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Review

The importance of adequate recognition of normal and dysplastic myelopoiesis for the diagnosis of myelodysplastic syndromes

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Summary. The diagnosis of myelodysplastic syndromes is based on the presence of cytopenias, dysplastic morphological features on peripheral blood (PB) and bone marrow (BM), cytogenetic abnormalities and requires to rule out other diseases resembling these conditions. Optical cytomorphology is the cornerstone of diagnosis of MDS. The recognition of cytological myelodysplasia has a crucial value in diagnosis and prognosis of MDS. Assessment of cytological morphology requires, like other diagnostic techniques (flow cytometry, cytogenetics, histological morphology), experienced observers and the availability of high quality and properly stained samples. The morphological analysis has shown moderate reproducibility among hematopathologists. The better characterization and standardization of morphological features has improved the reliability and reproducibility of MDS diagnosis. Maintaining the competence in morphology assessment requires experience and continuous training. For the correct assessment of cytologic myelodysplasia it is essential to keep in mind the morphology of normal myelopoiesis. To the extent of our knowledge there are no studies describing together morphological data on normal and dysplastic myelopoiesis in the framework of MDS. Therefore, by combining these data, this manuscript could serve as a useful tool for quotidian process of diagnosis.

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Introduction

The 2008 and 2017 WHO Classifications of Tumours of Haematopoietic and Lymphoid Tissues requires the recognition of dysplastic features for the diagnosis of myelodysplastic syndromes (MDS) and other myeloid neoplasms. The myelodysplastic syndromes are a group of acquired clonal hematopoietic stem cell disorders with very heterogeneous outcomes, characterized by ineffective hematopoiesis, manifested by morphologic dysplasia in hematopoietic cells, peripheral cytopenia(s) and an increased risk of development of acute myeloid leukemia (AML). The diagnosis of MDS is based on a combination of medical history, morphological features on peripheral blood (PB) and bone marrow (BM), cytogenetic and molecular data and it is mandatory to rule out other diseases. Optical cytomorphology is the cornerstone of diagnosis of MDS. The recognition of cytological myelodysplasia has a crucial value in diagnosis, classification and prognosis of MDS, although the presence of dysplasia is not in itself definitive evidence of a clonal disorder. It is very important to consider that myelodysplasia is not synonymous of MDS; no pathognomonic data of MDS exist and it is imperative to exclude other causes of

transient dysplasia (vitamin B12 and folic acid deficiency, viral infections, ethanol, exposure to lead and other heavy metals, particularly arsenic and several commonly used antibiotics, drugs and biological agents among others (Brunning et al., 2008; Hasserjian et al., 2017). Such conditions should be ruled out by a careful clinical history and physical and laboratory examinations. The diagnosis of MDS should not be established while the patient is on growth factor therapy, including erythropoietin (Brunning et al., 2008; Hasserjian et al., 2017). Assessment of cytological morphology requires, like other diagnostic techniques (flow cytometry, cytogenetics, and histological morphology), experienced observers and the availability of high quality and properly stained samples (Bennett et al., 1984; Brunning et al., 2008; Béné and Zini, 2017; Hasserjian et al., 2017). Poor quality smears may result in misinterpretation which is particularly notorious in assessing neutrophilic granulation. Slides should be made from freshly obtained specimens (peripheral blood exposed to anticoagulants for more than two hours is unsatisfactory) (Brunning et al., 2008; Hasserjian et al., 2017). The morphological analysis requires identification of blast cells in BM and PB, type and degree of dysplasia and presence of ring sideroblasts. As defined in the 2008 and 2017 WHO classifications (Brunning et al., 2008; Hasserjian et al., 2017), for enumeration of the blasts counting at least 200 cells in blood smears and 500 cells in BM smears is required. To assess dysplasia at least 200 neutrophils, 200 erythroid precursors and 30 megakaryocytes should be evaluated in bone marrow. The threshold used for considering a myeloid cell line as dysplastic is the presence of 10% abnormal cells in the corresponding myeloid lineage (Brunning et al., 2008; Hasserjian et al., 2017). Identification of dysplasia is not always satisfactorily reproducible among hematopathologists (Font et al., 2013; Senent et al., 2013). Several studies have been published in an attempt to improve identification of dysplasia of different cell types, including the following: i) precise definition of myeloblasts, promyelocytes and ring sideroblasts (Mufti et al., 2008), ii) the proposal to refine the definition of dysgranulopoiesis (Goasguen et al., 2014), the proposal to refine the definition of dyserythropoiesis (Goasguen et al., 2018), iii) identification of megakaryocytes and monocytic cells (Goasguen et al., 2009, 2016) and iiii) a proposal for a morphological score to detect bone marrow dysplasia (Della Porta et al., 2015). The better characterization and standardization of morphological features has improved the reliability and reproducibility of MDS diagnosis (Della Porta et al., 2015; Hasserjian et al., 2017). Although sometimes challenging to interpret, the morphologic features of MDS are well described, so that on the recent WHO revision for MDS, significant changes related to morphologic criteria have not been proposed (Hasserjian et al., 2017). For assesing cytologic myelodysplasia it is essential to keep in mind the morphology of normal myelopoiesis. It is also important to remember that the normal maturational process of myeloid cells is continuous, with a gradual transition from one stage to the next, and therefore the exact maturation stage of maturation may be difficult to establish. To the extent of our knowledge there are no studies describing together morphological data on normal and dysplastic myelopoiesis in the framework of MDS. Therefore, it seemed interesting to gather in single article different images of normal and dysplastic myelopoiesis (erythroblastic, granulocytic, megakaryocytic, and monocytic lineages) (Bennett et al., 1982; Woessner and Florensa, 2006; Brunning et al., 2008; Hasserjian et al., 2017). In addition, few ultrastructural (US) images that may be helpful for the understanding of some morphological data have been included.

Major manifestations of dysplasia are depicted (Brunning et al., 2008; Hasserjian et al., 2017) in Table 1.

Morphological characteristics of myelopoiesis

General characteristics of normal erythroid differentiation

During the maturation process we can observe the following changes: a decrease in cell size, a reduction of the nucleo-cytoplasmic ratio and a progressive nuclear

Table 1. Major manifestations of dysplasia.

Morphological manifestations of dysplasia.
Dyserithropoiesis
Nuclear budding
Internuclear bridging
Karyorrhesis
Multinuclearity
Nuclear hyperlobulation
Megaloblastoid changes
Citoplasmic
Ring sideroblasts
Vacuolitation
Periodic acid Shiff (PAS) positivity
Dysgranulopoiesis
Small or unusually large size
Nuclear hyposegmentation (pseudoPelger-Hüet)
Nuclear hypersegmentation
Decreased granules; agranularity
Peudo-Chediak-Higashi granules
Döhle bodies
Auer rods
Macropolicyte
Dysmegakarycytopoiesis
Micromegakaryocytes
Nuclear hypolobulation
Multinucleation (normal megakaryocytes are uninucleate with lobulated nuclei)
Megakaryocytes with large peripheral cytoplasmic dilatations cytoplasm completely devoid of granulation with persistent basophilia

condensation with a disappearance of nucleoli and an expulsion or enucleation of the nucleus (when the nucleus becomes pyknotic). Gradual changes of cytoplasmic tonality from basophilic to acidophilic (due to haemoglobin) clearly define the passage from one maturation stage to the next. The different morphological categories are depicted in Fig. 1: *Proerythroblast* is a large cell with a regular round shape. The nucleus is large, centrally placed, surrounded by a thin rim of deeply basophilic cytoplasm; its chromatin is a delicate and homogeneous network and the nucleoli are clearly visible. The nuclear/cytoploplasmic ratio is very high. The cytoplasm sometimes displays a lighter juxtanuclear area with a semilunar shape that correspond to the Golgi apparatus. (Fig. 2A-C). *Basophylic erythroblast*, has a reduced cell

Stages of maturation of the normal erythroid lineage

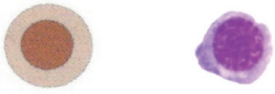
	Cell	Size	Nucleus	Visible nucleoli	Nucleus cytoplasmic ratio	Cytoplasm
Proerythroblast		20-25 µm	Round	1-2	Very high	Strong basophilic
Basophilic erythroblast		16-18 µm	Round	No	High	Basophilic
Polychromatic erythroblast		8-12 µm	Round	No	Low	Acidophilic
Orthochromatic erythroblast		7-10 µm	Round	No	Very low	Strong acidophilic
Reticulocyte		8-9 µm				Basophilic
Erythrocyte		7 µm				Acidophilic

Fig. 1. General characteristics of erythroid differentiation.

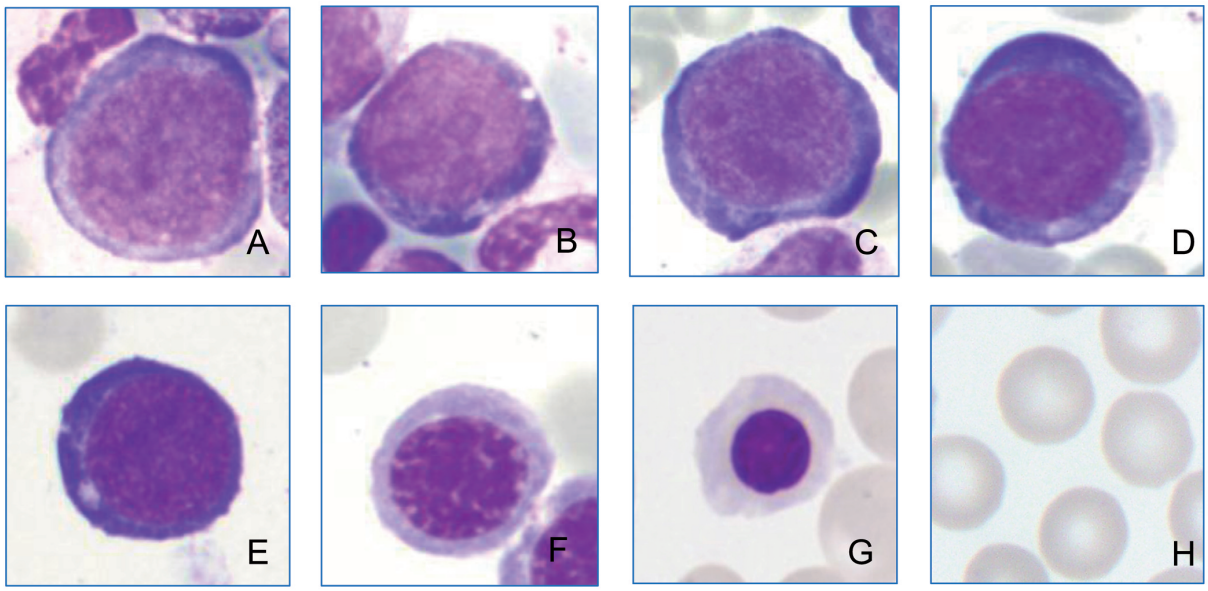


Fig. 2. Normal BM smears. May-Grünwald Giemsa stain. **A, B.** Proerythroblasts, with visible nucleoli. **C-E.** Basophylic erythroblasts, with a gradual maturational transition, the nucleo-cytoplasmic ratio is high and without visible nucleoli. **F.** Polychromatic erythroblast. **G.** Ortochromatic erythroblast. **H.** Erythrocyte.

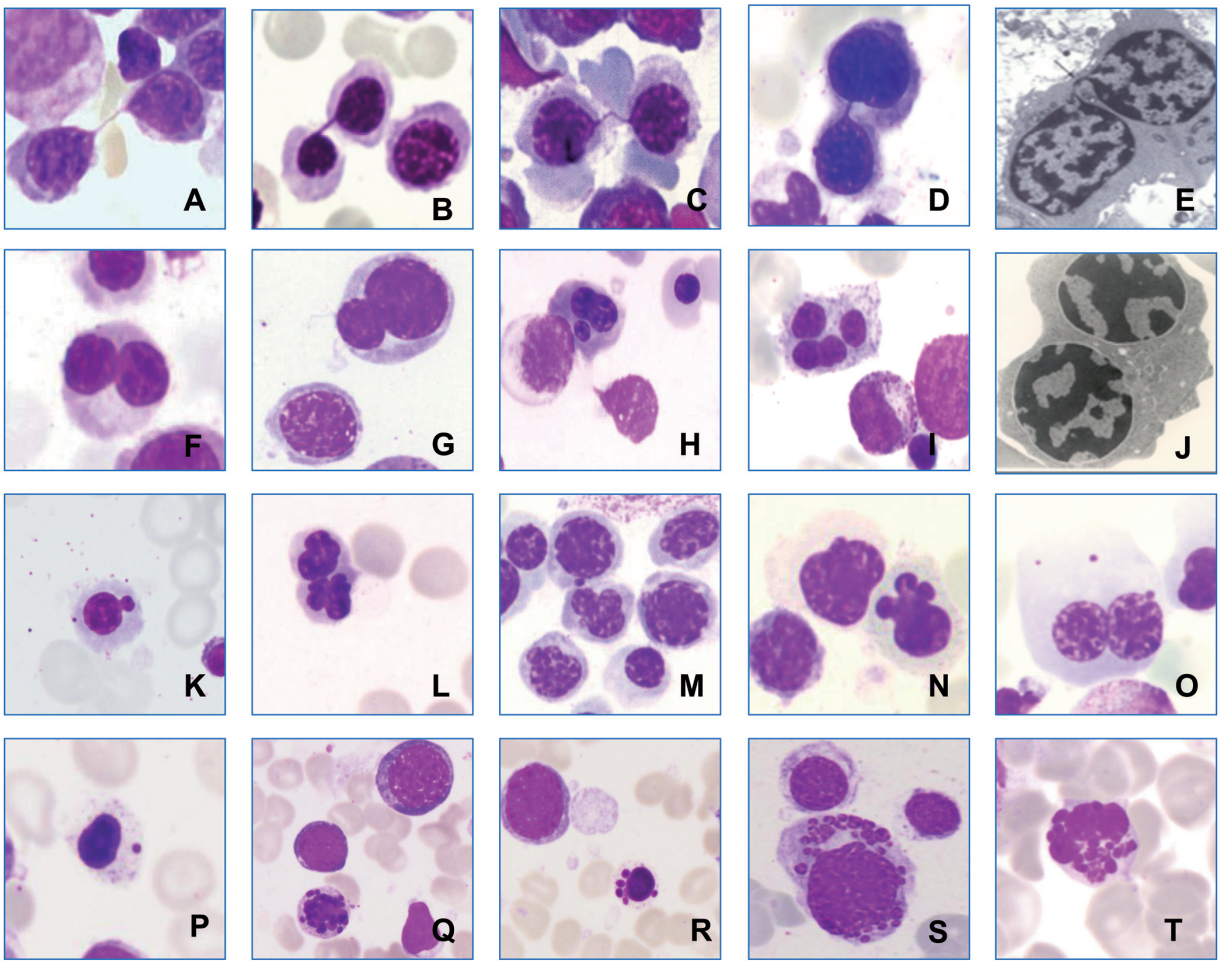


Fig. 3. Bone marrow smear from patients with MDS. May-Grünwald-Giemsa stain. **A-D.** Erythroblasts with internuclear bridging. **E.** Ultrastructural aspect of an erythroblast with a chromatin bridge connecting two nuclei. **F, G.** Binucleated erythroblasts. **H.** Erythroblast with three nuclei. **I.** Erythroblast with four nuclei. **J.** Ultrastructural aspect of a binucleated erythroblasts. **K-N.** Erythroblasts with irregular nuclear outline. **O-S.** Several erythroblasts with loss of chromatinic material (Howell Jolly bodies). **T.** An abnormal mitosis of an erythroblast.

size compared to that of its antecedent cell. The nucleus is round, centrally located, without visible nucleoli and a more condensed chromatinic network than in the previous stage of maturation. The cytoplasm is homogeneously blue and the nucleo-cytoplasmic ratio is high (Fig. 2D-E). *Polychromatic erythroblast;* the reduction in size is evident. The nucleus is round with its chromatin clumped into large dark masses alternating with clear areas. Nucleoli are not apparent. The cytoplasm is no longer blue since acidophylic staining of haemoglobin is superimposed producing a reddish or greyish color. The nucleo-cytoplasmic ratio is low (Fig. 2F). *Ortho-chromatic erythroblast;* the reduction in size continues and the nucleus becomes pyknotic (darker). Nucleoli are not visible. Acidophilia dominates in the cytoplasm with a persistence of certain greyish tinge of poly-chromatophilia in the youngest orthochromatic erythroblasts. The nucleo-cytoplasmic ratio is very low (Fig. 2G). *Reticulocyte;* this late stage of maturation takes place when the nucleus of an orthochromatic erythroblast is extruded (enucleation). Remnants of certain cytoplasmic organelle can be demonstrated by vital stains. *Erythrocyte;* the final stage, is an anuclear element coloured pinkish-grey with a clear area corresponding to the central physiological depression (Fig. 2H).

We depict the main cytological traits of dyserythropoiesis affecting the nucleus and the cytoplasm. Erythroblast with internuclear bridging (chromatin bridge connecting two nuclei of two erythroblasts completely separated or two nuclei inside the same erythroblast (binucleated erythroblast) (Fig. 3A-E); Binucleated erythroblast (Fig. 3F,G,J). Multinucleated erythroblast with several nuclei (Fig. 3H,I), three nuclei (Fig. 3H), four nuclei (Fig. 3I). Erythroblasts with abnormal nuclear shape, irregular nuclear outline, with single, double or triple indentations resulting in two or more nuclear lobes of equal or unequal size or with an irregular outline (Fig. 3K-N). Erythroblast with nuclear fragments of various sizes (Howell-Jolly bodies) (Fig.

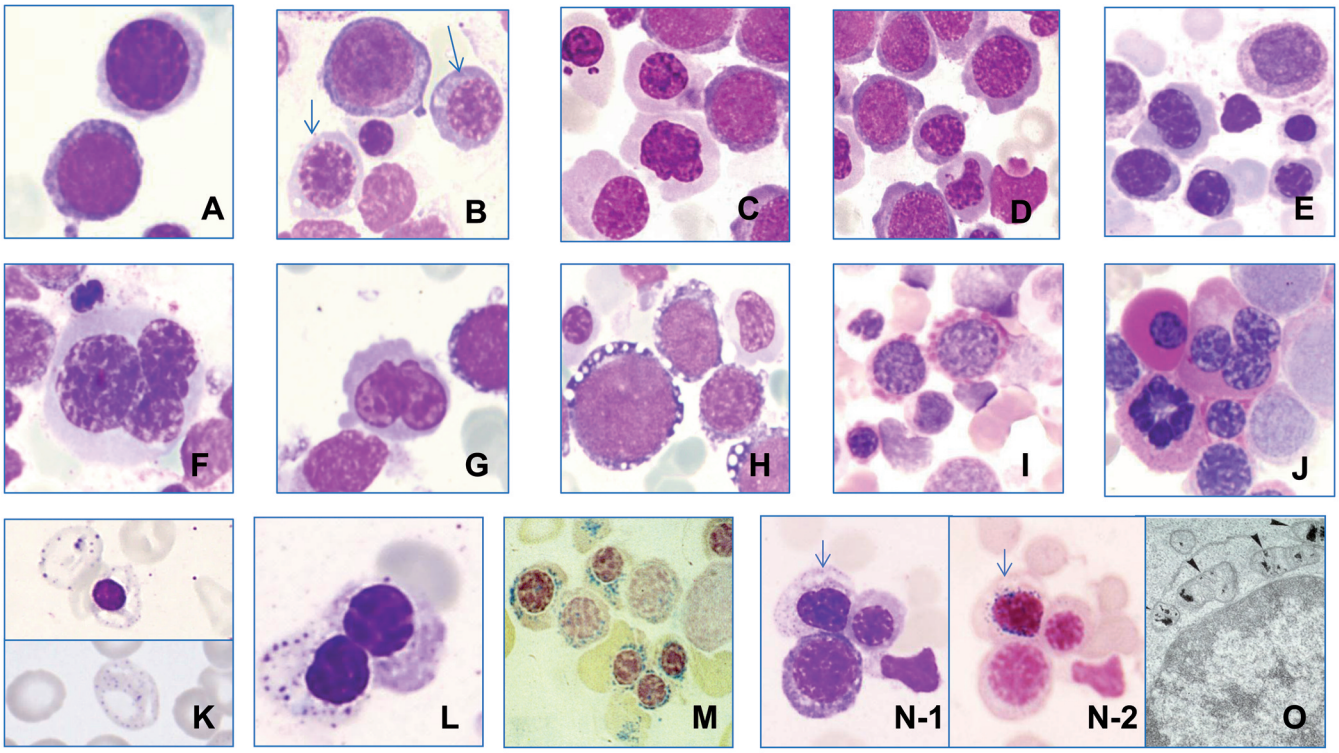


Fig. 4. Bone marrow smears from patients with MDS. May-Grünwald-Giemsa stain. **A.** Presence of a basophylic erythroblast and a mature erythroblast (top) with maturational asynchrony, with a cell size similar to basophylic erythroblast and persistence of cytoplasmatic basophilia. **B.** Basophylic erythroblast (top) and two mature erythroblasts with maturational asynchrony (arrows). **C-G.** Erythroblasts with megaloblastoid changes. **H.** Several erythroid precursors with cytoplasmic vacuoles. **I, J.** Several erythroid precursors with granular and diffuse cytoplasmic PAS positivity (Periodic acid-Shiff reaction). **K.** Erythroblasts with anomalous distribution of hemoglobin (upper). and erythrocyte with coexistence of anomalous distribution of hemoglobin and basophilic stippling (bottom). **L.** Erythroblasts with coexistence of anomalous distribution of hemoglobin and basophilic stippling. **M.** Ring sideroblasts (Perls stain). **N-1.** Erythroblast with coexistence of anomalous distribution of hemoglobin and basophilic stippling (arrow) (May-Grünwald-Giemsa stain). **N-2.** Perls staining applied on the same preparation previously discolored. **N-1.** certifies that this abnormal erythroblast is a ring sideroblast. **O.** Ultrastructural aspect of mitochondrial ferritin accumulation in a ring sideroblasts.

3O-S). Anomalous mitosis (Fig. 3T). Erythroblast with nuleocytoplasmic asynchronic maturation. These erythroblasts have a mature nucleus and cytoplasm with signs of immaturity (marked basophilia), generally with a cell size larger than that of normal (Fig. 4A,B). When an asynchrony is not marked it is difficult to detect and therefore not always reproducible among different observers. Erythroblast with megaloblastoid changes, erythroblasts with delayed nuclear maturation due to defective DNA synthesis, contain large immature nuclei, the chromatin is finely dispersed and has an opened stippled appearance (Fig. 4C-G). Erythroblast with cytoplasmic vacuoles that can be Periodic acid-Shiff reaction positive (PAS) (Fig. 4H). Erythroblast with cytoplasmic PAS positivity (Fig. 4I,J). Erythroblast with irregular density of the cytoplasmatic staining or anomalous distribution of hemoglobin (Fig. 4K,L). Erythroblast with basophilic stippling (Fig. 4K,L). Ring

Stages of maturation of the normal granulocytic lineage

Cell	Size	Nucleus	Visible nucleoli	Cytoplasm	Granules
Myeloblast	10-15 µm	• Round • Chromatin not condensed	2-3	Basophilic	Generally absent
Promyelocyte	16-20 µm	• Rounded • Not centrally located • Chromatin with irregular condensation	0-1	Basophilia partially replaced by acidophilia	Azurophilic granules
Myelocyte	13-18 µm	• Rounded • Condensed chromatin	No	Reduced basophilia	Predominance of specific granules (neutrophilic, eosinophilic, basophilic)
Metamyelocyte	12-15 µm	• Curved • Condensed chromatin	No	Reduced basophilia	Plenty specific granules
Band form	12-14 µm	• Curved • Condensed chromatin	No	Reduced basophilia	Abundant specific granules
Polysegmented	12-14 µm	• Nucleus segmented into 3 to 4 lobes connected by fine chromatin bridges • Condensed chromatin • Two nuclear lobes in eosinophils	No	Reduced basophilia	Abundant specific granules

Fig. 5. General characteristics of granulocytic differentiation.

sideroblast, erythroblast with ≥5 siderotic granules covering at least a third of the circumference of the nucleus revealed by Perls staining (Brunning et al., 2008; Mufti et al., 2008; Hasserjian et al., 2017) (Fig. 4M). As our group could demonstrate, the presence in May-Grünwald-Giemsa stained smears of erythroblasts with coexistence of anomalous distribution of hemoglobin and basophilic stippling is highly indicative of the presence of ring sideroblasts (visualized with the Perls stain) (Acin et al., 1995). The ring sideroblasts are the consequence of an abnormal deposition of ferritin between the mitochondrial ridges, a phenomenon clearly observable with the electron microscope (Fig. 4N1,N2,O).

General characteristics of granulocytic differentiation

The maturation process is characterized by a similar cell size in all differentiation phases except in the promyelocyte that is larger than its predecessor; a condensation of nuclear chromatin and reduction of the nuclear area is observed. Indentation and segmentation of the nucleus, loss of cytoplasmic basophilia and appearance of specific granules become evident (neutrophilic, eosinophilic, basophilic). The different morphological categories are shown in Fig. 5: *Myeloblast*, that is a rounded cell with a very high nuclear/cytoplasmic ratio. The nucleus is large and occupies the greater part of the cell and is generally centrally located; nuclear shape is variable, its chromatin is finely reticular and homogeneous and contains two or more visible nucleoli often not strongly demarcated by surrounding chromatin condensation. The cytoplasm is scarce and evenly uniformly and moderately basophilic without granules (agranular myeloblast) (Fig. 6A). Sometimes myeloblasts contain a small number of azurophilic granules (primary granules) but without a

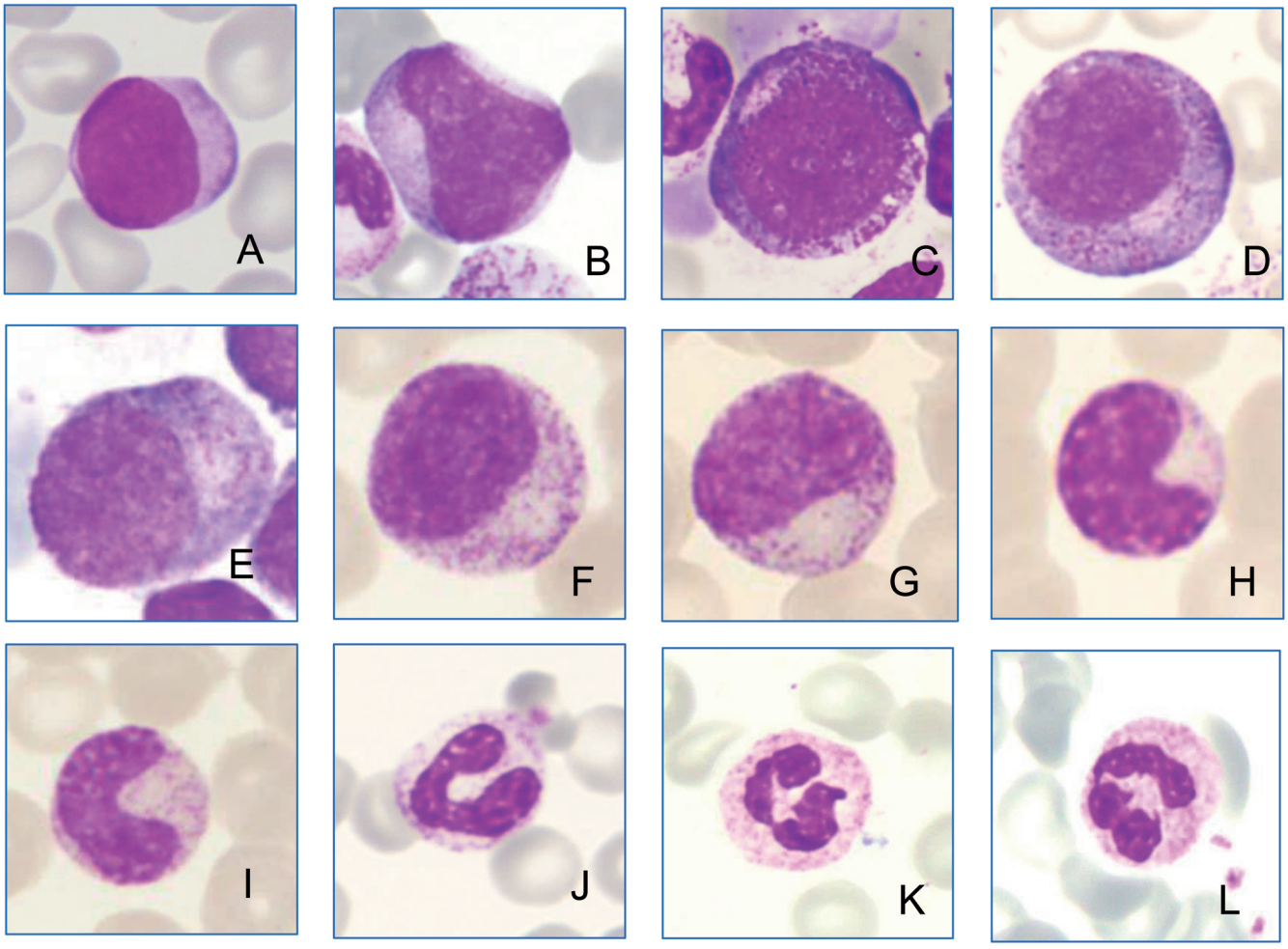


Fig. 6. Normal BM smears. Normal neutrophilic differentiation. May-Grünwald-Giemsa stain. **A.** Agranular myeloblast. **B.** Myeloblast with incipient granulation (granular myeloblast). **C.** Granular myeloblast. **D, E.** Promyelocytes. **F, G.** Myelocytes. **H.** Metamyelocyte. **I.** Metamyelocyte with more advanced maturation. **J.** Band form. **K, L.** Mature granulocyte.

clear area corresponding to the Golgi apparatus (granular myeloblast) (Fig. 6B,C). Granular blasts must be distinguished from promyelocytes. *Promyelocyte* is the largest cell of the granulocytic series with a lower nuclear/cytoplasmic ratio than myeloblasts. The rounded nucleus is generally eccentric, displaced by a lightly staining centrosomic area corresponding to the non-staining Golgi apparatus. Its chromatin is usually finely reticular. Nucleoli are fewer and smaller than in myeloblasts though still detectable. The cytoplasmic basophilia starts to be replaced by acidophilia and abundant azurophilic granules become clearly visible (Fig. 6D,E). *Myelocyte* is smaller than promyelocyte, round or slightly oval in shape. The chromatin is coarse, nucleoli are not visible and the cytoplasm is completely acidophilic as a consequence of the disappearance of ribosomes and the presence of increasing amounts of secondary specific granules (eosinophylic, basophylic and neutrophylic) (Fig. 6F,G). *Metamyelocyte* differs from myelocyte mainly by their nuclear shape, which is kidney bean shape or indented. The chromatin is condensed and the cytoplasm is acidophilic and packed

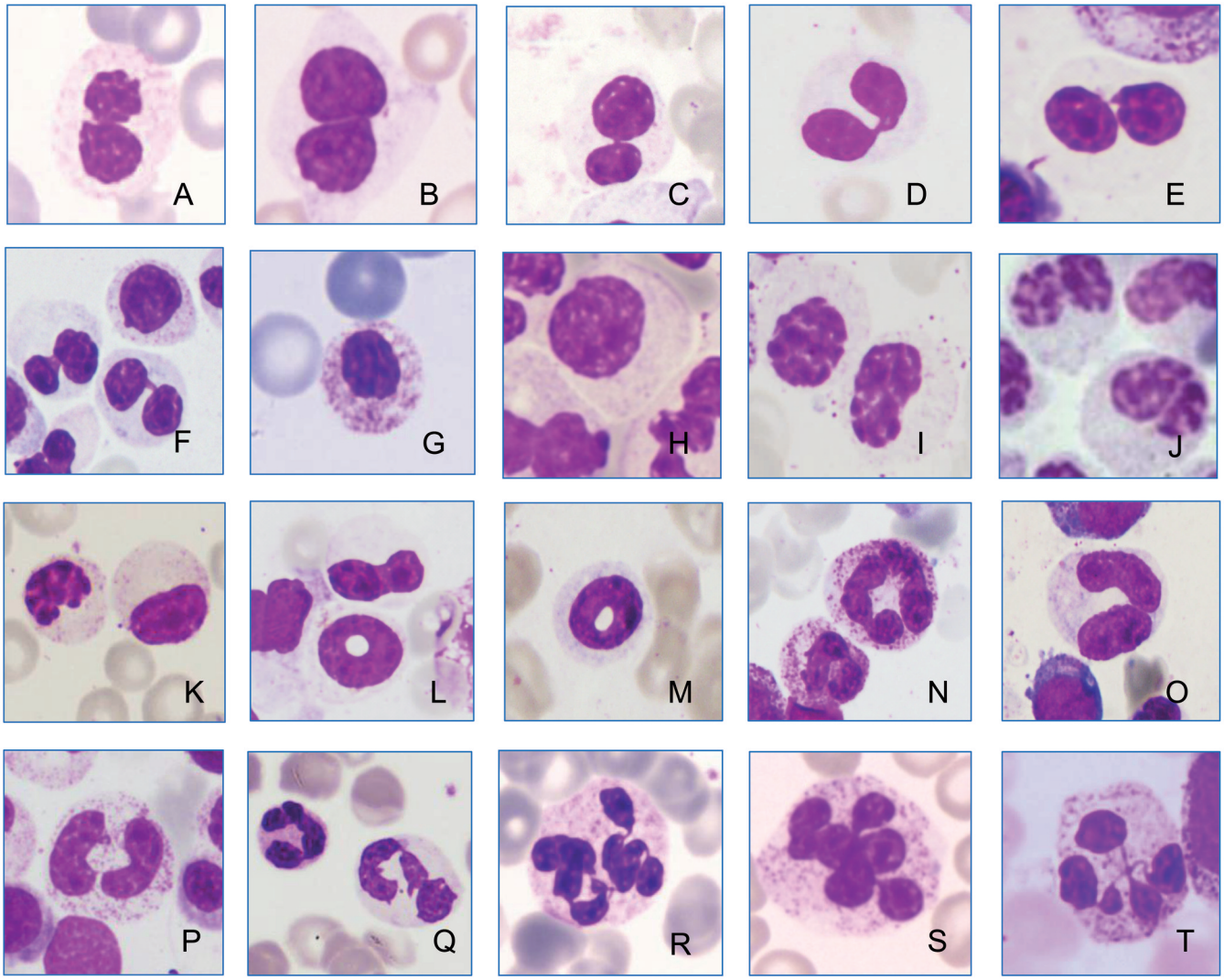


Fig. 7 Bone marrow smears from patients with MDS. May-Grünwald-Giemsa stain. **A, B.** Neutrophil granulocytes with nuclear hiposegmentation. **C-F.** Neutrophil granulocytes with pseudo Pelger-Huët anomaly with bilobulated nuclei (two equal nuclear masses linked by a very thin filament). **G, H.** Neutrophils granulocytes with a round nucleus without segments. **I-K.** Neutrophils granulocytes with degranulated cytoplasm, nuclear hiposegmentation and exaggerated chromatin clumping with large blocks separated by clear zones. **L, M.** Neutrophil granulocytes with alteration of nuclear segmentation, ring shaped. **N-Q.** Macropolicyts (double normal size). **R, S.** Macropolicyts with nuclear hypersegmentation. **T.** Neutrophil granulocyte with hypersegmentation and a radial distribution of the nuclear fragments joined by fine filaments (typically observed in myelocathexis).

with secondary granules (Fig. 6H,I). Band form, the nucleus becomes ribbon shaped. The nucleus of this cell has parallel borders for most of its length, like a band, and the cytoplasmic characteristics are similar to those of the metamyelocyte (Fig. 6J). *Mature granulocyte;* the nucleus shows 3-4 lobes connected by chromatin bridges and the cytoplasm is filled with secondary neutrophilic, eosinophilic or basophilic granules (Fig. 6K,L).

We depict the main cytological traits of dysgranulopoiesis affecting cell size, nucleus and cytoplasm of neutrophilic granulocytes. In many cases there are two or more alterations in the same cell. Neutrophilic granulocytes with nuclear hypo-segmentation (Fig. 7A,B), that is different from that observed in Pelger-type hyposegmentation. Neutrophilic granulocytes with pseudo Pelger-Huët anomaly characterized by nuclear hiposegmentation, with bilobulated nuclei (two equal nuclear masses linked by a

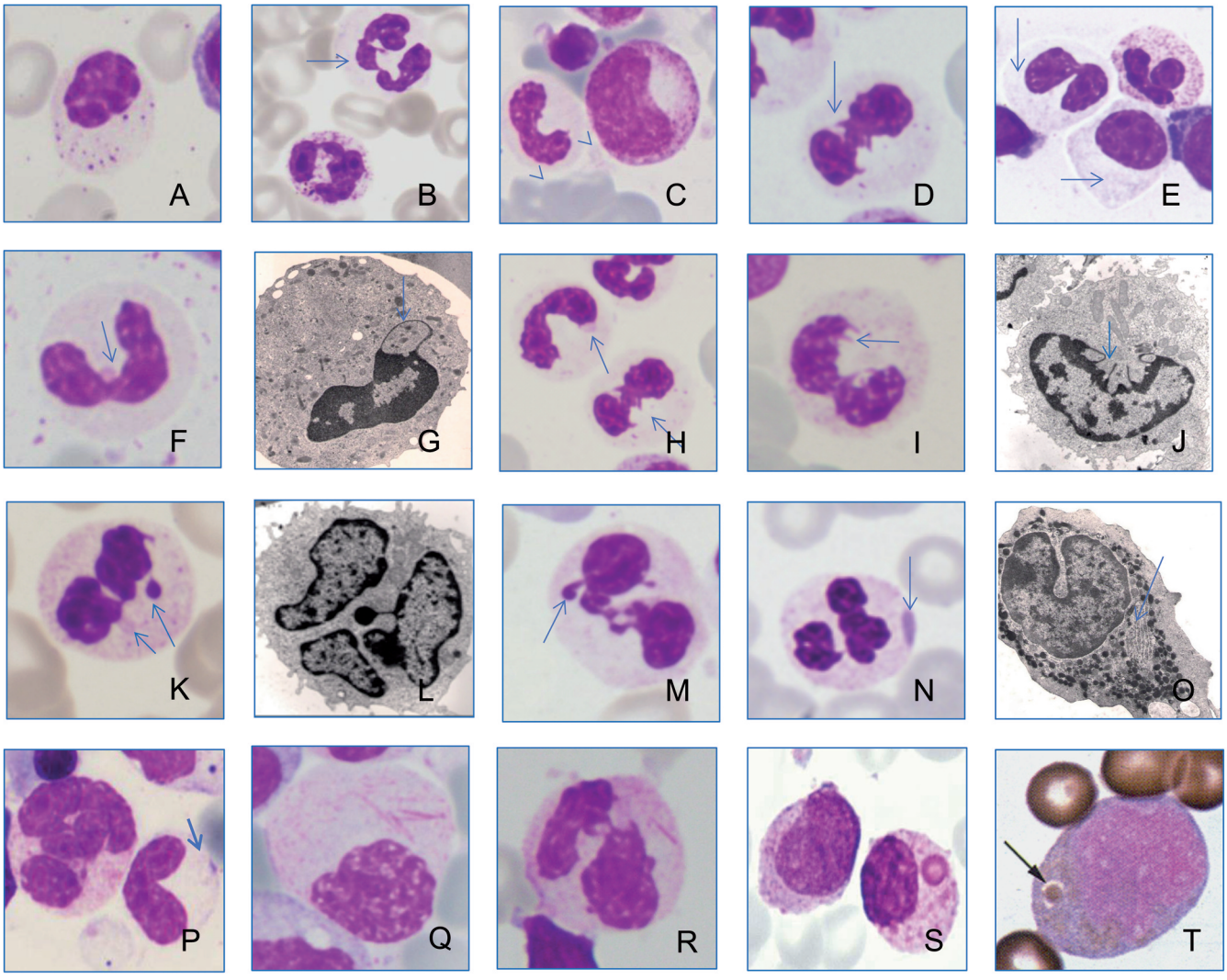


Fig. 8. Bone marrow smears from patients with MDS. May-Grünwald Giemsa stain. **A.** Neutrophil granulocyte with hypogranulation (2/3 reduction of cytoplasmic granules). **B-E.** Neutrophils granulocytes degranulated (arrow). **F.** Neutrophil granulocyte degranulated with a nuclear pocket (arrow). **G.** Nuclear pocket visualized by US (arrow). **H.** Neutrophil granulocyte degranulated with a nuclear pocket (long arrow) and neutrophil granulocyte degranulated with nuclear projections (short arrow). **I.** Neutrophil granulocyte degranulated with nuclear projections or appendices. **J.** Nuclear projections or appendices visualized by US. **K.** Neutrophil with a drumstick (long arrow) and a nuclear pocket (short arrow). **L.** A drumstick visualized by US. **M.** Neutrophil granulocyte degranulated with an abnormal nuclear shape and with a drumstick (arrow). **N.** Neutrophil granulocyte degranulated with a Döhle body. **O.** Döhle body visualized by US (arrow). **P.** Macropolycytes (left) and neutrophil granulocyte degranulated with a Döhle body (arrow). **Q, R.** Neutrophil granulocyte degranulated with Auer rods. **S.** Pseudo Chediack granules. **T.** Atypical primary granules containing myeloperoxidase (arrow).

very thin filament) (Fig. 7C-F) or with a round nucleus without segments and a chromatin structure coarser than that of a normal neutrophil (Fig. 7G-I). Neutrophilic granulocytes with abnormal condensation of chromatin (clumping), with large blocks of heterochromatin separated by clear zones, corresponding to euchromatin, usually associated with nuclear hyposegmentation (Fig. 7J,K). Neutrophilic granulocytes with bizarre nuclear configurations such as ring shaped (Fig. 7L,M). Macropolicytes are neutrophils of double normal size with a proportionate increase in the size of the nucleus and the degree of nuclear lobulation (Bain, 2011; Goasguen et al., 2014) (Fig. 7N-S). They can present different anomalies such as nuclei of bizarre shapes, two nuclei facing one another in the form of a mirror (Fig. 7O,P), nuclear hypersegmentation (more than five segments), (Fig. 7R,S) and hypersegmentation of the nuclear fragments joined by fine filaments, occasionally with a radial distribution (typically observed in myelocathexis) (Fig. 7T). Neutrophils with hypogranulation (2/3 reduction of cytoplasmic granules) (Goasguen et al., 2014) (Fig. 8A) and degranulation (Fig. 8B-H). Poor quality smears may result in misinterpretation in assessing neutrophilic granulation. To verify this data it is important to compare it with granulated neutrophils in the same preparation (double population of neutrophils) (Fig. 8B,C). In degranulated neutrophils nuclear pockets and nuclear appendices can frequently be observed. Nuclear pockets are situated beside the nucleus containing cytoplasmic material and are surrounded by elements of the nuclear envelope, an image that can be clearly visualized by US (Fig. 8F,G,H,K). The nuclear projections or appendices are small nuclear masses attached to the body of the nucleus by means of a coarse filament (a cell with more than 4 projections is considered dysplastic) (Goasguen et al., 2014), projections can be clearly visualized by US (Fig. 8H-J). These must be differentiated from a drumstick that is a chromatinic mass attached to the body of the nucleus by means of a thin filament (Fig. 8K-M).

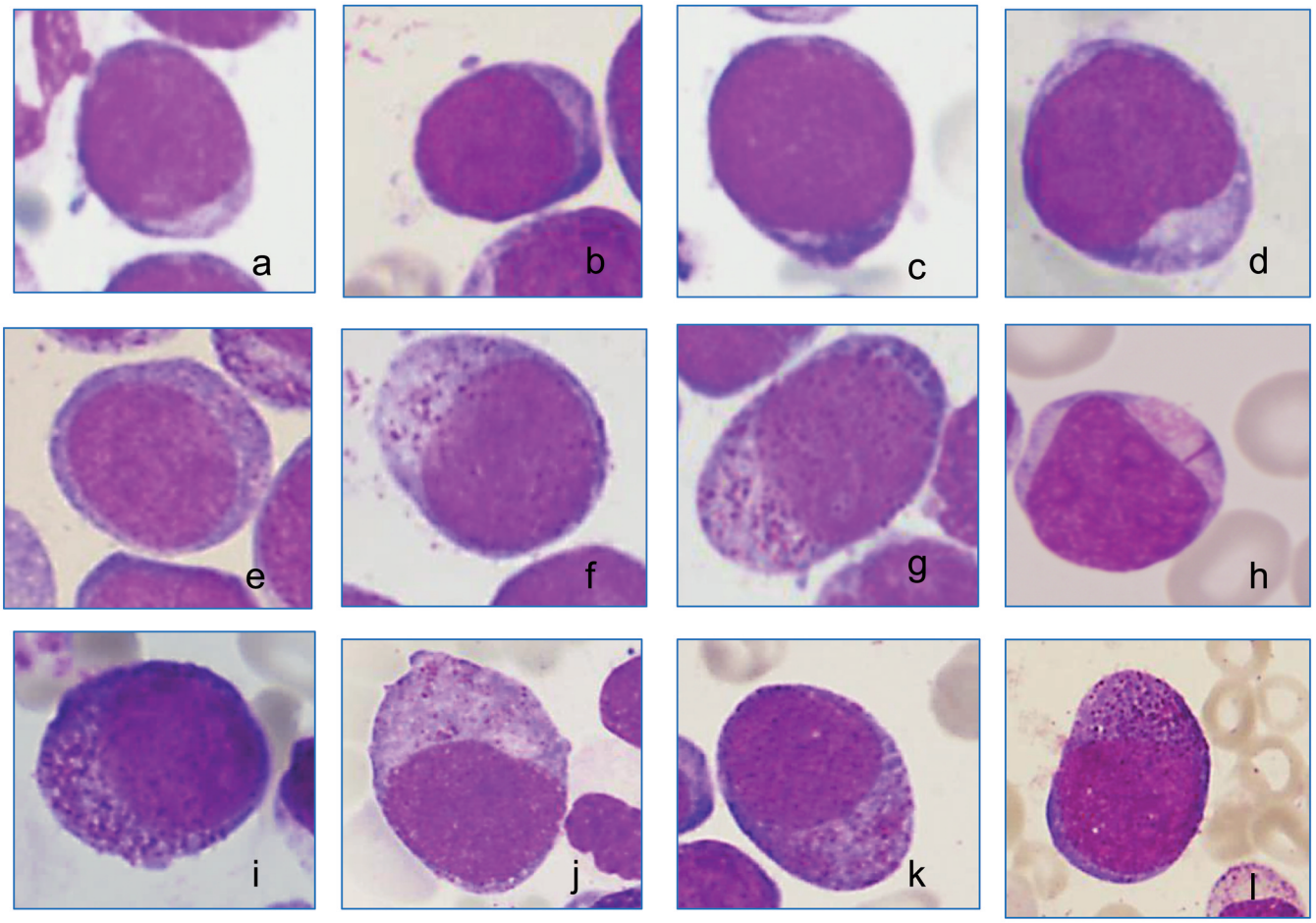


Fig. 9. Bone marrow smears from patients with MDS. May-Grünwald-Giemsa stain. **A-D.** Agranular myeloblast. **E-G.** Myeloblast with granulation. **H.** Myeloblast with an Auer rod. **I-L.** Promyelocytes.

Neutrophilic granulocytes with Döhle bodies, oval, basophilic inclusion usually located in the cytoplasmatic periphery of a granulocyte (Fig. 8N-P). Auer rods (Fig. 8Q,R) and pseudo Chediak granules (Fig. 8S,T) both are atypical primary granules containing myeloperoxidase.

Enumeration of blast cells is critical for both diagnosis and prognostic stratification of MDS. Myeloblasts can be agranular or granular. The agranular blast corresponds to the type I blast of the FAB classification (Bennett et al., 1982) (Fig. 9A-D). Granular blasts are cells that have the nuclear features of blast cells but have also cytoplasmic granules (Fig. 9E-

Stages of maturation of the normal megakaryocytic lineage

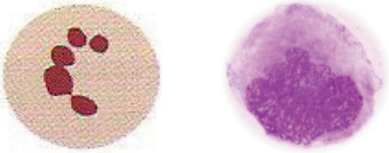
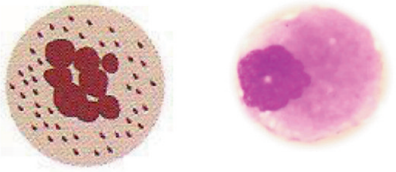
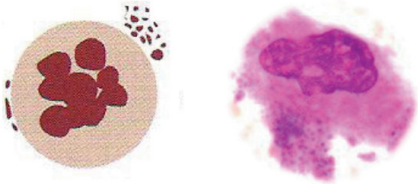
	Cell	Size	Nuclear morphology	Cytoplasm	
				Stain	Granules
Megakaryoblast		15-50 µm	Condensed bilobulated	Basophilic	Scarce
Promegakaryocyte		20-80 µm	Horseshoe shaped	Basophilia with central acidophilic area	Progressive increase
Granular megakaryocyte		50-80 µm	Multilobulated	Progressively acidophilic	Abundant
Mature megakaryocyte releasing platelets		20-150 µm	Condensed but highly lobulated	Acidophilia	Abundant
Nacked nucleus		Variable	Very condensed chromatin	-	-
Platelet		2-3 µm		Hyalinic peripheral zone	Azurophilic granules centrally located

Fig. 10. Morphological aspects of normal megakaryocytic differentiation.

G) and or Auer rods (Fig. 9H) and correspond to type II blasts of the FAB classification (Bennett et al., 1982). Granular blasts must be distinguished from dysplastic promyelocytes, charaterized by a reduced or irregular cytoplasmic basophilia, a poorly developed Golgi zone, hypergranularity or hypogranularity and irregular distribution (clumps) of granules (Brunning et al., 2008; Mufti et al., 2008; Hasserjian et al., 2017) (Fig. 9I-L).

Morphological aspect of normal megakaryocytic differentiation

In this evolutionary process, with a gradual transition from one stage to the next, occurs an increase in size and ploidy of the nucleus by endomitosis. Several events took place: formation of nuclear lobes that are always interconnected, increase in volume and granulation of the cytoplasm and finally detachment of cytoplasmic areas that correspond to proplatelets and newly formed platelets. Different morphologic categories exist (Fig. 10). *Megakaryoblast* is a cell with blastic appearance, large immature nucleus with loose chromatin and visible nucleoli, sometimes bilobulated that can be differentiated of atyipical binucleated megakaryocyte (Fig. 11A-D). The cytoplasm is scanty, basophilic or weakly basophilic and shows frequently cytoplasmic protrusions (projections). Rarely emperipolesis (presence of an intact cell within the cytoplasm of a predominantly mature megakaryocyte) can be observed (Fig. 1E). Megakaryoblast can be identified by means of immunocytochemistry. *Promegakaryocyte*, synonym of basophilic mega-

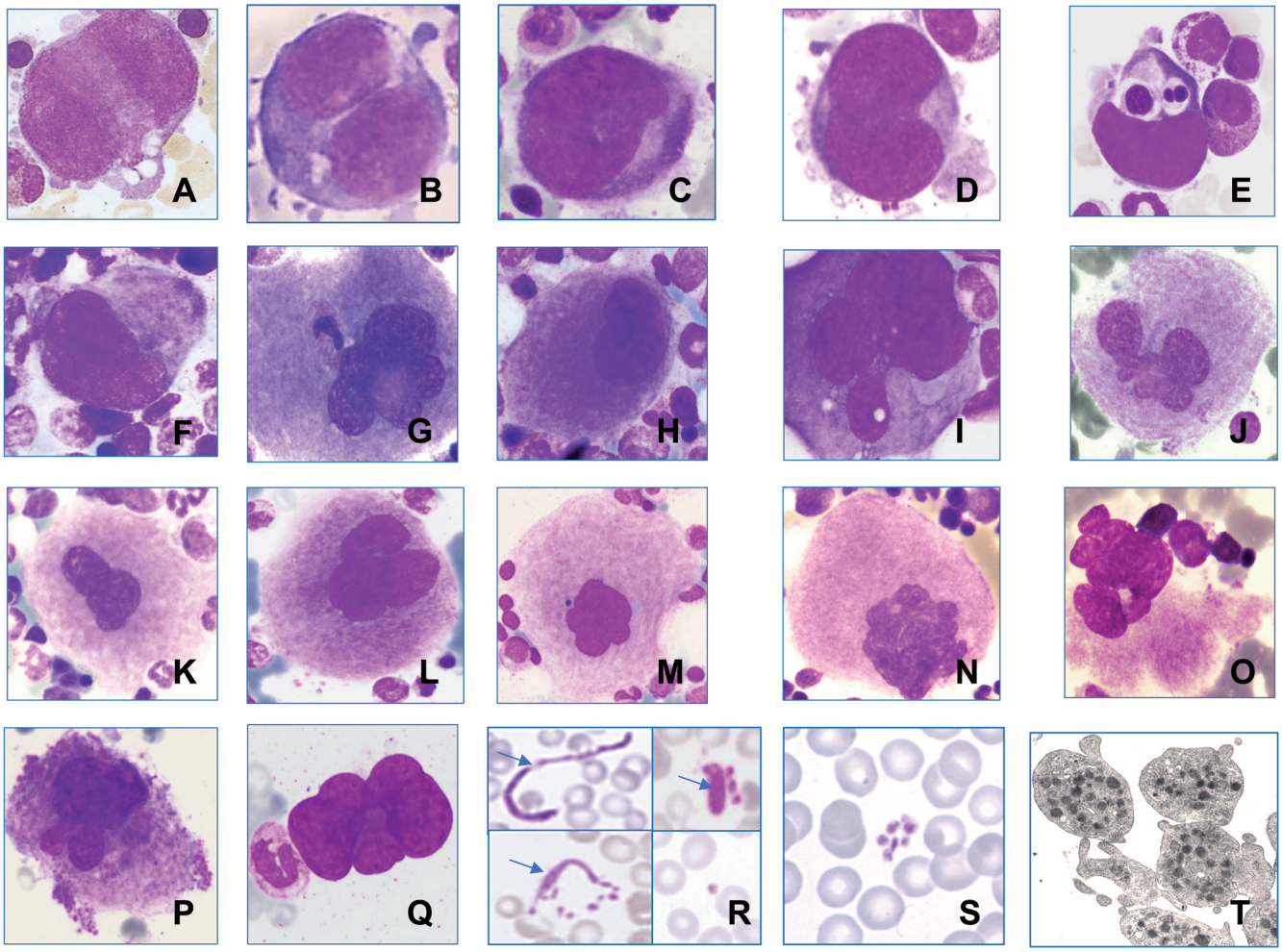


Fig. 11. Normal bone marrow smear. May-Grünwald-Giemsa stain. **A-E.** Megakaryoblasts. **F-I.** Promegakaryocytes or basophilic megakaryocyte or immature megakaryocyte. **J-L.** Granular megakaryocyte. **M-P.** Mature megakaryocyte with strands of platelets detaching from the periphery of the cytoplasm (proplatelets). **Q.** Bare nucleus. **R.** Several proplatelets (arrow) and platelets. **S.** Platelets. **T.** Ultrastructural aspect of sevaral platelets.

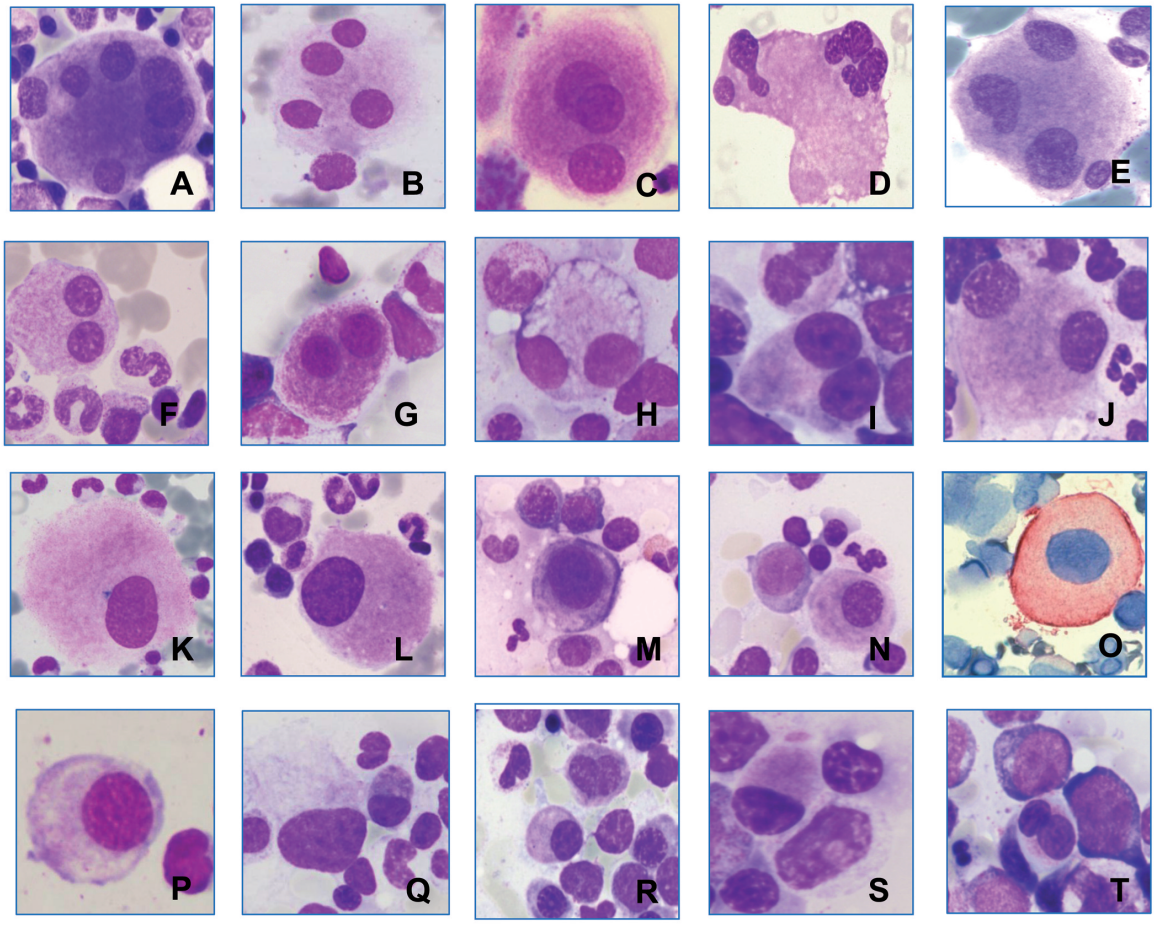


Fig. 12. Bone marrow smears from patients with MDS. May-Grünwald-Giemsa stain. **A-E.** Multinucleated megakaryocytes with scattered nuclei without connection among them. **F-J.** Binucleated megakaryocytes with different cell size. **K-N.** Non lobulated megakaryocyte or mononuclear megakaryocytes with different cell size or non lobulated **O.** Megakaryocyte immunostained with CD61 (GPIIIa membrane glycoprotein). **P.** Non lobulated megakaryocyte with cytoplasmic abnormalities. **Q-T.** Micromegakaryocytes (arows).

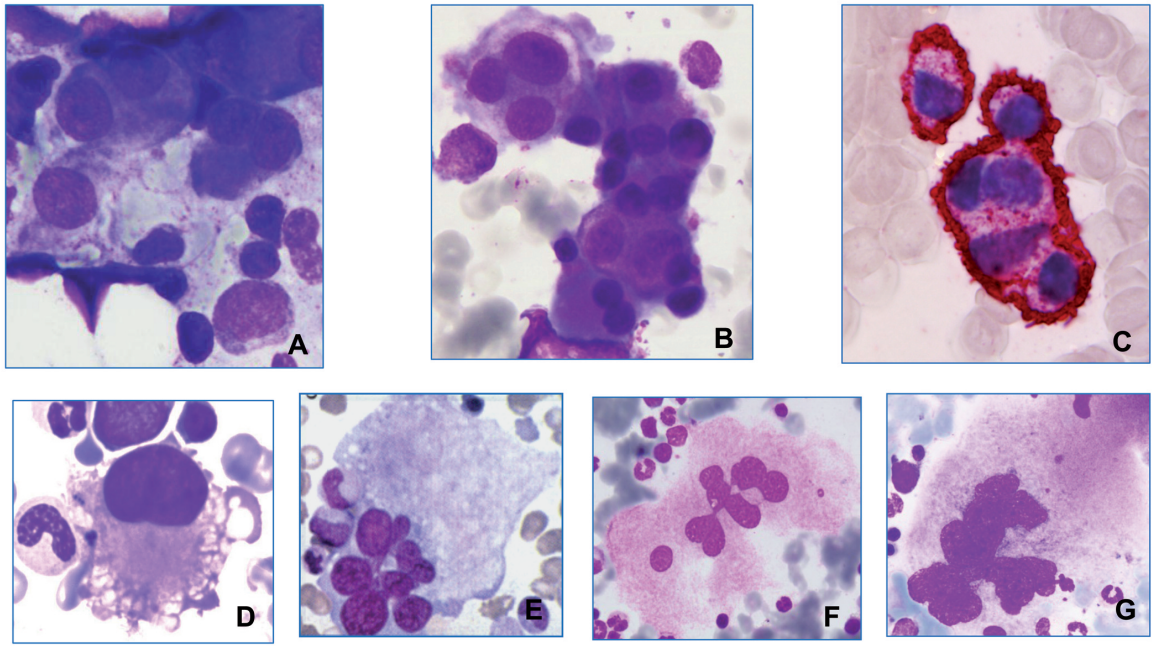


Fig. 13. A.B. Sheets of micromegakaryocytes with small lymphocyte-sized nuclei. **C.** Sheet of micromegakaryocytes immunostained with CD61 (GPIIIa membrane glycoprotein). **D.** Megakaryocyte with large peripheral cytoplasmic dilatations. **E.** Megakaryocyte with cytoplasm completely devoid of granulation with persistent basophilia. **F, G.** Giant and hyperlobulated megakaryocytes.

karyocyte and of immature megakaryocyte, is a cell variable in size with a lobulated nucleus (sometimes horseshoe-shaped) with a rather low ploidy and a deep basophilic cytoplasm in which progressive appearance of pseudo-granules can be seen (Fig. 11F-I). *Granular megakaryocyte* is a large cell (diameter even larger than 150 μm) with a nucleus, with elevated ploidy that is formed by large unequal lobes joined by bands of chromatin. The cytoplasm is abundant showing increasing acidophilia (Fig. 11J-L). *Mature megakaryocyte;* large cell whose cytoplasm is strongly eosinophilic and divided into numerous platelet fields corresponding to future platelets (Fig. 11M-P). Sometimes strands of platelets detaching from the periphery of the cytoplasm can be seen (proplatelets). These proplatelets are fragmented into platelets (Fig. 11O,P).

Bare nucleus; nucleus with or without remnants of residual cytoplasm represents the last stage of megakaryocytic differentiation (Fig. 11Q). *Platelets* or thrombocytes are small particles (without nucleus) detached from areas of the megakaryocytic cytoplasm. They show a hyalinic peripheral zone (hyalomere) and a centrally located acidophilic granular zone (chromomere). Some proplatelets can also be observed in peripheral blood (Fig. 11R-T).

We depict the main cytological traits of dysmegakaryopoiesis affecting nucleus and cytoplasm. Multinucleated megakaryocyte, megakaryocyte with scattered nuclei without connection among them (Fig. 12A-E). This cell should be differentiated from osteoclasts that are also giant cells with a finely grained cytoplasm that present several nuclei totally individualized, with condensed chromatin and a single nucleolus; these nuclei are usually grouped. Binucleated megakaryocyte with different cell sizes and with two

Stages of maturation of the normal monocytic lineage

Cell	Size	Nucleus	Visible nucleoli	Cytoplasm
Monoblast	15-20 µm	• Large and round • Not condensed chromatin	3-4	Abundant basophilia (strong leaden-blue)
Promonocyte	15-25 µm	• Irregular border with folds • Chromatin scarcely condensed	1-2	Abundant fine azurophilic granules
Monocyte	15-30 µm	• Central • Irregular with folds • Compacted stripped chromatin	No	Abundant azurophilic granules, sometimes with small vacuoles
Histiocyte/macrophage*				

*Can adopt different morphological aspects depending on the organ or tissue where it is finally located and on the nature of the engulfed or treasured material.

Fig. 14. General characteristics of monocytic differentiation.

separate mature nuclei (Fig. 12F-J). Non lobulated megakaryocyte or mononuclear megakaryocytes with varying cell sizes and a non-lobulated nucleus with regular profile (Fig. 12K-P). These megakaryocytes must be differentiated from normal megakaryocytes with low ploidy and superimposed nuclei that presents a discrets irregular profile (Fig. 12K). Micromegakaryocyte. Its size is equal or smaller than a promyelocyte (Fig. 12Q-T). They usually are mononucleated or binucleated with scanty basophilic cytoplasm or with many granules in those with a more mature appearance (Matsuda et al., 2007; Brunning et al., 2008; Goasguen et al., 2016; Hasserjian et al., 2017). Sometimes they present small hyperchromatic nuclei with a cytoplasm with platelet fragmentation. Sheets of micromegakaryocytes with small lymphocyte-sized nuclei can also be observed (Fig. 13A,B). The immunostaining with CD61 (GPIIIa membrane glycoprotein) allows the correct identification of these dysmorphic elements (Fig. 13C). Megakaryocytes with cytoplasmic abnormalities, such as large peripheral cytoplasmic dilations (Fig. 13D) or with cytoplasm completely devoid of granulation with persistence of basophilia (Fig. 13E). Large megakaryocytes with hypersegmented nuclei (Fig. 13F,G).

Morphological characteristics of monopoiesis

General characteristics of monocytic differentiation

The maturation process is characterized by small changes in cell size in all differentiation phases. The morphological categories are depicted in Fig. 14: *Monoblast;* large cell with a round nucleus and a very loose chromatin network. Nucleoli are variable in number and usually very obvious. The cytoplasm with a regular border is voluminous and basophilic with rare azurophilic granules (Fig. 15A). Sometimes the monoblast has a morphology indistinguishable from the myeloblast. *Promonocyte;* large cell with a low nucleus/cytoplasmic ratio. The nucleus is large and irregular in shape with a folded or convoluted outline. The chromatin is reticular but shows some spots of condensation. There are one or two nucleoli. The cytoplasm is abundant, weakly basophilic, containing delicate azurophilic granules (Fig. 15B). *Immature monocyte;* displays a convoluted or indented nucleus, the chromatin appears more condensed with rare nucleoli.

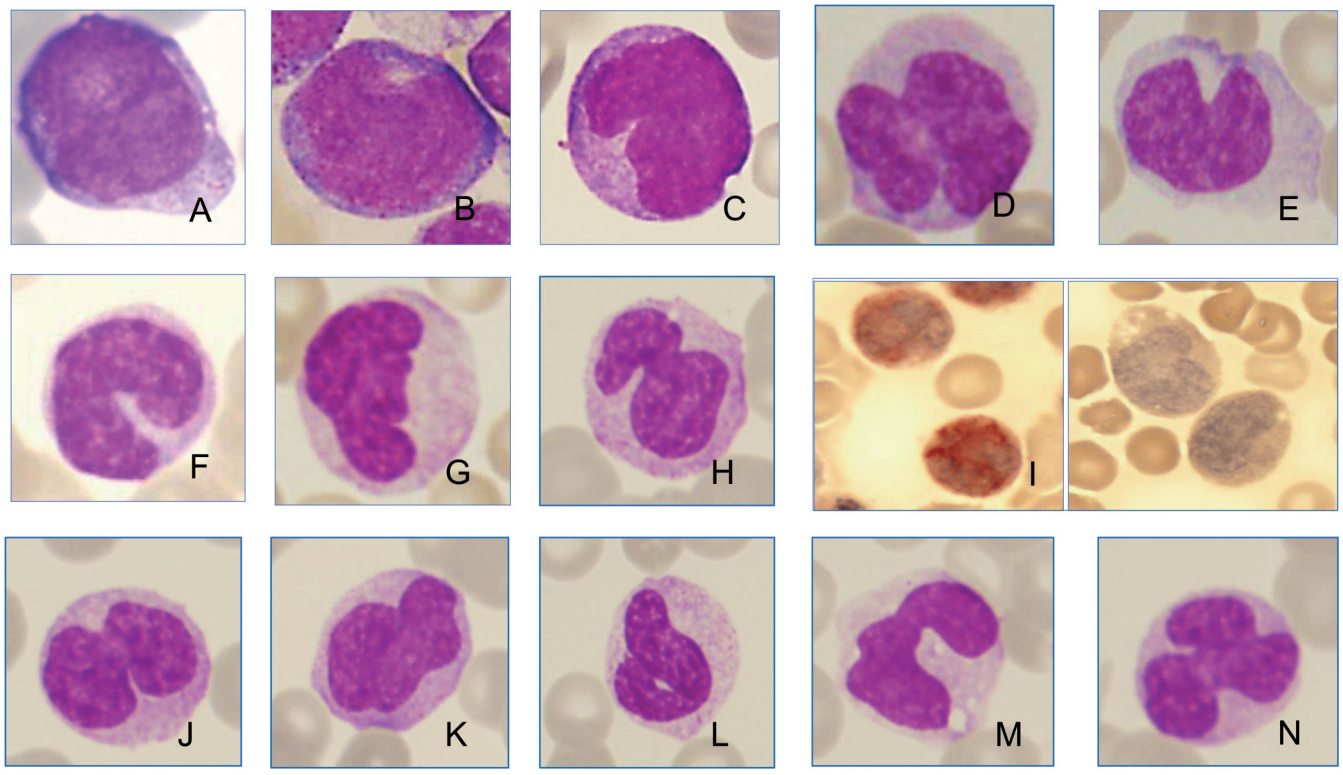


Fig. 15. General characteristics of monocytic differentiation. Bone marrow smears. May-Grünwald-Giemsa stain. **A.** Monoblast. **B.** Promonocyte. **C.** Inmature monocyte. **D-H.** Mature monocytes. **I.** Monocyte whith Napthyl butyrate esterase activity; this activity is inhibtable by FNa incubation. **J-N.** Monocytes from peripheral blood of a patient with inflammatory disease.

The degree of cytoplasmic basophilia is intermediate between that of promonocytes and that of *mature monocytes* (Fig. 15C). Monocyte is the largest cell of normal blood. The nucleus is large and of a variable shape (round, oval, reniform, folded). The chromatin appears spongy, lightly stained with a very characteristic spaced pattern. Nucleoli are not visible. The cytoplasm is voluminous with a ruffled border or shows fine projections. Its cytoplasm is light blue or grayish containing numerous minute azurophilic granules. Small vacuoles are common (Fig. 15D-N). *Histiocyte/macrophage;* this final stage of monocytic differentiation is not present in peripheral blood. Their morphological characteristics are variable and depending on the tissue where they are finally located and on the characteristics of the phagocytosed or stored material.

The morphological subtypes of normal monocytic cells do not differ from those observed in infected patients, or in those with inflammatory disease or with leukemic proliferation. The identification of immature monocytes and their distinction from promonocytes is of critical importance in making the distinction between acute myeloid leukemia and chronic myelomonocytic leukemia (CMML). In these patients promonocytes (blast equivalent cells) must be separated from monocytes, which can have abnormal features in CMML and for this reason a precise morphological evaluation is essential (Fig. 16) (Brunning et al., 2008; Hasserjian et al., 2017).

Final remark

In spite of the advances in flow cytometry, cytogenetics and molecular biology, the optical

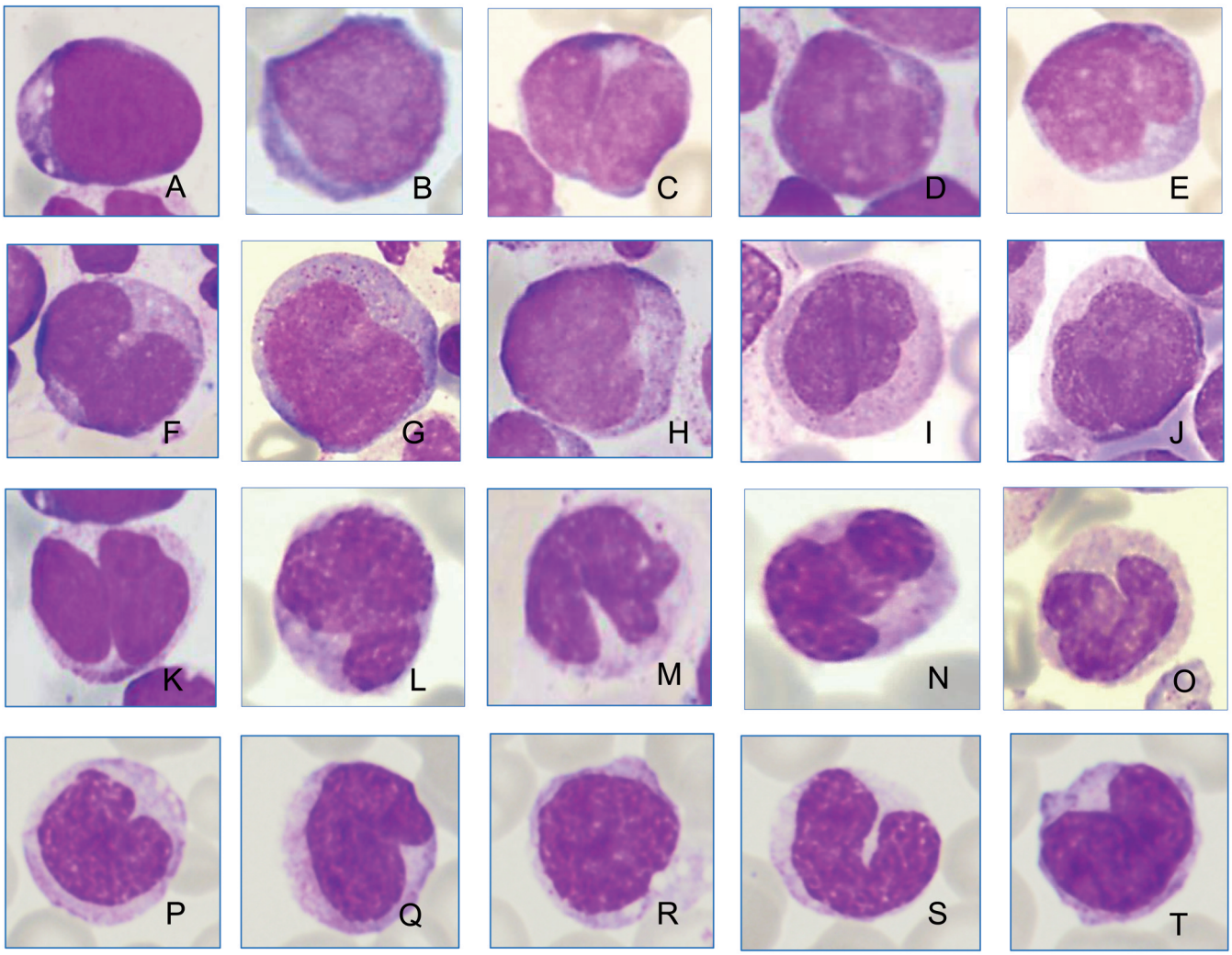


Fig. 16. Bone marrow smear from patient with chronic myelomonocytic leukemia. May-Grünwald-Giemsa stain. **A, B.** Monoblasts. **C-J.** Promonocytes. **K.** Inmature monocyte. **L-T.** Mature monocytes.

cytomorphology remains as the cornerstone of diagnosis of MDS, having a crucial value in diagnosis, classification and prognosis (Brunning et al., Della Porta 2015; Hasserjian et al., 2017). The importance of maintaining the competence in morphology assessment requires experience and continuous training. Reports offering together normal and dysplastic myelopoiesis could serve as a useful tool for quotidian process of diagnosis. Therefore, we expect that this manuscript will be useful for the characterization of morphological myelodysplasia.

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