SUPPLEMENTARY MATERIALS

Detailed methods

Patients and selection

A total of 718 patients who participated in a prospective cohort study in patients with early gastric cancer to detect prodromal Parkinson's disease (PD) (EGC-PPD cohort study) were included.[1] Two neurologists (J. S. and C. S.) retrospectively administered the Chart-Based Instrument for Delirium During Hospitalization (CHART-DEL) to all patients to determine whether they developed postoperative delirium (POD) after operation for EGC.[2]

Clinical assessment

Initial evaluation and annual telephone follow-up were performed according to the EGC-PPD cohort design.[1] We developed a Screening Questionnaire for Clinical Prodromal Markers (SQ-CPM) for the cohort (Supplementary Tables 1 and 2 in the online-only Data Supplement). The SQ-CPM questionnaire includes questions about well-known prodromal non-motor symptoms (hyposmia, constipation, and rapid eye movement sleep behavior disorder (RBD) [RBD1Q][3]) and validated motor symptoms for a survey of parkinsonism (slowness, stooped posture, rigidity, and gait instability).[4,5] Patients who were suspected of having developed or compounded motor symptoms during the annual telephone follow-up were evaluated by movement disorder specialists to confirm whether they had PD or another synucleinopathy, according to the diagnostic criteria.[6,7]

Additional detailed clinical evaluations were performed when a clinical diagnosis of overt parkinsonian syndromes was established. The original version of the Unified Parkinson's Disease Rating Scale was used to evaluate motor function and general Parkinsonian status.[8] Non-motor symptoms were evaluated using the Montreal Cognitive Assessment-Korean version (MoCA-K),[9] Frontal Assessment Battery (FAB),[10] Non-Motor Symptoms Scale,[11] RBD Screening Questionnaire (RBDSQ),[12] International Prostate Symptom Score (IPSS),[13] International Consultation on Incontinence Questionnaire (ICIQ),[14] Overactive Bladder Symptom Score,[15] and Brief Smell Identification Test (B-SIT).[16]

Specimen selection

Both proximal and distal marginal blocks of the patients' stomach specimens were used, resulting in two formalin-fixed paraffin-embedded blocks from each patient. The patients agreed to the pathological evaluation of their specimens for research purposes.

Immunohistochemistry

Serial 3-μm sections were cut from each surgical block for immunohistochemistry (IHC). The paraffin sections were mounted on a glass slide, de-waxed, rehydrated, and incubated with primary antibodies on automated machines as previously described.[17,18] A primary antibody to phosphorylated AS (pAS; 1/1,000 anti-pAS at serine 129 monoclonal Ab [EP1536Y]; Abcam ab51253, Cambridge, UK) was used in conjunction with the Leica Bond Max (Leica Microsystems GmbH, Wetzlar, Germany) system, in accordance with the manufacturer's instructions. Bound antibodies were detected using a Bond Polymer Refine Detection System (Leica Biosystems, Wetzlar, Germany).

Pathologic evaluation

The clinical information of the patients and staining methods were anonymized before presentation to the raters to prevent bias during pathologic evaluation. All the stained slides were scanned using a Leica slide scanner (Aperio AT2, Leica Biosystems, Wetzlar, Germany). Anonymized digital slides were evaluated using the Pathology Slide Viewing Software (Aperio ImageScope ver. 12.4, Leica Biosystems, Wetzlar, Germany).

The pAS positive findings were conservatively defined according to our previous study[17] as follows: 1) pAS IHC showing definite and clear staining such as "dots and fiber" or "Lewy body-like staining" pattern, as suggested in a consensus paper[19] and 2) localization in neural structures that was confirmed with anatomic inspection and neurofilament staining of adjacent slide.[20] The pAS positive findings were semi-quantitatively rated as Grade 1, 2, and 3, which corresponded to sparse, moderate, and frequent, respectively, in the multicenter study.[17,19]

A neuropathologist (SI.K.) and neurologist (C.S.), who were blinded to the anonymization procedure, separately examined the slides. C.S. has participated in our previous studies on gastrointestinal synucleinopathy,[17,18,20] and both raters have undergone the

training program in the microscopic reading of peripheral AS pathology of the Systemic Synuclein Sampling Study.[21] Any discrepancy between the two raters was resolved via a consensus meeting with independent investigators (S. P. and B. J.).

Statistical analysis

Statistical analysis was limited to the performance of a descriptive analysis because of the small number of patients with POD in this study.

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