hematologists seem to favor the diagnosis of TTP, whereas nephrologists tend to call it HUS. There is no distinct boundary between TTP and HUS: diagnosis of TTP is preferred for cases with neurologic disturbances, whereas cases involving predominantly renal impairment are classified as HUS. No information is given in the study of van der Plas[3] regarding the clinical symptoms and criteria, according to which the TMA in their patients had been classified as TTP. It is obvious (Table 1) that the creatinine level was normal in patients with classic TTP (median value, 76 μmol/L), but significantly increased ($P = .019$) in patients with BMT-associated TTP (median value, 178 μmol/L). Therefore, the TMA in the BMT group should be classified as HUS rather than as TTP.

In our study on vWF-cleaving protease in patients with TTP and HUS,[1] we observed deficient or strongly decreased protease activity in patients with familial and nonfamilial TTP, but normal protease activity in patients classified as having HUS. It should be added that 2 patients were included into the latter study who presented with symptoms of TMA after BMT: the patient no. 24A, classified as having TTP, had 25% protease activity, whereas the symptoms in the patient no. 35A were compatible with the diagnosis of HUS and the patient had normal activity of vWF-cleaving protease. Although there were no neurological symptoms observed in patient no. 24A, his diagnosis was apparently misclassified as TTP by the participating investigator, because the creatinine levels had been only moderately increased.

It appears that the discrepancy between conclusions of our report[1] and the report of van der Plas et al[3] only arises from the differential diagnosis of the TMA in BMT patients. If the BMT cases had been classified by van der Plas et al[3] as HUS, which may be appropriate regarding the laboratory data, their report would perfectly confirm our observation that the vWF-cleaving protease is deficient in patients with TTP but present in patients with HUS.

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