PROGRESS REPORT

Start project	**:** 01-Jul-2024
Period progress report	**:** 01-Jul-2024 – 30-Jun-2025
Date progress report	**:** 30-Jul-2025

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1. GENERAL DATA

Applicants	**:** Dr. H.G. Huizinga and Prof. Dr. B.C. Jacobs
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Project title	: Biomarkers for paranodal injury in Guillain-Barré syndrome

2. OVERVIEW OF SCIENTIFIC PROGRESS IN THE PROJECT

In this project we investigate serum neurofascin (sNF) and serum contactin-1 (sCNTN1) which function as paranodal cell adhesion molecules connecting the myelin and axon, and are essential for nerve conduction, as potential new prognostic biomarkers in Guillain-Barre syndrome (GBS).

The first aim of the study was to set up an assay to measure neurofascin in serum. We synthesized recombinant NF-155 and NF186 and tested whether these proteins could be measured in a commercial ELISA kit but found no signal using concentrations up to 100 ng/ml. NF was also not detectable in a few sera from patients with GBS or healthy controls. Next, we determined the reactivity of two monoclonal anti-neurofascin antibodies, as well as the reagents provided in the ELISA kit. While the antibodies from the ELISA kit did not stain NF155 or NF186 expressed as full-length protein by HEK293 cells, the

two monoclonals recognized only NF186. Using these monoclonal antibodies in a sandwich assay together with a polyclonal anti-NF antibody did however not result in detection of recombinant NF isoforms. Possibly antibodies are not compatible or antibodies recognize epitopes of NF that are only expressed intracellularly. In any case, the currently available antibody reagents seem not suitable for sensitive detection of extracellular NF.

For CNTN1, we used an assay that was already validated and performed a pilot study to assess sCNTN1 concentrations at study entry (n=63) and at follow up time points (n=16). We analyzed their association with clinical parameters, including nerve conduction studies (NCS), GBS disability scores (GBS-DS), disease outcome, and related biomarkers. No significant differences were found between patients with GBS and healthy controls. However, the results suggest that patients with an equivocal NCS classification had significantly lower sCNTN1 concentrations than those with demyelinating or axonal subtypes. Additionally, lower baseline sCNTN1 levels were significantly associated with greater disease severity. Longitudinal analysis revealed heterogeneous dynamics in sCNTN1 over the first month. Patients experiencing an early increase in sCNTN1 were significantly more likely to regain the ability to walk compared to those with a decline. We also observed strong correlations between sCNTN1 and serum albumin, potentially related to systemic inflammation. These findings propose sCNTN1 as a promising serum biomarker for monitoring disease activity and predicting recovery in GBS.

3. <u>WORK PLAN FOR THE NEXT PERIOD:</u>

For the remaining part of the project, we propose to expand the study cohort for sCNTN1 and measure additional serum samples, especially follow up samples, to find out whether the observed associations with clinical parameters can be confirmed. For this, no additional funds are required but the budget will be used to measure CNTN1 instead of NF. We kindly ask the committee to approve this change in study plan for the rest of the grant period.

We thank the GBS/CIDP Foundation International for generously supporting our project.