REVIEW ARTICLE

Morphology of leukaemias

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Abstract Acute leukaemias are characterised by uncontrolled proliferation of immature blood cells with lymphoid or myeloid lineage. Morphological classification is based on the identification of the leukaemia cell line and its stage of differentiation. The first microscopic descriptions dating from the 1930s pointed to 2 different types of leukaemia cells: lymphoid and myeloid. In 1976, the consensus that led to the French-American-British (FAB) classification was achieved. This includes criteria for identifying myeloid and lymphoid leukaemias, and gives a list of morphological subtypes, describing how these affect the patient's prognosis. Today, despite new classifications based on sophisticated studies, FAB classification is widely used by experts due to its technical simplicity, good diagnostic reliability and cost-effectiveness.

Morfología de las leucemias

Resumen Las leucemias agudas se caracterizan por proliferación descontrolada de células progenitoras de estirpe linfoide o mieloide. La clasificación morfológica se basa en la identificación de la línea celular de la célula leucémica y de la etapa de diferenciación. Desde las primeras descripciones microscópicas durante el primer tercio del siglo XIX se plantearon dos tipos distintos de las células leucémicas: linfoides y mieloides. Fue hasta 1976 cuando se logró el consenso que dio origen a la clasificación Franco-Americano-Británica (FAB) que conserva la gran división entre mieloides y linfoides, además de asignar subtipos a partir de características morfológicas, destacando la traducción directa que tienen sobre el pronóstico del paciente. Hoy

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Background

The word leukaemia (originally *leukämie* in German) comes from the Greek words *leukos* (λευκός): white and *haima* (αἷμα): blood. The modern term leukaemia was first proposed by Rudolf Virchow in 1846.[1] Unsurprisingly, the term was suggested after examining infected blood under the microscope, an instrument that was first invented in the 17th century, but only perfected 2 centuries later and used by physicians to identify alterations in tissues and cells.

The famous French physician Alfred Armand Louis Marie Velpeau (1795–1867) is credited with providing the first anatomical and pathological description of leukaemia. The patient was a 63-year-old man called Monsieur Vernis, who earned his living selling flowers and lemonade in Paris, and complained of fever, weakness, and an enlarged liver and spleen. Velpeau performed the autopsy on the patient, observing a greatly enlarged spleen weighing 4.5 kg and noting that the blood was very thick with a consistency similar to that ''of porridge''. Under the microscope, he observed a large quantity of ''globules of pus in the blood''. Velpeau reported the histological and anatomical findings in this patient to the Royal Academy of Medicine in Paris, and the article was published in March of 1827. A few years later, in 1839, Alfred François Donné, also a Paris-based physician and an enthusiast of microscopy as a diagnostic tool, described the case of a woman with splenomegaly and increased levels of ''mucous globules'' in the blood. The case caught the attention of one of Donné's foreign disciples, the English pathologist John Hughes Bennett (1812–1875). On retuning to his native land, Bennett came across a similar case of organ enlargement and changes in the consistency and colour of the blood, which he described in detail in the *Edinburgh Medical and Surgical Journal* (1845). Six years later, Bennett dubbed this pathology ''leukocytosis''.[1,2]

Five or six weeks after publication of Bennett's report, a report titled *Weisses Blut* (white blood, in German) was published in the German journal *Neue Notizen aus Gebiete der Natur-und Heilkunde*. The article reported the case of a 50-year-old cook by the name of María Straide, with a 4-year history of chronic fatigue and abdominal distension. On physical examination, the patient presented an enlarged, slightly tender spleen, oedema of the lower limbs, and diarrhoea. She had complained of epistaxis that was lasting for 8 days, rash, and weakness. The autopsy showed loss of colour in all organs, ''white spots'' on the liver, and a greatly enlarged spleen. Microscopic findings were ''everywhere in the blood vessels a mass closely resembling pus was found, with colourless corpuscles''. The author of the report was the young 24-year-old German pathologist Rudolf Virchow (1821–1902). These observations gave rise to
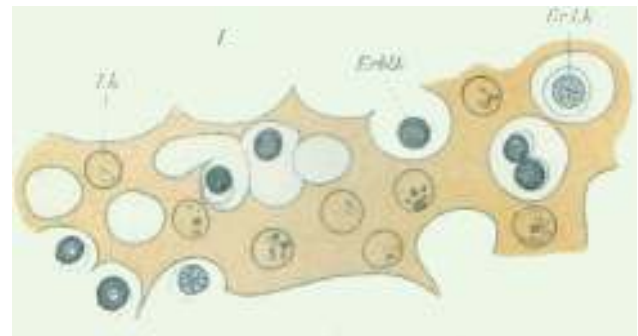

Figure 1   First published sketch of the origin of leukaemic cells, by Ernst C. Neumann. Public domain image, taken from: Wikimedia Commons.

the term leukaemia (*leukämie*). Although Virchow was quick to praise Bennett and addressed him with great respect, the appearance of his report so soon after Bennett's, proposing a different name for the same condition, sparked considerable controversy.[2,3]
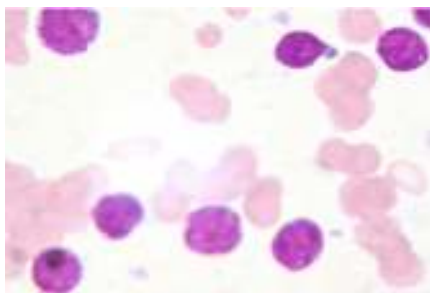
The next major step in the story of leukaemia morphology occurred in 1869, when both blood and bone marrow were studied under the microscope. Studying bone fragments, Franz Ernst C. Neumann (1834–1918), a German pathologist born in Königsberg, pupil of Hermann Ludwig F. von Helmholtz (1821–1894) and Virchow, was the first to show that haematopoiesis takes place in the bone marrow. He hypothesised that blood originates from a common precursor, which he called a stem cell (*Stammzelle* in German) (Fig. 1).[4,5] Neumann also suggested that, in addition to the lymphatic and splenic leukaemias proposed by Virchow, there was another type of leukaemia that originated in the bone marrow. He called this ''myeloid leukaemia'', and his theory was later validated by Bizzozero.[1,3]

The last breakthrough in this brief history of the morphology of leukaemia was spearheaded by Paul Ehrlich (1854–1915). While still a medical student, Ehrlich developed the technique of cell staining, and observed for the first time the cytoplasmic components and different types of nuclei in blood cells, for which he coined the terms acidophils and basophils.[6]

Morphology of acute leukaemias

Acute leukaemias are characterised by the uncontrolled proliferation of myeloid or lymphoid progenitor cells.[1] Their morphological classification is based on the identification of the leukaemia cell line and stage of cell differentiation.[1,6]

FAB classification of lymphoblastic leukaemia

L1   Lymphoblastic leukaemia with
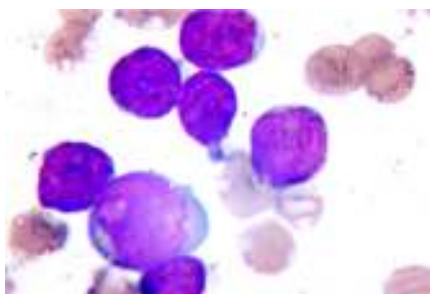   homogeneous structure

Frequency:

Between 25% and 30% of cases in adults,
and 85% of cases in children.

Morphology:

Blasts are homogeneous, nucleus is regular,
chromatin is homogeneous,
small or no nucleoli, scanty cytoplasm,
and mild to moderate basophilia.

Immunophenotype

B:	T:
• CD19	•CD3
•CD22	•CD7
•CD79a	•CD5
•CD10	•CD2
•CD20	•CD4
•Cytoplasmic or superficial immunoglobulin	

L2   Lymphoblastic leukaemia with
         varied structure
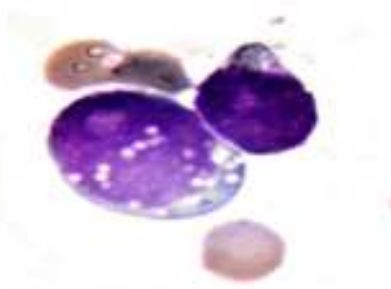
Frequency:

Accounts for 70% of cases in
adults, and 14% in children.

Morphology:

Nucleus is irregular, heterogeneous chromatin
structure, large nucleoli.

Immunophenotype

B:	T:
•CD19	•CD3
•CD22	•CD7
•CD79a	•CD5
•CD10	•CD2
•CD20	•CD4
•Cytoplasmic or superficial immunoglobulin	

L3   Burkitt's leukaemia

Frequency:

Rare subtype, accounting for
less than 1% to 2% of cases.

Morphology:

Large blasts, prominent nucleoli, stippled
homogeneous chromatin structure, abundant
cytoplasm, abundant cytoplasmic vacuolation
(bubble type) covering the nucleus.

Immunophenotype

B:	T:
•CD19	•CD3
•CD22	•CD7
•CD79a	•CD5
•CD10	•CD2
•CD20	•CD4
•Cytoplasmic or superficial immunoglobulin	

Figure 2   French-American-British (FAB) classification of acute lymphoblastic leukaemia. FAB classification of lymphoblastic leukaemia.

During the latter half of the 20th century, a number of leukaemia subgroups were defined, based on the correlation between their morphology and clinical characteristics, laboratory findings, and response to treatment. A number of classifications were proposed and later discarded as unsuitable for all types of patients. In some of these, the criteria were ambiguous; some used complicated and scarcely reproducible methods, while others used different terminology to describe the same type of leukaemia, thus creating an atmosphere of confusion. In 1976, this situation prompted 7 haematologists to come together to form an international cooperative group that gave rise to the **French-American-British (FAB)** classification system, which divided leukaemia in 2 types: myeloid and lymphoblastic.[7] The classification system can only be used in previously untreated patients, as chemotherapy distorts both normal and malignant cells.[8]

The following is a summary, with photos, of the FAB acute leukaemia classification system.

FAB classification of acute lymphoblastic leukaemia

Fig. 2 shows the subtypes, morphology, immunophenotye and prevalence of acute lymphoblastic leukaemias (ALL).[9]

Due to difficulties in distinguishing L1 and L2 subtype cells, the FAB group published an amended version of their classification system in 1981. In this, they proposed a scoring system for identifying L1 and L2 leukaemias on the basis of 4 cytological characteristics: nucleus:cytoplasm ratio, presence of nucleoli, characteristics of the nuclear membrane, and cell size (see Table 1).[10]
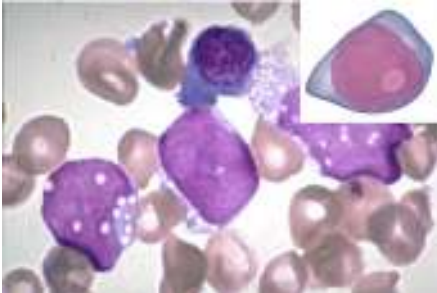
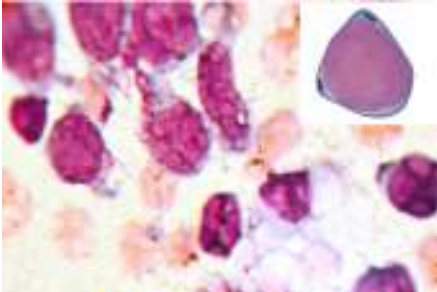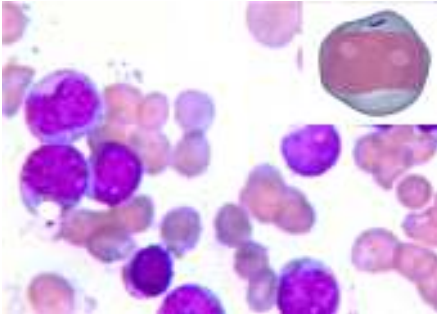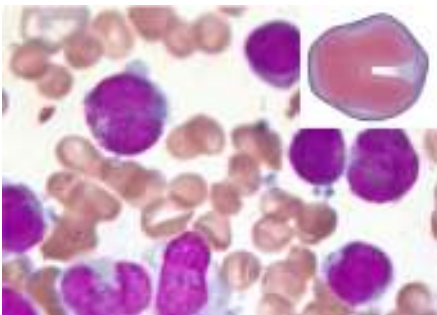## FAB classification of acute myeloblastic leukaemia

M0   Acute myeloblastic leukaemia
with minimal differentiation

Morphology:

Can resemble LLA-L2 blasts. Medium-sized
blasts, rounded nucleus, fine chromatin,
basophilic non-granular cytoplasm,
prominent nucleoli.

Immunophenotype

- CD13 +
- CD33 +
- CD11b +
- CD11c +
- CD14 +
- CD15 +

Photo courtesy of: Acute myeloid leukemia pathophysiology, 2012

M1   Acute myeloblastic leukaemia
without maturation

Morphology:

Medium-sized blasts with high nucleo:cytoplasm (n:c)
ratio, rounded nuclei with immature, dispersed chromatin
with one or more prominent nucleoli. Blasts can show
fine azurophilic granulation or isolated Auer rods in
the cytoplasm in 5% to 10% of cases

Immunophenotype

- MPO +
- CD13 +
- CD33 +
- CD117+
- •CD34 +/-

M2   Acute myeloblastic leukaemia
with maturation

Morphology:

Small to medium-sized blasts with high nucleo:
cytoplasm (n:c) ratio and rounded nuclei sometimes
located in a corner of the cytoplasm. The nucleus
shows dispersed, immature chromatin with one or more
nucleoli. The cytoplasm is basophilic and can contain
traces of primary azurophilic granulation or isolated
Auer rods.

Immunophenotype

- MPO +
- CD34 +/-
- CD13 +
- CD15 +
- HLA-DR +/-
- Sudan black +
- CD117 +/-

M3   Promyelocytic leukaemia

Morphology:

Abundant, intensely azurophilic granulation.
The nucleus is usually monocytic in appearance
(reniform) and is either irregular or bilobed with a
deep cleft. Scarcely basophilic cytoplasm due to the
proliferation of azurophilic granulation. Some atypical
promyelocytes also contain elongated or splinter-
shaped crystalline cytoplasmic inclusions specific to
this type of leukaemia.These usually form clumps,
but differ from Auer rods in that they show a tubular
substructure on electronic microscopy.

Immunophenotype

- CD13 +
- CD33 +
- HLA-DR -
- CD34 -

Figure 3   French-American-British (FAB) classification of acute myeloblastic leukaemia. FAB classification of acute myeloblastic leukaemia.

FAB classification of acute myeloblastic leukaemia

Figs. 3 and 4 show the immunophenotype and morphology of
acute myeloblastic leukaemias (AML), together with micro-
scope images and a brief summary.[9,11,12]

M0 Acute myeloblastic leukaemia

Myeloblastic leukaemia with minimal differentiation, or
M0-AML, is particularly difficult to diagnose from the mor-
phological point of view because the blasts present both
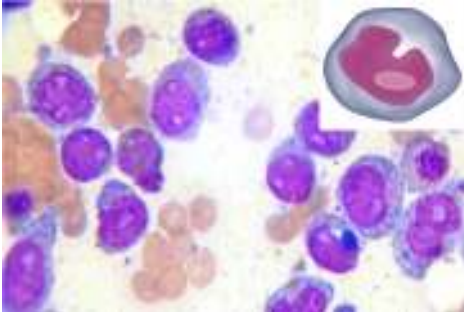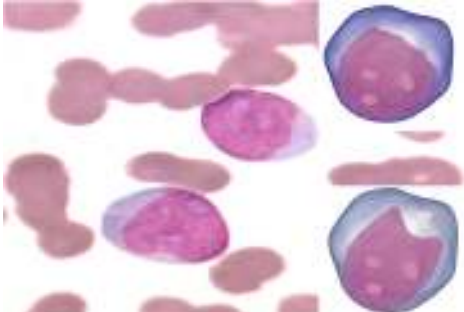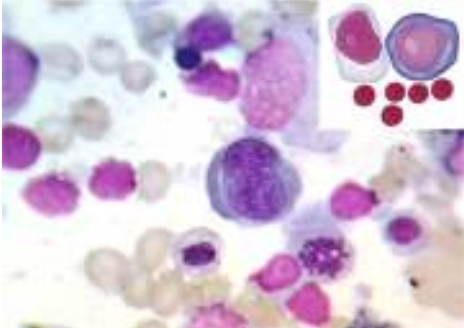
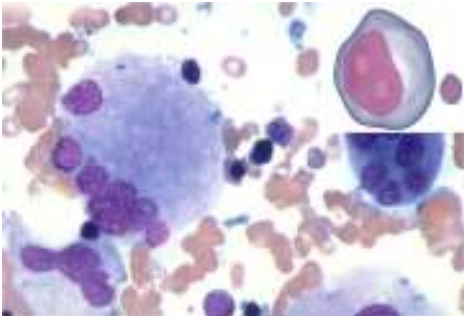## FAB classification of acute myeloblastic leukaemia

M4   **Acute myelomonocytic leukaemia**

Morphology: *Immunophenotype*

Large blasts, moderate nucleo:cytoplasm
(n:c) ratio and variable basophilia. The
nucleus may be rounded, kidney-shaped
or irregular. Nucleoli are usually prominent.

- CD13 +
- CD15 +
- CD33 +
- CD11b +
- CD11c +
- CD14 +
- CD64 +
- CD4 +

M5   **Acute monocytic leukaemia**

Immunophenotype

M5a acute monoblastic leukaemia:
Large blasts with rounded nucleus and dispersed,
immature chromatin (1-3 nucleoli) and moderately large
and intensely basophilic cytoplasm. The cytoplasm may
show some Auer rods and/or prolongations
and granulations.
M5b acute monocytic leukaemia
Promonocytes have a rounded or kidney-shaped
nucleus with a less basophilic cytoplasm that is
more highly granulated than monoblasts and contains
some vacuoles. A findings of erythrophagocytosis
together with monocytic blasts suggests a t(8;16)
translocation.

- CD14 +
- CD68 +
- CD4 +
- CD11c +
- HLA-DR +
- CD64 +

M6   **Acute erythroid leukaemia**

M6a erythroid leukaemia with proliferation of mixed blasts:
Over 50% erythroid precursors and around 30% myeloblasts.
Morphology of erythrocytes in peripheral blood is greatly changed,
with schistocytes, "pincered" or mushroom-shaped cells,
and spiculated echinocyte and acanthocyte cells.
M6b pure erythroid leukaemia:
Erythroids make up 80% of bone marrow
cells, with less than 3% myeloid cells.
Erythrocytes in peripheral blood consist
of macrocytes, basophilic stippling,
Howell-Jolly bodies or Cabot rings.

Immunophenotype

- CD13 +
- CD33 +
- CD15 +
- Glycophorin A +
- Glycophorin C +

M7   **Acute megakaryocytic leukaemia**

Morphology: *Immunophenotype*

Highly immature, polymorphic blasts. The nucleus is
eccentric with dispersed, reticulated chromatin and
1-3 prominent nucleoli. The cytoplasm is non-granular,
basophilic, and very similar in appearance to platelets,
with pseudopods or granulations. Micromegakaryocytes
and fragments of megakarioblasts are seen in peripheral
blood (giant platelets, some highly degranulated).

- CD41 +
- CD61 +
- CD42 +
- CD13 +
- CD33 +
- CD34 +

Figure 4   French-American-British (FAB) classification of acute myeloblastic leukaemia (continued).

Table 1 FAB classification of acute lymphoblastic leukaemia.

Criterion	Classification
High nucleus:cytoplasm ratio > 75% of cells	+
Low nucleus:cytoplasm ratio > 25% of cells	−
Nucleoli: 0–1 (small) > 75% of cells	+
Nucleoli: 1 or more (prominent) > 25% of cells	−
Irregular nuclear membrane > 25% of cells	−
Megaloblasts > 50% of cells	−

Obtain 1 classification in each category, sum total of signs. Total classification (−4 to +2): 0 to +2 L1 acute lymphoblastic leukaemia; −1 to −4 L2 acute lymphoblastic leukaemia.

lymphoblastic and myeloblastic features. They account for only 5% of adult AMLs.[13]

M1 Acute myeloblastic leukaemia

In acute myeloblastic leukaemia without maturation (M1), cells are usually monomorphic, with myeloid blasts in peripheral blood and no cells beyond the myeloblast stage of differentiation. Leukocytes are present at diagnosis.

M2 Acute myeloblastic leukaemia

Acute myeloblastic leukaemia with maturation (M2) accounts for around 30% of all cases of AML. Over 10% of cells are at the post-myeloblast stage (promyelocytes, myelocytes and neutrophils). One-third of all cases of AML-M2 are associated with *t(8;21)* translocation

M3 Acute myeloblastic leukaemia

Acute promyelocytic leukaemia (M3) is usually accompanied by low peripheral blood leucocyte levels, which makes diagnosis difficult. The majority of cells have a very characteristic morphology called *atypical promyelocytes* (hypergranular cells). Symptoms can include severe haemorrhage due to disseminated intravascular coagulation. The cytogenic change characteristic of AML-M3 is *t(15;17)*, which leads to the fusion of the *PML* (*promyelocytic leukaemic*) and retinoic acid receptor-alpha (*RARα*) genes, resulting in the *PML-RARα* transcript.

Microgranular variant of AML-M3
This accounts for between 15% and 20% of all cases of M3. Prognosis is poorer than normal M3, and leukocytosis is usually found. Morphologically, it is characterised by sparsely granulated leukaemia cells. The cytoplasm is more basophilic that in normal M3, due to a lower concentration of azurophilic granules.

M4 Acute myeloblastic leukaemia

Acute myelomonocytic leukaemia (M4) accounts for 20% of all cases of AML. Clinically, 10% of patients present gingival hyperplasia. This type of leukaemia has a granulocyte component and a monocyte component in varying proportions and different degrees of maturation. Cytochemically, they are positive for chloroacetate esterase, and monocytes are positive for naphthol-AS-D-acetate esterase or α-naphthyl butyrate esterase.

M4Eo variant: this occurs when at least 5% of cells are abnormal eosinophils, presenting monocytoid nuclei and atypical granules

M5 Acute myeloblastic leukaemia

This represents around 15% of all cases of AML. The leukaemic cells are of a monocytic lineage (monoblasts and promonocytes). This class is divided into: *M5a* or acute monoblastic leukaemia, in which monoblasts predominate, and accounts for between 5% and 8% of all AMLs; and *M5b* or acute monocytic leukaemia, in which a high proportion of promonocytes and monocytes are found, in addition to monoblasts. This type comprises 3% to 6% of AMLs.

The monoblastic form is usually associated with *t(9;11)*, *t(6;11)* and *t(8;16)* genetic translocations and *11q23* rearrangements (AML with recurrent genetic abnormalities according to the World Health Organization [WHO]).[9]

M6 Acute myeloblastic leukaemia

The M6 erythroid sub-type is defined by the FAB as a proliferation of dysplastic erythroid elements with a proliferation of blasts of myeloid origin. The WHO classification characterises 2 sub-types: erythroleukaemia (M6a), described as a mixed proliferation of myeloid and erythroid blasts, and can be secondary to a previous myelodysplastic syndrome. M6a accounts for 5–6% of all AMLs. The other subtype is the pure erythroid variant (M6b), which occurs when dyserythropoiesis is prominent.

M7 Acute myeloblastic leukaemia

Acute myeloblastic leukaemia (AML-M7) represents between 3% and 5% of all AMLs.[9]

Conclusions

Other leukaemia classifications have now gained prominence, such as that proposed by the European Group for the Immunological Characterization of Leukaemias (EGIL), based on immunophenotyping criteria,[14,15] or more recently, the classification drawn up by the World Health Organisation in 2001, updated in 2008, which combines morphology cytochemistry, immunophenotyping, clinical presentation, and genetic abnormalities. The WHO classification is now regarded as the gold standard for leukaemia diagnosis.[9,16] Despite the benefits of the FAB classification, molecular biology and immunophenotyping studies are essential for reaching an accurate diagnosis, choosing the right therapy, and establishing a prognosis. For these reasons, these tests are wholly justified, despite the high cost for both the patient and the hospital.[17]

For specialists, the purely morphological criteria, in other words, Wright's stained smears, of the FAB

classification are a powerful diagnostic tool. Browman was one of the first to evaluate intra-observer (64.8% and 70.5%) and inter-observer (63% and 72%) concordance between 2 experts. He found that concordance increased to 89% when cytochemistry studies were added to FAB, and reached 99% when immunophenotyping information is available.[18] Similar findings were reported in subsequent studies.[19] Curiously, a study performed in our hospital by Sales-Carmona et al. found that inter- and intra-observer concordance was good when using morphological criteria, while the addition of cytochemistry tests actually increased diagnostic disagreement.[20]

With this review, we hope to give clinicians an introduction to the morphological classification of leukaemias. We believe that far from underestimating these systems or considering them antiquated, haematologists should be trained in their use. After all, as in any human activity, practice makes perfect.

Conflict of interest

The authors declare that they have no conflict of interests.

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