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 Rümke focusing 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. Interestingly, in that report, 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.

Major incongruities are also found in another report on quantitative determinations of blood CD34 cells published in Blood. Siena et al. 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.

STEFAN SERKE
DIETER HUHN
Universitätsklinikum Rudolf Virchow-Charité
Abteilung Innere Medizin und Poliklinik
Hämatologisches Zentrallabor
Berlin, Germany

REFERENCES

5. Serke S, Säuberlich S, Huhn D: Multiparameter flow-cytometrical quantitation of circulating CD34-positive cells: Corre-
We would like to respond to the letter by Serke and Huhn regarding our recent manuscript published in Blood.1

Serke and Huhn comment on the identification and quantification of CD34+ cells by the immunoperoxidase slide technique used in our study. In addition, they criticize the way Siena et al2 have analyzed CD34+ cells by flow cytometry. We will focus on the issues relevant to our report.

We described the differential mobilization of hematopoietic progenitor cells into the peripheral blood after a standard-dose chemotherapy regimen with VP16, ifosfamide, and cisplatin (VIP) and the administration of granulocyte-macrophage colony-stimulating factor (GM-CSF) and interleukin-3 (IL-3) + GM-CSF. CD34+ cells were evaluated either by flow cytometry or by an immunoperoxidase staining method. This method was originally described by Bross et al1978; the adhesion slides are now commercially available.

For the studies performed by our group, the use of the immunoperoxidase staining technique offers several advantages when compared with flow cytometry:

1. It can be easily applied at very low numbers of absolute white blood cell counts in the peripheral blood, drawing only 2 mL of blood. Because kinetic analyses for the recruitment of peripheral blood progenitor cells were one of the major objectives of our study, it was important to detect the increase of progenitor cells as early as possible. Those cells started to increase at day 7, when peripheral blood white blood cell counts still were less than 1,000/µL.

2. In addition to the specific staining with the monoclonal antibody, all cells present on the adhesion slide can be analyzed with respect to their morphology. Thus, there are no false positive results due to the unspecific binding of anti-CD34 antibodies to immature granulocytes or monocytes. It is well established that there are overlapping gates for hematopoietic progenitors and monocytic cells as analyzed by flow cytometry.

3. At least 10,000 cells (as enumerated with the help of a grid) are attached on each spot of the slides and at least two separate spots were analyzed for CD34 expression. All positive cells can be easily detected and identified by the brown membrane staining and the typical morphology. The assay is reproducible and reliable.

4. The percentage of CD34+ cells at the day of maximal numbers of progenitor cells was up to 42%, with a median value of 18% (range, 5% to 42%), as measured by the immunoperoxidase method. Therefore, we are not looking at cells below the percentage of 0.01, as mentioned by Serke and Huhn.

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 Blüme.3,4

WOLFRAM BRUGGER
KLAUS BROSS
ROLAND MERTELSMANN
LOTHAR KANZ
Klinikum der Albert-Ludwigs-Universität Freiberg
Medizinische Universitätsklinik und Poliklinik
Abteilung Innere Medizin I
Hämato logic, Onkologie
Freiberg, Germany

REFERENCES

4. Fauser AA, Kanz L, Bross KJ, Löhr GW: T cells and probably...


Quantitation of CD34+ cells [letter; comment]

S Serke and D Huhn