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Research in morphology and flow cytometry is at the heart of hematology

Marie C. Béné[1] and Gina Zini,[2] EHA Scientific Working Group "Diagnosis", European LeukemiaNet WP10.

[1]Hematology Biology, Nantes University Hospital, France and [2]Blood Bank and UNICATT Cord Blood Bank, Fondazione Policlinico Gemelli, Università Cattolica del S. Cuore, Roma, Italy

E-mail: mariecbene@gmail.com *doi:10.3324/haematol.2016.163147*

Through its inherent implication in such major physiological systems as oxygenation, coagulation, protection against infections and tumor proliferation, hematology is a nearly boundless field for research and discovery. Indeed, hematology has been at the heart of such fundamental work as the understanding of iron regulation in red blood cell mediated tissue oxygenation,[1] or the deciphering of the intricate molecular interactions between endothelial cells, platelets and plasmatic proteins in the early stages of hemostasis.[2] Deeper insights into cell biology have also been attained through the use of the easily available blood or bone marrow cells and cell lines derived from hematological malignancies. Moreover, the notion of the importance of the microenvironment of many cells has certainly found fertile ground in the study of the bone marrow or diseased lymph nodes involved in leukemia and myeloproliferative or lymphoproliferative disorders.[3-5] Hematology is thus entertaining strong transdisciplinary interactions with pathology, immunology, biochemistry, cytogenetics, molecular biology and also, more recently, advanced imaging techniques.[6] These various aspects deal with the physiological maintenance of homeostasis, a precise and tightly regulated phenomenon in an extremely active system producing and eliminating trillions of cells every day. They are also at the heart of our increasing understanding of the mechanisms of disease and targeted therapeutic approaches.

In the field of hematology, two sub-disciplines are at the basis of diagnosis for malignant and non-malignant processes, i.e., the morphology of hematopoietic cells and flow cytometry. Both are considered indispensable, but it is sometimes forgotten that both represent true specialties, requiring thorough training and experience. It could be argued that the progresses of automation could alleviate these prerequisites, but the reality seems more subtle. Indeed, blood cell counters performing a complete blood count (CBC) are becoming more and more sophisticated, as previously underscored in the 2016 editorial from our scientific working group on innovation.[7] With different technological approaches, latest generation instruments perform accurate and reproducible quantitative measurements of peripheral blood cell composition, and have good sensitivity and specificity for flagging the presence of abnormal cells.[8] Understanding the flags or messages generated by these intelligent machines, in particular through morphological cell identification on a stained blood smear, still requires the knowledge initially placed in the design of automated interpretation. In addition, as with most expert systems, when the machine is at a loss, only the brain of the biologist/hematologist can come to the rescue, a task made increasingly difficult by the lack of experience in normal or benign situations taken care of by the instrument. Furthermore, cytomorphological analysis of bone marrow aspirates remains a cornerstone in the most recent WHO classification, and requires additional skill and knowledge.

The same is true for flow cytometry, with new instruments managing the whole process of handling samples

from HIV patients for instance.[9] In most cases, the robot is performing and provides straightforward and accurate results. But all biologists who have ever confronted this specific population know that some patients' samples refuse to behave as predicted, thus requiring the skills of the scientist to solve the riddle of, for instance, red blood cells not lysed efficiently. More is to be expected along these lines with the development of antibody cocktails adapted to specific disorders, where patients, in spite of all hope, cannot be standardized. What would happen, for example, in the case of double clones of lymphoproliferative disorders with an entirely automated system? Indeed, research is required in these fields to find the best compromise between time- and cost-saving robust methodology and hematological expertise in interpretation as well as "plan B" technology for unconventional cases.

Thus, research belongs at the heart of the hematology platform, dealing with the most widely prescribed blood test, CBC, and its flow cytometric corollaries.

But such a limited view would be far too reductionist. Indeed, both morphology and flow cytometry are also at the heart of clinical research. How would any clinical trial in malignant hematology be possible without an accurate diagnosis which initially relies on these tests? How would the hematological toxicity of sometimes daring/drastic therapies be interpreted without repeated CBC and blood and bone marrow cell examinations? How would the large amount of recently publicized[10] searches for fast chemosensitivity and high sensitivity minimal residual disease assessment be possible without flow cytometry? Indeed, although often not financed, these tests are at the core of evaluations in malignant, and also non-malignant, hematological diseases. The progress of clinical research in the field of myelodysplastic syndromes (MDSs) also rely on both morphology and flow cytometry. Indeed, a Perls prussian blue staining and microscopic evaluation is far easier and more broadly performed than the search for *SF3B1* mutations, even if the latter can be a surrogate classifier in cases where there are less than 5% ring siderob-lasts.[11] The growing literature underscores the value of searching for immunophenotypic anomalies in cytopenias with suspected MDS.[12]

In a broader sense of appreciation, it can also be pointed out that these "basic" methods are also extremely useful and necessary in more fundamental research. Examples are numerous, but some can be drawn from the latest meeting of the American Society of Hematology. In an exciting zebra fish model allowing for the manipulation of hematopoietic stem cells, Leonard Zon showed how the addition of mutations could indeed lead to the development of leukemia in these animals, as demonstrated morphologically by the clonal proliferation of leukocytes.[13] Similarly, in the Ernest Beutler lecture, Hugues de Thé and Zhu Chen reviewed how all-trans retinoic acid (ATRA) and arsenic can drive the leukemic cells of promyelocytic leukemia to proceed to morphological and immunophenotypic differentiation.[14] Even in the blossoming field of chimeric antigen receptor (CAR) T cells, the characterization of the most efficient cell products relies on flow cytometric assessment of their immunophenotype and pluripotency in chemokine secretion.[15]

Therefore, each year brings more knowledge and understanding in the complex world of hematology. Research progresses fast, using sophisticated new technology to unravel molecular mechanisms from bioinformatics analyses of huge sequences databases. Yet, in parallel, other methods are developed to get a better grasp on the "physical" structure/morphology of organelles such as ribosomes, i.e., with cryo-electron microscopy.[16] Meanwhile, classical methods retain their intrinsic value to check on the morphology of manipulated cells or to use antibodies to assess their immunophenotypic or secretory profile.

In the expanding field of hematological research, which apparently is still far from having unveiled all its idiosyncrasies, the importance of maintaining the competence of experts in morphology and flow cytometry appears to be as crucial as ever in the daily process of diagnosis.

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