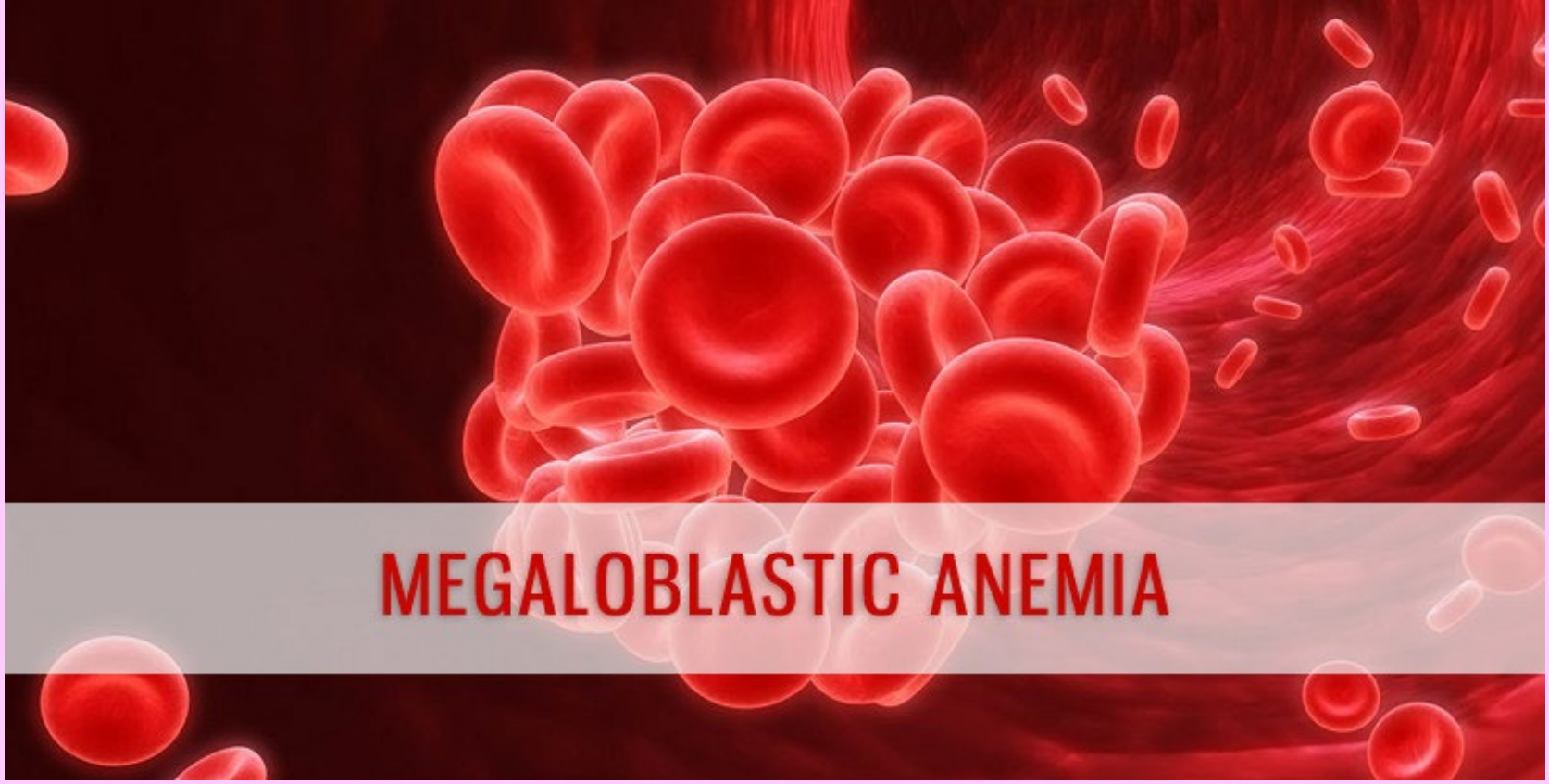


Aplastic anaemia

Dr. Mousa Qasim Hussein
Professor Internal Medicine

This results from a deficiency of vitamin B12 or folic acid, or from disturbances in folic acid metabolism.

Folate is an important substrate of, and vitamin B12 a co-factor for, the generation of the essential amino acid methionine from homocysteine

This reaction produces tetrahydrofolate,which is converted to thymidine monophosphate for incorporation into DNA.

Deficiency of either vitamin B12 or folate will therefore produce high plasma levels of homocysteine and impaired DNA synthesis.

The end result is cells with arrested nuclear maturation but normal cytoplasmic development: so-called nucleo-cytoplasmic asynchrony.

The high proliferation rate of bone marrow results in striking changes in the haematopoietic system in megaloblastic anaemia.

1-Cells become arrested in development and die within the marrow; this ineffective erythropoiesis results in an expanded hypercellular marrow.

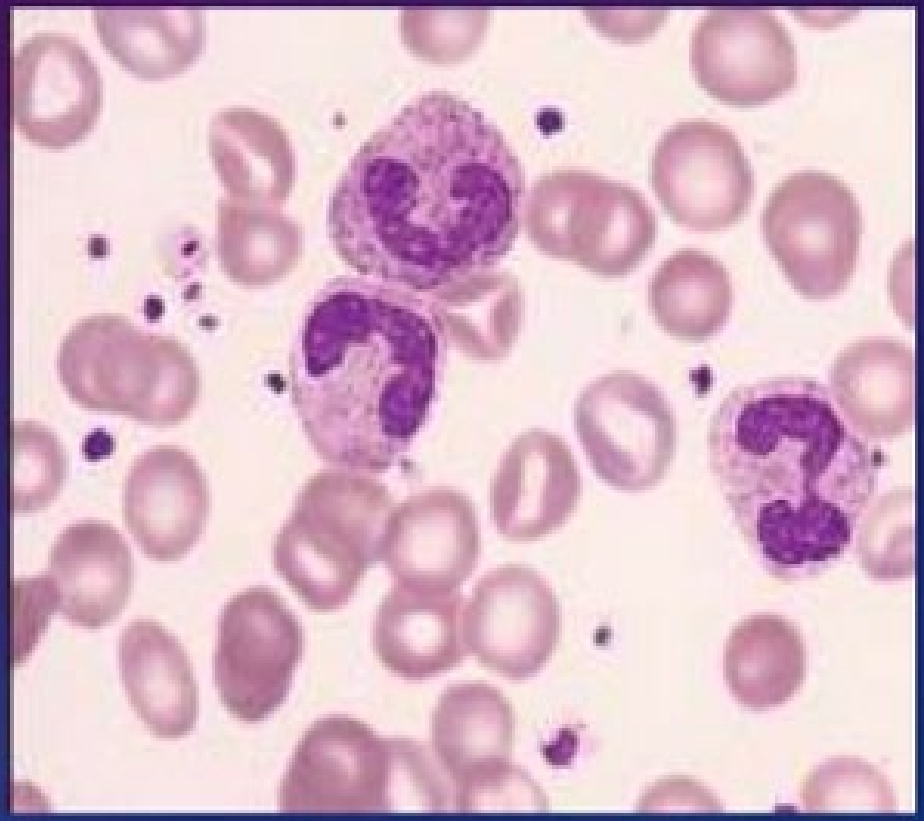
2-The megaloblastic changes are most evident in the early nucleated red cell precursors, and intramedullary haemolysis results in a raised bilirubin and lactate dehydrogenase (LDH) but no reticulocytosis

3-The mature neutrophils show hypersegmentation of their nuclei, with cells having six or more nuclear lobes.

4-If severe, a pancytopenia may be present in the peripheral blood.

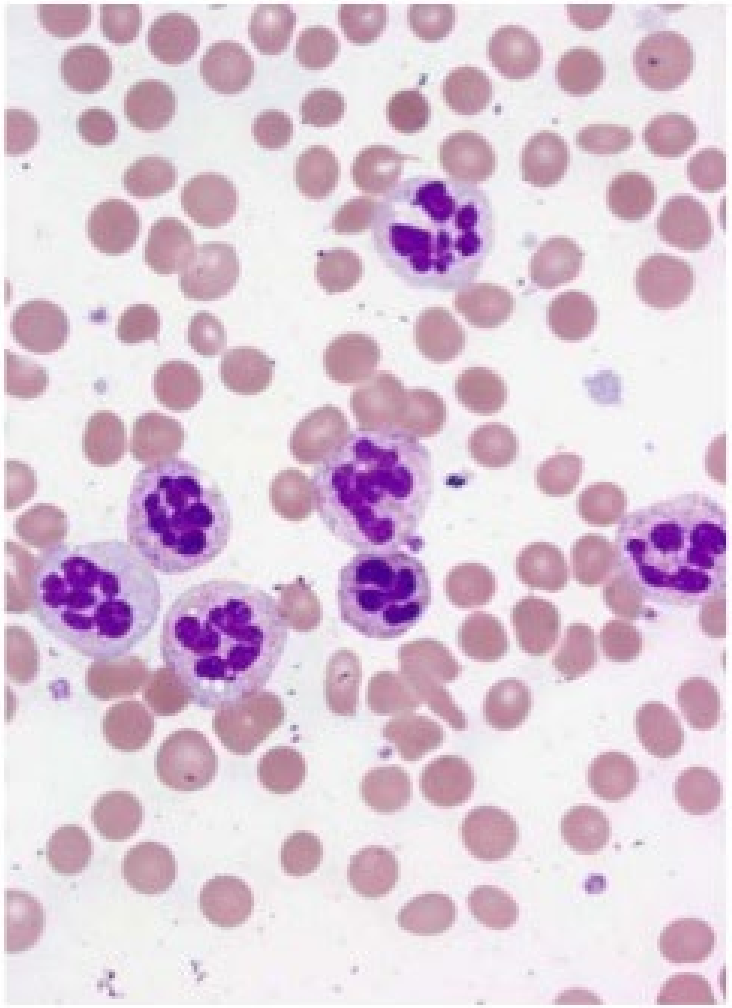
POLYMORPHONUCLEAR NEUTROPHILS

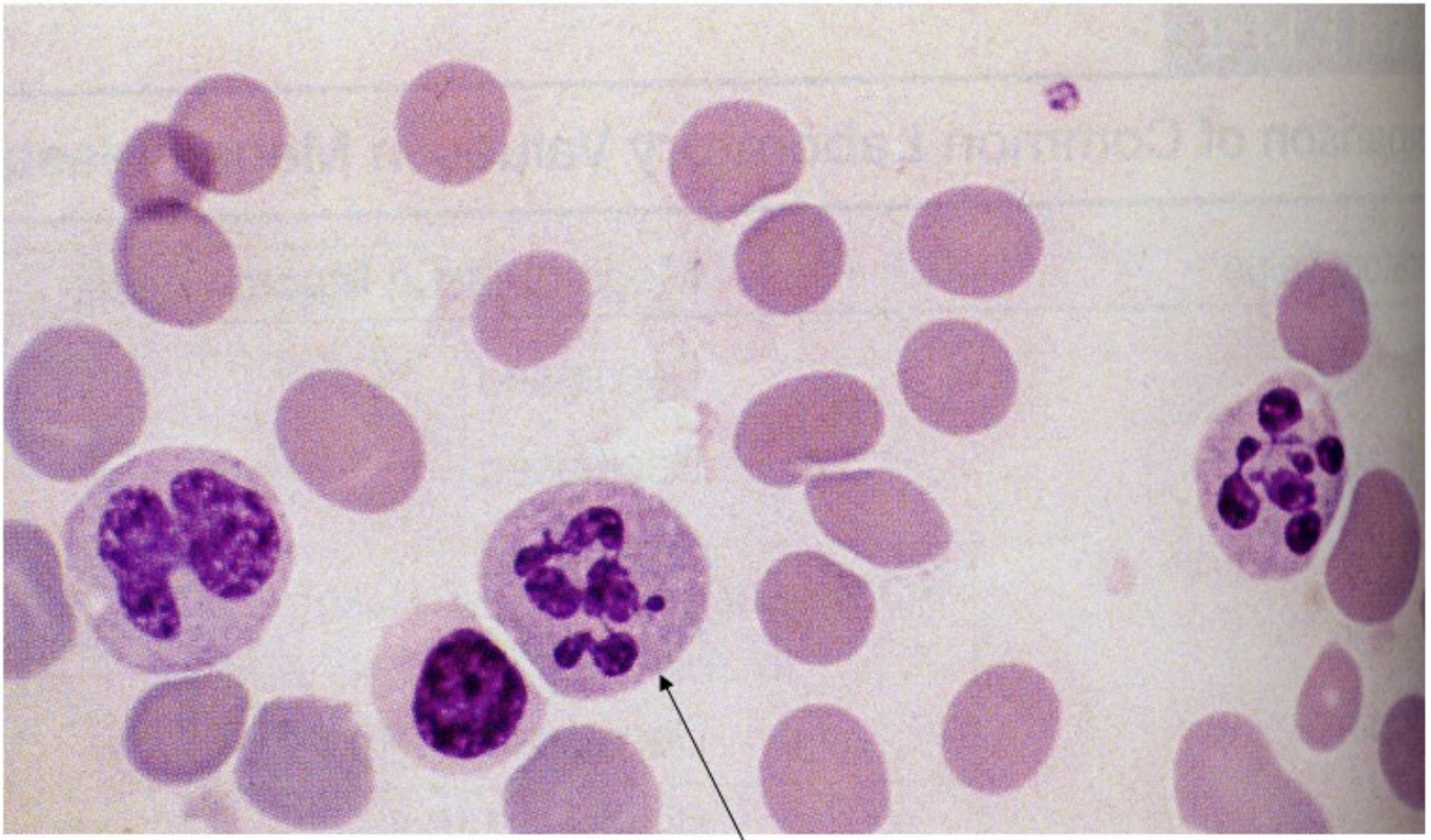
- 40 to 80 percent of total WBC count(2.0–7.0 $\times 10^9$/l)
- Diameter - 13 µm
- segmented nucleus and pink/orange cytoplasm with fine granulation(0.2-0.3µm) stain tan to pink with Wright's
- Lobes -2-5
- Neutrophils usually have trilobed nucleus.
- small percent has four lobes and occasionally five lobes.



Hypersegmented neutrophils

- Presence of even a single neutrophils with six or more lobes or the presence of more than 5% of neutrophils with five lobes.
- Seen in Megaloblastic anemia
  - Uraemia
  - Drugs-cytotoxic treatment with methotrexate
  - Hydroxycarbamide
  -




Hypersegmented neutrophil

Vitamin B_{12} deficiency, but not folate deficiency, is associated with neurological disease in up to 40% of cases.

The main pathological finding is focal demyelination affecting the:

spinal cord

peripheral nerves

optic nerves

cerebrum.

The most common manifestations are sensory, with peripheral paraesthesiae and ataxia of gait.

Clinical features of megaloblastic anaemia

Symptoms

- Malaise (90%)
- Breathlessness (50%)
- Paraesthesiae (80%)
- Sore mouth (20%)
- Weight loss
- Impotence
- Poor memory
- Depression
- Personality change
- Hallucinations
- Visual disturbance

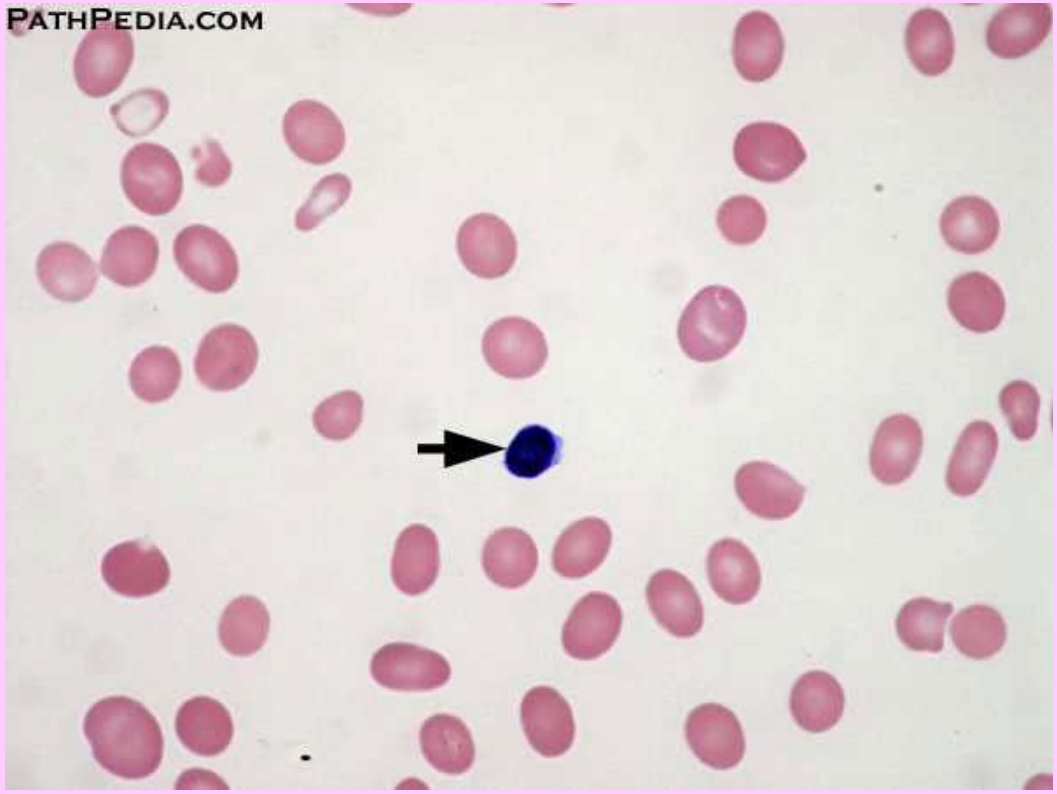
Signs

- Smooth tongue
- Angular cheilosis
- Vitiligo
- Skin pigmentation
- Heart failure
- Pyrexia

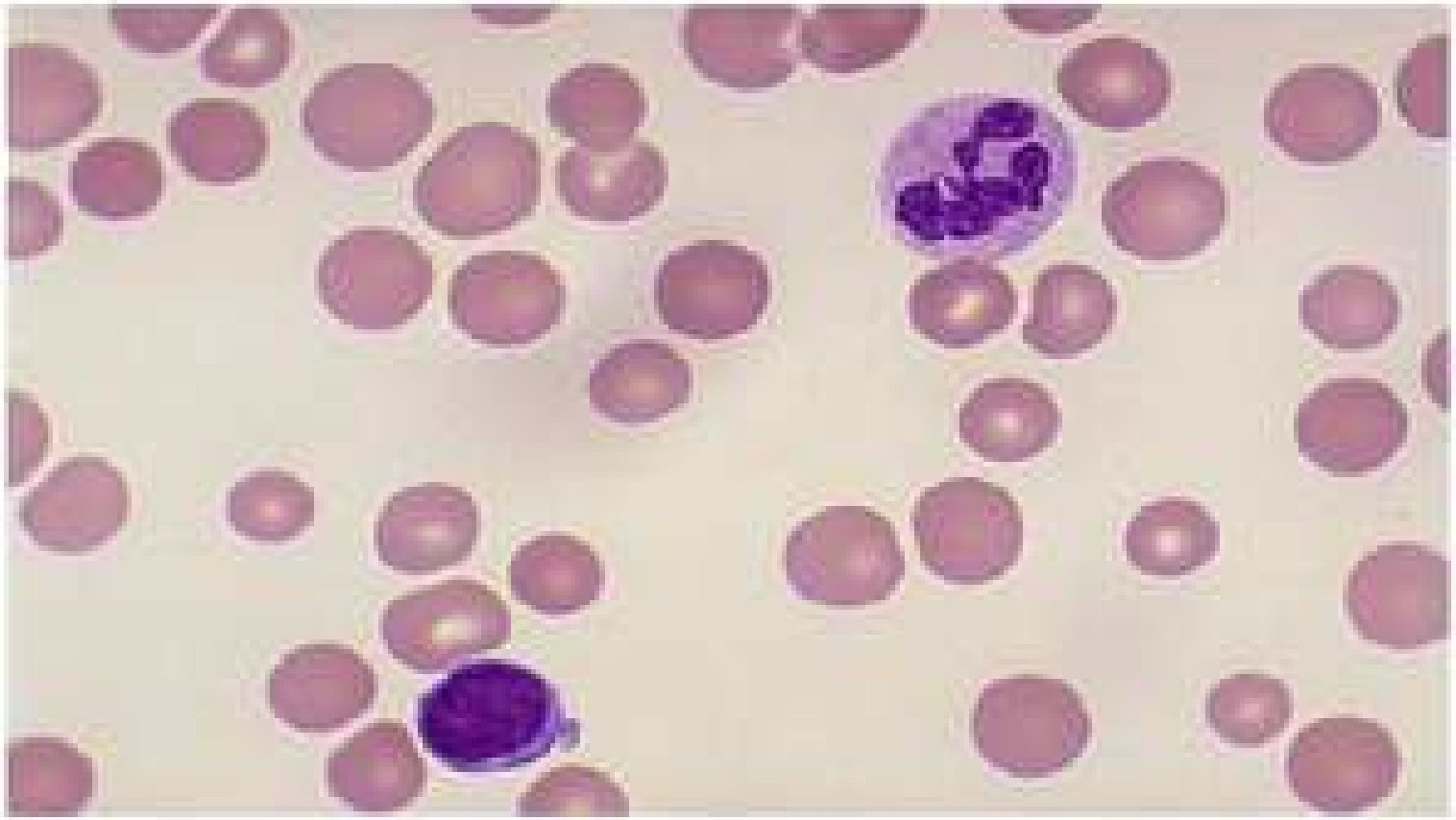
DIAGNOSTIC FEATURES OF MEGALOBLASTIC ANAEMIA :

Investigation	Result
Haemoglobin	Often reduced, may be very low
MCV	Usually raised, commonly > 120 fl
Erythrocyte count	Low for degree of anaemia
Blood film	Oval macrocytosis, poikilocytosis, red cell fragmentation, neutrophil hypersegmentation
Reticulocyte count	Low for degree of anaemia
Leucocyte count	Low or normal
Platelet count	Low or normal
Bone marrow	Increased cellularity, megaloblastic changes in erythroid series, giant metamyelocytes, dysplastic megakaryocytes, increased iron in stores, pathological non-ring sideroblasts
Serum ferritin	Elevated
Plasma LDH	Elevated, often markedly

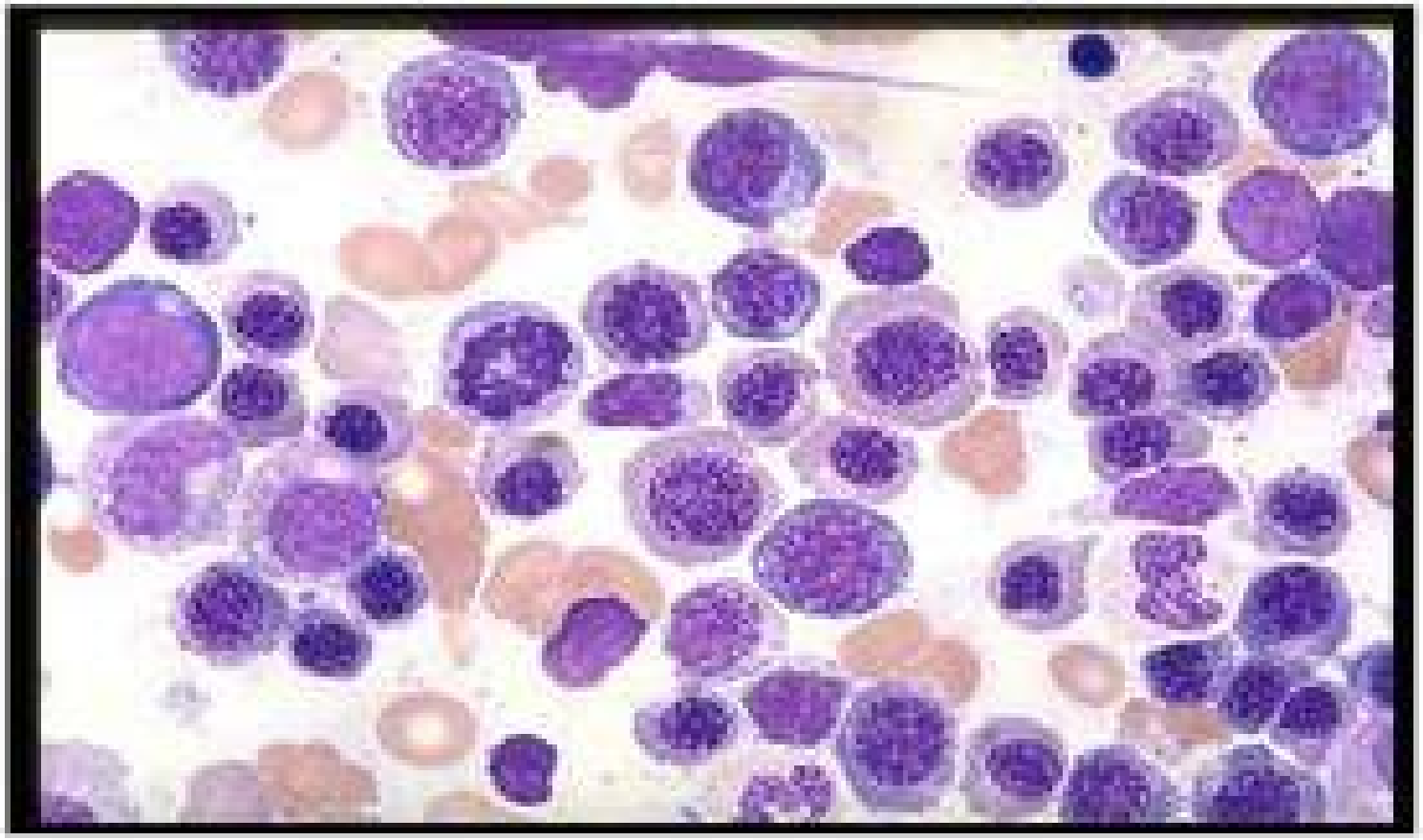
The increase in cell size can easily be calibrated using the nucleus of a small lymphocyte. A normal red blood cell is usually the same size as the nucleus of a small lymphocyte. Note that most red cells in this picture are larger than the nucleus of a small lymphocyte.



Megaloblastic Anemia
B-12 Deficiency



Megaloblastic Anemia
B-12 Deficiency
(Bone Marrow)



VITAMIN B12

NEUROLOGICAL FINDINGS IN B12 DEFICIENCY

Peripheral nerves

Glove and stocking paraesthesiae

Spinal cord

Subacute combined degeneration

Posterior columns-diminished vibration and proprioception

Corticospinal tracts-upper motor neuron signs

Cerebrum

Dementia

Optic atrophy

Autonomic neuropathy

B12 deficiency:

The average daily diet contains 5-30 µg of vitamin B12 mainly in meat, fish, eggs and milk-well in excess of the 1 µg daily requirement

Vitamin B12 absorption:

In the stomach, gastric enzymes release vitamin B12 from food and at gastric pH it binds to a carrier protein termed R protein.

The gastric parietal cells produce intrinsic factor, a vitamin B12-binding protein which optimally binds vitamin B12 at pH 8.

As gastric emptying occurs, pancreatic secretion raises the pH and vitamin B12 released from the diet switches from the R protein to intrinsic factor.

Bile also contains vitamin B12 which is available for reabsorption in the intestine. The vitamin B12 intrinsic factor complex binds to specific receptors in the terminal ileum and vitamin B12 is actively transported by the enterocytes to plasma, where it binds to transcobalamin II, a transport protein produced by the liver, which carries it to the tissues for utilisation.
The liver stores enough vitamin B12 for 3 years and this together with entrahepatic circulation,mean that take years to become.

Blood levels of vitamin B_{12} provide a reasonable indication of tissue stores and are usually diagnostic of deficiency and remain the first line test for most laboratories, Additional tests have been evaluated, including:

measurement of methylmalonic acid, holotranscobalamin and plasma homocysteine levels, but do not add much in most clinical situations. Levels of cobalamins fall in normal pregnancy. Reference ranges vary between laboratories, but levels below 150 ng/L are common and, in the last trimester, 5%–10% of women have levels below 100 ng/L. Spuriously low B12 values occur in women using the oral contraceptive pill and in patients with myeloma, in whom paraproteins can interfere with vitamin B12 assays.

Causes of vitamin B12 deficiency:

1-Dietary deficiency: strict vegans onset of clinical features can occure at any age between 10 -80 years.

Less strict vegetarians often have slightly low vitamin B12 levels but are not tissue vitamin B12-deficient.

2-Gastric pathology:

Release of vitamin B_{12} from the food requires normal gastric acid and enzyme secretion, and this is impaired by hypochlorhydria in elderly patients or following gastric surgery.

Total gastrectomy invariably results in vitamin B_{12} deficiency within 5 years, often combined with iron deficiency; these patients need life-long 3-monthly vitamin B_{12} injections.

After partial gastrectomy vitamin B_{12} deficiency only develops in 10-20% of patients by 5 years; an annual injection of vitamin B_{12} should prevent deficiency in this group.

Bariatric surgery: Patients may develop B12 deficiency after certain types of bariatric surgery

3-Pernicious anaemia:

This is an autoimmune disorder in which the gastric mucosa is atrophic with loss of parietal cells causing intrinsic factor deficiency. In the absence of intrinsic factor less than 1% of dietary vitamin B12 is absorbed

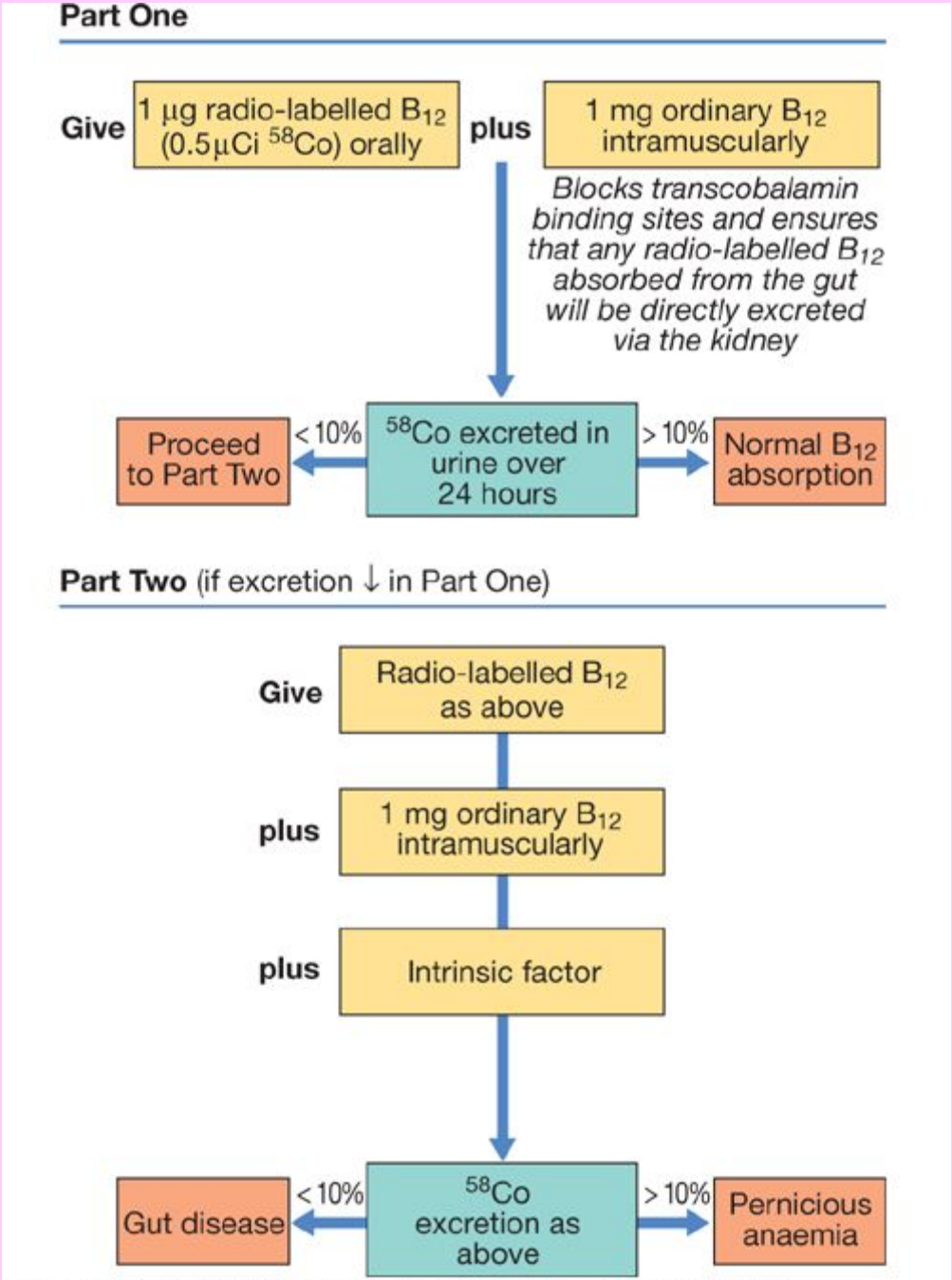
incidence of 25/100 000 population over the age of 40 years in developed countries, but an average age of onset of 60 years.

It is more common in individuals with other autoimmune disease (Hashimoto's thyroiditis, Graves' disease, vitiligo, hypoparathyroidism or Addison's disease) or a family history of these or pernicious anaemia

The finding of anti-intrinsic factor antibodies in the context of B12 deficiency is diagnostic of pernicious anaemia without further investigation.

Antiparietal cell antibodies are present in over 90% of cases but are also present in 20% of normal females over the age of 60 years; a negative result makes pernicious anaemia less likely but a positive result is not diagnostic.

The Schilling test, involving measurement of absorption of radio-labelled B12 after oral administration before and after replacement of intrinsic factor.



Colledge et al: Davidson's Principles and Practice of Medicine, 21st Edition

4- Small bowel factors:

a. One-third of all patients with pancreatic insufficiency fail to transfer dietary vitamin B12from R protein to intrinsic factor. This usually results in slightly low vitamin B12 values

b. Motility disorders or hypogammaglobulinaemia can result in bacterial overgrowth and the resulting competition for free vitamin B12can result in deficiency. This will be corrected to some extent by a course of antibiotics

c. fish tapeworm

d. Inflammatory disease of the terminal ileum, such as Crohn's disease, may impair the interaction of the vitamin B12- intrinsic factor complex with its receptor, as will surgery on this part of the bowel. Both may result in vitamin B12 malabsorption.

Management of megaloblastic anaemia

If a patient with a severe megaloblastic anaemia is very ill and treatment must be started before vitamin B12 and red cell folate results are available, that treatment should always include both folic acid and vitamin B12.

The use of folic acid alone in the presence of vitamin B12 deficiency may result in worsening of neurological features.

Rarely, if severe angina or heart failure is present, transfusion can be used.
The cardiovascular system is adapted to the chronic anaemia present in megaloblastosis and the volume load imposed by transfusion may result in decompensation and severe cardiac failure. In such circumstances,exchange transfusion or slow administration of 1 U of red cells with diuretic cover may be given.

TREATMENT

Vitamin B12 deficiency is treated with hydroxycobalamin. In cases of uncomplicated deficiency, 1000 µg IM for 6 doses 2 or 3 days apart, followed by maintenance therapy of 1000 µg every 3 months for life, is recommended.

In the presence of neurological involvement, a dose of 1000 µg on alternate days until there is no further improvement, followed by maintenance as above, is recommended.

The reticulocyte count will peak by the 5th–10th day after starting replacement therapy. The haemoglobin will rise by 10 g/L every week until normalised.
The response of the marrow is associated with a fall in plasma potassium levels and rapid depletion of iron stores. If an initial response is not maintained and the blood film is dimorphic (i.e. shows a mixture of microcytic and macrocytic cells), the patient may need additional iron therapy.
A sensory neuropathy may take 6–12 months to correct; long-standing neurological damage may not improve


B12
B12
B12
B12

Folate absorption

Folates are produced by plants and bacteria; hence dietary leafy vegetables(spinach, broccoli, lettuce), fruits (bananas, melons) and animal protein (liver, kidney) are a rich source. An average Western diet contains more than the minimum daily intake of 50 µg, but excess cooking destroys folates.

Most dietary folate is present as polyglutamates; these are converted to monoglutamate in the upper small bowel and actively transported into plasma.

Plasma folate is loosely bound to plasma proteins such as albumin and there is an enterohepatic circulation.

Total body stores of folate are small and deficiency can occur in a matter of weeks.

Folate defciency

Edentulous older people or psychiatric patients are particularly susceptible to dietary defciency and this is exacerbated in the presence of gut disease or malignancy. Pregnancy-induced folate defciency is the most common cause of megaloblastosis worldwide and is more likely in the context of twin pregnancies, multiparity and hyperemesis gravidarum.

Serum folate measurement is very sensitive to dietary intake; a single folate-rich meal can normalise it in a patient with true folate defciency, whereas anorexia, alcohol and anticonvulsant therapy can reduce it in the absence of megaloblastosis. For this reason, red cell folate levels are a more accurate indicator of folate stores and tissue folate defciency.

CAUSES OF FOLATE DEFICIENCY

Diet

•Poor intake of vegetables

Malabsorption

•e.g. Coeliac disease

Increased demand

•Pregnancy
•Cell proliferation, e.g. haemolysis

***Drugs**

•Certain anticonvulsants (e.g. phenytoin (
•Contraceptive pill
•Certain cytotoxic drugs (e.g. methotrexate (

Investigations of folic acid deiciency:

1- diagnostic findings

-low serum folate level(fasting blood sample)

-red cell folate levelindicate prolonged folate deficiencyand are probably the most relevant measure

2-corroborative findings

-macrocytic dysplastic blood picture.

-megaloblastic marrow

TREATMENT

Oral folic acid (5 mg daily for 3 weeks) will treat acute deficiency and 5 mg once weekly is adequate maintenance therapy.

Prophylactic folic acid in pregnancy prevents megaloblastosis in women at risk, and reduces the risk of fetal neural tube defects .

Prophylactic supplementation is also given in Chronic haematological disease associated with reduced red cell lifespan (e.g. haemolytic anaemias). There is some evidence that supraphysiological supplementation (400 µg/day) can reduce the risk of coronary and cerebrovascular disease by lowering plasma homocysteine levels. This has led the US Food and Drug Administration to introduce fortification of bread, flour and rice with folic acid .


PREMIUM
Nature's Way
QUALITY
Folic Acid
800 mcg
DAILY ESSENTIAL
100 Capsules / Certified Potency
DIETARY SUPPLEMENT
SOLARAY
DIETARY SUPPLEMENT
Folic Acid
800 MCG
IN A WHOLE RICE FOOD BASE
WITH ALOE VERA
100 VEGETARIAN CAPSULES
NATURE'S BOUNTY
Folic
Acid
400mcg
Promotes
Cardiovascular
Health*
B
250 tablets VITAMIN SUPPLEMENT

Aplastic anaemia

A disorder of hematopoiesis characterized by marked reduction or absence of erythroid, granulocytic and megakaryocytic cells in the bone marrow with resultant pancytopenia.

Primary idiopathic acquired aplastic anaemia

This is a rare disorder in Europe and North America, with 2–4 new cases per million population per annum.

The basic problem is failure of the pluripotent stem cells because of an autoimmune attack, producing hypoplasia of the bone marrow with a pancytopenia in the blood.

The diagnosis rests on exclusion of other causes of secondary aplastic anaemia and rare congenital causes, such Fanconies anemia

Clinical features and investigations

Patients present with symptoms of bone marrow failure, usually anaemia or bleeding, and less commonly, infections.

An FBC demonstrates pancytopenia, low reticulocytes and often macrocytosis. Bone marrow aspiration and trephine biopsy reveal hypocellularity. The severity of aplastic anaemia is graded according to the Camitta criteria

Camitta criteria

Severe AA (SAA)

• Marrow cellularity < 25% (or 25–50% with < 30% residual haematopoietic cells), plus at least two of:
Neutrophils < 0.5 × 109/L
Platelets < 20 × 109/L
Reticulocyte count < 20 × 109/L

Very severe AA (VSAA)

• As for SAA but neutrophils < 0.2 × 109/L

Non-severe AA (NSAA)

• AA not fulfilling the criteria for SAA or VSAA

Lymphadenopathy and splenomegaly are highly atypical of aplastic anemia. Café au lait spots and short stature suggest Fanconi's anemia.

Investigations:

1.full blood count:

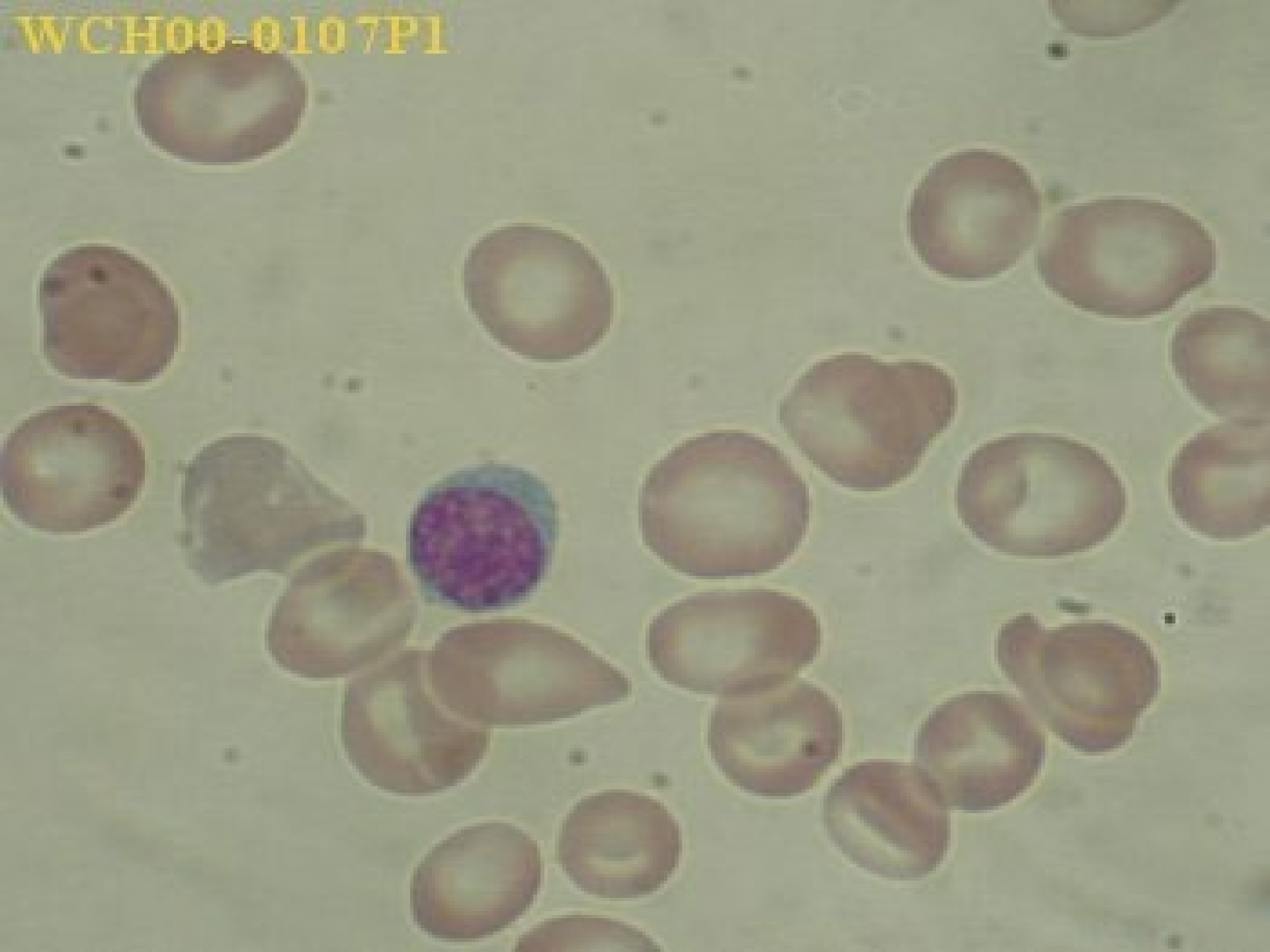
pancytopenia, reticulocytopenia, normochromic normocytic or slightly macrocytic anemia

2.bone marrow aspirate:

Numerous spiculs with empty fatty spaces and few hematopoietic cells

3.bone marrow biopsy :

allows a better assessment of the cellularity and permits evaluation for the presence of tumour cells haivy cells and fibrosis.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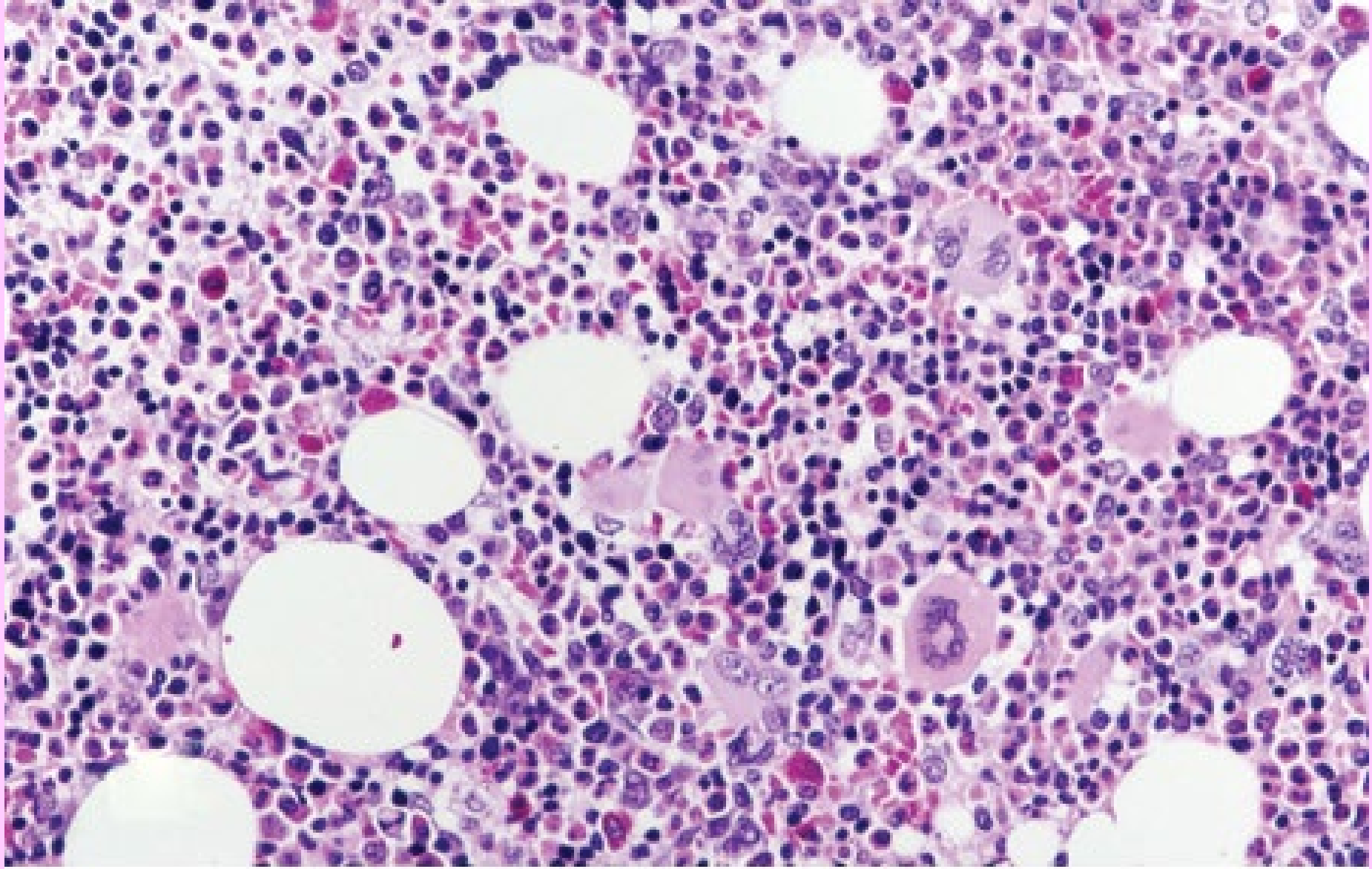

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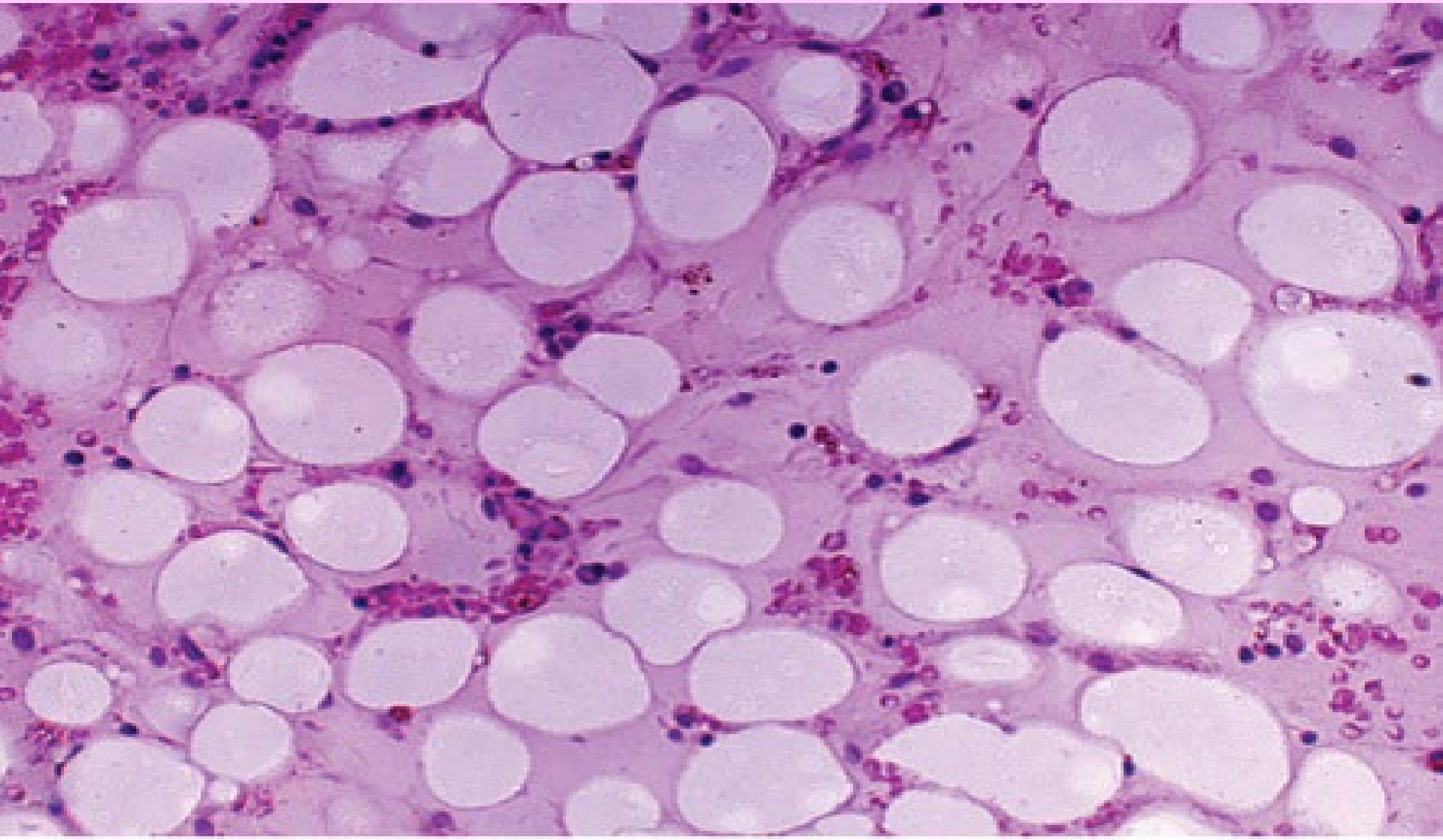

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Source: Lichtman MA, Kipps TJ, Seligsohn U, Kaushansky K, Prchal JT: *Williams Hematology, 8th Edition:* http://www.accessmedicine.com

Management

All patients will require blood product support and aggressive management of infection. The prognosis of severe aplastic anaemia managed with supportive therapy only is poor and more than 50% of patients die, usually in the first year.

The curative treatment for patients under 35 years of age with severe idiopathic aplastic anaemia is allogeneic HSCT if there is an available sibling donor . Older patients (35–50) may be candidates if they have no comorbidities .

Those with a compatible sibling donor should proceed to transplantation as soon as possible; they have a 75–90% chance of long-term cure.

In older patients and those without a suitable donor, immunosuppressive therapy (IST) with anti-thymocyte globulin (ATG) and ciclosporin is the treatment of choice and gives 5-year survival rates of 75%.

The thrombopoietin receptor agonist eltrombopag has produced trilineage responses in patients who fail immunosuppressive therapy and is licensed for this indication. Non-transplanted patients may relapse or other clonal disorders of haematopoiesis may evolve, such as paroxysmal nocturnal haemoglobinuria , myelodysplastic syndrome and AML , Patients with aplastic anaemia must be followed up long-term.

Causes of secondary aplastic anaemia

- Drugs
  - Cytotoxic drugs
  - Antibiotics – chloramphenicol, sulphonamides
  - Antirheumatic agents – penicillamine, gold, phenylbutazone, indometacin
  - Antithyroid drugs
  - Anticonvulsants
  - Immunosuppressants – azathioprine
- Chemicals
  - Benzene toluene solvent misuse – glue-sniffing
  - Insecticides – chlorinated hydrocarbons (DDT), organophosphates and carbamates
- Radiation
- Viral hepatitis
- Pregnancy
- Paroxysmal nocturnal haemoglobinuria

The clinical features and methods of diagnosis are the same as for primary idiopathic aplastic anaemia. An underlying cause should be treated or removed, but otherwise management is as for the idiopathic form.

THANK YOU