Supplementary Material to the Methods

Transglutaminase ELISAs

TG2 antibodies of both IgA and IgG isotypes were first screened with AESKU Celicheck new generation kit; positives were quantified using the Quanta-Flash h-tTG IgG and IgA assays, with a threshold of 20 AU or higher.

Anti-TG3 IgA (Immundiagnostik) was considered positive above 22 AU/mL (recommendation of the manufacturer). Anti-TG6 ELISAs (Zedira) defined positivity as titers exceeding 4.0 AU/mL for IgA and 5.1 AU/mL for IgG. Titers ranging from 2.6 to 4.0 AU/mL (IgA) and 3.3 to 5.1 AU/mL (IgG) were classified as equivocal (recommendations of the manufacturer).

Other laboratory examinations with reference ranges in parentheses

Deamidated gliadin IgA and IgG antibodies (<20 AU), total serum IgA (0.70–4.00 g/L), total serum IgG (7.00–16.00 g/L), antithyroid peroxidase antibodies (ATPO, <5.60 U/mL), antithyroglobulin antibodies (ATG, <115.0 UI/mL), thyroid-stimulating hormone receptor antibodies (TRAK, gray zone: 1.22–1.75 NE/L, positive >1.75 NE/L), TSH (0.350–4.940 mU/L), RBC (4.00–5.20 T/L), hemoglobin (120–150 g/L), mean corpuscular volume (MCV, 80.0–99.0 fL), and serum iron levels (12.5–32.2 μmol/L) were evaluated.

Ethics

The Semmelweis University Regional and Institutional Committee of Science and Research Ethics approved the study protocol and usage of register-based data (9/2021.). All participants provided written informed consent, and the use of their anonymized, aggregated data for publication. All procedures conformed to the Declaration of Helsinki.

Statistical analysis

All statistical analyses were performed using GraphPad Prism 8 (San Diego, CA, USA ; $p< 0.05$ was considered significant.

Normality was assessed using the Kolmogorov-Smirnov (KS), D'Agostino-Pearson, and Shapiro-Wilk tests. Non-parametric tests were applied when normality was not met.

Comparisons of anti-TG2, anti-TG6, and anti-DGP IgG/IgA by neurological comorbidities and/or GFD adherence used Kruskal-Wallis with Dunn's post-hoc test. Anti-TG3 IgA and GFD adherence were analyzed by two-sided Chi-square test. Mann-Whitney U tests compared antibody levels across GFD adherence groups; Kruskal-Wallis with Dunn's test was applied post-hoc. Spearman correlation analysis assessed relationships between transglutaminase antibody isotypes levels. For comparisons of TSH values by a GFD Mann-Whitney U test was used. Effect of hormone replacement therapy as assessed by Kruskal-Wallis test with Dunn's multiple comparisons test. Some groups contained a limited number of subjects, potentially limiting statistical power.