Morphology of Blood Disorders
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Second Edition

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Preface

This is a translation from the Italian of the second edition of a textbook on the morphology of the blood and bone marrow in blood diseases. Morphological details are supplemented by detailed descriptions of the output and role of automated instruments in disorders of the blood. Despite the enormous advances made in immunophenotyping and in cytogenetic and molecular aspects of haematology, morphology of the blood and marrow remains fundamental in haematological diagnosis. The authors are renowned in this field. I have translated the text into English so that their expertise can be shared with a wider audience.

Barbara J. Bain
London, January 2014
Biology of haemopoiesis

In the second half of the 19th century, developments in microscopy and in staining techniques permitted the observation of various types of blood cell, leading to a developing understanding of their heterogeneity, morphological characteristics and manner of proliferating and differentiating. The German pathologist, Neumann, recognised that the formation of blood cells from a single progenitor cell took place in the bone marrow. From this recognition, together with the work of the Russian scientist, Maximov, evolved the monophyletic or unitary theory of haemopoiesis, of which Adolfo Ferrata became one of the most convinced supporters (Fig. 1.1). It was the studies of McCulloch and Till, at the beginning of the 1960s, that provided the necessary experimental evidence with their demonstration of the capacity of bone marrow cells to form multilineage myeloid colonies in the spleen when injected into animals rendered aplastic by irradiation. This experiment was repeated in man with the first transplants of bone marrow between twins and into workers accidentally exposed to nuclear irradiation.

The blood, understood as both the haemopoietic matrix and the circulating cells, is a complicated tissue in which numerous elements are selected, organized and regulated like the instruments of a symphonic orchestra, to provide a harmonious, stable and effective result. The formation of the blood depends on the existence of a multipotent stem cell, recognised by its capacity to establish long-term cultures in vitro. This cell is at the top of a tree with increasing numbers of branches towards the base. The initial division is into two progenitors that differ in their potential, restricted respectively to lymphopoiesis and myelopoiesis. Successive branches give rise to other progenitors, organised in a hierarchy, with the capacity for differentiation becoming more limited and specific. This process culminates in the production of the population of morphologically recognisable haemopoietic cells of the bone marrow and ultimately of the mature elements that circulate in the peripheral blood.

Stem cells

Haemopoietic stem cells are multipotent and it is from them that all the circulating blood cells derive. Haemopoiesis is the well-organised and carefully controlled process by which this fundamental capacity is realised. The adjective ‘stem’ derives from the Latin stamen, meaning thread, to which, figuratively speaking, is bound the life of every man: the thread in classical mythology is spun and then cut off by the Fates. The stamen in botany, moreover, is the flower’s male organ. While this concept was used in the 19th century in Darwin’s evolutionary theory to indicate the unicellular ancestor of all living creatures, it was only at the beginning of the 20th century that the term ‘stem cell’ was attributed to the common precursor of all the cells of the blood.

The haemopoietic stem cells have two essential properties (Fig. 1.2):

- They are multipotent, that is capable of differentiating into all the various types of blood cell; this differentiation is associated with the loss of multilineage potential and the formation of committed haemopoietic progenitors;
- They are capable of indefinite self-renewal, maintaining themselves through many generations as a small constant percentage of daughter cells with their multilineage potential conserved.

The maintenance of stem cell numbers is necessary to maintain effective multilineage haemopoiesis for the life of an individual. In stable conditions (homeostasis), the majority of the rare mitoses seen in stem cells are symmetrical, producing two identical daughter cells, which are destined to give rise to differentiated progeny. The maintenance of a stable number of stem cells is achieved by the simultaneous occurrence of a more limited number of asymmetric divisions, in which a single stem cell produces two non-identical daughter cells, one of which maintains the original multipotent capacity while the other takes the path of differentiation. This asymmetric mitosis could be predetermined at the moment of mitosis, as is suggested by the polarized distribution of regulatory molecules, messenger RNA and individual proteins, which are not distributed equally between the daughter cells. In other instances, the factors determining that one of the daughter cells takes the pathway of differentiation could reside in the microenvironment.

The multipotent long-term culture initiating haemopoietic stem cells (LT-HSC) can be identified and enriched in the
capacity of stem cells can be demonstrated by culture, i.e. they can give rise to long-term cultures in vitro. The morphology of these forms is similar to that of small blast cells, with a very high nucleocyttoplasmic (N:C) ratio, scanty weakly basophilic cytoplasm and homogeneous nuclear chromatin, which is fairly compact (Figs 1.3, 1.4 and 1.5). In some cells the cytoplasm is more abundant and basophilic. The nucleus can have visible nucleoli of variable prominence.

The haemopoietic niche and the microenvironment

Stem cells and haemopoietic progenitors, from birth onwards, are located in the bone marrow where, in stable conditions, they divide very slowly (less than 10% enter cell cycle each day), with a delicate balance maintained between self-renewal, differentiation and quiescence. Alterations in the regulation of this carefully balanced system underlie the majority of haemopoietic neoplasms.

Stem cells self-renew and differentiate into progenitors and precursors at specific sites in the medullary cavity. These sites are known as haemopoietic niches. It is possible that there are different types of haemopoietic niche. They can be located close to the endosteum, which covers the trabecular surface of the bones, where the major influence on them is that of the osteoblasts, derived from mesenchymal stem cells, or alternatively in a perisinusoidal position, in the centre of the intertrabecular space, where the major influence is that of the endothelium.

The stem cell niche can be defined as a localised microenvironment with optimal conditions for self-renewal. They are sites where largely quiescent stem cells accumulate and are maintained in a dormant state, i.e. differentiation is inhibited, by being anchored to the osteoblasts that surround the niche by adhesion molecules (N-cadherin, integrin). In the presence of external stimuli, such as those produced by blood loss or growth factor therapy, the interaction with signalling molecules and the resultant modification of transcription factors and other cytoplasmic proteins favour the re-awakening of the dormant cells and their entry into the cell cycle. The ultimate result is the reconstitution of the integrity of the body’s supply of mature blood cells.

The stem cell niche has a heterogeneous cellular composition, including not only differentiating haemopoietic cells and macrophages, the latter also derived from haemopoietic stem cells, but also numerous stromal elements. The osteoblastic niches contain osteoblasts, fibroblasts, osteoclasts, neurons of the sympathetic nervous system, and even mesenchymal stem cells, which give origin to cells of the bone and connective tissue and are implicated in maintaining the migration of the haemopoietic stem cells. The perisinusoidal niches contain primarily endothelial cells and reticulum cells, in addition to numerous megakaryocytes.

Stem cells are characterised by striking mobility: they are capable of repeatedly entering, exiting and re-entering the haemopoietic niches in response to specific signals. This phenom-
Fig. 1.2 Schematic diagram of the haemopoietic compartments and their principal functional characteristics. Cells of the multipotent stem cell compartment have the maximum self-renewal capacity, proliferate quite slowly and only show differentiation to a minimal extent. Cells of the compartment of haemopoietic progenitors, while maintaining the morphological uniformity of the undifferentiated cells, are more heterogeneous and organised in a hierarchical manner (see Fig. 1.6): the capacity for self-renewal is reduced, while the cells proliferate and differentiate. The cells of the maturing-proliferating compartment, which correspond to the morphologically recognisable multilineage haemopoietic cells of the bone marrow, have lost the capacity for self-renewal. From this compartment originate the mature cells that pass into the peripheral blood: these are destined to carry out their functions without dividing again (with the exception of the lymphocytes).

Fig. 1.3 The haemocytoblasts of Ferrata (original illustration from Ferrata A, Le emopatie, 1933). The cells numbered from 8 to 10 particularly evoke the morphology and size of stem cells and haemopoietic progenitors as one recognises them today (see Figs 1.4 and 1.5). The other cells are more similar to myeloblasts, with an angulated nuclear outline (e.g. 1 and 2), or lymphoblasts.

Fig. 1.4 Morphology of haemopoietic stem cells. (A) Cytocentrifuge preparation of mononuclear cells harvested for peripheral blood stem cell transplantation on a cell separator (stem cell apheresis) from a donor treated with granulocyte colony-stimulating factor (G-CSF). There are numerous monocytes, some partly vacuolated neutrophils, a metamyelocyte on the lower edge and, just to the left of the centre, an undifferentiated haemopoietic progenitor, which has a high nucleocytoplasmic (N:C) ratio and the appearance of a small blast cell; it has dispersed chromatin, small nucleoli and a thin rim of basophilic agranular cytoplasm, which has a pale paranuclear area. (B) Haemopoietic stem cells, CD34 positive, harvested by stem cell apheresis and purified by an immunomagnetic method. The nucleus occupies nine tenths of the cell and has dispersed homogeneous chromatin; the top right cell has a visible nucleolus. The cytoplasm shows no features of differentiation.
The haemopoietic progenitors were initially given the generic designation ‘colony-forming units’ (e.g. CFU-S, the cells first described by McCulloch that give rise to spleen colonies). It is now known that each progenitor cell stage corresponds to an individual cell type, which has specific immunophenotypic and cultural characteristics, and can give rise to a well-defined limited repertoire of mature cells. At each stage, each cell undergoes multiple cellular divisions in the course of differentiation, thus amplifying the number of progenitors and, in turn, the number of haemopoietic precursors. Furthermore, with each transition from one stage to the next, a complex series of molecular events further restricts the capacity for differentiation. Thus, the first progenitors still have multilineage potential, albeit confined to the lymphoid (common B and T progenitor) or myeloid (CFU-GEMM; granulocyte, erythroid, macrophage, megakaryocyte) lineage. The CFU-GM, in turn, gives rise to progenitors with bilineage potential (CFU-GM for granulocytes and monocytes/macrophages; CFU-ME for megakaryocytes and erythroid cells). In the final stage, unilineage progenitors develop from the CFU-GM and CFU-ME; in appropriate cultural conditions, these can produce colonies composed of morphologically recognisable haemopoietic cells of a specific lineage. Each type of progenitor is controlled by glycoprotein growth factors binding to surface membrane receptors.

Haemopoietic progenitors

The term ‘progenitor’ refers to cells derived directly from haemopoietic stem cells and morphologically similar to them, but which have taken the first steps in differentiation (commitment) and are thus directed towards the production of an increasingly more limited range of mature haemopoietic cells (Fig. 1.6).

Erythroid progenitors

Cells that form pure erythroid colonies arise from the common myeloid progenitor, the CFU-GEMM, by means of an intermediate stage with bilineage erythroid/megakaryocyte potential (the CFU-ME; also known as the megakaryocyte-erythroid progenitor [MEP]). These erythroid-committed progenitors have

Fig. 1.5 Haemopoietic stem cells and progenitors from cord blood. (A) Cytocentrifuge preparation of cord blood stem cells after enrichment by magnetic immunoselection on a column using anti-CD34. The morphological features are fairly uniform: all the cells are small to medium sized and lack any morphological signs of differentiation. (B) Morphological differentiation of the same cord blood stem cells after two weeks in semiliquid culture in the presence of G-CSF. The majority of the cells now have cytoplasmic granules, indicating neutrophilic differentiation. Top left, a cell in anaphase. The most heavily granulated cell also shows chromatin condensation, with the morphological features of a promyelocyte or neutrophil myelocyte.
not been identified morphologically, but by their capacity to produce, in vitro, in the presence of the growth factor erythropoietin, clonal colonies composed of erythroblasts capable of synthesising haemoglobin (see Chapter 2). Two different types of erythroid progenitor can be identified, representing successive maturation stages:

- Burst-forming unit erythroid (BFU-E): these give rise in 14–16 days to large ‘explosive’ colonies, which have small numbers of erythropoietin receptors on their surface membrane; the morphology of these cells resembles that of very immature blast cells, with moderately basophilic cytoplasm, sometimes with pseudopods, and very delicate nuclear chromatin with visible nucleoli;

- Colony-forming unit erythroid (CFU-E): in about a week these give rise to small colonies of 16 or 32 haemoglobinised erythroblasts; these cells are rich in erythropoietin receptors and are now close to becoming proerythroblasts, even if their morphology is still that of an immature cell with delicate nuclear chromatin, a high N:C ratio and basophilic cytoplasm with pseudopods. On electron microscopy these cells can be seen to have many mitochondria and pinocytotic vesicles.

**Megakaryocyte progenitors**

These originate from the common megakaryocyte-erythroid progenitor (MEP) which, like the stem cell, has no defining morphological features, has receptors for growth factors of both lineages and is starting to show weak expression of CD41, CD61 and CD42. Two types of progenitor have also been identified for the megakaryocyte series, both of which have unilineage differentiation potential:

- BFU-MK, which in 21 days form colonies of hundreds of cells.
- CFU-MK, which in 10–12 days form colonies of 3–50 mature megakaryocytes.

These cells are CD34 negative and have specific markers of the megakaryocyte lineage. The main growth factor for megakaryocytes is thrombopoietin, which binds the receptor encoded by the MPL gene. MPL is present and active on megakaryocyte progenitors: it is a 70-kDa glycoprotein that is similar to erythropoietin. Numerous other cytokines have a positive effect on megakaryocytopoiesis (interleukin [IL]-3, IL-6, IL-11, granulocyte-macrophage colony-stimulating factor [GM-CSF], stem cell factor [SCF], leukaemia inhibitory factor [LIF]), while transforming growth factor (TGF) beta, interferon alpha and the proteins of platelet granules (PF4, beta-thromboglobulin) have, conversely, inhibitory effects.

**Granulocyte and monocyte/macrophage progenitors**

The differentiated progenitors of neutrophil granulocytes (CFU-G) and monocytes/macrophages (CFU-M) originate from a common precursor (CFU-GM), which in its turn has differentiated from a CD34-positive haemopoietic stem cell. The growth factors for these lineages are glycoproteins, which interact with specific receptors on the various committed progenitors: GM-CSF stimulates the production of mixed colonies;
G-CSF leads to the production of granulocyte colonies containing myeloblasts, promyelocytes and more mature granulocytes; M-CSF induces the formation of macrophage colonies. These glycoproteins are widely used in therapy.

The principle growth factor for eosinophil progenitors is IL-5. Progenitors of basophil granulocytes and mast cells also originate from a CD34-positive haemopoietic stem cell. They differentiate along two distinct maturation pathways, influenced by multiple and different growth factors (SCF, IL-3, IL-6, GM-CSF). According to recent investigations, there may also exist a common basophil-megakaryocyte progenitor; a possible hybrid eosinophil-basophil progenitor has also been described.18

**Lymphocyte progenitors**

The production of diverse populations of T and B lymphocytes is a complex phenomenon, which starts with the first lymphocyte-committed progenitor and continues in the primary and secondary lymphoid organs. The differentiation of stem cells and lymphocyte progenitors is directed by their interaction with stromal cells and with appropriate growth and differentiation factors. Development into T lymphocytes occurs in the microenvironment of the thymus and into B lymphocytes in the microenvironment of the bone marrow. The differentiation of B lymphocytes occurs in two phases. The first phase is antigen independent and involves the differentiation of stem cells and progenitors into pro-B progenitors and then into immature B cells, which express immunoglobulin M. The second phase is antigen dependent and involves the transformation of an immature B cell into a plasma cell. The production of T lymphocytes starts with the migration of a subpopulation of common lymphoid progenitors into the thymus: there they differentiate into multipotent T-cell progenitors from which in turn develop CD4-positive and CD8-positive subpopulations. One of the functions of the Notch receptors, which belong to a family of surface membrane proteins with an intracellular domain, is a key role in the development of T and B lymphocytes, acting directly at the level of transcription of specific genes. Their interactions with various specific molecules have a determining effect, particularly in the early phase of T-cell maturation and in the regulation of the T-cell immune response.19

The study of surface markers is fundamental in following the stages of maturation of the lymphocyte subclasses.

**Technical aspects of haematological cytology**

The biological and morphological study of blood cells is facilitated by the ease with which a blood sample can be obtained by venepuncture. In addition, the aspiration of haemopoietic tissue, with its complex and fascinating heterogeneity, from the bone marrow cavity is a minimally invasive biopsy procedure.

Morphological analysis is based on microscopy of stained films of blood and bone marrow. The techniques for making these haematological preparations are widely known and the reader is referred to texts on haematological techniques for the technical details.20,21 The interpretation of morphology, however, requires knowledge of certain basic methods, including the correct manner of preparing a film, suitable staining methods to demonstrate cellular structures optimally and the most efficient methods of microscopic examination.

**Peripheral blood film**

**Preparation of the film**

For making a blood film the most widely practised technique is that of making a film on a glass slide using either a drop of capillary or venous blood without anticoagulant or a drop of EDTA-anticoagulated venous blood from a specimen tube: the latter method has the advantage of avoiding platelet aggregation, while blood films of non-anticoagulated blood are optimal for the observation of erythrocyte morphology. The film can be made using a cover-slip that is narrower than the glass slide itself22 (all photographs of peripheral blood films that appear in this book have been prepared using this simple method). Automated instruments are available to make blood films using the double slide technique: in general, these provide good quality films in a reproducible manner. Alternatively the spreading of a blood film can be automated using an instrument based on centrifugation: the films thus obtained are more homogeneous with regard to thickness and the distribution of cells, but the technical problems related to the dispersion of aerosols means that they have not been widely employed.

For blood specimens from patients with leucopenia, and also when searching for pathological cells present in low numbers, it is useful to make a film from a drop of blood taken directly from the white cell layer formed after centrifugation, i.e. from the buffy coat. In these samples the haematocrit is very high and can be reduced by diluting the sample in a physiological solution or, better, in compatible plasma of blood group AB.20 Another method of leucocyte enrichment is cytocentrifugation of the mononuclear cell fraction that has been separated on a Ficoll-Hypaque density gradient: this method can increase the sensitivity of a search for pathological erythroblasts, lymphoma cells and blast cells of various types.23

The films must be left to dry in air. Subsequently, they can be fixed in methanol or stained directly with an alcohol-based stain.

**Staining**

The staining that is practised in haematology laboratories represents the perfecting of the mixture of acid and basic dyes that Paul Ehrlich first developed and which enabled him, in 1879, to publish the first detailed description of the different types of circulating leucocytes and their precursors in the bone marrow.

Ten years later, the Russian protozoologist, Romanowsky, whose work was aimed principally at visualising intra-erythrocytic malaria parasites, mixed methylene blue and eosin to obtain the
chromatic results that still carry his name (the Romanowsky effect). These two dyes, which are respectively a deep blue and an intense orange, when applied to a biological substrate produce a typical chromatic spectrum with these two colours at the extremes as well as a range of blues, reds and subtle intermediate colours, depending on the composition and the physicochemical characteristics of the cellular constituents. The dyes used most widely today in Europe (May-Grünwald-Giemsa) and in the United States of America (Wright) are an aqueous or alcoholic mixture of two stains or groups of stains: polychromed methylene blue and eosin Y. The first, which is a mixture of various oxidation derivatives of methylene blue, is basic, cationic; its fundamental active component is azure B, the colour of which is seen in almost pure form in the strongly basophilic cytoplasm of plasma cells or immature erythroblasts. Eosin Y is acidic, anionic, and its intense orange colour is seen in almost pure form in the granules of eosinophils.

The staining of cellular structures is a physicochemical phenomenon that depends on the interaction of the cationic molecule of azure blue with acidic basophilic substances such as cytoplasmic RNA, and on the interaction of the anionic eosin molecule with basic acidophilic substances such as the major basic protein of the eosinophils granules or the haemoglobin of the erythrocytes. The Romanowsky effect, that is, the typical colour, neither blue nor orange, of individual cellular components, such as the nucleus, depends on the combined complex chemical interaction of the two stains with cellular structures. In the nucleus, for example, the azure B molecule binds to the phosphoric groups of DNA, while the eosin molecule binds to cationic sites on nuclear proteins, together giving the reddish purple colour characteristic of chromatin. Basophil granules, however, show the phenomenon of metachromasia, in which binding to a blue dye, such as azure B or toluidine blue, conveys a different colour to the structure with which it has affinity, such as a very dark red, almost black, with azure blue staining or a vivid red with toluidine blue. The primary granules of the neutrophil series show a typical metachromatic affinity for azure B, which gives them a very intense vivid red colour: they are therefore designated azurophilic.

The results with various manual staining techniques are poorly reproducible, particularly because of the variable composition of different batches of commercially available stains. The International Council for Standardization in Haematology (ICSH) has proposed a reference staining method based on the use of purified forms of azure B and eosin Y. This method provides excellent results both with regard to quality and reproducibility.

Nowadays numerous film staining instruments are available, which in general produce good quality staining, even if the heterogeneity of the results makes it imperative for the user to carry out a preliminary evaluation of the chromatic range and the appearance of fine cytological details (chromatin network, cytoplasmic granules) in comparison with the manual method previously in use.

Microscopic examination

Naked eye analysis of a stained blood film permits the detection of technical imperfections and also irregular distribution of cells, such as that due to erythrocyte agglutination. Staining anomalies, such as increased blue staining attributable to a marked increase in the concentration of basophilic plasma proteins or the reddish colouration of a specimen that has been taken into heparin, can also be detected. The presence of small particles resembling small bone marrow particles in the tail or the body of the film may be the result of cryoglobulin precipitates, gross red cell agglutination, clumps of white cells and platelets or, exceptionally, the presence of large masses of neoplastic cells.

Blood films are generally observed at medium power (×20, ×25, ×40 or ×50 objective combined with ×8 or ×10 eye pieces). A quick but careful scan of the total film is particularly useful for choosing the optimal area to carry out a white cell differential count, this being the zone where the red cells touch each other without being superimposed, where morphology is optimal (Fig. 1.7). In well-made films, the distribution of white cells is uniform, with crowding confined to the tail and the edges, which is the area where neutrophils and monocytes tend to be most numerous. Observation at low or medium power also permits the detection of artefacts and anomalies of a generalised nature (Fig. 1.8), such as increased background staining and the presence of precipitates linked to hyperproteininaemia or the presence of a paraprotein (Figs 1.9; see also Figs 2.7, 9.1, 9.2 and 9.3) or cryoglobulin (Figs 1.10 and 1.11), platelet agglutination (Fig. 1.12; see also Fig. 1.27), leucocyte aggregation (Fig. 1.13) or red cell agglutination (see Fig. 2.54); a tendency to red cell stacking (rouleaux formation) (see Figs 2.7, 2.55 and 9.1); or the presence of fibrin strands (Fig. 1.14).

The peripheral blood film and automated instrument analysis of blood cells should be seen as complementary investigations (see Report 1.1).

Bone marrow aspirate

Morphological examination of a bone marrow aspirate remains even today the most important means of evaluating the function of the bone marrow and anomalies of haemopoiesis. According to a classical, but still valid, affirmation, it takes second place only to examination of the peripheral blood.

The bone marrow of an adult constitutes 4.7 ± 1.3% of total body weight (equivalent to 1.5–3.7 kg). The red or haemopoietic marrow, which represent about a quarter of this volume (500–700 g), is principally located within the central axial skeleton, in the flat bones and in the epiphyses of the long bones. Its structure is a network of trabecular bone lined by endosteum (formed from a single layer of flattened cells, associated with osteoblasts and osteoclasts), which crosses and delimits a cavity that is rich in sinusoids, reticular stoma, fat cells and small islands and cords of haemopoietic cells. There are around 10^12 haemopoietic cells throughout the body (0.4 × 10^12 erythroblasts; 0.5 × 10^12 granulocytic series; 1 × 10^9 megakaryocytes). The reticular cells
Fig. 1.7 Morphology of peripheral blood films according to the area of the film selected for examination. (A) Optimal distribution for observation of cytological details. The red cells show minimal superimposition and their morphology appears well preserved. The white cell characteristics are clearly displayed: the nuclei of the three neutrophils on the right, for example, do not show any superimposition of lobes and their granules are clearly seen; the monocyte at bottom left also does not show any distortion. The platelets are separated from each other and well distributed. (B) A thinner area in which the morphology of the erythrocytes is partly altered (there are some echinocytes). The white cells (from the left, a neutrophil with a small nuclear appendage, a lymphocyte with plentiful cytoplasm and a monocyte with a number of small vacuoles) appear well spread and their characteristics are recognisable. The platelets, which appear hypochromatic, are clearly seen. (C) A thicker area of the film. The red cells are stacked and their morphological characteristics cannot be readily identified. The platelets are numerous and hyperchromatic. The three granulocytes appear compressed, but are still readily identifiable because of the characteristics of their granules: from the left, an eosinophil, a neutrophil and a basophil.
Fig. 1.8 Salivary material in a peripheral blood film. This artefact is caused by a cough, a sneeze or some other vigorous respiratory activity on the part of the operator during the spreading of the film. (A) Low power: there is the appearance of a pseudocyst, with a pale circumference and within this, superimposed on the red cells, which are still visible because the overlying cells are transparent, are numerous large cells with dark nuclei and plentiful pale thin cytoplasm. (B) In this case, at higher power, proteinaceous material representing mucus is visible within the pale circle; the background red cells are not visible. There are numerous lymphocytes, visible as hyperchromatic nuclei, and large epithelial cells of oral mucosal origin; the latter are polygonal with a central dark round nucleus. (C) The epithelial cells desquamated from the oral mucosa of the operator making the film may be seen as isolated cells, as in this case. (D) This epithelial cell from the operator’s oral mucosa, which has contaminated the peripheral blood film, has its cytoplasm invaded by numerous cocciform bacteria, sometimes forming short chains.

Fig. 1.9 Artefact due to precipitation of protein in the peripheral blood film. (A) Cloudy violaceous masses with indistinct margins in a patient with polyclonal hypergammaglobulinaemia. (B) Bow- or comma-shaped protein deposits in a patient with hepatic cirrhosis and dysproteinaemia.
Fig. 1.10 Amorphous cryoglobulin deposits in a peripheral blood film. (A) Coalescent globular masses of amorphous pink material, with partly adherent peripheral platelets. There is a normal appearing lymphocyte. (B) A neutrophil granulocyte has phagocytosed a spherule of precipitated cryoglobulin. The cytoplasm is heavily vacuolated. (C) Top left, satellitism of platelets around an amorphous deposit of cryoglobulin. Bottom right, a monocyte with plentiful heavily vacuolated cytoplasm.

Fig. 1.11 Crystalline cryoglobulin deposits in peripheral blood films. (A) Crystals of various shapes, some refractile. (B) Small crystalline precipitates above and below a monocyte in the blood film of a different patient.
Fig. 1.12 Platelet agglutination. Bottom right, a small mass of platelets, which appear to have adhered to each other, although the individual platelets are still easily discerned. This appearance, which can be associated with a factitiously low platelet count and a ‘flag’ on automated instruments, is typical of pseudothrombocytopenia caused by EDTA-dependent platelet antibodies. On the left, a neutrophil band form.

Fig. 1.13 White cell agglutination in peripheral blood films. (A) This large agglutinate includes two monocytes, in addition to numerous very granular neutrophils. The platelets, clearly visible on the right, appear hyperchromic and heterogeneous in size: one giant platelet is visible towards the lower pole of the agglutinate, immediately above a small lymphocyte. (B) A large agglutinate of neutrophils, top left. A smaller agglutinate of platelets is seen near the bottom right corner. (C) Two small agglutinates of very granular neutrophil band forms. The individual cells seem to have adhered over a limited part of the cell circumference, which is straight in the area of contact. Cell morphology is well preserved. (D) Small flower-like aggregate of lymphocytes in an HIV-positive patient, in the absence of a lymphoproliferative disorder. The cells appear to have adhered at their poles.
spread their long cytoplasmic processes over the internal surfaces of the trabeculae and over the adventitial tissue that invests the sinusoids.

**Obtaining a bone marrow aspirate and preparing films**

The aspiration of bone marrow is usually performed, with informed consent, using an appropriate single-use needle, from the posterior superior iliac spine. This route of access to the marrow cavity allows a second needle to be immediately inserted to obtain a core biopsy of bone and bone marrow with a low frequency of complications and minimal inconvenience to the patient. Other sites can be used in particular cases, such as the sternum in obese patients and in those who have had pelvic irradiation or who are immobile, or the vertebral spinous processes or the proximal epiphysis of the tibia in children.

A high quality bone marrow aspirate requires that dilution of the sample by peripheral blood be kept to a minimum; this dilution increases in proportion to the volume of the aspirate. For this reason the maximum volume of fluid that should be aspirated must not be more than 0.2–0.3 ml. If it is necessary to have more material for further investigation, such as immunophenotyping or cytogenetic analysis, a second sample should be taken into a separate syringe.

There are numerous techniques described for dealing with aspirated bone marrow. The most widely employed, and the one used in the laboratory of the authors, is that described in the classic manual, *Dacie and Lewis Practical Hematology*, which is based on a film being made from a drop of particulate aspirate, after having disposed of surplus fluid by inclining the slide. The fragments of bone marrow are dragged along the glass slide, depending on their dimensions and density, to the tail of the preparation, leaving behind them cell-rich trails, which are optimal for microscopic examination. The quantity and quality of cells suitable for examination in these preparations can be increased by the delicate apposition and spreading of isolated particles, gathered with forceps or with the angle of a cover-slip, and placed on another slide. An alternative equally valid technique in expert hands and recommended for the quality of the cytological detail and for the absence of peripheral blood dilution, is based on the crushing of fragments between a slide and a cover-slip.

The methods used for staining bone marrow films are not dissimilar to those used for staining blood films. The stained films can be protected by permanent mounting of a suitable cover-slip. In addition to panoptic staining such as May-Grünwald-Giemsa, it is useful to routinely perform an iron stain using the Perls’ method. Cytochemical stains such as peroxidase, esterases, toluidine blue and periodic acid-Schiff (PAS) can be reserved for specific indications in selected cases.

**Pre-microscopic and microscopic examination**

Evaluation of a bone marrow aspirate must follow a systematic approach, which permits an assessment of the quality of the preparation and helps to validate the conclusions that are reached. Evaluation involves a pre-microscopic phase, which includes the acquisition of clinical information, noting the diagnostic question that is being asked, an assessment of potentially relevant therapeutic information (chemotherapy, iron, vitamins, growth factors, corticosteroids), examination of the full blood count and a peripheral blood film, consideration of information gained at the time of obtaining the sample (such as reduced or increased hardness of the bone), and the results of physical examination (such as the presence of splenomegaly or cutaneous lesions).

Examination of bone marrow films must first be done at low power (×10 objective). These important preliminary procedures guide the subsequent morphological assessment, which uses an approach that is both rigorous and systematic (Fig. 1.15):

1. Evaluation of whether or not the sample is representative: a bone marrow film can be considered representative of the actual cells in the marrow if fragments are present, if the films have been prepared in the correct manner and are suitable for cytological assessment, and if the majority of cells appear intact (Fig. 1.16).

2. Semiquantitative evaluation of cellularity, in relation to the stroma and fat cells in the particles (Figs 1.17 and 1.18). A correct estimate of cellularity is dependent on careful consideration of many factors (the quality of the material, adequacy of the aspirate, suitability of the films and age of the patient). This initial assessment is fundamental for reaching the desired objective, a valid microscopic assessment. The evaluation is subjective and difficult, but is effective. It does not provide a numerical estimate of cellularity (which is achieved by histological sections), but nevertheless can be expressed in seven semiquantitative descriptive terms (normal; slightly reduced, moderately or markedly reduced; slightly increased, moderately or markedly increased).

![](Fig. 1.14 Fibrin strands in a peripheral blood film.)
**Fig. 1.15** Schematic representation of the process of evaluating bone marrow aspirate films. The performance of a differential count (a myelogram) is obligatory only when particles are present, when the quality is acceptable and when the number of cells is sufficient. In other cases, if the presence of megakaryocytes and haemopoietic precursors indicates that the material is bone marrow, it is possible to make a qualitative evaluation.

**Fig. 1.16** Fragments or particles of marrow in bone marrow aspirate films, low power. (A) The fragment has been dragged along the slide, using a spreader at an angle of 45°, to the distal extremity or tail of the film. The particle visualised here is compact and very cellular. The area of choice for cytological evaluation at higher power is where cells have been left behind the fragment as the film is spread: in this case, top right. (B) In this small fragment, fat cells predominate and surround a dense mass of unidentifiable material. The cellularity of the preparation, judging from the trail behind the fragment, appears low.
3. Cytological assessment of large cells present in small numbers: presence, number, disposition and morphological characteristics of megakaryocytes (Fig. 1.18); identification of other large cells that are easily recognisable, which can be normal marrow constituents, such as osteoblasts and osteoclasts, plasma cells or mast cells, or groups or clumps of cells, which in their turn may be normal, reactive or neoplastic (Fig. 1.19).

4. Selection of the best and most representative area for high power examination: the choice is necessarily usually the trails of cells left behind a particle. The fundamental principle is that the area selected must be where the cells are regularly distributed, close together without being superimposed, with optimal preservation and visibility of cellular structures, and without an excess of damaged cells. Diagnostic errors, sometimes serious, can result from an inadequate cytological evaluation of a non-ideal preparation or a non-ideal area of the film, in which it is impossible to appreciate fine details such as chromatin structure, nucleoli or the characteristics of granules (Fig. 1.20).

Diagnostic cytohaematology is based essentially on observation at high power with an oil immersion objective (×50 and ×100). A qualitative morphological analysis of the cellular characteristics is normally accompanied by a quantitative estimate of the proportion of cells belonging to the various haemopoietic lineages plus an assessment of the proportion showing specific
**Fig. 1.18** General evaluation of cellularity and identification of large cells in an optimal area of bone marrow films; low to medium power.

(A) Preparation with optimal cellularity, distinguished by heterogeneous differentiation with all stages of maturation being present. One observes, in particular, the predominance of granulocyte precursors, with a reduced proportion of mature neutrophils (a useful observation for excluding dilution by peripheral blood, which here is minimal). At bottom right and bottom left are mature megakaryocytes with well-lobated nuclei; in the case of the cell on the right, the nucleus is probably hyperlobated: this is potentially a dysplastic feature which requires verification by examining a larger number of megakaryocytes, as discussed in Chapter 3.

(B) Hypercellular film in which the cytomorphological feature are well preserved. In this case erythroid cells predominate in all areas of the field; they show normal maturation with good representation of all maturation phases. Mature neutrophils are relatively less numerous, guaranteeing that dilution by peripheral blood is minimal. The large megakaryocyte shows plentiful, fully mature cytoplasm which is ready to form platelets, the granules being dense and numerous demarcation zones becoming apparent. The nucleus is multilobated and hyperdiploid, as is usual, with a rounded detached lobe in the lower left part of the cytoplasm: slight suggestions of dysmorphism of this type are observed in a reasonable proportion of normal subjects.

(C) A very cellular and heterogeneous marrow, with granulocytic and erythroid precursors showing well-preserved maturation. The violaceous filaments visible at the top and at the bottom are composed of nuclear material derived from cells that were damaged during the spreading of the film. No megakaryocytes are seen but there are numerous masses of well-formed platelets.
roblasts (the myeloid:erythroid or M:E ratio) varies in normal subjects from 1.5 to 4.5; lower values suggest erythroid hyperplasia or granulocytic hypoplasia, while higher values have the opposite significance. In addition to determining the percentage of cells of various haemopoietic lineages and the M:E ratio, classical evaluation of the marrow required assessment of various quantitative parameters which are now little used. The execution of the so-called maturation curve can occasionally give useful information: it permits an evaluation, with the help of a graphical representation, of the various maturation phases of a specific haemopoietic lineage, with a shift to the left indicating the presence of an increased proportion of less differentiated anomalies, such as dysplastic features. A differential count – a myelogram (Tables 1.1 and 1.2) – can be performed in various ways. Assuming that there is a good quality preparation (presence of fragments and adequate cellularity), the percentage of cells in various categories, including mature cells, such as segmented neutrophils, can be calculated: this procedure avoids arbitrary modifications of the evaluation and permits an assessment, based on the percentage of mature neutrophils (which generally is not greater than 10–15%), of the representative nature of the aspirate. It is necessary to count at least 500 cells, using two different films. On this basis the relationship between total cells belonging to the granulocytic series and total erythroblasts (the myeloid:erythroid or M:E ratio) varies in normal subjects from 1.5 to 4.5; lower values suggest erythroid hyperplasia or granulocytic hypoplasia, while higher values have the opposite significance. In addition to determining the percentage of cells of various haemopoietic lineages and the M:E ratio, classical evaluation of the marrow required assessment of various quantitative parameters which are now little used. The execution of the so-called maturation curve can occasionally give useful information: it permits an evaluation, with the help of a graphical representation, of the various maturation phases of a specific haemopoietic lineage, with a shift to the left indicating the presence of an increased proportion of less differentiated.
World Health Organization (WHO) classification of acute myeloid leukaemia and the myelodysplastic syndromes is based on these assessments. All pathological cells (blast cells, lymphoid cells, plasma cells) and also parasites must be analysed and if possible quantified, with their morphological characteristics being described.

The report (Table 1.3) must contain information on the quality of the preparation and the type of evaluation that has been possible. The myelogram must be reported for all samples with adequate and representative cellularity (according to the algorithm shown in Fig. 1.15). The conclusions can be confined to an accurate description of what has been observed or, when the morphological picture makes it possible, can include a definitive diagnosis, which in the majority of cases will be based on an integration of immunophenotypic, cytogenetic, molecular and histological data.

Determining the percentage of megakaryocytes is not useful. It is generally preferable to make a semiquantitative assessment in terms of the number of megakaryocytes seen in a low power microscopic field; now almost forgotten, but perhaps worth re-evaluating in the context of thrombocytopenia or thrombocytosis, is the differential count of megakaryocytes in different stages of maturation.

Determining the percentage of blast cells is of considerable importance as is the detection of dysplasia in any lineage; the forms, or to the right, of an increased proportion of cells at a more advanced stage of maturation. Determining the percentage of mitotic figures can be of interest (on average 0.5% in the granulocytic series and 1–2% in the erythroid series, with higher values in women than in men), although the karyological curve, which shows graphically the proportions of cells in various phases of the cell cycle, is no longer performed. Measuring the percentage of megakaryocytes is not useful. It is generally preferable to make a semiquantitative assessment in terms of the number of megakaryocytes seen in a low power microscopic field; now almost forgotten, but perhaps worth re-evaluating in the context of thrombocytopenia or thrombocytosis, is the differential count of megakaryocytes in different stages of maturation.

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Chapter 1

Histological examination

Bone marrow core biopsies are nowadays obtained with a suitable needle as part of a single procedure with the bone marrow aspirate. The biopsy specimens are fixed, sectioned and stained with haematoxylin and eosin (H&E), by the Giemsa technique and with a stain for reticulin fibres (e.g. a Gomori stain). The sections can also be used for immunohistochemistry, a fundamental procedure for investigating many pathological processes that cannot be recognised by morphology alone (Fig. 1.21).

The indication for histological examination depends on patient characteristics, but often it is necessitated by the suspicion of bone marrow fibrosis, hypocellularity of irregularly distributed focal lesions. In practice, the pathological processes in which a core biopsy is necessary, and indeed is quite often more useful than the aspirate, are lymphomas and lymphoproliferative disorders (to evaluate the type of infiltration, which is often nodular or focal), myeloproliferative neoplasms (to grade fibrosis and identify the characteristics of the megakaryocytes), multiple myeloma and other plasma cell disorders, solid tumours with the suspicion of bone marrow invasion and granulomatous conditions such as sarcoidosis.37,38 Above all, in patients in whom the preceding bone marrow aspiration has been a ‘dry tap’ or has yielded only a few drops of blood or diluted marrow without particles, it is very useful to utilise a core biopsy to make imprints for cytological examination.

Principles of haematological cytomorphology

The quantitative and qualitative characteristics that blood cells manifest when blood and bone marrow films are examined microscopically are the result of the simultaneous action of three biological forces, which act synergistically to produce adequate numbers of functioning circulating cells to meet the biological needs of the body. The first force is the reproductive force, manifest as proliferation of haemopoietic cells: these

### Table 1.1 Myelogram and maturation curve

<table>
<thead>
<tr>
<th></th>
<th>Myelogram (%) (of all cells)</th>
<th>Maturation curve (%) (with regard to the relevant lineage)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasts (myeloblasts)</td>
<td>1–3</td>
<td>1–4</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>1–7</td>
<td>5–10</td>
</tr>
<tr>
<td>Neutrophil myelocytes</td>
<td>5–20</td>
<td>20–40</td>
</tr>
<tr>
<td>Neutrophil metamyelocytes</td>
<td>10–30</td>
<td>20–40</td>
</tr>
<tr>
<td>Segmented neutrophils and band forms</td>
<td>5–25</td>
<td>10–35</td>
</tr>
<tr>
<td>Eosinophil series</td>
<td>1–4</td>
<td>–</td>
</tr>
<tr>
<td>Total granulocytic series</td>
<td>35–65</td>
<td>–</td>
</tr>
<tr>
<td>Proerythroblasts</td>
<td>1–5</td>
<td>2–5</td>
</tr>
<tr>
<td>Basophilic erythroblasts</td>
<td>2–8</td>
<td>10–20</td>
</tr>
<tr>
<td>Polychromatic erythroblasts</td>
<td>2–20</td>
<td>30–60</td>
</tr>
<tr>
<td>Orthochromatic erythroblasts</td>
<td>2–10</td>
<td>20–40</td>
</tr>
<tr>
<td>Total erythroid series</td>
<td>15–35</td>
<td>–</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0–3</td>
<td>–</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>3–15</td>
<td>–</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0–3</td>
<td>–</td>
</tr>
<tr>
<td>Macrophages, reticular cells</td>
<td>&lt;2</td>
<td>–</td>
</tr>
<tr>
<td>Mast cells</td>
<td>&lt;1</td>
<td>–</td>
</tr>
<tr>
<td>Megakaryocyte series</td>
<td>1–2 per field**</td>
<td>–</td>
</tr>
<tr>
<td>Myeloid:erythroid ratio*</td>
<td>1.5–4.5</td>
<td>–</td>
</tr>
</tbody>
</table>

*Myeloid:erythroid or granulocyte:erythroid ratio: calculated as the ratio between total cells of the granulocytic series, including the mature granulocytes and monocytes, and total cells of the erythroid series.

**Low power (×10 objective)

NB. The values reported were obtained by counting all nucleated cells, mature and immature, in cellular bone marrow films.

### Table 1.2 Myelogram: median values obtained in 50 healthy volunteers between the ages of 21 and 50 years³⁵

<table>
<thead>
<tr>
<th></th>
<th>Reference interval (%) (central 95%)</th>
<th>Median value (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blasts (myeloblasts)</td>
<td>0–3.0</td>
<td>1.4</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>3.2–12.4</td>
<td>7.8</td>
</tr>
<tr>
<td>Neutrophil myelocytes</td>
<td>3.7–10.0</td>
<td>7.6</td>
</tr>
<tr>
<td>Neutrophil metamyelocytes</td>
<td>2.3–5.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Segmented neutrophils and band forms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>21.9–42.3</td>
<td>32.1</td>
</tr>
<tr>
<td>Women</td>
<td>28.8–45.9</td>
<td>37.4</td>
</tr>
<tr>
<td>Eosinophil series</td>
<td>0.7–6.3</td>
<td>3.5</td>
</tr>
<tr>
<td>Basophil series</td>
<td>0–0.4</td>
<td>0.1</td>
</tr>
<tr>
<td>Erythroblasts (total)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>16.2–40.1</td>
<td>28.1</td>
</tr>
<tr>
<td>Women</td>
<td>13.0–32.0</td>
<td>22.5</td>
</tr>
<tr>
<td>Monocytes</td>
<td>0–2.6</td>
<td>1.3</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>6.0–20.0</td>
<td>13.1</td>
</tr>
<tr>
<td>Plasma cells</td>
<td>0–1.2</td>
<td>0.6</td>
</tr>
<tr>
<td>Macrophages</td>
<td>0–1.3</td>
<td>0.4</td>
</tr>
<tr>
<td>M:E ratio</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>1.1–4.1</td>
<td>2.1</td>
</tr>
<tr>
<td>Women</td>
<td>1.6–5.2</td>
<td>2.8</td>
</tr>
</tbody>
</table>

NB. The volume of aspirate was 0.1–0.2 ml and the films were made using the standard wedge-spread technique. Small difference between men and women were recognised in mature neutrophils, erythroblasts and the myeloid:erythroid (M:E) ratio. With regard to Table 1.1, one notes particularly the higher values for promyelocytes, neutrophils and lymphocytes.
increase in number as a result of mitotic divisions co-ordinated
by the combined action of growth factors. The second force,
tightly linked to the combined first, is that of evolution and differentiation:
this induces the cell, at the same time as it is proliferating, to acquire certain functional abilities together with corresponding morphological changes, becoming increasingly close to the mature end cell. These first two processes, in the healthy adult, occur in the haemopoietic tissue of the marrow and in lymphoid organs. The third force is that driving migration, which leads to the entry of mature cells into the circulation and also, in the case of granulocytes, lymphocytes and monocytes, to their exit from the circulating blood into extravascular tissues. The subtle hormonal equilibrium that co-ordinates this orderly process is now in large part well known.39

The proliferation and maturation occur in a somewhat similar manner in all haemopoietic lineages: in each cell lineage, for example, the most immature stages, in which proliferative forces dominate, show functional and volumetric hypertrophy of the nucleus and its structures and immature chromatin with nucleoli, all aimed at division into two cells. The drive to differentiation, on the other hand, is seen throughout the entire sequence of maturation, there being two simultaneous processes. The first process is the reduction in function, size and structure of the nucleus, in which the nucleoli become less evident and finally disappear and the chromatin becomes dense and compact; this process is maximally expressed in erythroblasts, thanks to the final asynchronous division of the orthochromatic erythroblast, which liberates the nucleus. The second process, at the level of the cytoplasm, is the acquisition of the cells’ specific functions, which leads to the progressive loss of basophilia and the appearance of new structures that profoundly alter the original staining characteristics and appearance, such as the appearance of haemoglobin and of the primary and secondary granules.

The morphological study of blood cells, bearing in mind the increasingly numerous biotechnological methods which are being developed and put at the service of diagnostic medicine, unites the great advantages of simplicity and relatively low costs with the indispensable need for a high level of competence and experience. Despite its apparent methodological poverty, morphological study has a complex multifaceted aim, no less than the analysis of the three forces described above and the results of their complex interactions on the quantitative and qualitative characteristics of the bone marrow. To the initial indispensable quantitative assessment, including the myelogram, morphological evaluation adds careful and accurate assessment of single cells, including the structural details and staining characteristics of their visible constituents. This qualitative assessment permits the recognition of minimal deviations from normal, such as asynchrony in maturation, or major deviations from normal, such as the replacement of the balanced heterogeneity of the normal blood and bone marrow by a monomorphic population of more or less atypical immature or abnormally maturing cells. Morphological abnormalities must be used to throw light on functional or pathological processes which involve the ultrastructure, biochemistry and genetics of the cell. Some morphological-functional correlations are simple and immediately apparent, such as the relationship between cytoplasmic basophilia and the presence of RNA or the recognition of the clear juxtanuclear zone as the negative image of the Golgi apparatus. In other areas, increasing knowledge has enhanced the ability to interpret morphological features, so that their description is more precise and informative, making it possible to use morphology to predict molecular abnormalities.

The description of morphological features involves not insignificant semiological difficulties, i.e. putting into words what is seen. Morphological interpretation is based on sensory perceptions of the observer, which determine the manner, in part subjective, of the approach. Communication of experience in this field is based on descriptions that are mainly visual (shape and colour), but also tactile (density and the impression of a smooth or rough surface). For this reason, the description in words of such individual and specific sensations may be more or less effective depending on individual ways of appreciating what is seen; this verbal communication must be accompanied, moreover, by a rich collection of images, which serve to decodify the verbal message, and thus permit the construction of a personal code for cytological identification. One must also mention the numerous semantic traps in this field: the term
pean study, in which 28 expert morphologists participated, consensus terminology was established and a glossary divided according to cell lineage was drawn up (Table 1.4). A selection of various examples of individual cell types, drawn from the collection of images used for this cooperative study, and the tabulated definitions of these cell types, were established by consensus and can be consulted on the website of the European LeukemiaNet and that of the European Hematology Association. On the site of the Società Italiana di Ematologia (SIE), in the section ‘Attività formative’ there is also an online test for self-validation in cytomorphology, based on the identification of normal and pathological cells, and also a selection of clinical cases based on cytomorphology.

‘myeloid’, for example, is used by some as a synonym for ‘medullary’, thus including the three haemopoietic (non-lymphoid) lineages (and this is the usage in the WHO 2008 classification), and by others is used in contrast to ‘erythroid’, thus limiting it to the granulocytic lineages.

Another terminological difficulty is the naming and interpretation of single cell types. This is linked in large part to different scientific and didactic traditions which have become established during the last two centuries in various parts of the world. Today, this can be overcome by international harmonisation both of teaching and of laboratory reporting, aided by information technology that permits the exchange of images and makes possible an interpretive exercise: in a recent multicentric European study, in which 28 expert morphologists participated, consensus terminology was established and a glossary divided according to cell lineage was drawn up (Table 1.4). A selection of various examples of individual cell types, drawn from the collection of images used for this cooperative study, and the tabulated definitions of these cell types, were established by consensus and can be consulted on the website of the European LeukemiaNet and that of the European Hematology Association. On the site of the Società Italiana di Ematologia (SIE), in the section ‘Attività formative’ there is also an online test for self-validation in cytomorphology, based on the identification of normal and pathological cells, and also a selection of clinical cases based on cytomorphology.