Detection Of Human Nerve-Binding Antibodies As A Potential Biomarker In Immune-Mediated Neuropathies

poster 412

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introduction

BACKGROUND

- Immune-mediated peripheral neuropathies are a clinically heterogeneous group of rare and disabling disorders in which an aberrant immune response targets various components of the peripheral nervous system
- Although more than 20 specific autoantibodies have been identified over the past three decades, the relevant target antigens and diagnostic antibodies for AIDP variant of GBS and for CIDP remain unknown[1].
- According to current guidelines for GBS[2] and CIDP[3], diagnosis relies primarily on clinical and electrophysiological criteria that are intentionally broad to include patients who may benefit from immunomodulatory treatment. As a result, these categories likely encompasses a spectrum of heterogeneous entities with distinct underlying mechanisms.

ROLE OF ANTIBODIES

- The critical role of circulating humoral factors – at least in a patient subpopulation – is strongly supported by experimental models of passive serum transfer[4], the established efficacy of plasma exchange in GBS[2], and the the results of the ADHERE trial leading to the approval of efgartigimod (an FcRn inhibitor that selectively reduces IgG levels) in CIDP[5].
- However, in the ADHERE trial, 34% of patients did not respond[5] and emerging real-world data have described cases of clinical worsening following transition from stable IVIg therapy to efgartigimod[6]. Whether this reflects the presence of non-IgG-mediated pathogenic mechanisms in some patients (IgM-mediated processes, IgG-independent complement activation, or cellular immune responses) or a potentially avoidable immunomodulatory gap arising from suboptimal timing of the transition from IVIg to efgartigimod remains unclear, creating challenges for decision-making in routine clinical practice.
- A potential biomarker could be the detection of serum IgM and IgG binding directly to human peripheral nerve structures, irrespective of whether the specific target antigen has been identified.

INDIRECT IMMUNOFLUORESCENCE

- Indirect immunofluorescence is a cornerstone for identifying novel autoantibody reactivities in chronic neuropathies.[1,7,8]
- Although significant progress was achieved using animal tissue as a substrate, detecting antibodies to unknown antigens could be hindered by interspecies molecular discrepancies.
- Despite the limited availability of normal human nerve tissue, a methodology for the systematic testing of sera from patients with paraproteinemia on frozen sections of normal human peripheral nerve was described more than 30 years ago.[8,9].

OBJECTIVE

This study aims to visualize antibody binding to normal human peripheral nerve via indirect immunofluorescence in patients with suspected immune-mediated neuropathy at our centre.

methods

NATIONAL REGISTRY AND BIOBANK

- A Czech national registry for patients with immune-mediated neuropathies (RESTAND) was launched in April 2024[10].
- As a pilot subproject, a biobank for collecting blood samples was launched in one of the centres (University Hospital Brno) in October 2024.
- Since January 2025, blood samples are taken from all patients with suspected immune-mediated neuropathy referred to our centre and willing to participate in our study, starting with treatment-naive samples.

INDIRECT IMMUNOFLUORESCENCE

- We followed the 1995 protocol for testing sera of patients with paraproteinemia[8], with minor modifications to align with our laboratory practices.
- Sections (12 µm) of normal human peripheral mixed nerve were cut, mounted on slides, air-dried, and fixed in acetone-methanol (1:1) at room temperature. For visualization of IgM binding, the sections were incubated with patient serum overnight at room temperature. For visualization of IgG binding, the sections were incubated with purified IgG for 30 min at room temperature. IgG was purified using Protein G SpinTrap™ columns according to the manufacturer's instructions and further processed as previously described[4]. Then, the sections were washed three times in phosphate-buffered saline (PBS) and subsequently incubated with primary antibodies specific for human IgM and IgG at room temperature in the dark, further incubated with fluorescent secondary antibodies rinsed in distilled water and mounted with glycerol.
- Healthy control samples and samples from patients with confirmed IgG and IgM antibodies targeting the nerve structures were used as reference patterns for indirect immunofluorescence evaluation (see Figure 1 and Figure 2).
- In all patient samples, two independent evaluators determined nerve binding IgG/IgM reactivity as positive or negative. Only if they agreed, the results were given as positive/negative, otherwise it was described as borderline result.

IgG visualization

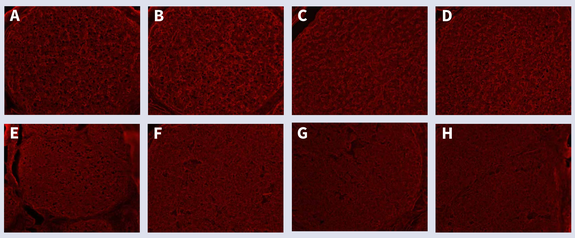

Figure 1: Nerve binding IgG visualization. (A) anti-GQ1b IgG positive, (B) anti-NF155/186 IgG positive, (C) anti-NF186 IgG positive, (D) anti-GM1 IgG positive, (E) negative control incubation without blood sample, (F-H) healthy controls.

IgM visualization

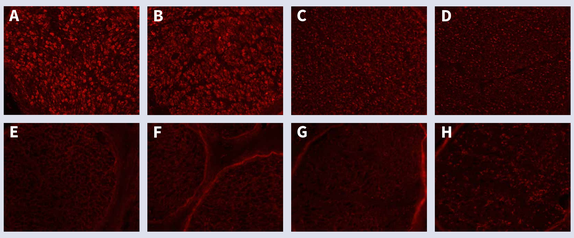

Figure 2: Nerve binding IgM visualization. (A-B) anti-MAG neuropathy, (C-D) multifocal motor neuropathy, (E) negative control incubation without blood sample, (F-H) healthy controls.

results

RESULTS

- **As of May 2026, we had collected samples and performed indirect immunofluorescence testing in a total of 34 patients and 6 healthy controls.**
- **Final diagnoses were nodopathy in 2 patients, anti-MAG neuropathy in 5 patients, GBS in 12 patients, and CIDP in 15 patients, including 3 patients initially diagnosed as GBS who were subsequently reclassified as acute-onset CIDP.**
- **The evaluators agreed that IgG binding could be clearly distinguished between positive control and healthy control samples (see Figure 1). In contrast, pronounced IgM staining was observed in 3 of 6 healthy controls (see Figure 2 – E), and the evaluators agreed that they were currently unable to reliably assess IgM reactivity as positive or negative in patient samples. The only consistently identifiable IgM pattern was anti-MAG IgM binding (see Figure 2 – A and B).**
- **All treatment-naive samples from patients with GBS collected at symptom onset exhibited an IgG staining pattern consistent with that observed in positive controls and were classified as positive. In a limited number of follow-up samples available, this IgG reactivity became undetectable within 3-8 months after symptom onset (see Figure 3).**
- **Among the 15 samples from patients with CIDP, evaluators classified IgG binding as positive in 7 samples, borderline in 2 samples, and negative in 6 samples (see Figure 4).**
- **At present, we are unable to reliably assess IgM positivity or negativity in patients with GBS and CIDP.**

Guillain-Barré syndrome

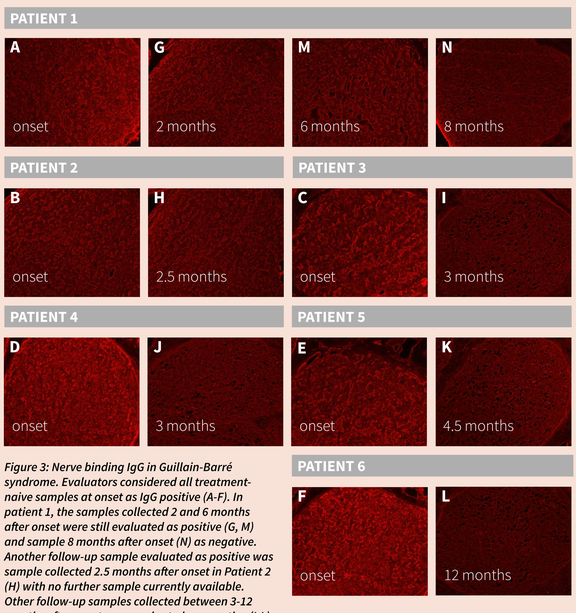

Figure 3: Nerve binding IgG in Guillain-Barré syndrome. Evaluators considered all treatment-naive samples at onset as IgG positive (A-F). In patient 1, the samples collected 2 and 6 months after onset were still evaluated as positive (G, M) and sample 8 months after onset (N) as negative. Another follow-up sample evaluated as positive was sample collected 2.5 months after onset in Patient 2 (H) with no further sample currently available. Other follow-up samples collected between 3-12 months after onset were evaluated as negative (I-L).

Chronic inflammatory demyelinating polyneuropathy

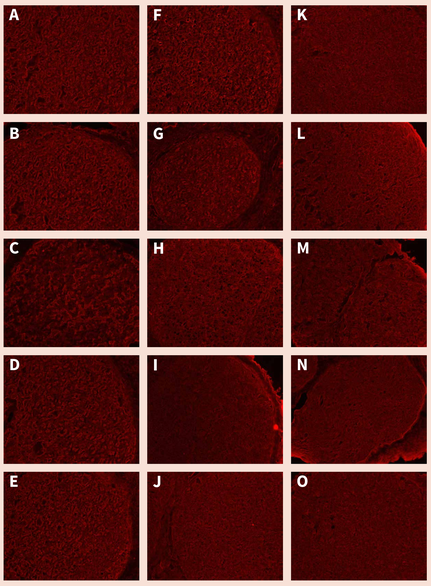

Figure 4: Nerve binding IgG in Chronic inflammatory demyelinating polyneuropathy . Evaluators considered samples A-G as positive, H-I as borderline and J-O as negative.

HIGHLIGHTS

- **We detected IgG binding in all samples from patients with GBS, and the limited number of follow-up samples available suggested that the IgG reactivity became undetectable in 3 - 8 months.**
- **We detected IgG binding in 7 of 15 patients with CIDP, while 2 samples were classified as borderline, and 6 as negative.**
- **Further methodology adjustments are needed to improve the detection of IgM binding.**

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Limitations

- Due to the absence of a gold standard or established reference method, we are unable to directly assess the validity of our methodology or perform a direct comparison with a validated method.
- Additionally, the IgG purification step may introduce bias and potential loss of IgG, including low-abundance or low-affinity antibodies, which could affect the sensitivity and representativeness of the detected IgG repertoire.
- Finally, we are currently unable to reliably distinguish IgM binding in patients from that observed in healthy controls.

Abbreviations

AIDP, acute inflammatory demyelinating polyradiculoneuropathy; **CIDP**, Chronic inflammatory demyelinating polyneuropathy ; **FcRn**, neonatal Fc receptor; **GBS**, Guillain-Barré syndrome; **HC**, healthy controls; **IVIg**, intravenous immunoglobulins; **MAG**, myelin-associated glycoprotein; **MMN**, multifocal motor neuropathy.

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Disclosures

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