J Neurol (2017) 264:1968–1977
DOI 10.1007/s00415-017-8582-0

ORIGINAL COMMUNICATION

High prevalence of neuronal surface autoantibodies associated with cognitive deficits in cancer patients

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Received: 25 March 2017 / Revised: 25 July 2017 / Accepted: 26 July 2017 / Published online: 7 August 2017

Abstract The recent discovery of neuronal cell-surface antibodies profoundly expanded the clinical spectrum of paraneoplastic neurological syndromes. Many of these syndromes are associated with impaired cognitive function, a clinical symptom that is of increasing concern in cancer patients. However, the frequency of these antibodies in cancer patients and their relation to clinical syndromes is currently unknown. Here, we investigated the prevalence of neuronal cell-surface antibodies and associated paraneoplastic neurological syndromes in 323 patients with different cancer types and in 105 controls. Cerebrospinal fluid and serum samples were analysed for a large panel of anti-neuronal antibodies and all patients were screened for cognitive deficits. Blood–brain barrier integrity was assessed using the age-normalized albumin cerebrospinal fluid/serum ratio. Anti-neuronal autoantibodies were observed in 24.5% of cancer patients (in contrast to 3.1% in neurological control patients without cancer and 2.5% in healthy controls) and were almost exclusively detected in serum. The majority of antibodies were directed against cell-surface antigens (75.9%), most frequently IgA/IgM isotypes targeting the *N*-methyl-D-aspartate (NMDA) receptor. Cognitive deficits and cerebellar syndromes were significantly more prevalent in antibody-positive in comparison with antibody-negative patients (21 vs. 7%, $p = 2.7 \times 10^{-4}$; 11 vs. 2%, $p = 3.0 \times 10^{-3}$). Antibody-positive patients with cognitive deficits had a significantly increased albumin cerebrospinal fluid/serum ratio in comparison with antibody-positive patients with other neurological deficits, indicating blood–brain barrier dysfunction (49.1×10^{-3} vs. 12.0×10^{-3}; $p = 0.036$). Our results show that anti-neuronal antibodies have a high prevalence in a wide range of different tumour types and are associated with distinct neurological deficits. Specifically, the results suggest a so far undefined cognitive paraneoplastic syndrome in patients with antibodies targeting neuronal surface antigens and concurrent blood–brain barrier dysfunction. Anti-neuronal antibodies might thus serve as a biomarker for potentially treatment-responsive cognitive impairments in cancer patients.

Keywords Cognitive impairment · Dementia · Autoantibodies · NMDA receptor · Cancer · Paraneoplastic syndrome

Introduction

Paraneoplastic neurologic syndromes (PNS) are immune-mediated disorders of the peripheral or central nervous system that are frequently associated with autoantibodies against neural antigens expressed by the tumour [1, 2]. These syndromes cause severe neurological deficits and can antedate clinical tumour manifestation [3]. Discovery of antibodies against intracellular epitopes 30 years ago supported the proposed autoimmune pathophysiology of PNS and facilitated their clinical diagnosis [4]. The recent description of autoantibodies targeting neuronal cell-surface

Electronic supplementary material The online version of this article (doi:10.1007/s00415-017-8582-0) contains supplementary material, which is available to authorized users.

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antigens profoundly expanded the clinical spectrum of PNS and helped to define several new distinct clinical syndromes in which these autoantibodies are directly pathogenic [5, 6]. These autoimmune encephalopathies are associated with autoantibodies of the IgG class and can occur with or without tumour, with antibody prevalence depending on tumour type [7–9]. Recent studies showed that IgA and IgM antibodies similarly cause neurological symptoms, receptor downregulation, and electrophysiological effects [10–12] and that IgA NMDAR antibodies can indicate the presence of an underlying tumour [13]. Most importantly, the majority of patients with IgG isotype anti-neuronal cell-surface antibodies (ANSAb) respond well to tumour resection and immunotherapy, contrasting the poor prognosis in PNS patients with antibodies against intracellular epitopes. In addition, a response to immunotherapy has also been shown in selected patients with IgA NMDAR antibodies [10, 11].

Here, we aimed to (1) investigate the prevalence of neuronal autoantibodies in patients with different cancer types and (2) study their relation to neurological deficits. We report that 24.5% of patients with different tumours harbour anti-neuronal autoantibodies mainly targeting cell-surface structures. We show that ANSAb seropositivity is associated with cognitive deficits and that the presence of these cognitive deficits is associated with blood–brain barrier integrity. These results thus indicate the existence of a cognitive paraneoplastic syndrome that is associated with neuronal surface antibodies.

Methods

Patients and control subjects

Serum and CSF samples from all patients with a tumour diagnosis (solid tumours and haematological malignancies) referred to the Department of Neurology at Charité University Hospital Berlin between 2003 and 2009 were analysed for anti-neuronal antibodies. Of 474 identified patients, both CSF and serum were available for antibody testing from 323 patients (mean age ± SD, 60.7 ± 13.7 years; 57% female) with the following tumour diagnoses: breast cancer ($n = 82$), lung cancer (75), non-Hodgkin lymphoma (40), prostate cancer (23), gastro-intestinal cancers (21), renal and urothelial cancer (15), acute myeloid leukaemia (17), ovarian cancer (10), cervical cancer (9), chronic lymphocytic leukaemia (10), cancer of unknown primary (9), malignant melanoma (7), and Hodgkin's lymphoma (5). Demographic and clinical features of patients are listed in Table 1 and Supplementary Table 1. Patients were referred for work-up and treatment of neurological disorders unrelated to cancer, suspected or proven leptomeningeal carcinomatosis, or suspected paraneoplastic syndromes; an overview of the neurological diagnoses is given in Table 2. All patients underwent full neurological examination. Charts of all patients were reviewed to obtain clinical information on tumour diagnosis, staging, treatment, CSF parameters, and neurologic/neuropsychological examination. Patients with classical paraneoplastic neurological syndromes (PNS, e.g., limbic encephalitis, paraneoplastic cerebellar degeneration) were identified based on established diagnostic criteria [1]. Furthermore, we identified patients with neurological syndromes that are frequently observed in PNS and might be indicative of an undetected PNS, i.e., cerebellar syndrome, epileptic seizures, polyneuropathy, and cognitive deficits. Cognitive deficits were defined to include (1) impairment of higher cognitive functions (e.g., attention, memory, or executive function) as independently assessed from patient documentation, referring to MMSE scores <25 or MoCA scores <26, (2) behavioural changes (such as irritability, agitation, or disinhibition), or (3) new-onset psychosis. Patients with cerebral metastasis were tested for antibodies, but excluded from

Table 1 Demographic data

	All	Antibody negative	Antibody positive	*p* value
Cases	323 (100%)	244 (75.5%)	79 (24.5%)	
Age, mean (range)	60.7 (8–94)	60.1 (8–94)	62.3 (25–86)	0.230†
Sex				
Male	139 (43.0%)	106 (43.4%)	33 (41.8%)	0.794χ
Female	184 (57.0%)	138 (56.6%)	46 (58.2%)	
Karnofsky index, mean (range)	67.6 (5–100)	68.6 (5–100)	64.6 (10–100)	0.297†
Chemotherapy	174 (53.9%)	136 (55.7%)	38 (48.1%)	0.170χ
Radiotherapy	106 (32.8%)	83 (34.0%)	23 (29.1%)	0.649χ
Metastases (noncerebral)	111 (34.4%)	79 (32.4%)	32 (40.5%)	0.993χ
Brain metastases	29 (9.0%)	18 (7.4%)	11 (13.9%)	0.077χ
Leptomeningeal carcinomatosis	61 (18.9%)	45 (18.4%)	16 (20.3%)	0.945χ

n (%) or mean (range) as indicated; † *t* test; χ Chi-square test

Table 2 Neurological diagnoses

Neurological diagnosis	Patients with cancer ($n = 323$)	Neurological control patients without cancer ($n = 65$)	p value
Cognitive deficits	37 (11.5%)	11 (16.9%)	0.222$^{\chi}$
Limbic encephalitis	2 (0.6%)	0 (0.0%)	1.000‡
Cerebellar syndrome	22 (6.8%)	5 (7.7%)	0.484‡
Polyneuropathy	47 (14.6%)	1 (1.5%)	**0.002**‡
Seizures	28 (8.7%)	1 (1.5%)	0.065‡
Mononeuropathies and radiculopathies	29 (9.0%)	7 (10.8%)	0.650$^{\chi}$
Stroke	28 (8.7%)	6 (9.2%)	0.884$^{\chi}$
Headache and pain syndromes	20 (6.2%)	5 (7.7%)	0.588‡
Infectious neurological diseases	8 (2.5%)	2 (3.1%)	0.677‡
Psychiatric diseases	5 (1.5%)	0 (0.0%)	0.595‡
Muscular disease	0 (0.0%)	1 (1.5%)	0.168‡
Multiple sclerosis and other neuroimmunological diseases	2 (0.6%)	5 (7.7%)	**0.002**‡
Movement disorders	2 (0.6%)	8 (12.3%)	**<0.001**‡
Other neurological diseases	93 (28.8%)	13 (20.0%)	0.147$^{\chi}$

Bold values indicate a significant group difference ($p < 0.05$)

Other neurological diseases include e.g., myelitis, amyotrophic lateral sclerosis, Leber's hereditary optic neuropathy (LHON), Fabry's disease, Alzheimer's disease, Still's disease, leukencephalopathy, nonvasculitic autoimmune meningoencephalopathy, Creutzfeld–Jacob disease, etc

‡ Fisher's exact test; $^{\chi}$ Chi-square test

analysis of clinical symptoms; cerebral metastases were detected using MRI. In addition, 65 age-matched patients with various neurological disorders, but without a history of cancer (mean age ± SD, controls vs. patients: 58.7 ± 15.2 vs. 60.7 ± 13.7 years, $p = 0.32$; Table 2) and 40 age-matched healthy blood donors (58.1 ± 14.6 vs. 60.7 ± 13.7 years, $p = 0.31$) were recruited as control subjects. The Charité University Hospital ethics committee approved the study and all patients gave informed written consent for research and publication.

CSF analysis and assessment of blood–brain barrier integrity

For all patients, CSF leukocyte count, lactate, and total protein were analysed. In addition, albumin, total IgG, IgA, and IgM were examined in serum and CSF. Calculation of the albumin CSF/serum ratio (Q_{Alb}) allowed for an assessment of blood–brain barrier integrity in all patients and the age-dependent reference range was calculated using the formula: $Q_{Alb} < [(\text{age in years}/15) + 4] \times 10^{-3}$ [14, 15].

Detection of anti-neuronal antibodies

Serum and CSF samples of all patients were tested for the presence of a large panel of anti-neuronal antibodies by indirect immunofluorescence as follows: biochip mosaics (Euroimmun, Lübeck, Germany) contained cryosections of brain tissue (rat hippocampus, rat cerebellum, and monkey cerebellum) and recombinant cell substrates expressing different neural antigens (NMDAR-NR1a, NMDAR-NR1a/NR2b, AMPAR-GluR1/GluR2, DPPX-IF1, DPPX-IF2, GABAR-B1/B2, LGI1, CASPR2, GLRA1b, mGluR1, mGluR5, MOG, Tr/DNER, AQP4, GAD65, GAD67, ZIC, and ARHGAP26). Goat FITC-labeled anti-human-IgG, -IgA, and -IgM antibodies were used as secondary antibodies (Euroimmun). The following autoantibodies were tested by immunoblotting, using recombinant antigens as antigenic targets: Yo, amphiphysin, Hu, Ri, Ma1, Ma2/Ta, CV2, Sox-1, Recoverin, and Titin. For additional immunohistochemistry, PFA-fixed and unfixed 20 µm rat and unfixed mouse brain sections were cut on a cryostat and mounted on glass slides. Fixed tissue was permeabilized in 0.1% Triton X-100 and unfixed and fixed tissues were blocked in 5% normal goat serum and 2% bovine serum albumine for 1 h. Serum was diluted 1:100 and sections incubated for 15–24 h at 4 °C, washed in PBS, and labeled with a secondary FITC goat anti-167 human antibody (Dianova).

Statistical analysis

Group differences in categorical variables were assessed using Pearson Chi-Square or Fisher's Exact tests and Cramer's Phi (φ) was calculated to assess effect size. One-way ANOVAs and Student's t test were used to analyse CSF data and to determine the relationship between PNS and the albumin CSF/serum ratio. All statistical tests performed were two-sided. Correlation of antibody positivity

and age was assessed using logistic regression. p values ≤ 0.05 were considered significant. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 22.0 (IBM Corp., Armonk, NY, USA).

Results

Anti-neuronal autoantibodies were detected in 79 of 323 tumour patients (24.5%; Fig. 1; Table 3). Antibodies

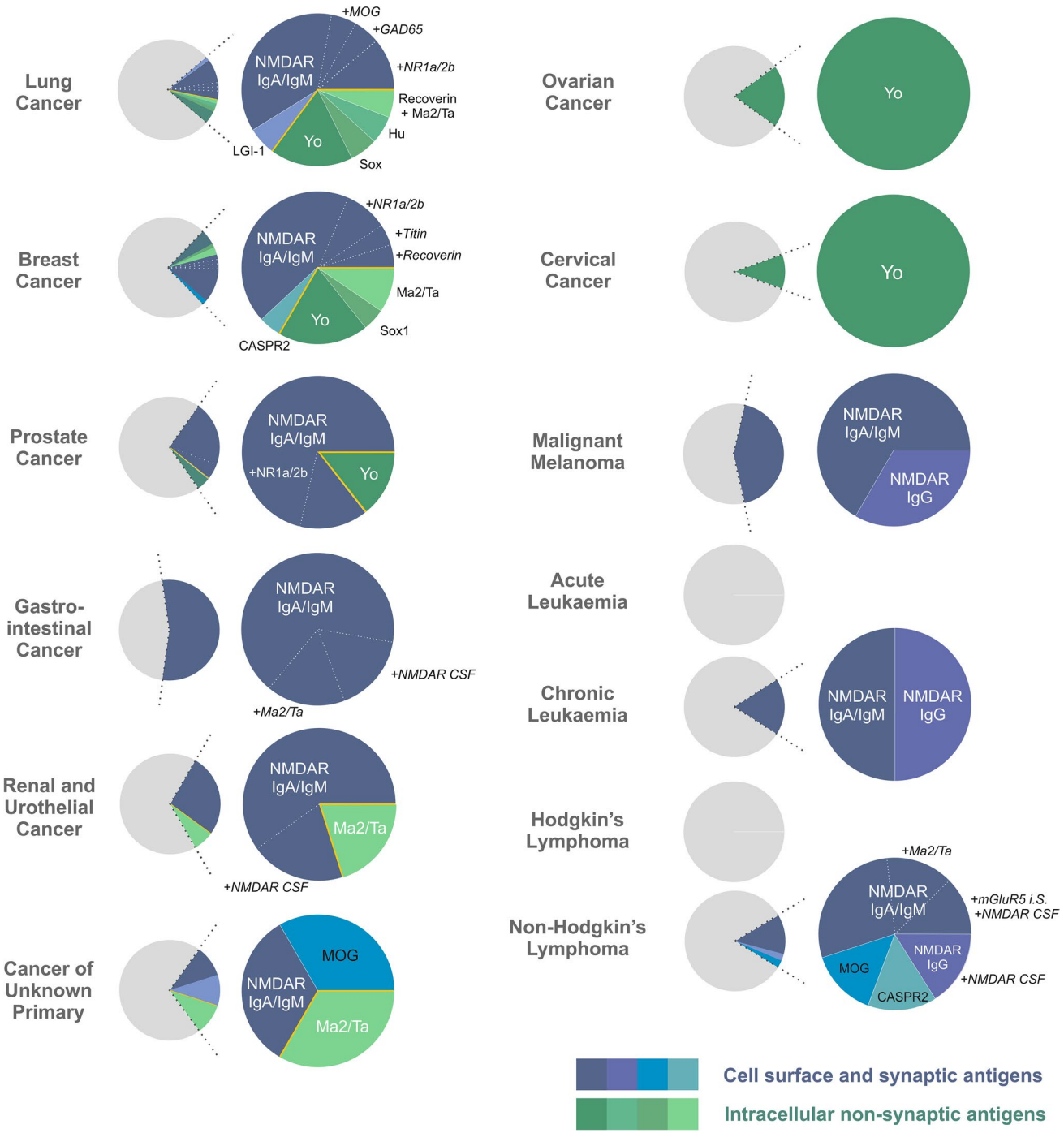

Fig. 1 Antibody Frequency. Frequency of neuronal surface and synaptic antibodies (*blue colours*) and antibodies against intracellular non-synaptic antigens (*green colours*) is shown for all investigated tumour entities. The *smaller circle* depicts overall prevalence for the given tumour type, while the *larger circle* details observed antibody types. *CUP* cancer of unknown primary, *CSF* cerebrospinal fluid, *CASPR2* contactin-associated protein 2, *LGI1* leucine-rich glioma inactivated 1, *MOG* myelin oligodendrocyte glycoprotein, *GAD65* glutamic acid decarboxylase 65, *mGluR5* metabotropic glutamate receptor 5, *Sox1* sex determining region Y-Box 1

Table 3 Antibody frequency

	Lung cancer	Breast cancer	Ovarian cancer	Cervical cancer	Prostate cancer	GIT cancer	Renal and urothelial cancer	Malignant melanoma	Acute leukaemia	Chronic leukaemia	Hodgkin's lymphoma	Non-Hodgkin lymphoma	CUP	Total
Cases	75	82	10	9	23	21	15	7	17	10	5	40	9	323
Antibody positive	17 (22.7%)	21 (25.6%)	2 (20%)	1 (11.1%)	7 (30.4%)	11 (52.4%)	5 (33.3%)	3 (42.9%)	0	2 (20%)	0	7 (17.5%)	3 (33.3%)	79 (24.5%)
Surface and synaptic	11 (14.7%)	14 (17.1%)			6 (26.1%)	11 (52.4%)	4 (26.7%)	3 (42.9%)		2 (20%)		7 (17.5%)	2 (22.2%)	60 (18.6%)
NMDAR	10 (13.3%)	13 (15.9%)			6 (26.1%)	11 (52.4%)	3 (20%)	3 (42.9%)		2 (20%)		5 (12.5%)	1 (11.1%)	54 (16.7%)
IgG								1 (14.3%)		1 (10%)		1 (2.5%)		3 (0.9%)
IgA	4 (5.3%)	5 (6.1%)				2 (9.5%)		1 (14.3%)				1 (2.5%)		13 (4%)
IgM	5 (6.7%)	5 (6.1%)			1 (4.3%)	3 (14.3%)	3 (20%)	1 (14.3%)		1 (10%)		2 (5%)	1 (11.1%)	22 (6.8%)
IgA+IgM	1 (1.3%)	3 (3.7%)			5 (21.7%)	6 (28.6%)						1 (2.5%)		16 (5%)
NMDAR in CSF						2* (+NMDAR)	1 (6.7%)					2* (+NMDAR)		1 (0.3%) [+ 4]
CASPR2		1 (1.2%)										1 (2.5%)		2 (0.6%)
LGI1	1 (1.3%)													1 (0.3%)
NR1a/2b	2* (+NMDAR)	2* (+NMDAR)			5* (+NMDAR)									[+9]
MOG	1* (+NMDAR)											1 (2.5%)	1 (11.1%)	2 (0.6%) [+1]
GAD65	1* (+NMDAR)													[+1]
mGluR5												1* (+NMDAR)		[+1]
Intracellular	6 (8%)	7 (8.5%)	2 (20%)	1 (11.1%)	1 (4.3%)		1 (6.7%)						1 (11.1%)	19 (5.9%)
Hu	1 (1.3%)													1 (0.3%)
Yo	3 (4%)	4 (4.9%)	2 (20%)	1 (11.1%)	1 (4.3%)									11 (3.4%)
Titin		1* (+NMDAR)												[+1]
Recoverin		1* (+NMDAR)												[+1]
Recoverin+Ma2/Ta	1 (1.3%)													1 (0.3%)
Ma2/Ta		2 (2.4%)				2* (+NMDAR)	1 (6.7%)					1* (+NMDAR)	1 (11.1%)	4 (1.2%) [+3]
Sox1	1 (1.3%)	1 (1.2%)												2 (0.6%)

Absolute number (percentage) of patients tested positive for a given antibody

Annotations: [*n*]*(+NMDAR): number of patients positive for given antibody in addition to NMDAR ab positivity

GIT gastro-intestinal tract, *CUP* cancer of unknown primary, *NMDAR N*-methyl-D-aspartate receptor, *Ig* immunoglobulin, *CSF* cerebrospinal fluid, *CASPR2* contactin-associated protein 2, *LGI1* leucine-rich glioma inactivated 1, *MOG* myelin oligodendrocyte glycoprotein, *GAD65* glutamic acid decarboxylase 65, *mGluR5* metabotropic glutamate receptor 5, *Sox1* sex determining region Y-Box1

were almost exclusively found in serum with detection of anti-NMDAR antibodies in both CSF and serum in three patients and in CSF only in one case. Of all patients, 60 (18.6%) had ANSAb or antibodies targeting synaptic antigens and 19 (5.9%) had antibodies against intracellular antigens (AICAb). Antibodies to the NMDAR were the most prevalent and observed in 54 patients (16.7%). Ig classes of NMDAR antibodies were IgM (6.8%), IgA (4%), combination of IgA and IgM (5%), and IgG (0.9%) with titres ranging from 1:10 to 1:3200 (Suppl. Figure 1). Further antibodies were directed against Yo (11 patients), Ma2/Ta (4), CASPR2 (2), MOG (2), SOX1 (2), LGI1 (1), and Hu (1). Multiple antibodies were observed in patients with anti-NMDAR antibodies that were additionally positive for antibodies against NR1a/2b (9), MOG (1), GAD65 (1), mGluR5 (1), Titin (1), Recoverin (1), and Ma2/Ta (3). A combination of anti-recoverin and anti-Ma2/Ta antibodies was observed in one patient. There was no correlation of antibody positivity and age ($b = 0.01$, Wald = 1.44, $p = 0.23$; Suppl. Figure 2) and there was no significant difference in antibody frequency between female (25%) and male (23.7%) patients ($p = 0.79$). In addition, Ab-positive patients did not differ from Ab-negative patients with regard to cancer treatment modality and presence of metastases (Table 1). Antibodies were differentially distributed across tumour types (Table 3). Only ANSAb were found in GIT cancer, malignant melanoma, chronic leukaemia and in non-Hodgkin lymphoma, while in ovarian and cervical cancer, only anti-Yo antibodies (AICAb) were detected. In the remaining tumour entities, both ANSAb and AICAb were observed. CSF analysis revealed elevated mean levels for leukocytes (27.5 ± 5.5/µl), lactate (2.7 ± 0.1 mmol/l), total protein (113.9 ± 13.1 mg/dl), albumin (54.5 ± 5 mg/dl), and albumin CSF/serum ($16.9 \pm 1.8 \times 10^{-3}$; Suppl. Table 2) in patients. CSF parameters were not significantly different between patients without antibodies, patients with ANSAb, and patients with AICAb (all $p > 0.17$). In the two control groups, serum anti-neuronal antibodies were observed in two of 65 control patients with neurological disorders (3.1%; 1 IgA NMDAR ab, 1 IgM NMDAR ab) and in one of 40 healthy controls (2.5%; 1 IgA NMDAR ab).

Sera with the highest IgA NMDAR antibodies showed a somatodendritic binding pattern on granule cells of the dentate gyrus and pyramidal neurons in the cornu ammonis (Suppl. Figure 3). Consistent staining required serum incubation times of >12 h which is in line with previous stainings of human IgA antibodies on brain sections [12] and exceeds the times commonly used in routine diagnostic laboratories for IgG antibodies. Binding of IgA NMDAR antibodies is thereby different to the neuropil distribution known from IgG antibodies of patients with NMDAR encephalitis, but resembles the pattern seen with commercial anti-NR1 antibodies.

We hypothesized that tumour patients with anti-neuronal antibodies (Ab-positive patients) would suffer more frequently from PNS or neurological symptoms that may indicate a PNS than Ab-negative patients. Indeed, PNS and suggestive neurological deficits were significantly more prevalent in Ab-positive patients in comparison with Ab-negative patients (53 vs. 24%; $p = 3.0 \times 10^{-6}$) with significantly more cognitive deficits and cerebellar syndromes in Ab-positive patients in contrast to Ab-negative patients (21 vs. 7%, $p = 2.7 \times 10^{-4}$; 11 vs. 2%, $p = 3.0 \times 10^{-3}$; Fig. 2). With respect to antibody type, AICAb-positive patients were more prone to develop a cerebellar syndrome than Ab-negative and ANSAb-positive patients (26 vs. 2 and 5%; $p = 2.1 \times 10^{-4}$ and $p = 0.02$). In ANSAb-positive patients, cognitive deficits were significantly more frequent than in AICAb-positive and Ab-negative patients (27 vs. 5 and 7%; $p = 0.048$ and $p = 1.1 \times 10^{-5}$). In addition, limbic encephalitis was more frequent in ANSAb-positive than in antibody-negative patients (4 vs. 0%; $p = 0.04$). Given that the majority of ANSAb-positive patients had IgA or IgM NMDAR antibodies, a subgroup analysis with these patients was performed. Compared to Ab-negative patients, cognitive deficits were significantly more frequent in IgA and IgM NMDAR ab-positive patients (31 and 24 vs. 7%; $p = 4.7 \times 10^{-5}$ and $p = 4.9 \times 10^{-4}$) and in the whole IgA/IgM NMDAR ab-positive group (26 vs. 7%; $p = 7.3 \times 10^{-5}$).

Next, we investigated the relationship between blood–brain barrier (BBB) integrity and presence of PNS/suggestive neurological deficits in the Ab-positive group, given that antibodies were predominantly detected in the patients' serum. Intriguingly, albumin CSF/serum ratio was significantly higher in Ab-positive patients with cognitive deficits in comparison with Ab-positive patients without PNS/suggestive neurological deficits (49.1×10^{-3} vs. 12.0×10^{-3}; $p = 0.036$; age-dependent reference value 9.07×10^{-3}; Fig. 3), suggesting an association of BBB disruption with neuronal antibody-associated cognitive deficits. In contrast, cognitive deficits did not correlate with albumin CSF/serum ratio in Ab-negative patients ($p = 0.17$).

About one-fifth of patients had leptomeningeal carcinomatosis, a common cause of cognitive impairment in cancer patients, that was associated with impaired BBB integrity (i.e., increased albumin CSF/serum ratio; $p = 7.6 \times 10^{-9}$, for both Ab-negative and Ab-positive patients). We, therefore, investigated the association of neuronal autoantibodies and cognitive deficits in this subgroup of patients. The antibody frequency in patients with and without leptomeningeal carcinomatosis was not different (26.2 vs. 25.8%; $p = 0.945$). We found a significant correlation of leptomeningeal carcinomatosis and cognitive deficits ($p = 0.015$; effect size $\varphi = 0.16$). However, this correlation was only observed in Ab-positive patients ($p = 0.03$; effect size $\varphi = 0.30$), but not in Ab-negative patients ($p = 0.19$; $\varphi = 0.09$), suggesting that

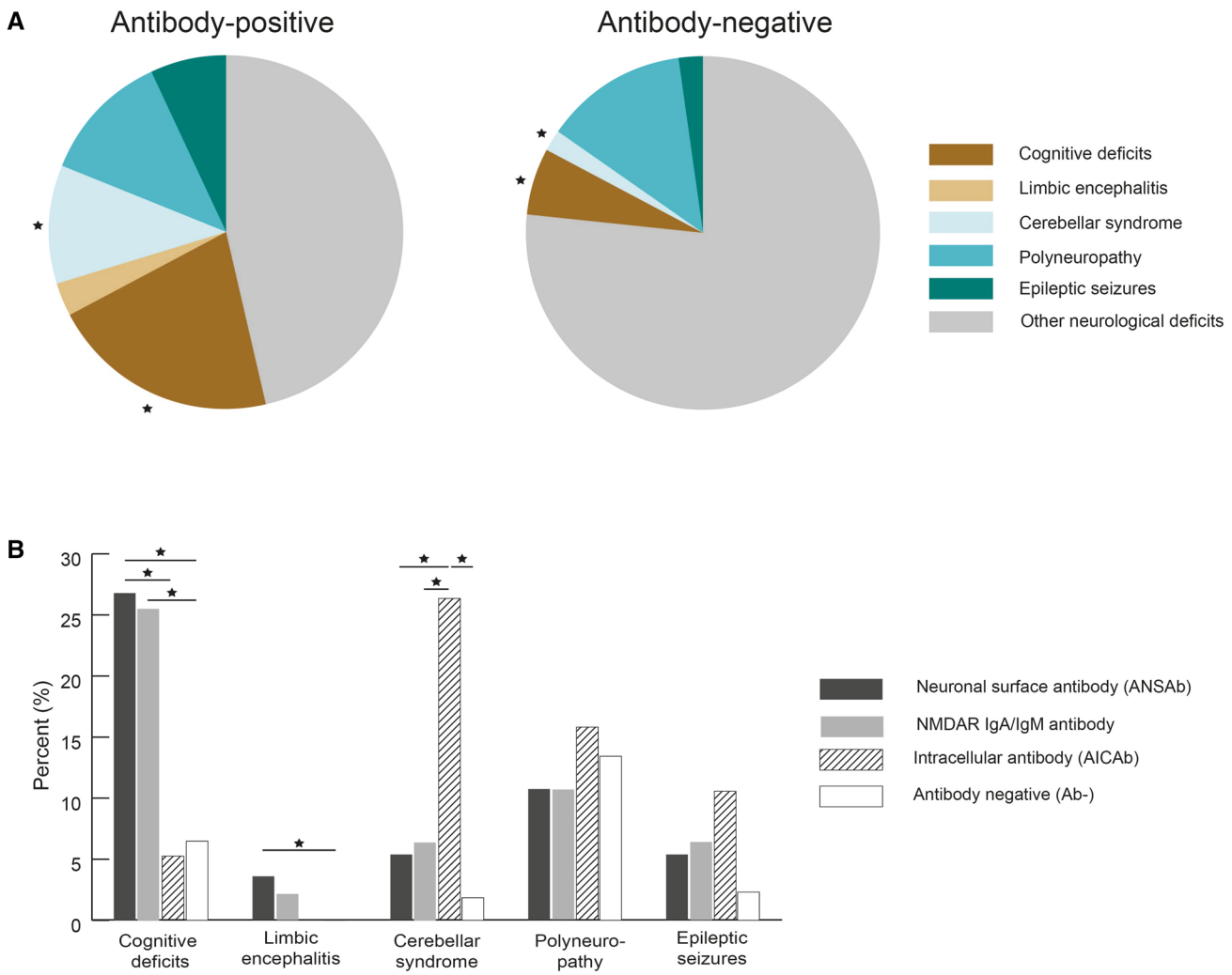

Fig. 2 Neurological deficits in antibody-positive and antibody-negative cancer patients. **a** Neurological deficits in antibody-positive (*left*) and antibody-negative patients (*right*). *Asterisks* indicate significant differences of corresponding neurological deficits. Note the significantly higher frequency of cognitive deficits and cerebellar syndromes in antibody-positive compared to antibody-negative patients. **b** Frequency of neurological deficits in patients with anti-neuronal surface antibodies (ANSAbs), anti-NMDAR IgA/IgM antibodies, anti-intracellular antibodies (AICAbs), and patients without antibodies (Ab−). Anti-NMDAR IgA/IgM antibodies are shown separately as they constitute the largest group of ANSAbs. In ANSAb-positive patients, cognitive deficits were significantly more frequent than in AICAb-positive and Ab-negative patients, and limbic encephalitis was more frequent compared to Ab-negative patients. Similarly, cognitive deficits were significantly more frequent in patients with IgA/IgM NMDAR antibodies compared to Ab-negative patients. Cerebellar syndrome was more frequent in AICAb-positive compared to ANSAb-positive, NMDAR IgA/IgM-positive, and Ab-negative patients

anti-neuronal autoantibodies might contribute to the development of cognitive deficits in patients with leptomeningeal carcinomatosis.

Discussion

Cognitive deficits affect a large proportion of cancer patients and their number is constantly rising given the increase in long-term survivors. Here, we report a high prevalence of neuronal surface antibodies in different tumour entities that were associated with cognitive deficits. Of pathogenic relevance, antibody-associated neurological deficits were linked to blood–brain barrier dysfunction. These results thus might indicate a cognitive paraneoplastic syndrome in ANSAb-positive tumour patients.

IgG NMDAR antibodies are the hallmark of anti-NMDAR encephalitis, an acute and severe neuropsychiatric disease mainly observed in young women that is associated with an ovarian teratoma in about 60% of adult patients [16–19]. Patients suffer from cognitive deficits that frequently persist after the acute disease stage [17, 20, 21]. In vitro and

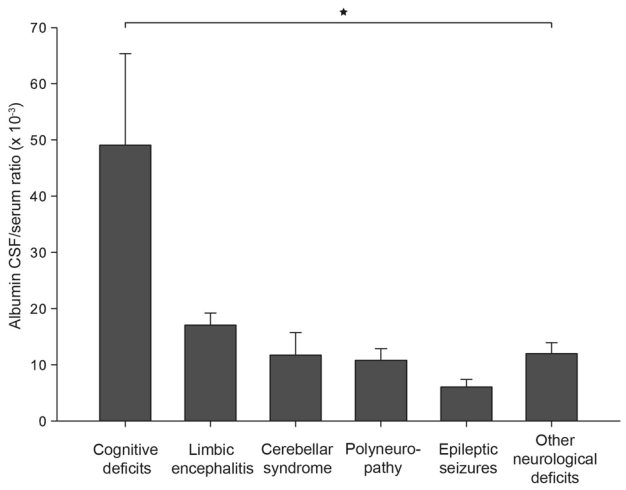

Fig. 3 Blood–brain barrier integrity in antibody-positive patients. Albumin CSF/serum ratio as measure of blood–brain barrier integrity in Ab-positive patients (mean + SEM). In Ab-positive patients with cognitive deficits, a significantly higher albumin CSF/serum ratio indicating impaired blood–brain barrier integrity was observed in comparison with Ab-positive patients without PNS/suggestive neurological deficits

in vivo studies have established that IgG antibodies downregulate neuronal NMDAR and induce electrophysiological dysfunction, thus causing the severe symptoms of the disease [5, 22]. In contrast, in the majority of antibody-positive patients of the present study, serum NMDAR antibodies of the IgA and IgM isotype were found. Recent studies have shown that IgA and IgM NMDAR antibodies can likewise be of pathogenic significance. Serum IgA and IgM NMDAR antibodies were detected in patients with slowly progressive cognitive deficits that significantly improved following immunotherapy [10, 11]. Moreover, IgM and purified IgA NMDAR antibodies reduced the density of NMDAR and other synaptic proteins in a titre-dependent manner and induced a profound decrease of NMDAR-mediated currents [10]. A recent study demonstrated that IgG, IgA, and IgM alike cause NMDAR internalization and reduction of glutamate currents [23]. Here, we observed a high prevalence of serum IgA and IgM NMDAR antibodies in tumour patients, with a prevalence of up to 50% in some tumour types, thus exceeding the prevalence in neurological patients without cancer and in other patient cohorts, including schizophrenia, dementia, and healthy subjects [11, 24–26]. Immunostaining showed that IgA NMDAR antibody-positive (but not control) serum recognized hippocampal neurons, yielding a pattern that is distinct from IgG NMDAR antibodies, thus indicating binding to different epitopes. Tumour patients with ANSAb, and more specifically patients with IgA and IgM NMDAR antibodies, had significantly more cognitive deficits in comparison with AICAb-positive and Ab-negative patients. With regard to current criteria for the diagnosis of paraneoplastic neurological syndromes, the identification of a non-classical clinical syndrome (i.e., cognitive deficits) together with the detection of potentially paraneoplastic neuronal antibodies (i.e., NMDAR antibodies) allows for the proposal of a distinct cognitive paraneoplastic syndrome, which resembles a mild cognitive impairment [1]. Importantly, the here observed neuropsychiatric symptoms of tumour patients might respond to immunotherapy given the good treatment response in patients with IgG ANSAb-mediated disorders that was also reported for selected patients with IgA/IgM-associated cognitive deficits [10, 11, 17, 18].

Since CSF and serum were available from all patients, albumin CSF/serum ratios could be determined to assess individual blood–brain barrier (BBB) integrity. Importantly, an association of the effect of anti-neuronal antibodies and BBB integrity was observed. In Ab-positive patients (but not in Ab-negative patients), albumin CSF/serum ratio was significantly higher in patients with cognitive deficits in comparison with patients with other neurological deficits and more than five-fold increased relative to the age-dependent reference value for our patient cohort. It is thus tempting to speculate that serum anti-neuronal antibodies play a role in cognitive deficits selectively in those patients, where they can access brain antigens because of BBB disruption, as suggested previously [24, 27]. For example, a recent study found increased stroke lesion size in anti-NMDAR-Ab-positive patients with leaky BBB [27]. As an alternative explanation, an impaired BBB integrity could allow for the recognition of neuronal antigens in patients with neuronal decline, leading to the formation of antibodies targeting these brain-restricted antigens [28]. We, furthermore, observed that leptomeningeal carcinomatosis was associated with BBB disruption in all tumour patients. Interestingly, a significant correlation between leptomeningeal carcinomatosis and cognitive deficits was found only in patients harbouring anti-neuronal antibodies, and not in patients without antibodies.

Cognitive deficits in cancer patients are of increasing concern, since they interfere with the social and professional function of patients and significantly reduce their quality of life [29, 30]. Affected cognitive domains include attention, memory, and executive functions, and impairments have been attributed to chemotherapy, fatigue, and to the cancer itself. Of note, it has been shown that cognitive deficits can occur independently from treatment and that they can already be present before initiation of therapy [29]. Proposed mechanisms include a deregulated immune response with triggering of neurotoxic cytokines; however, the exact pathophysiology remains poorly understood [31]. The here observed circulating serum ANSAb might, therefore, contribute to cognitive deficits in cancer patients and may also explain the observed heterogeneity of cognitive dysfunction in these patients. It might further be speculated that anti-neuronal antibodies contribute to the differential

susceptibility to chemotherapy and radiation-induced cognitive impairment in tumour patients, particularly in patients with impaired BBB integrity. These are important open questions that need to be addressed in future prospective studies.

A limitation of the present study is that sample selection was restricted to tumour patients referred to neurology. Nevertheless, we clearly show that neuronal antibody-associated neurological deficits affect a large proportion of cancer patients, likely contributing to their disease severity and conferring a significant reduction in quality of life. Due to the retrospective nature of the present study, we cannot assess whether immunotherapy would have improved the cognitive impairment; however, previous experience with the same ANSAb in related diseases suggests that cognitive deficits might respond well to immunotherapy. We, furthermore, show that BBB dysfunction is related to the pathophysiology of serum ANSAb-mediated cognitive deficits. Future studies are needed that prospectively assess neurological deficits in cancer patients with neuronal antibodies [32]. These studies will allow to disentangle antibody effects on specific cognitive domains, to control for effects of disease activity and cancer therapy, to investigate the response to immunomodulatory treatment, and to evaluate the potential of these antibodies as biomarkers for oncological outcome.

Acknowledgements CF is supported by the Berlin School of Mind and Brain.

Compliance with ethical standards

Conflicts of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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