ABSTRACT

To validate the diagnostic utility of a conventional enzyme-linked immunosorbent assay (ELISA) for determination of anti-NF155 antibodies, sera from 191 patients with CIDP were examined with ELISA using human recombinant NF155. 15(8%) CIDP patients were IgG4 anti-NF155-positive, which were confirmed by western blot, cell-based assay, and immunohistochemistry studies. None of disease controls or healthy subjects had positive results. The anti-NF155 activities on ELISA were significantly positively-correlated with those on CBA (p<0.01). Clinical features of anti-NF155-positive CIDP were consistent with those in previous studies. The ELISA combined with determination of the IgG4 subclass is a simple and reliable method for initial screening for anti-NF155 antibodies. (Ref. 1)

METHODS

1. Serum samples and ELISA

# Subjects: 191 patients with EFNS/PNS criteria-met CIDP, 20 healthy controls, 99 disease controls

# ELISA: 2) Antigens: recombinant human NF protein which corresponds to isoform NF155 and recombinant rat NF 155 (200 ng/well). Patient serum diluted 1:200 with 1% BSA in PBS. Anti-human IgG-Fc ab diluted to 1:500. Optical density (OD) was measured at 490 nm. IgG subclass was examined.

2. Verification of ELISA results

Western blot (WB): patient serum (1:200) and anti-human IgG-Fc ab (1:5000) were used. Cell-based assay (CBA): NF155-turbo GFP-transfected and naive HEK293 cells were used. Flow cytometric assay was performed as reported elsewhere. 3) IHC on rat sciatic nerves (frozen section): rabbit polyclonal anti-contactin-associated protein 1 (Caspr1) ab and patient serum (1:160) were used.

3. Analyses of clinical findings and statistical analysis

Clinical data were collected from questionnaires sent by attending physicians. A two-tailed p < 0.05: significant.

CONCLUSIONS

# 8% of CIDP patients were IgG4 anti-NF155-positive.

# Human NF155-based ELISA combined with determination of the IgG4 subclass is a simple and reliable method for initial screening of anti-NF155 antibodies.

# We confirmed IgG4 anti-NF155(+) CIDP is a distinct subtype from typical CIDP. 3, 4)

REFERENCES