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der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

Neuronale Autoantikörper und kognitive Beeinträchtigung in Patienten mit gastrointestinalem Tumor

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Neuronal Autoantibodies and Cognitive Impairment in Patients with Gastrointestinal Cancer

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List of Abbreviations

AICAbs	Neuronal autoantibodies targeting intracellular neuronal antigens
BDI	Beck Depression Inventory
CRCI	Cancer-Related Cognitive Impairment
ECOG	Eastern Cooperative Oncology Group, score to describe performance status
FACIT	Functional Assessment of Chronic Illness Therapy, score to describe the fatigue status
ICCTF	International Cancer and Cognition Task Force
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IQ	Intelligence Quotient
LPS	*Leistungsprüfungssystem*
MoCA	Montreal Cognitive Assessment
MWT-A	*Mehrfachwahl-Wortschatz-Intelligenztest-A*
NED	No Evidence of Disease
NMOSD	Neuromyelitis Optica Spectrum Disorder
NSAbs	Neuronal autoantibodies targeting neuronal surface epitopes
PNS	Paraneoplastic Neurological Syndromes
ROCF	Rey–Osterrieth Complex Figure
SCLC	Small Cell Lung Cancer
SD	Standard Deviation
SF-12	Short-Form-12, questionnaire about physical and mental health
TAP	*Testbatterie für Aufmerksamkeit*
TIA	Transient Ischemic Attack
VLMT	Verbal Learning and Memory Test

Abstract

Introduction

Cancer-related cognitive impairment (CRCI) is a common complication in patients with gastrointestinal cancer, but the causes for CRCI are still unknown. Previous studies have suggested that neuronal autoantibodies may be related to CRCI. Neuronal autoantibodies are associated with tumors, as well as with paraneoplastic neurological syndromes (PNS). Case studies on patients with gastrointestinal cancer, neuronal autoantibodies, and PNS indicate that neuronal autoantibodies may be associated with CRCI. However, the frequency of neuronal autoantibodies and their effect on cognition in patients with gastrointestinal cancer has not been systematically analyzed. Therefore, the aim of this study was to investigate the relation between neuronal autoantibodies and cognitive function in patients with gastrointestinal cancer.

Methods

Serum samples of 158 patients with gastrointestinal cancer were tested for neuronal autoantibodies using cell-based assays and immunohistochemical tissue staining. Furthermore, after applying exclusion criteria, 90 patients participated in a set of neuropsychological tests to assess cognitive function. In order to exclude other possible factors for cognitive dysfunction, exclusion criteria entailed prior neurological diseases (e.g., brain metastases, stroke/TIA), psychiatric diseases (e.g. depressive symptoms), age over eighty years, self-reported reduced general condition, insufficient German language fluency, and declined participation. Cognitive impairment was defined using the International Cancer and Cognition Task Force (ICCTF) criteria by Wefel et al. (1). Cognitive function was compared between antibody-positive and antibody-negative patients. In addition, other clinical factors were assessed for an association with cognitive performance in patients with gastrointestinal cancer.

Results

Neuronal autoantibodies were found in 32/158 (20.3%) of patients with gastrointestinal cancer. Primarily, IgA and IgM antibodies against the NMDA receptor were found (10.8%, 17/158). More than half of the patients (54/89 (60.7%)) were cognitively impaired according to the ICCTF criteria. The affected cognitive domains included attention, executive function, as well as working, verbal, and visuospatial memory. Antibody-positive patients performed significantly worse in tests for verbal fluency (ab- n=68 mean 24.4 words vs ab+ n=13 mean 18.3, U=225.000, Z=-2.796, p=0.005). Other factors associated with impaired cognition included age, level of intelligence, years of education, and abuse of alcohol. Interestingly, chemotherapy was not associated with cognitive impairment.

Conclusion

This study shows that CRCI is common in patients with gastrointestinal cancer and that neuronal autoantibodies may play a major role in impaired executive function in these patients.

Abstrakt

Einleitung

Krebsbedingte kognitive Beeinträchtigungen (CRCI) sind eine häufige Komplikation bei Patient*innen gastrointestinalem Tumor, wobei die Ursachen unbekannt sind. Frühere Studien legen nahe, dass neuronale Autoantikörper mit CRCI in Verbindung stehen. Neuronale Autoantikörper werden sowohl mit Tumoren als auch mit paraneoplastischen neurologischen Syndromen (PNS) assoziiert. Fallstudien über Patient*innen mit gastrointestinalen Tumoren, neuronalen Autoantikörpern und PNS deuten darauf hin, dass neuronale Autoantikörper mit CRCI zusammenhängen können. Die Häufigkeit von neuronalen Autoantikörpern und ihre Auswirkungen auf die Kognition bei Patient*innen mit gastrointestinalen Tumoren wurde bisher nicht systematisch untersucht. Ziel dieser Studie war es, den Zusammenhang zwischen neuronalen Autoantikörpern bei Patient*innen mit gastrointestinalen Tumoren und der kognitiven Funktion zu untersuchen.

Methoden

Serumproben von 158 Probanden mit gastrointestinalem Tumor wurden mit zellbasierten Assays und immunhistochemischen Gewebefärbungen auf neuronale Autoantikörper untersucht. Zudem nahmen 90 Patient*innen an neuropsychologischen Tests zur Bewertung der kognitiven Funktion teil. Folgende Ausschlusskriterien wurden angewandt: Frühere neurologische Erkrankungen, psychiatrische Erkrankungen, ein Alter von über achtzig Jahren, ein eingeschränkter Allgemeinzustand, Sprachfähigkeit ohne fließendes Deutsch und die Ablehnung der Teilnahme. Die kognitive Beeinträchtigung wurde anhand der Kriterien der International Cancer and Cognition Task Force (ICCTF) von Wefel et al. klassifiziert (1). Die kognitive Funktion wurde zwischen Antikörper-positiven und Antikörper-negativen Patient*innen verglichen. Zudem wurden andere klinische Faktoren auf einen Zusammenhang mit der kognitiven Leistung bei Patient*innen mit gastrointestinalem Tumor untersucht.

Ergebnisse

Neuronale Autoantikörper wurden bei 32/158 (20,3%) der Patient*innen mit gastrointestinalem Tumor festgestellt. In erster Linie wurden IgA- und IgM-Antikörper gegen den NMDA-Rezeptor gefunden (10,8%, 17/158). Mehr als die Hälfte der Untersuchten (60,7%, 54/89) waren kognitiv beeinträchtigt. Zu den beeinträchtigten kognitiven Bereichen gehörten die Aufmerksamkeit, die Exekutivfunktion, das Arbeitsgedächtnis, sowie das verbale und visuell-räumliche Gedächtnis. Antikörper-positive Patient*innen schnitten bei dem Test *Verbal Fluency* signifikant schlechter ab (ab- n=68, M 24,4 Wörter vs ab+ n=13, M 18,3 Wörter, U=225,000, Z=-2,796, p=0,005). Ansonsten wurde das Alter, das Intelligenzniveau, die Anzahl der Bildungsjahre und ein Alkoholmissbrauch mit einer beeinträchtigten Kognition assoziiert. Chemotherapie wurde nicht mit kognitiven Beeinträchtigungen assoziiert.

Zusammenfassung

Diese Studie zeigt, dass CRCI bei Patient*innen mit gastrointestinalem Tumor häufig vorkommt und dass neuronale Autoantikörper eine wichtige Rolle bei der Beeinträchtigung der Exekutivfunktion spielen können.

Introduction

Paraneoplastic Neurological Syndromes (PNS) and Neuronal Antibodies

Paraneoplastic neurological syndromes (PNS) describe autoimmune processes in cancer patients leading to neurological symptoms that are not caused by the tumor itself, or by metastasis, infection, ischemia or metabolic disruption (2,3). Being part of the autoimmune process, autoantibodies targeting neuronal structures can be detected in serum samples or cerebrospinal fluid (2). A common hypothesis outlines that these autoantibodies share antigens that are expressed not only by the tumor but also the nervous system (4). There are two types of autoantibodies, targeting either intracellular neuronal antigens (AICAbs) or neuronal surface epitopes (NSAbs) (5). Onconeural autoantibodies (also known as "high risk" or "intermediate risk" antibodies) against intracellular antigens are often associated with an underlying tumor and are often detected preceding tumor diagnosis by up to several months or even years. Among the well-characterized intracellular antigens are the following: Hu, Yo, Ma, Ta, Ri, and Tr. Examples of extracellular neuronal surface structures that are targeted by known autoantibodies are AMPAR, GABAbR, CASPR2, LGI1, and NMDAR. It is of note that an increasing number of NSAbs are being found (5). As part of these discoveries, new diseases such as anti-NMDA receptor encephalitis have been described (6). Here, immunoglobulin G (IgG) NMDAR antibodies have been well characterized as an underlying pathological effect. In contrast, immunoglobulin A (IgA) and immunoglobulin M (IgM) antibodies are associated with slow cognitive impairment and dementia (7,8). While PNS are most often associated with small cell lung cancer (SCLC), other tumor types might also be associated with PNS. Finke et al. found a high prevalence of NSAbs in patients with different types of cancer which were associated with cognitive impairment (9). In a subsequent prospective study by Bartels et al., autoantibodies primarily against neuronal surface proteins were found to be associated with cognitive impairment in patients with melanoma (10). Furthermore, disorders that were associated with neuronal autoantibodies responded well to immunosuppressive therapy and may therefore be treatable (7).

At the same time, cancer-related cognitive impairment (CRCI) is a common complication in cancer patients. However, the reasons as to why exactly patients with cancer also suffer from cognitive impairment are not yet clear. One important cause of cognitive impairment in patients with cancer may be autoantibodies against neuronal structures.

Gastrointestinal Cancer, Cognitive Impairment and PNS

Gastrointestinal Cancer and Cognitive Impairment

Cancer-related cognitive impairment (CRCI) is a common condition in cancer patients (11). The first studies on this topic dealt with breast cancer patients being treated with chemotherapy (12). However, CRCI affects patients with various cancer types, including patients with gastrointestinal cancer (11). Importantly, CRCI also affects patients independent of chemotherapy (13).

There has been extensive research concerning patients with gastrointestinal cancer suffering from cognitive impairment. For instance, van Deudekom et al. investigated patients with esophageal cancer and identified nineteen articles reporting cognitive impairment being associated with adverse health outcomes (14). However, they did not report the prevalence or type of cognitive impairment. Furthermore, Visovatti et al. found patients with colorectal cancer to be vulnerable to cognitive dysfunction (15).

Chemotherapy is arguably one of the reasons for cognitive impairment. Sales et al. found patients with colorectal cancer to perform worse in executive function tasks after they had received chemotherapy (16). Moreover, Cruzado et al. observed that patients with colon cancer performed worse in tasks concerning verbal memory after they had received chemotherapy (17). Dhillon et al. outlined that patients with colorectal cancer reported more cognitive symptoms after they had received chemotherapy (18). However, they did not find an association between perceived cognitive impairment and the results of neuropsychological testing (18). Furthermore, chemotherapy might also have long term effects, such as increasing the risk of dementia in patients with colorectal cancer, as outlined by Du et al. (19).

Vardy et al. conducted a large prospective study and found more cognitive impairment in patients with colorectal cancer who had not received any cancer treatment compared to a healthy control group (20,21). Nonetheless, they did not find an association between chemotherapy and cognitive impairment, but outline that the concrete mechanisms and reasons for cognitive impairment in patients with colorectal cancer remain largely unknown (20). Ahles and Saykin further state that the mechanism of CRCI is not yet fully understood (22).

As a result, the specific reasons are still subject to debate and research. Dwek et al., for instance, are conducting a study to research the reason for worse cognitive functioning in patients with colorectal cancer (23). They are looking at chemotherapy, but also the disease itself, and other factors. Visovatti et al. state that other factors such as age and lower levels of education may further increase the likelihood of worse cognitive performance in patients with colorectal cancer (15). Regier et al. outline that cognitive impairment in patients with oral-digestive cancer is more often found in older patients than in younger ones (24).

One possible mechanism for cognitive impairment in patients with gastrointestinal cancer might involve autoantibodies against neuronal structures. These autoantibodies are associated with paraneoplastic syndromes. The following will elaborate on the current state of research concerning paraneoplastic neurological syndromes in patients with gastrointestinal cancer.

Gastrointestinal Cancer and PNS

In their retrospective study, Finke et al. found a high prevalence of anti-neuronal antibodies in different types of tumors which were associated with cognitive deficits. This study suggests potential cognitive paraneoplastic syndromes in patients with neuronal autoantibodies. The study also examined 21 samples from patients with gastrointestinal cancer out of which more than 50% (11) proved to be antibody-positive (9). Furthermore, Linnoila et al. identified gastrointestinal cancer in five of seven (71%) cases of patients with antibodies of purkinje cell cytoplasmic antibody type I (PCA-1-IgG), also known as anti-Yo (25). Here, antibodies might be predictive of gastrointestinal cancer.

Moreover, several case studies of patients with gastrointestinal cancer and paraneoplastic syndromes including GABA-B-Receptor encephalitis, limbic encephalitis, paraneoplastic myelopathy, Guillain-Barré syndrome, anti-Hu syndrome, and opsoclonus myoclonus syndrome have been published:

Table 1: Case Studies of Paraneoplastic Syndromes in Patients with GI Cancer

Tumour Type	Paraneoplastic Neurological Syndrome	Study/Authors
esophageal cancer	GABA-B-receptor encephalitis with the symptoms of acute vertigo, nausea, vomiting, facial palsy, dysarthria, dysphagia, ataxia, and respiratory failure	Mundiyanapurath et al. (26)
esophageal cancer	limbic encephalitis	Menezes et al. (27)
esophageal cancer	first autopsied case of a patient with paraneoplastic myelopathy	Urai et al. (28)
esophageal cancer	Guillain-Barré syndrome	Zilli and Allal (29)
esophageal small cell carcinoma	paraneoplastic encephalomyelitis caused by an anti-Hu syndrome	Shirafuji (30)
squamous cell carcinoma of the esophagus	opsoclonus myoclonus syndrome	Rossor et al. (31)
esophagogastric squamous cell carcinoma	limbic encephalitis	Mc Cormack et al. (32)
adenocarcinoma of the gastro-esophageal junction	limbic encephalitis	Pathak et al. (33)
gastric cancer	opsoclonus, limbic encephalitis, and anti-Ma2 antibodies	Biotti et al. (34)
gastric cancer	paraneoplastic encephalomyelitis	Storstein et al. (35)
gastric cancer	limbic encephalitis	Uneno et al. (36)
gastric cancer	paraneoplastic neurological syndrome	Murakami et al. (37)
gastric adenocarcinoma	anti-GABA-B receptor encephalitis	Jia et al. (38)
gastric adenocarcinoma and a skin cancer relapse	Guillain-Barré syndrome	Colantuoni et al. (39)

colorectal adenocarcinoma	distal acquired demyelinating symmetric neuropathy	Ayyappan et al. (40)
colon adenocarcinoma with liver metastases	sensory neuropathy and limbic encephalitis	Sio et al. (41)

Overall, there tend to be fewer case studies reporting paraneoplastic syndromes in patients with colorectal carcinoma compared to patients with esophageal or gastric carcinoma. Nevertheless, the overall number of case studies of patients with gastrointestinal cancer and paraneoplastic syndromes suggests a high clinical relevance. However, there is no systematic assessment of neuronal antibody prevalence in this patient group. Moreover, the mechanisms of CRCI in patients with gastrointestinal cancer are not yet fully understood. One potential underlying mechanism of CRCI might be neuronal antibodies. Yet, no studies have systematically investigated the effects of the neuronal antibodies on cognition in patients with gastrointestinal cancer. Therefore, the aim of this study is to investigate the following research questions.

Questions and Hypotheses

1.) What is the seroprevalence of neuronal antibodies in patients with gastrointestinal tumors?

2.) What types of antibodies can be identified?

3.) What are their associated cognitive and neurological dysfunctions?

4.) What other factors may be associated with cognitive performance?

It is hypothesized that (1) neuronal autoantibodies are found in a substantial proportion of patients with gastrointestinal cancer. Furthermore, (2) it is hypothesized that the autoantibodies are directed against neuronal intracellular und extracellular structures; that (3) antibodies are associated with worse cognitive and neurological function of patients with gastrointestinal cancer; and (4) that other factors such as demographic factors, mental and physical health, medical history, treatment factors or tumor stage may influence cognition.

Therefore, the blood serum of a large group of patients with gastrointestinal cancer are tested for the seroprevalence and type of neuronal antibodies. After exclusion of patients due to prior neurological diseases, psychiatric diseases, and other factors (such as high age, self-reported reduced general condition, insufficient German language fluency, and declined participation), a subset of patients participate in a set of neuropsychological tests and are neurologically examined. The results are then analyzed for group differences between antibody-positive and antibody-negative patients to investigate the associations between antibodies and cognitive as well as neurological disorders. Moreover, clinical data on the patients is collected to study other factors which may have an impact on cognition.

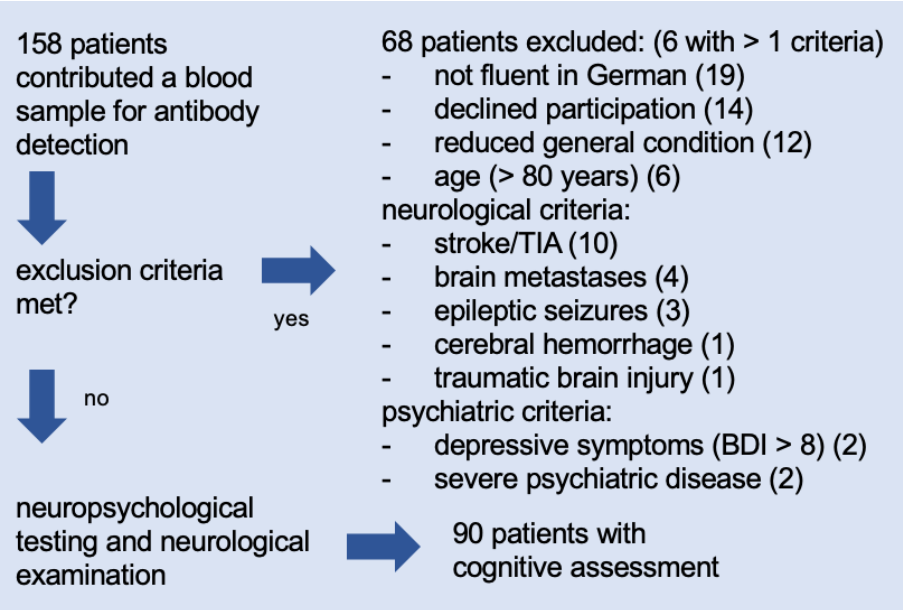
Materials and Methods

Recruitment of Patients

In total, 158 patients with gastrointestinal cancer were recruited at the Department of Oncology at the Charité (Campus Virchow and Mitte) in Berlin, Germany. Patients with gastrointestinal cancer of the upper and lower tract (esophageal cancer, carcinoma of the gastroesophageal junction, gastric cancer and colorectal carcinoma) were included, with no prior determined size of each subgroup. Out of the 158 patients, 58 patients (37%) had gastric adenocarcinoma, 36 (23%) adenocarcinoma of the gastroesophageal junction, 31 (20%) colorectal adenocarcinoma, 30 (19%) esophageal cancer, and 3 patients (2%) had another gastrointestinal cancer, such as a gastric stump carcinoma or a Krukenberg carcinoma. The median age of the patients was 66 years, with a range between 25 to 87 years. Out of the 158 patients, 48 (30%) were female.

Blood samples were taken from all patients. Furthermore, if no exclusion criteria were met, neuropsychological testing and a neurological examination were carried out. Exclusion criteria entailed severely reduced general condition (indicated by treating physicians), insufficient German language fluency, declined participation, and prior neurological or psychological impairment. For further details, please refer to the following chart or a more detailed description further below:

Figure 1: Flowchart of Patients



The recruitment of patients, including collecting the blood samples and completing the neuropsychological testing, was done during an internship. As part of the doctoral thesis, the patients' medical records were then reviewed to obtain clinical data, including information on tumor stage, treatment, and medical history. Additionally, all data was combined in a clinical database. All patients gave written informed consent. The study was approved by the Ethics Committee of the Charité.

Antibody Detection

Serum samples from all 158 patients were tested for neuronal autoantibodies. Antibody analysis was blinded to clinical and neuropsychological patient information. The testing was carried out in collaboration with the Institute of Experimental Immunology (Euroimmun AG) in Lübeck, Germany. To this end, indirect immunofluorescence with neuronal tissue and cell-based assays were used.

Indirect immunofluorescence was applied using biochip mosaics. A biochip mosaic consists of incubation fields with a mosaic of different substrates (42). Here, the biochip mosaics contained brain tissue from a rat (hippocampus and cerebellum), a monkey (cerebellum), and cell-based assays with plasmid transfected HEK293 cells. Human embryonic kidney (HEK) cells are cells which have been transfected with DNA of an adenovirus (43). HEK293 cells produce proteins from plasmid vectors. Here, the HEK293 cells expressed the following neuronal antigens:

Table 2: Neuronal Antigens Expressed by HEK293 Cells

Antigen	Further Description
AMPA1/2	α-amino-3-hydroxy- 5-methyl-4-isoxazolepropionic acid
Amphiphysin	
AQP4	aquaporin-4
ARHGAP26	rho GTPase activating protein 26
CARPVIII	carbonic anhydrase-related protein VIII
CASPR2	contactin-associated protein-2
CV2	
DNER	delta and notch-like epidermal growth factor-related receptor
DPPX	dipeptidyl-peptidase-like protein-6

GABA-a (GABARA1+GABARB3)	binding gamma-aminobutyric acid receptor a
GABA-b (GABAR-B1/B2)	binding gamma-aminobutyric acid receptor b
GAD65/67	glutamic acid decarboxylase isoforms with 65 kDa and 67kDa
GRM1/5	glutamate receptor, metabotropic
pre-GLRA1b	glycine receptor subunit alpha-1
Homer3	homer protein homolog 3
Hu	antibody: antineuronal nuclear antibody type 1 ANNA-1
IgLON5	IgLON family member 5
ITPR1	inositol 1,4,5-trisphosphate receptor type 1
LGI1	leucine-rich, glioma inactivated 1
Ma2	synonym: Ta
MOG	myelin oligodendrocyte glycoprotein
NMDAR-NR1a/NR1a	*n*-methyl-d-aspartic acid
Recoverin	
Ri	
Yo	antibody: purkinje-cell-autoantibody PCA-1
ZIC4	zic family member 4

First, the biochip mosaics were incubated with the patient serum, then washed, and then incubated with secondary fluorescein-labeled antibodies (using anti-IgG, and additionally anti-IgM and anti-IgA for NMDAR). Lastly, fluorescence microscopy was used to evaluate binding of the fluorescein-labeled antibodies to the cell-based assays or tissue. Additionally, to confirm intracellular antigens, immunoblot assays were used for the antigens Amphiphysin, CV2, Ma2, Ri, Yo, Hu, Recoverin, SOX1, Zic4, GAD65, DNER. Here, antigens on a membrane help to detect antibodies as they bind to the antigens (44). Additionally, a second antibody labelled with alkaline phosphatase is added and binds to the first antibody (44). A color reaction between the alkaline phosphatase and added nitro blue tetrazolium chloride/5-bromo-4-chloro-3-indolyl phosphatase indicates the antigen position (44). Please note that the methods used here were also previously described by Bartels et al. (10).

Neuropsychological Assessment

After exclusion criteria were applied, 90 patients participated in neuropsychological testing to assess their cognitive function. Test data was incomplete in 4 patients due to

refusal to participate in all tests. Assessment of cognitive performance was blinded to the antibody results.

To minimize potential confounder variables regarding neuropsychological function, the following exclusion criteria were applied: age over 80 years (6 patients), inability to participate due to severely reduced general condition as stated by treating physicians (12 patients), insufficient German language fluency (19 patients), or declined participation in neuropsychological testing (14 patients). Neurological exclusion criteria entailed stroke/TIA (10 patients), cerebral hemorrhage (1 patient), brain metastases (4 patients), traumatic brain injury (1 patient), or epileptic seizures (3 patients). Finally, psychiatric exclusion criteria included depressive symptoms measured with the Beck Depression Inventory (BDI) questionnaire (score > 8) (2 patients), paranoid psychosis (1 patient), and schizoaffective disorder (1 patient).

The following tests were applied to examine 6 cognitive domains:

1) For verbal memory, the Verbal Learning and Memory Test (VLMT) test was carried out. The VLMT is a standardized test where patients listen to and restate words for multiple rounds (45). Furthermore, recall after distraction, delayed recall and recognition are tested.

2) Visuospatial memory was tested with the Rey–Osterrieth Complex Figure (ROCF) test. This test consists of a figure with geometric elements, whereby patients are asked to draw the figure first with a template, then without the template, and a last time after a break (46).

3) For working memory, the Digit Span Forward and Backward test was used. As part of the Wechsler Adult Intelligence Scale, it is a very common test where the number of digits patients are able to restate (as heard and in reverse order) is examined (47).

4) For fluid intelligence, the subtest 3 of the Leistungsprüfungssystem (LPS) test was used. Here, inferential thinking is tested using geometric figures with irregularities (48).

5) Examining attention, the Testbatterie für Aufmerksamkeit (TAP) Alertness and Divided Attention test was used. In the alertness test, the reaction time after a stimulus is measured. In the divided attention test, patients completed dual tasks, paying attention to two stimuli at the same time (49).

6) For executive functions, a different subset of TAP tests was used. Patients are asked to observe stimuli which then require either a reaction or a suppression of a reaction (TAP test Go/NoGo), name the color of letters where the word itself has the meaning of a different color (Stroop test), and state as many words belonging to a group (e.g. animals) as the patient can think of in a specific timeframe (Regensburger Verbal Fluency test) (49–51).

For each test, a reference group with healthy participants was established. Additionally, patients participated in the Mehrfachwahl-Wortschatz-Intelligenztest-A (MWT-A) test for crystallized intelligence (52). Furthermore, patients filled out the (Short-Form-12) SF-12 questionnaire to obtain information about the physical and mental health status, the FACIT questionnaire in order to assess the fatigue status, and the Beck Depression Inventory-Fast Screen (BDI-FS) questionnaire to assess symptoms of depression (53–55). Patients were asked to rate their Eastern Cooperative Oncology Group score (ECOG) (56).

Neurological Examination

Finally, a full physical neurological examination was carried out on all patients who had also participated in the neuropsychological testing (n=90). This included the examination of the cranial nerves, motor function, sensory function, and coordination and gait.

Statistical Analysis

As part of the doctoral thesis, descriptive analysis including assessment of frequency, distribution, and prevalence was performed for demographic, antibody, and neuropsychological data. Group differences of categorical data were analyzed using the Chi-square or Fisher's exact tests. For continuous data, a Mann-Whitney test was applied.

Assessing the hypotheses, and therefore the relationship of antibody prevalence and cognitive impairment, it was first assessed if the criteria of a cognitive deficit was fulfilled. Cognitive deficits were defined using the International Cognition and Cancer Task Force

(ICCTF) criteria by Wefel et al. (1). Three different definitions of cognitive deficits were used in this study:

1.) Cognitive impairment was defined by a test score 1.5 standard deviations (SD) below the reference control group of healthy participants in at least two tests.

2.) Additionally, a second less sensitive definition was established where the test scores had to be from different domains. In the second definition, cognitive impairment was defined by a test score 1.5 SD below the reference control group in at least two tests from different cognitive domains. Assessing each domain separately, a cognitive deficit in one domain was defined by a test score being at least 1.5 SD below the reference group in at least one test.

3.) Cognitive impairment was defined by a test score 2 SD below the reference control group in at least one test.

Group differences between antibody-positive and antibody-negative patients as well as the cognitive deficits were then analyzed using the Chi-square/ Fisher's exact tests.

Please note that antibody-positive patients were defined as patients with an antibody detected using the cell-based assay. Further differentiation was made between neuronal surface antibodies (NSAbs) and antibodies against intracellular antigens (AICAbs.)
Patients with staining in only one of the three used brain tissues (hippocampus from a rat, cerebellum from a rat, cerebellum from a monkey) or another positive tissue staining (which was not assignable to the brain tissues on the mosaic chip, such as Golgi 3200/100, Myelin 1000 etc.) were not considered antibody-positive. Nevertheless, they were also excluded from the antibody-negative group when analyzing for group differences to ensure a true negative control group excluding antibodies as well as tissue staining.

Using the neuropsychological test scores, group differences between antibody-positive and antibody-negative patients were analyzed using a Mann-Whitney test. Nonparametric tests were used due to the data not being normally distributed. In addition, to compare different cognitive performances using tests with different scales and directions, the test scores were transformed to z-scores using the mean and standard deviation (SD) of the antibody-negative patients as a reference. A composite cognitive

score (being the mean of the z-scores) was computed for all cognitive domains and the overall cognitive performance.

Additionally, other factors potentially associated with cognitive impairment were analyzed. These included demographic factors (such as age, sex, IQ, and years of education), the mental and physical health status (using the FACIT, ECOG, BDI, and SF12 status), medical history (such as former abuse of alcohol and sedating medication), tumor therapy (treatment-naive, chemotherapy, antibody therapy, radiotherapy or surgery of the primary tumor), and tumor stage (no evidence of disease (NED) or metastasis). Group differences were analyzed using the Chi-square or Fisher's exact tests for categorical data, the Mann-Whitney test for continuous outcome test scores with a categorical predictor variable, or the Spearman correlation for continuous outcome variables with continuous predictor variables.

Generally, all tests were two-sided and p-values ≤ 0.05 were considered significant. Considering the fact that each individual hypothesis test was of interest in the present explorative study, no adjustment for multiple testing was applied. The software SPSS by IBM was used, Version 25 released in 2017 in Armonk, NY. Tables were created using EXCEL by Microsoft, Version 16, and graphs using the software PRISM by GraphPad, Version 8 or R by the R Core Team, and Version 3.6 for the forest plot using the z-scores.

Results

Descriptive Analysis

Demographics

Demographic data will be displayed for the total cohort of 158 patients [and in square brackets for the subgroup of 90 patients who participated in neuropsychological testing and received cognitive assessment].
Regarding tumor subtypes, 58/158 patients (37%) [34/90 (38%)] had gastric adenocarcinoma, 36/158 (23%) [21/90 (23%)] adenocarcinoma of the gastroesophageal junction, 31/158 (20%) [18/90 (20%)] colorectal adenocarcinoma, 30/158 (19%) [15/90 (17%)] esophageal cancer, and 3/158 patients (2%) had another gastrointestinal cancer, such as a gastric stump carcinoma or a Krukenberg carcinoma. A total of 48/158 (30%) [32/90 (36%)] were female. Current evidence of tumor disease was present in 135/158 (85%) [79/90 (88%)]. Eighty-eight (56%) [51/90 (57%)] had metastases to distant sites, including peritoneal, liver, lung, bones, ovary, brain, pancreas, intestine, pleura, adrenal gland, bone marrow, gallbladder, and testicle metastases. Thirty-three (21%) [20/90 (22%)] were treatment-naive, while the remaining had received the following therapies: 114/158 (72%) [66/90 (73%)] were treated with chemotherapy; 33/158 (21%) [16/90 (18%)] were treated with radiotherapy; 73/158 (46%) [39/90 (43%)] underwent surgery of the primary tumor; and 47/158 (30%) [29/90 (32%)] received antibody therapy, including Bevacizumab, Cetuximab, Pantitumumab, Pembrolizumab, Pertuzumab, Ramucirumab, and Trastuzumab.

As expected, patients who were excluded from cognitive assessment were older (mean 67.7 years for excluded patients versus 61.2 years for patients with cognitive assessment) and more often had a neurological disease (32.4% of excluded patients versus 11.1% of patients with cognitive assessment). Prevalence of esophageal adenocarcinoma was lower in the subgroup of patients with cognitive assessment (2.2% of patients with cognitive assessment versus 10.3% of patients excluded from cognitive assessment). Further demographic information is provided in Table 9.

Antibodies and Tissue Staining

Out of all 158 patients, 32 patients (20.3%) showed an antibody-positive result. In the 90 patients with neuropsychological testing, neuronal antibodies were detected in 13 patients (14.4%). Most antibodies (19/158 (12%) in the total cohort and 9/90 (10%) in the subgroup with cognitive assessment) were directed against neuronal surface antigens (NSAbs) - mainly against the NMDA receptor of the IgM/IgA isotype. Less frequently, NSAbs included antibodies against the NMDA receptor of IgG isotype, and antibodies against MOG, or GlyR. Antibodies against intracellular antigens (AICAbs) were found in 14 of 158 (8.9%) of patients or in 4 of 90 (4.4%) patients with cognitive assessment, which were mainly targeted against ARHGAP26, Ma2(Ta), and Yo. Less frequently observed AICAbs included the ones targeting CARPVIII and SOX1.

Table 3: Antibody-Positive Patients

	All patients (*n*=158)		Patients with neuropsychological testing (*n*=90)	
	No.*	Percent*	No.*	Percent*
Antibody-positive	32	20.3	13	14.4
One antibody only	31	19.6	13	14.4
Combination of two antibodies†	1	0.6	0	0.0
Surface antigen(s)	19	12.0	9	10.0
NMDAR	17	10.8	8	8.9
NMDAR IgM	8	5.1	4	4.4
NMDAR IgA†	8	5.1	3	3.3
NMDAR IgG	1	0.6	1	1.1
MOG	1	0.6	0	0.0
GlyR	1	0.6	1	1.1
Intracellular antigen(s)	14	8.9	4	4.4
ARHGAP26†	6	3.8	1	1.1
Ma2 (Ta)	3	1.9	1	1.1
Yo	3	1.9	0	0.0
CARPVIII	1	0.6	1	1.1
SOX1	1	0.6	1	1.1

*IgM, immunoglobulin M; IgA, immunoglobulin A; IgG, immunoglobulin G; *Numbers do not add up due to combination of two antibodies. †Combinations of two antibodies: ARHGAP26+NMDAR IgA (*n=*1).*

Antibody prevalence in the subgroup with cognitive assessment was significantly lower compared to the subgroup excluded from cognitive assessment: whereas 19 out of 68 patients (27.9%) without neuropsychological testing were found to be antibody-positive, only 13 out of 90 patients (14.4%) with cognitive assessment were found to be antibody-positive (χ^2 (1) = 4.47, p=0.035, φ = 0.18). Similarly, the prevalence of AICAbs in the subgroup with cognitive assessment was significantly lower: whereas 10 out of 68 patients (14.7%) without neuropsychological testing were found to be positive, only 4 out of 90 patients (4.4%) had an AICAbs (χ^2 (1) = 5.53, p=0.018, φ = 0.21) (see Table 9).

The reasons for the antibody-positive patients being excluded from neuropsychological testing were age over 80 years (n=2), self-reported reduced general condition (n=2), insufficient German language fluency (n=9), declined participation (n=1), and neurological diseases (brain metastases (n=1), traumatic brain injury (n=1), history of stroke (n=2), or TIA (n=3)). One patient fulfilled multiple exclusion criteria, including insufficient German language fluency, age over 80 years, and a history of TIA.

Comparing antibody prevalence between different tumor entities, patients with esophageal cancer had a lower antibody prevalence (6.7% of patients with esophageal cancer were antibody positive vs 26.3% of patients with other tumors; p=0.021) (Table 7). Next, the association of sex with antibody prevalence was analyzed. Here, male patients were more often antibody-positive compared to female patients (26.7% of male patients vs 11.6% of female patients; p=0.046) (Table 7).

Table 4: Demographics of all Patients (n=158) and Comparison of Antibody-Negative vs Antibody-Positive Patients

	All *(%)* (*n*=158)†	Ab- *(%)* (*n*=112)†	Ab+ *(%)* (*n*=32)†	*p*
Age				
Mean ± SD (years)	64 ± 12	63.7 ± 11.8	65.2 ± 13.3	.39U
Sex				
Male	110 *(69.6)*	74 *(66.0)*	27 *(84.4)*	**.046**X
Gastrointestinal cancer subtype				
Esophageal cancer	30 *(19.0)*	28 *(25.0)*	2 *(6.3)*	**.021**X
Adenocarcinoma	9 *(5.7)*	9 *(8.0)*	0 *(0.0)*	.21‡
Squamous epithelial carcinoma	21 *(13.3)*	19 *(17.0)*	2 *(6.3)*	.16‡
Adenocarcinoma of the gastroesophageal transition	36 *(22.8)*	28 *(25.0)*	6 *(18.8)*	.46X
Gastric adenocarcinoma	58 *(36.7)*	35 *(31.3)*	15 *(46.9)*	.10X
Gastric stump adenocarcinoma	2 *(1.3)*	1 *(0.9)*	0 *(0.0)*	/
Krukenberg adenocarcinoma	1 *(0.6)*	1 *(0.9)*	0 *(0.0)*	/
Colorectal adenocarcinoma	31 *(19.6)*	19 *(17.0)*	9 *(28.1)*	.16X
Clinical stage				
Current tumor disease	135 *(85.4)*	95 *(84.8)*	26 *(81.3)*	.63X
Metastasized	88 *(55.7)*	58 *(51.8)*	19 *(59.4)*	.45X
Current recurrence (relapse)	29 *(18.4)*	19 *(17.0)*	5 *(15.6)*	.86X
Local	6 *(3.8)*	4 *(3.6)*	1 *(3.1)*	1.0‡
Distant	23 *(14.6)*	15 *(13.4)*	4 *(12.5)*	1.0‡
NED - Currently in aftercare	23 *(14.6)*	17 *(15.2)*	6 *(18.8)*	.63X
Site of metastases	**n*=156	**n*=111	**n*=31	
Peritoneal	38 *(24.4)*	24 *(21.6)*	7 *(22.6)*	.91X
Liver	38 *(24.4)*	23 *(20.7)*	9 *(29.0)*	.33X
Lung	19 *(12.2)*	12 *(10.8)*	6 *(19.4)*	.23‡
Bones	9 *(5.8)*	6 *(5.4)*	2 *(6.5)*	1.0‡

Ovary	4 *(2.6)*	3 *(2.7)*	1 *(3.2)*	1.0‡
Brain	4 *(2.6)*	3 *(2.7)*	1 *(3.2)*	1.0‡
Pancreas	3 *(1.9)*	1 *(0.9)*	1 *(3.2)*	.39‡
Intestine	3 *(1.9)*	2 *(1.8)*	0 *(0.0)*	1.0‡
Pleura	2 *(1.3)*	2 *(1.8)*	0 *(0.0)*	/
Adrenal gland	1 *(0.6)*	1 *(0.9)*	1 *(3.2)*	/
Bone marrow	1 *(0.6)*	1 *(0.9)*	0 *(0.0)*	/
Gallbladder	1 *(0.6)*	0 *(0.0)*	0 *(0.0)*	/
Testicle	1 *(0.6)*	0 *(0.0)*	0 *(0.0)*	/
Treatment				
Treatment-naive	33 *(20.9)*	25 *(22.3)*	6 *(18.8)*	.67χ
Chemotherapy (prior and/or current)	114 *(72.2)*	80 *(71.4)*	23 *(71.9)*	.96χ
Current chemotherapy	**n*=157 58 *(36.9)*	**n*=111 39 *(35.1)*	12 *(37.5)*	.81χ
Radiotherapy (prior and/or current)	33 *(20.9)*	26 *(23.2)*	7 *(21.9)*	.87χ
Local radiation (primary tumor)	31 *(19.6)*	24 *(21.4)*	7 *(21.9)*	96χ
Current local radiation	7 *(4.4)*	7 *(16.3)*	0 *(0.0)*	.35‡
Brain radiation	2 *(1.3)*	2 *(1.8)*	0 *(0.0)*	/
Current brain radiation	2 *(1.3)*	2 *(1.8)*	0 *(0.0)*	/
Surgery of primary tumor	73 *(46.2)*	45 *(40.2)*	20 *(62.5)*	**.025**χ
Surgery of metastases	20 *(12.7)*	14 *(12.5)*	5 *(15.6)*	.77‡
Antibody therapy	47 *(29.8)*	29 *(25.9)*	13 *(40.6)*	.11χ
Current antibody therapy	**n*=154 24 *(15.6)*	**n*=109 14 *(12.8)*	**n*=31 5 *(16.1)*	.77‡
Other targeted therapy	1 *(0.6)*	0 *(0.0)*	1 *(3.1)*	/
Other current/prior malignancy in history				
Yes	22 *(13.9)*	15 *(13.4)*	5 *(15.6)*	.77‡
Simultaneous second carcinoma	3 *(1.9)*	3 *(2.7)*	0 *(0.0)*	1.0‡
Lung cancer	2 *(1.3)*	2 *(1.8)*	0 *(0.0)*	/
Urothelial carcinoma	1 *(0.6)*	1 *(0.9)*	0 *(0.0)*	/

Prior malignancy in history	21 *(13.3)*	14 *(12.5)*	5 *(15.6)*	.77‡
Medical history				
Neurological disease§	32 *(20.3)*	18 *(16.1)*	10 *(31.3)*	.056χ
Psychiatric disease#	10 *(6.3)*	6 *(5.4)*	1 *(3.1)*	1.0‡
Alcohol abuse in history	**n*=115 9 *(7.8)*	**n*=84 8 *(9.5)*	**n*=20 1 *(5.0)*	1.0‡

*χ: Chi-square test; ‡: Fisher's exact test; U: Mann-Whitney U test; /: No significance test conducted; †: Numbers do not add up because: tissue- or other staining-positive patients were excluded from antibody-negative group. *: If data is missing, the n of patients with known data is displayed. Percentages are computed for the known data. § includes: peripheral neuropathy (10 patients), stroke (8 - out of which 1 is hemorrhagic), s/p TIA (4), spinal stenosis with disc prolapse (3), peripheral nerve affection (2), peripheral nerve paresis (2), epilepsy (2), suspected epilepsy (1), absence-epilepsy during childhood (1), s/p acoustic neuroma (1), migraine (1), Parkinson's suspected (1), restless leg syndrome (1), s/p traumatic brain injury (1); # includes: depression (5), burnout syndrome (3), anxiety disorder (2), schizoaffective disorder (1), paranoid psychosis (1).*

Table 5: Demographics of Patients Included in Neuropsychological Testing (n=90) and Comparison of Antibody-Negative vs Antibody-Positive Patients

	All *(%)* (*n*=90)†	Ab- *(%)* (*n*=69)†	Ab+ *(%)* (*n*=13)†	*p*	Intracellular Ab+ *(%)* (*n*=4)†	*p*
Age						
Mean ± SD (years)	61.2 ± 11.7	62 ± 11.3	58.2 ± 14.6	.40U	67.5 ± 15.0	.23U
Range	28-80	31-80	28-76		45-76	
Sex						
Male	58 *(64.4)*	42 *(60.9)*	11 *(84.6)*	.12‡	4 *(100.0)*	.29‡
Gastrointestinal cancer subtype						
Esophageal cancer	15 *(16.7)*	14 *(20.3)*	1 *(7.7)*	.45‡	1 *(25.0)*	1.0‡
Adenocarcinoma	2 *(2.2)*	2 *(2.9)*	0 *(0.0)*	/	0 *(0.0)*	/
Squamous epithelial carcinoma	13 *(14.4)*	12 *(17.4)*	1 *(7.7)*	.68‡	1 *(25.0)*	.55‡
Adenocarcinoma of the gastroesophageal junction	21 *(23.3)*	17 *(24.6)*	3 *(23.1)*	1.0‡	1 *(25.0)*	1.0‡
Gastric adenocarcinoma	34 *(37.8)*	25 *(36.2)*	4 *(30.8)*	1.0‡	1 *(25.0)*	1.0‡
Gastric stump adenocarcinoma	1 *(1.1)*	0 *(0.0)*	0 *(0.0)*	/	0 *(0.0)*	/
Krukenberg adenocarcinoma	1 *(1.1)*	1 *(0.0)*	0 *(0.0)*	/	0 *(0.0)*	/
Colorectal adenocarcinoma	18 *(20.0)*	12 *(17.4)*	5 *(38.5)*	.13‡	1 *(25.0)*	.55‡

Clinical stage						
Current tumor disease	79 *(87.8)*	59 *(85.5)*	12 *(92.3)*	1.0‡	3 *(75.0)*	.49‡
Metastasized	51 *(56.7)*	35 *(50.7)*	10 *(76.9)*	.08ˣ	1 *(25.0)*	.62‡
Current recurrence (relapse)	18 *(20.0)*	11 *(15.9)*	3 *(23.1)*	.69‡	1 *(25.0)*	.52‡
Local	2 *(2.2)*	1 *(1.5)*	1 *(7.7)*	/	1 *(25.0)*	/
Distant	16 *(17.8)*	10 *(14.5)*	2 *(15.4)*	1.0‡	0 *(0.0)*	1.0‡
NED - Currently in aftercare	11 *(12.2)*	10 *(14.5)*	1 *(7.7)*	1.0‡	1 *(25.0)*	.49‡
Site of metastases						
Peritoneal	23 *(25.6)*	15 *(21.7)*	4*(30.8)*	.49‡	0 *(0.0)*	.58‡
Liver	22 *(24.4)*	14 *(20.3)*	5 *(38.5)*	.17‡	1 *(25.0)*	1.0‡
Lung	12 *(13.3)*	9 *(13.0)*	2 *(15.4)*	1.0‡	0 *(0.0)*	1.0‡
Bones	5 *(5.6)*	3 *(4.4)*	1 *(7.7)*	.51‡	0 *(0.0)*	1.0‡
Ovary	3 *(3.3)*	2 *(2.9)*	1 *(7.7)*	.41‡	0 *(0.0)*	1.0‡
Adrenal gland	1 *(1.1)*	0 *(0.0)*	1 *(7.7)*	/	0 *(0.0)*	/
Bone marrow	1 *(1.1)*	1 *(1.5)*	0 *(0.0)*	/	0 *(0.0)*	/
Intestine	1 *(1.1)*	1 *(1.5)*	0 *(0.0)*	/	0 *(0.0)*	/
Pancreas	1 *(1.1)*	1 *(1.5)*	0 *(0.0)*	/	0 *(0.0)*	/
Testicle	1 *(1.1)*	0 *(0.0)*	0 *(0.0)*	/	0 *(0.0)*	/
Treatment						
Treatment-naive	20 *(22.2)*	16 *(23.2)*	2 *(15.4)*	.72‡	1 *(25.0)*	1.0‡
Chemotherapy (prior and/or current)	66 *(73.3)*	49 *(71.0)*	11 *(84.6)*	.50‡	3 *(75.0)*	1.0‡
Current chemotherapy	**n*=89 37 *(41.6)*	**n*=69 27*(39.1)*	6 *(46.2)*	.67ˣ	1 *(25.0)*	1.0‡
Radiotherapy (prior and/or current)	16 *(17.8)*	12 *(17.4)*	4 *(30.8)*	.27‡	0 *(0.0)*	.16‡
Local radiation (primary tumor)	16 *(17.8)*	12 *(17.4)*	4 *(30.8)*	.27‡	2 (50.0)	.16‡
Current local radiation	4 *(4.4)*	4 *(5.8)*	0 *(0.0)*	1.0‡	0 *(0.0)*	1.0‡
Surgery of primary tumor	39 *(43.3)*	26 *(37.7)*	8 *(61.5)*	.11ˣ	3 *(75.0)*	.29‡
Surgery of metastases	14 *(15.6)*	9 *(13.0)*	4 *(30.8)*	.21‡	1 *(25.0)*	.45‡
Antibody therapy	29 *(32.2)*	19 *(27.5)*	7 *(53.9)*	.10‡	2 *(50.0)*	.57‡
Current antibody therapy	**n*=88 17 *(19.3)*	**n*=68 11 *(16.2)*	**n*=12 3 *(25.0)*	.43‡	1 *(25.0)*	.53‡

Other targeted therapy	1 *(1.1)*	0 *(0.0)*	1 *(7.7)*	/	0 *(0.0)*	/
Other current/prior malignancy in history						
Yes	9 *(10.0)*	9 *(13.0)*	0 *(0.0)*	.34‡	0 *(0.0)*	1.0‡
Simultaneous second carcinoma	3 *(3.3)*	3 *(4.4)*	0 *(0.0)*	1.0‡	0 *(0.0)*	1.0‡
Lung cancer	2 *(2.2)*	2 *(2.9)*	0 *(0.0)*	/	0 *(0.0)*	/
Urothelial carcinoma	1 *(1.1)*	1 *(1.5)*	0 *(0.0)*	/	0 *(0.0)*	/
Prior malignancy in history	8 *(8.9)*	8 *(11.6)*	0 *(0.0)*	.34‡	0 *(0.0)*	1.0‡
Medical history						
Neurological disease§	10 *(11.1)*	7 *(10.1)*	1 *(7.7)*	1.0‡	1 *(25.0)*	.39‡
Psychiatric disease#	5 *(5.6)*	2 *(2.9)*	1 *(7.7)*	.41‡	0 *(0.0)*	1.0‡
Alcohol abuse in history	6 *(6.7)*	5 *(7.3)*	1 *(7.7)*	1.0‡	0 *(0.0)*	1.0‡
Sedating medication at time of examination						
Yes	25 *(27.8)*	20 *(29.0)*	5 *(38.5)*	.52‡	1 *(25.0)*	1.0‡
Opioid analgesics	15 *(16.7)*	13 *(18.8)*	2 *(15.4)*	1.0‡	0 *(0.0)*	1.0‡
Antidepressants	5 *(5.6)*	3 *(4.4)*	2 *(15.4)*	.18‡	0 *(0.0)*	1.0‡
Anticonvulsant drugs (co-analgesics)	3 *(3.3)*	1 *(1.5)*	2 *(15.4)*	.06‡	1 *(25.0)*	1.1‡
Sleeping drugs	3 *(3.3)*	2 *(2.9)*	1 *(7.7)*	.41‡	0 *(0.0)*	1.0‡
THC pain medication	2 *(2.2)*	2 (2.9)	0 *(0.0)*	/	0 *(0.0)*	/
Muscle relaxant	1 *(1.1)*	1 *(1.5)*	0 *(0.0)*	/	0 *(0.0)*	/
ECOG performance status						
0	46 *(51.1)*	38 *(55.1)*	5 *(38.5)*	.27X	2 *(50.0)*	1.0‡
1	38 *(42.2)*	27 *(39.1)*	6 *(46.2)*	.64X	1 *(25.0)*	1.0‡
2	6 *(6.7)*	4 *(5.8)*	2 *(15.4)*	.24X	1 *(25.0)*	.25‡
FACIT fatigue score	**n*=89	**n*=68				
Mean ± SD	36.6 ± 9.9	36.8 ± 10.3	37.2 ± 8.5	.98U	40.3 ± 9.2	.53U
Range	10-52	10-52	20-49		27-47	
BDI-FS depression score	**n*=89	**n*=68				
Mean ± SD	2.2 ± 2.0	2.3 ± 2.2	1.8 ± 1.7	.47U	1.0 ± 0.8	.29U
Range	0-7	0-7	0-6		0-2	

SF-12 physical health score	*n=88	*n=67				
Mean ± SD	39.2 ± 10.6	40.1 ± 10.8	37.1 ± 9.6	.31^{U}	41.6 ± 7.7	.84^{U}
Range	19-61	19-61	23-54		32-50	
SF-12 mental health score	*n=88	*n=67				
Mean ± SD	47.4 ± 11.4	47.6 ± 11.6	49.9 ± 12.3	.55^{U}	59.9 ± 3.0	**.02^{U}**
Range	18-64	18-64	28-63		57-63	
Years of education						
Mean ± SD	14.8 ± 3.2	14.7 ± 3.3	15.3 ± 3.2	.42^{U}	14.8 ± 3.5	1.0^{U}
Range	10-23	10-23	11-21		11-19	
IQ (derived by MWT-A test score)	*n=86	*n=67	*n=12		*n=3	
Mean ± SD	107.5 ± 16.1	107.8 ± 16.0	107.8 ± 14.4	.99^{U}	118.7 ± 16.2	.26^{U}
Range	70-143	70-139	81-128		100-128	

*χ: Chi-square test; ‡: Fisher's exact test; U: Mann-Whitney U test; /: No significance test conducted; †: Numbers do not add up because: tissue- or other staining-positive patients were excluded from antibody-negative group. *: If data is missing, the n of patients with known data is displayed. Percentages are computed for the known data. § includes: peripheral neuropathy (6 patients), peripheral nerve affection (2), absence-epilepsy during childhood (1), migraine (1), restless leg syndrome (1); # includes: depression (2 - current BDI depression score not above 7 points), burnout syndrome (3), anxiety disorder (1).*

Table 6: Demographics of Patients Included in Neuropsychological Testing (n=90) vs Patients Excluded from Neuropsychological Testing (n=68)

	All *(%)* (*n*=158)	Included in neuropsychological testing *(%)* (*n*=90)	Excluded from neuropsychological testing *(%)* (*n*=68)	*p*
Age				
Mean ± SD (years)	64 ± 12	61.2 ± 11.7	67.7 ± 11.4	**.001^{U}**
Sex				
Male	110 *(69.6)*	58 *(64.4)*	52 *(76.5)*	.10^{X}
Gastrointestinal cancer subtype				
Esophageal cancer	30 *(19.0)*	15 *(16.7)*	15 *(22.1)*	.39^{X}
Adenocarcinoma	9 *(5.7)*	2 *(2.2)*	7 *(10.3)*	**.04‡**
Squamous epithelial carcinoma	21 *(13.3)*	13 *(14.4)*	8 *(11.8)*	.62^{X}

Adenocarcinoma of the gastroesophageal junction	36 *(22.8)*	21 *(23.3)*	15 *(22.1)*	.85[x]
Gastric adenocarcinoma	58 *(36.7)*	34 *(37.8)*	24 *(35.3)*	.75[x]
Gastric stump adenocarcinoma	2 *(1.3)*	1 *(1.1)*	1 *(1.5)*	/
Krukenberg adenocarcinoma	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
Colorectal adenocarcinoma	31 *(19.6)*	18 *(20.0)*	13 *(19.1)*	.89[x]
Clinical stage				
Current tumor disease	135 *(85.4)*	79 *(87.8)*	56 *(82.4)*	.34[x]
Metastasized	88 *(55.7)*	51 *(56.7)*	37 *(54.4)*	.78[x]
Current recurrence (relapse)	29 *(18.4)*	18 *(20.0)*	11 *(16.2)*	.54[x]
Local	6 *(3.8)*	2 *(2.2)*	4 *(5.9)*	.40‡
Distant	23 *(14.6)*	16 *(17.8)*	7 *(10.3)*	.19[x]
NED - Currently in aftercare	23 *(14.6)*	11 *(12.2)*	12 *(17.6)*	.34[x]
Site of metastases	**n*=156			
Peritoneal	38 *(24.4)*	23 *(25.6)*	15 *(22.1)*	.68[x]
Liver	38 *(24.4)*	22 *(24.4)*	16 *(23.5)*	.99[x]
Lung	19 *(12.2)*	12 *(13.3)*	7 *(10.3)*	.61[x]
Bones	9 *(5.8)*	5 *(5.6)*	4 *(5.9)*	1.0‡
Ovary	4 *(2.6)*	3 *(3.3)*	1 *(1.5)*	.64‡
Brain	4 *(2.6)*	0 *(0.0)*	4 *(5.9)*	**.03‡**
Pancreas	3 *(1.9)*	1 *(1.1)*	2 *(2.9)*	.57‡
Intestine	3 *(1.9)*	1 *(1.1)*	2 *(2.9)*	.57‡
Pleura	2 *(1.3)*	0 *(0.0)*	2 *(2.9)*	/
Adrenal gland	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
Bone marrow	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
Gallbladder	1 *(0.6)*	0 *(0.0)*	1 *(1.5)*	/
Testicle	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
Treatment				
Treatment-naive	33 *(20.9)*	20 *(22.2)*	13 *(19.1)*	.64[x]
Chemotherapy (prior and/or current)	114 *(72.2)*	66 *(73.3)*	48 *(70.6)*	.70[x]
Current chemotherapy	**n*=157 58 *(36.9)*	**n*=89 37 *(41.6)*	**n*= 68 21 *(30.9)*	.17[x]

Radiotherapy (prior and/or current)	33 *(20.9)*	16 *(17.8)*	17 *(25.0)*	.27[x]
Local radiation (primary tumor)	31 *(19.6)*	16 *(17.8)*	15 *(22.1)*	.50[x]
Current local radiation	7 *(4.4)*	4 *(4.4)*	3 *(4.4)*	1.0‡
Brain radiation	2 *(1.3)*	0 *(0.0)*	2 *(2.9)*	/
Current brain radiation	2 *(1.3)*	0 *(0.0)*	2 *(2.9)*	/
Surgery of primary tumor	73 *(46.2)*	39 *(43.3)*	34 *(50.0)*	.41[x]
Surgery of metastases	20 *(12.7)*	14 *(15.6)*	6 *(8.8)*	.21[x]
Antibody therapy	47 *(29.8)*	29 *(32.2)*	18 *(26.5)*	.43[x]
Current antibody therapy	**n*=154 24 *(15.6)*	**n*=88 17 *(19.3)*	*n=66 7 *(10.3)*	.14[x]
Other targeted therapy	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
Other current/prior malignancy in history				
Yes	22 *(13.9)*	9 *(10.0)*	13 *(19.1)*	.10[x]
Simultaneous second carcinoma	3 *(1.9)*	3 *(3.3)*	0 *(0.0)*	.26‡
Lung cancer	2 *(1.3)*	2 *(2.2)*	0 *(0.0)*	/
Urothelial carcinoma	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
Prior malignancy in history	21 *(13.3)*	8 *(8.9)*	13 *(19.1)*	.06[x]
Medical history				
Neurological disease[§]	32 *(20.3)*	10 *(11.1)*	22 *(32.4)*	**.001[x]**
Psychiatric disease[#]	10 *(6.3)*	5 *(5.6)*	5 *(7.4)*	.75‡
Alcohol abuse in history	**n*=115 9 *(7.8)*	6 *(6.7)*	**n*=25 3 *(12.0)*	.41‡
Neuronal autoantibodies	**	**	**	
Antibody-positive	32 *(20.3)*	13 *(14.4)*	19 *(27.9)*	**.035[x]**
One antibody only	31 *(19.6)*	13 *(14.4)*	18 *(26.5)*	.06[x]
Combination of two antibodies[†]	1 *(0.6)*	0 *(0.0)*	1 *(1.5)*	/
Surface antigen(s)	19 *(12.2)*	9 *(10.0)*	10 *(14.7)*	.24[x]
NMDAR	17 *(10.8)*	8 *(8.9)*	9 *(13.2)*	.26[x]
NMDAR IgM	8 *(5.1)*	4 *(4.4)*	4 *(5.9)*	.71[‡]
NMDAR IgA[†]	8 *(5.1)*	3 *(3.3)*	5 *(7.4)*	.26[‡]
NMDAR IgG	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
MOG	1 *(0.6)*	0 *(0.0)*	1 *(1.5)*	/

GlyR	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
Intracellular antigen(s)	14 *(8.9)*	4 *(4.4)*	10 *(14.7)*	**.018χ**
ARHGAP26†	6 *(3.8)*	1 *(1.1)*	5 *(7.4)*	.09‡
Ma2 (Ta)	3 *(1.9)*	1 *(1.1)*	2 *(2.9)*	.58‡
Yo	3 *(1.9)*	0 *(0.0)*	3 *(4.4)*	.08‡
CARPVIII	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
SOX1	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
Tissue staining	**	**	**	
Tissue staining positive	14 *(8.9)*	5 *(5.6)*	9 *(13.2)*	.06χ
Ab- in rec. cells (UFO)	8 *(5.1)*	4 *(4.4)*	4 *(5.9)*	.71χ
Ab+ in rec. cells***	6 *(3.8)*	1 *(1.1)*	5 *(7.4)*	**.040‡**
Hippocampus (rat)	4 *(2.6)*	0 *(0.0)*	4 *(5.9)*	**.033‡**
Cerebellum (rat)	11 *(7.0)*	4 *(4.4)*	7 *(10.3)*	.12‡
Cerebellum (monkey)	13 *(8.2)*	5 *(5.6)*	8 *(11.8)*	.11χ
Number of stained tissues††				
One	4 *(2.6)*	1 *(1.1)*	3 *(4.4)*	.32‡
Two	6 *(3.8)*	4 *(4.4)*	2 *(2.9)*	.70‡
Three	4 *(2.6)*	0 *(0.0)*	4 *(5.9)*	**.033‡**
Other staining				
Other staining positive	8 *(5.1)*	5 *(5.6)*	3 *(4.4)*	1.0‡
Golgi 3200/1000	3 *(1.9)*	2 *(2.2)*	1 *(1.5)*	1.0‡
Myelin 1000	1 *(0.6)*	0 *(0.0)*	1 *(1.5)*	/
Fine cytoplasmic fluorescence IgG IFT 1000	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
Granular cytoplasmic fluorescence 3200	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
Myositis EUROLINE Ro52+++				
Other antibodies: Islet cells 100	1 *(0.6)*	1 *(1.1)*	0 *(0.0)*	/
Immunoblot Titin borderline	1 *(0.6)*	0 *(0.0)*	1 *(1.5)*	/

*χ: Chi-square test; ‡: Fisher's exact test; U: Mann-Whitney U test; /: No significance test conducted; *: If data is missing, the n of patients with known data is displayed. Percentages are computed for the known data. All patients n=158: § includes: peripheral neuropathy (10 patients), stroke (8 - out of which 1 is hemorrhagic), s/pTIA (4), spinal stenosis with disc prolapse (3), peripheral nerve affection (2), peripheral nerve paresis (2), epilepsy (2), suspected epilepsy (1),*

*absence-epilepsy during childhood (1), s/p acoustic neuroma (1), migraine (1), Parkinson's suspected (1), restless leg syndrome (1), s/p traumatic brain injury (1); # includes: depression (5), burnout syndrome (3), anxiety disorder (2), schizoaffective disorder (1), paranoid psychosis (1); Included in neuropsychological testing n=90: § includes: peripheral neuropathy (6 patients), peripheral nerve affection (2), absence-epilepsy during childhood (1), migraine (1), restless leg syndrome (1); # includes: depression (2 - current BDI depression score not above 7 points), burnout syndrome (3), anxiety disorder (1); Excluded from neuropsychological testing n=68: § includes: peripheral neuropathy (4 patients), stroke (8 - out of which 1 is hemorrhagic), s/p TIA (4), spinal stenosis with disc prolapse (3), peripheral nerve paresis (2), epilepsy (2), suspected epilepsy (1), s/p acoustic neuroma (1), Parkinson's suspected (1), s/p traumatic brain injury (1); # includes: depression (3), anxiety disorder (1), schizoaffective disorder (1), paranoid psychosis (1). IgM, immunoglobulin M; IgA, immunoglobulin A; IgG, immunoglobulin G; **Numbers do not add up due to combination of two antibodies or tissue staining. † Combinations of two antibodies: ARHGAP26+NMDAR IgA (n=1). ***Ab+ in rec. cells: ARHGAP26+NMDAR IgA (n=1), CARPVIII (n=1), NMDAR IgM (n=1), Ma2(Ta) (n=1), MOG (n=1), Yo (n=1). ††Combinations of tissue staining include: All patients (n=158): One tissue: CM (n=3), CR (n=1), HR (n=0). Two tissues: CR+CM (n=6), CR+HR (n=0), CM+HR (n=0). Patients with neuropsychological testing (n=90): One tissue: CM (n=1), CR (n=0), HR (n=0). Two tissues: CR+CM (n=4), CR+HR (n=0), CM+HR (n=0). (CM=Cerebellum monkey, CR=Cerebellum rat, HR=Hippocampus rat, Ab+ = Antibody-positive in recombinant cells, Ab- = Antibody-negative in recombinant cells, UFO= unknown fluorescence object). All patients (n=158): Golgi 1000 (n=2), Golgi 3200 (n=1). Patients with neuropsychological testing (n=90): Golgi 1000 (n=2).*

Fourteen out of 158 patients (8.9%) and 5 out of 90 patients (5.6%) with neuropsychological testing showed a positive tissue staining (Table 4).

Table 7: Tissue-Staining Positive Patients

	All patients (*n*=158)		Patients with neuropsychological testing (*n*=90)	
	No.*	Percent*	No.*	Percent*
Tissue staining positive	14	*8.9*	5	*5.6*
Ab- in rec. cells (UFO)	8	*5.1*	4	*4.4*
Ab+ in rec. cells**	6	*3.8*	1	*1.1*
Hippocampus (rat)	4	*2.5*	0	*0.0*
Cerebellum (rat)	11	*7.0*	4	*4.4*
Cerebellum (monkey)	13	*8.2*	5	*5.6*
Number of stained tissues†				
One	4	*2.5*	1	*1.1*
Two	6	*3.8*	4	*4.4*
Three	4	*2.5*	0	*0.0*

**Numbers do not add up due to combination of tissue staining. **Ab+ in rec. cells: ARHGAP26+NMDAR IgA (n=1), CARPVIII (n=1), NMDAR IgM (n=1), Ma2(Ta) (n=1), MOG (n=1), Yo (n=1). †Combinations of tissue staining include: All patients (n=158): One tissue: CM (n=3), CR (n=1), HR (n=0). Two tissues: CR+CM (n=6), CR+HR (n=0), CM+HR (n=0). Patients with neuropsychological testing (n=90): One tissue: CM (n=1), CR (n=0), HR (n=0). Two tissues: CR+CM (n=4), CR+HR (n=0), CM+HR (n=0). (CM=Cerebellum monkey, CR=Cerebellum rat, HR=Hippocampus rat, Ab+ = Antibody-positive, Ab- = Antibody-negative, UFO= unknown fluorescence object).*

Eight out of all 158 patients (5.1%) patients and 5 out of 90 patients (5.6%) with neuropsychological testing showed a staining pattern that was not assignable to the three brain tissues (i.e., cerebellum monkey, cerebellum rat, hippocampus rat) on the mosaic chip, including Golgi 3200/1000, Myelin 1000, and Cytoplasmatic fluorescence (for details, see Table 5).

Table 8: Patients with Tissue Staining Patterns Other than Cerebellum (Monkey or Rat) or Hippocampus (Rat)

	All patients (*n*=158)		Patients with neuropsychological testing (*n*=90)	
	No.	Percent	No.	Percent
Other staining positive	8	*5.1*	5	*5.6*
Golgi 3200/1000	3	*1.9*	2	*2.2*
Myelin 1000	1	*0.6*	0	*0.0*
Fine cytoplasmic fluorescence IgG IFT 1000	1	*0.6*	1	*1.1*
Granular cytoplasmic fluorescence 3200, Myositis Ro52+++	1	*0.6*	1	*1.1*
Other antibodies: Islet cells 100	1	*0.6*	1	*1.1*
Immunoblot Titin borderline	1	*0.6*	0	*0.0*

*All patients (*n=*158): Golgi 1000 (*n=*2), Golgi 3200 (*n=*1). Patients with neuropsychological testing (*n=*90): Golgi 1000 (*n=*2)*

Cognitive Impairment

Subjective cognitive impairment was reported by 28 out of 88 examined patients (31.8%) (missing data for two patients). Objective measurement of cognitive function was performed following the ICCTF criteria outlined in the Methods section. The categorical variable of cognitive impairment was only defined for 89 patients due to incomplete data. Nevertheless, the available data of the performed tests was used for analyses of the raw test scores.

Applying the first definition of the ICCTF criteria, 54 out of 89 patients (60.7%) had cognitive impairment with a test score 1.5 SD below the reference control group in at least two tests. Following our added definition, 43 out of 89 patients (47.2%) showed a test score 1.5 SD below the reference control group in at least two tests from different domains. Based on the last definition, 58 out of 89 patients (65.2%) had a test score 2 SD below the reference control group in at least one test. The affected domains here included attention (43.8%), executive function (24.7%), working memory (18.9%), verbal learning (17.2%), visuospatial memory (3.4%), and fluid intelligence (0%).

Neurological Examination

Overall, 62% of the 90 patients undergoing a neurological examination showed an abnormal neurological function in at least one examination of the cranial nerves, motor function, sensory function, or coordination. Cerebellar symptoms, including dysmetria, dysdiadochokinesia, a positive Romberg trial, and balancing insecurity, were the most common dysfunction, affecting 33% of the examined patients overall, and 30% of the antibody-positive, and 33% of the antibody-negative patients. Furthermore, 30% of all patients, 46% of antibody-positive patients, and 28% of the antibody-negative patients showed polyneuropathic symptoms including pallhypesthesia, distal symmetric hypesthesia, or combinations. Antibody-positive patients more often showed an abnormal function with 9/13 (69%) compared to antibody-negative patients with 40/66 (60%) ($p=0.756$, Fisher's exact test). However, no significant difference between antibody-negative and antibody-positive patients was found (see Table 10).

Table 9: Neurological Examination

Neurological examination (*n*=90)	Total No. *(%)*	N.	D.	C.	Ab- (*n*=69) *(%)*	Antibody and/or Tissue + (*n*=17) *(%)*	p	Antibody + (*n*=13) *(%)*	p
Abnormal neurological examination	54 *(62.1) n*=87	7	9 *n*=12	44 *n*=63	40 *(60.6) n*=66	11 *(64.7)*	0.757	9 *(69.2)*	0.756
Cranial nerves									
Abnormal oculomotor function†	1 *(1.1) n*=87	1 n=9	1 *n*=12	0 *n*=64	1 *(1.5) n*=66	0 *(0.0)*	/	0 *(0.0)*	/
Trigeminal hypesthesia	0 *(0.0)*	0	0	0	0 *(0.0)*	0 *(0.0)*	/	0 *(0.0)*	/
Uni/bilateral hypoacusis	7 *(7.8)*	1	3	5	7 (10.1)	0 *(0.0)*	0.336	0 *(0.0)*	0.590
Cranial nerve pathology#	1 *(1.1) n*=88	0	0 *n*=12	1 *n*=65	0 (*0.0) n*=67	0 *(0.0)*	/	0 *(0.0)*	/
Motor function									
Reduced muscle strength/paresis	2 *(2.4) n*=85	1	1	1 *n*=62	1 *(1.5) n*=65	0 *(0.0)*	/	0 *(0.0)*	/
Abnormal muscle tone (rigor/spasticity)	1 (1.1)	0	0	0	1 *(1.4)*	0 *(0.0)*	/	0 *(0.0)*	/
Asymmetric reflexes	2 *(2.2) n*=89	0	0	2 *n*=65	2 (*2.9) n*=68	0 *(0.0)*	/	0 *(0.0)*	/
Missing reflexes	11 *(12.4) n*=89	1	2	10 *n*=65	9 *(13.2) n*=68	2 *(11.8)*	1	1 *(7.7)*	1
Babinski positive	0 *(0.0)*	0	0	0	0 *(0.0)*	0 *(0.0)*	/	0 *(0.0)*	/
Sensory function									
Pallhypesthesia	24 *(27.6) n*=87	3 n=9	6 *n*=12	19 *n*=64	18 *(26.9) n*=67	6 *(35.3)*	0.552	5 *(38.5)*	0.505
Hypesthesia upper/lower limb	4 *(4.5) n*=89	1	1 *n*=12	3	3 *(4.4) n*=68	1 *(5.9)*	1	1 *(7.7)*	0.511
Polyneuropathic symptoms§	26 *(29.5) n*=88	4	6 *n*=12	21 *n*=65	19 *(27.9) n*=68	7 *(41.2)*	0.289	6 *(46.2)*	0.206
Coordination									
Dysmetria in finger-nose-test	0 *(0.0) n*=88	0	0 *n*=12	0 *n*=65	0 *(0.0) n*=67	0 *(0.0)*	/	0 *(0.0)*	/
Balancing insecurity	23 *(28.4) n*=81	4 n=9	5 *n*=10	18 *n*=59	18 *(29) n*=62	5 *(29.4)*	1	4 *(30.8)*	1
Romberg trial positive	2 *(2.3) n*=87	0	0 *n*=12	2 *n*=64	1 *(1.5) n*=66	0 *(0.0)*	/	0 *(0.0)*	/
Cerebellar symptoms‡	27 *(32.5) n*=83	4 n=9	6 *n*=11	21 *n*=60	21 *(32.8) n*=64	5 *(29.4)*	0.789	4 *(30.8)*	1

Neurological examination (*n*=90)	Intracellular Antibody + (*n*=4) *(%)*	p	Surface Antibody + (*n*=9) *(%)*	p	NMDAR + (*n*=8) *(%)*	p	Tissue + (*n*=5) *(%)*	P
Abnormal neurological examination	3 *(75.0)*	1	6 (66.7)	1	5 *(62.5)*	1	3 *(60.0)*	1
Cranial nerves								
Abnormal oculomotor function†	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/
Trigeminal hypesthesia	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/
Uni/bilateral hypoacusis	0 *(0.0)*	1	0 *(0.0)*	1	0 *(0.0)*	1	0 *(0.0)*	1
Cranial nerve pathology#	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/
Motor function								
Reduced muscle strength/paresis	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/
Abnormal muscle tone (rigor/spasticity)	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/
Asymmetric reflexes	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/
Missing reflexes	0 *(0.0)*	1	1 *(11.1)*	1	1 *(12.5)*	1	1 (*20.0)*	0.532
Babinski positive	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/
Sensory function								
Pallhypesthesia	2 *(50.0)*	0.314	3 *(33.3)*	0.701	2 *(25.0)*	1	2 *(40.0)*	0.613
Hypesthesia upper/lower limb	0 *(0.0)*	1	1 *(11.1)*	0.398	1 *(12.5)*	0.365	0 (0.0)	1
Polyneuropathic symptoms§	2 *(50.0)*	0.575	4 *(44.4)*	0.439	3 *(37.5)*	0.684	2 *(40.0)*	0.621
Coordination								
Dysmetria in finger-nose-test	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/
Balancing insecurity	3 *(75.0)*	0.091	1 *(11.1)*	0.428	1 *(12.5)*	0.433	2 *(40.0)*	0.631
Romberg trial positive	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/	0 *(0.0)*	/
Cerebellar symptoms‡	3 *(75.0)*	0.122	1 *(11.1)*	0.262	1 *(12.5)*	0.421	2 *(40.0)*	1

N.: Neurological disease (n=10), D.: Diabetes (n=13), C.: Chemotherapy (n=66).*Neurological diseases include: peripheral polyneuropathy, migraine, restless legs syndrome, injury of the radialis nerve, injury of the femoralis nerve, epilepsy during childhood. Bold numbers represent a significant effect. P-values are computed with Chi-square test/Fisher's exact test; †: includes: saccadic pursuit movements, congenital nystagmus, diplopia or combinations; #: includes: anisocoria, trigeminal hypesthesia, facial paresis, diplopia or combinations; §: includes: PNP diagnosis, pallhypesthesia, distal symmetric hypesthesia or combinations; ‡: includes: dysmetria, dysdiadochokinesia, Romberg trial positive, balancing insecurity or combinations.*

Antibodies and Cognitive Impairment

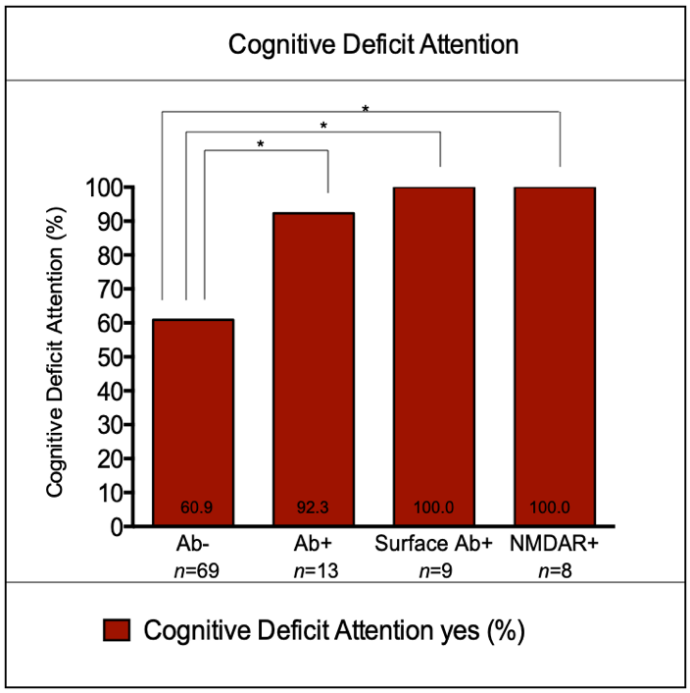
Cognitive Deficits

Applying the ICCTF definitions for a cognitive deficit (see Methods section), no significant difference between antibody-positive and antibody-negative patients was found. Nevertheless, 10/13 (77%) of the antibody-positive patients were cognitively impaired according to the first definition compared to 39/68 (57%) of the antibody-negative patients (χ^2 (1) = 1.75, p=0.186**,** φ = 0.15). Additionally, using the second definition, 8/13 (62%) of the antibody-positive patients were cognitively impaired compared to 32/68 (47%) of the antibody-negative patients (χ^2 (1) = 0.92, p=0.339**,** φ = 0.11). Lastly, according to the third definition, 9/13 (69%) of the antibody-positive patients were cognitively impaired compared to 44/69 (64%) of the antibody-negative patients (p=1, Fisher's exact test).

Looking at the different domains, a cognitive deficit in one domain was defined by a test score being at least 1.5 SD below the reference group in at least one test. Applying this additional explorative definition, antibody-positive patients had a higher frequency of a deficit in the attention domain compared to antibody-negative patients (ab+ 92.3% vs ab- 60.9%, Fisher's exact test: p=0.030) (Figure 2). Similarly, patients with NSAbs also had a higher rate of cognitive deficits in the attention domain compared to antibody-negative patients (ab+ 100% vs ab- 60.9%, Fisher's exact test: p=0.024) (Figure 2). This association was also found for patients with antibodies targeted against the NMDA receptor (ab+ 100% vs ab- 60.9%, Fisher's exact test: p=0.045) (Figure 2).
Concerning the other cognitive domains, there was no significant difference between antibody-positive and antibody-negative patients. Nonetheless, antibody-positive patients had a higher rate of a cognitive deficit in the domain verbal fluency (ab+ 39% vs ab- 24%, Fisher's exact test: p=0.315), and in the domain short-term memory (ab+ 31% vs ab- 23%, Fisher's exact test: p=0.725). Antibody-positive and antibody-negative patients had similar frequencies of a cognitive deficit in the domain visuospatial memory (ab+ 7.7% vs ab- 7.4%, Fisher's exact test: p=1), and in the domain fluid intelligence (ab+ 0% vs ab- 1.4%, Fisher's exact test: p=1). Interestingly, antibody-positive patients had a cognitive deficit less often in the domain executive function compared to antibody-negative patients (ab+ 39% vs ab- 50%, χ^2 (1) = 0.58, p=0.446**,** φ = 0.09).

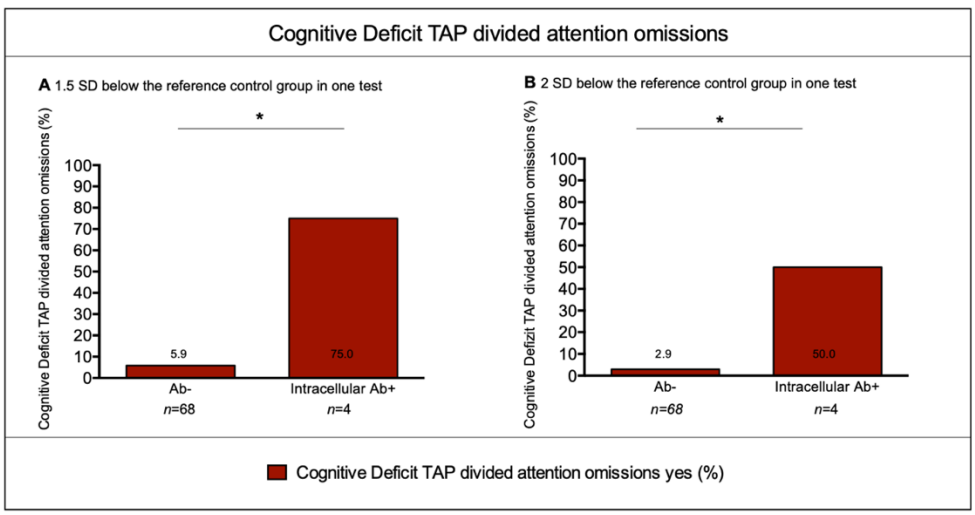
Looking at each test independently from the ICCTF criteria, a cognitive deficit was defined by a test score being either 1.5 or 2 SD below the reference control group. Here, intracellular antibody-positive patients had a deficit significantly more often in the test TAP Divided Attention Omissions (1.5 SD: AICAbs+ 75% vs ab- 5.9%, Fisher's exact test: p=0.002; 2 SD: AICAbs+ 50% vs ab- 2.9%, Fisher's exact test: p=0.014) (Figure 3).

Figure 2: Cognitive Deficit Attention



Ab+ patients showed a cognitive deficit significantly more often in the domain attention compared with ab- patients [92.3% (ab+) versus 60.9% (ab-), Fisher's exact test: p=*0.030]; Surface Ab+ patients showed a deficit significantly more often in the domain attention compared with ab- patients [100.0% (surface ab+) versus 60.9% (ab-), Fisher's exact test:* p=*0.024]; NMDAR+ patients showed a cognitive deficit significantly more often in the domain attention compared with ab- patients [100.0% (surface ab+) versus 60.9% (ab-), Fisher's exact test:* p=*0.045]; ': Cognitive deficit in the domain attention was considered when the ICCTF criteria 1 was fulfilled, which refers to at least two tests with a score 1.5 standard deviations below the reference control group; *: p<0.05; ab, antibody; NMDAR, anti-NMDA receptor.*

Figure 3: Cognitive Deficit Test TAP Divided Attention Omissions



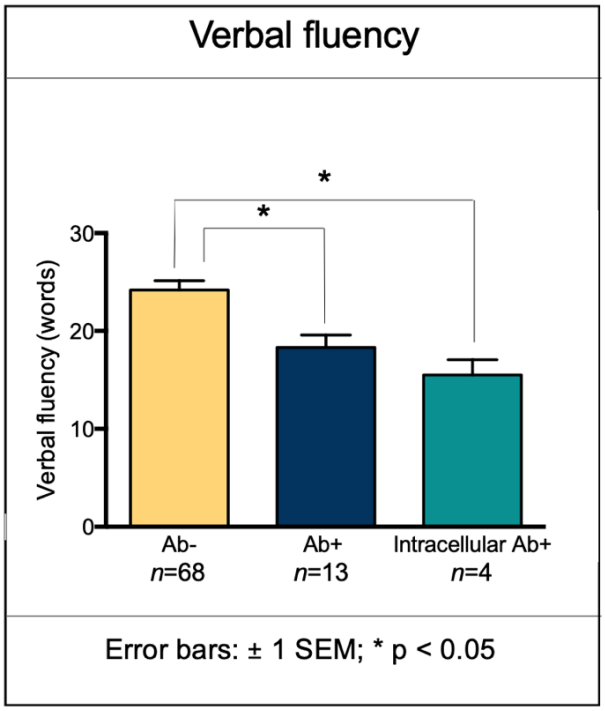
*Intracellular Ab+ patients showed a cognitive deficit significantly more often in the test TAP divided attention omissions compared with ab- patients [A 75.0% (intracellular ab+) versus 5.9% (ab-), Fisher's exact test: p=0.002; B 50.0% (intracellular ab+) versus 2.9% (ab-), Fisher's exact test: p=0.014]; ': Cognitive deficit in the test TAP divided attention omission was considered when the ICCTF criteria 1 was fulfilled, which refers to at least two tests with a score 1.5 standard deviations below the reference control group; '': Cognitive deficit in the test TAP divided attention omission was considered when the ICCTF criteria 2 was fulfilled, which refers to at least one test with a score 2 standard deviations below the reference control group; *: p<0.05; ab, antibody.*

Test Scores

Using the raw test scores, antibody-positive patients scored significantly worse in the test verbal fluency (ab- n=68 (1 missing) mean 24.4 words vs ab+ n=13 mean 18.3, *U*=225.000, *Z*=-2.796, p=0.005) (Figure 4 and Table 11). Similarly, patients with AICAbs scored significantly worse in the test verbal fluency (ab- n=68 (1 missing) mean 24.4 words vs AICAbs+ n=4 mean 15.5, *U*=35.000, *Z*=-2.486, p=0.009) (Figure 4 and Table 11).

Overall, antibody-positive and antibody-negative patients scored similar results in the remaining tests (Table 11). While antibody-positive patients scored consistently worse in the tests for verbal memory and visuospatial memory compared to antibody-negative patients, only small differences can be noted (Table 11). In the tests for short-term memory and fluid intelligence, antibody-positive patients even scored slightly better compared to antibody-negative patients (Table 11). In the tests for attention and executive function, there was a marginally better score in some tests for antibody-positive patients, and in other tests for antibody-negative patients (Table 11). Yet, in the test for divided attention omissions, antibody-positive patients had omissions more often (and therefore a worse test result) than antibody-negative patients (ab+ mean 4 vs ab- mean 2.3, *U*=573.000, *Z*=1.717, p=0.09).

Figure 4: Verbal Fluency



*Ab+ significantly named less words compared to ab- [mean 18.3 words (ab+) versus mean 24.4 words (ab-), Mann-Whitney U test: p=0.005]; Intracellular ab+ significantly named less words compared to ab- [mean 15.5 words (intracellular ab+) versus mean 24.4 words (ab-), Mann-Whitney U test: p=0.009]; Error bars: ±1SEM; *: p<0.05; ab, antibody.*

Table 10: Neuropsychological Test Results

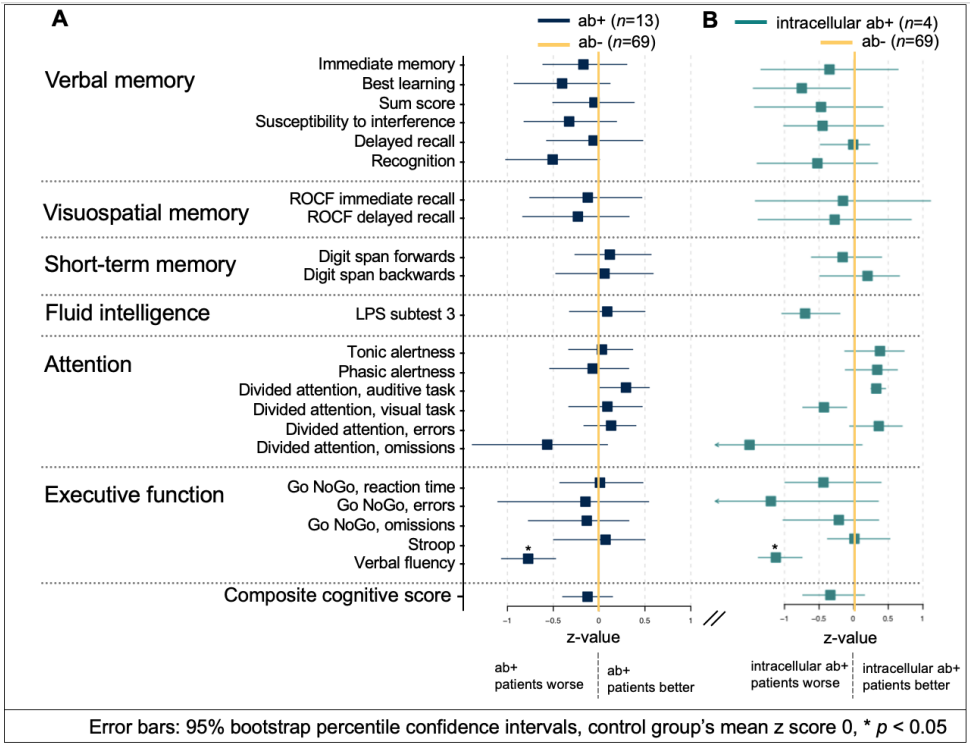
Neuropsychological results (*n*=90)	Ab- mean *(SD)* (*n*=69)	Ab+ mean *(SD)* (*n*=13)	p^U	Intracellular Ab+ mean *(SD)* (*n*=4)	p^U
Verbal memory (VLMT)					
Sum score (points)	47.0 *(11.2)* (*n*=68)	46.5 *(9.7)*	.72	41.8 *(1.5)*	.36
Trial 1 - Immediate memory/Supraspan (points)	6.5 *(2.3)*	6.2 *(2.0)*	.64	5.8 *(2.6)*	.63
Trial 5 - Best learning (points)	11.6 *(2.5)* (*n*=68)	10.6 *(2.5)*	.17	9.8 *(2.2)*	.14
Trial 6 - Susceptibility to interference (points)	9.4 *(3.1)*	8.4 *(3.0)*	.14	8.0 *(2.7)*	.19
Delayed recall (points)	9.3 *(3.2)* (*n*=68)	9.1 *(3.2)*	.64	9.3 *(1.5)*	.90
Recognition (points)	11.9 (*3.2)* (*n*=67)	10.3 *(3.1)*	.07	10.3 *(3.5)*	.28
Visuospatial memory (ROCF)					
Immediate recall (points)	20.32 *(6.9)*	19.3 *(8.4)*	.80	19.0 *(10.4)*	.88
Delayed recall (points)	20.2 *(7.0)* (*n*=68)	18.3 *(8.0)*	.48	17.9 *(9.1)*	.61
Short-term and working memory					
Digit span forwards (points)	7.4 *(2.2)*	7.6 *(1.8)*	.56	7.0 *(1.4)*	.86
Digit span backwards (points)	5.6 *(2.2)*	5.7 *(2.2)*	.68	6.0 *(1.4)*	.47
Fluid intelligence					
LPS (points)	20.7 *(6.0)*	21.2 *(4.8)*	.57	16.5 *(3.1)*	.12
Attention					
tonic alertness (ms)	320.4 *(93.3)*	312.7 *(62.5)*	.65	285.0 *(46.2)*	.58
phasic alertness (ms)	321.0 *(96.3)*	327.8 *(80.4)*	.43	288.3 *(43.8)*	.68
divided attention, auditive task (ms)	654.3 *(166.3)* (*n*=68)	605.4 *(82.7)* (*n*=12)	.53	599.7 *(18.8)* (*n*=3)	.76
divided attention, visual task (ms)	907.8 *(211.7)* (*n*=68)	888.8 *(162.8)*	.84	998.8 (84.8)	.09
divided attention (errors)	4.2 *(6.0)* (*n*=68)	3.4 *(3.4)*	.81	2.0 *(2.8)*	.32
divided attention (omissions)	2.3 *(2.9)* (*n*=68)	4.0 *(4.2)*	.09	6.8 *(6.6)*	.07
Executive function					
Go/NoGo task (ms)	627.0 *(98.3)* (*n*=68)	626 *(84.7)*	.69	670.0 *(82.9)*	.32
Go/NoGo task (errors)	1.0 *(1.5)* (*n*=68)	1.2 *(2.4)*	.64	2.8 *(3.6)*	.28
Go/NoGo task (omissions)	0.8 *(2.2)* (*n*= 68)	1.1 *(2.4)*	.76	1.3 *(1.9)*	.44
Stroop (sec)	146.7 *(41.3)* (*n*=68)	143.9 *(40.7)*	.86	146.3 *(21.3)*	.66
Verbal fluency (words)	24.4 *(7.9)* (*n*=68)	18.3 *(4.6)*	**.005**	15.5 *(3.1)*	**.009**

*U: Mann-Whitney U test; *: If data is missing, the n of patients; §: was calculated by averaging all 22 z-transformed subtests from the 6 different cognitive domains*

Z-Scores

Antibody-positive patients showed significantly lower z-scores for the verbal fluency test compared to antibody-negative patients (ab- n=68 (1 missing) z-score 0.0 vs ab+ n=13 z-score -0.77, *U*=225.000, *Z*=-2.796, p=0.005) (Figure 5 and Table 12). Likewise, patients with AICAbs had significantly lower z-scores for the verbal fluency test compared to antibody-negative patients (ab- n=68 (1 missing) z-score 0.00 vs AICAbs+ n=4 z-score -1.13, *U*=35.000, *Z*=-2.486, p=0.009) (Figure 5 and Table 12). Moreover, antibody-positive patients showed a non-significant trend to score worse in the memory domain (e.g. in the recognition test: ab- n=67 (2 missing) z-score 0.0 vs ab+ n=13 z-score -0.51, *U*=308.500, *Z*=-1.671, p=0.095) and had numerically lower composite cognitive scores (ab- z-score 0.0 vs ab+ z-score -0.13, *U*=363.500, *Z*=-1.079, p=0.28).

Figure 5: Forest Plot Z-Scores



Comparison of neuropsychological test results between ab- and ab+ (A) and intracellular ab+ (B) using z-scores. Z-scores were calculated using group means and SD of antibody-negative (antibody-, tissue-, other staining-negative) patients as a reference group. Test scales with lower values representing a better performance were multiplied by -1 so that lower values stand for lower performance. The composite cognitive score was calculated using the average mean on obtained z-values from 22 tests. *: Significant differences (p < 0.05; Mann-Whitney U test). (A) Ab+ patients scored significantly worse in the verbal fluency test (z-scores: -0.8 ± 0.6 (ab+) versus 0.0 ± 1.0 (ab-), p=0.005, Mann-Whitney U test). (B) Intracellular ab+ patients scored significantly worse in the verbal fluency test (z-scores: -1.1 ± 0.4 (intracellular ab+) versus 0.0 ± 1.0 (ab-), p=0.009, Mann-Whitney U test). Error bars: 95% bootstrap percentile confidence intervals; ab, antibody; LPS, Leistungsprüfssystem; ROCF, Rey-Osterrieth Complex Figure.

Table 11: Z-Scores

Z-scores (calculated using the neuropsychological test results) (n=90)	Ab- mean *(SD)* (*n*=69)	Ab+ mean *(SD)* (*n*=13)	p^U	Intracellular Ab+ mean *(SD)* (*n*=4)	p^U
Composite cognitive score (z-value)§	0.0 *(0.6)*	-0.13 *(0.5)*	.28	-0.3 *(0.6)*	.19
Verbal memory (VLMT)					
Average VLMT performance (z-value)	0.0 *(0.8)*	-0.25 *(0.8)*	.16	-0.4 *(0.7)*	.26
Sum score (z-value)	0.0 *(1.0)* (*n*=68)	-0.1 *(0.9)*	.72	-0.5 *(1.0)*	.36
Trial 1 - Immediate memory/Supraspan (z-value)	0.0 *(1.0)*	-0.7 *(0.9)*	.64	-0.4 *(1.2)*	.63
Trial 5 - Best learning (z-value)	0.0 *(1.0)* (*n*=68)	-0.4 *(1.0)*	.17	-0.8 *(0.9)*	.14
Trial 6 - Susceptibility to interference (z-value)	0.0 *(1.0)*	-0.3 *(1.0)*	.14	-0.5 *(0.9)*	.19
Delayed recall (z-value)	0.0 *(1.0)* (*n*=68)	-0.1 *(1.0)*	.64	0.0 *(0.5)*	.90
Recognition (z-value)	0.0 (1.0) (*n*=67)	-0.5 *(1.0)*	.07	-0.5 *(1.1)*	.28
Visuospatial memory (ROCF)					
Average visuospatial memory performance (z-value)	0.0 *(1.0)*	-0.2 *(1.2)*	.56	-0.2 *(1.4)*	.75
Early recall (z-value)	0.0 *(1.0)*	-0.1 *(1.2)*	.80	-0.2 *(1.5)*	.88
Delayed recall (z-value)	0.0 *(1.0)* (*n*=68)	-0.2 *(1.1)*	.48	-0.3 *(1.3)*	.61
Short-term and working memory					
Average short-term memory (z-value)	0.0 *(0.9)*	0.1 *(0.8)*	.71	0.0 *(0.5)*	.74
Digit span forwards (z-value)	0.0 *(1.0)*	0.1 *(0.8)*	.56	-0.2 *(0.6)*	.86
Digit span backwards (z-value)	0.0 *(1.0)*	0.1 *(1.0)*	.68	0.2 *(0.7)*	.47
Fluid intelligence					
Average fluid intelligence (z-value)	0.0 *(1.0)*	0.1 *(0.8)*	.57	-0.7 *(0.5)*	.12
LPS (z-value)	0.0 *(1.0)*	0.1 *(0.8)*	.57	-0.7 *(0.5)*	.12
Attention					

Average attention performance (z-value)	0.0 *(0.7)*	0.0 *(0.4)*	.51	-0.1 *(0.5)*	.57
tonic alertness (z-value)	0.0 *(1.0)*	0.0 *(0.7)*	.65	0.4 *(0.5)*	.58
phasic alertness (z-value)	0.0 *(1.0)*	-0.1 *(0.8)*	.43	0.3 *(0.5)*	.68
Average alertness performance (z-value)	0.0 *(1.0)*	0.0 *(0.7)*	.50	0.4 *(0.5)*	.65
divided attention, auditive task (z-value)	*0.0 (1.0)* (*n*=68)	0.3 *(0.5)* (*n*=12)	.53	0.3 *(0.1)* (*n*=3)	.76
divided attention, visual task (z-value)	0.1 *(1.0)* (*n*=68)	0.1 *(0.8)*	.84	*-0.4 (0.4)*	.09
divided attention, errors (z-value)	0.0 *(1.0)* (*n*=68)	0.1 *(0.6)*	.81	0.4 *(0.5)*	.32
divided attention, omissions (z-value)	0.0 *(1.0)* (*n*=68)	-0.6 *(1.4)*	.09	-1.5 *(2.3)*	.07
Average divided attention performance (z-value)	0.0 *(0.6)* (*n*=68)	-0.1 *(0.6)*	.97	-0.4 *(0.7)*	.26
Executive function					
Average executive function performance (z-value)	0.0 *(0.7)* (*n*=68)	-0.2 *(0.8)*	.47	-0.6 *(0.8)*	.10
Go/NoGo task (z-value)	0.0 *(1.0)* (*n*=68)	0.0 *(0.9)*	.69	-0.4 *(0.8)*	.32
Go/NoGo task, errors (z-value)	0.0 (1.0) (*n*=68)	-0.1 *(1.6)*	.64	*-1.2 (2.5)*	.28
Go/NoGo task, omissions (z-value)	0.0 *(1.0)* (*n*= 68)	-0.1 *(1.1)*	.76	-0.2 *(0.9)*	.44
Average Go/NoGo function performance (z-value)	0.0 *(0.8)* (*n*=68)	-0.1 *(0.9)*	.91	-0.6 *(1.1)*	.21
Stroop (z-value)	0.0 *(1.0)* (*n*=68)	0.1 *(1.0)*	.86	0.0 *(0.5)*	.66
Verbal fluency (z-value)	0.0 *(1.0)* (*n*=68)	-0.8 *(0.6)*	**.005**	-1.1 *(0.4)*	**.009**

*U: Mann-Whitney U test; *: If data is missing, the n of patients; §: was calculated by averaging all 22 z-transformed subtests from the 6 different cognitive domains.*

Other Factors Associated with Cognitive Impairment

To assess other factors potentially influencing cognition, the association between cognitive performance (applying the ICCTF criteria for a definition of a cognitive deficit and comparing the raw test scores) and demographic factors, mental/physical health, medical history, treatment of tumor, and tumor stage were investigated.

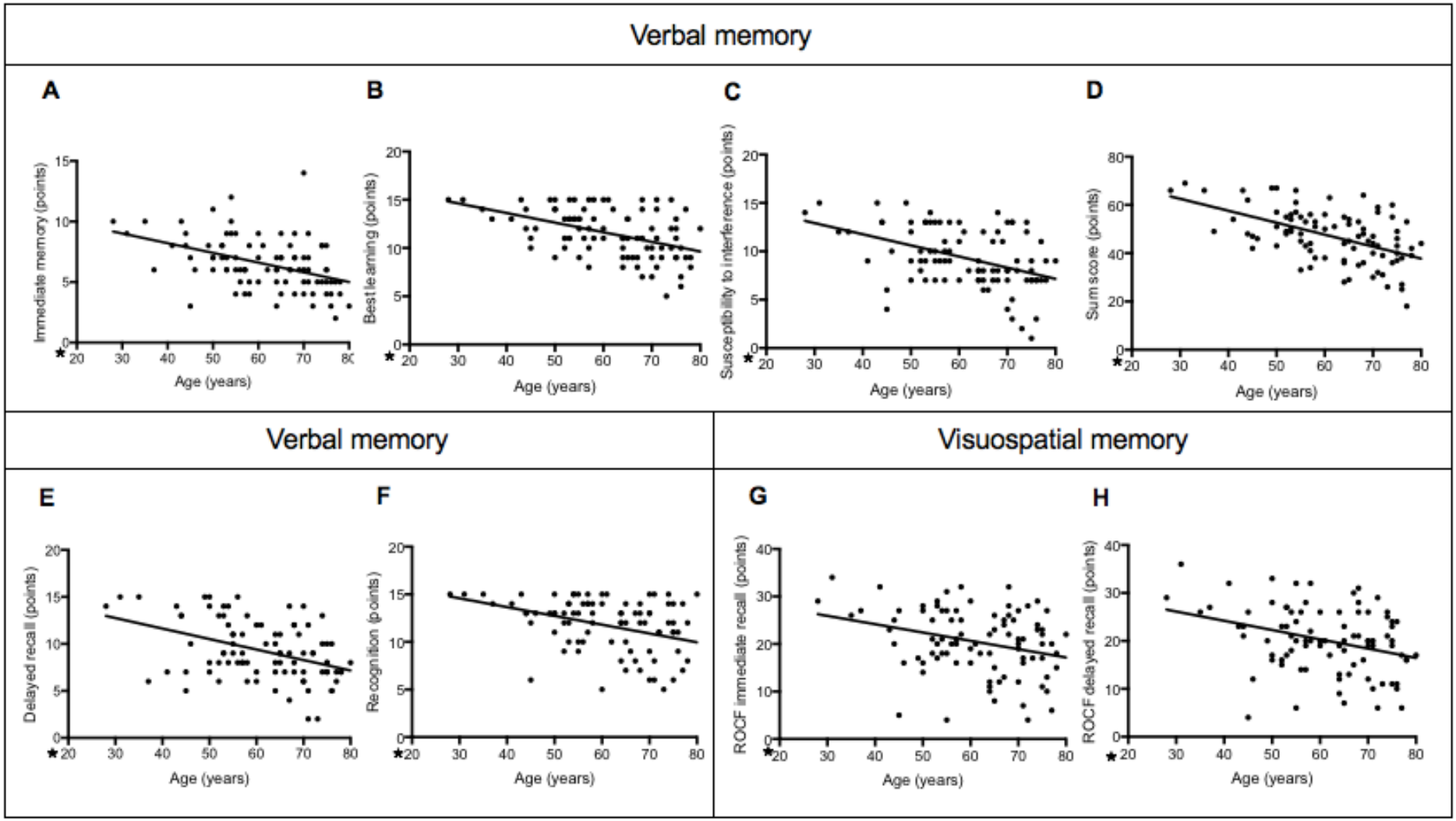
Demographic Factors

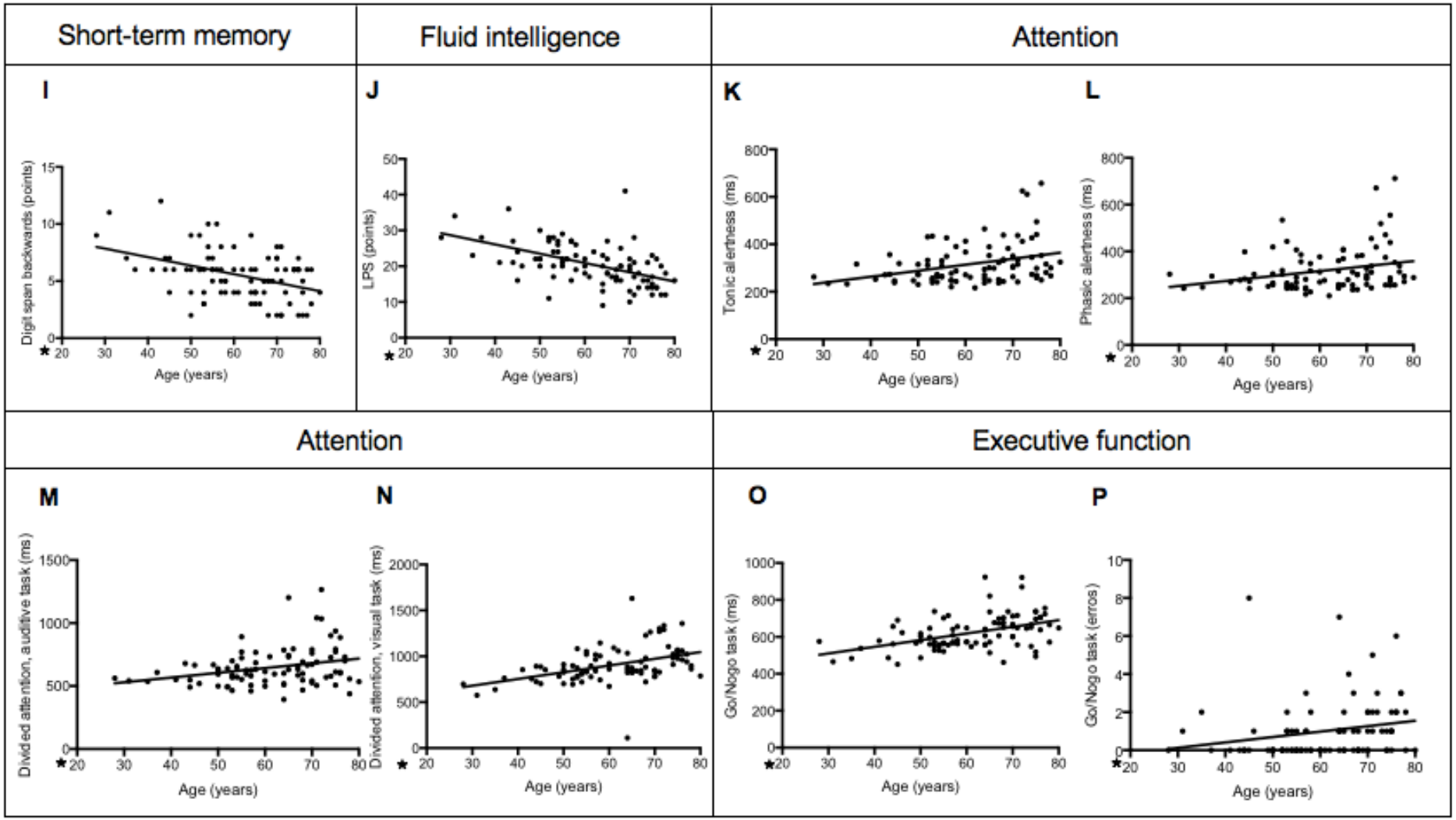
Significant correlations were found between higher age and worse test results in almost all tests, covering the domains memory, fluid intelligence, attention, and executive function. Higher age significantly correlated with a lower composite cognitive score (ρ=-.548, p<0.001; for more details, please refer to Figure 6, Tables 6 and 13).

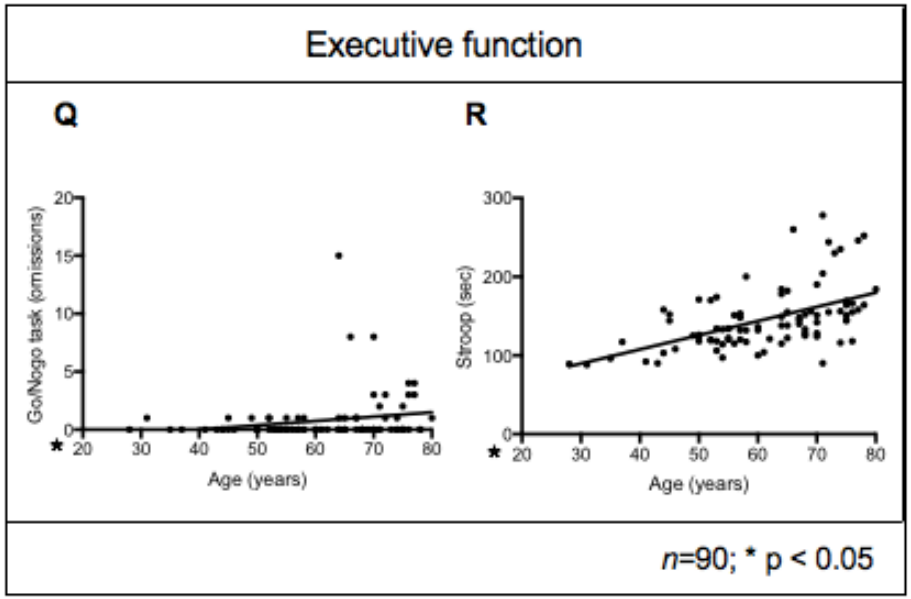
Furthermore, higher intelligence level and more years of education correlated with better results in a high number of tests in the domains memory, fluid intelligence, attention, and executive function, and also a higher composite cognitive score (level of intelligence: ρ=.433, p<0.001, years of education: ρ=.301, p<0.004; for more details, please refer to Figure 7, Tables 6 and 13).

Female patients scored better in one test in the domain verbal memory (best memory: female mean 12.4 words vs male mean 11.07 words, U=1,189.500, Z=2.522, p=0.012), and worse in one test in the domain attention (phasic alertness: female mean 335 seconds vs male mean 308 seconds, U=1,192.500, Z=2.230, p=0.026). However, there was no significant difference between sex and the composite cognitive score (U=961.000, Z=-.278, p=0.781).

Figure 6: Influence of Age on Cognition

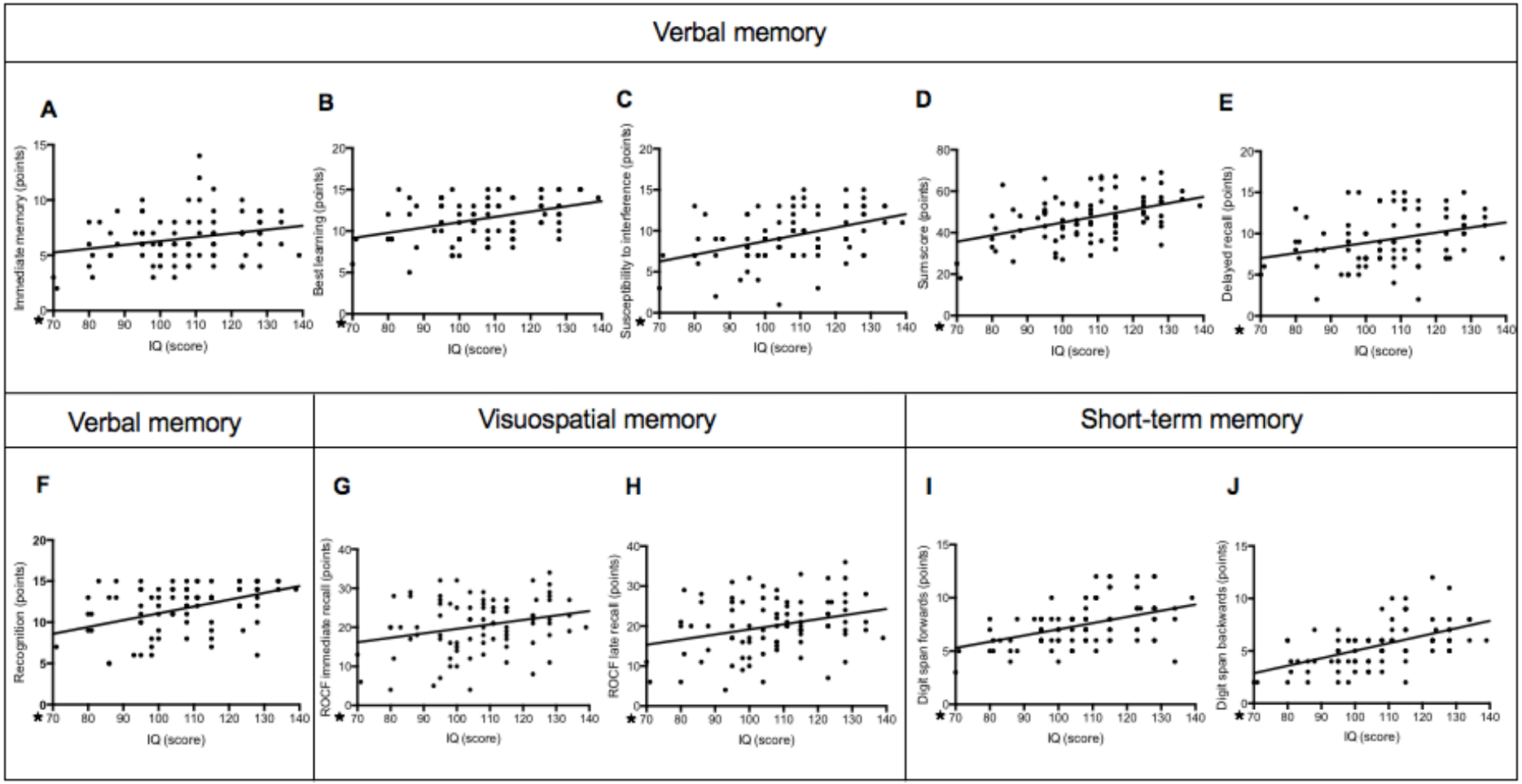


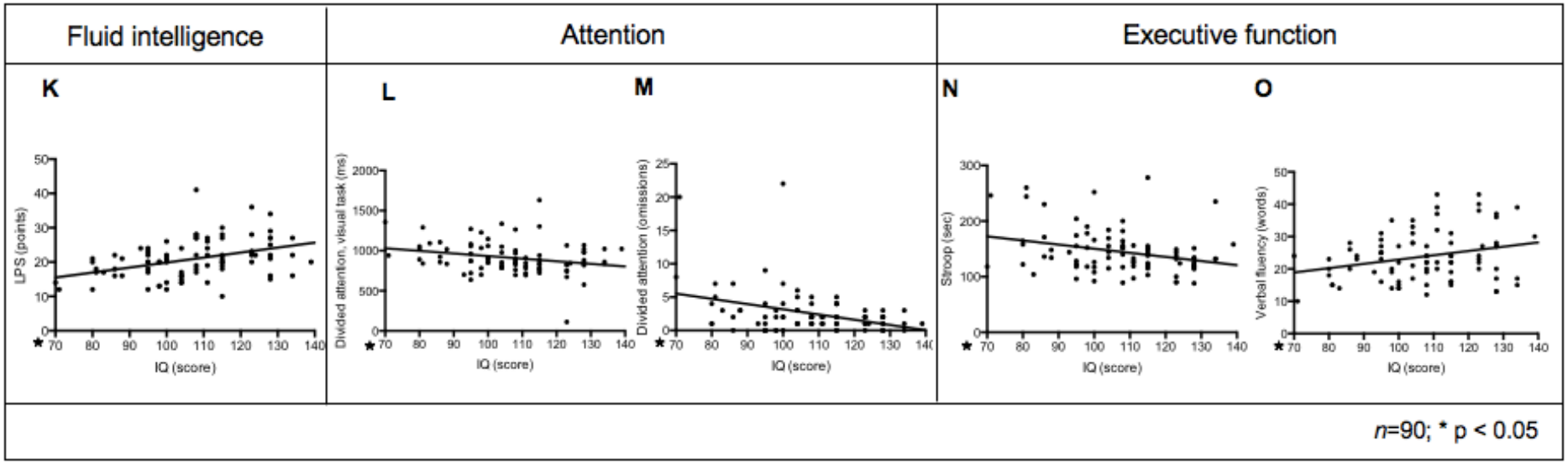

Short-term memory
Fluid intelligence
Attention
I
J
K
L
Digit span backwards (points)
LPS (points)
Tonic alertness (ms)
Phasic alertness (ms)
Age (years)
Attention
Executive function
M
N
O
P
Divided attention, auditive task (ms)
Divided attention, visual task (ms)
Go/Nogo task (ms)
Go/Nogo task (errors)
Age (years)



Using Spearman correlation, age (in years) was a significant factor in 18 out of 22 overall performed tests from all six cognitive domains. The older the patient, the worse the patient scored in the domain. Verbal memory: (A) VLMT Immediate memory, p<0.001 (B) VLMT Best learning, p<0.001 (C) VLMT Susceptibility to interference, p<0.001 (D) VLMT Sum Score, p<0.001 (E) VLMT Delayed recall, p<0.001 (F) VLMT Recognition, p=0.001; Visuospatial memory: (G) ROCF immediate recall, p=0.011 (H) ROCF delayed recall, p=0.006; Short-term memory: (I) Digit span backwards, p=0.001; Fluid intelligence (J) LPS, p<0.001; Attention: (K) Tonic alertness, p=0.001 (L) Phasic alertness, p=0.013 (M) Divided attention, auditive task, p=0.012 (N) Divided attention, visual task, p<0.001; Executive function: (O) Go/NoGo task, p<0.001 (P) Go/NoGo task, errors, p=0.001 (Q) Go/NoGo task, omissions, p=0.013 (R) Stroop, p<0.001; n=90; p < 0.05; ROCF, Rey-Osterrieth Complex Figure.*

Figure 7: Influence of IQ on Cognition





Using Spearman correlation, the IQ score (derived by MWT-A test score) was a significant factor in 15 out of 22 overall performed tests from all six cognitive domains. The higher the IQ score, the better the patient scored in the domain. Verbal memory: (A) VLMT Immediate memory, p=0.025 (B) VLMT Best learning, p=0.001 (C) VLMT Susceptibility to interference, p<0.001 (D) VLMT Sum Score, p<0.001 (E) VLMT Delayed recall, p=0.002 (F) VLMT Recognition, p<0.001; Visuospatial memory: (G) ROCF immediate recall, p=0.035 (H) ROCF delayed recall, p=0.014; Short-term memory: (I) Digit span forwards, p<0.001 (J) Digit span backwards, p<0.001; Fluid intelligence (K) LPS, p<0.001; Attention: (L) Divided attention, visual task, p=0.013 (M) Divided attention, omissions, p=0.011; Executive function: (N) Stroop, p=0.010 (O) Verbal fluency, p=0.049; n=90; p < 0.05; ROCF, Rey-Osterrieth Complex Figure.*

Mental/Physical Health

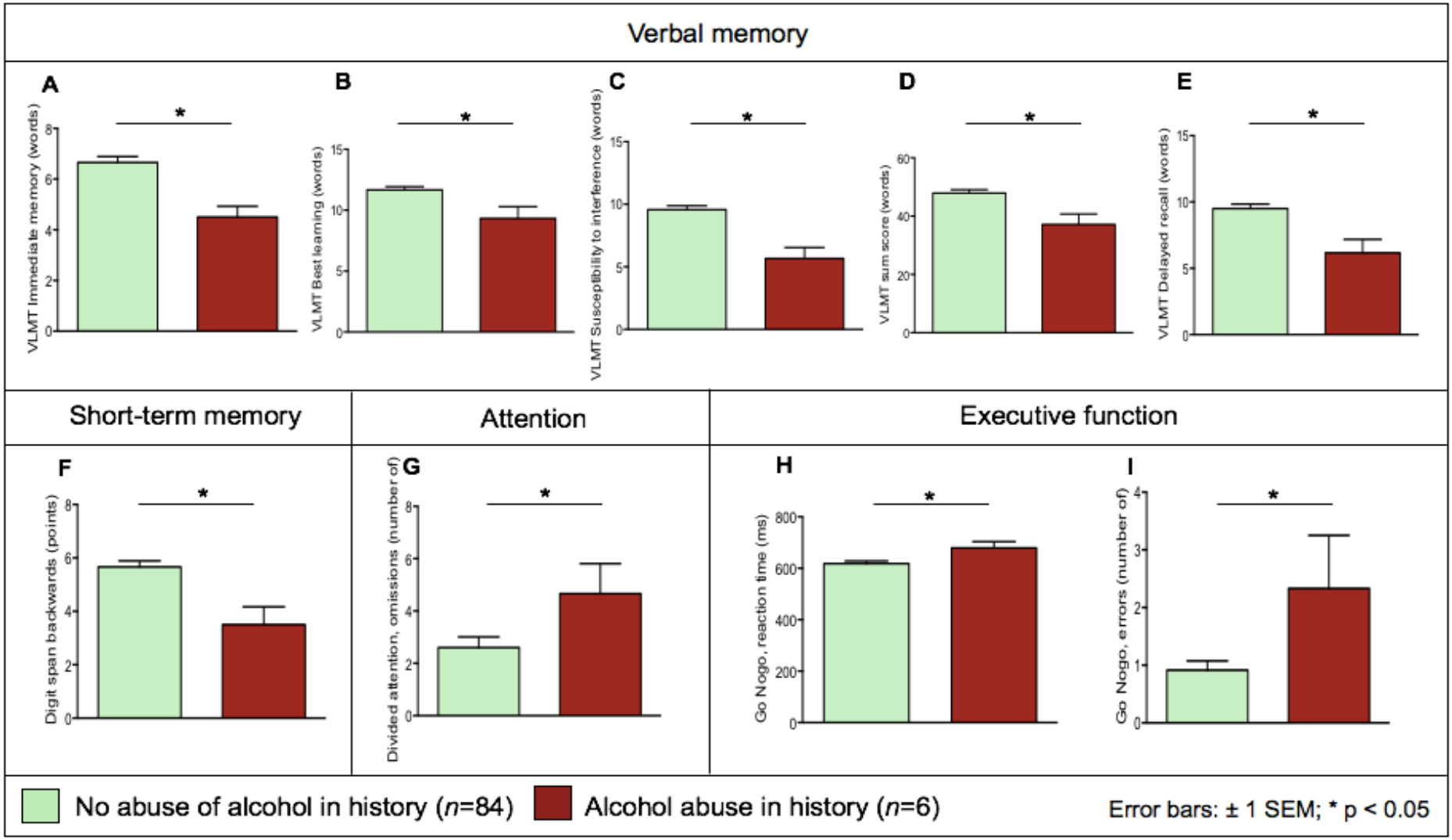
Patients with cognitive impairment had similar test scores for depression and quality of life to patients without cognitive impairment (see Tables 6 and 13). Patients with a higher score in the FACIT questionnaire scored better in the test digit span backwards in the domain short-term memory (ρ=.212, p=0.046), but no significant correlation between the FACIT score and the composite cognitive score was found (ρ=.118, p=0.272). A higher ECOG score correlated with a worse test result in the test divided attention omissions of the attention domain (ρ=.215, p=0.043) but no significant correlation between the ECOG score and the composite cognitive score was found (ρ=-.193, p=0.069).

Medical History

Patients with former use of alcohol performed worse in nine tests in the domains memory, attention, and executive function and also correlated with a lower composite cognitive score (*U*=413.000, *Z*=-2.604, p=0.009; for more details, please refer to Figure 8, Tables 6 and 13).

The use of sedating medication was associated with a worse test result in the delayed recall test for verbal memory (with sedating medication mean 8.2 words vs no sedating medication 9.7 words, *U*=580.500, *Z*=-2.015, p=0.044), but no significant difference concerning the composite cognitive score was found (*U*=683.500, *Z*=-1.167, p=0.243).

Figure 8: Influence of Former Abuse of Alcohol on Cognition



Compared with patients with no abuse of alcohol in history, patients with abuse of alcohol in history named significantly less words in (A) VLMT Immediate memory (6.65 ± 2.16 words (no history of alcohol abuse) versus 4.50 ± 1.05 words (history of alcohol abuse), p=0.009, Mann-Whitney U test), (B) VLMT Best learning (11.69 ± 2.45 words (no history of alcohol abuse) versus 9.33 ± 2.34 words (history of alcohol abuse), p=0.033, Mann-Whitney U test), (C) VLMT Susceptibility to interference (9.58 ± 2.98 words (no history of alcohol abuse) versus 5.67 ± 2.16 words (history of alcohol abuse), p=0.003, Mann-Whitney U test), (D) VLMT Sum score (47.88 ± 10.81 words (no history of alcohol abuse) versus 37.17 ± 8.73 words (history of alcohol abuse), p=0.019, Mann-Whitney U test), and (E) VLMT Delayed recall (9.51 ± 3.07 words (no history of alcohol abuse) versus 6.17 ± 2.48 words (history of alcohol abuse), p=0.017, Mann-Whitney U test), which indicates worse verbal memory. Compared with patients with no abuse of alcohol in history, patients with abuse of alcohol in history scored significantly less points in the (F) Digit span backwards (5.67 ± 2.07 points (no history of alcohol abuse) versus 3.50 ± 1.64 points (history of alcohol abuse), p=0.014, Mann-Whitney U test), which indicates worse short-term memory. Compared with patients with no abuse of alcohol in history, patients with abuse of alcohol in history scored a significantly higher number of omissions in the (G) Divided attention (2.60 ± 3.74 omissions (no history of alcohol abuse) versus 4.67 ± 2.81 omissions (history of alcohol abuse), p=0.030, Mann-Whitney U test), which indicates worse attention. Compared with patients with no abuse of alcohol in history, patients with abuse of alcohol in history needed a significantly higher number of milliseconds in the (H) Go NoGo (618.05 ± 94.66 ms (no history of alcohol abuse) versus 679.17 ± 59.30 ms (history of alcohol abuse), p=0.033, Mann-Whitney U test), and scored a significantly higher number of errors in the (I) Go NoGo (0.92 ± 1.47 errors (no history of alcohol abuse) versus 2.33 ± 2.25 errors (history of alcohol abuse), p=0.044, Mann-Whitney U test), which indicates worse executive function; Error bars: +1SEM, p < 0.05; ROCF, Rey-Osterrieth Complex Figure.*

Treatment Factors

Surprisingly, patients with chemotherapy performed better in six tests for the domains memory, attention, and executive function (for more details, please refer to Tables 6 and 13). However, there was no significant difference between patients with chemotherapy and the composite cognitive score (*U*=887.000, *Z*=-1.871, p=0.061).

Patients being treated with antibody therapy had higher scores in the test for fluid intelligence (LPS: antibody therapy mean 23.1 points vs no antibody therapy mean 19.4 points, *U*=606.500, *Z*=-2.406, p=0.016). Nevertheless, there was no significant difference between patients with antibody therapy and the composite cognitive score (*U*=784.500, *Z*=-.863, p=0.388).

There was no significant difference between being treatment-naive, receiving radiotherapy, or surgery and cognitive performance.

Tumor Stage

No evidence of disease (NED) was associated with a lower prevalence of cognitive deficits using the definition of at least one test score being 2 SD below the reference control group (NED cognitive deficit 4/11 (36.4%) vs no NED cognitive deficit 54/79 (68.4%), p=0.049, Fisher's exact test, Tables 6 and 14). Nonetheless, there was no significant difference between patients with NED and the composite cognitive score (*U*=455.500, *Z*=.259, p=0.796). Patients with metastases scored better results in two tests in the domains fluid intelligence (LPS: metastases mean 22.0 points vs no metastases 18.8 points, *U*=661.000, *Z*=-2.722, p=0.006), and executive function (go/nogo omissions: metastases mean 0.78 omissions vs no metastases mean 0.79 omissions, *U*=1,210.000, *Z*=2.498, p=0.012). However, there was no significant difference between patients with metastases and the composite cognitive score (*U*=831.000, *Z*=-1.331, p=0.183).

For more detailed results of the testing, please refer to Table 6 below (and Tables 13 and 14):

Table 12: Other Factors Associated with Cognitive Impairment Version A

	Significant Difference	Direction of significant difference	Number of subtests with difference[T]	Domains with significant difference[D]	p-value, Test of significance against composite cognitive score
Demographic Factors					
Age	Yes[S]	The higher the age, the worse the test result	18	all	**<0.001[S]**
Sex (Female *n* =32)	Yes[U], No[X]	Women scored better	2	verbal memory, attention	0.781[U]
IQ *(derived by MWT-A test score) *n=86*	Yes[S]	The higher the IQ score, the better the test result	15	all	**<0.001[S]**
Years of education	Yes[S]	The more YoE, the better the test result	11	all	**0.004[S]**
Mental/Physical Health					
FACIT **n=89*	Yes[S]	The higher the FACIT score, the better the test result	1	short-term memory	0.272[S]
ECOG	Yes[S]	The higher the ECOG score, the worse the test result	1	attention	0.069[S]
BDI **n=89*	No[S]		0		0.811[S]
SF12 mental **n=88*	No[S]		0		0.340[S]
SF12 physical **n=88*	No[S]		0		0.933[S]
Medical History					
Former Abuse of Alcohol *n=6*	Yes[U], No[X]	Patients with former abuse of alcohol scored worse	9	verbal memory, short-term memory, attention, executive function	**0.009[U]**
Sedating Medication *n=25*	Yes[U], No[X]	Patients with sedating medication scored worse	1	verbal memory	0.243[U]
Treatment of Tumor					
Treatment naive *n=20*	No[X,U]		0		0.295[U]
Chemotherapy *n=66*	Yes[U], No[X]	Patients with chemotherapy scored better	6	verbal memory, attention, executive function	0.061[U]
Antibody Therapy *n=29*	Yes[U], No[X]	Patients with antibody therapy scored better	1	fluid intelligence	0.388[U]
Radiotherapy *n=16*	No[X,U]		0		0.483[U]
Surgery *(Primary Tumor) n=39*	No[X,U]		0		0.732[U]
Tumor Stage					
NED *n=11*	No[U], Yes[‡]	Patients with NED showed less often a cognitive deficit	CD[C]		0.796[U]
Metastasis *n=51*	Yes[U], No[X]	Patients with metastasis scored better	2	fluid intelligence, executive function	0.183[U]

χ: Chi-square test*; ‡: Fisher's exact test; U:* Mann-Whitney U test*; S: Spearman Correlation; *: If data is missing, the n of patients with known data is displayed. C: Cognitive Deficit with the criteria being at least one test score -2 standard deviations below the reference control group.* n *all=90; T: Overall 22 subtests; D: Overall 6 domains (verbal memory, visuospatial memory, short-term memory, fluid intelligence, attention, executive function).*

Please also additionally refer to Figures 6 - 8 for graphical illustration of these findings.

Table 13: Other Factors Associated with Cognitive Impairment Version B

	Results
Demographic Factors	
Age	The higher the age, the worse the test result in 18 subtests in the domains verbal memory, visuospatial memory, short-term memory, fluid intelligence, attention, executive function.[S]
Sex (Female *n*=32)	Women scored better in 1 subtest in the domain verbal memory, and worse in 1 subtest in the domain attention.[U] No significant difference.[X]
IQ *(derived by MWT-A test score) *n=86*	The higher the IQ score, the better the test result in 15 subtests in the domains verbal memory, visuospatial memory, short-term memory, fluid intelligence, attention, executive function.[S]
Years of education	The more years of education, the better the test result in 11 subtests in the domains verbal memory, visuospatial memory, short-term memory, fluid intelligence, attention, executive function.[S]
Mental/Physical Health	
FACIT **n=89*	The higher the FACIT score, the better the test result in 1 subtest in the domain short-term memory.[S]
ECOG	The higher the ECOG score, the worse the test result in 1 subtest in the domain attention.[S]
BDI **n=89*	No significant difference.[S]
SF12 mental **n=88*	No significant difference.[S]
SF12 physical **n=88*	No significant difference.[S]
Medical History	
Former Abuse of Alcohol *n=6*	Patients with former abuse of alcohol scored worse in 9 subtests in the domain verbal memory, short-term memory, attention, executive function.[U] No significant difference.[X]
Sedating Medication *n=25*	Patients with sedating medication scored worse in 1 subtest in the domain verbal memory.[U] No significant difference.[X]
Treatment of Tumor	
Treatment-naive *n=20*	No significant difference.[X,U]
Chemotherapy *n=66*	Patients with chemotherapy scored better in 6 subtests in the domains verbal memory, attention, executive function.[U] No significant difference.[X]
Antibody Therapy *n=29*	Patients with antibody therapy scored better in 1 subtest in the domain fluid intelligence.[U]

	No significant difference.[X]
Radiotherapy *n=16*	No significant difference.[X,U]
Surgery *(Primary Tumor) n=39*	No significant difference.[X,U]
Tumor Stage	
NED *n=11*	Patients with NED showed less often a cognitive deficit[C,‡] No significant difference.[U]
Metastasis *n=51*	Patients with metastasis scored better in 2 subtests in the domains fluid intelligence, executive function.[U] No significant difference.[X]

*χ: Chi-square test; ‡: Fisher's exact test; U: Mann-Whitney U test; S: Spearman Correlation; *: If data is missing, the n of patients with known data is displayed. C: Cognitive Deficit with the criteria being at least one test score -2 standard deviations below the reference control group. n all=90; Overall 22 subtests in 6 domains (verbal memory, visuospatial memory, short-term memory, fluid intelligence, attention, executive function).*

Table 14: Other Factors Associated with Cognitive Impairment - Cognitive Deficits

	Total *n=90*	ICCTF 1 yes *n=54**	ICCTF 1 no *n=35**	*p*	ICCTF 1.2 yes *n=42*	ICCTF 2 no *n=47**	*p*	ICCFT 2 yes *n=58**	ICCFT 3 no *n=31**	*p*
Person-Related Factors										
Age Mean ± SD (years)	61.2 ± 11.7	63.5 ± 10.52	57.5 ± 12.6		63.3 ± 11.1	59.1 ± 12.0		64.0 ± 10.5	55.7 ± 12.1	
Sex Male	58 (64.4%)	34 (63.0%)	24 (68.6%)	.587^{X}	27 (64.3%)	31 (66.0%)	.869^{X}	35 (60.3%)	22 (71.0%)	.32^{X}
IQ (derived by MWT-A test score)	107.5 ± 16.1 *n=86*	104.2 ± 16.3 *n=50*	112.0 ± 15.2		101.1 ± 15.7 *n=39*	112.8 ± 14.9 *n=46*		104.6 ± 16.5 *n=56*	113.1 ± 14.3 *n=29*	
Years of education Mean ± SD (years)	14.8 ± 3.2	14.9 ± 3.3	14.8 ± 3.1		14.4 ± 3.1	15.2 ± 3.3		14.6 ± 3.2	15.5 ± 3.3	
Mental/Physical Health										
FACIT Mean ± SD	36.6 ± 9.9 *n=89*	35.7 ± 10.2 *n=53*	37.6 ± 9.2		36.1 ± 9.1 *n=41*	36.7 ± 10.4		35.4 ± 10.7 *n=57*	38.6 ± 8.0	
ECOG										
0	46 (51.1%)	25 (46.3%)	20 (57.1%)	.317^{X}	17 (40.5%)	28 (59.6%)	.072^{X}	28 (48.3%)	18 (58.1%)	.379^{X}
1	38 (42.2%)	25 (46.3%)	13 (37.1%)	.394^{X}	21 (50.0%)	17 (36.2%)	.188^{X}	27 (46.6%)	11 (35.5%)	.315^{X}
2	6 (6.7%)	4 (7.4%)	2 (5.7%)	1‡	4 (9.5%)	2 (43%)	.415‡	3 (5.2%)	2 (6.5%)	1‡
BDI Mean ± SD	2.2 ± 2.0 *n=89*	2.1 ± 1.9 *n=53*	2.5 ± 2.2		2.1 ± 1.9 *n=41*	2.4 ± 2.1		2.2 ± 2.0 *n=57*	2.3 ± 2.1	
SF12 mental Mean ± SD	47.4 ± 11.4 *n=88*	45.9 ± 11.6 *n=52*	49.4 ± 11.1		46.4 ± 11.1 *n=40*	48.1 ± 11.8		46.2 ± 11.6 *n=56*	49.2 ± 11.0	
SF12 physical Mean ± SD	39.2 ± 10.6 *n=88*	38.9 ± 10.7 *n=52*	39.2 ± 10.5		38.3 ± 10.2 *n=40*	39.6 ± 10.9		38.6 ± 10.9 *n=56*	40.2 ± 10.4	
Medical History										
Former Abuse of Alcohol	6 (6.7%)	5 (9.3%)	1 (2.9%)	.397‡	5 (11.9%)	1 (2.1%)	.096‡	5 (8.6%)	1 (3.2%)	.661‡
Sedating Medication	25 (27.8%)	18 (33.3%)	7 (20.0%)	.172^{X}	15 (35.7%)	10 (21.3%)	.13^{X}	19 (32.8%)	6 (19.4%)	.18^{X}
Treatment of Tumor										
Treatment naive	20 (22.2%)	13 (24.1%)	7 (20.0%)	.653^{X}	11 (26.2%)	9 (19.1%)	.427^{X}	15 (25.9%)	5 (16.1%)	.295^{X}
Chemotherapy	66 (73.3%)	38 (70.4%)	27 (77.1%)	.482^{X}	28 (66.7%)	37 (78.7%)	.201^{X}	40 (69.0%)	25 (80.6%)	.237^{X}
Antibody Therapy	29 (32.2%)	15 (27.8%)	14 (40.0%)	.229^{X}	17 (40.5%)	17 (36.2%)	.445^{X}	16 (27.6%)	18 (58.1%)	.169^{X}
Radiotherapy	16 (17.8%)	10 (18.5%)	6 (17.1%)	.869^{X}	7 (16.7%)	9 (19.1%)	.761^{X}	10 (17.2%)	6 (19.4%)	.805^{X}
Surgery *(Primary Tumor)*	39 (43.3%)	21 (38.9%)	17 (48.6%)	.367^{X}	17 (40.5%)	21 (44.7%)	.689^{X}	24 (41.4%)	15 (48.4%)	.526^{X}
Tumor Stage										
NED	11 (12.2%)	5 (9.3%)	6 (17.1%)	.330‡	5 (11.9%)	6 (12.8%)	.902^{X}	4 (6.9%)	7 (22.6%)	**.044**‡
Metastasis	51 (56.7%)	29 (53.7%)	21 (60.0%)	.559^{X}	23 (54.8%)	27 (57.4%)	.799^{X}	33 (56.9%)	18 (58.1%)	.915^{X}

χ: *Chi-square test*; ‡: *Fisher's exact test*; *: If data is missing, the n of patients with known data is displayed. N=1 without assignment of ICCTF criteria. Percentages are computed for the known data

Discussion

In this study, over sixty percent of patients with gastrointestinal cancer were cognitively impaired when applying the ICCTF criteria. Twenty percent of all patients were seropositive for neuronal autoantibodies. These neuronal antibodies were associated with impaired verbal fluency and attention deficits, suggesting a potential important role in the context of cancer-related cognitive impairment. Other factors associated with cognitive impairment included age, years of education, and former alcohol abuse, while chemotherapy was not associated with worse cognitive performance.

Gastrointestinal Cancer and Cognitive Impairment

In the current investigation, patients with gastrointestinal cancer had a high prevalence of cognitive impairment. While about one-third of the patients participating in this study stated having a subjective cognitive impairment, in fact, more than half of the patients in this study had a cognitive deficit on objective neuropsychological assessment. This discrepancy between subjective and objective cognitive impairment is in line with previous studies outlining that perceived cognitive impairment and objective cognitive function may not correlate (18,21). When applying the ICCTF criteria, the main affected cognitive domains on objective assessment included attention, executive function, working memory, and verbal learning. Vardy et al. applied the same ICCTF criteria and demonstrated a similar rate of cognitive impairment in similar domains in patients with colorectal cancer (20,21). In their study, 45% of the participants with colorectal cancer had cognitive impairment in the domains attention, working memory, and verbal learning (20,21). Moreover, Regier et al. found comparable rates of cognitive impairment (48%) in patients with head, neck, and gastrointestinal cancer (24). However, they used the Montreal Cognitive Assessment (MoCA) screening tool. Additionally, while van Deudekom et al. found cognitive impairment to be associated with esophageal cancer (14), Visovatti et al. looked at patients with colorectal cancer and found these patients to perform worse in attention tasks compared to a healthy control group (15). These findings support the statement that CRCI is an important complication in gastrointestinal cancer patients (11).

It remains unclear what the mechanisms are behind cognitive impairment or cancer-related cognitive impairment (CRCI) in patients with gastrointestinal cancer (22). For instance, Visovatto et al. and Regier et al. found cognitive impairment to be associated with older age, lower level of education, and fatigue (15,24). So far, extensive research has focused on the role of chemotherapy (16–19). However, Vardy et al. showed cognitive impairment independent of and prior to chemotherapy (20). While they did find high levels of inflammatory cytokines in patients with colorectal cancer, they did not find an association between these cytokines and cognitive impairment (20). Yet, inflammatory mediators may play an important role (13). This may be in line with other immune processes such as autoantibodies against neuronal structures impacting the cognitive function in patients with gastrointestinal cancer (9). As a result, this study argues that patients with gastrointestinal cancer suffer from cancer-related cognitive impairment.

Gastrointestinal Cancer and Neuronal Autoantibodies

In this study, 20.3% of patients with gastrointestinal cancer were antibody-positive with 8.9% harboring antibodies against intracellular antigens and 12.0% against neuronal surface antigens. This is in line with the findings of a previous study by Finke et al. which found neuronal antibodies in 24.5% of more than 300 patients with different types of tumors (9). This sample included 21 patients with gastrointestinal cancer, out of which 11 (52%) were antibody-positive (9). In the current study, the prevalence of neuronal autoantibodies was lower. However, the retrospective study by Finke et al. might have overestimated the prevalence of neuronal antibodies as they had a smaller sample size of patients with gastrointestinal cancer and a potential selection bias when recruiting cancer patients with neurological problems. Moreover, the frequency of antibodies in this study was similar to other types of cancer such as melanoma (10).

Furthermore, while Linnoila et al. found anti-Yo antibodies only in men with gastrointestinal adenocarcinomas (25), no studies have systematically analyzed the prevalence difference of neuronal autoantibodies depending on the sex. In the current analysis, antibody-positive patients were more often male (84%) compared to antibody-negative patients (66%). Moreover, patients with esophageal cancer were less often antibody-positive (6%) compared to the other tumor entities (23%). So far, no study has

systematically investigated the prevalence of neuronal autoantibodies in patients with different tumor entities of gastrointestinal cancer. However, in the current analysis, antibody prevalence was not associated with tumor stage, site of metastases, chemotherapy, or prior neurological disease. In addition, no association between antibody prevalence and age was observed.

It is of note that many new antibodies have been identified in recent years. While onconeural antibodies (being antibodies against intracellular structures (AICAbs)) are associated with paraneoplastic neurological syndromes (5,57), new studies are investigating the effects of the latest identified antibodies. Among those are antibodies against neuronal surface antigens (NSAbs), which can occur with and without an underlying tumor disease. In this study, 10.8% of the patients had an antibody against the NMDA receptor. Interestingly, CSF antibodies against the NMDA receptor can cause NMDAR encephalitis (6). While this condition is associated with CSF IgG antibodies, IgA antibodies have also been found to be associated with cognitive decline and dementia (6–8). Prüss et al. reported patients with cognitive dysfunction to have IgA NMDAR antibodies (7). Doss et al. found IgA and IgM NMDAR antibodies in a high number of patients with dementia (8). The pathogenic effects of IgA and IgM NMDAR antibodies are currently being discussed (8,58,59). Castillo-Gómez et al. argue that all NMDAR autoantibodies have pathogenic potential (58). Hara et al. conclude that while IgG NMDAR antibodies may be specific for anti-NMDAR encephalitis, IgA and IgM antibodies are non-specific and occur in other diseases (59). Moreover, these antibodies have been associated with cognitive impairment in different types of cancer (9,10). Finke et al. observed neuronal antibodies in 25% of patients with different types of cancer, with IgA and IgM NMDAR antibodies being most frequent (9). They further described cognitive deficits to be more prevalent in antibody-positive patients (9). Bartels et al. showed that melanoma patients harbor neuronal antibodies associated with cognitive impairment (10). Here, 5.1% of the patients proved to have an antibody against the NMDA receptor type IgM and another 5.1% against the NMDA receptor type IgA. Only one patient (0.6%) had IgG NMDA receptor antibodies. In addition to NMDA receptor antibodies, AICAbs (mainly ARHGAP26, Ma2(Ta), and Anti-Yo antibodies) were detected. Previous studies on ARHGAP26 have shown an association with cerebellar ataxia (60–62). The Ma2(Ta) antibody has previously been associated with brainstem and limbic encephalitis in patients with lung, testicular, and breast cancer (63–66). Anti-Yo antibodies are

associated with cerebellar degeneration in patients with ovarian, uterine, and breast cancer (67,68).

Previous studies have outlined the importance of neuronal antibodies and paraneoplastic syndromes in patients with gastrointestinal cancer. Linnoila et al. suggest that Purkinje cell cytoplasmic antibody type I (or anti-Yo) may be predictive of gastrointestinal cancer (25). Other studies, however, have associated anti-Yo antibodies primarily with ovarian, uterine, and breast cancer (67,68). Nonetheless, various case reports have described paraneoplastic neurological syndromes in patients with gastrointestinal cancer including GABA-B-receptor encephalitis, limbic encephalitis (with anti-Ma2 antibodies), Guillain-Barré syndrome, and encephalomyelitis (due to anti-Hu antibodies) (26–41,69–72). While this study did not find any patients with anti-GABA-B or anti-Hu antibodies, it did find patients with antibodies targeting ARHGAP26, Ma2, or Yo. However, clinical data from the patients in this study did not suggest classic paraneoplastic syndromes as described in previous case studies. Paraneoplastic syndromes are generally rare, affecting less than 1 in 10,000 cancer patients (73). Yet, the number of the case studies and the high prevalence of neuronal antibodies does arguably suggest an important role of neuronal autoantibodies and potential paraneoplastic syndromes in patients with gastrointestinal cancer.

Therefore, this study suggests that autoantibodies against neuronal structures may play a relevant pathophysiological role in patients with gastrointestinal cancer. Furthermore, paraneoplastic neuronal syndromes associated with autoantibodies may be considered when patients with gastrointestinal cancer display neurological symptoms.

Neuronal Autoantibodies and Cognitive Impairment

This study found that patients with neuronal autoantibodies showed cognitive impairment, specifically in tests for verbal fluency. There are studies outlining worse verbal fluency test scores being associated with neurological and psychiatric diseases such as Parkinson’s disease, Alzheimer's disease, schizophrenia, and bipolar disorder (74–76). Furthermore, case studies outline the detrimental effect of neuronal antibodies against LGI1 and the NMDA receptor on the outcome in verbal fluency tests (77,78). Here, in the

subgroup of patients included in the neuropsychological assessment, primarily NSAbs which were mainly against the NMDA receptor were found. While no patient had LGI1 antibodies, antibodies against GlyR (belonging to NSAbs) and ARHGAP16, Ma2(Ta), CARPVIII, and SOX1 (belonging to AICAbs) were detected. Therefore, neuronal autoantibodies might be involved in CRCI pathophysiology.

This finding is in line with the results of antibodies being associated with cognitive deficits reported by Finke et al. (9). This might well be an essential finding, as CRCI as a potentially antibody-mediated effect might be treatment-responsive using immunosuppressive therapy (7,9). Blood-brain barrier dysfunction has been shown as a potential mechanism leading to cognitive impairment in patients with neuronal autoantibodies. (9). Therefore, the central nervous system might be exposed to potentially pathogenic antibodies. Moreover, a study assessing melanoma patients further argues that neuronal autoantibodies might contribute to CRCI (10). The study by Bartels et al. used the same methodology, had a similar cohort size with participants of similar ages, and with similar years of education as compared to this study (10). While they found a similar antibody prevalence with also primarily antibodies against the NMDA receptor, less patients were cognitively impaired overall compared to this study (10). However, the antibody-positive patients in the study by Bartels et al. were impacted in more domains, including memory, attention, and executive function, as compared to this study (10). When comparing the studies, further differences must be noted, such as more patients receiving chemotherapy, radiotherapy, and immunotherapy in the current study.

While this study observed significantly impaired verbal fluency in antibody-positive patients compared to antibody-negative patients, no difference between antibody-positive and antibody-negative patients regarding the prevalence of an overall cognitive deficit (as defined by the ICCTF) was found. It is of note that the prevalence of antibodies was lower in the subgroup of patients included in neuropsychological assessment (14.4%) compared to the whole cohort (20.3%). Sixty-eight patients were excluded from the neuropsychological testing, out of which 19 patients were antibody-positive. These patients were excluded due to age over 80 years, severely reduced general condition, insufficient German language fluency, or having declined participation. The reduced prevalence of antibodies in the subgroup of patients with cognitive assessment might be

a reason as to why differences in cognitive function did not meet the specified level of significance on statistical tests.

Other Factors Influencing Cognitive Impairment

The following section covers other relevant factors that can influence cognitive impairment in patients with gastrointestinal cancer. While tumor stage, depression, and quality of life were not associated with worse cognitive outcomes, this study found that evidence of active disease, higher age, lower levels of intelligence, fewer years of education, and a former abuse of alcohol were associated with worse cognitive performance. These findings are in line with previous studies investigating the mechanisms for CRCI. Indeed, it has been shown that cancer patients of higher age have an increased risk of cognitive impairment than younger patients (24,79,80). While there have been no studies to our knowledge that have systematically analyzed the association of levels of intelligence, years of education, and former use of alcohol in patients with CRCI, these factors are known to influence cognitive performance in otherwise healthy patients. For instance, fewer years of education are associated with worse cognitive performance (81,82). Moreover, alcohol is well known to cause brain damage and cognitive impairment (83–85).

Generally, most studies on CRCI have examined the effect of cancer treatments such as chemotherapy. Interestingly, chemotherapy was not associated with worse cognitive function in this study. This is in contrast to a number of studies focusing on chemotherapy causing cognitive dysfunction (16–19). Patients with gastrointestinal cancer receiving chemotherapy performed worse in neuropsychological testing, including a decline in executive function as well as verbal memory, and were associated with a higher risk of developing dementia when compared to patients without chemotherapy treatment (16–19). However, a study by Vardy et al. found a high prevalence of CRCI independent of chemotherapy (20). In the current large prospective study, patients with gastrointestinal cancer performed worse in the domains attention/working memory, verbal memory, and processing speed compared to a healthy control group (20). Therefore, cognitive impairment in cancer patients can occur before and independent of cancer treatment (20,86).

In conclusion, the underlying mechanisms of CRCI may include numerous potential factors. The exact causes of CRCI remain largely still unknown, indicating a need for further research. However, neuronal antibodies might represent one potential contributing factor to CRCI, in addition to other factors such as age, active tumor disease, education, and abuse of alcohol.

Limitations and Further Studies

The study entails several limitations and thus warrants further research. Firstly, while all patients participating in the neuropsychological testing were fluent in German, 13 participants were not native speakers. The reference groups established for each test consisted of native speakers who might have had a language advantage when completing the tests. While patients not speaking German as their native language did not have a cognitive deficit more often, they did score worse in two tests (divided attention omissions: not native speaker mean 5.4 omissions vs native speaker 2.3 omissions, U=696.500, Z=2.396, p=0.017, verbal fluency: not native speaker mean 17.2 words vs native speaker mean 24.6 words, U=207.500, Z=-3.333, p=0.001). Nonetheless, there is no significant difference concerning the prevalence of non-native German speakers between antibody-positive and antibody-negative patients (antibody-positive with 23% non-native speakers vs antibody-negative with 10% non-native speakers, p=0.192, Fisher's exact test). Moreover, there is still a significant difference between antibody-positive and antibody-negative patients in the test for verbal fluency even after excluding non-native German speakers from the analysis (U=155.500, Z=-2.475, p=0.013). In addition, the sample size included in neuropsychological assessment - especially of the subgroup of antibody-positive patients - is rather small, so further studies with larger sample sizes are needed to confirm the findings. Moreover, the patient cohort is inhomogeneous with respect to the types of gastrointestinal tumors. Further studies focusing on one specific tumor entity (e.g. gastric cancer) with a larger sample sizes are needed. Further studies should also specifically investigate demographic factors, mental and physical health, medical history, treatment of tumor, and tumor stage. Furthermore, other factors such as the motivation to participate could potentially be important confounder variables and have not been systematically examined. Currently, there is no longitudinal data on the antibody seroprevalence or the clinical data and, therefore,

follow-up research for long-term effects is needed. Finally, patients with positive tissue staining should be further investigated, potentially leading to the detection of novel autoantibodies with distinct clinical phenotypes.

Conclusion

Cognitive impairment is a common symptom in patients with gastrointestinal cancer. Here, over half of the patients had objective cognitive impairment when applying the ICCTF criteria, while one-third of the patients reported subjective cognitive impairment. Twenty percent of the patients were antibody-positive (with 8.9% against intracellular antigens and 12.0% NSAbs) and scored significantly worse in the test for verbal fluency. Therefore, neuronal autoantibodies might potentially be an important factor associated with cancer-related cognitive impairment. Other factors contributing to worse cognitive performance include higher age, lower educational level, former diseases, and cancer treatment. Importantly, CRCI-associated autoantibodies may be treatable with immunosuppressive therapy. Further research (e.g. with larger sample sizes) is needed to confirm and further develop the findings.

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„Ich, Leonie-Sophie Nabben, geb. Dudda, versichere an Eides statt, dass ich die vorgelegte Dissertation mit dem Thema: "Neuronale Autoantikörper und kognitive Beeinträchtigung in Patienten mit gastrointestinalem Tumor - Neuronal Autoantibodies and Cognitive Impairment in Patients with Gastrointestinal Cancer" selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

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Ich versichere ferner, dass ich die in Zusammenarbeit mit anderen Personen generierten Daten, Datenauswertungen und Schlussfolgerungen korrekt gekennzeichnet und meinen eigenen Beitrag sowie die Beiträge anderer Personen korrekt kenntlich gemacht habe (siehe Anteilserklärung). Texte oder Textteile, die gemeinsam mit anderen erstellt oder verwendet wurden, habe ich korrekt kenntlich gemacht.

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Weiterhin versichere ich, dass ich diese Dissertation weder in gleicher noch in ähnlicher Form bereits an einer anderen Fakultät eingereicht habe.

Die Bedeutung dieser eidesstattlichen Versicherung und die strafrechtlichen Folgen einer unwahren eidesstattlichen Versicherung (§§156, 161 des Strafgesetzbuches) sind mir bekannt und bewusst.“

CV LEONIE NABBEN

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

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Statistical Certification

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Bescheinigung

Hiermit bescheinige ich, dass Leonie Dudda innerhalb der Service Unit Biometrie des Instituts für Biometrie und klinische Epidemiologie (iBikE) bei mir eine statistische Beratung zu einem Promotionsvorhaben wahrgenommen hat. Folgende Beratungstermine wurden wahrgenommen:

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Folgende wesentliche Ratschläge hinsichtlich einer sinnvollen Auswertung und Interpretation der Daten wurden während der Beratung erteilt:

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