

Comparative analysis of paranodal antibody assays in autoimmune nodopathies: diagnostic accuracy and inter-laboratory agreement

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INTRODUCTION

Autoimmune nodopathies (AN) are a subgroup of neuropathies that harbour antibodies targeting paranodal/nodal proteins (PNAbs): neurofascin-155 (NF155), neurofascin 186 (NF186), contactin-1 (CNTN1) and contactin-associated protein 1 (CASPR1). However, laboratory strategies for their detection are not standardized.

AIMS

We aimed to evaluate the analytic performances and inter-laboratory discrepancies of three PNABs detection assays (commercial cell-based assay [C-CBA] and in-house assays: live cell-based assay [L-CBA] and enzyme-linked immunosorbent assay [ELISA]) in two expert Neuroimmunology Centres.

METHODS

A cohort of patients with chronic demyelinating polyneuropathy (CIDP) (n=43), along with pathological controls (n=36) and healthy controls (n=10), were tested in two laboratories (Montpellier and Pavia) using C-CBA, L-CBA, and ELISA. PNABs positivity required confirmation by both in-house tests in Pavia, or positivity to two or more tests in Montpellier. AN diagnosis was then made if clinical records showed a clinical phenotype consistent with AN.

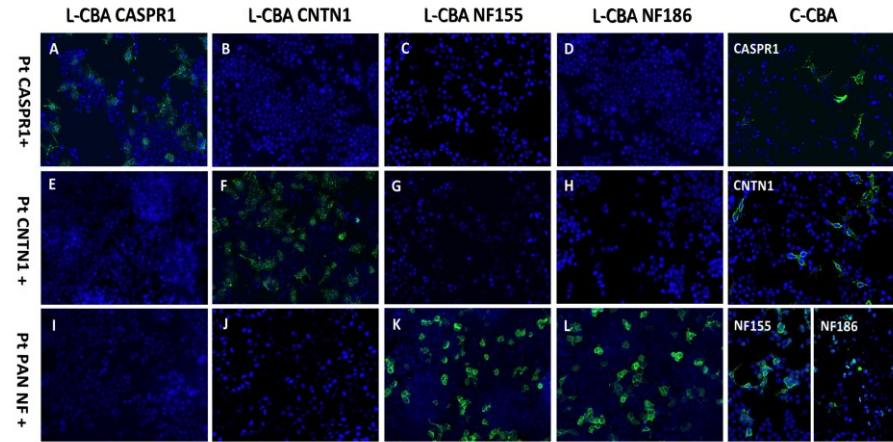


Figure 1. PNABs cell-based assays illustrating the results for three patients included in the study: one positive for CASPR1 autoantibodies (upper row), one for CNTN1 autoantibodies (middle row), and one for pan-neurofascin IgG (lower row). Autoantibodies specifically bind the specific target (green) on HEK293T transfected cells. DAPI stains cell nuclei. Scale bars: 10 µm. Abbreviations: C-CBA = commercial cell-based assay; CASPR1 = contactin-associated protein 1; CNTN1 = contactin-1; L-CBA = live cell-based assay; NF155 = neurofascin-155; NF186 = neurofascin-186; Pt = patient.

RESULTS

Among individual assays, ELISA showed the highest sensitivity but lowest specificity, while L-CBA and C-CBA showed comparable performances.

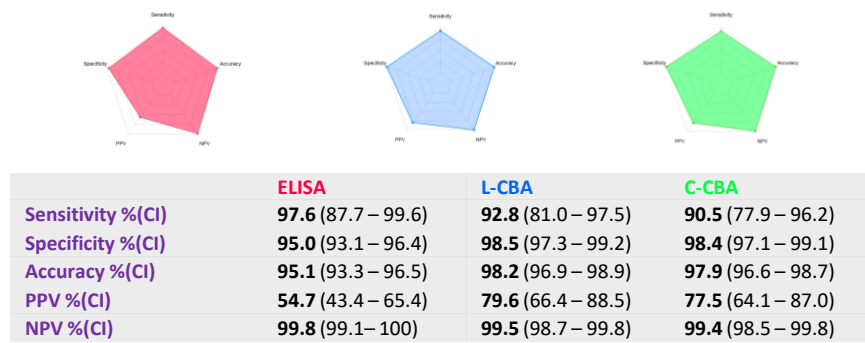


Figure 2 and Table 1. Spider plots and table showing analytic performances of three PNABs assays. Abbreviations: C-CBA = commercial cell-based assay; CI = confidence interval; ELISA = enzyme-linked immunosorbent assay; L-CBA = live cell-based assay; NPV = negative predictive value; PNABs = antibodies against nodal/paranodal proteins; PPV = positive predictive value.

Overall in-house assays' accuracy was 99.3% (sensitivity: 92.9%; specificity: 99.7%). C-CBAs exhibited a similar performance, with an accuracy of 97.9% (sensitivity: 90.5%; specificity: 98.4%).



Figure 3. Spider plots showing analytic performances of in-house assays (red) vs commercial assay (blue) for each antibody. Abbreviations: Caspr1 = contactin-associated protein 1; CNTN1 = contactin-1; NF155 = neurofascin-155; NF186 = neurofascin-186; NPV = negative predictive value; PPV = positive predictive value

In-house assays detected PNABs in 21 AN patients' samples (NF155 = 11, PanNF = 2, CNTN1 = 5, Caspr1 = 3). Substantial overall inter-laboratory agreement was observed (Fleiss' kappa: 0.735), with only 3/90 (3.3%) discordant samples (sample #4, #22 and #31 in Figure 4).

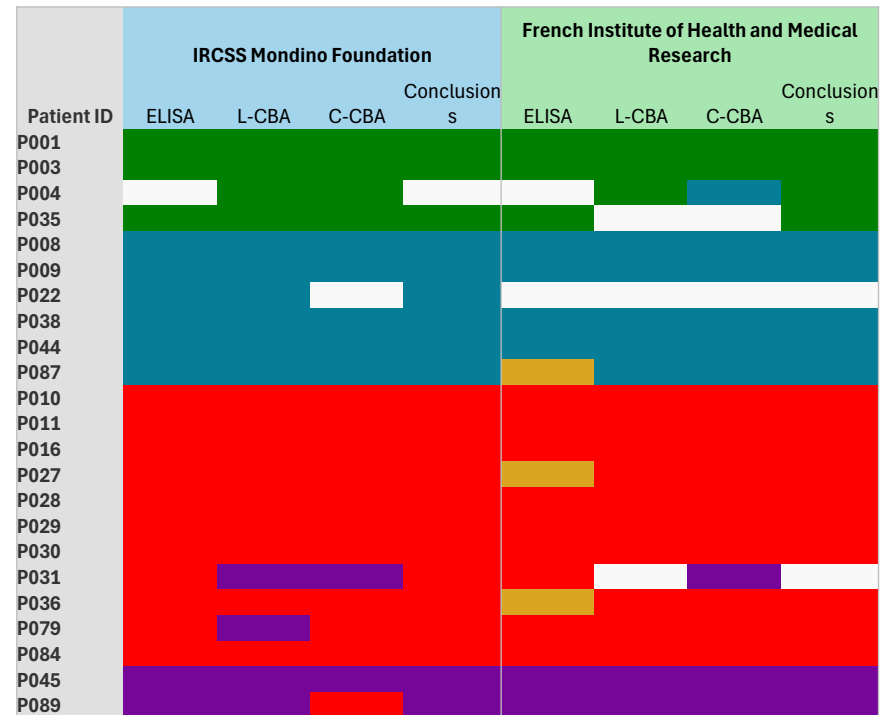


Figure 4. Heatmap showing the results (green = Caspr1, blue = CNTN1, red = NF155, purple = NF186, yellow = all PNABs, white = negative), across PNABs assays conducted in the two Neuroimmunology laboratories in Pavia and Montpellier, for patients considered PNAB-positive in at least one Centre. Abbreviations: C-CBA = commercial cell-based assay; Caspr1 = contactin-associated protein 1; CNTN1 = contactin-1; ELISA = enzyme-linked immunosorbent assay; L-CBA = live cell-based assay; NF155 = neurofascin-155; NF186 = neurofascin-186; PNABs = antibodies against nodal/paranodal proteins.

CONCLUSION

PNABs assays have overall good performances and reproducibility. Commercial CBA represent a reliable alternative to in-house assays for their detection.

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