ONION-BULB PATTERNS PREDICT ACQUIRED OR INHERITED DEMYELINATING POLYNEUROPATHY

JENNIFER A. TRACY, MD, PETER J. DYCK, MD, CHRISTOPHER J. KLEIN, MD, JANEAN K. ENGELSTAD, HT,
JANE E. MEYER, BS, and P. JAMES B. DYCK, MD

Department of Neurology, Mayo Clinic, 200 First Street SW, Rochester, Minnesota, 55905, USA

Accepted 23 February 2019

ABSTRACT: Introduction: Onion-bulbs (OB) are concentrically layered Schwann-cell processes, surrounding nerve fibers, occurring in both inherited and acquired demyelinating polyneuropathies. We investigated whether OB patterns (generalized, mixed, or focal) correlate with acquired or inherited neuropathies. Methods: One hundred thirty-one OB-rich nerve biopsies were graded for OB pattern and inflammation without knowledge of clinical history. We classified inherited (n = 49) or acquired (n = 82) neuropathies based solely on clinical history. Results: Fifty-one biopsies had generalized (34 inherited vs. 17 acquired, P < 0.001), 54 mixed (48 acquired vs. 6 inherited, P < 0.001), and 26 focal/multifocal (inherited [n = 9], acquired [n = 17]) OB. Inflammation occurred more frequently in acquired (n = 54) than inherited (n = 14) neuropathy (P = 0.004). Discussion: Generalized OB correlates with inherited neuropathy; mixed OB with acquired. Inflammation occurs more in acquired neuropathy cases. OB patterns are best explained by ubiquitous Schwann-cell involvement in inherited and multifocal Schwann-cell involvement in acquired neuropathies and predict the electrophysiology of uniform demyelination in inherited and unequal demyelination in acquired neuropathies.

Muscle Nerve 59:665–670, 2019

Hypertrophic neuropathy is caused by onion-bulb (OB) formation. OB are formed in nerve by repeated demyelination of axons, followed by abortive attempts at remyelination, resulting in multiple concentric layers of Schwann-cell processes and basement membranes surrounding the axons; these OB resemble an onion when viewed in transverse section. Other cell types, however, can also be present in OB. Despite the process of ongoing remyelination, axons often remain thinly myelinated or even unmyelinated. Demyelinating pathology is associated with characteristic electrophysiological alterations (slowing of conduction velocities, prolongation of distal latencies, F-wave latencies, and blink reflexes), and clinically by weakness and large myelinated fiber sensory loss. Although these findings may alert a clinician to the presence of a demyelinating polyneuropathy, the distinction between inherited and acquired causes can be elusive in many patients, particularly when genetic testing is unrevealing. The pathology may also be similar, with OB found in both inherited and acquired demyelinating neuropathies.

Over the years, we have observed that the pattern of distribution varies in nerve biopsies containing frequent, large OB. We recognize 3 major patterns of distribution: (1) generalized (OB surrounding nearly all myelinated nerve fibers [MF] or former sites of MF); (2) mixed (axons surrounded by large OB located immediately adjacent to axons without OB and normal myelin, in an apparently random and mixed pattern); and (3) focal (all MF in a region surrounded by OB, while MF in other regions are normal and without OB). We hypothesized that OB patterns are useful in determining whether the neuropathy is due to an inherited or an acquired etiology. We theorized that inherited demyelinating neuropathies would be more likely to show a generalized OB pattern (given that all cells share a common mutation), whereas acquired demyelinating neuropathies would be more likely to show a mixed or multifocal pattern (given that inflammatory demyelinating events are likely to occur in a patchy, multifocal distribution throughout the nerve). The aim of our study was to use the Mayo Clinic nerve biopsy experience to determine whether the pattern of OB formation (generalized, mixed, or focal) would predict whether a hypertrophic neuropathy was inherited or acquired.

METHODS

We screened the Mayo Clinic Peripheral Nerve Laboratory database to identify patients who had undergone a nerve biopsy in which “onion bulbs” were recorded as a pathological finding. We included patients with any type of nerve biopsy. These were most commonly sural or other whole distal cutaneous nerve, as well as fascicular nerve biopsies from proximal mixed motor and sensory nerves, in patients who also had detailed medical records available (either from our institution or from another institution with detailed clinical information included when the nerve biopsy specimen was processed). The Mayo Clinic Institutional Review Board approved this study, and participating patients (or their legally authorized representatives) provided written consent to use their medical information.

All biopsy specimens were reviewed by 2 of the authors (J.A.T. and P.J.B.D.) without knowledge of the clinical history; biopsies were excluded if OB were small (e.g., only 1 or 2 layers of Schwann-cell processes or only visible on electron microscopy) or uncommonly found. The included biopsies were then classified into 1 of 5 OB patterns: generalized, mixed, or focal (as described earlier). The average size and the frequency of OB were graded. The presence of nerve-fiber loss was recorded and characterized as

Abbreviations: CIDP, chronic inflammatory demyelinating polyneuropathy; HMSN, hereditary motor sensory neuropathy; MF, myelinated nerve fibers; MGUS, monoclonal gammopathy of undetermined significance; OB, onion-bulb; PMP-22, peripheral myelin protein-22

Key words: chronic inflammatory demyelinating polyradiculoneuropathy; demyelinating neuropathy; inherited neuropathy; onion bulbs; peripheral neuropathy; hypertrophic neuropathy

Funding: Alnylam Pharmaceuticals to P.J.D.; and NCI and NCATS to C.J.K.

Conflicts of Interest: None of the authors have any conflict of interest to disclose.

Correspondence to: P.J.B. Dyck; e-mail: dyck.pjames@mayo.edu

© 2019 Wiley Periodicals, Inc.
Published online 26 February 2019 in Wiley Online Library (wileyonlinelibrary.com). DOI 10.1002/mus.26452

Onion-Bulbs in Polyneuropathy
The presence of inflammation was noted and classified as epineurial, perineurial, or endoneurial, and as individual (<10 cells), small (10–49 cells), moderate (50–99 cells), or large (≥100 cells) in size. In a blinded fashion to the pathology, the medical records of the included patients were reviewed and the clinical (sometimes genetically proven) diagnoses recorded. Patients were classified into 1 of 4 categories based on the following criteria: definite inherited (genetic testing positive for a specific inherited neuropathy or at least 1 similar clinically affected first-degree relative), clinical neuropathy and no response to immunomodulatory therapy (if given); possible inherited (some family history and a clinical neuropathy or slowly progressive course [with or without hamartomies or high arches] or congenital neuropathy, and no response to immunomodulatory therapy [if given]); definite acquired (clinical neuropathy and good response to immunomodulatory therapy and not meeting criteria for inherited); and possible acquired (rapid progression of symptoms over <2 years or focal onset or possible response to therapy). Only after the pathological and clinical grading were completed, did we look at the correlation to see whether OB formation predicted an acquired or inherited cause of the neuropathy.

**RESULTS**

We identified 858 nerve biopsies with OB. From these, 131 biopsies had both frequent, large OB and sufficient clinical information available to classify the neuropathy as inherited or acquired.

Based on the clinical criteria previously described, 82 biopsies were classified as acquired (41 as definite acquired and 41 as possible acquired) and 49 biopsies as inherited (35 definite inherited and 14 as possible inherited). The 82 biopsies with acquired hypertrophic neuropathy had the following diagnoses: chronic inflammatory demyelinating polyradiculoneuropathy (CIDP), 66; inflammatory neuropathy with rheumatologic disease (4); monoclonal gammopathy of undetermined significance (MGUS) neuropathy (4); diabetic neuropathy (3); multiple-system atrophy with peripheral neuropathy (1); primary amyloidosis (1); and other acquired neuropathy (3). The 49 biopsies with inherited hypertrophic neuropathy had the following diagnoses: hereditary motor and sensory neuropathy type 1 (HMSN type 1, Charcot–Marie–Tooth type 1, 25); HMSN type 3 (Dejerine–Sottas neuropathy, 11); inherited neuropathy with insensitivity to pain (1); and other inherited (12). Nine of the 35 biopsies with definite inherited neuropathy had positive genetic testing (8 for peripheral myelin protein-22 [PMP-22] duplications and 1 with a PMP-22 point mutation). For statistical analysis, we consolidated the definite and possible categories together into 2 groups, acquired and inherited. The age at biopsy was significantly lower for patients with inherited (29.6 ± 16.2 years) than for acquired (46.8 ± 19.1 years) disorders (P = 0.0015), and there were significantly more women in the inherited group (Table 1). Three patients had 2 nerve biopsies performed. All 3 had inherited neuropathy (2 HMSN type 3 and 1 HMSN type 1), and all 6 biopsies showed the generalized OB pattern.

The pathology of the OB formation showed 51 biopsies with generalized OB, 54 with mixed OB, and 26 with focal OB. Inflammatory changes (small or larger collections) occurred in 68 biopsies, and multifocal fiber loss was present in 36 patients. When correlation between the clinical and pathological findings was made, interesting patterns emerged.

The generalized OB pattern (Fig. 1) was significantly more frequent in the inherited group (n = 34) than in the acquired group (n = 17) (P < 0.001). In genetically proven (via PMP-22 duplications) inherited neuropathies, 7 of 8 had a generalized pattern of OB formations, and only 1 had a mixed pattern. The mixed OB pattern (Fig. 2) was significantly more frequent in the acquired group (n = 48) than in the inherited group (n = 6) (P < 0.001). There was no significant difference in frequency of the focal OB pattern between the inherited group (n = 9) and the acquired group (n = 17) (P = 0.82) (Fig. 3). Multifocal fiber loss was more frequent in the acquired group (n = 30) than the inherited group (n = 6) (P = 0.006). Inflammation (small collections or larger in size) occurred more frequently in the acquired group (n = 54) than in the inherited group (n = 14) (P = 0.004) (Fig. 4). The presence of endoneurial inflammation of any size (individual cells or larger collections) was more common in acquired (n = 50) than in inherited (n = 13) cases (P < 0.001). The appearance, composition, and average size of the OB formations themselves did not seem to differ between the inherited and acquired cohorts (Fig. 5).

**DISCUSSION**

Making a specific diagnosis in chronic hypertrophic neuropathies can be difficult even after detailed clinical, laboratory, and electrophysiologic evaluation. Although
genetic testing is advancing rapidly, it is expensive, and the genetic abnormalities for many of the inherited neuropathies remain unknown. Therefore, identifying specific patterns of OB formation to help differentiate inherited from acquired hypertrophic neuropathies is clinically relevant, useful information and an important diagnostic aid to neuromuscular physicians.

Many gene abnormalities have been found in inherited demyelinating polyneuropathies. In general, inherited demyelinating neuropathies are slowly progressive, with distal lower extremity weakness, thin calves, high arches, and hammertoes. Nerve conduction studies typically show features of diffuse demyelination with uniform slowing of conduction velocities and lack of temporal dispersion or conduction block. Acquired demyelinating neuropathies, such as CIDP, usually present later in life (adulthood), may be relapsing–remitting or slowly progressive, and may have proximal weakness. Nerve conduction studies also show demyelinating features, but, in acquired cases, temporal dispersion, conduction block, and prolonged F-wave latencies (out of proportion to the F-wave estimate implying proximal slowing) are more frequent than in inherited neuropathy. Classifications for CIDP focus largely on nerve-conduction criteria to diagnose definite CIDP. Other studies have noted that some forms of inherited neuropathy also have temporal dispersion and conduction block.

Although OB are the pathological hallmark of hypertrophic neuropathies, other histological features may include increased fascicular area and increased collagen. Endoneurial edema has been reported in CIDP. Inflammation in nerve has been one of the

FIGURE 1. Generalized onion-bulbs (OB) seen in nerve biopsy epoxy sections stained with methylene blue, showing OB surrounding almost all fibers (generalized) as seen in (A) hereditary motor sensory neuropathy (HMSN) type 1 (Charcot–Marie–Tooth, inherited neuropathy), note the normal myelin thickness; (B) HMSN type 3 (Dejerine–Sottas, inherited neuropathy), note the hypomyelination (thin myelin); and in (C) CIDP (acquired), note most OB do not have a myelinated fiber at their centers. The generalized pattern is found more frequently in inherited neuropathies.

FIGURE 2. Mixed onion-bulbs (OB) seen in nerve biopsy epoxy sections stained with methylene blue, showing OB surrounding some fibers, whereas other myelinated fibers do not have OB, as seen in (A) chronic inflammatory demyelinating polyneuropathy (CIDP) (acquired) with biopsy from nerve root; (B) CIDP (acquired); and (C) focal hypertrophic neuropathy of the median nerve (focal CIDP, acquired). The mixed pattern is found much more commonly in acquired neuropathies.
The main ways to distinguish acquired neuropathies from inherited ones. However, other studies have found little difference in the amount of inflammation from nerves of CIDP patients compared with chronic idiopathic axonal neuropathies.

In the present study, we have shown that the OB patterns in hypertrophic neuropathies correlate strongly with the neuropathy subtype; namely, that inherited demyelinating neuropathies are significantly more likely to have a generalized pattern of OB formation, whereas acquired demyelinating neuropathies are significantly more likely to have a mixed pattern of OB formation. Focal patterns of OB formation were seen both in inherited and acquired disease. Pathophysiologically, these findings are likely explained by the fact that all cells in inherited neuropathies possess the same gene mutation and, thus, the same susceptibility to the defect in myelination. Over time, Schwann cells and their axons face the same stresses and are likely to develop demyelination–remyelination at approximately the same rate, developing generalized OB. This pattern of generalized OB explains the nerve-conduction findings in inherited neuropathies—the electrophysiological correlate of the generalized OB pattern is the uniform slowing of conduction velocities observed in inherited neuropathies. In contrast, in acquired demyelinating neuropathies, inflammatory or immune-mediated demyelination occurs, and local factors are important. Thus, some myelinated nerve fibers are affected, whereas immediately adjacent myelinated fibers are unaffected. This multifocal pathophysiology explains the development of the mixed OB pattern—the electrophysiological correlate of the mixed OB pattern is unequal demyelination demonstrated by temporal dispersion and conduction block seen in acquired demyelinating neuropathies. Although we predicted that the focal pattern of OB formation would occur more in the acquired than in the inherited neuropathies because of a multifocal inflammatory/immune attack, this is not what we found. There was no significant difference in the rates of focal OB between the 2 groups (inherited vs. acquired). We do not have a good explanation for the focal pattern that occurs in some cases of inherited neuropathies. It may be due to a mosaicism.

As expected, inflammatory infiltrates, specifically endoneurial inflammation, were significantly more common in acquired demyelinating neuropathies. Inflammatory infiltrates were also found in some inherited neuropathy biopsies, making the OB pattern even more useful in discriminating acquired from inherited causes of neuropathy. Multifocal fiber loss was also significantly more common in acquired neuropathies, likely representing the effects of local factors in a patchy fashion rather than an underlying genetic defect affecting all nerve fibers equally.

In general, nerve biopsies are not essential to determine acquired vs. inherited demyelinating neuropathy.
However, there remain cases without a defined gene abnormality, with equivocal electrophysiological testing, an unexpected rate of disease progression, or other factors that obscure the clinical distinction. Making the correct diagnosis becomes particularly important as the arsenal of immunomodulatory therapies for acquired diseases becomes more elaborate, with worrisome side-effect profiles and high costs. Gene-silencing therapies are also becoming available for some inherited neuropathies (such as hereditary transthyretin amyloidosis\cite{17,18}) and such therapy may become available for some hypertrophic inherited neuropathies in the

**FIGURE 4.** Inflammation and onion-bulbs (OB) seen in transverse paraffin sections from 2 patients with CIDP. Serial sections stained with (A) hematoxylin–eosin and (B) CD45 show a perivascular endoneurial collection of inflammatory cells and background OB in a patient with CIDP. Three consecutive paraffin cross-sections show: (C) hematoxylin-eosin stain a large inflammatory collection in epineurium adjacent to the perineurium that (D) reacts to a T-cell preparation (CD3) and (E) OB are confirmed by their reactivity to a Schwann-cell preparation (S-100). Inflammatory infiltrates are more common in acquired neuropathy.

**FIGURE 5.** Serial transverse paraffin sections taken from a fascicular sciatic biopsy of a patient with CIDP showing the architecture of onion-bulbs (OB). The OB are composed of whorled Schwann-cell cytoplasmic processes (A) (hematoxylin–eosin stain) that react to Schwann-cell immunostaining (B) (S-100), but not to perineurial immunostaining (C) (epithelial membrane antigen).
future. Misdiagnosis can be particularly risky in cases perceived as inflammatory disease refractory to treatment, for which progressively more toxic agents may be prescribed. In such cases, nerve biopsy still may be an appropriate tool to determine whether the neuropathy is acquired or inherited.

There are limitations to this study. The results apply only to patients in whom the OB formations are frequent and large. We specifically excluded biopsies with only few or small OB, given that these features present in many different types of neurogenic disorders, including primarily axonal neuropathies. These biopsies would have to be graded as mixed pattern, and hence the mixed pattern would lose its specificity and significance. The results of this study should be extrapolated only to biopsy specimens with large and frequent OB. In cases of CIDP without OB, other techniques (in particular, teased fiber analysis) may be more helpful to determine the presence of demyelination.

Another limitation is that the designation of appropriate clinical diagnoses for the patients in this study was based upon retrospective chart review. Although performed as accurately as possible, it still is imperfect.

Yet another limitation centers on the pathological grading. The graders were blinded to the final diagnosis, but the coexisting findings of inflammatory infiltrates and multifocal fiber loss, which are more common in acquired neuropathies, may have inadvertently caused them to become partially unblinded while grading OB patterns.

The main finding of our study is that the generalized pattern of OB formation was associated with inherited neuropathies, whereas the mixed pattern of OB formation was associated with acquired neuropathies. The generalized pattern is suggestive but not specific for inherited neuropathy, and some patients with acquired neuropathies had diffuse, generalized OB. In contrast, the mixed pattern of OB formation is quite specific for acquired demyelinating neuropathies, and few inherited neuropathies showed mixed OB. This is important because a patient presenting with mixed OB likely has an acquired neuropathy and may deserve a trial with immunotherapy.

As noted, we only included large OB for our study; all patients in the study had long-term neuropathy. One could ask whether the OB pattern is just a reflection of chronicity of the disease (the more chronic the more generalized the OB pattern). However, we do not believe this to be the case; already in early childhood type 1 and type 3 HMSN the OB are well formed, very large, and in a generalized pattern. Another potentially troubling question is why some patients with acquired neuropathies have mixed OB whereas others have generalized OB. The answer to this question may lie in the temporal evolution of the disease. In early acquired demyelinating neuropathy, patients do not have OB and only have segmental demyelination (such patients are not included in this study). In later stages, the inflammatory demyelinating process may be quite spotty, and some myelinated fibers may be normal, whereas other adjacent fibers may have large OB (the mixed OB pattern). As the disease progresses to the end stages, all myelinated fibers may become affected and develop the generalized OB pattern.

Ethical Publication Statement: We (the authors) confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

REFERENCES