To the Editor:

The recent study by Brugger et al. reflects the impact of quantitative determinations of circulating hematopoietic cells identified as CD34+ cells. However, the goal of reliable determinations on a parameter such as CD34 cells/µL of blood stresses the need for proper quantitative methods.

Brugger et al. report a mean number of 300 CD34 cells/mL of blood in steady-state healthy adults, with levels up to 2 × 10^6/mL of blood in patients after chemotherapy and treatment with cytokines. The method used for the quantitation is reported to be microscopical evaluation of slides after immunocytochemistry (using CD34 antibody) of peripheral blood mononuclear cells. Although they are essential for proper understanding of the data, no mention is made of the total number of cells evaluated nor of the percentage ("permillages") of cells considered to be CD34+. Therefore, we have made our own calculations. Given a mean number of about 2,500 lymphocytes/µL of blood (there is no absolute leukocyte count given in the report) and the reported mean number of 0.3 CD34 cells/µL of blood, it can be deduced that Brugger et al detected 1 cell considered CD34+ from approximately 7,500 cells looked at. In view of the classical reports of Rümke2,3 focussing on the 95% confidence limits when performing microscopical differentials, one may question the reproducibility and reliability of the data reported by Brugger et al observing less than 0.01% of cells. Furthermore, it is noteworthy that Brugger et al assign a sensitivity of 0.01% to their microscopical method. The assignment of this high degree of sensitivity is directly linked to a previous report in which Bross, one of Brugger’s coauthors, coauthored.4 Interestingly, in that report,4 no hint is given to suggest such a high degree of sensitivity.

Furthermore, the authors state that the mean numbers of colony-forming units granulocyte-macrophage (CFU-GM) per milliliter of blood and of burst-forming units-erythroid (BFU-E) per milliliter of blood determined from steady-state healthy adults were 130 and 85, respectively. In view of the mean number of 300 CD34 cells/mL of blood, a cloning efficiency of about 75% can be deduced. This, in fact, is in some contrast to the clonogenities reported by others applying flow cytometry-based determinations of blood CD34 cells.5,7

Major incongruities are also found in another report on quantitative determinations of blood CD34 cells published in Blood.7 Siena et al.2 stated that with single samples of 50 µL of blood they performed the flow cytometrical analyses of CD34 cells with 10,000 cells per determination in each case. This volume (50 µL) of blood was considered necessary samples with leukocyte counts down to 150/µL of blood. Perhaps we are wrong in calculating, but we think that 150 × 50 = 7,500. So, with samples with a leukocyte count less than 200/µL of blood there will not even be the 10,000 cells reported when drawing the 50 µL, not to think of cell loss associated with centrifugation and the setting of the gate on the cytometer.

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RESPONSE

We would like to respond to the letter by Serke and Huhn regarding our recent manuscript published in Blood. In our study, the number of CD34+ cells was considerably low only under steady-state conditions (0 to 30 cells per spot). To calculate a cloning efficiency of CD34+ cells under steady-state hematopoiesis by dividing the median number of CD34+ cells by the median number of clonogenic progenitors (as suggested by Serke and Huhn) is very errorsome, because under baseline conditions the numbers of CD34+ cells, as well as clonogenic progenitor cells (as shown in our report), are very low and, thus, the variation might be very high. Due to this fact, the calculated cloning efficiencies from different investigators might be different. Serke and Huhn should be aware that the only way to reliably determine the cloning efficiency in this setting is by sorting CD34+ cells and to subsequently determine their clonogenic capacity.

(5) The sensitivity of flow cytometry is approximately 0.5% to 1%; cell populations of less than 0.5% are inadequately estimated by flow cytometry as to draw major conclusions from whether a population comprises 0.1% or 0.3%, for example.

Interestingly, using flow cytometry, Serke and Huhn report a nearly identical percentage of CD34+ cells in normal volunteers when compared with our study using the immunoperoxidase technique!

In our lab, we routinely use flow cytometry to determine the timing for the routine harvest of peripheral blood progenitor cells in our patients. Moreover, we apply this simple technique for dual- and triple-color analyses of CD34+ cells.

We have studied in detail both methods in comparison for CD34+ cell populations greater than 0.5% and found that the results are nearly identical at percentages greater than 1% CD34+ cells.

In summary, we have used a very sensitive, reliable and reproducible method to detect circulating CD34+ cells. The method and its application has been published in numerous international journals since its original publication by Bross and Blume.

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Quantitation of CD34+ cells [letter; comment]
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