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### Peripheral Blood Smear: Diagnostic Clues and Algorithms

Announcer:

Welcome to CME on ReachMD. The following CME activity is proved by TOPEC and the Roswell Park Comprehensive Cancer Center through the generous support of BlueCross BlueShield of Western New York.

Dr. Neppalli:

Hi, my name is Dr. Vishala Neppalli. I'm the head of Division of Diagnostic Hematology at Roswell Park Cancer Institute. In this presentation today, I'm going to discuss the importance of peripheral blood smear evaluation in the context of blood disorders.

This presentation has no affiliation or conflict of interest.

I would like to acknowledge the contribution of some of the images from a fellow hematopathologist, Dr. Nancy Rosenthal. I would also like to thank my bone marrow laboratory at Roswell Park Cancer Institute for their contribution and support to patient care and educational activities.

After completion of this activity, the participants will be able to:

- discuss the importance of peripheral blood smear review,
- discuss the differential diagnosis related to red blood cell indices in a hemogram report,
- discuss the differential diagnosis of abnormal white blood cell count indices and morphology in a hemogram report.

Typically, the peripheral blood is sent to the clinical labs in a tube that contains an anticoagulant in order to prevent clotting of blood, and the blood is put through two different diagnostic processes. The first process includes running the sample on an automated hemogram analyzer, which produces numerical values in the peripheral blood. The second process is to take a portion of this blood sample, put it on a glass slide, make a smear, and the cartoon is a depiction of the peripheral blood smear, and that peripheral blood smear is subjected to a few stains that is evaluated under the microscope by a highly trained clinical technologist and/or a hematopathologist.

So, in the first process there is review of the numerical data that is produced by the machine. So the review can be from a single time point, or it can be from multiple time points. The single time point assay typically involves where a peripheral blood from a clinical practice is submitted to the laboratory to evaluate single time point assay. A multiple time point assay typically would be sending peripheral blood, which are multiple samples from the same patient at different time points. It can be one sample a day, it can be one sample every other day, and these kind of time point assays are performed mostly on inpatients within the hospitals. The importance of it is that we are looking at the numerical counts, and the numerical counts for each of the cellular elements in the peripheral blood gives us diagnostic clues about the underlying disease process. So, in this particular slide, you will see that white blood cell counts—they may be within the normal range, they may be increased, or they may be decreased. Similarly, the red blood cells, they may be increased; they may be decreased. And we are also evaluating hemoglobin, which is oxygen-carrying protein in the red blood cells, and that gives us a clue about the type of anemia in patients. And in platelets, we are evaluating for an increase, a normal platelet count or a decreased platelet count, and by morphology we look at the morphology of the platelets on a glass slide where the platelets may be

large in size, they may be small in size, or they may or may not have any granules in them.

So, how is a peripheral blood smear made? This is a cartoon depiction of how we actually prepare a peripheral blood smear. It requires a lot of expert technique in which you just take a single drop of blood, put it on a glass slide, and a small amount of pressure is applied, and the smear is made on the glass slide. So you will see that towards the closer end where the blood is dropped on the glass slide is called a thick area, and next, adjacent to that is a thin area, and beyond that is called a featheredge. The thick area is not the optimal place for a pathologist to look for cells because the morphology is not very crisp. If you look at the thin area of the glass slide, which is right here, the morphology of the cells, they are distributed evenly, and it is very crisp. In the featheredge, you have cells that are actually fragmented or they are very heavy cells that tend to accumulate towards the featheredge or along the edges of the glass slide. So, however, it is of great importance for the evaluating pathologist to screen the entire glass slide so that we capture all of the information that's present on the specimen.

So, what you're seeing on the left side here in the image—actually, sorry, on the right side in the image is a typical morphology of a peripheral blood smear. The pink discs that are present and large number of them are your red blood cells. That is a major component of the peripheral blood. You have nucleated cells, which are your white blood cells, and this is a lymphocyte, and this is a granulocyte, which has multiple segments, and you also have small snippets of cytoplasm which are dark blue that are floating around, and these are the platelets. And on the left side of the screen, you are seeing a magnified view of a monocyte, a basophil and the eosinophil, and this is typically how we evaluate the cells under a microscope.

So, this table actually gives us an idea as to what does it actually mean when the numbers of these are changed, when they are outside of the normal range. So, if you start with the red blood cell indices and if the hemoglobin is increased, it comes with a differential diagnosis. So, typically, when we are evaluating peripheral blood and we are looking at individual cell elements and we are saying they are in the normal range, they are increased or decreased, what we are saying is the peripheral blood might by, in itself, be able to give you a diagnosis, or it might be the beginning of a very long diagnostic process, so it's a window into a different, into a huge differential diagnosis. In that situation, in the second scenario, we need additional studies. We do additional laboratory evaluation, we may get radiology, or we may have to subject the patient to other testing strategies to arrive at a final diagnostic call. So, evaluation of peripheral blood is extremely important.

So, in hemoglobin, if it is increased, we are thinking of polycythemia vera where the red blood cell count is increased. Polycythemia vera is a malignant condition, and secondary polycythemia is a reactive condition, so in each of these categories, when an individual cell count is increased or decreased, you will see that there is a reason for that. It could be a reactive reason, or it could be a malignant reason, so just because a cell count is increased, we cannot jump to a neoplastic diagnosis. It's very important to remember that there are many reactive conditions that can mimic malignant conditions, and it's very important for us to differentiate what is a reactive condition from a malignant condition in each of these entities.

And going on to the different components of WBCs, you'll see the increased eosinophil differentials and the basophil differential diagnoses, and also, the same thing applies for platelets as well. But the other important criteria, when we are looking at peripheral blood, is something called pancytopenia. What does pancytopenia mean? It means that all the cell components that are present in the white blood cell are equally decreased to some extent in a variable extent, so this complete reduction of cellular elements in the peripheral blood is considered pancytopenia, and that also includes reactive conditions and malignant conditions. Say the patient's bone marrow, which is a place where all these cells are produced, is affected by either drugs or toxins or either chemotherapy. For whatever reason the bone marrow is not functioning at capacity and it's not producing these cells. So, in other conditions—like malignant conditions, prostate cancer, or you have breast cancer—that travels to the bone marrow and makes a home for itself in the bone marrow, it is destroying the bone marrow's capacity to generate these peripheral blood cellular elements, and hence, you see pancytopenia. So pancytopenia may not entirely because of blood cancers or cancerous conditions related to hematopoietic condition, but it can also be caused by other malignancies that affect bone marrow functioning capacity.

So, morphologic descriptions are some things that we often times read in our reports, and I'll try to explain what these terminologies mean. So when we are looking at red cell size, we refer to them as microcytic, which means the red cell size is small. Immediately I'm thinking of iron deficiency, or I'm thinking of thalassemia in the picture, or otherwise, there is something called microcytosis. What comes into my mind is vitamin B12 and folate. When I'm thinking normocytic and the patient is anemic, usually it's anemia of chronic disease. So, these are the differentials that come automatically into these terminologies. And the other terms that we use is based on the color of the red blood cells. If the red blood cells are normochromic, which is normal color, then anemia of chronic disease is normochromic. So, if you are thinking of hypochromic, when the color is decreased and the red blood cells look very pale—in the next slide you're going to see an example of that—it's iron deficiency anemia is very typical for that.

So, leading to the next slide, you can see, in the middle, you can see hypochromia where you have the central pallor. There's only thin

rim of pink cytoplasm in these cells. This is profound iron deficiency in the patient, and these are the red blood cells that are deficient in iron. So, automatically when you get a report that reads microcytic hypochromic anemia, you're thinking of iron deficiency. The next question you have to ask is: What is causing this iron deficiency? Patients are losing iron, which is required for formation of hemoglobin, which means there's chronic blood loss, or the patients probably sometimes don't consume it, or the patients are consuming it but they're not able to absorb it because there's a malabsorption syndrome, such as sprue. So what we typically do is that we go ahead and do some laboratory studies to look at the storage of iron or availability of iron. The gold standard for looking for iron is a bone marrow, but that's not the usual practice. Conducting these particular tests for serum ferritin or serum iron or transferrin saturation will tell us whether the patient is iron-deficient or not.

The next category quickly to talk about will be megaloblastic anemia. This is a condition where the red cell size is increased, because usually it is in case of vitamin B12 and folate deficiencies. The size of these red cells is enormous, and they have this oval morphology to it, and you can take a normal red blood cell and you can put three of those inside this red blood cell. That is megaloblastic—mega is big. And because it is affecting the nucleic acid formation, which is a DNA synthesis, it affects every dividing cell in the body. So, because red blood cells are dividing and the white blood cells are heavily dividing cells, it affects their nuclear configuration, so we tend to see several nuclear lobes in a neutrophil, which is called a hypersegmented neutrophil. So these are called diagnostic giveaways in peripheral blood. When we look at this, immediately we are suspecting vitamin B12 and folate. Now, are these things unique to only reactive conditions and vitamin deficiencies? No. Sometimes you can see these in neoplastic conditions as well, which are called myelodysplastic syndromes. So, this is how the differential is generated, and this table is a snapshot of what causes vitamin B12 deficiency, and what are the most frequent causes of folate deficiencies in patients.

Then, now changing the topic from red blood cell disorders, we'll move to the white blood cell disorders. So, the most, the predominant component of the white blood cells is neutrophils, and the neutrophils in this picture are normal-appearing. And when the number of neutrophils is increased beyond the normal range, it is called neutrophilia. So, here you can see that the cutoff for that would be 7,000 per microliter is considered... Anything before that is considered neutrophilia. Neutrophilia-reactive causes can be sometimes acute infection, they could be chronic inflammations, or there can be some drugs that can cause that, or you can have malignant conditions that can cause as well, leading to an increase of these mature neutrophils in the peripheral blood, and those cancerous conditions of the blood are—because they are chronic problems; they fester for a very long time—they are regarded as chronic myeloid neoplasm. So, if you ever get a report that says neutrophilia and you cannot explain away by any reactive conditions, any drugs, and the patients continue to have this problem, then you need to start thinking am I dealing with an unusual malignant situation, and it requires further workup.

Now, the second component that can be increased or decreased is another component of the white blood cell. It is called lymphocytes. So, lymphocytosis is a very interesting condition because lymphocytosis signifies that your lymphocyte count, the absolute number of lymphocytes, are increased in the peripheral blood. Now, they can also be increased because of reactive conditions. The most common reactive condition that affects an increase in the lymphocytes are viral infections, and in young adults the most common condition is infectious mononucleosis. So that is something you should always consider when you have a very young patient visiting the clinic—they are having lymphocytes. Look at their morphology, have a hematopathologist evaluate it, and they will be able to tell you whether the lymphocytes are reactive from the get-go by looking at the glass slide. And in a young patient, a morphology like this is a high index of suspicion for a viral infection.

In an older patient, and I will show you some examples of malignant as well. So, usually, these are the clues that we derive from morphology and the counts put together in the peripheral blood that is taking us in the direction of reactive or direction of neoplastic.

Now, this is an example of a leukemic process, which is a malignant condition, and it is a chronic process. This is the peripheral blood, and in the peripheral blood—these 2 slides are going to show you different components in the peripheral blood. So, what we are seeing is that they are mature neutrophils, they have more bands here, and there are some immature which should not be in the peripheral blood are present in the peripheral blood. And in this condition called chronic myelogenous leukemia, there are too many of these cells in the peripheral blood, and this is an abnormal feature. And this particular morphology is a textbook description of chronic myelogenous leukemia as soon as we see it. And another feature that we see in patients, whether they are symptomatic or not, is basophils. You can see a cell with very dark granules on the left side of the screen. The arrow is pointing to it. And those basophils are increased in number when you count them. It's called absolute basophilia, and that's an abnormal sign. With a picture that looks like this, immediately I ask for additional studies. I ask for some genetic testing, which comes with 9;22 translocation, and it's a chronic myeloid leukemia, and the patients will require referral. They may also have a big spleen in that situation, and that requires for us to seriously think of a neoplastic process.

Now, we talked about the myeloid process. We talked about the neutrophils. What about the lymphocytes if the lymphocytes get malignant? So, this is an example of a chronic lymphocytic leukemia. I showed you an example of reactive. Now, if you compare the

morphology with this morphology, you're going to be looking at chronic lymphocytic leukemia. This is a malignant condition of the lymphocytes. And if you look at all these lymphocytes, they all resemble each other. They are mirror images of each other to some degree, and they are very close in morphologic resemblance, and these are mature lymphocytes. And recognizing the presence of these lymphocytes and too many of them circulating in the peripheral blood is a morphologic very high index of suspicion of a disease called chronic lymphocytic leukemia. When we see something like this, we subject this blood to other specialized testing in the lab in order to establish the diagnosis.

Now come the acute leukemias. Acute leukemias are conditions which actually present suddenly. They are sudden in onset, as the name goes, and they can be of two types. They can be derived from the lymphoid lineage, or they can be derived from the myeloid lineage. So, one of the conditions I'm going to show you is called ALL, which is acute lymphoblastic leukemia, and all these leukemias, acute leukemias, result in bone marrow failure, so normal blood cells are absent in the peripheral blood. You start to see abnormal cells in the peripheral blood. And acute myeloid leukemia is where the abnormal accumulation of immature neutrophils and everything that you normally see in the bone marrow start to appear in the peripheral blood. So that is a distinction between lymphoid and myeloid. One is of lymphoid lineage, and one is of myeloid lineage, and in diagnosis it's extremely critical for us to distinguish between the two at ground zero, which means at the point the patient walks into your clinic or into the hospital, we need to make this distinction whether it's lymphoid leukemia or myeloid leukemia because the treatment strategies are extremely different between the two.

This is a peripheral blood smear on the acute myeloid leukemia. So, in this particular situation you have a lot of abnormal cells, and these kind of cells should never be present in the peripheral blood. If you have numerous cells which are present in the peripheral blood and we do absolute counts, and if they are greater than 20% of all the nucleated cells in the peripheral blood it constitutes acute leukemia, because these are blasts. But the presence of a specific structure in the cytoplasm called an Auer rod helps me to make a diagnosis of acute myeloid leukemia. So, essentially, patients with acute myeloid leukemia will have variable decrease in the number of normal cells in the peripheral blood, and the abnormal cells tend to increase in the peripheral blood, and that is a very abnormal finding, and almost the diagnosis can be made by this particular image in the morphology. There could be additional testing that is required in these patients from genetic perspective in order to see how they would respond to treatment, what their prognosis is, but this in itself makes a diagnosis of AML, or acute myeloid leukemia.

Acute lymphoblastic leukemia is usually a disease of the children. It is a clonal lymphoid process which is arising from a very immature lymphoid cell. It can be a B-cell, it can be a T-cell, or it can be B-cell precursors, and it also leads to suppression of the bone marrow of its normal function because the marrow is taken over by these cells, by lymphoblasts. So, the example of what lymphoblasts look like by morphology is just like this. There are numerous of these cells in the peripheral blood that you will see, greater than 20% of all the white blood cells in the peripheral blood, very high nuclear to cytoplasmic ratio. It's very difficult to see. It's a thin rim of cytoplasm. They don't have any granules, and importantly, we do look for Auer rods, which we found in the previous slide are absent in these cells.

So, when I look at something like this, the first thing I would call, if the cells are enormously increased, is the patient is having acute leukemia, period. A diagnosis is already made, but then I have to distinguish whether it's a lymphoblastic. You can have some myeloid leukemias that resemble lymphoblasts. So, it's important for me to do other complex testing in order to arrive and distinguish between myeloid and lymphoid in that category, and complex testing like flow cytometry is the most common test that we use that allows us to make that distinction. It tells us the proteins that are present on these cells specifically that help us to make that diagnosis. But these two diagnoses, when you have increased number of blasts reported, or if you have any number of blasts reported, it's an immediate referral to a tertiary care center because these are diseases of immediate urgency in patients.

And, to conclude, you have seen in this, different aspects of variations in cellular elements and what it means to arriving at a differential diagnosis, and what it also means to arriving at a specific diagnosis in the peripheral blood. So from this you have seen review of hemogram data, either a single time point or multiple time point data, and peripheral blood smear morphology evaluation in combination are initial steps in the diagnostic pathways of blood disorders. They are very important in the diagnostic pathway. Peripheral blood smear morphology in conjunction with relevant laboratory data—so it's not just one test and you run with the diagnosis—it tells you you have to do other things in combination. It gives you a diagnostic tree, and you need to follow that pathway. So that's what we do with some of the examples I showed you. Evaluation of peripheral blood smear morphology is a critical initial step in the evaluation of neoplastic hematologic processes. It's very easy to get. It's a very quick thing to do. Morphologic review takes less than one hour for us to conduct, staining the slide and looking at it and coming up... but it requires that this testing be performed in the hands of an expert hematopathologist.

And, finally, peripheral blood smear morphology provides guidance in the selection of appropriate, definitive, diagnostic testing strategies, specifically in blood disorders. There are numerous diagnostic tests that are present at our disposal, which are extremely complex. You are thinking of next-generation sequencing. You're thinking of chromosomal assays. And irrespective of whatever, there

is a diagnostic pathway, and the first step in the diagnostic pathway is peripheral blood smear evaluation and analysis of the numerical changes in the peripheral blood, and the next step leads to the choice of—informed choice--of complex testing so that we are not lost in the process of making a diagnosis.

Finally, thank you all for listening to this presentation, and you can reach out to me in my e-mail, and I'd be happy to answer any of your questions.

Announcer:

The preceding activity was provided by TOPEC and the Roswell Park Comprehensive Cancer Center through the generous support of BlueCross BlueShield of Western New York.

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