Education Activity Case Study, Presentation

On the way to a family reunion, two sisters driving in the same vehicle are involved in an automobile accident. Many vehicles are involved in this accident as a consequence of slippery roads due to snow and ice. As one of the sisters is pregnant, at approximately 34-weeks gestational age, they are brought to the Emergency Department.

Carol, the pregnant sister, is 24 years of age, and Beatrice is 29-years-old. Following the hospital protocol, a type and screen is obtained from both of them.

Carol is O Rh negative with a positive antibody screen. Her sister is also O Rh negative with a positive antibody screen. Anti-D is the only antibody identified for both sisters.

Carol is admitted for observation, since she begins to have contractions. As prenatal care was performed elsewhere, pertinent information is collected. Carol had received prophylactic Rh immune globulin (RhIG) at week 28. Carol relays that her husband, the father, is blood type A Rh positive. The older sister Beatrice has had three children. The first child was normal and from a normal delivery. The second child developed hemolytic disease of the fetus and the newborn (HDFN). The third child required intensive treatment and the baby stayed in the neonatal intensive care unit (NICU) for several weeks because of HDFN. Beatrice and Carol have never been transfused and/or admitted to a hospital, with the exception of the time that Beatrice delivered her children.

After twelve hours of active labor, Carol delivers (vaginal) a baby boy.

RhIG is administered to the mother less than 24-hours after delivery, and the dose is calculated based on the results of the Kleihauer-Betke test.

At 24-hours the baby is jaundiced. A blood smear review reports the presence of spherocytes. A direct antiglobulin test (DAT) performed on cord blood is positive. A red cell eluate from cord blood shows the specificity of the antibody to be anti-A. The bilirubin is measured at 15 mg/dL and is predominantly indirect.

INTRODUCTION

Hemolytic disease of the fetus and the newborn (HDFN), previously known as erythroblastosis fetalis (reflecting the presence of large numbers of nucleated red blood cells [NRBCs] in severe cases), is presently classified as an alloimmune hemolytic disorder.

HDFN is caused by the transplacental passage of maternal antifetal red cell antibodies. Currently, this disorder is generally referred to as hemolytic disease of the fetus and newborn (HDFN) and from now on we will use HDFN in this education activity. In other words, HDFN occurs when fetus / newborn red cells possess an antigen that the mother lacks and the mother generates an antibody against the antigen. The
trigger red cell antigen in the fetus/newborn is inherited from the father. The antibody must cross the placenta (IgG crosses the placenta, but IgM and IgA do not). More than 50 different RBC antigens can cause HDFN. Most frequently, HDFN results from incompatibility in Rh and ABO red cell antigens between mother and fetus. However, many other red cell antigens, in a much lower frequency, can be responsible for this disorder. Over 30 years ago, this condition was synonymous with Rh D alloimmunization and was a common occurrence. Moreover, prenatal care had little effect on the outcome and it resulted in a significant number of babies with considerable morbidity and mortality. The introduction in the late 60s and 70s of prophylactic anti-D immunoglobulin (IG) for Rh D negative women has changed the landscape of HDFN and counts as one of the great success stories in modern medicine.

HYPERBILIRUBINEMIA IN THE NEWBORN

When considering HDFN we have to account for the mother’s care as well as the baby’s clinical presentation. Moreover, most of the medical progress achieved in the last several decades resulted from measures to prevent sensitization of the mother to Rh D antigen and/or counteract the fetal red cell hemolysis by intensive and close monitoring of the pregnant mother identified at risk for HDFN.

Increased newborn bilirubin is a very common occurrence. Initial evaluation requires consideration of the age of the infant, associated clinical findings, and most importantly, if the bilirubin is primarily unconjugated (indirect) or conjugated (direct).

Increased conjugated bilirubin is always a condition of concern in the newborn, and the differential diagnosis includes biliary atresia or obstruction, hepatitis, and Alpha 1 antitrypsin deficiency. Increased unconjugated bilirubin is most commonly physiologic (generally breast feeding related), but may be also a manifestation of HDFN, red cell hemolysis, sepsis, Gilbert disease, Crigler–Najjar syndrome, or many other inherited conditions.

ALLOIMUNIZATION AND PATHOPHYSIOLOGY

The pathophysiology of HDFN requires initial sensitization, that is, the mother must be exposed (sensitized) to a red cell antigen that she lacks. The fetus’ red cells must have the antigen to which the mother is sensitized.
The most common mechanisms for maternal sensitization include previous pregnancy and transfusion. During pregnancy and at delivery, small amounts of fetal red cells may enter the maternal circulation. The risk of sensitization is usually related to the volume of fetal blood that reaches the mother and the red cell antigens responsible for the incompatibility.

Although several classes of immunoglobulins may be produced (IgG, IgM, IgA) as part of the immunization response, only IgG has the ability to cross the placenta and cause HDFN.

**ABO Related**

Most ABO related situations, like the one presented in the Blood Cell Identification challenges, occur in group O mothers with group A or group B infants. It is important to remember that in ABO-HDFN, the mother already has the naturally occurring antibodies (anti-A and anti-B), and consequently previous sensitization is not required. Therefore, the clinical syndrome may present during the first pregnancy.

In contrast, in Rh (D) related HDFN the mother is Rh negative (D negative) and the baby is Rh positive (D-positive). The first born is usually not affected, unless there was previous sensitization by a previous pregnancy or transfusion.

Although ABO incompatibility occurs in about 15% of group O pregnancies, HDFN secondary to ABO incompatibility is estimated to occur in only 3% of all births. This mechanism is now the most common HDFN presentation.

HDFN with ABO incompatibility is less severe than the one observed with Rh D incompatibility. Occasionally, the diagnosis is suggested by the presence of unexplained hyperbilirubinemia in a group A or B infant with a group O mother, as was seen in the case history associated with the Blood Cell Identification challenges BCK/BCP-21 through BCK/BCP-25. In contrast to Rh D mediated disease, spherocytosis in ABO may be prominent.

**Rh Related**

Two separate genes located on the short arm of chromosome 1 encode Rh proteins. Rh antigens exist in three loci: Cc, Dd, and Ee. Rh expression is limited to mature RBCs with no Rh antigen expression on RBC progenitors (young NRBCs).

The risk of Rh immunization when immunophylaxis is not administered is estimated to be:

- 16% if Rh positive fetuses are ABO compatible with their Rh negative mothers
- About 2% if they are ABO incompatible
- About 5% after therapeutic abortion
- 2% after spontaneous abortion
The ABO incompatible RBCs are rapidly destroyed in the maternal circulation, reducing the likelihood of exposure to the immune system.

In regard to positive RhD expression, about 45% of individuals are homozygous (DD) and 55% are heterozygous (Dd). The Rh negative phenotype represents absence of D protein on RBCs, and most commonly results from deletion of the RhD gene on both chromosomes. Rh negative individuals (d/d) comprise about 15% of Caucasians, 5% of African Americans, and less than 1% of Asians.

Individuals with partial or weak D phenotype express normal but reduced quantities of D antigen on the RBC surface, and most (90%) cannot be sensitized to produce anti-D. However, there is a small group of individuals that are part of the partial D phenotype that can make anti-D, and consequently are at risk for HDFN. As most women with partial D phenotype are classified as Rh negative during routine prenatal evaluation, they are eligible for Rh immune globulin treatment (RhIG).

**CLINICAL AND LABORATORY HIGHLIGHTS THAT IMPACT MANAGEMENT**

The clinical presentation of HDFN has a wide spectrum that varies from mild jaundice and anemia to hydrops fetalis (abnormal accumulation of fluid in tissues and body cavities).

**Fetal Stage**

As the placenta and mother contribute to clearing the fetal bilirubin, the main problem for the fetus is anemia. Extramedullary hematopoiesis occurs as a fetal response to the anemia resulting in hepatosplenomegaly. In the most severe situations, heart failure occurs when large amounts of fluid build-up in many locations, creating hydrops.

Currently, intrauterine transfusions are utilized to correct severe fetal anemia and prevent fetal death in utero. This allows for continuation of pregnancy to a point where delivery by gestational age is safe.

The severity of HDFN disease is related to the antigen specificity of the antibody involved, the strength of expression of the antigen on fetal red cells, and the amount of maternal antibody produced and transported across the placenta.

**HDFN Due to Anti-D**

HDFN due to anti-D was the most common trigger for HDFN. However, since RhIG was approved by the FDA in 1968 for prophylaxis, the incidence is much lower. D antigen is a very potent immunogen, and since 85% of the population is D-positive, an Rh negative mother has a high chance of bearing an Rh positive fetus.
HDFN Due to Other Antigens

HDFN due to ABO is usually mild, but occasionally may require an exchange transfusion after delivery. As the B and A antigens are not well developed at birth, when exchange transfusion is needed, it is usually more required by the degree of hyperbilirubinemia than the anemia. When D antigen and ABO incompatibility occur together, there is a protective effect of the latter as it results in a lower risk of Rh sensitization for the D antigen.

Up to 5% of the HDFN cases are related to antibodies other than ABO or anti-D. Antibodies against other Rh antigens (anti-c, anti-E), Kell, Kidd, Duffy, MNS, Diego, and other rare ones may be involved. Of this list, anti-Kell, anti-E and anti-c are the most frequently involved. Of importance is that Kell is also expressed in the maturing nucleated red cells as opposed to Rh antigens that are not expressed on maturing fetal red cells. Consequently, Kell related HDFN anemia has a component of erythropoietic suppression along with the hemolysis as mechanisms for the fetal anemia. Therefore, antibody titers for anti-Kell are not as predictable of the fetal anemia and the level of bilirubin pigment present in the amniotic fluid as when a different antigen is involved.

After Delivery

Close to 60% of normal newborns experience jaundice sometime during the first week of life. Unconjugated high bilirubin is commonly the result of the physiologically immature liver not being able to clear the bilirubin in a timely manner. The presence of jaundice requires monitoring as physiologic states must be distinguished from pathologic ones. At high levels, bilirubin is toxic to the central nervous system and may produce permanent neurological damage. This condition is known as kernicterus (also known as bilirubin encephalopathy).

The newborn has a blood-brain barrier that is more permeable to bilirubin than in older infants and adults. Bilirubin that is neither bound to albumin nor conjugated is capable of crossing the blood-brain barrier. As a consequence, the newborn stage is the time with the highest risk for kernicterus when there is a rapidly rising indirect bilirubin, regardless of the etiology.

With HDFN, the newborn will frequently have a positive direct antiglobulin test (Coombs test) and jaundice. The severity of the anemia varies and the findings in the peripheral smear include the presence of spherocytes (more pronounced with ABO incompatibility), circulating NRBCs, increased number of reticulocytes, polychromasia, and red cell fragments.

Consequently, the peripheral smear findings are not specific, but along with jaundice and the positive direct Coombs, they may point the clinician towards the possibility of HDFN if prenatal care did not previously uncover the risk for HDFN.

A positive Coombs test does not in itself confirm the diagnosis of HDFN as occasionally false positives may occur. DAT with strong positive results (4+) have the best predictive value for identifying neonates that
will require management. In situations in which the mother was given prophylactic RhIG, the newborn may have a positive DAT related to passive transfer of the anti-D administered to the mother.

The differential diagnosis of pathologic jaundice related to hemolysis (in the absence of a positive Coombs test) in the newborn period includes disorders affecting red cell membrane (hereditary spherocytosis and elliptocytosis), and red cell enzyme deficiencies (glucose-6-phosphate dehydrogenase and pyruvate kinase). Also, hydrops may be related to non immune disorders that result in severe anemia like hemoglobinopathies, cardiac failure, congenital heart defects, and infections.

**NEWBORN MANAGEMENT OF HDFN**

Phototherapy and exchange transfusions are the most common interventions for newborn management of HDFN.

Assessment of the infant’s rising bilirubin is of utmost importance in deciding the best treatment. Phototherapy makes use of light at a wavelength of 420 – 480nm to convert unconjugated bilirubin to the water soluble biliverdin, which is excreted by the newborn’s kidneys. Exchange transfusion is used in severe cases and when there is impending danger for kernicterus. It has the advantage of removing the maternal antibody and the sensitized red cells. The RBCs used for exchange should be compatible with the baby, should be antigen negative for any antibodies present in the maternal serum, and also should be irradiated prior to infusion (to prevent graft-versus-host disease).

**COMPLICATIONS**

Unfortunately, kernicterus still occurs in some situations, and neurological damage becomes permanent. The degree of severity varies from case to case and it may be obvious early in life or become apparent as the child develops.

Ultimately, in some cases with significant hemolytic disease, late anemia of infancy develops and it may require transfusion.

**PRENATAL CARE**

**Unsensitized Mother**

The availability of prenatal care to the mother is crucial in the prevention of sensitization as well as proper management of individuals that are at risk for HDFN. In the USA, a blood type and antibody screen is usually obtained at the first obstetric visit.

A women that types as Rh (D) negative and has a negative antibody screen will follow the prevention route and will receive RhIG at specified times during the pregnancy and always at the time of delivery. Also, if
there is any situation that indicates the possibility of feto-maternal hemorrhage (FMH) (fetal blood gets into the maternal circulation), prophylaxis is warranted. Some degree of FMH takes place during delivery in most pregnancies. However, abdominal trauma, abortion, placental abruption, amniocentesis, or other invasive procedures may also precipitate FMH. The rosette test and Kleihauer-Betke acid elution test are techniques for detecting fetal cells in the maternal circulation.

The rosette screening test uses mother’s RBCs, anti-D, along with D-positive indicator cell reagents. The indicator RBCs will form rosettes around any antibody coated D-positive fetal RBCs present, giving a qualitative indication of evidence for FMH.

The Kleihauer-Betke acid elution test is based on eluting hemoglobin A from adult RBCs on an alcohol fixed blood smear. As this will not affect hemoglobin F, when the preparation is stained and reviewed the fetal RBCs appear pink in contrast with adult ghost red cells. The fetal red cells can be counted and reported as a percentage of maternal RBCs. This important information reported by the clinical laboratory is used to calculate the amount of RhIG to be given to the mother to prevent sensitization. Flow cytometry analysis to determine the number of fetal red cells in maternal circulation is replacing the Kleihauer-Betke acid elution test in large high volume medical laboratories.

In pregnancies complicated by red cell alloimmunization, the major morbidity is the risk of progressive hemolytic anemia in the fetus. As this may lead to fetal death as early as the 17th week of gestation, early diagnosis and careful management are of utmost importance. Fetal survival is currently over 90% if anemia is treated with intrauterine blood transfusions. It is important to point out that these high risk pregnancies are best managed by specialized perinatology obstetricians and require the support of specialized testing, including the laboratory.

RhIG is dispensed in 300-ug vials, each of which theoretically suppresses the immune response to 15 mL of Rh positive red blood cells or 30 mL of Rh positive whole blood.

RhIG is made from a human source for intramuscular injection. RhIG may suppress the primary response to Rh D in most women. However, it is of no value in a woman that has already been sensitized to the D antigen and/ or has evidence of active anti-D production. RhIG must be given during each pregnancy to an Rh-negative mother carrying an Rh-positive fetus. Prophylactic treatments at 28 weeks of gestation and again within 72 hours following delivery are the standard indications.

**Sensitized Mother**

If an antibody is detected during pregnancy, at any time, its specificity should be characterized and periodic titers should be obtained.

Currently, anti-D is still one of the most common antibodies found in pregnant women, followed by anti-K, anti-c, and anti-E. Of those fetuses who require intrauterine transfusions, 85% are due to anti-D, 10% due to anti-K, and 3.5% due to anti-c.
Several modalities are available to monitor the pregnancy and identify the need for intrauterine transfusion. For many years the standard was the performance of serial amniocentesis for the determination of bilirubin levels in amniotic fluid. This is based on the rationale that fetal hemolysis results in the progressive accumulation of bilirubin in amniotic fluid and the degree of accumulation correlates with the severity of the hemolysis and degree of anemia.

The bilirubin level in amniotic fluid is measured by spectrophotometry and expressed as the change in optical density at a wavelength of 450nm (delta OD 450). These changes are then plotted on a chart originally crafted by Liley and later modified by Queenan and colleagues. By plotting these curves, patients can be classified at different levels of severity and managed according to their assumed severity. However, performance of this test carries the morbidity of amniocentesis, which may result in membrane rupture, infection, and fetal loss.

Ultrasonographic measurements of the fetal liver and spleen that indirectly assess the level of extramedullary hematopoiesis as well as gestational age and presence of hydrops have also been used in lieu of the invasive amniocentesis.

Most recently, the peak systolic middle cerebral artery Doppler velocity measurement of fetal blood flow has been popularized as a screening tool for fetal anemia. Fetuses with anemia have high cardiac output and decreased blood viscosity, resulting in high blood-flow velocities. This is becoming the most frequently used method and in many situations replaces the need for amniocentesis.

**Summary**

An alloimmunized mother who produces an IgG antibody against a fetal red blood cell antigen may develop hemolytic anemia in the fetus / newborn. Hemolysis can cause severe disease (hydrops fetalis), moderate disease requiring treatment (intrauterine and / or newborn exchange transfusion), or mild disease requiring phototherapy or no therapy after delivery.

RBC alloimmunization during pregnancy continues to occur in spite of directed prenatal care to prevent it. In regards to the Rh D antigen, this may be related to inadvertent omissions in RhIG administrations, sensitization prior to the standard 28-week administration or may be related to a known small failure rate of the intervention. As there are not immune globulins to prevent exposure to RBC antigens, with the exception of D antigen, routine prenatal screening to detect the presence of a maternal circulating antibody becomes the trigger point for planning further maternal, fetal, and newborn medical care.
Summary, cont’d
The clinical laboratory has a crucial role in the recognition of unexpected situations as well as a critical supporting role in the monitoring and managing of mothers and babies at risk for HDFN. The significant progress made in the care of this disorder is responsible for the greater than 85% current perinatal survival and babies that are usually free of neurological damage.

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<tr>
<th>Consider the possibility of HDFN when one or more of the following situations is encountered:</th>
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<tbody>
<tr>
<td>▪ Newborn has rapidly developing or severe hyperbilirubinemia not predicted by maternal prenatal antibody screening</td>
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<td>▪ Positive maternal antibody screening (indirect Coombs) and or confirmation of a severely anemic/hydropic fetus</td>
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<td>▪ A positive direct antiglobulin test (Coombs test) in the baby</td>
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<td>▪ Laboratory evidence of newborn hemolysis including blood smear review</td>
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<td>▪ Prolonged newborn hyperbilirubinemia</td>
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<th>Laboratory findings in HDFN include:</th>
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<tr>
<td>▪ Anemia</td>
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<td>▪ Reticulocytosis</td>
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<td>▪ Hyperbilirubinemia</td>
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<td>▪ Increased number of NRBCs in circulation</td>
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<td>▪ Positive direct antiglobulin test (DAT- Direct Coombs)</td>
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<td>▪ Rh negative mother blood type or ABO incompatibility between mother–child</td>
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<td>▪ Smear: polychromasia, NRBCs, spherocytes (may be absent), fragmented cells (may not be present)</td>
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References

William Koss, MD, FCAP: William Koss, MD, is Director of Clinical Pathology, Scott & White Memorial Hospital and Scott & White Clinic in Temple, TX. He is CP/AP and Hematology Board Certified by the American Board of Pathology. He is also the Director of the Hematopathology Fellowship at Scott & White and Texas A&M Medical School where he is a Professor of Pathology and Laboratory Medicine. Dr. Koss is also a member of the Hematology and Clinical Microscopy Resource Committee for the College of American Pathologists (CAP).
### Case History

This peripheral blood smear is from a 73-year-old man with a history of stage IV mycosis fungoides who presents with leukocytosis and recent progression of his tumors. Laboratory data includes: WBC = 25.6 x 10^9/L; RBC = 4.34 x 10^12/L; HGB = 12.5 g/dL; HCT = 39.5%; MCV = 91 fL; and PLT = 413 x 10^9/L.

### DISCUSSION

The clinical case history provided indicates that this elderly man has been previously diagnosed with a skin lymphoma called mycosis fungoides (MF) and that the lymphoma has recently progressed. The complete blood count (CBC) shows a slight anemia, a normal platelet count, and an increased number of white blood cells. Based on the provided images (BCK/BCP-26 through BCK/BCP-30) of the blood smear, it is clear that the increased white blood cell (WBC) count is primarily due to the presence of abnormal mononuclear cells in the blood.

The abnormal mononuclear cells are larger than normal lymphocytes (Image 1 in the Appendix) and have folded and convoluted nuclei, which impart a cerebriform appearance to the nuclei. The abnormal mononuclear cells do not resemble large granular lymphocytes (Image 2 in the Appendix) or reactive lymphocytes, which are often present in viral infections such as infectious mononucleosis (Image 3 in the Appendix). The abnormal cells are not monocytes; they lack the abundant amount of cytoplasm as well as the granules found in a normal monocyte (Image 4 in the Appendix). In addition, the chromatin of the abnormal cells is more condensed than the chromatin found in monocytes. The abnormal cells also are not blasts. Blasts classically have more dispersed chromatin (Image 5 in the Appendix). However, blast morphology is quite variable and some (particularly lymphoblasts) can have chromatin, which is less dispersed than is typically present in myeloblasts. With respect to differentiating between blasts and lymphoma cells, careful attention to the other WBC parameters can be useful. For instance, the platelet count is normal in this case history. A normal platelet count is more likely observed with circulating lymphoma cells than with an acute leukemia (acute leukemia would be very likely in this case if all the abnormal cells are blasts). However, this is a general guideline, since exceptions of acute leukemias associated with normal or increased numbers of platelets occur.

It should be clear that these mononuclear cells are not normal WBCs even if their specific identity is uncertain. All cases with abnormal cells in the blood need to be correlated with the clinical findings in order to perform an accurate assessment. In this case history, given the clinical history of preexisting mycosis fungoides (MF), the established propensity of lymphoma cells from this skin lymphoma to circulate within the blood (see discussion below), and the morphology of the mononuclear cells, it is a logical conclusion that these abnormal cells are lymphoma cells. In this particular case history, the lymphoma cells are called Sézary cells. Sézary cells are a specific type of lymphoma cells with convoluted nuclei that are present in the blood smear of patients with Sézary syndrome. Sézary syndrome (SS) is the leukemic phase of the skin lymphoma, mycosis fungoides. SS can occur in patients with a well established history of MF, as in this case, or as the initial sign of lymphoma in some patients. Since this patient has a well established history of MF, this case is best classified as secondary SS.
All cases with abnormal cells in the blood smear need to be correlated with the clinical findings in order to perform an accurate assessment.

Secondary SS should be distinguished from primary SS, which develops in patients who have no prior history of a skin lymphoma. A concurrent skin biopsy from patients with primary SS may not show classic features of MF, even though the skin is involved by the lymphoma. However, both primary and secondary SS are T-cell lymphomas, which involve the skin and the blood. Classically, all patients with SS have generalized erythroderma (red skin), which involves the entire skin including the palms of the hands and soles of the feet, as well as itching and abnormal dryness. Although information about these clinical features is not provided for this case, their presence is helpful to establish the diagnosis of SS.

Guidelines have been proposed for the diagnosis of SS and also to help determine the extent of the disease. One of the guidelines is morphologic evaluation of the blood smear to determine the number of lymphoma cells that are present in the blood. Less than 5% tumor cells may represent no blood involvement or very low tumor burden. More than 5% lymphoma cells in the blood may indicate low or high tumor burden, depending on the absolute number of lymphoma cells that are present. Sézary cells ≥1000/uL in the blood is regarded as high tumor burden. Thus, it is important that the lymphoma cells in this case are recognized and that the clinical care team is notified of their presence in the blood. Of note, in some instances, Sézary cells are smaller that those illustrated in this case. However, the typical morphologic features such as the convoluted/folded nuclei with condensed chromatin are still present.

Ancillary techniques including flow cytometric immunophenotyping and molecular analysis are also among the suggested guidelines to evaluate for the lymphoma cells in the blood. These techniques are often required to confirm the morphologic impression in cases with low numbers of tumor cells in the blood, since very low numbers of abnormal appearing lymphocytes that resemble Sézary cells can occur in reactive conditions. In SS cases, flow cytometry of the blood would be expected to demonstrate that the abnormal mononuclear cells are mature T-cells, which are most often helper-inducer (CD4+) type. In addition, the lymphoma cells may have an abnormal phenotype such as absence of a key surface marker (often CD26, CD7, or CD3) which is present on normal T-cells. However, the flow cytometric findings are not always definitive for several reasons, including the fact that all the lymphoma cells may not have the same phenotype, making them difficult to detect.

Molecular analysis of blood involved by SS is expected to show T-cells with monoclonal rearrangement of the T-cell receptor gene. However, the testing methods currently used for molecular analysis are extremely sensitive and may detect a very small population of monoclonal T-cells, which does not always represent SS. Therefore, results from molecular analysis must always be correlated with the morphologic findings in the blood smear. For this reason, detection of monoclonal T-cells in the blood is most useful for the diagnosis of SS when the same population of monoclonal T-cells is also present in skin lesions from the patient.
Morphological evaluation of the blood smear, clinical history of the patient, flow cytometry, cytogenetic analysis, and molecular analysis of the blood are helpful when evaluating and diagnosing Sézary syndrome.

To summarize, the abnormal cells in this case are Sézary cells, which are lymphoma cells with convoluted nuclei present in the blood of patients with SS. SS can occur in patients with a well established history of MF or as the initial sign of lymphoma in some patients. When the phenotype of Sézary cells is abnormal by flow cytometry, this can be used to help establish the diagnosis of SS. Molecular analysis that demonstrates monoclonal T-cells in the blood can also support a diagnosis of SS. Finally, results of ancillary studies must be correlated with the morphologic findings in the blood smear in all cases.

The morphologic and clinical findings in this case are classic, making diagnosis of SS non-problematic. However, many different types of lymphoma cells can circulate in the blood, and they display a wide morphologic spectrum, (Image 6 in the Appendix). In some instances, the lymphoma cells may show overlapping morphologic features with Sézary cells. For example, adult T-cell lymphoma/leukemia (ATLL) and T-cell prolymphocytic leukemia (TPLL) cells can closely mimic Sézary cells. In addition, skin lesions are frequently present in patients with ATLL, although they are different from those in SS. This is one reason why it is important to correlate morphologic and clinical findings when evaluating abnormal blood smears. When all the features such as the clinical presentation, physical findings in the patient, and morphology of the cells in the blood are correlated, it is usually possible to have a very good idea what type of lymphoma cells are in the blood. A variety of special studies can be used to confirm the impression arrived at from these findings. For example, demonstration of HTLV-1 viral genome within the lymphoma cells helps to support ATLL and exclude SS. While TPLL lack a specific viral etiology, the clinical presentation of the patient as well as results of various ancillary findings, such as cytogenetic analysis, are usually helpful to distinguish TPLL from SS.
References


Appendix

**Image 1. Normal Mature Lymphocyte**

This normal lymphocyte is small, and the nucleus is round with condensed chromatin.

**Image 2. Large Granular Lymphocyte**

This large granular lymphocyte has condensed chromatin with an abundant amount of cytoplasm and visible azurophilic granules.

**Image 3. Reactive Lymphocyte**

This reactive lymphocyte has abundant pale blue cytoplasm, round to oval nuclei and moderately condensed chromatin. The cytoplasm hugs adjacent red blood cells and shows a basophilic rim at their margins.

**Image 4. BCK/BCP-29 Normal Monocyte**

This normal monocyte has an abundant amount of cytoplasm, which contains variable numbers of small azurophilic granules as well as vacuoles. The nucleus is large and indented or folded with condensed chromatin and does not contain a nucleolus.
These blasts show variable morphology. The nucleus of one blast (A) is round and the chromatin is fine with three visible nucleolus. The chromatin in the other blast (B) is less dispersed, the nucleus is folded, and at least one nucleolus is visible. The cytoplasm is scant in both blasts, but is more abundant in the blast depicted in image A.
Image 6. Lymphoma Cells: Figure Legend

A. Adult T-cell lymphoma/leukemia cell: This lymphoma cell has a folded nucleus, condensed chromatin, and a small amount of cytoplasm containing vacuoles. The cell closely resembles a Sézary cell.

B. T-cell prolymphocytic leukemia cells: Several lymphoma cells are illustrated in this image since the WBC count was very high (~500 x 10^9/L). The lymphoma cells are small with condensed chromatin, scant cytoplasm, and most have irregular nuclei. The lymphoma cells closely resemble Sézary cells and could not be distinguished based on morphology alone. A constellation of findings (increased WBC, flow cytometric analysis, and genetic studies) established the diagnosis.

C. Follicular lymphoma cell: This lymphoma cell is depicted in the blood of a 55-year-old man who had a long history of follicular lymphoma. The cell is large with a deeply indented nucleus, condensed chromatin, one visible nucleolus, and a small amount of cytoplasm. The Sézary cell nucleus lacks the deep nuclear indentations present in this lymphoma cell.

D. Mantle cell lymphoma: This lymphoma cell is present in the blood of an 80-year-old man. He had no prior history of lymphoma but had a high WBC count (21 x 10^9/L), slight anemia, and a normal platelet count. This lymphoma cell has irregular nuclear contours, condensed chromatin, visible nucleolus, and very scant cytoplasm.
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