Letter to the editor:
Lange T et al. Quantitative reverse transcription polymerase chain reaction should not replace conventional cytogenetics for monitoring patients with chronic myeloid leukemia during early phase of imatinib therapy. Leukemia 2004; 89: 49-57

Haematologica 2004; 89:(7)e93-e94

Sir,

regarding the above mentioned manuscript we have to express our deep concern on the validity of the data which the authors obtained by their quantitative real-time RT-PCR technique for the following reasons:

1. Lange et al. state that processing of blood samples was performed up to 24 hours after collection. It could be shown that RNA transcripts change rapidly within hours after phlebotomy.[1] In a study investigating AML1/ETO fusion transcripts in acute leukemia ABL as well as AML1/ETO transcripts in blood samples decreased equally by 0.5 log after 24 h at room temperature.[2] Thus, the sample processing interval in the present study appears to be inappropriately long.

2. The authors state that they collected 20 mL of peripheral blood and subsequently reverse transcribed 0.5 mg RNA. While the average RNA content of a mammalian cells is roughly 20 pg leucocytes contain approximately 2 pg RNA per cell whereas only a fraction (~25%) is mRNA. Even by the most sensitive acid guanidinium thiocyanate-phenol-chloroform technique a maximum of 10 µg RNA can be extracted from 1 mL peripheral blood of individuals exhibiting a normal WBC. As it must be assumed that after 3 months of imatinib therapy most patients did not have excessive leucocyte numbers one has to speculate that 0.5 mg reverse transcribed RNA is a misprint.

3. The authors do not provide information about the individual bcr/abl transcript type of their patients. Although the most common fusion transcripts a2b2 and a3b2 are detected by their RT-PCR a2b3 and a3b3 fusions which have an incidence of 5% among CML patients cannot be amplified by the oligonucleotides given in the paper.

4. Concerning details of the RT reaction the authors cite a previous work of them.[3] In this work no further information is given instead a publication of another group is cited.[4] Here also no details are provided but a further reference given.[5] Finally, in this ten years old reference 50 times less RNA (10 µg) than in the present paper were reverse transcribed into cDNA making both procedures incomparable. If it is true that 0.5 mg reverse transcribed RNA is a misprint it is likely that 0.5 µg was meant. However, in this case the RT reaction would have contained 20 times less RNA than in the cited work by Cross et al. As detailed information concerning the RT volume are not given in the reference it can only be speculated that the final cDNA concentration in the current manuscript was either 20 µg/µL or 20 ng/µL, respectively (0.5 mg or 0.5 µg RNA in a RT reaction volume of 25 µL).

5. Concerning details of the quantitative RT-PCR the authors again refer to a previous work of them[6] where 2 µL of the RT reaction were used for the RT-PCR. Thus, with respect to the calculations described above 2 µL of the cDNA preparation represent either 2×10^7 cells (in the unlikely case of 0.5 mg reverse transcribed RNA) or 2×10^4 cells (in the case of 0.5 µg reverse transcribed RNA) per reaction, respectively. The latter amount of cells is certainly not sufficient for a sensitive technique such as RT-PCR.

6. The authors used glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as reference transcript. It is meanwhile broadly accepted that GAPDH is unsuitable for this purpose as its expression varies enormously (up to 17-fold) and approximately 52 processed pseudogenes are known.[7] Moreover, a recent study using cDNA microarray technology failed to identify GAPDH as a housekeeping/maintenance gene.[8] Thus, GAPDH is now generally excluded as control gene for quantitative RT-PCR.[9]

7. It has been shown that GAPDH is at least as strong expressed as β-actin in peripheral blood leucocytes[10] whereas the absolute expression of β-actin is approximately 500 molecules per cell.[11,8] Lange et al. state that they excluded samples with GAPDH levels below 6022 molecules per µL. Given a total RT-PCR reaction volume of 25 µL the minimum GAPDH content was therefore 1.5×10^5 which again corresponds to 300 cells. Independent from our above mentioned calculations the authors prove hereby that they analyzed an unacceptable low amount of amplifiable cDNA. In contrast to the manuscript the reference which is given by the authors[12] does not provide any further information concerning the exclusion of low quality samples.

Summing up, the data provided by Lange et al. are difficult to interpret as important details concerning the quantitative RT-PCR are apparently wrong or misleading. Furthermore, their experimental setting cannot be properly reproduced due to lacking information. Finally, as far as one can reproduce the results it is obvious that the described quantitative RT-PCR method is insufficient for the purpose of the presented work. Thus, in our eyes it is illegitimate that the authors come to the conclusion which is anticipated in the title of the manuscript.

Yours sincerely

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