MEGAKARYOCYTIC IDENTIFICATION

To the Editor:

In the article by Levine, Hazzard, and Lamberg, a flaw due to an inadequate number of experimental animals and human subjects studied might have produced a phenomenon of an apparently discriminative visual threshold value that separated most of the megakaryocyte population from most of the other bone marrow cells. In Fig. 2, size histograms of megakaryocytes were obtained from marrow suspensions pooled from only two guinea pigs of unspecified strain, which is an inappropriately small number of animals for a threshold value determination study. One would reasonably expect that the size trough observed between the megakaryocyte and nonmegakaryocyte subpopulations would tend to level out with a more representative pool of cells being analyzed. Alternatively, the latter might have given a different and more realistic cut-off value. In accurate threshold value determinations, a large number of individual cell measurements cannot fully compensate for a nonrepresentative sample.

The authors examined human megakaryocytes in marrow smears from only three patients. No hint was given as to their diagnoses, received therapy, and thrombocyte counts, respectively. On marrow smears (lower part of Fig. 4), a zone, rather than "a point," of overlap between the two populations studied shifted, due to flattening of cells, from 14 μm to 19–23 μm. However, about 50% of all megakaryocytes of maturation stage I fell within this range. Such a degree of overlap would apparently obliterate any advantage of relying upon a visual threshold value, since the probability of distinguishing correctly between the megakaryocytes of this stage and nonmegakaryocytes would be expected to be around ½.

In preleukemic and overtly leukemic patients with affected megakaryocytopenia (hypolobulation of micromegakaryocytes), there is a large number of atypical blasts that may have a high nuclear-cytoplasmic ratio and bi- or multilobed nucleus (e.g., T-cell leukemias). Since not all of the small (either normal or abnormal) megakaryocytes show clearly bilobed nuclei, but may, instead, display either folding and/or irregularity of the nuclei, a confusion with blast cells, particularly in M4 and M5 A leukemias, seems inevitable. Even in rare cases of megakaryocytic leukemia, morphological separation of malignant blasts from the early normal megakaryocytic precursors is extremely difficult by resorting only to the standard morphological criteria (which duly include those recommended by the authors). The blast cells transitional to immature megakaryocytes are frequently observed intermingled with a greater number of blast cells widely varying in diameter, which tends to further "homogenize" the cell size distribution. In patients with deranged folate metabolism (as is common in both untreated and treated leukemias) and megaloblastic hematopoiesis, asynchronous maturation of all cell lines in the marrow would be expected to additionally "blur" any meaningful clear-cut visual threshold value. Therefore, introducing one would lead to unwarranted oversimplifications with a consequent wrong discrimination between the cells. Specific cytochemical staining (acetylcholinesterase) would be mandatory in such patients.

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To the Editor:

Dr. G. M. Janković’s fear that an introduction of a visual threshold value in megakaryocyte–nonmegakaryocyte discrimination on marrow smears may be potentially hazardous indicates a misreading of our paper, “The Significance of Megakaryocyte Size,” which appeared in the November 1982 issue of Blood.

Similar size distributions to that shown in Fig. 2 have been obtained from a great many examinations of normal guinea pig, rat, mouse, monkey, and human marrow, some of which were previously published. The flow cytometric results on isolated megakaryocytes and on unseparated marrow cells confirmed the separate, slightly overlapping size distributions of megakaryocytes and nonmegakaryocytes. Our interpretation of these data was not to recommend “relying upon a visual threshold value,” which he laments, but to examine the easily detected cells noticeably larger than the general marrow population for specific morphological criteria of megakaryocytes. This point was stated clearly in the abstract, results, and discussion sections. The definitely larger marrow cells (>20 μm in diameter) are virtually always megakaryocytes. In the questionable range between the peak of nonmegakaryocytes and the obvious megakaryocytes (i.e., 14–20 μm), the great majority of cells (not just half) are not megakaryocytes but can now generally be distinguished without difficulty.

The data presented were of cells from normals, and no quantitative extrapolation to the variety of pathologic states considered by Dr. Janković was suggested. We might point out that megakaryocyte lobulation is more akin to polymorphonuclear leukocyte lobulation and distinct from the indentations of T cells and other lymphocytes, the infoldings of monocytes, the irregular nuclear contours of monocytes, leukemic blasts, etc. These differences may be subtle and can be made more apparent on Feulgen stains of Carnoy’s fixed cells.

We wished to provide a simple approach to detecting the significant fraction of smaller megakaryocytes that were known to exist by electron microscopy but not often appreciated clinically. As we stated in the conclusion, we do not yet know what size threshold could be used as a sole criterion for the identification of megakaryocytes. Furthermore, specific immunofluorescence with antiplatelet antibodies (acetylcholinesterase activity is not present in humans) seems to detect, in preliminary studies, only a few percent more megakaryocytes than are found by the simpler approach considered in our paper. Only if one wishes certain identification of specific cells are special studies necessary.

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Megakaryocytic identification [letter]
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