THE DIAGNOSIS OF PAROXYSMAL NOCTURNAL HEMOGLOBINURIA: ROLE OF FLOW CYTOMETRY

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Paroxysmal nocturnal hemoglobinuria (PNH) is an acquired clonal disorder typically affecting young adults, which involves the phosphatidylinositol glycan anchor biosynthesis class A gene (PIGA) coded in chromosome X, in virtually every case. The altered gene encodes for a defective protein product, the pig-a enzyme which is involved in the early steps of the synthesis of glycosylphosphatidylinositol (GPI). This later molecule (GPI) acts as an anchor of a wide range of proteins to the cell surface. At present a large number of GPI-anchor associated proteins have been described which are expressed by one or multiple distinct compartments of hematopoietic cells. The altered PIGA gene leads to an altered GPI anchor which leads to defective expression of GPI-AP on the cytoplasmic membrane, on the cell surface. Among other GPI-AP, expression of the CD55 and CD59 complement regulatory molecules is downregulated; such defective expression of the CD55 and CD59 molecules, leads to an increased sensitivity of blood cells to complement-induced cell lysis; this contributes to a large extent to the clinical manifestations and complications of the disease. Such increased susceptibility of blood cells to complement-induced cell lysis has long been used as the basis for classical diagnostic tests based on the degree of hemolysis achieved after complement activation, e.g. the Ham test. Despite this, such methods are associated with a relatively low sensitivity and specificity which has limited their diagnostic utility due to previous transfusions, the relatively low sensitivity of detection of hemolysis and to other causes of hemolysis, among other reasons. Increased knowledge about the physiopathology of the disease, leaded to the demonstration of defective expression of both CD59 and CD55 on one or multiple populations of peripheral blood red cells and/or leucocytes of PNH patients, this translating into a new diagnostic test. Because of this flow cytometry became an essential tool in the diagnosis of PNH. In turn, it could be also demonstrated that investigation of the presence of GPI-deficient cells among circulating mature neutrophils and monocytes, increases the sensitivity of red blood cell screening because of the shorter lifetime of both populations of leucocytes. Despite all the above, routine assessment of CD55 and CD59 is also associated with several limitations due to distinct patterns of expression among different cell populations, dim and heterogeneous expression among normal individuals and the lack of experience in many labs as regards the normal patterns of expression of both markers in distinct populations of hematopoietic cells. In recent years this has led to the investigation of the potential clinical utility of other GPI-associated markers together with the development of a unique fluorescent reagent based on a bacterial toxin –aerolysin-. This reagent, FLAER, is a fluorochrome-conjugated modified bacterial toxin which has the property of specifically binding to GPI domains on the surface of white blood cells and platelets, in the absence of cytotoxic effects. Thus, this single reagent allows simultaneous evaluation of the presence of GPI-deficient cells in multiple populations of leucocytes coexisting in a sample, including mature neutrophils and monocytes. However, it can not be reliably used to identify GPI-deficient red blood cells due to unspecific binding (most probably to glycophorin A) on the surface of erythrocytes. In turn, the most informative CD markers for the detection of GPI-deficient neutrophils and monocytes, include CD24, CD16 and/or CD66b and
CD14, respectively. In order to avoid false positive results due to inappropriate flow cytometric gating of these cell populations, additional markers are typically used for the selection of neutrophils (e.g. CD15 and CD45) and monocytes (CD64 and/or CD33 and CD45). Altogether, the combination of FLAER and the above cited markers allows unequivocal identification of GPI-deficient cells in patients suspected of carrying a GPI-deficiency in multicentric settings, with virtually no false-positive and false negative cases. For this purpose, screening of peripheral blood over bone marrow samples is preferred. Despite all these advances, few markers are still available for the assessment of the degree of involvement of red blood cells and CD59 remains as the most relevant marker to distinguish between normal and GPI-deficient erythrocytes; in addition, usage of appropriate sensitive clones/fluorochrome-conjugated reagents, allows discrimination between type II and type III GPI-deficient red cells. Noteworthy, CD55 also appears to be highly informative as regards the detection of type II cells among mature neutrophils. Despite this it should be noted that the clinical significance of the presence of type II cells still remains to be fully established.

In summary, combined flow cytometric assessment of FLAER and GPI-associated markers on mature peripheral blood neutrophils and monocytes and/or red cells is currently recommended for the diagnostic screening of subjects suspected of PNH. In the near future, refinement of the flow cytometric data analysis strategies used for the identification of the distinct subpopulations of white blood cells, together with more efficient algorithms to select for patients who should be screened for the presence of GPI-deficient cells, will shorten time from symptoms to diagnosis and increase the efficiency of PNH diagnosis particularly among patients presenting with non-classical forms of the disease.