OBSERVATION

Peripheral Autoimmune Neuropathy Assessed Using Corneal In Vivo Confocal Microscopy

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Background: Corneal nerves can be examined using in vivo confocal microscopy (IVCM). This new technique permits sequential observation of the corneal subbasal nerve plexus and detects early signs of diabetic peripheral neuropathy.

Objective: To describe a patient with autoimmune peripheral neuropathy followed up using corneal IVCM.

Design: Case report.

Setting: Clinic of neurology, Geneva, Switzerland.

Patient: A 56-year-old man with peripheral neuropathy diagnosed as anti–myelin-associated glycoprotein neuropathy. His symptoms initially worsened despite the administration of intravenous immunoglobulins and plasma exchange. Evolution was eventually favorable after rituximab and corticosteroids were given. At 1-year follow-up, clinical recovery was almost complete, and the patient was stable according to the results of clinical and electrophysiologic assessments.

Main Outcome Measure: Corneal nerve measurement by IVCM.

Results: Examination of corneal nerves using IVCM at 2 different times during the patient's clinical evolution (peak disease and recovery phase) demonstrated histologic signs that correlated with the results of clinical and electrophysiologic assessments.

Conclusion: This observation supports the hypothesis that corneal IVCM could also be helpful for the early detection or follow-up of autoimmune peripheral neuropathy.

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THE CORNEA IS ONE OF THE most densely innervated parts of the human body. In vivo confocal microscopy (IVCM) provides the opportunity to examine living human cornea nerves at the cellular level. The noninvasive nature of IVCM allows multiple examinations of the same tissue across time. This technique has been successfully used to examine nerves in healthy corneas[1] and to assess their nerve changes in patients with leprosy[2] or diabetes mellitus.[3-5] We herein describe a patient with anti–myelin-associated glycoprotein (anti-MAG) peripheral neuropathy followed up using corneal IVCM. Examination of corneal stroma demonstrated alteration of the nerves that correlated across time with the results of clinical and electrophysiologic assessments.

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REPORT OF A CASE

Across a 2-month period, a 56-year-old man developed progressive ascending sensory loss in both legs, associated with distal weakness. On hospital admission, neurologic examination of the lower limbs revealed distal and symmetrical weakness (grade 4 on the Medical Research Council scale) associated with alteration of touch and pain sensations. Tendon jerks were absent. The remaining results of his neurologic examination were normal.

Results of routine laboratory blood tests and cerebrospinal fluid examination were normal. Immunologic tests revealed a paraproteinemia IgM kappa (monoclonal gammopathy of undetermined significance) associated with anti-MAG antibodies to 7916 arbitrary units ($N < 1000$). Paraneoplastic antibodies (amphiphysin, CV2/CRMP5, GAD, HU, Ri, and Yo) and antiganglioside antibodies (GM1, GM2, GD1a, GD1b, and GQ1b) were absent. Results of serologic testing were negative (human immunodeficiency virus, hepatitis B and C, *Borrelia burgdorferi*, syphilis, and *Campylobacter jejuni*) or were not suggestive of an acute infection (herpes virus types 1-6). Findings from spinal magnetic resonance imaging and thoracic computed tomography were normal. Bone marrow biopsy results were normal except for mild polyclonal plasmocytosis.

Despite the initiation of intravenous immunoglobulin therapy and, 1 month later,

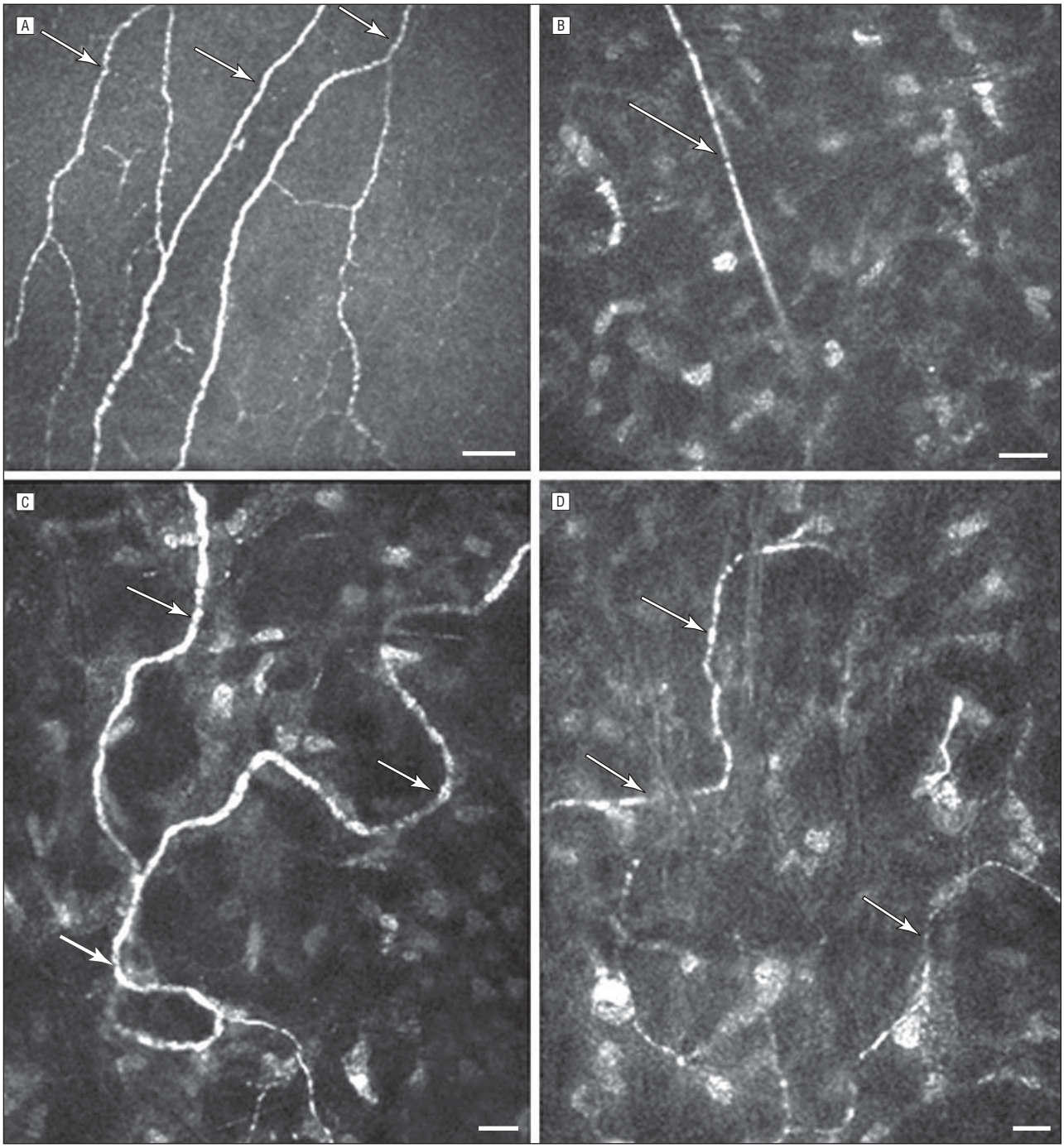

Figure. Corneal nerve examination by means of in vivo confocal microscopy (left eye; bar=50 µm). A, Basal epithelial nerve bundles are normal in our patient (arrows). B, Stromal nerves in a control patient with typically linear morphologic features (arrow). C, Stromal nerves are thickened and tortuous during clinical worsening (arrows). D, One year after escalation of therapy, including rituximab dosage, the stromal nerves are thinner and less tortuous (arrows).

plasma exchange, the clinical course was marked by rapid progression of distal lower limb weakness and sensory ataxia, which necessitated assistance with walking. Electroneuromyography of the lower limbs at this stage showed reduced amplitudes, increased latencies, severe temporal dispersion of distal motor responses with marked slowing of nerve conduction velocities, and disappearance of F waves.

The patient had no personal or family history of eye disease and no history of contact lens wear, ocular trauma or surgery, or systemic diseases that might have affected the cornea. Results of an ophthalmic evaluation, including visual acuity and anterior segment, fundus, and corneal sensitivity using cotton-wool stimulus all over the corneal surface, were clinically normal.

Corneal IVCM was performed using a Heidelberg Retina Tomograph II Rostock Cornea Module (Heidelberg Engineering GmbH, Dossenheim, Germany). After the application of topical anesthesia (oxybuprocaine, 0.4%) (Novartis Pharma Schweiz AG, Bern, Switzerland), Lacryvisc gel (Alcon Laboratories Inc, Hünenberg, Zug, Switzerland) was applied before aligning the

lens. Raw, full-screen images were captured throughout the cornea of both eyes at the time of the aggravation. Images are presented without further digital treatment. Examination of the density and morphologic features of the stromal nerves revealed abnormally thickened (mean diameter of nerve fibers, 10 μm) and tortuous nerves (**Figure**, C), whereas in the normal cornea, the stromal nerves appear as linear bundles (mean diameter of nerve fibers, ≤5 μm) (Figure, B). In contrast, the basal epithelial nerve bundles revealed no abnormality (Figure, A).

Treatment using rituximab (anti-CD20) associated with pulse methylprednisone and azathioprine was initiated. Rapid and dramatic improvement was observed, with diminished weakness and sensory symptoms of the lower limbs. One year later, the patient was receiving prednisone, 20 mg/d by mouth, and azathioprine. Flow cytometry demonstrated a persistent effect of rituximab on B-cell depletion (<1% $CD19^+$ B cells). Clinical recovery persisted with no new symptoms or signs, and the patient could walk for more than 1 hour without assistance. A control electroneuromyogram showed increased distal motor response amplitudes and reappearance of F waves. Follow-up of the neuropathy using corneal IVCM revealed dramatic histologic improvement marked by decreased thickness (mean diameter of nerve fibers, 5 μm) and reduced tortuosity of the stromal nerves (Figure, D).

COMMENT

Anti-MAG neuropathy is an autoimmune, antibody-mediated, demyelinating neuropathy. The monoclonal anti-MAG antibodies are directed against the myelin sheath and are believed to be pathogenic.[6] The case reported herein illustrates an anti-MAG neuropathy requiring polyimmunosuppressive therapy[7] eventually associated with a favorable clinical evolution.

The cornea is one of the most densely innervated parts of the human body. The nerve bundles lose their perineurium at the limbus and continue centrally and anteriorly in the cornea surrounded by Schwann cell sheaths.[8] The nerve bundles then penetrate the Bowman layer, turn abruptly, and continue parallel to the cornea surface, forming the subbasal nerve plexus. Corneal nerve architecture in humans has been studied using light and electron microscopy. However, these studies have the intrinsic limitation that they were performed on corneal tissue obtained from cadavers or enucleated eyes and, therefore, were likely to be affected by artifacts that result from tissue processing and postmortem, or ex vivo, nerve degeneration. In vivo confocal microscopy provides a unique opportunity to examine the living human cornea at the cellular level.[1] The noninvasive nature of this technique means that multiple examinations may be performed on the same tissue across time. In addition, this technique provides similar or even superior information to that obtained by means of histopathologic examinations, enabling quantification of corneal nerve morphologic features.[9]

The extent of nerve damage and repair in the cornea of this patient, visualized using IVCM, correlated in time with the results of clinical and electrophysiologic assessments. Corneal nerves showed alterations in the stroma but not in the basal epithelium. This may be related to the fact that, in contrast with epithelial axons, stromal nerves are surrounded by Schwann cells[2] susceptible to autoantibodies. The histologic description of anti-MAG neuropathy classically reveals abnormal thickening of the myelin sheath associated with myelin-bound autoantibodies.[6] Therefore, this observation is consistent with the histologic picture observed using corneal IVCM in the present patient with thickened and tortuous stromal nerves.

In conclusion, we believe that corneal IVCM, a noninvasive surrogate marker of nerve fiber abnormalities, may be useful for assessing peripheral autoimmune demyelinating conditions such as anti-MAG neuropathy. This new technique might help physicians detect autoimmune neuropathy earlier and follow disease progression or response to therapeutic intervention by means of repeated assessment.

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