Understanding and Recognizing the Pelger-Huët Anomaly

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Abstract

The Pelger-Huët anomaly (PHA) is a recognized morphologic variant affecting all granulocytes but is most evident in polymorphonuclear neutrophils (PMNs). PHA is caused by a decreased amount of the lamin B receptor (LBR). Recognition of PHA morphologic features serves as a marker for mutations in the LBR gene. This review summarizes the history of PHA and the current knowledge of the functions of the LBR. Guidance is given for distinguishing PHA from other hematologic disorders in which granulocytes may show similar changes. Recognition of PHA in the laboratory should prompt communication to the patient’s physician about the possible clinical significance of this finding and the recommended screening for the anomaly in other family members by CBC and review of a peripheral blood smear.

The human polymorphonuclear leukocyte (PMN) is unusual among eukaryotic cells in that it has a lobulated nucleus containing condensed chromatin. The segmented nucleus allows for rapid diapedesis through vessel walls and chemotaxis through interstitial spaces. The shape of the interphase nucleus is partly determined by the nuclear envelope (NE), a complex structure consisting of 2 unit membranes, the outer nuclear membrane (ONM) and the inner nuclear membrane (INM). The ONM faces the cytoplasm and is continuous with membranes of the rough endoplasmic reticulum, whereas the INM faces the nucleoplasm. The ONM and INM are continuous at nuclear pores, channels that allow communication and transport between the nucleoplasm and cytoplasm. The INM is supported by the nuclear lamina (NL), which consists of a network of intermediate filaments known as lamins. In humans there are A- and B-type lamins encoded by 3 genes; at least 7 different lamin proteins are produced through alternative splicing of these genes.1 It is believed that the B-type lamins are the building blocks of the NL since they are essential for survival, whereas the A-type lamins have more specialized functions.2

The NL forms a layer between the INM and membrane-associated heterochromatin, but also provides a structural link between chromatin and the INM. In doing so, the NL is responsible for organizing the interphase nucleus and for dissolving and reforming the NE during cell division. The NL also ensures correct positioning of nuclear pore complexes within the NE. Evidence supports a role for the NL in organizing the cytoskeleton as well. This additional role for the NL is important for cells that lack a cell wall, where the NL functions as the load-bearing structure with the ability to withstand forces of deformation.1

The NL performs its various functions through associations with a number of chromatin-associated proteins and NE.
transmembrane proteins. The dynamics of the NL and its associated proteins have gained intense scrutiny in recent years owing to the discoveries of a number of mutations in the lamins and/or associated proteins that are responsible for at least 20 different diseases encompassing striated muscle disorders, peripheral neuropathies, lipodystrophy, premature aging, and developmental abnormalities resulting in prenatal death. The diseases are known collectively as laminopathies (reviewed by Broers et al1 and Prokocimer et al3).

The lamin B receptor (LBR) is an INM protein that binds B-type lamins and heterochromatin; its role in nuclear segmentation of PMNs was demonstrated in retinoic acid–induced granulocytic maturation of the human myeloblastic HL-60 cells. Mutations in the LBR gene are responsible for the morphologic changes seen in leukocytes known as the Pelger-Huët anomaly (PHA). Current knowledge regarding the structure and function of LBR tentatively links LBR aberrations as laminopathies. Different mutations in the LBR gene have been linked to various phenotypes in humans ranging from benign disorders of abnormal nuclear shape, as in heterozygous PHA, to more serious consequences, as occurs in hydrops, ectopic calcifications, and moth-eaten (HEM)/Greenberg skeletal dysplasia, a prenatal lethal condition resulting from homozygous calcifications, and disproportionate body stature, macrocephalus, ventricular septal defect, polydactyly, and short metacarpals. In contrast, homoyzygous PHA in rabbits and mice is associated with skeletal abnormalities, early lethality, and ichthyosis (the latter occurring in mice). The phenotypic manifestations of Greenberg dysplasia are characterized by in utero lethality, severe hydrops, defects in chondro-osseous calcification, short-limbed dwarfism, polydactyly, and other skeletal malformations. The phenotypic manifestations of Greenberg dysplasia are similar to those present in homozygous PHA in rabbits.13

History

An anomaly of PMNs as being “short and compact” was described in 1928 by Karl Pelger in 2 patients with disseminated tuberculosis. Pelger believed that the anomaly was associated with a poor prognosis in patients with tuberculosis because both patients died. However, Pelger’s conclusions were refuted in 1932 by G.J. Huët, a Dutch pediatrician, who recognized the “Pelger anomaly” in a 7-year-old girl with tuberculosis who recovered from her illness. Furthermore, examination of peripheral blood smears revealed the Pelger anomaly in several of the child’s relatives, including the girl’s aunt who was one of the patients originally described by Pelger. Huët concluded that the aberrant PMN morphology, later termed the Pelger-Huët anomaly, was a benign autosomal dominant inherited trait rather than an acquired change associated with a poor prognosis for tuberculosis. It is now known that heterozygous mutations of LBR result in hyposegmented PMN nuclei known as PHA. The worldwide occurrence of PHA varies from 0.01% to 0.1% in diverse populations, with higher percentages (0.6% and 1.0%, respectively) in northeast

Sweden and southeast Germany. Subsequent population studies showed PHA to be present with a prevalence rate of 1 in 4,785 in the United States and 1 in 6,000 in Great Britain. In the heterozygous state, PHA presently serves as a marker for the carrier state of an LBR mutation with no adverse symptoms; patients exhibiting this anomaly are clinically normal with respect to neutrophil function. In vitro, PHA granulocytes exhibit no significant differences when compared with normal granulocytes regarding cytoplasmic granular enzymes, nitroblue tetrazolium reduction, superoxide production, phagocytosis, and chemotaxis, although a study by Park et al showed that PHA granulocytes from persons in the same family had slower migration through structural barriers than normal granulocytes. In 1952, a Dutch girl was described as having homozygous PHA with no overt skeletal abnormalities other than a mild short stature. In a review of 11 reported cases of homozygous PHA in humans, variable clinical conditions were described in some cases, while others had no observed anomalies. Described clinical conditions included psychomotor retardation, disproportionate body stature, macrocephalus, ventricular septal defect, polydactyly, and short metacarpals. In contrast, homozygous PHA in rabbits and mice is associated with skeletal abnormalities, early lethality, and ichthyosis (the latter occurring in mice). The clinical spectrum cannot be made in human homozygous PHA because molecular data are lacking compared to the animal studies. In 2003, a lethal phenotype attributed to homozygous mutations in the LBR gene was reported following studies on the autosomal recessive HEM/Greenberg dysplasia. Greenberg dysplasia is characterized by in utero lethality, severe hydrops, defects in chondro-osseous calcification, short-limbed dwarfism, polydactyly, and other skeletal malformations. The phenotypic manifestations of Greenberg dysplasia are similar to those present in homozygous PHA in rabbits.
To date, there are fewer reported cases of PHA homozygosity in humans than would be predicted by the prevalence of heterozygous PHA, raising the question of increased fetal loss in humans as observed in rabbits and mice. The question is currently not resolved, as the causes for the variability in the clinical manifestations in humans reported with homozygous PHA need clarification. Currently, 11 different mutations of the LBR gene have been discovered leading to human PHA, and the differences in genetic background, site of the mutation, and severity of the mutation may explain the lack of one specific phenotype associated with PHA. Until more is known about LBR and its functions, patients exhibiting PHA may benefit from counseling about the possible genetic consequences of this anomaly.

The Lamin B Receptor

The LBR is a multifunctional protein containing 2 subdomains. The amino terminal of approximately 200 amino acids faces the nucleoplasm and binds to the B-type lamins and HP-1 type chromatin. The amino terminal of LBR is important for the reassembly of the NE at the end of mitosis, in nuclear and chromatin organization, and in gene expression. The carboxyl terminal, composed of approximately 400 amino acids, is reputed to contain 8 transmembrane segments. Its gene sequence bears strong sequence similarities to 2 human sterol reductase genes and demonstrates C-14 sterol reductase activity. Therefore, the LBR protein belongs to the sterol reductase family of proteins. Sequence analysis of the human LBR gene indicates that the LBR gene has 13 exons; exons 1 through 4 encode the amino terminal, and exons 5 through 13 encode the C-terminus. The intron between exons 4 and 5 is large, suggesting that the LBR gene came from 2 separate primordial genes having different functions.

Possible Functions of LBR

The advent of tissue staining techniques in the second half of the 19th century enabled Paul Ehrlich to probably be the first to describe the neutrophil nucleus as being polymorphous in appearance. Further advances in ultrastructural techniques confirmed that the neutrophil nucleus is multilobulated with an abundance of peripheral heterochromatin. The distinctive nuclear shape is the differentiated result of 6 morphologically identified stages of granulopoiesis, beginning with the myeloblast containing a spherical, euchromatic nucleus that ends with the neutrophil displaying a heterochromatic, multilobulated nucleus. It is now well established that the presence of intact LBR is essential for normal neutrophil morphologic development, together with an intact microtubular network. Furthermore, the degree of nuclear segmentation depends on the number of functional copies of the LBR gene. Hence persons with PHA have hypersegmented nuclei corresponding to reduced amounts of functional LBR. Increased expression of LBR during granulopoiesis is implicated in the downregulation of lamin A/C, thereby contributing to the less rigid nuclear structure needed for the granulocyte’s ability of diapedesis. LBR may also be involved in the maintenance of the structure of the endoplasmic reticulum since a number of cell lines transfected with vectors containing mutant LBR constructs demonstrated profound changes in the morphology of the endoplasmic reticulum and the NE.

The mechanisms whereby LBR mutations maintain nuclear shape are not known. LBR, together with chromatin, lamins, and the INM, forms distinct microdomains in the nucleus, which are altered in heterozygous LBR-mutant mice. One can speculate that LBR, by virtue of its association with chromatin and other nuclear structures, may indirectly regulate gene expression, and this regulation may be tissue-specific depending on the LBR-associated proteins unique to that tissue. As mentioned, a second function of LBR is that of its sterol reductase activity in human cholesterol biosynthesis. This was a surprising function since, to date, all enzymes involved in cholesterol biosynthesis localize to the endoplasmic reticulum. Currently, it is debated whether diseases associated with LBR mutations, especially Greenberg dysplasia, should be classified as laminopathies or whether the phenotypic and pathologic symptoms result from aberrant sterol reductase activity separate from the role of LBR in nuclear morphology.

Classifying mutations in LBR as laminopathies is supported by the presence of 2 analogous sterol reductases in the endoplasmic reticulum that could substitute for the lack of sterol reductase activity resulting from aberrant or null existence of LBR. On the other hand, symptoms associated with Greenberg dysplasia are similar to other inherited skeletal dysplasias associated with aberrant cholesterol biosynthesis in humans. It is not known how compromised function of LBR’s sterol reductase activity contributes to Greenberg dysplasia; accumulation of abnormal or intermediate cholesterol metabolites can affect other nuclear and cytoplasmic structural and metabolic functions. It is now well established that mutations in the LBR gene that result in complete loss of its sterol reductase function is developmentally lethal in humans. In addition, the expression pattern of LBR during mouse prenatal development and the finding that LBR is expressed in human osteoclasts and osteoblasts indicate a role for LBR in cartilage and bone development. It should be noted that not all mutations in the LBR gene result in PHA, further supporting a separation of the structural and sterol reductase functions of LBR.

This brief summary on the history and functional aspects of LBR and PHA underscores the importance of correct clinical diagnosis of PHA. The following presents our findings on distinguishing PHA from other conditions resembling this anomaly.
Morphologic Discrimination of PHA From Pseudo-PHA and Leukemoid Reaction in Peripheral Blood Smears

Although PHA has been known as a morphologic variant of WBCs for decades, the anomaly can, at times, be confused with other hematologic disorders having similar WBC changes. Recent case reports have included the misinterpretation of PHA as a possible myeloproliferative disorder, leading to a bone marrow procedure and the initiation of clinical workup and treatment for suspected sepsis in a newborn with tachypnea and unrecognized PHA. In the latter case, a WBC differential count performed on a peripheral blood smear from the infant yielded an impression of a shift to the left in granulocytes. This observation, along with the initial tachypnea in the newborn, led to clinical initiation of a septic workup, including lumbar puncture and initiation of antibiotics. The anomaly was recognized after a family history was provided that the father, grandfather, and paternal uncle of the infant had PHA. Familiarity with the classic morphologic changes in the granulocytes of PHA can help avoid laboratory misinterpretation of granulocyte morphology as a leukemoid reaction, myeloproliferative neoplasm, or myelodysplastic syndrome.

The nuclei in patients with congenital PHA are characterized by a lower nuclear/cytoplasmic ratio, and the nuclear chromatin is coarse, densely clumped, and darkly stained. In the heterozygous phenotypes, 55% to 93% of the neutrophils show a bilobed nucleus. The lobes are symmetrical with a “dumbbell” or “pince-nez” appearance and are connected by a thin filament of chromatin Image 1. The PMN nuclei appear shorter and thicker than usual. Some cells may closely

| Table 1 |

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<tr>
<th>Nuclear Characteristics of PHA Granulocytes and Possible Cell Misidentification</th>
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<tr>
<td>Variants of PHA PMNs</td>
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<tr>
<td>PHA unilobed variant: Ovoid nucleus but chromatin more compact, condensed, and darkly stained than typical myelocyte; lower N/C ratio than typical myelocyte</td>
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<tr>
<td>PHA indented nucleus (peanut shape): Chromatin more dense than expected for apparent nuclear stage; nucleus more compact and smaller than typical metamyelocyte or band form</td>
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<tr>
<td>Bilobed PHA: Classic bilobed “pince-nez” appearance in some PMNs; lobes may be perfectly symmetrical or slightly asymmetric; neutrophils with more than 2 lobes rare</td>
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<td>PHA monocyte: Nucleus round to oval or has membrane irregularities</td>
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<td>PHA eosinophil: Unilobed nucleus</td>
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N/C, nuclear/cytoplasmic; PHA, Pelger-Huët anomaly, PMN, polymorphonuclear leukocyte.
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resemble myelocytes or metamyelocytes owing to an indented nuclear shape, but they differ in that overall the cells are smaller than usual and the nuclear chromatin pattern is markedly dense within a smaller-than-usual nucleus, as seen in a mature granulocyte Image 2. A small population (up to 4%) of neutrophils, known as “Stodtmeister cells” Image 3, may be present and are characterized by a nonlobulated or peanut-shaped nucleus. Overall cell size, appearance of cytoplasm, and staining quality of granules in PHA cells are similar to normal mature neutrophils. In homozygous PHA, Stodtmeister cells predominate, making up 94% to 96% of the total neutrophils.8

The nuclei of all leukocytes are affected in PHA, including the suppression of segmentation of granulopoiesis, along with changes in the nuclei of lymphocytes, monocytes, eosinophils, and basophils Image 4 and Image 5.8 When a high band count is seen in a WBC differential count, or

Image 2 A, Pelger-Huët anomaly (PHA) with a unilobed ovoid nucleus. This may be confused with a myelocyte, but the nucleus is smaller, with more compact and densely staining chromatin than a typical myelocyte (Wright-Giemsa, ×100, oil immersion). B, A typical myelocyte with an eccentric ovoid nucleus, a higher nuclear/cytoplasmic ratio, and a chromatin pattern more open than in a unilobed PHA variant (Wright-Giemsa, ×100, oil immersion).

Image 3 A, A slightly indented nucleus in Pelger-Huët anomaly (PHA). These cells may be confused with metamyelocytes, but the nucleus is smaller and the nuclear chromatin is dense and compact (Wright-Giemsa, ×100, oil immersion). B, A typical metamyelocyte. Note the higher nuclear/cytoplasmic ratio than in the PHA variant and the more open and lightly staining chromatin pattern (Wright-Giemsa, ×100, oil immersion).
when bands, myelocytes, and metamyelocytes are reported in a patient without inflammation or other known causes for the appearance of immature granulocytes in the peripheral blood, careful analysis of the peripheral smear should be undertaken to assess for possible PHA.

Bilobed neutrophils are also seen in acquired conditions and are known as pseudo-PHA. These acquired conditions include leukemoid reactions during severe bacterial infections, HIV, tuberculosis, and *Mycoplasma pneumoniae* infections, some medications, myelodysplastic syndromes, myeloproliferative neoplasms, and acute myeloid leukemia.²⁸ Thus, the distinction of PHA from pseudo-PHA is clinically important. Knowledge of morphology and correlation with clinical history can permit this distinction in most cases. Döhle bodies, cytoplasmic vacuoles, and toxic granulations common to granulocytes in leukemoid reactions may be present in PHA when concomitant inflammation is present. Review of the clinical history usually explains the presence of immature granulocytes in the peripheral blood during a leukemoid reaction. In these cases, lymphocytes and monocytes may also appear reactive, along with large platelets as a sign of bone marrow response.

**Image 4** A, A hypolobated eosinophil in Pelger-Huët anomaly. The nucleus is indented rather than displaying 2 distinct lobes and may be interpreted as dysplastic (Wright-Giemsa, ×100, oil immersion). B, Typical bilobed eosinophils (Wright-Giemsa, ×100, oil immersion).

**Image 5** A, A reactive monocyte with an irregular nuclear membrane contour in Pelger-Huët anomaly. Such changes, along with bilobed polymorphonuclear leukocytes, may be confused with myelodysplasia (Wright-Giemsa, ×100, oil immersion). B, A typical reactive monocyte with a convoluted but smooth nuclear membrane (Wright-Giemsa, ×100, oil immersion).
Acquired pseudo-PHA cell nuclei in the myeloproliferative neoplasms and myelodysplastic syndromes may be distinguished from congenital PHA by noting marked heterogeneity in nuclear lobations, which are not symmetrical Image 7. Other findings of myelodysplasia will also be present, including hypogranulation and an increased nuclear/cytoplasmic ratio of granulocytes, while the nuclear chromatin is not as condensed and dark as in PHA. Hypersegmented neutrophils and large granulocytes may be seen, and the pseudo-PHA cells are less than 25% of granulocytes. Another important clue is the presence of cytopenias and anemia that are found in the myelodysplastic syndromes, together with circulating blasts and nucleated RBCs frequently seen on a peripheral blood smear Image 8. Platelet and RBC anisocytosis may be prominent. The cause of the pseudo-PHA morphology in myelodysplasia is thought to be acquired clonal LBR mutations or reduced LBR expression in myelodysplasia. Another theory is that pseudo-PHA is a type of apoptotic neutrophil, as ultrastructurally, the cells resemble mature granulocytes undergoing apoptosis.

In acute care facilities, a patient with PHA can be experiencing an associated leukemoid reaction with true shift to the left in granulocytes, confounding the recognition of the concomitant disorders. In these cases (personal observation), more typical myelocytes and metamyelocytes may be observed Image 9 and Image 10, but more mature cells appearing as bands and neutrophils demonstrate typical PHA nuclear changes, notably dense, dark nuclear chromatin. Nuclei in these cells will appear more mature than the nuclear shape suggests, and the nuclear chromatin will be hyperchromatic and densely clumped. The majority of the segmented neutrophils will show symmetrical bilobed nuclei or, less frequently, the unilobed variants of homozygous PHA.
To distinguish the morphologic features of PHA and pseudo-PHA, a well-prepared and well-stained smear of peripheral blood is critical. Smears should be prepared promptly following collection because specimens older than 12 hours may show degenerated neutrophils with round, pyknotic nuclei, making assessment difficult.

Identification of PHA necessitates thorough analysis of all peripheral blood cell morphologic features and review of patient clinical and family history to separate PHA from pseudo-PHA. When heterozygous or homozygous PHA is identified and pseudo-PHA excluded, the finding should be communicated to the clinician and noted on a CBC or consultative report. A comment can be made that heterozygous PHA is usually clinically benign but may serve as a morphologic marker of defects in the LBR, and family screening for the disorder is recommended. This can be done by a pathologist or a hematologist familiar with granulocyte morphologic features to identify other affected family members, including the more uncommon homozygous PHA with possible associated clinical findings.

Future studies of LBR may lead to the explanation of abnormalities of WBC morphologic features in other hematologic disease states, such as hypersegmentation of neutrophils in megaloblastic anemia and marked nuclear condensation and clumping in chronic lymphocytic leukemia. Further understanding of the pathophysiology of these morphologic variants may lead to more accurate diagnosis and improved treatment of hematologic disorders.

References


