QUESTION 93
A 24-year-old woman, who has recently arrived in Australia from Vietnam, presents for evaluation of abnormal menstrual bleeding. There are no abnormalities on examination. Results of investigations are listed below.

Full blood count:
- haemoglobin: $113 \text{ g/L} [120-155]$  
- red cell count: $5.2 \times 10^{12} \text{ /L} [4.1-5.2]$  
- mean corpuscular volume (MCV): $71 \text{ fL} [80-95]$  
- mean corpuscular haemoglobin (MCH): $22.0 \text{ pg} [27.0-32.5]$  
- mean corpuscular haemoglobin concentration (MCHC): $310 \text{ g/L} [325-360]$  
- white cell count: $6.6 \times 10^9 \text{ /L} [3.5-9.5]$  
- platelet count: $212 \times 10^9 \text{ /L} [130-330]$  

Blood film shows red cell microcytosis and hypochromasia but is otherwise normal.

Haemoglobin (Hb) electrophoresis (cellulose acetate, pH 8.6):
- HbA$_2$: $2.7\% [1.8-3.5]$  
- HbF: $0.4\% [0-2.0]$  
- No abnormal bands

**HbH preparation:**

Sirum biochemistry:
- iron: $8 \text{ μmol/L} [7-32]$  
- transferrin: $3.2 \text{ g/L} [2.1-3.6]$  
- ferritin: $15 \text{ μg/L} [7-280]$  

The most likely diagnosis is:

A. homozygous alpha * thalassaemia (−α/−α).
B. early iron deficiency.
C. congenital sideroblastic anaemia.
D. sickle cell anaemia.
E. heterozygous beta thalassaemia.

**Evaluation of anaemia**

Overview — Erythropoiesis in the adult takes place within the bone marrow under the influence of the stromal framework, cytokines, and the erythroid specific growth factor, erythropoietin (EPO). EPO is a true endocrine hormone produced in the kidney by cells that sense the adequacy of tissue oxygenation relative to the individual's metabolic activity.

EPO enhances the growth and differentiation of the two erythroid progenitors: burst forming units-erythroid (BFU-E) and colony forming units-erythroid (CFU-E) into normoblasts of increasing maturity. When the normoblast extrudes its nucleus to form a red blood cell, it still has a ribosomal network which, when stained supravitally, identifies it as a reticulocyte, a cell still capable of a limited amount of hemoglobin and protein synthesis.

The reticulocyte retains its ribosomal network (and its staining characteristics) for about four days, of which three days are generally spent in the marrow and one day in the peripheral blood. The resulting mature RBC circulates for 110 to 120 days, after which it is removed from the circulation by macrophages that detect senescent signals, through mechanisms that are poorly understood.

Under steady state conditions, the rate of RBC production equals the rate of RBC loss. Assuming, as a first approximation, survival of mature RBC of 100 days, 1 percent of RBCs are removed from the circulation each day. To achieve a constant RBC mass, RBC losses must be replaced with an equal number of reticulocytes during the same time period.

Reticulocytes normally survive in the circulation for one day; after this time they lose their reticulum (RNA) and become mature red blood cells. Under steady-state conditions reticulocytes will represent approximately 1 percent of total circulating RBC. Since the normal RBC count is approximately 5 million/microL, the bone
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Marrow must produce approximately 50,000 reticulocytes/microL of whole blood each day in order to achieve a stable RBC mass. Lesser rates of RBC production, if persistent, lead to anemia.

The rate of red cell production increases markedly under the influence of high levels of erythropoietin (EPO). A normal bone marrow repelte with iron, folate, and cobalamin can increase erythropoiesis in response to EPO about 5-fold in adults and 7- to 8-fold in children. Thus, under optimal conditions, steady-state absolute reticulocyte counts as high as 250,000/microL are possible in the adult.

Volume status — HGB, HCT, and RBC count are all concentrations and dependent on the red blood cell mass (RCM) as well as the plasma volume. As a result, values will be reduced if the RCM is decreased and/or if the plasma volume is increased.

Symptoms — Symptoms related to anemia can result from two factors:
- decreased oxygen delivery to tissues, and,
- in patients with acute and marked bleeding, the added insult of hypovolemia. There is some reduction in blood volume but not plasma volume after acute severe hemolysis, due to the fall in RBC mass. In comparison, total blood volume remains normal in anemia due to chronic, low-grade bleeding, since there is ample time for equilibration with the extravascular space and renal retention of salt and water.

CAUSES OF ANEMIA — There are two general approaches one can use to help identify the cause of anemia:
- A kinetic approach, addressing the mechanism(s) responsible for the fall in hemoglobin concentration
- A morphologic approach categorizing anemias via alterations in RBC size (ie, mean corpuscular volume) and the reticulocyte response.

Kinetic approach — Anemia can be caused by one or more of three independent mechanisms:
- decreased RBC production,
- increased RBC destruction, and
- blood loss.

Decreased RBC production —
- Lack of nutrients, such as iron, B12, or folate. This can be due to dietary lack, malabsorption (eg, pernicious anemia, sprue), or blood loss (iron deficiency)
- Bone marrow disorders (eg, aplastic anemia, pure RBC aplasia, myelodyplasia, tumor infiltration)
- Bone marrow suppression (eg, drugs, chemotherapy, irradiation).
- Low levels of trophic hormones which stimulate RBC production, such as EPO (eg, chronic renal failure), thyroid hormone (eg, hypothyroidism), and androgens (eg, hypogonadism). A rare cause of anemia due to reduced EPO production has been described in patients with autonomic dysfunction and orthostatic hypotension
- The anemia of chronic disease/inflammation, associated with infectious, inflammatory, or malignant disorders, is characterized by reduced availability of iron due to decreased absorption from the gastrointestinal tract and decreased release from macrophages, a relative reduction in erythropoietin levels, and a mild reduction in RBC lifespan.

Increased RBC destruction —
- Hemolytic anemia will ensue when the bone marrow is unable to keep up with the need to replace more than about 5 percent of the RBC mass per day, corresponding to a RBC survival of about 20 days. Examples include:
  - Inherited hemolytic anemias (eg, hereditary spherocytosis, sickle cell disease, thalassemia major)
  - Acquired hemolytic anemias (eg, Coombs'-positive autoimmune hemolytic anemia, thrombotic thrombocytopenic purpura-hemolytic uremic syndrome, malaria)

Blood loss — Iron deficiency in the United States and Western Europe is almost always due to blood loss. Blood loss is the most common cause of anemia and may take any one of a number of forms:
- Obvious bleeding (eg, trauma, melena, hematemesis, menometrorrhagia)
- Occult bleeding (eg, slowly bleeding ulcer or carcinoma).
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- Induced bleeding (e.g., repeated diagnostic testing, hemodialysis losses, excessive blood donation)

In addition to the loss of RBCs from the body, which the bone marrow must replace, loss of the iron contained in these cells will ultimately lead to iron deficiency, once tissue stores of iron have been depleted. This usually occurs in males and females after losses of 1200 mL and 600 mL, respectively. However, since about 25 percent of menstruating females have absent iron stores, any amount of bleeding will result in anemia in this subpopulation.

Since availability of iron is normally rate-limiting for RBC production, iron deficiency associated with chronic bleeding leads to a reduced marrow response, worsening the degree of anemia.

Morphologic approach — The causes of anemia can also be classified according to measurement of RBC size, as seen on the blood smear and as reported by automatic cell counter indices. The normal RBC has a volume of 80 to 96 femtoliters (fL, 10⁻¹⁵ Liter) and a diameter of approximately 7 to 8 microns, equal to that of the nucleus of a small lymphocyte. Thus, RBCs larger than the nucleus of a small lymphocyte on a peripheral smear are considered large or macrocytic, while those that appear smaller are considered small or microcytic.

An increased RDW indicates the presence of cells of widely differing sizes, but it is not diagnostic of any particular disorder. However, some automatic cell counters have computer programs which "flag" for the presence of abnormalities such as:
- anisocytosis (cells of varying size),
- microcytosis, macrocytosis, and
- hypochromia (reduced hemoglobin content per cell)

Macrocytic anemia —
- characterized by an MCV above 100 fL (femtoliters) (An increased MCV is a normal characteristic of reticulocytes)
- Any condition causing marked reticulocytosis will be associated with an increased MCV.
- Abnormal nucleic acid metabolism of erythroid precursors (e.g., folate or cobalamin deficiency and drugs interfering with nucleic acid synthesis, such as zidovudine and hydroxyurea).
- Abnormal RBC maturation (e.g., myelodysplastic syndrome, acute leukemia, LGL leukemia). Other common causes include alcohol abuse, liver disease, and hypothyroidism.

Microcytic anemia —
- characterized by the presence of "small" RBCs (i.e., MCV below 80 fL).
- usually accompanied by a decreased hemoglobin content within the RBC, with parallel reductions in MCV and MCH, producing a hypochromic (low MCH) as well as a microcytic (low MCV) appearance on the blood smear
- The following pathologic processes lead to the production of hypochromic microcytic red cells:
  - Reduced iron availability —
    - severe iron deficiency,
    - the anemia of chronic disease,
    - copper deficiency
  - Reduced heme synthesis —
    - lead poisoning,
    - congenital or acquired sideroblastic anemia
  - Reduced globin production —
    - thalassemic states,
    - other hemoglobinopathies

The three most common causes of microcytosis in clinical practice are iron deficiency, alpha or beta thalassemia minor, and (less often) the anemia of chronic disease (anemia of chronic inflammation).

Normocytic anemia — By definition, the mean RBC volume is normal (MCV between 80 and 100 fL) in patients with normocytic anemia. Approach to this extremely large and amorphous category can be narrowed somewhat by examination of the blood smear to determine if there is a subpopulation of RBCs with distinctive size or shape.
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abnormalities which would place the patient in one of the above categories (ie, early microcytic or macrocytic anemia), or by use of the kinetic approach to determine the mechanism(s) underlying the anemia.

Systemic disorders — Anemia may be the first manifestation of a systemic disorder, along with other nonspecific complaints such as fever, weight loss, anorexia, and malaise. Simple laboratory tests may give additional clues toward the underlying disease process. These include abnormalities on the urinalysis or routine chest x-ray, liver or renal function tests, erythrocyte sedimentation rate, serum protein electrophoresis, WBC count and differential, and reduced (or increased) platelet counts. Anemia in the elderly is discussed separately (see "The elderly" above).

Anemia of chronic renal disease — Anemia is a common complication of renal disease, and may be multifactorial.

**homozygous alpha (+) thalassaemia (–α/–α)**
Alph (0) thalassemia refers to the more than 20 different genetic mutations of the alpha globin locus which result in the deletion of both alpha chain loci on one chromosome 16. Patients who carry alpha (0) gene mutations on both chromosomes cannot make alpha chains and are therefore unable to make any hemoglobin A, F, or A2. This condition is incompatible with extrauterine life.

Alpha (+) thalassemia — Alpha (+) thalassemia refers to the more than 15 different genetic mutations which result in decreased production of alpha globin, usually due to deletion of one of the two alpha chain loci in the affected chromosome. As a result, there are three general forms of alpha (+) thalassemia based upon the number of inherited alpha genes:

- Inheritance of three normal alpha genes (aa/a-a) has been termed alpha thalassemia minima, silent carrier of alpha thalassemia, alpha thalassemia-2 trait, or heterozygosity for alpha (+) thalassemia. Affected subjects are clinically normal and may also be hematologically normal; the diagnosis can be reliably made only via DNA analysis.
- Inheritance of two normal alpha genes has been termed alpha thalassemia minor or alpha thalassemia-1 trait, and is due either to heterozygosity for alpha (0) thalassemia (aa/-a) or homozygosity for alpha (+) thalassemia (a/-a). These subjects are clinically normal but may have minimal anemia along with reductions in mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH).
- Inheritance of one normal alpha gene (a/-/-) is termed hemoglobin H (HbH) disease, because of the formation of HbH, which is composed of tetramers of the resulting excess beta chains. These patients have moderate to severe degrees of lifelong hemolytic anemia, very modest degrees of ineffective erythropoiesis, splenomegaly, and variable bony changes.
- As mentioned above, inheritance of no alpha genes (-/-/-) is incompatible with extrauterine life, since the affected fetus will be unable to make any of the hemoglobins normally produced after birth (eg, hemoglobins A, F, and A2), all of which require the ability to produce alpha globin chains.

**Iron Deficiency Anaemia**

**CAUSES OF IRON DEFICIENCY**

- Blood loss — The major cause of iron deficiency in affluent countries is blood loss, either overt or occult.
- Decreased iron absorption — Gastrointestinal malabsorption of iron is a relatively uncommon cause of iron deficiency, although it may be observed in certain diseases associated with generalized malabsorption or achlorhydria. These diagnoses (ie, atrophic gastritis, Helicobacter pylori gastritis, sprue) should be considered in patients with otherwise unexplained iron deficiency, especially when there is refractoriness to oral iron therapy.
- There are several other uncommon causes of iron deficiency:
  - Intravascular hemolysis
  - Pulmonary hemosiderosis
  - Response to erythropoietin — A response to treatment with erythropoietin (EPO) for the anemia of chronic renal failure often leads to iron deficiency, since the iron requirements generated by this response can usually not be met by mobilization of the patient's iron stores alone.

**STAGES OF IRON DEFICIENCY**
Normal body iron content — The normal iron content of the body is 3 to 4 grams. It exists in the following forms:
- Hemoglobin in circulating red cells — approximately 2 grams
- Iron containing proteins, such as myoglobin, cytochromes, and catalase — 400 mg
- Iron bound to transferrin in plasma — 3 to 7 mg
- The remainder is storage iron in the form of ferritin or hemosiderin.

Storage iron in adult men has been estimated as being approximately 10 mg/kg, and is found mostly in liver, spleen, and bone marrow. Adult women have less storage iron, depending upon the extent of menses, pregnancies, deliveries, lactation, and iron intake.

For ferritin levels in the range from 20 to 300 ng/mL, there appears to be a direct quantitative relationship between the ferritin concentration and iron stores

Iron stores (mg) (8 to 10) x ferritin (ng/mL)

Progressive iron depletion —
- In the first stage, iron stores can be totally depleted without causing anemia. The storage iron pool, consisting primarily of ferritin and hemosiderin-bound iron within the monocyte-macrophage system chiefly in bone marrow, liver and spleen, contains approximately 0.8 to 1.0 g of iron in men and one-half this value in women. The storage pool can be looked upon as a reserve of iron that can be utilized when there is increased need for hemoglobin synthesis, as in acute blood loss, growth in children and adolescents, pregnancy, lactation and response to EPO. Once these stores are depleted, there is still enough iron present in the body within the "labile" iron pool from the daily turnover of red cells for normal hemoglobin synthesis, but the patient is now vulnerable to development of anemia should there be further iron losses
- Further loss of iron results in anemia, which is initially normocytic with a normal absolute reticulocyte count. This stage of iron deficiency is common in the United States.
- More profound deficiency results in the classical hypochromia and microcytosis of iron-deficient erythropoiesis.

Laboratory tests in iron deficiency of increasing severity

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>Fe deficiency without anemia</th>
<th>Fe deficiency with mild anemia</th>
<th>Severe Fe deficiency with severe anemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marrow reticulo-</td>
<td>2+ to 3+</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>endothelial iron</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>Serum iron, µg/dL</td>
<td>60 to 150</td>
<td>60 to 150</td>
<td>&lt;60</td>
<td>&lt;40</td>
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<tr>
<td>Iron binding capacity</td>
<td>300 to 360</td>
<td>300 to 390</td>
<td>350 to 400</td>
<td>&gt;410</td>
</tr>
<tr>
<td>(transferrin), µg/dL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saturation (SI/TIBC),</td>
<td>20 to 50</td>
<td>30</td>
<td>&lt;15</td>
<td>&lt;10</td>
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<tr>
<td>percent</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemoglobin, g/dL</td>
<td>Normal</td>
<td>Normal</td>
<td>9 to 12</td>
<td>6 to 7</td>
</tr>
<tr>
<td>Red cell morphology</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal or slight hypochromia</td>
<td>Hypochromia and microcytosis</td>
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<td>Plasma or serum</td>
<td>40 to 200</td>
<td>&lt;20</td>
<td>&lt;10</td>
<td>0 to 10</td>
</tr>
<tr>
<td>ferritin, ng/mL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythrocyte protoporphyrin, ng/mL</td>
<td>RBC 30 to 70</td>
<td>30 to 70</td>
<td>&gt;100</td>
<td>100 to 200</td>
</tr>
</tbody>
</table>
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<table>
<thead>
<tr>
<th>Other tissue changes</th>
<th>None</th>
<th>None</th>
<th>None</th>
<th>Nail and epithelial changes</th>
</tr>
</thead>
</table>

Note: Test results outlined in bold type are the ones most likely to define the various stages of iron deficiency. Thus, the presence or absence of iron stores (marrow reticuloendothelial iron) in a non-anemic patient serves to distinguish normal subjects from those with iron deficiency without anemia, respectively.

CLINICAL MANIFESTATIONS — The usual presenting symptoms in adults, as seen in current practice, are primarily due to anemia and include weakness, headache, irritability and varying degrees of fatigue and exercise intolerance. However, many patients are asymptomatic and present only with anemia.

Classic presentation — The following is a “classic” presentation of iron-deficiency anemia. The patient, a multigravid woman in her 40’s, presents with tiredness, fatigue, and chronic blood loss from menometrorrhagia. The following laboratory findings were noted:

- Hemoglobin was low at 8 g/dL; mean cell volume (MCV) was low at 75 fL
- The mean corpuscular hemoglobin (MCH) was low; a blood smear showed microcytic, hypochromic red cells
- The serum iron was low (10 microg/dL) and the total iron binding capacity (TIBC)/transferrin was elevated (400 microg/dL), resulting in a low transferrin saturation of 2.5 percent.
- The plasma ferritin concentration was markedly reduced (10 ng/mL)
- Iron stores were absent after performing the “gold standard” test of estimating iron stores via microscopic examination of the Prussian Blue reaction on an aspirate of the patient's bone marrow
- Finally, the patient responded briskly to a therapeutic trial of oral iron, with a reticulocytosis, followed by elevations in the hemoglobin concentration and hematocrit.

The current reality in developed countries is that this classic presentation is uncommon, and that the diagnosis and management of iron deficiency anemia is usually a good deal more complicated.

ESTIMATION OF IRON STORES — The history, complete blood count, red cell indices, and smear usually allow the clinician to make a presumptive diagnosis of iron deficiency anemia. This can be followed by a therapeutic trial of iron administration to provide both confirmation of the diagnosis and therapy.

Serum or plasma ferritin — The serum or plasma ferritin concentration is an excellent indicator of iron stores in otherwise healthy adults and has replaced assessment of bone marrow iron stores as the gold standard for diagnosis in most patients but

- Elevated in pregnancy
- Acute phase reactant in inflammatory states

Serum iron and transferrin (TIBC) — In iron deficiency anemia, the serum iron concentration (SI) is reduced, and the level of transferrin (also measured as total iron binding capacity [TIBC]) is elevated; the latter finding reflects the reciprocal relationship between serum iron and transferrin gene expression in most nonerythroid cells. The low SI and high transferrin/TIBC result in a low transferrin saturation or index (saturation = SI/TIBC x 100), often to levels less than 10 percent, compared to the normal value of 25 to 45 percent [16,42].

Bone marrow iron — Iron in bone marrow macrophages and erythroid precursors (sideroblasts) can be detected with the Prussian Blue stain on marrow spicules. Lack of stainable iron in erythroid precursors as well as marrow macrophages is considered by most clinicians to be the “gold standard” for the diagnosis of iron deficiency. In contrast, in uncomplicated anemia of chronic disease, iron is present in marrow macrophages but absent or reduced in erythroid precursors but

- Considered too invasive

Serum transferrin receptor — Circulating transferrin receptor (sTfR) is derived from bone marrow erythroid precursors. It provides a quantitative measure of total erythropoietic activity, since its concentration in serum is directly proportional to erythropoietic rate and inversely proportional to tissue iron availability. Thus, iron deficient patients should have increased levels of sTfR (
Red cell morphology and indices — Despite the classic description of iron deficiency as leading to a hypochromic, microcytic anemia, many iron deficient patients in western countries will have normal red cell morphology. Further, the finding of a hypochromic microcytic anemia is not pathognomonic of iron deficiency, with thalassemia and, less commonly, the anemia of chronic disease being the other common conditions encountered in daily practice. It is important to rule out these disorders before beginning a trial of iron therapy, since many such patients are already iron overloaded.

Reticulocyte indices — With the advent of automated counting of reticulocytes, several new reticulocyte parameters are available to clinicians and pathologists.

Red cell zinc protoporphyrin level — The last step in the biosynthesis of heme is the addition of iron to protoporphyrin IX. If iron is unavailable, zinc (Zn) substitutes, forming zinc protoporphyrin, which can be measured. This test measures the lack of iron, not why it is unavailable.

Red cell zinc protoporphyrin (FEP) is also elevated in lead poisoning.

**Congenital sideroblastic anaemia**

**Diagnosis**

**Complete blood count** —
- The anemia is usually moderate and normocytic or macrocytic, with a variable population of hypochromic cells on the blood smear.
- Particularly characteristic are occasional siderocytes: hypochromic red cells with basophilic stippling that stains positive for iron.
- Leukocyte and platelet counts are often within the normal range in patients with AISA. The presence of moderate leukopenia and/or thrombocytopenia tends to be associated with other myelodysplastic features, such as the pseudo-Pelger anomaly. Leukocytosis and/or thrombocytosis are least common, and may reflect the presence of a myeloproliferative disorder.
- Free erythrocyte protoporphyrin — The free erythrocyte protoporphyrin is characteristically increased, up to about 300 µg/dL (normal: 20 to 65). However, in some patients, values have ranged from 1055 to 10,514 µg/dL, and some have experienced photosensitivity.
- Iron studies — Serum iron and ferritin levels reflect the commonly associated iron overload, as in hereditary sideroblastic anemia.
- Bone marrow examination — Bone marrow aspiration shows the presence of erythroid hyperplasia, commonly with mild megaloblastic changes. The marrow macrophage iron content is increased and, in contrast to the hereditary form, ring sideroblasts are evident at all stages of maturation; their presence establishes the diagnosis.

**Sickle Cell Disease**

**Findings in sickle cell disease** —

The chronic hemolysis of sickle cell disease is usually associated with
- a mild to moderate anemia (hematocrit 20 to 30 percent),
- reticulocytosis of 3 to 15 percent (accounting for high or high-normal mean corpuscular volume [MCV]),
- unconjugated hyperbilirubinemia, and
- elevated serum LDH and low serum haptoglobin.

The peripheral blood smear reveals
- sickled red cells,
- polychromasia indicative of reticulocytosis, and
- Howell-Jolly bodies reflecting hyposplenism.

The red cells are
- normochromic unless there is coexistent thalassemia or iron deficiency.
- If the age-adjusted MCV is not elevated, the possibility of sickle cell-beta thalassemia, coincident alpha thalassemia, or iron deficiency should be considered.

- The Hb F level is usually slightly to moderately elevated and
- Hb A is absent).
The amount of Hb F is a function of the number of reticulocytes that contain Hb F, the extent of selective survival of Hb F-containing reticulocytes to become mature Hb F-containing erythrocytes, and the amount of Hb F per red cell.

This girl has
- Hb 113
- a microcytic hypochromic anaemia – consistent with iron deficiency
- normal HbA2 (absent in sickle cell) and Hb F (raised in sickle cell)
- No abnormal bands
- HbH preparation: HbH inclusions present
- Iron studies within normal range (would be raised in congenital sideroblastic anaemia – associated with iron overload)

A. homozygous alpha thalassaemia (−α/−α).

Alpha thalassemia-1 trait — Alpha thalassemia-1 trait, also called alpha thalassemia minor, resembles mild beta thalassemia trait.

The peripheral blood smear shows
- hypochromia,
- microcytosis, and
- target cells.
- The MCV is often less than 80 fL, but hemoglobin electrophoresis is normal.
- Elevation of Hb A2 does not occur in alpha thalassemia;
- slight elevations of Hb F have been reported.

The reduced synthesis of alpha globin chains leads to an accumulation of otherwise normal beta globin chains in adults and gamma chains in the fetus. Instead of forming alpha/beta dimers which then form normal HbA tetramers (α2β2), the excess beta globin chains assemble into beta-4 tetramers, called HbH hence the positive HbH inclusions and therefore the correct answer

B. early iron deficiency.
To have hypochromic cells indicates iron deficiency, if this were the cause there would be abnormalities in her iron studies especially ferritin level – see table above

C. congenital sideroblastic anaemia.
Normal iron studies – The iron studies would most likely be raised consistent with the common association of iron overload

D. sickle cell anaemia.
normal HbA₂ 2.7% [1.8-3.5] - would be low in sickle cell anaemia and HbF – would be raised in sickle cell

E. heterozygous beta thalassaemia.
The terms beta thalassemia minor and beta thalassemia trait are used to describe heterozygotes who carry one normal beta globin allele and one beta thalassemic allele. The vast majority of these patients are entirely asymptomatic, but do present an abnormal blood picture that is sometimes erroneously diagnosed as iron deficiency anaemia.

- Typically, the blood count and peripheral blood film exhibit features similar to those seen in iron deficiency anemia (eg, hypochromia and microcytosis).
- However, as a rule, the microcytosis is much more profound, and the anemia much milder, than that seen in iron deficiency anemia.
- Patients with beta thalassemia trait almost always have a hematocrit >30 percent, and a mean corpuscular volume of the red cells (MCV) <75 fL.
- The RDW in patients with thalassemia trait tends to be normal, since virtually all cells are hypochromic and microcytic.