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Commentary

Towards a New Diagnostic Standard for Niemann–Pick C Disease

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Niemann–Pick C (NPC) disease is rare, neurodegenerative, lysosomal cholesterol storage disorder. Diagnosis of the disease is often delayed due to disease heterogeneity, non-specific early visceral and neurological symptoms, and lack of a rapid and reliable diagnostic assay. As a result, the disease progresses and opportunities to intervene tragically are lost.

Until recently, the principal diagnostic test for NPC disease was filipin staining of unesterified cholesterol in cultured fibroblasts obtained from a skin biopsy. While the filipin assay is sensitive for diagnosis of "classical" disease, it is unable to provide a firm diagnosis in fibroblasts with "variant" phenotypes that represent >1/3 of NPC cases (Stampfer et al., 2013). Moreover, the test is invasive, performed only at specialized centers, is costly (3000 USD), and generally entails ~3 month turnaround time, all of which are disincentives for broad clinical deployment. Genetic analysis, involving sequencing of the NPC1 and NPC2 genes, is an important diagnostic tool, though, due to cost considerations, it is generally applied as a confirmatory rather than screening test. Furthermore, routine gene sequencing detects mutations on both alleles in only 85% cases and is confounded by the highly polymorphic nature of NPC1 (>400 known mutations), which makes interpretation of new mutations challenging (Stampfer et al., 2013).

Over the past several years, biomarker discovery efforts have led to identification of several promising NPC disease biomarkers. One of these markers, cholestane-3β,5α,6β-triol ("triol"), which is a cholesterol oxidation product, has emerged as a sensitive diagnostic for NPC disease (Porter et al., 2010). The application of this biomarker to rapid diagnosis of NPC has been demonstrated at multiple centers and on diverse platforms (Boenzi et al., 2014; Klinke et al., 2015; Pajares et al., 2015; Polo et al., 2015; Jiang et al., 2011). In this issue of *EBioMedicine*, Reunert et al. report the largest single site experience in prospective diagnostics for NPC disease using the triol assay (Reunert et al., 2016). From 1902 suspected NPC patients, 69 NPC1, 2 NPC2, and 12 NP-A/B patients were identified. Identification of 71 new NPC patients over a three-year period by a single clinical laboratory is unprecedented, demonstrating the utility of the triol assay as a rapid and reliable means to screen patients suspected of NPC. It is noteworthy that 40% of the diagnoses were among patients >20 years of age, confirming suspicions that the disorder is underdiagnosed in the adult population, and hinting that the incidence of NPC may be higher than previously reported. Bolstered by experience from more than two dozen laboratories worldwide, the report from Reunert et al. strongly support the authors' recommendation that the triol assay become the diagnostic standard for NPC screening, replacing the less sensitive, qualitative, and time-consuming filipin staining test as the first-line NPC diagnostic assay.

The growing experience with the triol biomarker in clinical laboratories also provides important insight into the limitations of the assay. Although triol is a highly sensitive marker for detection of NPC, it has broader specificity than originally reported, extending beyond NPC to related disorders of sterol metabolism. Plasma triol, which is generated by non-enzymatic oxidation of cholesterol, is similarly elevated in the setting of NP-A/B (ASMD, acid sphingomyelinase deficiency), cerebrotendinous xanthomatosis (CTX), and lysosomal acid lipase deficiency (LALD) (Klinke et al., 2015; Pajares et al., 2015; Reunert et al., 2016). In addition, the plasma triol assay may be falsely elevated in neonates with non-NPC cholestasis (Polo et al., 2015). Whether the elevated triol in these conditions is due to increased formation *in vivo*, endogenous interference, or artifact generated by sample handling or to generation of the labile 5,6-epoxycholesterol intermediate, which can be converted into non-endogenous triol during the storage and sample preparation (*e.g.*, by chemical derivatization), remains to be determined. Finally, approximately 25% of NPC carriers have plasma triol levels that overlap with NPC patients, confounding interpretation of test results (Jiang et al., 2011; Reunert et al., 2016).

What might we look for in an ideal NPC diagnostic test? Such a test would be based on an analyte that can be detected by mass spectrometry without need for derivatization, which can introduce artifact into the assay. The test would display exceptional sensitivity and specificity for NPC, and further permit unambiguous discrimination between NPC carriers and patients, and could be used for screening neonates with cholestasis, a population known to be enriched for NPC. Ideally, the analyte would be stable at room temperature and during long-term storage, which would simplify NPC diagnostics, allowing clinicians from anywhere in the world to send samples to clinical labs without need for

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dry ice or overnight shipments. A dried blood spot-based assay would also have the advantage of potential integration into existing newborn screening platforms.

In addition to triol, several other NPC disease markers have been described and explored as NPC diagnostics (Giese et al., 2015; Welford et al., 2014). Understanding of the relative merits of each marker awaits rigorous benchmarking in the clinical laboratory setting. Broad dissemination of assays based on this new generation of biochemical markers would be expected to eliminate the diagnostic delay that at present exceeds five years. Furthermore, newborn screens based on these biomarkers would provide more accurate assessment of the true disease incidence and facilitate therapeutic intervention in pre-symptomatic patients. Ultimately, advancement in diagnostics will enable initiation of therapy in neonates and change the natural history of the disease, prolonging the asymptomatic phase before onset of neurological symptoms, increasing lifespan, and improving quality of life.

Disclosure

D.O. holds a patent for use of oxysterol biomarkers in diagnosis and treatment of Niemann–Pick C disease.

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