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ORIGINAL ARTICLE

Clinical, serological and genetic predictors of response to immunotherapy in anti-IgLON5 disease

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Anti-IgLON5 disease is a newly defined clinical entity characterized by a progressive course with high disability and mortality rate. While precise pathogenetic mechanisms remain unclear, features characteristic of both autoimmune and neurodegenerative diseases were reported. Data on immunotherapy are limited, and its efficacy remains controversial. In this study, we retrospectively investigated an anti-IgLON5 disease cohort with special focus on clinical, serological and genetic predictors of the immunotherapy response and long-term outcome.

Patients were recruited from the GENERATE (German Network for Research on Autoimmune Encephalitis) registry. Along with clinical parameters, anti-IgLON5 immunoglobulin (Ig)G in serum and CSF, anti-IgLON5 $IgG_{1\text{-}4}$, IgA and IgM in serum, neurofilament light chain and glial fibrillary acidic protein in serum as well as human leukocyte antigen-genotypes were determined.

We identified 53 patients (symptom onset 63.8 ± 10.3 years, female:male 1:1.5). The most frequent initial clinical presentations were bulbar syndrome, hyperkinetic syndrome or isolated sleep disorder [at least one symptom present in 38% (20/53)]. At the time of diagnosis, the majority of patients had a generalized multi-systemic phenotype; nevertheless, 21% (11/53) still had an isolated brainstem syndrome and/or a characteristic sleep disorder only. About one third of patients [28% (15/53)] reported subacute disease onset and 51% (27/53) relapse-like exacerbations during the disease course. Inflammatory CSF changes were evident in 37% (19/51) and increased blood-CSF-barrier permeability in 46% (21/46). CSF cell count significantly decreased, while serum anti-IgLON5 IgG titre increased with disease duration. The presence of human leukocyte antigen-DRB1*10:01 [55% (24/44)] was associated with higher serum anti-IgLON5 IgG titres. Neurofilament light chain and glial fibrillary acidic protein in serum were substantially increased (71.1 ± 103.9 pg/ml and 126.7 ± 73.3 pg/ml, respectively). First-line immunotherapy of relapse-like acute-to-subacute exacerbation episodes resulted in improvement in 41% (11/27) of patients and early initiation within the first 6 weeks was a predictor for therapy response. Sixty-eight per cent (36/53) of patients were treated with long-term

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immunotherapy and 75% (27/36) of these experienced no further disease progression (observation period of 20.2 ± 15.4 months). Long-term immunotherapy initiation during the first year after onset and low pre-treatment neurofilament light chain were significant predictors for a better outcome.
In conclusion, subacute disease onset and early inflammatory CSF changes support the primary role of autoimmune mechanisms at least at initial stages of anti-IgLON5 disease. Early immunotherapy, prior to advanced neurodegeneration, is associated with a better long-term clinical outcome. Low serum neurofilament light chain at treatment initiation may serve as a potential biomarker of the immunotherapy response.

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Introduction

Anti-IgLON5 disease was first described in 2014 in patients suffering from distinctive rapid eye movement (REM) and non-REM sleep disorders and sleep disordered breathing,[1] in whom antineuronal autoantibodies targeting IgLON5 were identified. IgLON5 is a neuronal surface protein widely expressed and involved in neuronal development and synaptogenesis.[2,3] With more widespread testing available, further manifestations, including characteristic daily sleep attacks, progressive brainstem syndromes, gait abnormalities and neuropsychiatric symptoms have been reported in cohort studies[4,5] and case reports,[6–13] and the term 'anti-IgLON5 disease' was coined. Intriguingly, features characteristic of both autoimmune and neurodegenerative diseases were reported. On one hand, anti-IgLON5 autoantibodies in serum and CSF and a strong association with human leucocyte antigen (HLA)-DRB1*10:01 and HLA-DQB1*05:01 were found.[14] On the other, hyperphosphorylated three- and four-repeat tau protein accumulations, predominantly in the hypothalamus and tegmentum of the brainstem on histopathology[15] and limited or absent response to immunotherapy,[4,5] were reported. Some *in vitro* findings suggested a link between IgLON5 autoantibodies and secondary neurodegeneration,[16] and most researchers assume the disease to be primarily neuroinflammatory/antibody mediated. Yet, whether immunotherapy is beneficial (and if so at what stage) remains controversial, and predictors of treatment response and outcome are mostly unknown.

Mostly, a progressive disease course with a mortality rate of up to 34% despite immunotherapy has been described.[17] However, single cases with dramatic improvement under immunotherapy, particularly in patients with subacute disease onset, have been reported.[6,18–20] For this reason, we hypothesized that patients with subacute manifestation more frequently show findings suggestive of underlying neuroinflammation (e.g. inflammatory CSF findings), a higher frequency of complement-fixing anti-IgLON5 immunoglobulin (Ig) G_1 and a better response to immunotherapy. Therefore, we retrospectively investigated clinical and paraclinical biomarkers of neuroinflammation, serological and genetic patterns and their effect on immunotherapy response in a large, retrospective cohort of anti-IgLON5 disease patients.

Materials and methods

Design and patient recruitment

We performed a retrospective analysis of patients identified through the German network for research on autoimmune encephalitis (GENERATE; https://generate-net.de/) registry. Inclusion criteria were: (i) age ≥18 years; (ii) anti-IgLON5 IgG positivity in serum (cut-off >1:32) and/or CSF (≥1:1) by cell-based assay (CBA); and (iii) neurological deficits consistent with anti-IgLON5 disease. Patients diagnosed with anti-IgLON5 disease between 2014 and 2020 were included from 27 sites.

In addition to the GENERATE registry data available, treating physicians completed questionnaires based on local medical records and structured on-site or telephone-based patient interviews to specifically query the following: (i) comorbidities (malignancy, other autoimmune diseases); (ii) age at disease manifestation and diagnosis; (iii) time and number of physicians contacted until the correct diagnosis was made; (iv) clinical manifestation including main symptoms; (v) subacute or chronically progressive course of the disease at the initial stage [defined as the time until development of substantial deficits relevant for everyday life: subacute ≤4 weeks and progressive (>4 weeks)]; (vi) presence of further relapse-like episodes of deterioration during the disease course (defined as a rapid and significant clinical progression of neurological deficits within ≤4 weeks not attributable to other comorbidities or therapy); (vii) time point of initiation of immunotherapy and subjective impression of its efficacy by treating physician; and (viii) modified Rankin scale (mRS) at the last visit. Follow-up visits within the 12 months prior to analysis were performed in all patients if available. In rare cases, treating physicians were contacted to check for missing information.

Cognitive impairment was analysed based on available local neuropsychological testing. To correct for different protocols, only frequency of general performance that was below average [below 1 standard deviation (SD) of the age- and level of education-adjusted standard values] was analysed in comprehensive cognitive functional areas (attention/concentration, executive function, visuo-constructive/visuo-spatial function, verbal retention, non-verbal retention). Biomarkers of inflammation were analysed in the first available CSF sample. CSF changes were classified as inflammatory if white blood cell count above 4 cells/µl and/or isolated oligoclonal IgG bands in CSF were present. Age-adjusted blood–CSF-barrier (BCSFB) dysfunction (increased serum/CSF albumin-quotient in relation to their age-adjusted norm values),[21,22] total CSF protein level (cut-off 500 mg/dl), evidence of intrathecal IgG, IgM and IgA synthesis (serum/CSF immunoglobulin quotient)[21,22] and routinely determined neurodegeneration markers in CSF (pathological if total tau protein >614 pg/ml, phospho-tau >61 pg/ml, amyloid-β $(Aβ)_{1–42}$ <550 pg/ml and Aβ-quotient$_{1–42/1–40}$ <0.07) were acquired from patient files.

To analyse the response to immunotherapy, we differentiated between exacerbation-related immunotherapy with short acting substances/interventions [short-term immunotherapy (STIT): single courses of intravenous immunoglobulins (IVIG), intravenous high-dose corticosteroids (ivMP) and/or plasma exchange/immunoadsorption (PLEX)] and long-term (long-acting) immunotherapy (LTIT): azathioprine, rituximab, pulsed cyclophosphamide, repetitive IVIG, long-term oral low-dose or relapse-independent repetitive corticosteroids or regular repetitive PLEX. Estimating the efficacy of STIT was based on subjective assessment by the treating physicians and medical records (improvement versus no improvement). The efficacy of LTIT was estimated based on mRS.

The study was performed according to the Declaration of Helsinki and has been approved by the ethical committee of the University of Lübeck (vote-no. 13–162). All enrolled patients or their legal representatives gave written informed consent prior to enrolment in the registry.

Serological testing

All available CSF and serum samples were tested for IgLON5 antibodies with immunohistochemistry on sagittal rat brain sections and by immunofluorescence using live-CBA with HEK293T-cells transfected with human IgLON5 DNA in expression vectors as described elsewhere.[1] Titrations were performed using CBA employing anti-human IgG (H + L, goat Alexa488, LifeTechnologies, 1:1000) by two blinded investigators. IgLON5 antibody Ig-isotypes and IgG-subtypes were analysed by using the respective anti-human isotype specific secondary antibodies. Presence or absence of IgLON5 antibody isotypes and IgG-subtypes was evaluated at two standardized dilutions by two researchers independently, who were blinded to the results (Supplementary material).

Serological neurofilament light chains and glial fibrillary acidic protein

All available serum samples were analysed for concentrations of neurofilament light chain (NFL), glial fibrillary acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), and tau protein in pg/ml using a single molecule array technique with a commercially available 4-plex kit (Neurology 4-plex kit, Quanterix) on a HD-X platform. Results were quantified using calibration curves with known standards assayed in the same run. Assays were done according to the manufacturers' protocols, except for analysing singlicates (due to limited amount of serum) instead of duplicates after determining inter- and intra-assay coefficient of variance for NFL/GFAP high and low controls to be below 10% (Supplementary material).

Genetic analysis

We collected EDTA-anticoagulated blood, peripheral blood mononuclear cells or saliva for DNA isolation. Genotype data were derived from the Illumina Infinium Global Screening Array (GSA). HLA alleles were imputed using the R package HIBAG,[23] employing 7.164 quality-controlled variants in the HLA region with a GSA- and European population-specific prediction model.

Statistical analysis

Statistical analysis was performed using IBM® SPSS Statistics (version 27). The ranks of two groups were compared by Mann–Whitney U-test. The independence of two binary distributed variables was tested with the χ^2 test. In case of significant dependency, the odds ratio (OR) was calculated. Spearman correlation (r_{Sp}) was used to correlate two variables. All statistical tests were two-tailed.

We used binary logistic regression to identify response predictors for the immunotherapy with supposed short-term effects that were administered after subacute relapse-like disease exacerbations (substantial clinical relevant improvement versus no substantial clinically relevant improvement).

We used ordinal regression to identify efficacy predictors for long-term immunotherapy (last mRS). Afterwards, multiple regression was performed to identify the variables with the strongest influence for STIT and LTIT. The following independent variables were included in regression models: (i) disease manifestation (subacute versus slowly progressive); (ii) pre-treatment NFL value in serum; (iii) pre-treatment GFAP value in serum; (iv) delay since acute-to-subacute exacerbation episode for relapse-associated therapy or time from onset until long-term immunotherapy initiation (early—within the first year after disease manifestation, versus late—thereafter); and (v) extent of clinical involvement (restricted to brainstem and/or hypothalamus—isolated isolated brainstem and/or characteristic sleep disorder versus generalized clinical phenotype based on predominant tau protein aggregation in the hypothalamus and brainstem in histopathological studies.[15] We adjusted NFL and GFAP values for the age at sampling by entering the confounder (age) to the raw NFL and GFAP data in the regression analysis. As a diagnostic accuracy test, the area under the curve (AUC) of the receiver operating characteristic (ROC) curve analysis was evaluated. Patients with missing data were discarded from the respective analysis.

Data are shown as mean ± SD for interval-scaled variables or as median and range for ordinal-scaled variables. For all analyses, the significance threshold was set at P-value < 0.05.

Data availability

The data that support the findings of this study are available from the corresponding author, upon reasonable request. No de-identified patient data will be shared.

Results

Three of 56 patients were excluded from the study due to the lack of necessary information or withdrawal of consent (flow chart in Supplementary Fig. 1) and 53 patients with anti-IgLON5 disease were included in the final analysis.

Clinical presentation

The mean age at disease manifestation was 63.8 ± 10.3 years (range 40–82) and the time between onset of symptoms and diagnosis was 33.3 ± 37.5 months (females:males 1:1.5, age at last visit 67.9 ± 9.9 years). A median of four physicians including a median of two neurologists were consulted before the correct diagnosis was made. Twenty-eight per cent (15/53) of the patients had a subacute disease onset (≤4 weeks) which resulted in a significantly earlier diagnosis compared to patients with a slowly progressive manifestation (>4 weeks, latency from manifestation to diagnosis; 15.6 ± 25.1 versus 40.3 ± 39.5 months, U = 133.5, P = 0.003; Fig. 1A and B). Episodes of a subacute deterioration during the disease course were reported by 51% (27/53) patients (12/27 initially had a slowly progressive onset).

The most common initial clinical diagnoses/syndromes considered before detecting anti-IgLON5 disease were bulbar syndromes [17% (9/53), 7/9 were first diagnosed with a seronegative bulbar form of myasthenia gravis], followed by hyperkinetic syndromes in 11% (6/53) and 9% (5/53) each, sleep disorders (REM sleep behaviour disorder, daily sleep attacks, non-REM-parasomnia or sleep disorder breathing), cognitive impairment/dementia, polyneuropathy or hypokinetic-rigid syndromes (Table 1).

At the time of anti-IgLON5 disease diagnosis, most patients (79%, 42/53) already had a generalized multi-system phenotype with core symptoms of brainstem dysfunction, characteristic sleep disturbances/parasomnias as described elsewhere,[24,25] gait disorders, fasciculations, autonomic dysfunction, chorea and severe cognitive dysfunction sometimes with psychotic symptoms (Table 2). Twenty-one per cent (11/53) of patients still had a paucisymptomatic phenotype (isolated brainstem and/or characteristic sleep disorder only). Patients with a progressive onset significantly more often had a generalized phenotype compared to patients with a subacute onset [$\chi^2(1) = 4.71$, P = 0.03; Fig. 1C]. In total, 28/53 (53%) patients required assisted ventilation during the disease course.

Tumour association and comorbidities

Eight patients (15%) suffered from different types of malignancies. In four patients, the malignancy occurred before manifestation of anti-IgLON5 disease (prostate cancer, non-small cell lung cancer, breast cancer, bladder cancer); in four patients, it was diagnosed following symptom onset (osseous metastasized prostate cancer, breast cancer, malignant melanoma, neuroendocrine tumours of the ileum). None of these eight patients had a paraneoplastic neurologic syndrome (PNS)-care-score[26] >3, indicating a low likelihood of paraneoplastic aetiology and suggesting coincidence. Six patients (11%) had further non-neurological autoimmune diseases (psoriasis vulgaris, Graves' disease, autoimmune pancreatitis,

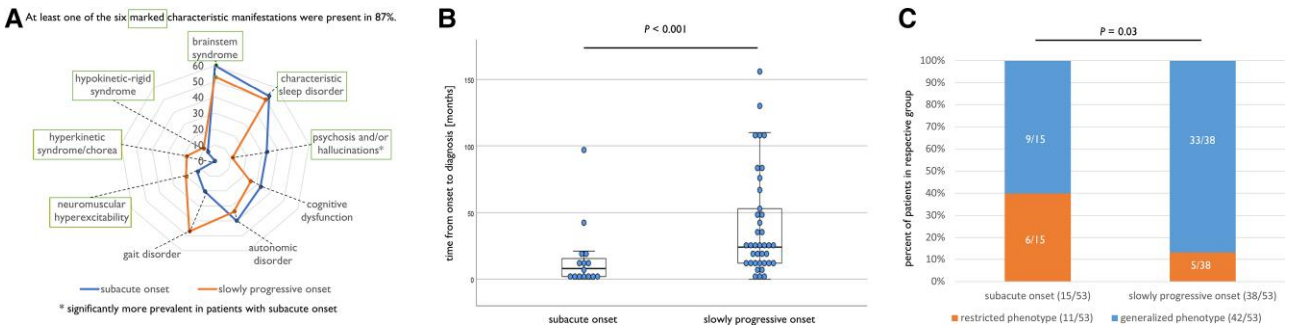

Figure 1 Differences in subacute and slowly progressive clinical manifestation. Fifteen patients (28%) reported a subacute disease onset (≤4 weeks), while 38 (72%) had a slowly progressive disease onset with (12/38, 32%) or without (26/38, 68%) overlapping relapse-like exacerbations. The clinical phenotype of patients with subacute and progressive onset differed significantly regarding the occurrence of psychosis and/hallucinations only. At least one of six characteristic manifestations was present in 87% at the time of diagnosis (**A**). Subacute disease onset was associated with significantly earlier correct diagnosis (**B**) and higher prevalence of a paucisymptomatic clinical phenotype (**C**) compared to slowly progressive manifestation.

Table 1 Initial clinical diagnosis by disease manifestation and before detecting anti-IgLON5 antibody

Clinical diagnosis before detecting anti-IgLON5 antibody	*n* (%)
Bulbar form of myasthenia gravis (7) or not further specified bulbar syndrome (2)	9/53 (17)
Hyperkinetic syndromes (e.g. choreatic and dystonic movements disorder; orofacial dyskinesia or myokymia in 4/6)	6/53 (11)
Isolated sleep disorders	5/53 (9)
Isolated cognitive impairment/dementia	5/53 (9)
Polyneuropathy	5/53 (9)
Hypokinetic-rigid syndrome, incl. Parkinson's disease (3) and progressive supranuclear palsy (2)	5/53 (9)
Benign fasciculation syndrome	4/53 (8)
Amyotrophic lateral sclerosis	4/53 (8)
Psychosis	2/53 (4)
Others[a]	8/53 (15)

[a]Others comprise atactic syndrome, Miller-Fisher syndrome, Lambert–Eaton myasthenic syndrome, mitochondriopathy, laryngospasm, trismus, hypoxic encephalopathy and cerebellar ataxia, neuropathy and vestibular areflexia syndrome.

atopic dermatitis, urticaria, vitiligo). Eight patients (15%) had other auto-antibodies, including anti-nuclear autoantibodies, anti-dsDNA, mitochondrial M2 protein as well as antineuronal antibodies targeting the GABA B receptor, recoverin, amphiphysin and N-type calcium channels. There were no clinical or paraclinical signs of limbic encephalitis, retinopathy, stiff-person syndrome or Lambert–Eaton syndrome in these patients.

Inflammatory and neurodegeneration markers in CSF and serum

For CSF analysis, two patients had to be excluded due to artificial blood contamination. In total, 37% (19/51) of patients had inflammatory changes in CSF: increased cell count in 33% (17/51, median 7 cells/µl, 5–56/µl) and CSF restricted oligoclonal bands in 13% (6/47). None of the patients had detectable intrathecal IgG ($n = 44$), IgM ($n = 38$) or IgA ($n = 40$) synthesis. Age-adjusted BCSFB dysfunction was evident in 46% (21/46, albumin quotient $13.2 \pm 5.4 \times 10^{-3}$) and CSF total protein was elevated in 47% (24/51, 862.7 ± 410.9 mg/dl). The CSF cell count was negatively correlated with the time from clinical manifestation to lumbar puncture ($r_{Sp} = -0.31$, $P = 0.027$; Fig. 2A). One of 25 and 3 of 20 patients had increased total-tau protein and phospho-tau protein in the CSF, respectively. Furthermore, 2 of 27 had a decreased $A\beta_{1-42}$ protein and 1 of 16 a decreased Aβ-quotient$_{1-42/1-40}$. None had a typical Alzheimer's disease CSF pattern (increased phospho-tau protein and decreased $A\beta_{1-42}$ protein). We measured NFL and GFAP levels in 41 sera (mean value of 71.1 ± 103.9 pg/ml and 126.7 ± 73.3 pg/ml, respectively, age at sampling 68.8 ± 9.5 years), 27 of them were taken therapy-naïve during the diagnostic work-up or shortly after diagnosis. In these pre-treatment samples, NFL and GFAP values were negatively correlated with the CSF cell count ($r_{Sp} = -0.51$, $P = 0.009$ and $r_{Sp} = -0.34$, $P = 0.034$, respectively, time between lumbar puncture and serum sampling 1.3 ± 2.1 months). Pre-treatment GFAP was significantly lower in patients with a subacute onset (107.1 ± 43.5 versus 177.4 ± 77.8 pg/ml, U = 39.0, $P = 0.010$; Fig. 2C) and with a paucisymptomatic phenotype (103.7 ± 24.9 versus 164.5 ± 79.2 pg/ml, U = 30.0, $P = 0.027$; Fig. 2D). We observed similar changes in NFL that did not reach the level of significance. Neither NFL nor GFAP values correlated with the time from disease onset (30.7 ± 36.8 months) or diagnosis (2.1 ± 1.3 months) to serum sampling (data not shown). Pre-treatment NFL and GFAP values were positively correlated ($r_{Sp} = 0.53$, $P = 0.004$). The results for pre-treatment tau protein were similar to those for NFL (data not shown), while both biomarkers were positively correlated ($r_{Sp} = 0.46$, $P = 0.029$). UCH-L1 was not associated with any clinical or para-clinical results of the described analyses and did not correlate with any other neurodegeneration markers (data not shown).

IgLON5 antibody testing

IgLON5 antibody was detected using routine clinical CBA diagnostics in 52/53 (98%) sera and 44/50 (88%) CSF samples at an initial diagnostic workup. The one serum-negative patient had IgLON5 antibodies only in the CSF. The patient died 1 month after onset and no follow-up serum was available.

We were able to re-test 62 sera (45 initial and 17 follow-up) and 17 CSF samples. All sera showed a typical staining pattern using rat brain immunohistochemistry (not shown). End point titrations were performed using live-cell based assays as previously described.[1] The initial median anti-IgLON5 IgG titre was 1:640 (range: 0–1:20 480) in serum and 1:16 (range: 0–1:256) in CSF. Analysis of anti-IgLON5 IgG subclasses in sera showed the known subtype specificities of IgLON5 antibodies[4,5]: IgG_1 in 42/45 patients, IgG_2 in 29/45, IgG_3 in 6/45 and IgG_4 in 40/45. A minority of patients had

Table 2 Clinical symptom groups at time of diagnosis in anti-IgLON5 disease

Symptom groups	Subcategories	Frequency (*n*/53)
Brainstem syndrome	Total	**55% (29)**
	Moderate to severe dysphagia	36% (19)
	Dysarthria	32% (17)
	Bilateral vocal cord paresis	9% (5)
	Gaze palsy	19% (10)
Sleep disorder	Total	**51% (27)**
	Moderate to severe characteristic sleep disturbances/parasomnia	45% (24)
	Irresistible sleep attacks[a]	30% (16)
Gait disorder	Total	**40% (21)**
	Limited walking distance	34% (18)
Neuromuscular hyperexcitability	Total fasciculations	**34% (18)**
	Clinical evidence of fasciculations and cramps	19% (10)
	Electromyography done	55% (29)
	Myographic evidence of fasciculations	52% (15/29)
Cognitive dysfunction	Clinically evident cognitive dysfunction	**28% (15)**
	Neuropsychological testing done	43% (23)
	Objective cognitive deficits	87% (20/23)
	Concentration/attention deficits	57% (13/23)
	Verbal learning deficits	61% (14/23)
	Executive deficits	61% (14/23)
Hyperkinetic syndrome/chorea	Total	**21% (11)**
	Facial localization	15% (8)
Psychosis and/or hallucinations	Total	**17% (9)**
Hypokinetic-rigid syndrome	Total	**9% (5)**
Autonomic dysfunction	Cardiac arrhythmias (e.g. nocturnal bradycardia, cardiac arrest), abnormal heart rate variability, obstipation, orthostatic dysregulation, hyperhidrosis	**36% (19)**

Prevalences of different symptom groups in the cohort are bolded.
[a]Irresistible sleep attacks during daytime in 16/53 (frequency of three attacks/day in median, range 1–20 attacks/day).

IgLON5-IgA (8/45) and -IgM (19/45). The total serum anti-IgLON5 IgG titre, but not the IgG titre in CSF, showed a weak positive correlation with the time from manifestation to sampling ($r_{Sp} = 0.29$, $P = 0.049$; Fig. 2B). Anti-IgLON5 IgG serum titres were significantly positively correlated with CSF anti-IgLON5 IgG titres ($r_{Sp} = 0.80$, $P < 0.001$). We found no association between the presence of IgG-subtypes, IgA or IgM and any clinical characteristic including the time from onset to serum sampling.

HLA-genotype-phenotype analysis

The HLA-DRB1*10:01 allele was found in 24 (55%) of 44 tested patients, all of whom were heterozygous. The HLA-DQB1*05:01 allele was present in 32 (73%) of 43 tested patients, and 3 of 24 re-tested patients were homozygous. Fifty-one per cent (22/43) of the patients were double positive for both haplotypes.

Both HLA-DRB1*10:01- and HLA-DQB1*05:01-positive patients were significantly younger at manifestation when compared to HLA-DRB1*non-10:01 and HLA-DQB1*non-05:01 (60.6 ± 7.8 versus 68.1 ± 11.4 years, U = 136.0, $P = 0.014$ and 61.9 ± 9.6 versus 71.3 ± 8.9 years, U = 89.5, $P = 0.016$, respectively). HLA-DRB1*10:01 carriers demonstrated in comparison to HLA-DRB1*non-10:01 carriers a significant association to higher anti-IgLON5 IgG [1:1280 (1:40–1:10 240) versus 1:480 (0–1:20 480), U = 120.0, $P = 0.049$], while the CSF cell count difference did not reach a level of significance (Supplementary Fig. 2). We found a significant association between both HLA alleles and characteristic sleep disorders: 71% (17/24) of the HLA-DRB1*10:01-positive and 30% (6/20) of the HLA-DRB1*10:01-negative patients suffered from characteristic sleep disorders, resulting in an OR of 5.7 for this syndrome [$\chi^2(1) = 7.29$, 95% confidence interval (CI) 1.545; 20.788, $P = 0.007$]. Similarly, 63% (20/32) of the HLA-DQB1*05:01-positive and only 18% (2/11) of the HLA-DQB1*05:01-negative patients suffered from characteristic sleep disorders resulting in an OR of 7.5 [$\chi^2(1) = 6.44$, 95%CI 1.382; 40.690, $P = 0.011$]. No other associations between HLA status and clinical manifestation were observed.

First-line immunotherapy with supposed short-term effects

Twenty-seven patients with a relapse-like rapid deterioration received first-line immunotherapy with supposed short-term effects (IVIG, ivMP, PLEX; Table 3). The mean latency between last clinical deterioration and STIT induction was 5.2 ± 10.7 months.

Eleven of 27 (41%) patients showed clinical improvement after STIT. Early induction of STIT after clinical deterioration (but none of the other predefined factors) was the only significant predictor for a positive treatment response (Nagelkerke's $R^2 = 0.442$, $P = 0.001$, OR = 0.428, 95%CI 0.195; 0.941; Fig. 3A). STIT induction in the first 6 weeks predicted clinical improvement with a sensitivity of 90% and specificity of 94% ($AUC_{ROC} = 0.859$, $P = 0.002$, 95%CI 0.697; 1).

Long-term immunotherapy

Seventy per cent (36/53) of the patients were treated with LTIT (Table 4). Total IgLON5 IgG titres of patients under LTIT were reduced by 1.5 titre levels in median ($n = 12$, follow-up time: 15.3 ± 9.3 months), while in patients not treated with LTIT, titres doubled over time in median ($n = 5$, follow-up-time: 3 ± 1 months, U = 11.0, $P = 0.04$; Fig. 3B). At the last visit, the group of patients under LTIT had a lower median mRS of 2 (0–6) compared with the untreated group, with a median mRS of 3 (1–6). This difference was not significant; however, the follow-up time was substantially shorter in untreated patients (9.4 ± 10.8 months versus 23.3 ± 15.1 months, U = 110.5, $P < 0.01$). The age of patients at diagnosis did not correlate with the last mRS.

The mean latency from disease onset to therapy start was 34.7 ± 34.8 months. The mean observation period under therapy was 20.2 ± 15.4 months. In total, 27/36 (75%) patients experienced no further disease progression under LTIT.

Univariable ordinal regression analysis (Table 5) revealed three potential predictors of treatment response, including a subacute disease manifestation (OR = 0.176, $P = 0.025$), lower pre-treatment NFL level (age adjusted, OR = 1.065, $P = 0.019$) and induction of immunotherapy within the first year after manifestation (OR = 0.095, $P = 0.003$). Neither lower pre-treatment GFAP level (OR = 1.013, $P =$

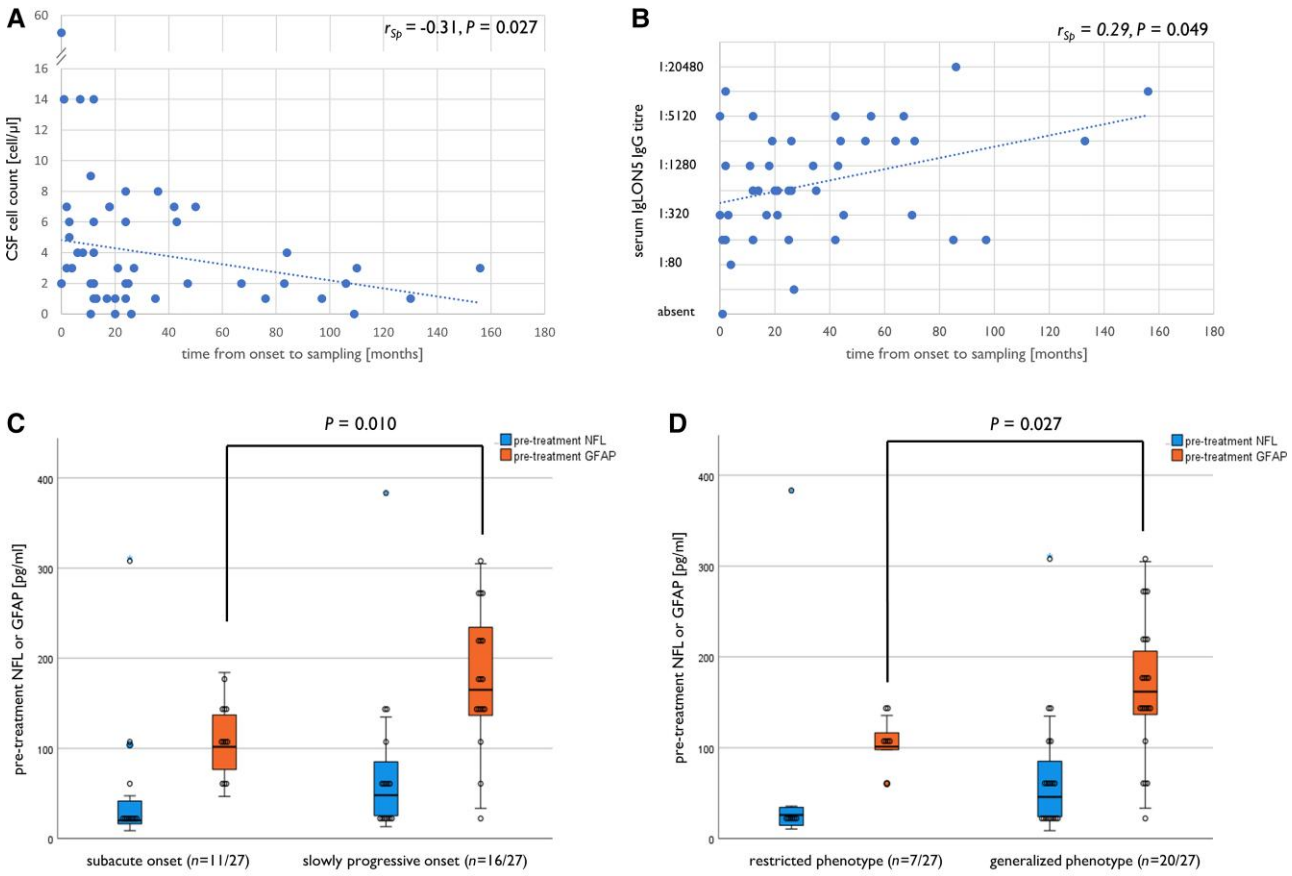

Figure 2 Inflammatory CSF changes, anti-IgLON5 IgG and biomarkers of neurodegeneration depending on disease duration. Time from disease manifestation to sampling correlated negatively with the CSF cell count (**A**) and positively with the total serum anti-IgLON5 IgG titre (**B**). Pre-treatment GFAP was significantly lower in patients with a subacute onset (**C**) and paucisymptomatic clinical phenotype (**D**). Pre-treatment NFL demonstrated similar changes, not reaching the level of significance. NFL and GFAP values are provided as raw data (not age-adjusted).

Table 3 First-line short-term immunotherapy

Short-term immunotherapy	*n* (%)	Clinical improvement, *n* (%)
ivMP	11/27 (40.7)	4/11 (36.4)
IVIG	7/27 (25.9)	4/7 (57.1)
PLEX	1/27 (3.7)	1/1 (100)
ivMP + IVIG	4/27 (14.8)	2/4 (50)
ivMP + PLEX	3/27 (11.1)	0/3 (0)
ivMP + IVIG + PLEX	1/27 (3.7)	0/1 (0)

0.093) nor paucisymptomatic clinical involvement (OR = 0.235, P = 0.064) were significant predictors of a better immunotherapy response.

Multivariable ordinal regression was able to explain a very good amount of variance, as shown by Nagelkerke's pseudo-R^2 = 0.779 (P < 0.001). Low pre-treatment NFL values (OR = 1.093, P = 0.024) and induction of LTIT within the first year after manifestation (OR = 0.005, P = 0.035) remained two significant independent predictors of treatment response (Fig. 3C–E).

Central hypoventilation and mortality rate

In total, ten patients died within a mean of 42.7 ± 37.4 months after disease onset. The causes of death were highly suggestive of anti-IgLON5 disease in at least six patients: four patients died due to central hypoventilation, one due to aspiration and one patient with gait disorder due to complications following a traumatic fall. Another patient died after cardiac arrhythmia of unknown origin, which could also potentially be associated with anti-IgLON5 disease. One patient died due to pulmonary embolism. The causes of death of the other two patients remained unclear; however at least one of them suffered from dysphagia. Six patients died irrespective of immunotherapy; in all of them, LTIT was started after the first year after onset. The NFL and GFAP concentrations in serum correlated with the last mRS (r_{Sp} = 0.52, P < 0.001 and r_{Sp} = 0.45, P = 0.003, respectively) and were substantially higher in patients who died (conservatively, irrespective of the cause of death) compared with surviving patients (187.8 ± 127.3 versus 51.0 ± 86.6 pg/ml, U = 14.0, P < 0.001, and 209.8 ± 72.6 versus 124.2 ± 66.6 pg/ml, U = 45.0, P = 0.027, samples taken 5.7 ± 6.1 months before death). A pre-treatment NFL concentration above 72.4 pg/ml within the first year after diagnosis had a sensitivity of 100% and specificity of 95.2% for death in our cohort (AUC_{ROC} = 0.984, P < 0.001, 95%CI 0.945; 1). GFAP had a less sufficient diagnostic accuracy (AUC_{ROC} = 0.786, P = 0.027, 95%CI 0.593; 0.978).

Discussion

Here, we characterized diagnosis and clinical course in a large anti-IgLON5 disease cohort. We focused on the clinical features

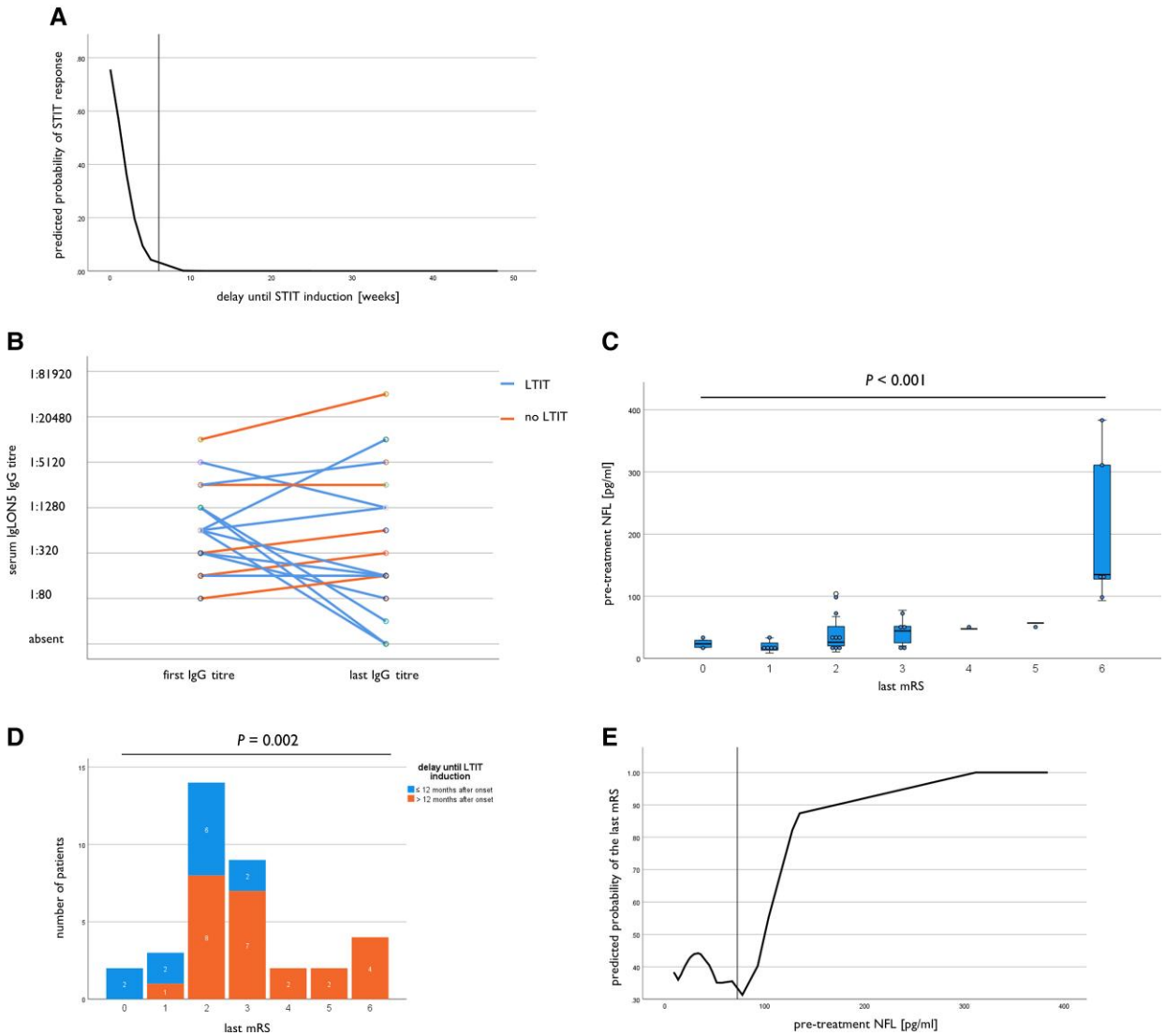

Figure 3 Treatment response. First-line STIT: predicted probability for STIT response after relapse-like deterioration based on therapy delay. STIT induction in the first 6 weeks predicted clinical improvement with a sensitivity of 90% and specificity of 93.7% (**A**). LTIT: antibody titre change in time depending on immunotherapy induction (**B**). Pre-treatment NFL concentration (**C**) in relation to last mRS. Delay of immunotherapy induction in relation of LTIT response (**D**). Predicted probability for LTIT response based on pre-treatment NFL concentrations. An NFL concentration above 72.4 pg/ml within the first year after diagnosis had a sensitivity of 100% and specificity of 95.2% for lethal outcome in our cohort (**E**).

Table 4 Long-term immunotherapy

Long-term immunotherapy	*n* (%)	Median last mRS, range	Stabilized or improved, *n* (%)
Rituximab	18/36	3, 1–6	13/18 (72.2)
Periodic IVIG	15/36	2, 0–6	13/15 (86.7)
Intravenous or oral corticosteroids	14/36	3, 2–6	9/14 (64.3)
Azathioprine	7/36	2, 1–6	6/7 (85.7)
Repetitive PLEX	4/36	2.5, 2–6	3/4 (75)
Cyclophosphamide	2/36	2 and 4	2/2 (100)

Combinations of different therapies were possible.

and laboratory findings indicating the inflammatory nature of the disease and performed an analysis of potential immunotherapy response predictors.

The time point of treatment initiation seems to be decisive in anti-IgLON5 disease. However, due to its rarity and clinical heterogeneity, an early diagnosis of this disease remains challenging. In the present study, the time between onset of symptoms and diagnosis was almost 3 years, while in median, four physicians were consulted before the correct diagnosis was made. A similar mean timeframe of 30 months from onset until correct diagnosis has been reported previously.[25] In the initial cohorts, Gaig *et al.*[4] and Honorat *et al.*[5] identified sleep disorders (22–75%), gait dysfunction (16–70%) and a bulbar syndrome (20–60%) as the most prevalent clinical presentations. Additional studies reported a high prevalence of hyperkinetic syndromes[27,28] and other rare manifestations (parkinsonism,[29] psychosis,[30] motor neuron disease-like phenotype[31]). However, there have been no studies systematically reporting the early disease manifestations. We identified three characteristic tentative initial diagnoses, including seronegative

Table 5 Simple and multiple regression analysis of long-term immunotherapy predictors

Variables	Odds ratio (95%CI)	*P*-value
Simple regression analysis		
Subacute disease manifestation	0.176 (−3.264; −0.215)	**0.025**
Pre-treatment NFL level	1.065 (0.010; 0.115)	**0.019**
Pre-treatment GFAP level	1.013 (−0.002; 0.029)	0.093
Paucisymptomatic clinical involvement	0.235 (−2.988; 0.087)	0.064
Immunotherapy within the first year after manifestation	0.095(−3.919; −0.800)	**0.003**
Multiple regression analysis		
Subacute disease manifestation	5.936 (−1.974; 5.537)	0.353
Pre-treatment NFL	1.093 (0.012; 0.166)	**0.024**
Immunotherapy within the first year after manifestation	0.005 (−10.048; −0.370)	**0.035**

NFL and GFAP were adjusted by entering the age at sampling to the raw NFL and GFAP values. Prevalences of different symptom groups in the cohort are bolded.

bulbar myasthenia gravis, hyperkinetic syndromes (mostly with facial localization) and isolated sleep disorders, suggestive of anti-IgLON5 disease in 38% of patients at disease onset. Besides cognitive impairment, gait instability and autonomic dysfunction being less specific in the elderly, progressive brainstem deficits (bulbar or oculomotor dysfunction, facial dyskinesia or dystonia), characteristic sleep disorder,[24,25] neuromuscular hyperexcitability, acute psychosis or hyperkinetic or hypokinetic-rigid syndrome were present in 87% of patients at the time of diagnosis. These signs could serve as diagnostic 'red flags' for anti-IgLON5 IgG testing if no alternative diagnosis explaining these deficits exists. At the time of correct diagnosis, only one in five patients (21%) still had isolated brainstem and/or hypothalamic deficits, regions that are supposed to be affected early on, according to histopathological data.[25] The majority of patients had developed a generalized phenotype, frequently with gait (50%) and cognitive impairments (36%), associated with increased overall disability. Intriguingly, we observed a correlation of serum GFAP levels with the extent of system-involvement. This could be due to astrocytosis in affected brain areas better mirroring the actual functional deficit due to neurodegeneration than NFL levels, yet this needs to be replicated in independent cohorts. Nevertheless, serum NFL and GFAP levels were considerably higher than in published control cohorts.[32,33] Prevalence of cognitive impairment is probably higher than clinically evident (28%). As many as 87% of patients who underwent neuropsychological testing showed some cognitive dysfunction. This included basic cognitive functions such as attention (tonic and focused) as well as more specific functions such as executive functions and verbal memory. The presence of generalized polytopic muscle fasciculations—detected clinically in 19% and by electromyography in 52%—is of particular interest. Being only occasionally reported previously, these signs turned out to be highly prevalent and indicate potential involvement of the anterior horn motor neuron soma or its axon, both expressing IgLON5 protein.[4,5,31,34] Electromyography seems to increase diagnostic accuracy and should thus be included in the diagnostic workup in anti-IgLON5 disease. Vice versa, anti-IgLON5 antibodies should be considered in patients with atypical amyotrophic lateral sclerosis.

The observed prevalence of tumour diseases in 15% of our cohort is in line with epidemiological data that indicate their presence in 14% of men and 12% of women in the same age group.[35] We could not find any association with either a specific tumour type or characteristic clinical phenotype in patients with a history of oncologic diseases. In accordance with recently published criteria for paraneoplastic diseases,[26] patients with coincident tumours in our cohort had a low PNS-care score ≤3, suggesting a non-paraneoplastic origin. Taken together, this suggests that tumours identified in patients with IgLON5 disease are rather coincidental and not causative. Similar to other autoimmune diseases, multiple antineuronal autoantibodies were identified in a few patients in our cohort and should not lead to diagnostic uncertainty.

Despite a presumed slowly progressive course of disease in the beginning, we identified an acute to subacute manifestation in 28% of our patients. Acute onset was associated with higher prevalence of a paucisymptomatic clinical phenotype and acute psychosis, resulting in an earlier definite diagnosis. Moreover, an additional 32% of the patients with a slowly progressive onset suffered from relapse-like exacerbations during the later disease course. Similarly, single cases have previously been reported by ourselves and others.[6,18–20,26,36] Inflammatory changes in CSF could be demonstrated in one-third of all IgLON5 patients. Even though only mild pleocytosis was evident,[37] an increased CSF cell count was associated with a subacute disease manifestation and negatively correlated with time from onset of first symptoms to CSF analysis. This implies that other patients might show similar inflammatory changes if tested earlier. Interestingly, CSF cell counts were negatively correlated with NFL levels. Taking into account the lack of inflammatory changes demonstrated in most autopsied patients, this supports the hypothesis of initial inflammatory processes leading to neurodegeneration largely independent of the neuroinflammation in anti-IgLON5 disease.[37]

The precise underlying inflammatory mechanisms are unknown; however, surface antigen localization and the presence of autoantibodies in CSF suggest that anti-IgLON5 IgG has a direct pathogenic role. Still, it remains unclear which subclass of anti-IgLON5 IgG is critical. Anti-IgLON5 IgG serum titres significantly increased with disease duration and were positively correlated with CSF anti-IgLON5 IgG titres. However, this finding was borderline significant and needs to be reproduced in independent series. We did not find an association between anti-IgLON5 IgG and NFL levels. In agreement with previous studies,[4,5] the majority of patients had autoantibodies of both IgG_1 and IgG_4 subclasses. It remains unclear which subclass of anti-IgLON5 IgG is critical; few available experimental studies have demonstrated a pathogenic role of IgG_1, but not IgG_4, autoantibodies in a cell culture model.[38] Yet, our data currently do not support strict sequential appearance of IgG_4 following initial IgG_1 autoantibodies, and we did not observe any association of subacute exacerbations or poorer long-term outcome with the presence of IgG_1. Interestingly anti-IgLON5 IgG titres decreased in 58% of patients receiving LTIT, but none without therapy.

We confirmed a high prevalence of the HLA-alleles DRB1*10:01 and DQB1*05:01 and their association with characteristic sleep disorders. In contrast to Gaig *et al.*,[14] we did not find an association with bulbar dysfunction or better cognitive status. Patients with the HLA-DRB1*10:01 genotype were younger and had higher total anti-IgLON5 IgG titre, suggesting this genotype has a possible pathogenic role. In line with this, Gaig *et al.*[14] reported two IgLON5 peptides strongly binding to HLA-DRB1*10:01.

Due to partly contradictory data, it is still under discussion whether anti-IgLON5 disease has primarily an autoimmune or neurodegenerative pathophysiology and if immunotherapy should be

administered in these patients. Strikingly, 41% of our patients benefited from exacerbation-related short-term immunotherapy and as much as 75% from long-term immunotherapy. An early start of immunotherapy was a significant treatment response predictor for both STIT and LTIT. Additionally, a low pre-treatment NFL value, probably reflecting less pronounced neuronal loss, was a further independent predictor for better treatment outcome. An acute to subacute disease onset and paucisymptomatic clinical involvement could be also associated with a beneficial effect of long-term immunotherapy. However, the effects of these two predictors seemed to be secondary and associated with an earlier treatment start. Larger studies are needed to corroborate these findings. These results are in line with previous experimental studies demonstrating IgG_1-dependent irreversible IgLON5 internalization followed by a rapid cytoskeletal disruption.[16,38,39] The irreversibility of the antibody internalization and probably secondary evolving neurodegeneration might explain a lower therapy response in later disease stages and therapeutic skepticism in earlier studies.[1,40]

In a recent study by Gaig *et al.*,[28] only 13% of patients showed improvement of movement disorders after immunotherapy; however, no data on treatment delay have been reported. Similar to other cohorts,[19] 19% of patients in our cohort died within 3 years after onset. Central hypoventilation and dysphagia were most often the causes of death, while the former was associated with later immunotherapy initiation. Moreover, higher serum NFL concentration in the first year after diagnosis predicted a lethal outcome with high accuracy.

Our study has several limitations. Firstly, due to the rarity of the disease, we had to perform a retrospective analysis. Anti-IgLON5 disease with a slowly progressive manifestation was associated with a later diagnosis. This form could generally be more often misclassified as neurodegenerative diseases and underrepresented in our cohort. Secondly, CSF testing and neuropsychological investigations were performed at different sites, although with standardized methods. However, we performed central re-testing of IgLON5 IgG and IgG_{1-4} subclasses, IgM and IgA as well as HLA-genotyping, GFAP and NFL measurement in all available samples. Even though we were able to increase the case numbers in our IgLON5 cohort compared with most other published cohorts, we had to limit the analysis to the main predictors for therapy response; predictors with a lower effect might have been missed in this analysis. Furthermore, the analysis of short-term response to immunotherapy was based on the physicians' judgment and not on functional assessment, which could have introduced a reporting bias. A number of our findings are in parallel with those published previously confirming the validity of our cohort/data. Still, our results should be interpreted with caution due to the retrospective nature of our study and need to be confirmed in further prospective international surveys. We specifically caution against using cut-offs of serum markers identified in our cohort for clinical purposes due to inter-instrument differences, lack of standardized calibrators and control material. We must state that details of a few patients have previously been published as case reports.[6–13]

In conclusion, along with characteristic sleep disorders, progressive oculomotor or bulbar dysfunction, facial dyskinesias as well as generalized fasciculations or unclear psychosis should raise the suspicion of underlying anti-IgLON5 disease. Early inflammatory CSF changes, a subacute disease manifestation and/or further relapse-like exacerbations are not rare and suggest an active role of autoimmune mechanisms at least at the initial stages of the disease. Both short-term exacerbation-related and long-term immunotherapies appear to be effective if started early, during the supposed inflammatory time window until widespread neurodegeneration occurs. The association of lower baseline NFL levels with a good treatment response give further support to this consideration.

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Competing interests

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Supplementary material

Supplementary material is available at *Brain* online.

Appendix 1

See the Supplementary material for further details.

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