Medicine and Cardiology Centre, University of Szeged, Hungary. His main field of interest is pathobiology and treatment of mantle cell lymphoma and multiple myeloma.

Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

References

1. Duyvené de Wit T, Hellström-Lindberg E, Jäger U, Evans-Jones G, Smand C. H-Net, the European Network for Harmonization of Training in Hematology, and its policy. Haematologica. 2012;97(12): 1776-8.
2. Ossenkoppele G, Evans-Jones G, Jaeger U, Hellström-Lindberg E; Curriculum Update Working Group. Towards a joint definition of European hematology. Haematologica. 2012;97(5):636-7.
3. Strivens J, Hellström-Lindberg E, Bjerrum OW, Toh CH. A European strategy for targeted education in hematology. Haematologica. 2013; 98(7):1000-2.
4. Ericsson KA. The road to excellence: The acquisition of expert performance in the arts, science, sports and games. Mahawah, NJ: Lawrence Erlbaum Associates; 1996.
5. Hanken IM. Teaching and learning music performance: The master class. The Finnish Journal of Music Education 2008;11:26-36.

Morphology and immunophenotyping issues in the integrated diagnosis of hematologic disorders of elderly patients

Gina Zini,[1] and Marie C. Béné[2]

[1]Università Cattolica, Rome, Italy; and [2]University Hospital and Faculty of Medicine, Nantes, France

E-mail: mariechristine.bene@chu-nantes.fr

doi:10.3324/haematol.2014.106724

In the middle of the 19th century, when Bennett and Virchow were trying to decide whether "leucocythemia" or "leukemia" would be the proper word to describe the recently discovered chronic myelogenous leukemia (CML), life expectancy was steadily rising from around 40 years of age in the previous century, to finally reach 50 years in 1900. This is to say that many hematologic disorders were extremely rare at that time. Nowadays, a newborn baby may expect to live up to 100 years old.[1] Among the myriad of challenges this perspective raises, that of an increase in chronic hematologic disorders is to be foreseen and in fact can already be perceived. Four major evolutions can be highlighted which will require the skill of trained morphologists and adapted flow cytometry studies, for integrated diagnoses and follow up, where cytogenetics has already an important place and where that of molecular and next generation sequencing (NGS) techniques will certainly find theirs. They are namely: i) nutritional deficiency-related and autoimmune disorders, mostly anemia;[2] ii) chronic myeloproliferative/myelodysplastic or lymphoid neoplasms; iii) therapy-related secondary leukemia/lymphomas; and iv) follow up of long-term survivors.

Nutrition is complicated for elderly people and related problems are often underestimated. Even when they are institutionalized in facilities where dietary concerns are taken care of, food intake is not necessarily well controlled in pre-senile or senile individuals. The issue is obviously even worse for people living on their own who sometimes have a low income or who fail to feed themselves properly. For Biermer disease or hemolytic anemia, the picture is made more complex by the increase in autoimmune diseases resulting from an aging immune system. As age and other diseases possibly find an equilibrium, several other conditions (renal failure, cardiovascular diseases) may lead to a decrease in hemoglobin levels associated with a variety of morphological anomalies of the erythroid lineage which have to be recognized.

Chronic proliferative diseases of the myeloid and lymphoid lineage will also likely be diagnosed with increased frequency in an aging population. Myeloproliferative neoplasms (MPN) are mostly diagnosed on the basis of increased blood counts in one or several lineages. Morphological examination of blood or bone marrow smears, together with cytogenetics findings, usually confirms the suspected diagnosis. Nowadays, molecular identification of *BCR-ABL* gene fusion, *Jak2* or calreticulin mutations can provide both the basis for the initiation of targeted therapy[3] and a means to follow minimal residual disease after allogeneic stem cell transplantation (alloSCT), now considered for fit patients who had previously been considered "old" only on the basis of their age.[4] Although immunophenotypic anomalies have been reported in these diseases, they are of little interest in the current management of such patients. Myelodysplastic syndromes (MDS), which are usually diagnosed by the discovery of one or several cytopenias around the age of 70 years, have long received only supportive care. The development of new drugs, likely to decrease transfusion dependency and its complications is changing this picture. Proper diagnosis and application of the International Prognostic Scoring System (IPSS) or the International Prognostic Scoring System Revised (IPSSR)[5] prognosis criteria require a combination of morphology and cytogenetics. In the past few years, the positive input of specific immunophenotypic exploration has been stressed by collaborative groups[6] and is now recommended in the most recent guidelines.[7] Therapeutic management of these diseases in elderly patients, however, raises a number of practical and organizational issues, not to mention the influence of comorbidities and an aged hematopoietic system possibly less and less responsive to stimulation.[8]

Chronic proliferations of the lymphoid lineage are also likely to cause greater concern. There remains uncertainty as to how to manage monoclonal B-cell lymphocytosis, diagnosed by cell count and immunophenotyping,[9] or low-grade chronic B-cell disorders such as marginal zone lymphoma[10] or hairy cell leukemia, even if the recent discovery of *BRAF* mutations targetable by vemurafemib provides a new opportunity for refractory patients, who are likely to be older.[11] Conversely, large trials and efficient therapeutic

approaches are now available for chronic lymphoid leukemia[10] and mantle cell lymphoma.[12] All these lymphoproliferative disorders rely heavily on immunophenotyping to characterize their lineage, apply proper scoring and define light chain restriction lineage.[13] These diseases are being diagnosed with increasing frequency, quite often fortuitously in elderly people hospitalized for a fall, a stroke or other unrelated reasons. Follow up after initiation of therapy is also increasingly benefitting from the efficient application of flow cytometry.[14]

In the WHO 2008 classification,[15] therapy-related myeloid neoplasms (t-MN) is a unique category including therapy-related acute myeloid leukemia (t-AML), t-MDS and t-MDS/MPN occurring as an evolution and/or a complication of cytotoxic chemotherapy and/or radiation therapy administered for a prior neoplastic or non-neoplastic disorder. They represent 10-20% of all hematologic malignancies. Transformation of MPN is definitively excluded from this category due to the lack of robust diagnostic criteria to identify a disease progression from a therapy-related neoplasm. In the 2008 WHO classification, the type of previous treatment is no longer considered, although it is suggested that information on previous treatment could be clinically useful. All subtypes of MDS and AML are reported as possibly therapy-related and, therefore, classical morphological and immunophenotypic diagnostic criteria apply.[13,16] Many patients will present in the stage of overt AML that differs from *de novo* AML primarily by the high incidence of trilineage involvement, difficulty in classification, frequent cytogenetic abnormalities and poor response to antileukemic therapy. The bone marrow is reported as hypercellular in up to 52% of patients, normocellularity or hypocellularity may occur at diagnosis, while marrow fibrosis is reported in approximately 15% of patients. Mild to marked dyserythropoietic changes are almost always present and ringed sideroblasts over 15% are reported in a high percentage of cases. Dysgranulopoiesis is almost always detected, while Auer rods are mainly observed in patients with t-AML. Myelodysplastic changes in megakaryocytes, variable in size and distribution, are very frequently detected. Increased bone marrow basophils and reactive plasmacytic infiltration are also reported in the literature. The prognosis appears to have little relationship to the stage of the disease; the morphological subclassification by the WHO guidelines of 2 subtypes of t-AML and t-MDS offers no prognostic information regarding disease progression or survival; morphological subclassification of t-MDS is not clinically useful for risk stratification whereas cytogenetic abnormalities are predictive of overall outcome. Immunophenotypic features are the same as for *de novo* AML. Simple and robust methods to measure sensitivity to chemotherapy could be useful if patients are fit enough to receive such treatment.[17] Depending on the age of onset, definition of "elderly" and fitness of the patients, alloSCT remains an option. Both after efficient chemotherapy or SCT, patients could benefit from follow up of minimal residual disease in flow cytometry.[18]

The issue of minimal residual disease is also finding new applications as the survival of patients with nearly all types of hematologic malignancies increases after therapy. The impressively high rate of prolonged complete remission in childhood acute lymphoblastic leukemia is probably one of the major breakthroughs of the 20th century allowing us to consider over 80% of the patients cured.[19] Although this is not the case in adults, improvements are also considerable in this population for most hematologic malignancies. In myeloma, for instance, the emergence of new drugs and associations has contributed to a significant improvement in survival in this disease.[20] In parallel, the introduction of targeted immunotherapy has revolutionized the management of patients with B-cell lymphomas.[21] These advances have two consequences. The first is that treated patients live longer and reach an older age where the surveillance of their disease becomes complicated by the morphological and perhaps immunophenotypic alterations related to age. The second is that patients who would not have been considered eligible for therapy are now enrolled in highly efficient clinical trials, and thus need to benefit from the best diagnosis available. In the past, such elderly patients who were ineligible for therapy would have only been minimally investigated and no follow up would have been considered.

In conclusion, increased survival of the general population is resulting in an increasing incidence of diseases formerly considered rather rare. Their diagnosis is not strikingly different from that of the same pathologies occurring at a younger age, and their management, although it must take care with regards to frailty and comorbidities, may also be similar to current approaches in fit patients. The development of more effective drugs will also increase the duration of follow up for patients with hematologic malignancies for whom it will be important to regularly verify that the disease remains under control. Such drugs are also making it possible to consider initiating therapy in elderly patients who had previously been considered only eligible for palliative therapy. The eternal human dream of immortality will remain a dream, but living longer with a decent quality of life, by mastering disease if it cannot be avoided, is steadily becoming more of a reality.

Gina Zini is Professor of Hematology, Head of the Blood Bank and Cord Blood Bank at the Catholic University, Rome, Italy. Marie Christine Béné is Professor of Hematology at the University Hospital and Faculty of Medicine, Nantes, France, Head of the Department of Hematology Biology. She is head of the European LeukemiaNet WP10 and the EHA Scientific Working Group (SWG), both on "Diagnosis: morphology and flow cytometry," co-chaired by Prof. Gina Zini.

Financial and other disclosures provided by the author using the ICMJE (www.icmje.org) Uniform Format for Disclosure of Competing Interests are available with the full text of this paper at www.haematologica.org.

References

1. Christensen K, Doblhammer G, Rau R, Vaupel JW. Ageing populations: the challenges ahead. Lancet. 2009;374(9696):1196-208.
2. Argento V, Roylance J, Skudlarska B, Dainiak N, Amoateng-Adjepong Y. Anemia prevalence in a home visit geriatric population. J Am Med Dir Assoc. 2008;9(6):422-6.
3. Breccia M, Tiribelli M, Alimena G. Tyrosine kinase inhibitors for elderly chronic myeloid leukemia patients: a systematic review of efficacy and safety data. Crit Rev Oncol Hematol. 2012;84(1):93-100.
4. Ramakrishnan A, Sandmaier BM. Optimizing reduced-intensity conditioning regimens for myeloproliferative neoplasms. Expert Rev Hematol. 2010;3(1):23-33.

5. Greenberg PL, Tuechler H, Schanz J, Sanz G, Garcia-Manero G, Solé F, et al. Revised international prognostic scoring system for myelodysplastic syndromes. Blood. 2012;120(12):2454-65.
6. Westers TM, Ireland R, Kern W, Alhan C, Balleisen JS, Bettelheim P, et al. Standardization of flow cytometry in myelodysplastic syndromes: a report from an international consortium and the European LeukemiaNet Working Group. Leukemia. 2012;26(7):1730-41.
7. Malcovati L, Hellström-Lindberg E, Bowen D, Adès L, Cermak J, Del Cañizo C, et al. Diagnosis and treatment of primary myelodysplastic syndromes in adults: recommendations from the European LeukemiaNet. Blood. 2013;122(17):2943-64.
8. Ria R, Moschetta M, Reale A, Mangialardi G, Castrovilli A, Vacca A, et al. Managing myelodysplastic symptoms in elderly patients. Clin Interv Aging. 2009;4:413-23.
9. Ghia P, Caligaris-Cappio F. Monoclonal B-cell lymphocytosis: right track or red herring? Blood. 2012;119(19):4358-62.
10. Shanafelt T. Treatment of older patients with chronic lymphocytic leukemia: key questions and current answers. Hematology Am Soc Hematol Educ Program. 2013;2013:158-67.
11. Machnicki MM, Stoklosa T. BRAF - A new player in hematological neoplasms. Blood Cells Mol Dis. 2014 pii: S1079-9796(14)00002-3. [Epub ahead of print]
12. Vose JM. Mantle cell lymphoma: 2013 Update on diagnosis, risk-stratification, and clinical management. Am J Hematol. 2013;88(12):1082-8.
13. Béné MC, Nebe T, Bettelheim P, Buldini B, Bumbea H, Kern W, et al. Immunophenotyping of acute leukemia and lymphoproliferative disorders: a consensus proposal of the European LeukemiaNet Work Package 10. Leukemia. 2011;25(4):567-74.
14. Rawstron AC, Böttcher S, Letestu R, Villamor N, Fazi C, Kartsios H, et al. Improving efficiency and sensitivity: European Research Initiative in CLL (ERIC) update on the international harmonised approach for flow cytometric residual disease monitoring in CLL. Leukemia. 2013;27(1):142-9.
15. Vardiman JW, Arber DA, Brunning RD, Larson RA, Matutes E, Baumann I, et al. Therapy-related myeloid neoplasms. In: Swerdlow H, Campo E, Harris NL et al. WHO Classifications of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC 2008.
16. Michels SD, McKenna RW, Arthur DC, Brunning RD. Therapy-related acute myeloid leukemia and myelodysplastic syndrome: a clinical and morphologic study of 65 cases. Blood. 1985;65(6):1364-72.
17. Lacombe F, Arnoulet C, Maynadié M, Lippert E, Luquet I, Pigneux A, et al. Early clearance of peripheral blasts measured by flow cytometry during the first week of AML induction therapy as a new independent prognostic factor: a GOELAMS study. Leukemia. 2009;23(2):350-7.
18. Freeman SD, Virgo P, Couzens S, Grimwade D, Russell N, Hills RK, et al. Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. J Clin Oncol. 2013;31(32):4123-31.
19. Silverman LB, Gelber RD, Dalton VK, Asselin BL, Barr RD, Clavell LA, et al. Improved outcome for children with acute lymphoblastic leukemia: results of Dana-Farber Consortium Protocol 91-01. Blood. 2001;97(5):1211-8.
20. Moreau P, Richardson PG, Cavo M, Orlowski RZ, San Miguel JF, Palumbo A, et al. Proteasome inhibitors in multiple myeloma: 10 years later. Blood. 2012;120(5):947-59.
21. McLaughlin P. Progress and promise in the treatment of indolent lymphomas. Oncologist. 2002;7:217-25.

Platelet count and aging

Carlo L. Balduini and Patrizia Noris

Department of Internal Medicine, University of Pavia - IRCCS Policlinico San Matteo Foundation, Pavia, Italy

E-mail: c.balduini@smatteo.pv.it

doi:10.3324/haematol.2014.106260

After Giulio Bizzozero identified platelets at the end of the 19th century,[1] many authors sought to define the normal concentration of these elements in human blood. However, methods to enumerate platelets remained inaccurate until the middle of the last century, and the proposed reference intervals for platelet count ranged from 130-350 to 500-900x10^9/L of whole blood.[2]

The development of the Coulter Principle in 1953[3] revolutionized blood counting and resulted in the development of the electronic instruments currently used in our laboratories. About thirty years ago, these instruments were used to study several thousands of blood samples of unselected donors thus defining the reference interval of platelet count as 150-450 or 150-400x10^9/L.[4,5] These values are still used today in most Western countries, although in the meantime several studies have indicated that platelet count varies according to age, sex and ethnicity. It is, therefore, appropriate to discuss whether a single reference interval for all people is still valid or whether new normal ranges taking into account these variables have to be used in clinical practice.

Aging and platelet count

The matter of age-related changes in platelet count was first examined in 1977 by Stevens and Alexander, who measured platelet count in 868 blood donors aged between 18 and 65 years and did not find any age-related differences.[6] In contrast, a progressive decline in platelet count with aging was shown a few years later in 477 ambulatory patients, with a difference of over 100x10^9/L between children aged 1-5 years old and seniors over the age of 71 years.[7] A correlation between platelet count and age was also found by a larger study that evaluated 12,142 adult inhabitants of the United States and found statistically significant differences between young and old individuals.[8] However, these differences were small: less than 30x10^9/L between people of 17-19 years and those over 70. Thus, until a few years ago, both the existence and the possible extent of age-related changes in platelet count were uncertain, but a series of cross-sectional studies performed recently in different Italian populations has definitively clarified this matter. Analysis of 12,517 inhabitants of Sardinian geographic isolates found that a 10-year increase in age corresponded to a 9x10^9/L decrease in platelet count.[9] Very similar results were obtained in 7266 inhabitants of five additional geographic isolates located in different Italian areas[10] and in the cohort of the Moli-Sani Project including 24,318 subjects from 30 Molise cities and villages.[11] Finally, a recent study put together all data of subjects enrolled in the three population-based studies referenced above and concluded that age-related changes were actually very large: platelet count in old age was reduced by 35% in men and by 25% in women with respect to early infancy.[12] As shown in Figure 1, most of this reduction occurred in childhood and in old age, with only minor changes in adulthood. Thus, there is no longer any doubt that age is a major determinant of platelet count in healthy people.

There is no proven explanation for these age-related changes, although it may be that the sharp decrease in