Guideline for investigation of adults and children presenting with Thrombocytosis

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Commissioned by the BCSH .

The purpose of this guideline is to provide an approach to the

Diagnosis,

Investigation

and management of patients with a thrombocytosis (i.e., a platelet count >450 × 10^9/l).

This will include advice on how to distinguish reactive thrombocytosis from true haematological disease and how to distinguish essential thrombocythaemia (ET) from other myeloproliferative neoplasms (MPN), myelodysplastic syndromes (MDS) or overlap syndromes.

Thrombocytosis is a common finding and is a frequent cause of referral for further investigation. There is a wide range of primary and secondary causes as well as false or ‘spurious’ conditions mimicking thrombocytosis .

Establishing the cause therefore requires consideration of

1- Clinical features,

2-Haematological parameters,

3-Bone marrow aspirate and trephine biopsy morphological features and

4- the presence or absence of clonal genetic abnormalities.

Primary	Secondary	Spurious
Essential thrombocythaemia	Infection	Microspherocytes (e.g. severe burns)
Polycythaemia vera	Inflammation	Cryoglobulinaemia
Primary myelofibrosis	Tissue damage	Neoplastic cell cytoplasmic fragments
Myelodysplasia with del(5q)	Hyposplenism	Schistocytes
Refractory anaemia with ring sideroblasts associated with marked thrombocytosis	Post-operative	Bacteria
Chronic myeloid leukaemia	Haemorrhage	Pappenheimer bodies

Iron deficiency

Atypical chronic myeloid leukaemia

Malignancy

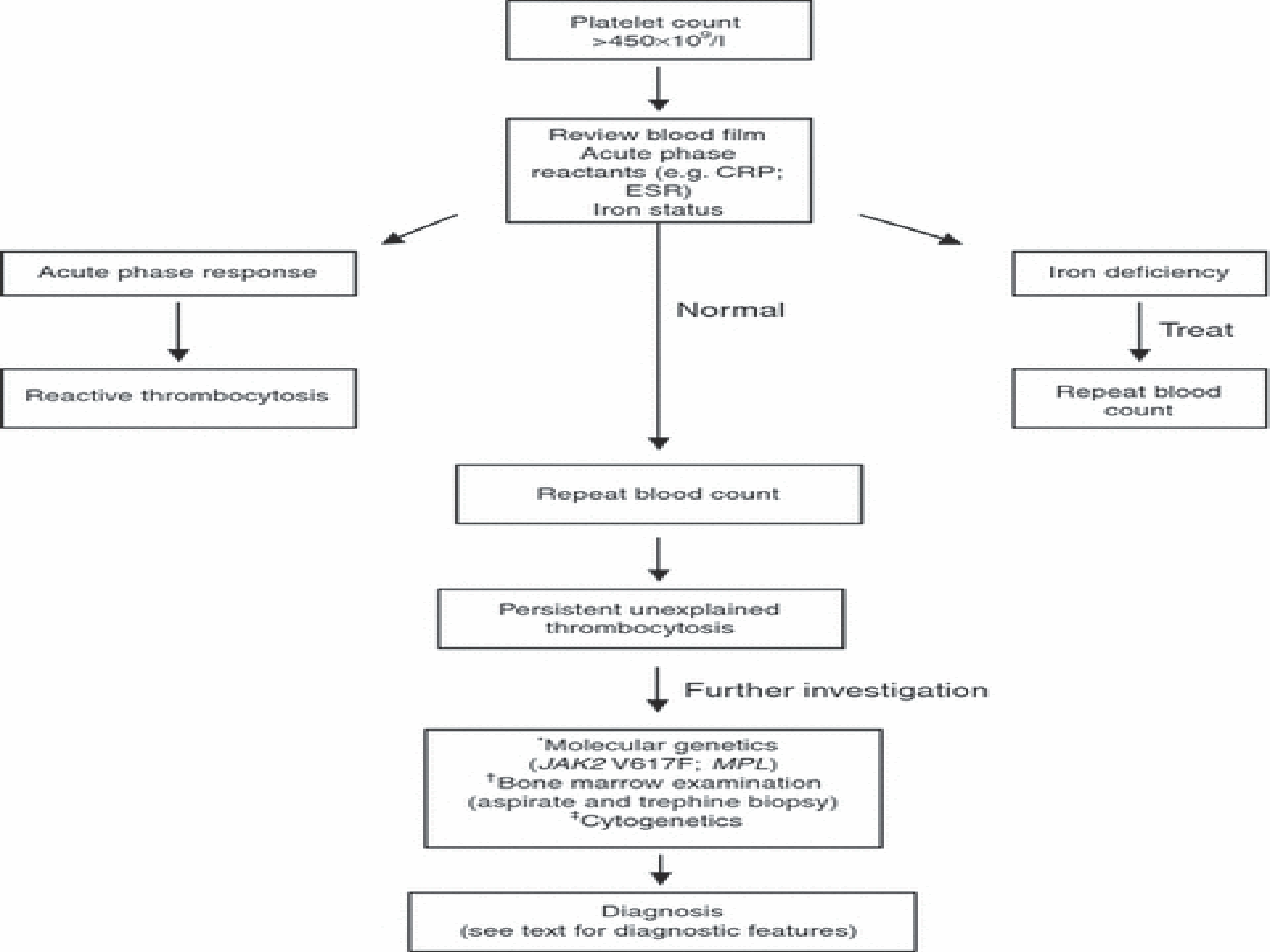
MDS/MPN-U

Haemolysis

Drug therapy (e.g. corticosteroids; adrenaline)

Cytokine administration (e.g. thrombopoietin)

Rebound following myelosuppressive chemotherapy


Platelet count >450×10⁹/l
Review blood film
Acute phase reactants (e.g. CRP; ESR)
Iron status
Acute phase response
Reactive thrombocytosis
Normal
Iron deficiency
Treat
Repeat blood count
Repeat blood count
Persistent unexplained thrombocytosis
Further investigation
*Molecular genetics (JAK2 V617F; MPL)
†Bone marrow examination (aspirate and trephine biopsy)
‡Cytogenetics
Diagnosis (see text for diagnostic features)

Haematological disease

primary thrombocytosis

Primary thrombocytosis (also referred to as essential thrombocytosis, essential thrombocythaemia and primary thrombocythaemia) is due to a failure to regulate the production of platelets (autonomous production) and is a feature of a number of myeloproliferative disorders.

About a third of patients are asymptomatic at the time of diagnosis.There is sustained megakaryocyte proliferation that causes an increased number of circulating platelets.

. It was seen as a monoclonal disorder that involved pluripotent stem cells but recent studies suggest that, in some patients, it may be polyclonal.

- The median age at diagnosis is 60 years but it can occur at any age and up to 20% of patients are younger than 40 years.
- It is rare in children.
- There is an equal sex ratio in the more common older group but, in younger patients, there is a female predominant. Overall, the female-to-male ratio is about 2:1.

Diagnosis requires A1–A3 or A1 + A3–A5

A1	Sustained platelet count >450 × 10^9/l
A2	Presence of an acquired pathogenetic mutation (e.g. in the *JAK2* or *MPL* genes)
A3	No other myeloid malignancy, especially PV , PMF , CML or MDS
A4	No reactive cause for thrombocytosis and normal iron stores
A5	Bone marrow aspirate and trephine biopsy showing increased megakaryocyte numbers displaying a spectrum of morphology with predominant large megakaryocytes with hyperlobated nuclei and abundant cytoplasm. Reticulin is generally not increased.

The blood film shows a thrombocytosis with varying degrees of platelet anisocytosis ,Increase MPV.

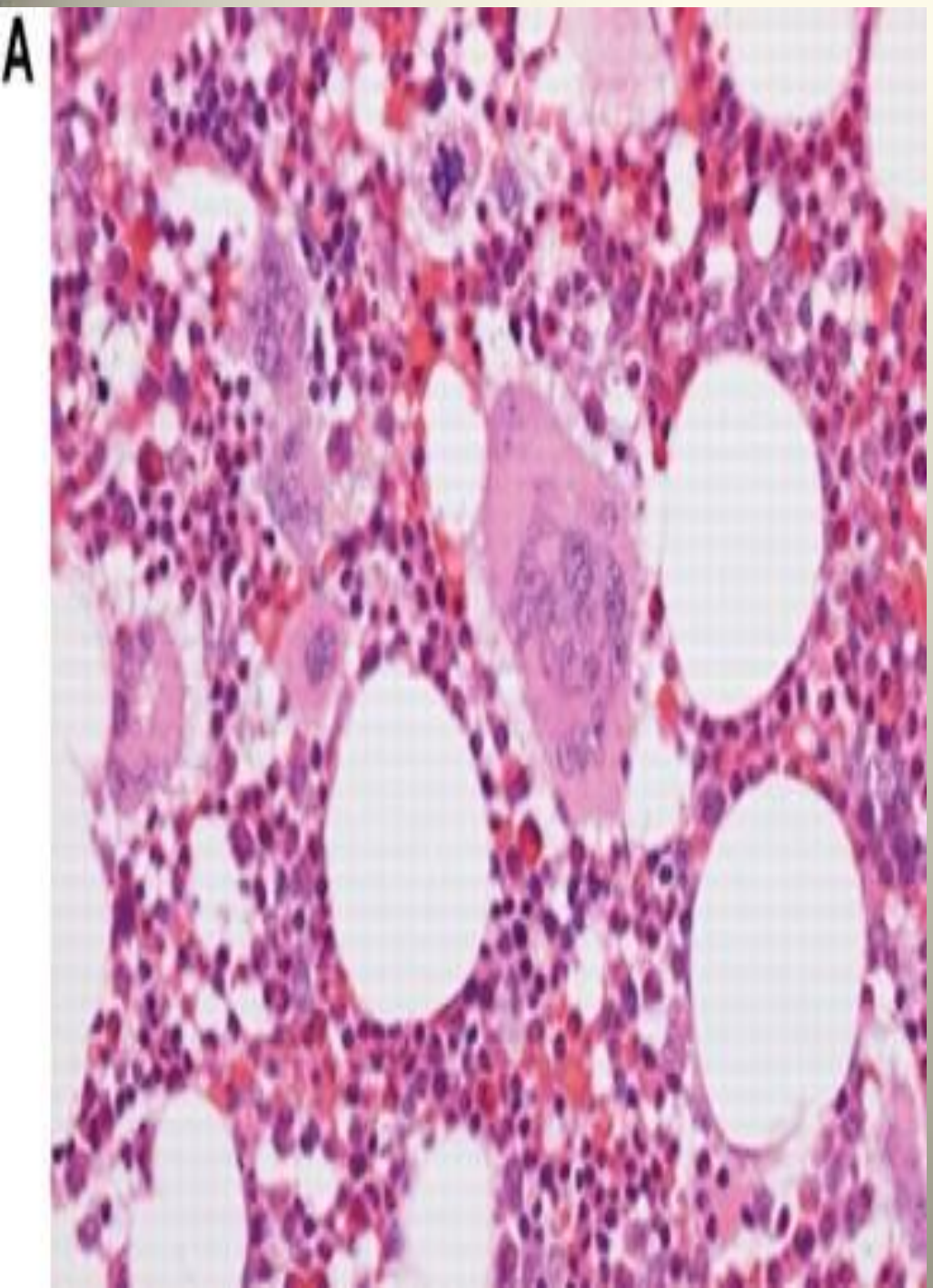
Platelet morphology can vary from those of normal size and granulation to larger atypical forms that may be hypogranular. .

In ET, the bone marrow is normocellular for age or mildly hypercellular.

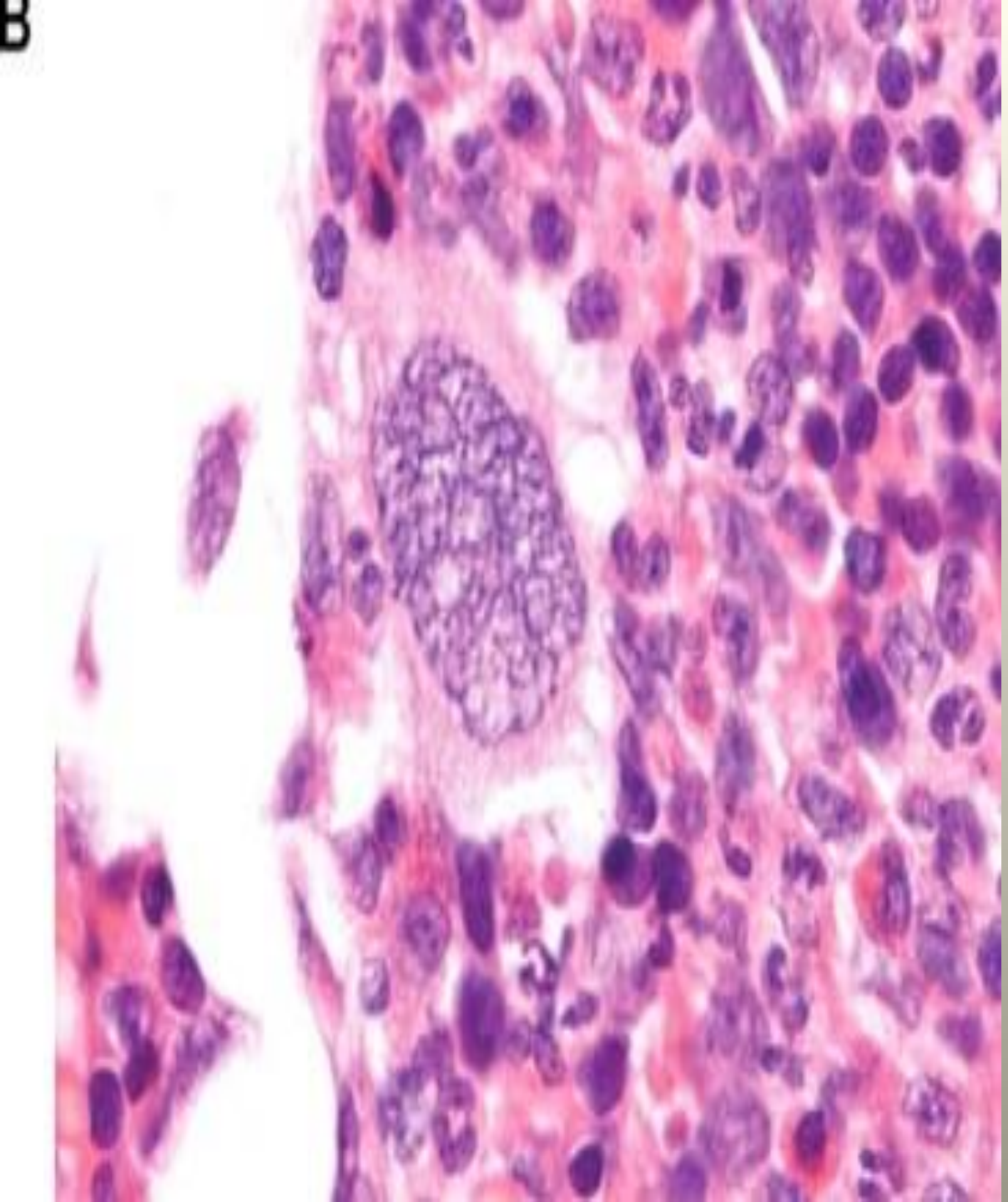
Megakaryocytes are increased in number .

The WHO Classification states that large or giant megakaryocytes predominate but smaller forms may also be seen, particularly if immunohistochemistry is used to aid megakaryocyte identification in trephine biopsy sections.

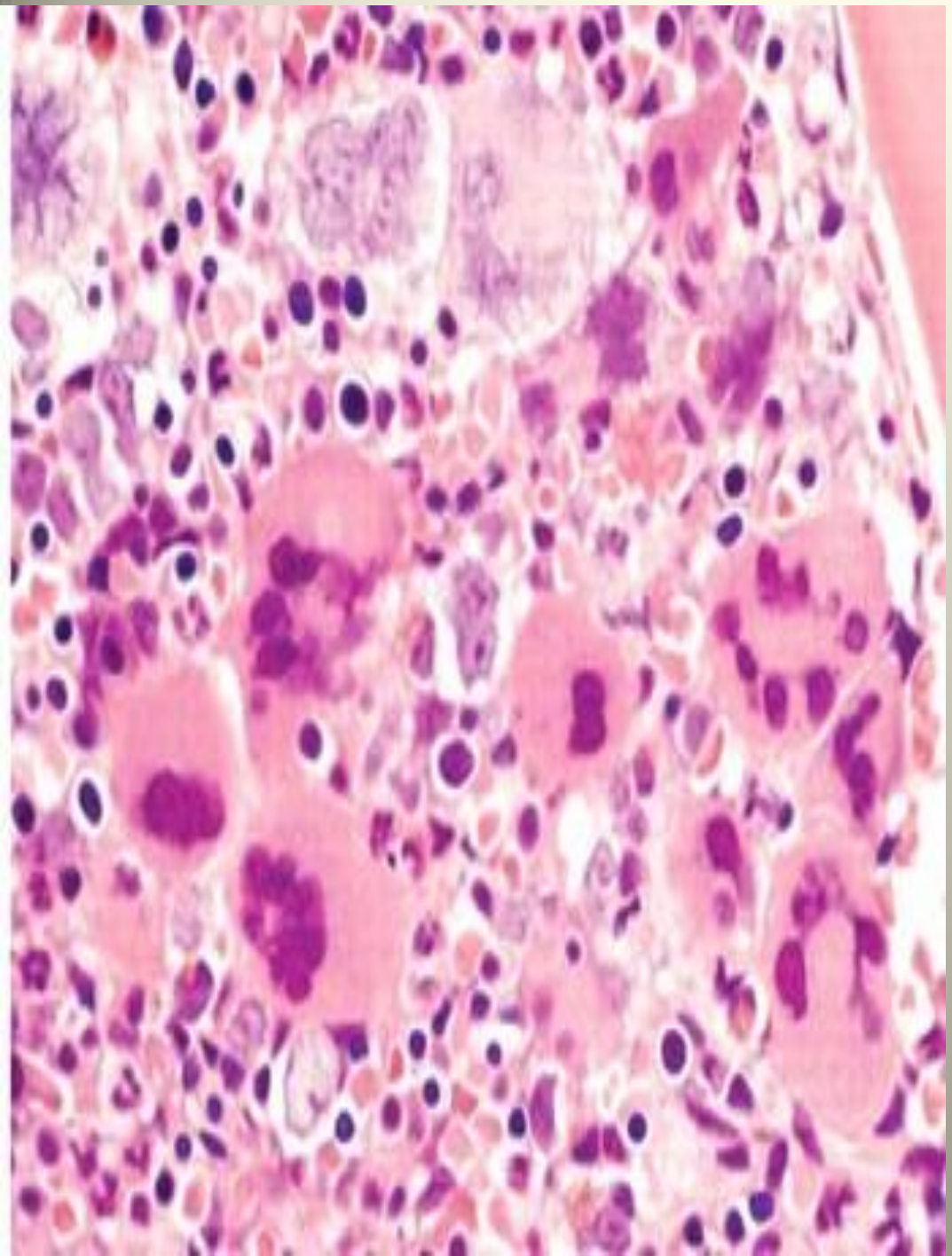
Megakaryocytes with normal morphology, including nearly-bare end-stage variants with pyknotic nuclei are also present. This spectrum of megakaryocyte morphology is typical of ET.



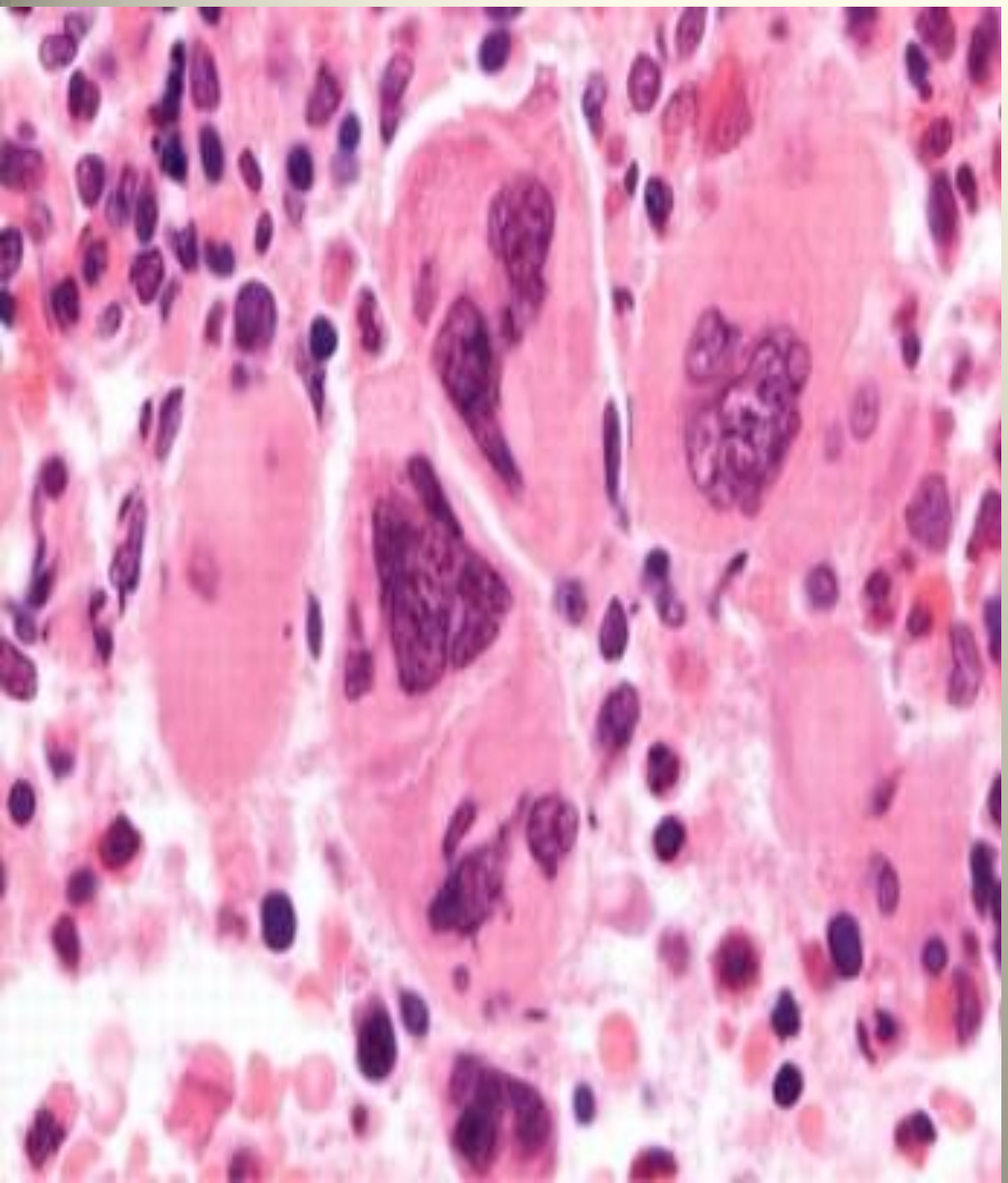
Examples of morphologic features scored on 370 diagnostic bone marrow trephine specimens from patients enrolled in prospective clinical trials of ET. (A) H&E-stained section (×400 magnification) showing a large, staghorn megakaryocyte in the center of the field.



H&E-stained section (×1000) showing a cloudlike megakaryocyte in a background of increased cellularity



A loose cluster of megakaryocytes showing other marrow cells between individual megakaryocytes



A tight cluster of megakaryocytes showing molding of the juxtaposed cell surfaces between adjacent megakaryocytes, and no intervening marrow cells.

A clonal genetic abnormality can be demonstrated in approximately 60% of cases of ET.
The *JAK2* V617F mutation is detectable in 50% and the *MPL* mutation in up to 10%.

Routine karyotyping is not always required but a karyotypic abnormality where present may be a useful marker for disease progression.

- Platelet aggregation studies are abnormal and show impaired platelet aggregation (to adrenaline, ADP and collagen but not to ristocetin and arachidonic acid).

- Some patients may have spontaneous platelet aggregation.

Polycythaemia vera (PV)

PV is characterized by an elevated haemoglobin, haematocrit and red cell mass, although up to 15% of patients may present with a marked thrombocytosis and clinical features such as pruritis would add weight to a diagnosis of PV.

Bone marrow examination (though not always essential) shows a pan-myelosis with normal erythroid and granulocytic differentiation but with spatial disorganisation.

The megakaryocytes show marked pleomorphism with a higher than normal nuclear:cytoplasmic ratio plus a characteristic mixture of large and small variants; loose clustering of megakaryocyte is usual.

The giant megakaryocytes with hyperlobated nuclei that are a feature of ET are not seen in PV.

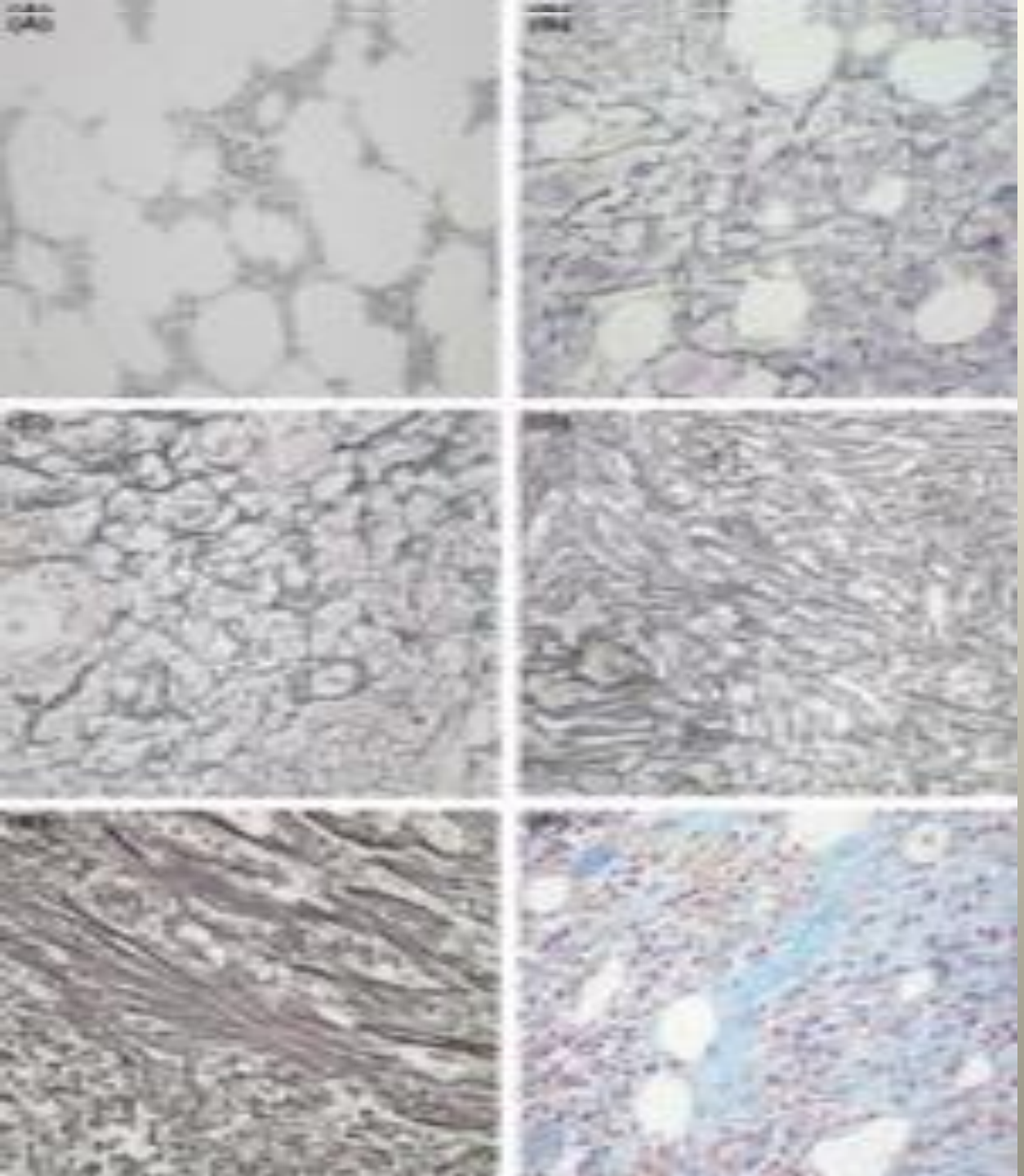
The *JAK2* V617F mutation is present in more than 97% of PV patients and a further 1–2% will have an exon 12 mutation of *JAK2* .

Exon 12 mutations have not been documented in the context of ET or PMF.

Myelofibrosis

The quantification of stromal reticulin fibres and detection of collagen fibrosis are fundamental to the classification and assessment of progression in MPN.

Several different semi-quantitative methods for grading reticulin and collagen fibrosis have been developed, ranging from 4 to 6 grades.



Reticulin grading: examples of reticulin stains. A. Grade 0/3 scattered linear reticulin with no intersections (cross-overs) corresponding to normal bone marrow; B. Grade 1/3 loose network of reticulin with many intersections, especially in perivascular areas; C. Grade 2/3 widespread and dense increase in reticulin with extensive intersections; D. Grade 3/3 diffuse and dense increase in reticulin with extensive intersections; E. Grade 3/3 coarse bundles of collagen demonstrated; F. Collagen demonstrated using MSB trichrome stain

WHO grading of bone marrow fibrosis

Grading	Description*
MF-0	Scattered linear reticulin with no intersections (cross-overs) corresponding to normal BM
MF-1	Loose network of reticulin with many intersections, especially in perivascular areas
MF-2	Diffuse and dense increase in reticulin with extensive intersections, occasionally with focal bundles of collagen and/or focal osteosclerosis
MF-3	Diffuse and dense increase in reticulin with extensive intersections and coarse bundles of collagen, often associated with osteosclerosis

*Fibre density should be assessed in areas showing active haematopoiesis

Myelodysplastic/myeloproliferative overlap syndromes

The existence of diseases that can demonstrate the clinical and laboratory features of both MDS and a chronic MPN, have been recognized within the WHO Classification of Tumours (Swerdlow, 2008).

These entities have been termed the myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPN). Care should be taken to ensure that those patients who have a clear history of a preceding myeloproliferative disorder with evidence of subsequent dysplastic transformation, are not included in this group, as the evidence base for their clinical management is different.

The MDS/MPN overlap category has been divided into four separate sub-groups:

chronic myelomonocytic leukaemia (CMML);
atypical CML (aCML);
juvenile myelomonocytic leukaemia (JMML); and
MDS/MPN, unclassifiable (MDS/MPN-U).

Within the latter designation, there has been recognition of the provisional entity of refractory anaemia with ringed sideroblasts associated with marked thrombocytosis (RARS-T).

Myelodysplastic/myeloproliferative neoplasm; provisional entity – refractory anaemia with ring sideroblasts associated with marked thrombocytosis (RARS-T).

This is a provisional entity which has features of

1- Both ET and refractory anaemia

2- Ring sideroblasts.

3-Platelet morphology is normal. Red cells are dimorphic

The bone marrow (aspirate and trephine biopsy) is

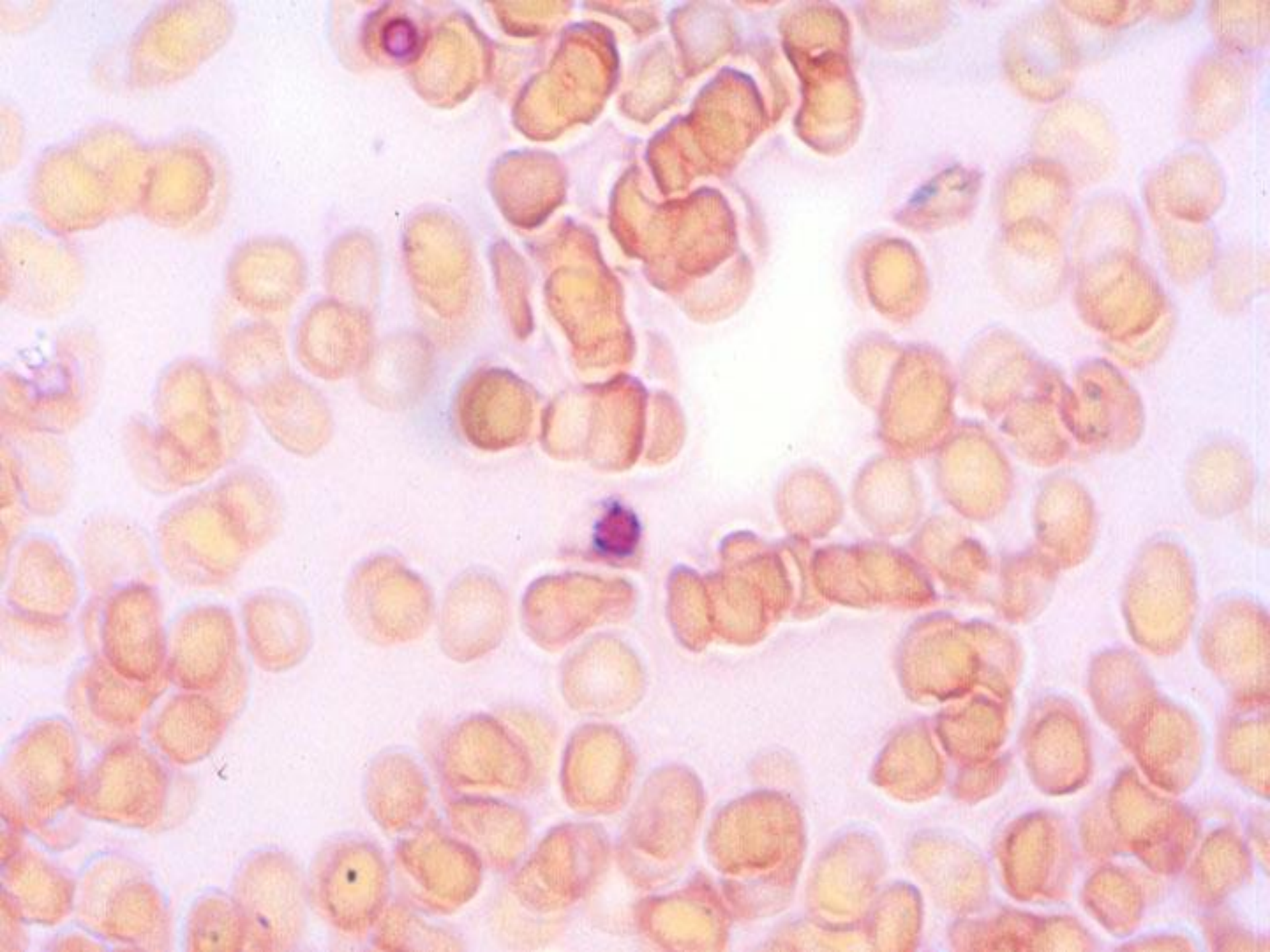
1-Hypercellular and shows increased megakaryocytes with morphological features similar to those seen in ET or PMF.

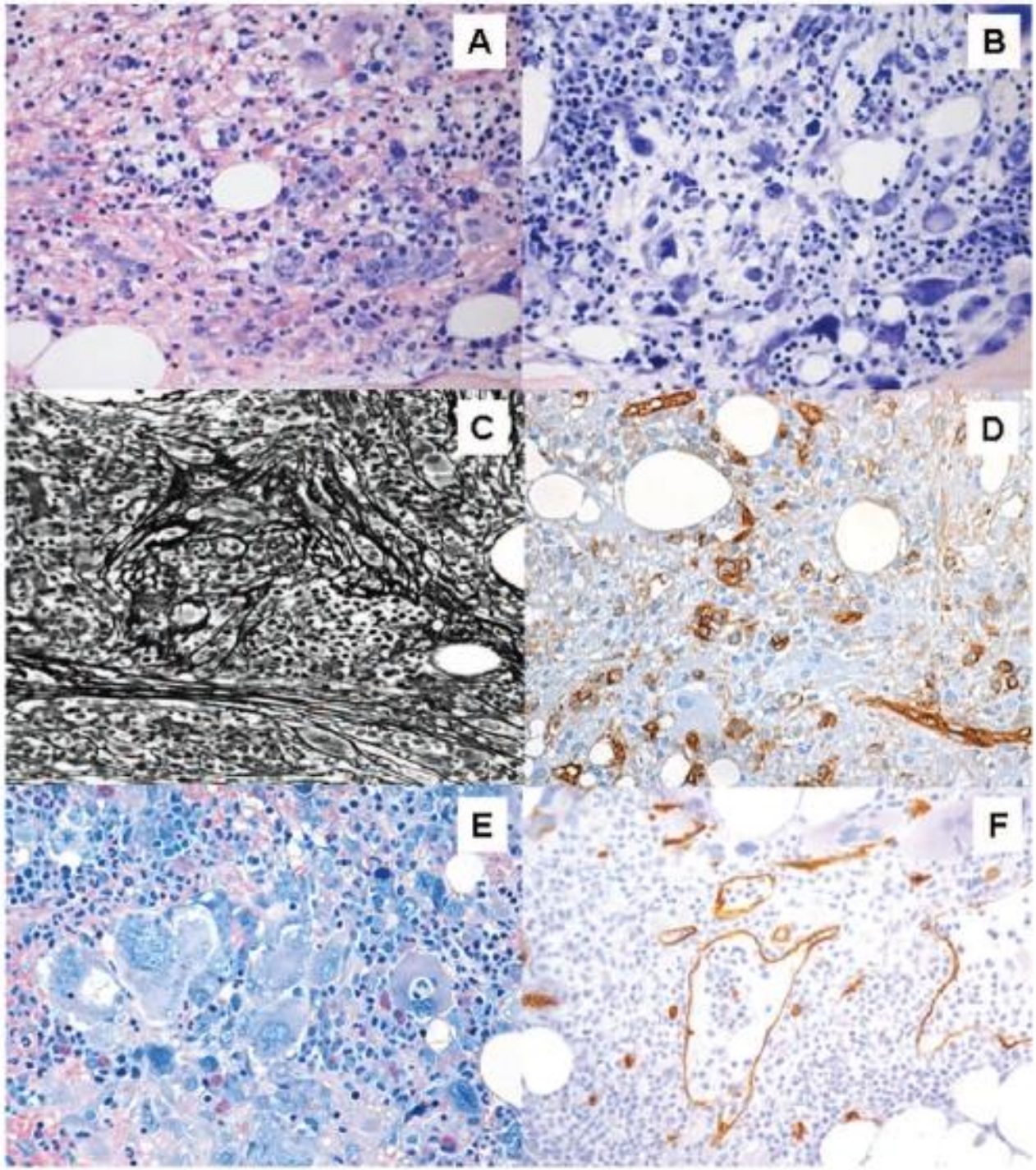
2- Megakaryocyte clustering can be present.

3-There is also increased erythropoiesis with dys-erythropoiesis and ring sideroblasts accounting for more than 15% of late normoblasts.

4-Reticulin varies, from normal to moderately increased.

5-The *JAK2* V617F mutation is present in more than 50% of patients





(A) increased bone marrow cellularity with erythroid hyperplasia, (B) dysplastic megakaryocytes (such as hypolobulated megakaryocytes).

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Myelodysplastic Syndrome associated with isolated del(5q).

Myelodysplastic syndrome with isolated del(5q) is commonly associated with thrombocytosis (30–50% of patients) and macrocytic anaemia.

The platelet morphology is unremarkable whilst the red cells are macrocytic, with mild poikilocytosis and minimal polychromasia.

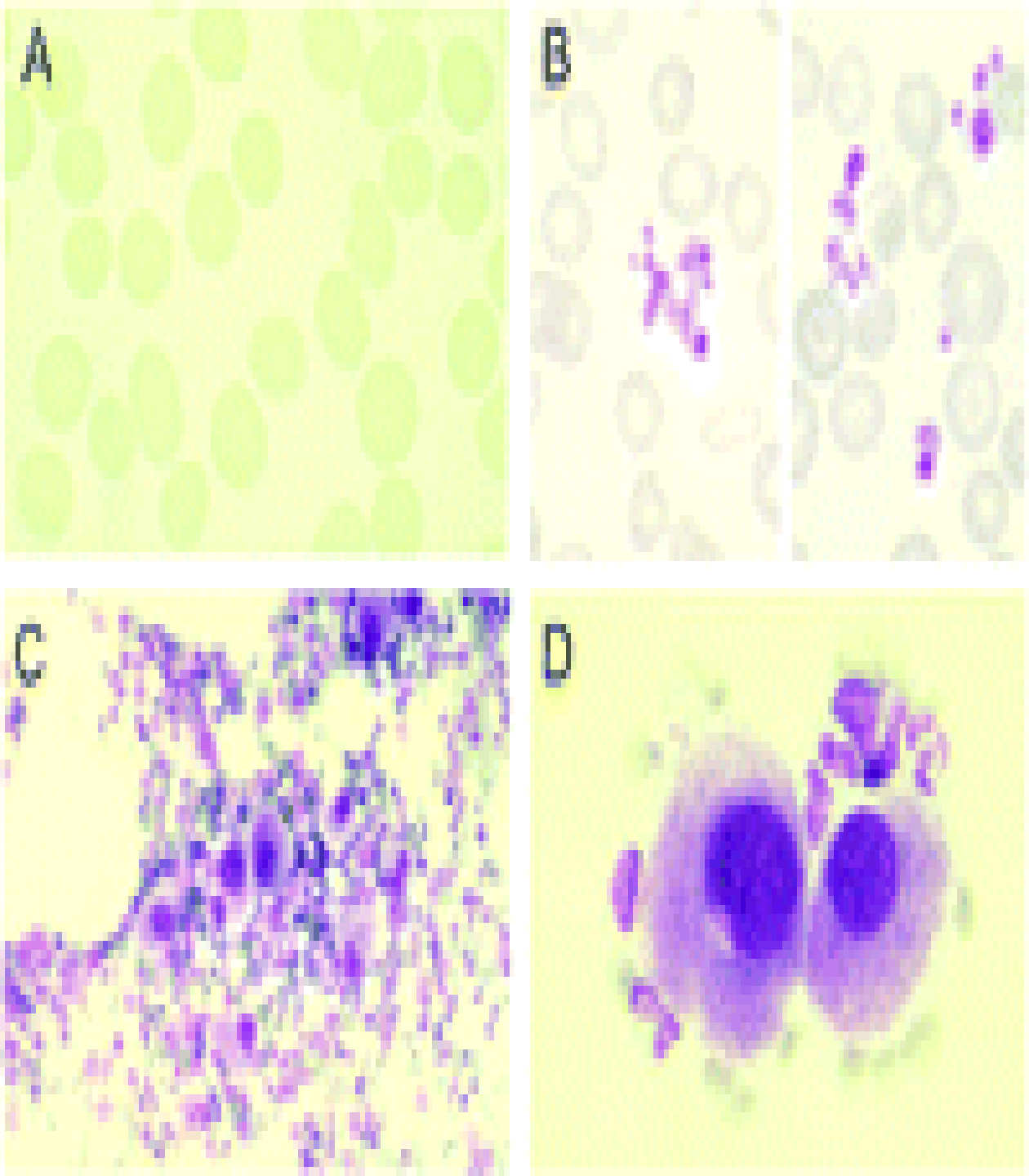
. The bone marrow is

1- normocellular, or mildly hypercellular, with Dyserythropoiesis.

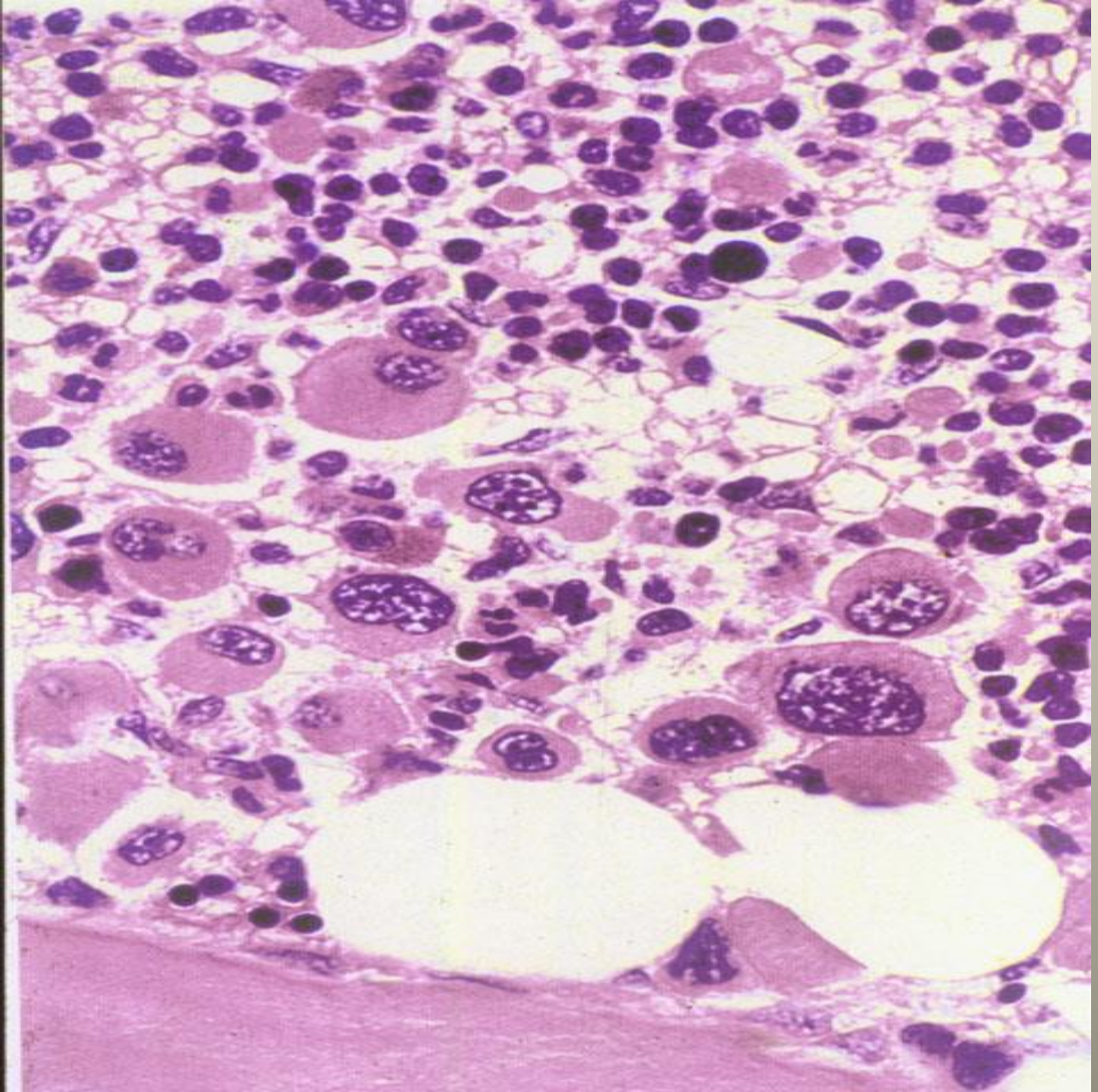
2-Megakaryocytes are increased in number and are present in the marrow interstitium; they are small or of normal size and have monolobed, or hypolobated nuclei that are characteristically eccentrically placed.

3-Cytogenetic demonstration of del(5q), is required to make the diagnosis.

A small number of patients may also have the *JAK2* V617F mutation



Peripheral blood smear and bone marrow aspirate from a patient with 5q- syndrome. (A) Oval macrocytes. (B) Numerous platelets. (C) and (D) Megakaryocytes with round, non-lobulated nuclei.



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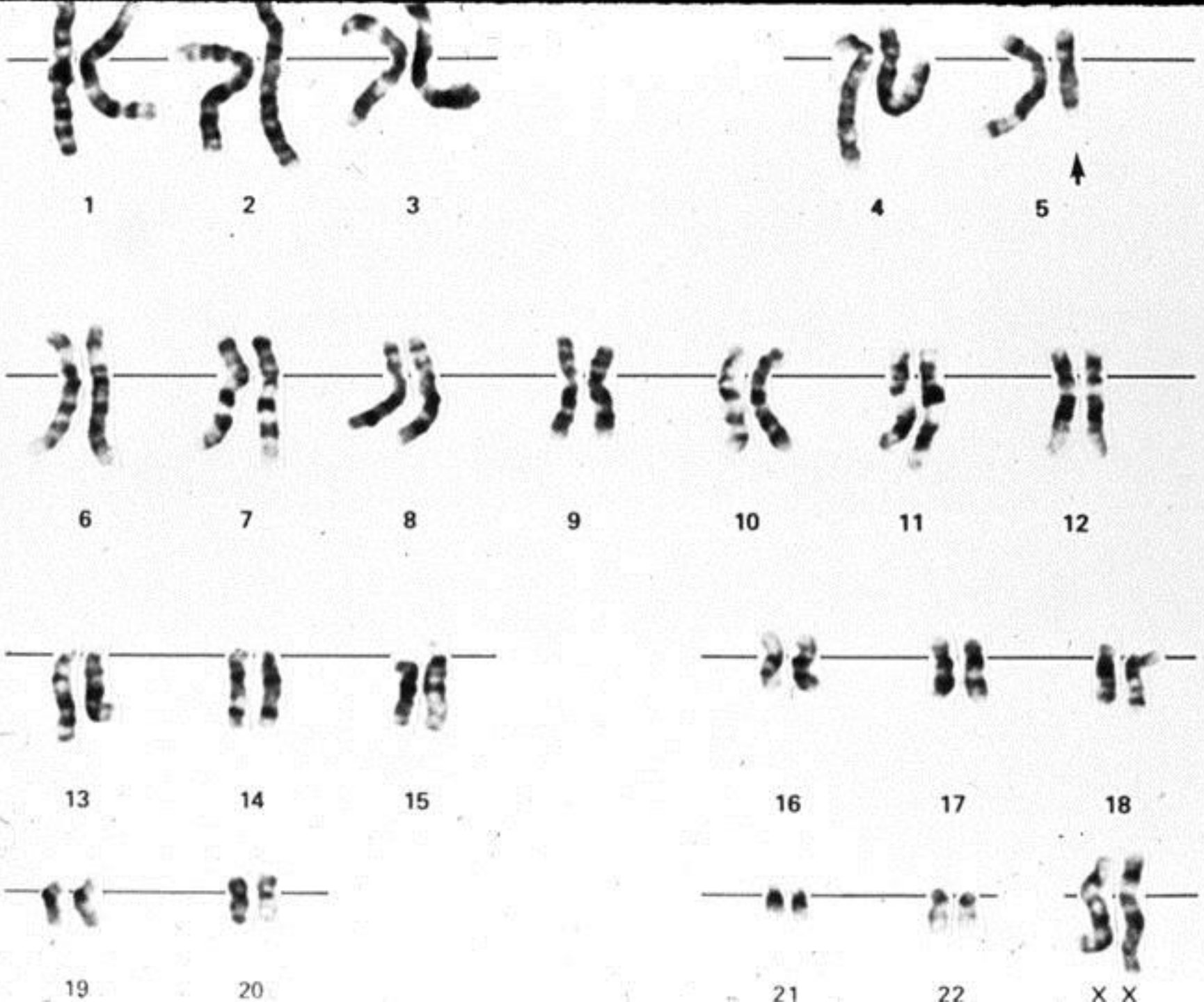
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Secondary thrombocytosis

The incidence is highest during the first 3 months of life, and preterm infants are more prone than term infants.

One meta-analysis found that 3-13% of hospitalised paediatric patients had a platelet count of more than 500 x 10^9/L.

Reactive thrombocytosis

This can be secondary to a number of conditions. It is an exaggerated physiological response to a primary problem, such as an infection.

The trigger factor (eg infection) results in the release of cytokines which mediate an increase in platelet production.

It is often a transient phenomenon which disappears when the underlying cause is resolved.

- . The following are likely to be raised in secondary thrombocytosis:
  - Erythrocyte sedimentation rate (ESR).
  - C-reactive protein (CRP).
  - Fibrinogen level.
  - Factor VIII procoagulant activity.
  - Von Willebrand antigen level.

The platelets are mostly small.

with a normal mean platelet volume MPV.

The blood film may show other features to indicate an underlying cause, A bone marrow aspirate or trephine is not usually required for reactive thrombocytosis.

If one has been performed due to diagnostic uncertainty, this will show megakaryocytic hyperplasia with normal mature and left-shifted megakaryocyte morphology.
The megakaryocytes will have a normal interstitial distribution and not show clustering