Analysis of Bone Marrow Aspiration Fluid Using Automated Blood Cell Counters

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KEYWORDS

• Bone marrow analysis • Automated blood cell counters • Reticulocytes
• Flow cytometry • Erythroblast count • Bone marrow aspirate • Toxicologic studies

KEY POINTS

- Automated bone marrow analysis using blood cell counters is not a substitute for microscopic evaluation of bone marrow cytology in aspirate films.
- Potential advantages of automated bone marrow analysis are increased throughput and efficiency, decreased manual labor, reduced costs, increased reproducibility, objectivity of measurements, and less variability due to the individual variation in interpretation and the quality of the smear.
- The main difficulties for bone marrow analysis with blood cell counters are the presence of heterogeneous and immature progenitors, fat droplets, microfibers, and cell aggregates.
- Original information can derive from the comparison of results obtained from the simultaneous analysis of peripheral blood and bone marrow.
- Bone marrow fluid can be used as a substitute for venous blood in emergency situations, at least for the measurement of red cell parameters.
- Automated bone marrow fluid analysis will be useful for repetitive tasks such as toxicologic studies in laboratory animals.

INTRODUCTION

Technology has revolutionized clinical laboratories. Almost all diagnostic tests are currently carried out using highly automated and robotized systems. In hematology, automated blood cell counters were introduced in the 1960s; after half a century of continuous improvements, the latest generation instruments produce in less than a minute dozens of results that are much more precise and accurate than those obtained with the manual techniques formerly in use. The development of increasingly sophisticated methods has transitioned the hematology laboratory from the centrifuge

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Abbreviations	
IRF	Immature reticulocyte fraction
LUC	Large unstained cell
MCV	Mean corpuscular volume
NRBC	Nucleated red blood cell
PANDA	Peroxidase activity and nuclear density analysis
PBS	Phosphate-buffered saline
TNCC	Total nucleated cell count
WBC	White blood cell

hematocrit to an in-depth scrutiny of multiple blood cell properties. Any single cell in a peripheral blood sample can now be identified and counted, so that accurate differential leukocyte count, subtle red blood cell features, and even proportions of more or less mature reticulocytes can be measured at a rhythm of hundreds of samples per hour.[1,2] Very few areas still hold out against the technological supremacy; among these, in particular, no significant changes have occurred until now as for bone marrow aspirate. Cytomorphological examination of aspirate smears remains a well-established, basic method to assess the state of hematological cell lineages, to diagnose hematologic disorders, and to evaluate treatment-related changes of the hemopoietic system. Although progress in immunophenotyping techniques using multiangle flow cytometry, together with the recognition of specific genetic and molecular markers, has greatly improved diagnostic sensitivity and accuracy for many hematological conditions, the microscope identification and counting of normal and abnormal cell populations remains an essential tool, and the starting point, for the great majority of hematological diagnoses.[3]

The attempts to obtain a precise quantification of bone marrow cell populations through the utilization of fully automated blood cell counters used in routine hematology laboratories have not been fully successful until now. The complex nature of bone marrow fluid, and the many differences of its composition in comparison to peripheral blood, for which all instruments originally were designed, can justify the present inadequacy of hematological instruments for bone marrow fluid analysis. This article will attempt to summarize the current state of research, the reasons for the presently disappointing results, and opportunities for new forthcoming achievements.

Bone Marrow Fluid

The bone marrow of an adult constitutes 4.7% plus or minus 1.3% of total body weight (equivalent to 1.5–3.7 kg). The red or hemopoietic marrow represents about a quarter of this volume. It is principally located within the central axial skeleton, in the flat bones, and in the epiphyses of the long bones. Its structure consists of a network of trabecular bone lined by endosteum, with multiple cavities rich in sinusoids, reticular stroma, fat cells, and small islands and cords of hemopoietic cells. Although normal peripheral blood only contains 5 classes of mature leukocytes, the bone marrow fluid, aspirated with different techniques, is composed by many different types of cells in different phases of maturation, often aggregated in particles of different size, with fat and fibers.[4,5] The various stages of the granulocyte and erythroid series show a heterogeneous spectrum of sizes and other properties, such as nucleocytoplasmic ratio, cytoplasmic content, and nuclear shapes. Megakaryocytes and other giant cells are dispersed between them. Cellular abnormalities in hematologic disorders, such as myelodysplastic syndromes, leukemia, and myeloproliferative syndromes, produce further changes in the qualitative and quantitative marrow cytology.