HEMATOLOGY CASE STUDY:
A Hypochromic, Microcytic Anemia

Course # DL-922

by
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1. a  b  c  d
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4. a  b  c  d
5. a  b  c  d
6. a  b  c  d
7. a  b  c  d
8. a  b  c  d
9. a  b  c  d
10. a  b  c  d

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HEMATOLOGY CASE STUDY: A HYPOCHROMIC, MICROCYTIC ANEMIA
Course Number: DL-922
1.0 CE
Level of Difficulty: Basic

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CASE: A 19 year old man (C.C.) had been competing as an amateur boxer. He was successful enough to turn professional. He found his stamina decreased in the longer pro-boxing bouts, making him less competitive in the professional ranks. Concern regarding his boxing future caused him to consult a physician. The physical exam was normal. His CBC yielded the following results:

<table>
<thead>
<tr>
<th>TEST</th>
<th>RESULTS</th>
<th>REF. RANGE*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CONVENT. UNITS</td>
<td>SI UNITS</td>
</tr>
<tr>
<td>WBC</td>
<td>9.5 x 10^3/µl</td>
<td>9.5 x 10^9/l</td>
</tr>
<tr>
<td>RBC</td>
<td>5.35 x 10^6/µl</td>
<td>5.35 x 10^12/l</td>
</tr>
<tr>
<td>Hgb</td>
<td>10.5 g/dl</td>
<td>105 g/l</td>
</tr>
<tr>
<td>Hct</td>
<td>36.0%</td>
<td>360 l/l</td>
</tr>
<tr>
<td>MCV:</td>
<td>67 fl</td>
<td>67 fl</td>
</tr>
<tr>
<td>MCH:</td>
<td>19.6 pg</td>
<td>19.6 pg</td>
</tr>
<tr>
<td>MCHC:</td>
<td>29.2 g/dl</td>
<td>292 g/l</td>
</tr>
<tr>
<td>RDW:</td>
<td>14.2%</td>
<td></td>
</tr>
<tr>
<td>Platelets</td>
<td>260 x 10^3/µl</td>
<td>260 x 10^9/l</td>
</tr>
<tr>
<td>MPV</td>
<td>10.5 fl</td>
<td></td>
</tr>
</tbody>
</table>

*Harmening (Ref. 1)

The RBC morphology on the peripheral blood smear showed microcytosis with slight hypochromia. A few target cells and slight anisocytosis were noted.

The physician, noting the low hemoglobin and hematocrit, prescribed oral iron and ordered a test for the most common source of unknown bleeding in adult males, a stool occult blood. The test was negative. After 2 months of iron therapy C.C. reported no improvement in his endurance. A repeat CBC at this time showed similar results to the first one. At this point the physician consulted with the hematology Clinical Laboratory Scientist before ordering additional tests.

Consider the questions
1. What are the causes of hypochromic, microcytic anemias?
2. What tests are used to differentiate among them?
COURSE OBJECTIVES: at the end of the course the participant will be able to
1. List the causes of hypochromic, microcytic anemias
2. Identify tests used to differentiate among hypochromic, microcytic anemias
3. State the globin chain composition of the various hemoglobins mentioned
4. Differentiate between the genetic causes of alpha thalassemia and beta thalassemia
5. Discuss the clinical manifestations of homozygous versus heterozygous states of the
defective genes
6. Describe the red cell morphology associated with thalassemia minor
7. Differentiate between alpha thalassemia minor and beta thalassemia minor
8. Evaluate and compare laboratory tests and morphology between thalassemia minor and iron
deficiency anemia

DISCUSSION: The causes of hypochromic, microcytic anemias are iron deficiency (the most
common), anemia of chronic disease, thalassemia, sideroblastic anemia, and lead poisoning.
They may be differentiated by the tests in the following Table 1:

<table>
<thead>
<tr>
<th></th>
<th>RDW</th>
<th>Serum Iron</th>
<th>TIBC</th>
<th>Ferritin</th>
<th>FEP</th>
<th>A2 level</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency</td>
<td>Inc</td>
<td>Dec</td>
<td>Inc</td>
<td>Dec</td>
<td>Inc</td>
<td>Nor</td>
</tr>
<tr>
<td>Chronic disease</td>
<td>Nor</td>
<td>Dec</td>
<td>Dec</td>
<td>Inc</td>
<td>Inc</td>
<td>Nor</td>
</tr>
<tr>
<td>αThalassemia trait</td>
<td>Nor</td>
<td>Nor</td>
<td>Nor</td>
<td>Nor</td>
<td>Nor</td>
<td>Nor</td>
</tr>
<tr>
<td>βThalassemia trait</td>
<td>Nor</td>
<td>Nor</td>
<td>Nor</td>
<td>Nor</td>
<td>Nor</td>
<td>Inc</td>
</tr>
<tr>
<td>Sideroblastic</td>
<td>Inc</td>
<td>Inc</td>
<td>Nor</td>
<td>Inc</td>
<td>Inc</td>
<td>Nor</td>
</tr>
<tr>
<td>Lead poisoning</td>
<td>Nor</td>
<td>Nor</td>
<td>Nor</td>
<td>Nor</td>
<td>Inc</td>
<td>Nor</td>
</tr>
</tbody>
</table>

RDW = red cell distribution width, TIBC = total iron binding capacity,
FEP = free erythrocyte protoporphyrin
*Adapted from Harmening (Ref. 1)

The physician ordered serum iron, ferritin, and FEP. The results were within reference
ranges, eliminating iron deficiency, anemia of chronic disease, sideroblastic anemia, and lead
poisoning. This resulted in a provisional diagnosis of Thalassemia minor. At this point, what
other information would be useful to confirm the diagnosis?
THALASSEMAIA

The Thalassemias are a heterogeneous group of hereditary diseases of hemoglobin synthesis involving decreased production of one of the hemoglobin globin chain types. Normal adult hemoglobin is composed of 95 - 97% Hb A (2α and 2β chains), 2 – 3% Hb A2 (2α and 2δ chains), and 2% Hb F (fetal hemoglobin, 2α and 2γ chains). The 2 principal types of Thalassemia are alpha Thalassemia and beta Thalassemia, depending on which chains are affected. The following shows a general classification:

**NORMAL**

<table>
<thead>
<tr>
<th>HEMOGLOBIN F</th>
<th>HEMOGLOBIN A</th>
</tr>
</thead>
<tbody>
<tr>
<td>α2γ2</td>
<td>α2β2</td>
</tr>
</tbody>
</table>

**ALPHA THALASSEMAIA**

\[ \alpha^2\gamma2 \]

excess gamma chains, Hb Bart’s

\[\alpha^2\beta2\]

excess beta chains, Hb H

**BETA THALASSEMAIA**

\[ \alpha^2\gamma2 \]

Hb F persists beyond infancy

\[ \alpha^2\beta2 \]

excess alpha chains ppt. as inclusions

<table>
<thead>
<tr>
<th>Condition</th>
<th>Genotype</th>
<th>Clinical Feature</th>
<th>Newborn Hb pattern</th>
<th>&gt;First Year Hb pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrops fetalis</td>
<td>-/-</td>
<td>Fetal or neonatal death</td>
<td>Hb Bart’s &gt;80%</td>
<td>--</td>
</tr>
<tr>
<td>Hb H disease</td>
<td>-/-α</td>
<td>Chronic hemolytic anemia</td>
<td>Hb Bart’s 20-40%</td>
<td>Hb H 5-30% Hb Bart’s--trace</td>
</tr>
<tr>
<td>Thalassemia minor</td>
<td>-/ααα or</td>
<td>Slight anemia, Micro, hypo RBC</td>
<td>Hb Bart’s 2-10%</td>
<td>Normal</td>
</tr>
<tr>
<td>Silent Carrier</td>
<td>αα/-α</td>
<td>No hematologic or clinical abnormal</td>
<td>Hb Bart’s 1%</td>
<td>Normal</td>
</tr>
<tr>
<td>Normal</td>
<td>αα/αα</td>
<td>No hematologic or clinical abnormal</td>
<td>Hb Bart’s 0-trace</td>
<td>Normal</td>
</tr>
</tbody>
</table>

**TABLE 2**

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Deletion of all 4 genes is incompatible with life. Hb H disease has some production of Hb A but Hb H ($\beta^4$) is unstable and precipitates in the cells, causing increased hemolysis of RBCs.

Alpha thalassemia minor can be caused by both genes deleted on one chromosome or 1 gene deleted on both chromosomes. Deletion of only one gene causes no apparent consequences, a condition called a silent carrier.

Alpha thalassemia is more commonly found in Southeast Asia, less commonly in the Mediterranean, and sporadically in other parts of the world.

Other genetic abnormalities that cause alpha chain elongation, such as Hb Constant Spring, Hb Seal Rock, Hb Koya Dora, or Hb Icaria, result in decreased alpha chain production with effects similar to alpha gene deletion. Other genetic causes of decrease in alpha chain production have been identified, giving geneticists much fodder for investigation.

**BETA THALASSEMIA**

There are 2 genes for production of beta chains, one on each chromosome 11. The genetics of decreased production of beta chains is more complex than that found in alpha thalassemia. Beta-thalassemias are heterogeneous at the molecular level. More than 200 disease-causing mutations have been identified to date. The large majority of mutations are simple single-nucleotide substitutions, or deletion or insertion of oligonucleotides leading to a frameshift. Rarely, the $\beta$-thalassemias are the result of gross gene deletion. A number of different genetic backgrounds have been described, usually associated with a different geographic area. Beta thalassemia is commonly found in the Mediterranean Sea area (‘thalassa’ means sea). It is particularly common in northern Italy, Greece, Algeria, and Saudi Arabia and can also be found across southern Asia to Southeast Asia. The clinical severity of beta thalassemia is variable, depending on the type of genetic defect or the combination of defects. Severe beta thalassemia is not evident until the infant is several months old since Hb F is produced in adequate quantities until then. The main categories of genetic defects are $\beta^-$ and $\beta^+$. $\beta^-$ gene produces no beta chains. $\beta^+$ gene produces variable amounts of beta chains depending on the specific genetic inheritance. There are several other genetic defects: Hb Lepore, which results from unequal crossover between delta and beta genes, and $\delta\beta$Thal, a combined defect of delta and beta chain synthesis. These are less common and will not be discussed further. The following table gives a brief overview of beta thalassemias:

**TABLE III-A  Severe thalassemia**

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Genotype</th>
<th>Hb pattern</th>
<th>Clinical feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta^-$ thalassemia</td>
<td>$\beta^-\beta^-$</td>
<td>No HbA, var. Hb A2 remaining is HbF</td>
<td>Thalassemia major</td>
</tr>
<tr>
<td>$\beta^+$ thalassemia</td>
<td>$\beta^+\beta^+$</td>
<td>↓Hb A, ↑Hb F, variable Hb A2</td>
<td>Thalassemia major or intermedia</td>
</tr>
<tr>
<td>$\beta^-\beta^+$ heterozygote</td>
<td>$\beta^-\beta^+$</td>
<td>↓↓Hb A, ↑↑Hb F, variable Hb A2</td>
<td>Thalassemia major</td>
</tr>
</tbody>
</table>

**TABLE III-B  Thalassemia minor**

<table>
<thead>
<tr>
<th>Syndrome</th>
<th>Genotype</th>
<th>Hb pattern</th>
<th>Clinical feature</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\beta^-$ thal minor</td>
<td>$\beta^-\beta$</td>
<td>Hb A, ↑Hb A2, slight ↑Hb F</td>
<td>Thalassemia minor</td>
</tr>
<tr>
<td>$\beta^+$ thal minor</td>
<td>$\beta^+\beta$</td>
<td>Hb A, ↑Hb A2, slight ↑Hb F</td>
<td>Thalassemia minor</td>
</tr>
</tbody>
</table>
LABORATORY FINDINGS IN THALASSEMIA

The main focus of this course is thalassemia minor, but a brief discussion of the more severe thalassemias follows:

**Thalassemia major:**
Anemia is profound – Hb 2-3 g/dl (Hb 20 to 30 g/L). Hematocrit and RBC count are also decreased, hence the indices are uniformly depressed. The MCV, MCH and MCHC are all decreased. The RDW is increased due to anisocytosis. The blood smear shows marked hypochromia and microcytosis with extreme anisocytosis and poikilocytosis with bizarre shapes, target cells, ovalocytosis, Cabot rings, Howell Jolly bodies, nuclear fragments, basophilic stippling, siderocytes, and often large number of nucleated RBCs.

**Hemoglobin H disease:**
The peripheral smear shows hypochromia and microcytosis, target cells, mild to moderate anisopoikilocytosis. Incubation of blood with brilliant cresyl blue supravital stain will cause precipitation of Hb H in the erythrocytes seen as multiple “golf ball like” inclusion bodies.

**Thalassemia minor:**
Thalassemia minor is common, particularly in areas where there are people of Mediterranean, Southeast Asian, and African ancestry. As was illustrated by the case study, the causes of a low hemoglobin and hematocrit must be differentiated. In the absence of clinical symptoms, giving a course of oral iron therapy and evaluating the result is not a recommended procedure to assure quality patient outcomes. Because iron overload may result, assessment of serum iron, ferritin, TIBC, and FEP will better determine the probable cause. Decreased serum iron would indicate iron deficiency or anemia of chronic disease; increased serum iron would indicate sideroblastic anemia and increased FEP along with normal serum iron would be characteristic of lead poisoning. Again, referring to the question posed at the end of the case study, what other information would be useful to confirm the diagnosis?

In this patient, a healthy active young man, anemia of chronic disease and lead poisoning are unlikely. Iron deficiency is ruled out by the unresponsiveness to iron therapy. Knowledge of the individual’s racial background might be useful. In this case, he was of Italian descent. This particular ancestry coupled with the decreased MCV and MCH that are not corrected with iron therapy targets a diagnosis of Thalassemia minor. The next step is to determine the type of thalassemia. Hemoglobin electrophoresis may be useful in demonstrating the type of thalassemia by showing the presence of Hb A2, Hb F, Hb H, Hb Constant Spring, Hb Lepore or other structurally abnormal hemoglobins. In this case Hb A2 was increased (5%) and Hb F was 4.5%. Thus, there is corroboration of beta thalassemia minor.

Diagnosis of thalassemia minor is important in order to reassure the patient that the levels of hemoglobin and hematocrit are normal for him and he should not be placed on iron therapy, which could lead to iron overload. Also the patient needs to be counseled that if he marries a woman who is a carrier of beta thalassemia, hemoglobin E or hemoglobin S, there may be significant consequences in their children.

Alpha thalassemia minor is harder to diagnose than beta thalassemia minor because the levels of Hb A2 and Hb F are not increased. Frequently it is a diagnosis made by exclusion. Again knowing the patient’s racial background is useful. The hematological values along with other clues, such as racial background, will help.
There are several ways a laboratorian may suspect that the patient has a thalassemia minor from the initial CBC. In particular it is important to differentiate between thalassemia minor and iron deficiency. In thalassemia minor the hemoglobin and hematocrit are decreased but the RBC count is not correspondingly low and frequently is in the normal range, resulting in discordance in the indices. (The MCV is slightly decreased and the MCH is decreased but the MCHC is near normal). Also the cells tend to be a similar size so the RDW is normal. In contrast, in iron deficiency the RBC count is usually relatively lower and there is significantly more anisocytosis, thus the indices are in concordance and the RDW is increased. A mathematical manipulation of the indices has been used to help differentiate between thalassemia minor and iron deficiency. One of the formulas is Mentzer’s, as follows:

<table>
<thead>
<tr>
<th>MCV</th>
<th>If the result is &lt;13, then thalassemia minor</th>
</tr>
</thead>
<tbody>
<tr>
<td>RBC</td>
<td>If the result is &gt;13, then iron deficiency</td>
</tr>
</tbody>
</table>

The red cell morphology on the blood smear may also give indication of whether the patient has thalassemia minor or iron deficiency. The morphology seen in thalassemia minor is hypochromic, microcytic with slight anisocytosis, mild to moderate poikilocytosis, target cells and frequently basophilic stippling. The smear is characterized by having a majority of similar appearing red blood cells. The morphology in iron deficiency shows hypochromia, microcytosis, moderate anisocytosis, mild to moderate poikilocytosis—ovalocytes, “pencil cells” (long elliptical forms), folded cells, usually no basophilic stippling.

The differences are that thalassemia minor has similar sized cells, usually no pencil shaped cells and may show basophilic stippling while iron deficiency has moderate anisocytosis, more poikilocytosis, especially pencil shaped cells, and no basophilic stippling. An individual’s iron stores must be determined. Serum ferritin is a good indicator of the level of stored iron. In iron deficiency there are decreased stores of iron as indicated by decreased serum ferritin. In thalassemia minor there are normal iron stores. (refer to Table I)

CONCLUSION
In this course we have discussed the causes of hypochromic, microcytic anemias: iron deficiency, α thalassemia, β thalassemia, anemia of chronic disease, sideroblastic anemia, and lead poisoning. These anemias may be differentiated by the laboratory tests shown in Table I along with clinical history. Emphasis was placed on the causes and identification of thalassemias, in particular the types of thalassemia minor. Identifying and differentiating thalassemia minor from iron deficiency anemia may be done by evaluating the serum iron and ferritin levels, the FEP, and TIBC. Cellulose acetate electrophoresis may differentiate between α thalassemia and β thalassemia.

REFERENCES
REVIEW QUESTIONS
Select the one best answer.

1. A patient with hypochromic, microcytic anemia has increased serum iron, increased RDW, increased ferritin, increased FEP. What is the most probable diagnosis?
   a. lead poisoning
   b. sideroblastic anemia
   c. anemia of chronic disease
   d. thalassemia minor

2. Using Table I, which test would differentiate between alpha thalassemia minor and lead poisoning?
   a. Hgb A2
   b. TIBC
   c. ferritin
   d. FEP

3. At birth in alpha thalassemia there is an increase of Hb Bart’s. Hb Bart’s is composed of which of the following?
   a. 4 α chains
   b. 4 γ chains
   c. α2γ2
   d. 4 δ chains

4. Severe beta thalassemia is not diagnosed until after the infant is several months old because
   a. Hb F is present in sufficient quantities in young infants.
   b. The extra hemoglobin in newborns takes 2 months to decrease.
   c. Hb A2 is present in sufficient quantities to substitute for HbA
   d. Hemoglobin from the mother lasts for about 2 months.

5. The genetic mechanism associated with alpha thalassemia is
   a. mutation in intervening sequences in the gene
   b. includes unequal crossover (Hb Lepore)
   c. deletion of one or more genes
   d. mutation in the promoter area

6. A useful test to differentiate between alpha thalassemia minor and beta thalassemia minor is
   a. RDW
   b. FEP
   c. Hb A2
   d. presence of stippled cells on blood smear
7. A patient has the following values on the CBC:

- RBC: $4.02 \times 10^{12}/l$ (4.2 x $10^6/\mu l$)
- MCV: 79.6 fl
- Hgb: 90 g/l (9.0 g/dl)
- MCH: 22.4 pg
- Hct: 320 l/l (32.0%)
- MCHC: 281 g/l (28.1/dl)
- RDW: 16.2%

The differential diagnosis is iron deficiency anemia or thalassemia minor. Which of the values is the most help in differentiating between the two?

a. RDW  
b. MCV  
c. Hct  
d. MCH

8. A Mentzer calculation of the values from the patient in Question #7 would indicate that patient has

a. an iron deficiency anemia  
b. thalassemia minor  
c. thalassemia major  
d. anemia of chronic disease

9. Differences in RBC morphology between thalassemia minor and iron deficiency anemia include evaluating

a. the amount of microcytosis  
b. presence of target cells  
c. presence of ovalocytes  
d. presence of stippled cells

10. Inheritance of $\beta^+$ genes results in which of the following clinical conditions in the individual?

a. thalassemia major  
b. thalassemia intermedia  
c. thalassemia minor  
d. no clinical abnormality