Go with the flow: perforin and CD107a in HLH

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In this issue of Blood, Rubin et al describe the diagnostic accuracy of measuring perforin and CD107a expression in patients with primary hemophagocytic lymphohistiocytosis (HLH) and compare the performance of these assays to quantification of natural killer cell cytotoxicity.1 Data from this study support the incorporation of these assays into future diagnostic algorithms for primary HLH.

HLH comprises a heterogeneous group of disorders characterized by the excessive activation of CD8+ T cells and macrophages that secrete high levels of proinflammatory cytokines. Inherited or “primary” HLH is caused by the presence of germline mutations that negatively affect the killing activity of cytotoxic lymphocytes (CD8+ T and natural killer [NK] cells). In contrast, sporadic or “secondary” HLH is associated with similar clinical features, but patients lack identifiable germline mutations. Prompt diagnosis of HLH is essential because it allows for the rapid institution of life-saving immunosuppressive therapies. It is also important to distinguish between the primary and secondary forms of the disease because the former can only be cured by allogeneic hematopoietic stem cell transplantation. Nevertheless, the signs and symptoms of HLH are nonspecific, making it challenging to differentiate it from other more common disorders such as sepsis. Similarly, the lack of rapid, robust, and reliable clinical tests hampers the timely identification of individuals with primary HLH. In this study, Rubin et al provide convincing data that it is time to rethink how we diagnose patients with primary HLH.

There are currently 7 known genes responsible for primary HLH or the related pigmentary disorders (Griscelli syndrome type 2, Chediak Higashi syndrome, and Hermansky Pudak syndrome type 2) in which HLH may also develop.2,3 These genes include PRF1, which encodes the pore-forming protein perforin, and UNC13D, STX11, STXBPD, RAB27A, LYST, and AP3B1, which encode proteins essential for the proper movement and extrusion of perforin-containing granules (see figure).1 Because of the presence of germline biallelic loss of function mutations, cytotoxic lymphocytes from primary HLH patients exhibit profound reductions in, or even absence of, target cell killing. In contrast, cytotoxic lymphocytes from secondary HLH patients generally exhibit normal or only transiently reduced cytotoxicity.4 Patients with X-linked lymphoproliferative syndromes (XLP) 1 and 2 (caused by germline mutations in SH2D1A and XIAP, respectively) may also develop hyperinflammation; however, it remains less well understood how mutations in these genes contribute to HLH.

Reduced NK cell cytotoxicity has long been considered a standard component of HLH diagnostic criteria.2 However, in recent years, new flow cytometry–based methods have been developed and used to investigate specific NK cell defects; namely, measurement of intracellular perforin levels5 and quantification of NK cell degranulation via upregulation of CD107a,6 a protein found in the membrane of perforin–containing cytolytic vesicles. When these vesicles fuse with the plasma membrane of cytotoxic lymphocytes after contact with a target cell, CD107a is exposed on the cytotoxic lymphocyte surface and can be quantified by staining using an anti-CD107a antibody (see figure).6,7

The report by Rubin et al is the first to comprehensively evaluate the diagnostic accuracy of NK cell cytotoxicity, intracellular perforin expression, and CD107a upregulation in patients with HLH. In this large, retrospective study, the sensitivity and specificity of these assays were evaluated in patients with and without HLH–associated germline mutations. Through this study, the authors make several important observations. First, assessment of NK cell cytotoxicity has poor diagnostic accuracy for identifying individuals with primary HLH. Notably, 17% of patients with bi-allelic PRF1 or degranulation gene mutations exhibited normal NK cell function, whereas individuals who lacked biallelic mutations, carried monoallelic mutations, or harbored variants of uncertain significance exhibited impaired NK cell killing. It remains unclear why some of the patients with biallelic mutations had normal NK cell function, although it is possible that their NK cells used alternative perforin–independent mechanisms to mediate target cell killing.7 NK cell viability and cytotoxic activity can be negatively affected by exposure to steroids,8 which are commonly used to treat HLH, and by delayed transit time between collection and testing.1 The results of the cytotoxicity assay can also be skewed when the absolute number of NK cells is low (a common finding seen in cytopenic HLH patients). In addition, and as noted before, NK cell cytotoxicity assays are often temporarily abnormal in patients with secondary HLH.4 Therefore, it is understandable why patients who lacked biallelic mutations might have exhibited normal NK cell killing. Despite the NK cytotoxicity assay’s lower sensitivity, specificity, and positive predictive value, Rubin et al find that it has excellent negative predictive value. Therefore, this assay may still be helpful in ruling out certain forms of primary HLH. Second, Rubin et al found that low perforin expression was extremely sensitive and specific (96.6 and 99.5%, respectively) for the detection of primary HLH when caused by biallelic PRF1 mutations. Similarly, reduced CD107a expression exhibited good sensitivity and specificity (93.8% and 72%, respectively) for primary HLH due to cytotoxicity gene defects. Third and last, the authors show that by combining perforin and CD107a expression testing, the diagnostic accuracy of these tests for primary HLH is very good and only marginally improved through the addition of NK cell cytotoxicity testing.

Although sequencing of known genes remains the gold standard for establishing a diagnosis of primary HLH, the use of flow cytometry–based assays cannot be underestimated, especially in patients for whom genetic testing is unrevealing or provides partial or uncertain information.
Indeed, the same holds true for measuring expression of SAP and XIAP, the proteins defective in XLP1 and XLP2. The excellent reproducibility, increasing availability, and short turnaround time of these assays allow for a rapid determination of the possible heritable nature of HLH using a minimal volume of blood. The fact that these assays are not affected by HLH-directed treatment makes them even more appealing.

Importantly, early diagnosis is expected to inform the appropriate initiation of HLH-directed therapy, which may lessen morbidity and mortality by reversing immune activation before significant organ damage occurs. The results provided by Rubin et al argue that it is time to “go with the flow” and include reduced perforin and CD107a expression among future diagnostic criteria for primary HLH.

**Conflict-of-interest disclosure:** The authors declare no competing financial interests.

### REFERENCES


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