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Neuronal autoantibodies associated with memory impairment
in prostate cancer patients

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Abbreviations

ADT	Androgen deprivation therapy
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor
ANXA11	Annexin A11
ARHGAP26	Rho GTPase Activating Protein 26
BDI-FS	Beck Depression Inventory-Fast Screen
CUP	Carcinoma of unknown primary
Caspr2	Contactin-associated protein-like 2
CI	Confidence interval
CLL	Chronic lymphocytic leukemia
CNS	Central nervous system
CRCI	Cancer-related cognitive impairment
CRMP5	Collapsin Response Mediator-Protein 5
CSF	Cerebrospinal fluid
EEG	Electroencephalogram
FACIT-F	Functional Assessment of Chronic Illness Therapy-Fatigue
GABAB receptor	γ-aminobutyric acid receptor B
GAD 65	Glutamic acid decarboxylase 65
GlyR	Glycine receptor
GLRA1	Glycine receptor alpha 1b subunit gene
GnRH	Gonadotropine releasing hormone
HEK cells	Human Embryonic Kidney cells
ICCTF	International Cognition and Cancer Task Force
ITPR1	Inositol 1,4,5-Trisphosphate Receptor Type 1
IgA	Immunoglobulin A

IgG	Immunoglobulin G
IgM	Immunoglobulin M
IMP1	Insulin-like growth factor 2 mRNA-binding protein 1
IMP3	Insulin-like growth factor 2 mRNA-binding protein 3
KOC	K-homology domain-containing protein overexpressed in cancer
LEMS	Lambert-Eaton myasthenic syndrome
LGI1	Leucine-rich glioma-inactivated protein 1
LPS	Leistungsprüfsystem
mGluR5	metabotropic glutamate receptor 5
MMSE	Mini Mental Status Examination
MoCA	Montreal Cognitive Assessment
MOG	Myelin oligodendrocyte glycoprotein
MRI	Magnetic Resonance Imaging
MWT-A	Mehrfachwahl-Wortschatz-Intelligenztest-A
NART	National adult reading test
NMDAR	N-methyl-d-aspartate receptor
PERM	Progressive encephalomyelitis with rigidity and myoclonus
PND	Paraneoplastic disorder
PNS	Paraneoplastic syndrome
PRDX6	Peroxiredoxin 6
PSA	Prostate-specific antigen
ROCF	Rey Osterrieth Complex Figure
SCLC	Small cell lung cancer
SD	Standard deviation
SEER	Surveillance, Epidemiology, and End Results Program

SEM	Standard error of the mean
SF-12	Short Form 12 Health Survey
TAP	Tests of Attentional Performance
VGCC	Voltage-gated calcium channel
VLMT	Verbal Learning Memory Test
WMS-R	Wechsler Memory Scale-Revised
ZIC 4	Zic family member 4

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Abstract

Background and Objective

Due to the increasing number of long-term survivors, cancer-related cognitive impairment is attracting growing attention in prostate cancer patients. Although primarily studied in the context of androgen deprivation therapy, cognitive impairment frequently occurs independently of cancer treatment. In recent years, new neuronal autoantibodies directed against cell-surface antigens have been identified. Specifically, antibodies targeting the NMDA receptor are highly prevalent in cancer patients and have been associated with cognitive impairment and dementia.

Therefore, this study aims to assess the prevalence of these antibodies and evaluate their role as potential mediators of cancer-related cognitive impairment in prostate cancer.

Patients and Methods

A total of 187 patients with prostate cancer were recruited at the Department of Urology, Charité-Universitätsmedizin Berlin and local outpatient clinic between February and September 2015. Neuronal autoantibody testing was performed using a well-established commercial assay. A subgroup of 102 patients without confounding factors for cognitive dysfunction underwent neurological examination and comprehensive neuropsychological assessment using a detailed neuropsychological test battery. Prevalence of cognitive impairment was assessed following the definition of the "International Cognition and Cancer Task Force" and compared between antibody-positive and antibody-negative patients, as well as between antibody subgroups.

Results

Neuronal autoantibodies were detected in 18.2% (34/187) of prostate cancer patients. The majority of antibodies were directed against neuronal surface antigens (27/187 [14.4%]), predominantly antibodies of the IgA and IgM isotype directed against the NMDA receptor (26/187 [13.9%]). Antibodies directed against intracellular epitopes were identified in 5.3% (10/187) of patients.

Forty-eight percent (49/102) of patients presented cognitive impairment. Antibody-positive patients had 4-fold higher odds to have a verbal memory deficit compared to antibody-negative patients (33.3% [6/18] vs. 10.7% [9/84]; OR 4.2, 95% CI 1.3-13.8, p=0.014). The subgroup of patients with IgA/IgM NMDA receptor antibodies similarly showed a significantly higher frequency of verbal memory deficits (46.2% [6/13] vs.

10.7% [9/84]; OR 7.1, 95% CI 2.0-26.0, p=0.001). In addition, patients with IgA NMDA receptor antibodies showed reduced performance on visuospatial memory tasks. Logistic and linear regression models confirmed IgA/IgM NMDA receptor antibodies as an independent predictor of memory impairment.

Conclusion

Neuronal autoantibodies, especially antibodies targeting neuronal surface epitopes, occur in a large number of prostate cancer patients. IgA and IgM NMDA receptor antibodies are associated with increased odds for memory impairment. Therefore, these neuronal antibodies, indicative of a tumor-associated immune-response against the nervous system, may represent an important pathogenic factor in cancer-related cognitive impairment in prostate cancer.

Kurzfassung

Hintergrund und Zielsetzung

Tumor-assoziierte kognitive Defizite werden zunehmend als relevante Komplikation von Tumorerkrankungen erkannt. Bei Patienten mit Prostatakarzinom spielt dies eine zunehmende Rolle aufgrund der steigenden Lebenserwartung und des hierdurch vermehrten Langzeitüberlebens. Bisher wurden kognitive Defizite bei Prostatakarzinom-Patienten vorwiegend im Rahmen einer Hormontherapie untersucht. Relevante kognitive Defizite können jedoch auch unabhängig von der Tumortherapie auftreten. In den letzten Jahren wurden neue neuronale Antikörper gegen Zelloberflächenantigene identifiziert. Insbesondere Antikörper gegen den NMDA-Rezeptor zeigen eine hohe Prävalenz in Tumorpatienten und wurden im Zusammenhang mit kognitiven Defiziten und Demenz beschrieben.

In dieser Studie sollen daher die Prävalenz neuronaler Autoantikörper und deren Assoziation mit kognitiven und neurologischen Defiziten bei Patienten mit Prostatakarzinom untersucht werden.

Methoden

Insgesamt wurden 187 Patienten mit Prostatakarzinom zwischen Februar und September 2015 in der Klinik für Urologie, Charité-Universitätsmedizin Berlin und in einer urologischen Fachpraxis eingeschlossen. Die Antikörperdiagnostik erfolgte mittels standardisierter kommerzieller Verfahren. Nach Ausschluss von Patienten mit möglichen Stör- und Einflussfaktoren auf die kognitive Funktion, wurde eine Untergruppe von 102 Patienten neuropsychologisch und neurologisch untersucht. Die Prävalenz kognitiver Defizite wurde nach den standardisierten Kriterien der „International Cognition and Cancer Task Force" bewertet und zwischen Antikörper-positiven und Antikörper-negativen sowie zwischen Antikörper Subgruppen verglichen.

Ergebnisse

Neuronale Antikörper wurden im Serum von 18.2% (34/187) der Prostatakarzinom-Patienten detektiert. Der Großteil der Antikörper war gegen Oberflächenantigene (14.4% [27/187]) gerichtet, am häufigsten fanden sich NMDA-Rezeptor Antikörper (13.9% [26/187]) vom IgA- und IgM-Subtyp. Antikörper gegen intrazelluläre Antigene wurden bei 5.3% (10/187) der Patienten nachgewiesen.

Ein kognitives Defizit wurde in 48% (49/102) der Prostatakarzinom-Patienten identifiziert. Antikörper-positive Patienten zeigten im Vergleich zu den Antikörper-

negativen Patienten signifikant häufiger ein Defizit im verbalen Gedächtnis (33.3% [6/18] vs. 10.7% [9/84]; OR 4.2, CI 1.3-13.8, p=0.014). Die Subgruppe von Patienten mit IgA/IgM NMDA-Rezeptor-Antikörpern zeigte ebenfalls signifikant häufiger ein Gedächtnisdefizit im Vergleich zu den Antikörper-negativen Patienten (46.2% [6/13] vs. 10.7% [9/84]; OR 7.1, 95% CI 2.0-26.0, p=0.001). Zudem zeigte die Patientengruppe mit IgA NMDA-Rezeptor-Antikörpern im Vergleich zu den Antikörper-negativen Patienten ein signifikant schlechteres Testergebnis im Bereich von verbalem und räumlichem Gedächtnis. IgA und IgM NMDA-Rezeptor-Antikörper konnten mittels logistischer und linearer Regression als unabhängige Prädiktoren für Gedächtnisdefizite bestätigt werden.

Fazit

Neuronale Autoantikörper, insbesondere Antikörper gegen neuronale Oberflächenantigene, treten in einem hohen Anteil von Prostatakarzinom-Patienten auf. IgA und IgM NMDA-Rezeptor-Antikörper zeigen einen Zusammenhang mit Gedächtnisdefiziten. Diese neuronalen Oberflächen-Antikörper, hinweisend für eine tumor-induzierte Immunantwort gegen das Nervensystem, stellen deshalb möglicherweise einen wichtigen pathogenetischen Faktor in der Entstehung von tumor-induzierten Gedächtnisdefiziten in Prostatakarzinom-Patienten dar.

1. Introduction

1.1 Cancer-related cognitive impairment

The significant improvement of therapeutic options and survival rates for many types of cancers has resulted in an increasing number of long-term survivors. Therefore, long-term complications after successful cancer treatment are of growing concern. Complications affecting the central nervous system are of particular importance as they significantly impair well-being, functional independence and overall quality of life (1). Cognitive dysfunction in cancer patients with non-central nervous system malignancies, commonly referred to as cancer-related cognitive impairment (CRCI), occurs in up to 75% of cancer patients during or after treatment and can persist for months or years (2). It most frequently affects the cognitive domains of memory, executive function, processing speed and attention. The pathophysiology of CRCI still remains unclear. In 1983, Silberfarb first described cognitive disorders in cancer patients receiving chemotherapy (3). He proposed a multifactorial etiology including concomitant medication, infections, and adverse events of therapy as well as nutritional, metabolic and endocrine disorders. This "chemotherapy-related cognitive impairment" was organized into three categories: cognitive impairment directly related to chemotherapy, cognitive impairment resulting from the cancer itself and cognitive impairment resulting from concurrently administered medications. Since then, CRCI has predominantly been studied in the context of chemotherapy toxicity in breast cancer patients, frequently referred to as "chemobrain" (4, 5). Recently, it has also been described in the context of other cancer treatments such as hormone therapy and targeted therapies (6). In particular, in prostate cancer patients, cognitive impairment following androgen deprivation therapy (ADT) is of particular interest. However, CRCI has also been reported independently of cancer therapy and many patients present impaired cognitive function before start of treatment. In a series of breast cancer patients presented by Wefel et al. (7), 33% exhibited cognitive impairment prior to systemic therapy. Other studies have reported CRCI in up to 40% of breast cancer patients before initiation of chemotherapy (1, 8, 9). Furthermore, multiple neuroimaging studies have revealed functional neurophysiological differences between breast cancer patients prior to systemic treatment and healthy controls (10). In contrast, research on CRCI independent of cancer treatment in other tumor entities remains sparse. While inflammation with increased levels of proinflammatory

cytokines, genetic factors and hormonal status have been suggested as potential pathogenic factors contributing to neurotoxicity in cancer patients (2, 6, 11), the precise underlying mechanisms of CRCI remain unclear.

1.2 Paraneoplastic syndromes

Paraneoplastic syndromes describe a group of disorders associated with an underlying malignancy that are not directly induced by the tumor itself or by its metastases. These disorders can arise from tumor secretion of functional hormones, peptides and cytokines or from immune cross-reactivity between a tumor and healthy organs and tissues (12, 13). Paraneoplastic syndromes have been described in up to 8% of cancer patients (14) and most frequently affect the endocrine system, the nervous system, the hematological system, the immune system and the skin. Paraneoplastic syndromes affecting the nervous system are referred to as paraneoplastic neurological disorders (PND) and are thought to mostly be immune-mediated (15). The ectopic expression of neuronal proteins by a tumor induces an antineuronal immune response associated with a heterogeneous group of disorders involving the central, peripheral and autonomic nervous system.

Most commonly, PND are associated with tumors expressing neuroendocrine proteins such as small cell lung cancer (SCLC) and neuroblastoma, tumors affecting organs with immunoregulatory properties such as thymomas, tumors containing neuronal tissue such as teratomas and tumors deriving from immunoglobulin producing cells such as plasma cell tumors and B-cell lymphomas. While the prevalence of PND ranges from 3-20% in these tumors, they occur in less than 1% of other cancers (16). Classic paraneoplastic neurological syndromes include encephalomyelitis, limbic encephalitis, subacute cerebellar degeneration, opsoclonus-myoclonus, subacute sensory neuronopathy, chronic gastrointestinal pseudo-obstruction, Lambert-Eaton myasthenic syndrome (LEMS) and dermatomyositis (17). These classic paraneoplastic syndromes are induced by well-characterised antibodies directed against intracellular nuclear and cytoplasmatic proteins such as Hu, Yo, Ri and Ma2 or antibodies directed against intracellular synaptic proteins such as glutamic acid decarboxylase (GAD65) and Amphiphysin.

A new group of neuronal antibodies targeting cell-surface and synaptic proteins was first identified by Dalmau in 2007 (18). Important targets of these novel antibodies include the N-methyl-d-aspartate receptor (NMDAR), the α-amino-3-hydroxy-5-methyl-

4-isoxazolepropionic acid receptor (AMPAR), the γ-aminobutyric acid receptor B (GABAB receptor), the leucine-rich glioma-inactivated protein 1 (LGI1), the contactin-associated protein-like 2 (Caspr2), the glycine receptor (GlyR) and the metabotropic glutamate receptor 5 (mGluR5). These cell-surface antibodies are associated with different neurological disorders with distinct characteristic clinical symptoms, disease course and response to treatment (19). The classic PND associated with antibodies directed against intracellular antigens and mediated by a cytotoxic T-cell response often progress rapidly and do not respond to cancer treatment and immunotherapy (16, 20, 21). On the other hand, PND associated with antibodies targeting cell-surface antigens often occur independently of malignancy and are often highly responsive to cancer treatment and immunotherapy (16, 20, 21).

Typical neurological disorders associated with these cell-surface antibodies include anti-NMDA receptor encephalitis caused by NMDA receptor antibodies of the IgG subtype (22). In contrast, serum NMDA receptor antibodies of the IgA isotype were identified in association with slowly progressive cognitive impairment (23) and serum IgA/IgM NMDA receptor antibodies were shown to occur in a significant number of patients with dementia, with up to 60% in the group of patients with "unclassified dementia" (24). These serum NMDA receptor antibodies were significantly more prevalent in dementia patients (16.1% all NMDA receptor antibody isotypes; 9.5% IgM, 4.9% IgA, and 1.7% IgG) compared to cognitively healthy controls (2.8% all NMDA receptor isotypes; 1.9% IgM and 0.9% IgA).

In 2004, before the discovery of neuronal cell surface antibodies, diagnostic criteria for classic paraneoplastic neurological syndromes were developed in order to help clinicians diagnose and classify these disorders and thereby improve the research in this field. Paraneoplastic disorders were divided into the two categories of "definite" and "possible" PNS depending on the presence or absence of cancer, the presence of a "classical" syndrome and the detection of "well-characterized" onconeural antibodies (17).

Diagnostic criteria for paraneoplastic neurological syndromes (PNS) (17)

<u>Definite PNS</u>

1. A classical syndrome and cancer that develops within five years of the diagnosis of the neurological disorder.
2. A non-classical syndrome that resolves or significantly improves after cancer treatment without concomitant immunotherapy provided that the syndrome is not susceptible to spontaneous remission.
3. A non-classical syndrome with onconeural antibodies (well characterized or not) and cancer that develops within five years of the diagnosis of the neurological disorder.
4. A neurological syndrome (classical or not) with well-characterized onconeural antibodies (anti-Hu, -Yo, -CV2, -Ri, -Ma2, or -amphiphysin), and no cancer.

<u>Possible PNS</u>

1. A classical syndrome, no onconeural antibodies, no cancer but at high risk to have an underlying tumour.
2. A neurological syndrome (classical or not) with partially characterized onconeural antibodies and no cancer.
3. A non-classical syndrome, no onconeural antibodies, and cancer present within two years of diagnosis.

However, with the detection of cell-surface antibodies and distinct associated neurological symptoms, expansion and modification of these criteria became necessary. In 2005, seven patients had already been described with a new form of limbic encephalitis, which was shown to be associated with undefined antibodies targeting unknown cell-surface proteins (25). The progressive characterization of the associated antibody targets enabled the classification of these syndromes. Dalmau first described the clinical characteristics of anti-NMDA receptor encephalitis, the best studied group of these disorders to date (22). In 2012, Zuliani et al. provided a detailed description of the clinical features of further well-defined syndromes including limbic encephalitis, Morvan's syndrome, progressive encephalomyelitis with rigidity and myoclonus (PERM) and cerebellar ataxia (26). Furthermore, the authors suggested an updated diagnostic approach and classification founded on the prior criteria by Graus et al. (17), based either on the presence of one of these well-defined neuronal surface antibody syndromes or, in the case of uncharacteristic symptoms, on the presence of the following three criteria: acute/subacute onset of symptoms, evidence of CNS inflammation and exclusion of other causes (26). Diagnostic classification probability was determined by clinical presentation, antibody testing and response to immunotherapy. However, this classification was deemed too reliant on antibody

testing and response to immunotherapy, information not available at the time of disease diagnosis (27). Therefore in 2016, Graus et al. proposed a new algorithm for the diagnosis of these disorders, previously defined as "autoimmune encephalitis" (28), with a more accessible, practical, clinical, syndrome-based approach (27). In order to accelerate diagnosis and improve the outcome, these new guidelines were founded on conventional clinical and neurological assessment and standard diagnostic tests (MRI, CSF, EEG). It was proposed that antibody results may subsequently be used to refine diagnosis and treatment.

However, the constantly emerging detection of new antibodies will potentially require further updates of these diagnostic recommendations.

1.3 Prostate cancer

1.3.1 Epidemiology

As prostate cancer represents one of the most common non-skin cancers in men worldwide and remains among the leading causes of cancer deaths, it poses an important public health concern (29). Prostate cancer mostly occurs in patients over 60 years, with highly variable prevalence, incidence and mortality rates between regions and countries. This is partly linked to the high prevalence of occult disease with diagnosis dependent on detection efforts such as early detection and screening programs (29). Overall, the widespread introduction of prostate-specific antigen screening (PSA) has led to a significant increase of prostate cancer incidence (30, 31). In addition, the increasing proportion of early stage cases is responsible for the continuously growing gap between incidence and mortality. The most recent statistics by the "Surveillance, Epidemiology, and End Results Program" (SEER) reported the rate of new cases at 109.8 per 100,000 men per year and the death rate at 19.0 per 100,000 men per year in the United States with around 192,000 new cases (10.6% of all new cancer cases) and 33,000 deaths (5.5% of all cancer deaths) registered for 2020. The 5-year relative survival rate was estimated at 97.8% and the lifetime risk of developing prostate cancer was estimated at 12.1%. The median age at diagnosis was 66 years with the diagnosis of prostate cancer most frequently occurring among men aged 65-74 years (32). In the current German guidelines on prostate cancer published in May 2019, prostate cancer was reported as the most frequently diagnosed cancer in men in Germany (25.4% of cancers) with a yearly incidence of approximately 60.000

cases per year and as the second deadliest cancer (11.3% of all cancer deaths) with approximately 12.000 deaths per year. Prostate cancer was estimated to be the sixth most frequent cause of death over all (3.1% of deaths). The median age of diagnosis was 69 years (33).

In localized prostate cancer, treatment options include prostatectomy, radiation therapy and active surveillance. In early stages, conservative management including a watchful waiting approach without curative intent often achieves cancer control, especially in patients with coexisting comorbidities limiting treatment options. In locally advanced prostate cancer, either primary radiation therapy combined with androgen deprivation therapy or surgical treatment followed by adjuvant radiation with or without androgen deprivation therapy, are recommended. In advanced prostate cancer requiring systemic treatment, androgen deprivation therapy, chemotherapy or immunotherapy represent possible treatment options (34).

1.3.2 Cognitive impairment, dementia and androgen deprivation therapy in prostate cancer

Besides the well-known association between CRCI and chemotherapy, more recent investigations have also shown cognitive impairment following other cancer treatments, such as hormone therapies and targeted therapies (6). In light of the significant rise in ADT, CRCI has drawn increased attention in prostate cancer patients. Whereas ADT was historically limited to advanced inoperable or metastatic disease, the introduction of widespread PSA testing, the earlier detection and the downward stage migration, have led to the extension of indications for ADT to earlier disease stages (35-37). Shahinian et al. reported a substantial increase of gonadotropin-releasing hormone (GnRH) agonist treatment over the period 1991-1999 (3.7% to 30.9%) across all disease stages and histologic grades of prostate cancer, mostly in patients over 80 years (38). Cooperberg et al. described a significant decrease in watchful waiting for low-risk disease concomitant with an increase in the primary use of ADT (19.4% from 1993-1995 vs. 8.3% from 1999-2001) (37).

Similar to the symptoms of age-related decline in testosterone levels, the side effects of ADT include vasomotor symptoms, osteoporosis, anemia, gynecomastia, depression, sexual dysfunction and overall decrease in quality of life (39). However, considering that over half of all prostate cancer diagnoses occur in men aged over 65 years, the association of ADT with cognitive dysfunction and risk of dementia are

currently a central research focus (40). Due to physiological cognitive ageing, this patient population is already at high risk of developing cognitive decline and dementia. In their detailed review, McHugh et al. reported a multitude of biological research studies supporting the neurophysiologic role of androgens in brain regions critical for memory and higher order cognitive function, as well as the impact of age-related decline in testosterone on cognitive function and association with dementia. Furthermore, they presented multiple neuroimaging studies demonstrating the structural and functional effects of ADT on the brain. In one study, applying blood oxygen level dependent functional MRI, ADT was associated with impaired neurovascular responses (41). Another study showed a significant reduction in grey matter volume under ADT, determined by voxel based morphometry (42).

This evidence supports the hypothesis of a potential adverse effect of ADT on cognition. However, to date, the results of the multiple studies aiming to determine the true impact of ADT on cognitive dysfunction and dementia remain controversial and inconclusive.

1.3.3 Paraneoplastic syndromes in prostate cancer

Prostate cancer is the second most common urological malignancy to be associated with paraneoplastic syndromes after renal cell carcinoma. Although paraneoplastic syndromes generally occur in advanced stages of prostate cancer, mostly of the neuroendocrine or small cell carcinoma histological subtype, they often present the first clinical manifestation of the disease which consequently lead to the initial cancer diagnosis. Around one hundred cases of paraneoplastic syndromes associated with prostate cancer have been reported in the literature including endocrine, dermatological and neurological manifestations (43). To date, PND have only been described in association with onconeural antibodies targeting intracellular epitopes. In the case study by Storstein et al., a total of 37 prostate cancer with paraneoplastic neurological disorders were identified (44). Paraneoplastic cerebellar degeneration, paraneoplastic encephalomyelitis, paraneoplastic limbic encephalitis and subacute sensory neuronopathy were the most frequent clinical manifestations. Anti-Hu antibodies, present in 74% of antibody-positive patients, were the most frequently identified antibodies. Anti-Yo, anti-CV2/CRMP5, anti-Amphiphysin and anti-VGCC antibodies were among the other antibodies detected in a minority of patients. In this cohort of patients, the diagnosis of PND preceded the cancer diagnosis in 50% of

cases. Other case studies of paraneoplastic neurological disorders in prostate cancer reported on a patient with a paraneoplastic brainstem syndrome associated with unknown antibodies (45), paraneoplastic cerebellar degeneration associated with anti-Yo antibodies (46), paraneoplastic cerebellar degeneration associated with anti-mGluR1 antibodies (47) and paraneoplastic limbic encephalitis associated with anti-Hu antibodies (48).

1.4 Relationship between neuronal autoantibodies, cancer and cognitive impairment: New cognitive paraneoplastic syndrome?

The newly identified association between neuronal antibodies targeting cell-surface antigens and cognitive impairment led to the investigation of their role as a potential pathogenic factor in the development of cancer-related cognitive impairment.

In a retrospective study (49), neuronal antibodies were detected in 24.5% of cancer patients including breast cancer, lung cancer, non-Hodgkin lymphoma, prostate cancer, gastrointestinal cancers, renal and urothelial cancer, cervical cancer, chronic lymphocytic leukemia, cancer of unknown primary, malignant melanoma and Hodgkin's lymphoma. In 75.9% of cases, antibodies were directed against cell-surface antigens, most frequently antibodies of the IgA and IgM isotype targeting the NMDA receptor (68%). In the control group, the prevalence of anti-neuronal antibodies was only 5.6%, with 3.1% in neurological control patients without cancer and 2.5% in healthy controls. Cognitive deficits, defined as impairment of higher cognitive functions (independently assessed by patient documentation or determined by „Mini Mental Status Examination" (MMSE) scores < 25/Montreal Cognitive Assessment (MoCA) scores < 26), behavioral changes or new-onset psychosis as well as cerebellar symptoms, were shown to be significantly more frequent in antibody-positive patients. Finke et al. therefore proposed a distinct cognitive paraneoplastic syndrome, resembling mild cognitive impairment, associated with neuronal surface antibodies, warranting further prospective studies to characterize neurological deficits in cancer patients with anti-neuronal antibodies.

In a first prospective study on melanoma patients to further characterize these cognitive syndromes, the association between antibody-positivity and cognitive impairment was confirmed, specifically affecting memory, attention and executive function, with three-fold higher odds in antibody-positive patients. Neuronal

autoantibodies were detected in 22.3% of patients, of which 71.4% were directed against the NMDA receptor, most frequently of the IgA and IgM isotype (50).

To date, studies addressing this question in other tumor entities have not yet been reported.

1.5 Aim of the study

The pathogenic mechanisms of cancer-related cognitive impairment independent of cancer treatment are not yet clearly understood. The aim of this prospective study was to assess the association between neuronal autoantibodies and cognitive impairment in prostate cancer.

To this end, a consecutive cohort of prostate cancer patients was recruited and underwent multiple tests including evaluation of the prevalence of neuronal autoantibodies, detailed neuropsychological assessment of cognitive function and quality of life, and a complete neurological examination.

2. Methods

2.1 Patients

One hundred and eighty-seven patients with prostate cancer were consecutively recruited at the Department of Urology, Charité-Universitätsmedizin Berlin, Germany and at a local outpatient clinic between February and September 2015. All patients with histologically confirmed prostate cancer were enrolled, irrespective of tumor stage and prior to surgical treatment. Sociodemographic data, tumor and treatment details, and medical history were collected by review of patient's charts. The median age was 66.9 years (range: 47-88), the median PSA level at enrollment was 15.8 ng/ml (range: 0.01-159.8), 93.0% (174/187) of patients underwent surgical treatment and 12.3% (23/187) received androgen deprivation therapy. Detailed demographic and clinical data including patient age, tumor staging and grading, prostate cancer treatment, prior malignancies and medical history are provided in Table 1. The study was approved by the ethics committee of Charité-Universitätsmedizin Berlin and all patients provided written informed consent in accordance with institutional and federal guidelines.
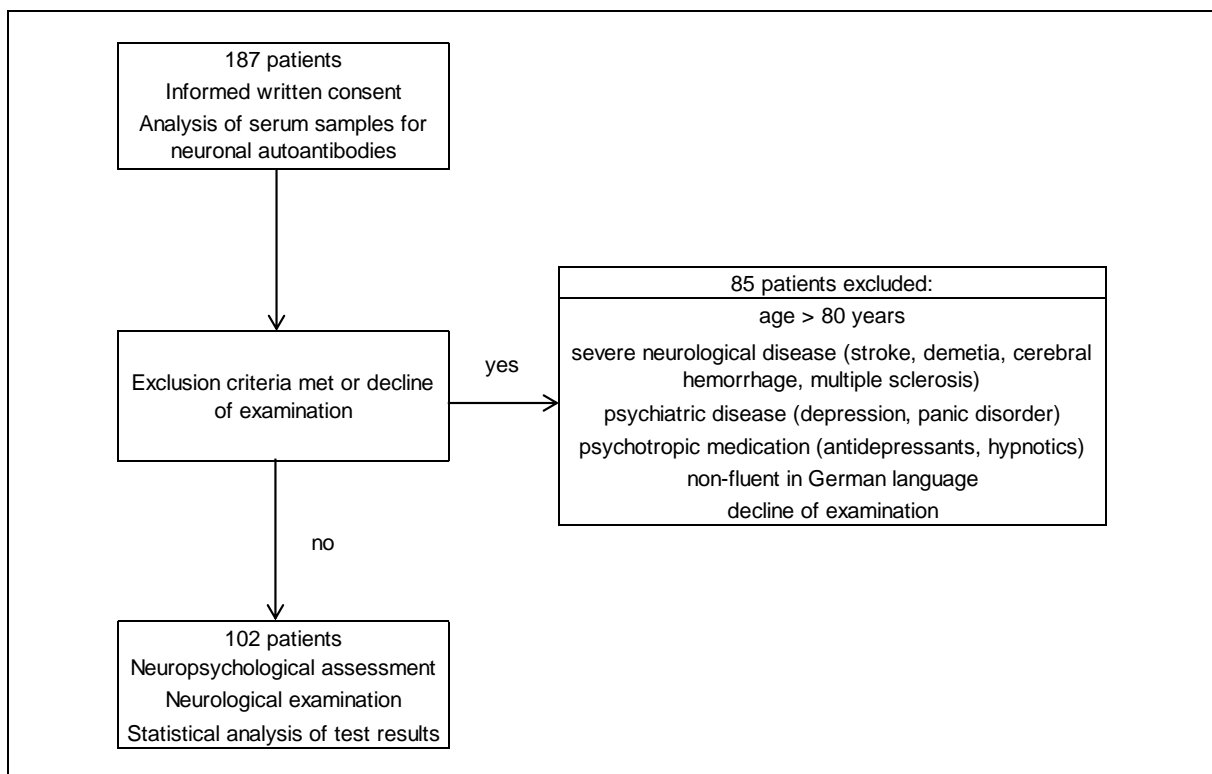
Figure 1. Flow chart patient selection
Graphic representation of the patient recruitment and enrollment process.

Table 1. Demographics of all patients and comparison of antibody-positive vs. antibody-negative patients.

	All patients *(%)* (n=187)	Ab- (%) (n=153)	Ab+ (%) (n=34)	p
Age (years)				
Mean	66.9	66.7	67.5	0.682†
Median	67.0	67.0	67.0	
Range	47-88	47-88	53-84	
T category of primary tumor				
Unknown	7 (3.7)	5 (3.3)	2 (5.9)	
T1	2 (1.1)	2 (1.3)	0	
T2	103 (55.1)	86 (56.2)	17 (50.0)	0.726ˣ
T3	75 (40.1)	60 (39.2)	15 (44.1)	
N category				
Unknown	37 (19.8)	30 (19.6)	7 (20.6)	
N0	121 (64.7)	100 (65.4)	21 (61.8)	0.909ˣ
N1	29 (15.5)	23 (15.0)	6 (17.6)	
M category				
Unknown/Metastasis possible	20 (10.7)	16 (10.5)	4 (11.8)	
M0	150 (80.2)	124 (81.0)	26 (76.5)	0.801ˣ
M1	17 (9.1)	13 (8.5)	4 (11.8)	
M1a (non-regional lymph nodes)	1 (0.5)	1 (0.7)	0	1.000‡
Lymph node metastases	5 (2.7)	4 (2.6)	1 (2.9)	1.000‡
M1b (bone)	11 (5.9)	11 (7.2)	2 (5.9)	0.217‡
Bone metastases	13 (7.0)	11 (7.2)	2 (5.9)	1.000‡
M1c (other sites with or without bone disease)	5 (2.7)	1 (0.7)	4 (11.8)	**0.004‡**
Lung	2 (1.1)	1 (0.7)	1 (2.9)	0.328‡
Liver	3 (1.6)	0	3 (8.8)	**0.005‡**
Multiple metastases	5 (2.7)	3 (2.0)	2 (5.9)	0.220‡
Pretreatment serum PSA level (ng/ml)				
Mean	15.8	14.4	22.3	0.115†
Median	8.4	8.1	10.0	
Range	0.01-159.8	0.01-159.8	0.15-134.0	
Gleason score				
3+3=6	13 (7.0)	11 (7.2)	2 (5.9)	
3+4=7	71 (38.0)	54 (35.3)	17 (50.0)	
4+3=7	50 (26.7)	44 (28.8)	6 (17.6)	0.678ˣ
4+4=8	9 (4.8)	7 (4.6)	2 (5.9)	
4+5=9	40 (21.4)	34 (22.2)	6 (17.6)	

5+5=10	1 (0.5)	1 (0.7)	0	
Unknown	3 (1.6)	2 (1.3)	1 (2.9)	
Grade group				
Grade group 1 (GS ≤ 6)	13 (7.1)	11 (7.3)	2 (6.1)	
Grade group 2 (GS 3+4=7)	71 (38.6)	54 (35.8)	17 (51.5)	
Grade group 3 (GS 4+3=7)	50 (27.2)	44 (29.1)	6 (18.2)	0.572^x
Grade group 4 (GS 8)	9 (4.9)	7 (4.6)	2 (6.1)	
Grade group 5 (GS 9 or 10)	41 (22.3)	35 (23.2)	6 (18.2)	
AJCC prognostic stage group (Eighth Edition 2017)/UICC stage				
I	5 (2.7)	4 (2.6)	1 (2.9)	
II	60 (32.1)	50 (32.7)	10 (29.4)	
IIA	2 (1.1)	2 (1.3)	0	
IIB	37 (19.8)	29 (19.0)	8 (23.5)	
IIC	21 (11.2)	19 (12.4)	2 (5.9)	
III	40 (21.4)	33 (21.6)	7 (20.6)	
IIIA	2 (1.1)	1 (0.7)	1 (2.9)	0.865^x
IIIB	25 (13.4)	20 (13.1)	5 (14.7)	
IIIC	13 (7.0)	12 (7.8)	1 (2.9)	
IV	36 (19.3)	28 (18.3)	8 (23.5)	
IVA	19 (10.2)	15 (9.8)	4 (11.8)	
IVB	17 (9.1)	13 (8.5)	4 (11.8)	
X	46 (24.6)	38 (24.8)	8 (23.5)	
L category (infiltration of lymphatic vessels)				
Unknown	15 (8.0)	11 (7.2)	4 (11.8)	
L0	149 (79.7)	126 (82.4)	23 (67.6)	0.149^x
L1	23 (12.3)	16 (10.5)	7 (20.6)	
V category (infiltration into vein)				
Unknown	16 (8.6)	12 (7.8)	4 (11.8)	
V0	164 (87.7)	136 (88.9)	28 (82.4)	0.565^x
V1	7 (3.7)	5 (3.3)	2 (5.9)	
Perineural invasion				
Yes	139 (74.3)	118 (77.1)	21 (61.8)	
No	38 (20.3)	28 (18.3)	10 (29.4)	0.173^x
Unknown	10 (5.3)	7 (4.6)	3 (8.8)	
Lymphocytic infiltration of primary site				
Yes	63 (33.7)	52 (34.0)	11 (32.4)	
No	71 (38.0)	55 (35.9)	16 (47.1)	0.405^x
Unknown	53 (28.3)	46 (30.1)	7 (20.6)	
Prostate cancer treatment				

Surgical treatment: Radical prostatectomy	174 (93.0)	144 (94.1)	30 (88.2)	0.259‡
Resection margin R0	104 (55.6)	87 (56.9)	17 (50.0)	
Resection margin R1	66 (35.3)	53 (34.6)	13 (38.2)	0.557ˣ
Resection margin RX	4 (2.1)	4 (2.6)	0	
Radiation therapy: External beam radiation therapy or brachytherapy	9 (4.8)	6 (3.9)	3 (8.8)	0.214‡
Androgen deprivation therapy	23 (12.3)	16 (10.5)	7 (20.6)	0.144‡
Current ADT	19 (10.2)	14 (9.2)	5 (14.7)	0.349‡
Chemotherapy	1 (0.5)	0	1 (2.9)	0.182‡
Antibody therapy	1 (0.5)	1 (0.7)	0	1.000‡
Other current/prior malignancy in history				
Yes	29 (15.5)	24 (15.7)	5 (14.7)	0.886ˣ
Hematological malignancies (CLL, lymphoma)	3 (1.6)	2 (1.3)	1 (2.9)	
CLL	1 (0.5)	0	1 (2.9)	
Lymphoma	2 (1.1)	2 (1.3)	0	
Gastrointestinal cancer	6 (3.2)	5 (3.3)	1 (2.9)	
Esophageal squamous cell carcinoma	1 (0.5)+	1 (0.7)	0	
Colon cancer	2 (1.1)	2 (1.3)	0	
Pancreas cancer	1 (0.5)	1 (0.7)	0	
Rectum carcinoma	1 (0.5)	0	1 (2.9)	
Stomach cancer	1 (0.5)	1 (0.7)	0	
Urological cancer	9 (4.8)	8 (5.2)	1 (2.9)	
Bladder carcinoma	5 (2.7)+	5 (3.3) +	0	
Renal cell carcinoma	1 (0.5)	1 (0.7)	0	
Testicular cancer	3 (1.6)	2 (1.3)	1 (2.9)	0.177ˣ
Skin cancer	8 (4.3)	8 (5.2)	0	
Basal cell carcinoma	7 (3.7) +	7 (4.8)	0	
Malignant melanoma	3 (1.6) +	3 (2.0)	0	
Parotid gland carcinoma	1 (0.5)	1 (0.7)	0	
Thyroid carcinoma	1 (0.5)	0	1 (2.9)	
CUP	1 (0.5)	0	1 (2.9)	
Medical history				
Arterial hypertension	105 (56.1)	89 (58.2)	16 (47.1)	0.238ˣ
Diabetes mellitus type 2	27 (14.4)	20 (13.1)	7 (20.6)	0.307ˣ
Urologic and nephrological disease	48 (25.7)	37 (24.2)	11 (32.4)	0.324ˣ
Cardiovascular disease	49 (26.2)	40 (26.1)	9 (26.5)	0.969ˣ
Pulmonary disease	16 (8.6)	14 (9.2)	2 (5.9)	0.741‡

Gastroenterological disease	39 *(20.9)*	32 *(20.9)*	7 *(20.6)*	0.966$^\chi$
Neurological disease *	34 *(18.2)*	27 *(17.6)*	7 *(20.6)*	0.688$^\chi$
Psychiatric disease §	12 *(6.4)*	10 *(6.5)*	2 *(5.9)*	1.000‡
Autoimmune diseaese	7 *(3.7)*	5 *(3.3)*	2 *(5.9)*	0.613$^\chi$
Rheumatic disease	5 *(2.7)*	5 *(3.3)*	0	0.587$^\chi$
Thyroid disease	17 *(9.1)*	13 *(8.5)*	4 *(11.8)*	0.518‡

χ: Chi-square test; ‡: Fisher's exact test; †: Mann-Whitney U test; +: multiple prior malignancies; *: includes (multiple diseases possible): stroke (9 patients), TIA (3), mild cognitive impairment (1), cerebral hemorrhage (2), former hypoxic ischemic encephalopathy (1), pituitary adenoma (1), former/current cluster headache syndrome (2), former/current migraine (3), multiple sclerosis (1), meningoradiculoneuritis due to Lyme disease (2), intervertebral disc disorder with radiculopathy/myelopathy (9), spinal stenosis (3), polyneuropathy (2), essential tremor (2). §: includes: former/current depression (10), former panic disorder (1), burnout (1)

2.2 Autoantibody testing

All patient's sera were tested for a large panel of neuronal autoantibodies directed against intracellular non-synaptic antigens (Ma2, Yo, Hu, ZIC4, ARHGAP26, ITPR1), intracellular synaptic antigens (GAD65, GAD67, Homer3, Amphiphysin) and surface antigens (NMDAR-NR1a/NR1a, pre-GLRA1b, MOG). Antibody analysis was performed by indirect immunofluorescence using well-established commercial cell-based assays (Institute of Experimental Immunology, Euroimmun AG, Lübeck, Germany).

For the BIOCHIP Technology, cover glasses coated with biological substrates (cultured cells or tissue sections) are cut into millimeter-sized fragments (BIOCHIPs). Multiple BIOCHIPs coated with different substrates are then arranged in one reaction field (BIOCHIP Mosaics) so antibodies against various antigens can be investigated simultaneously. In this study, the BIOCHIPs contained both frozen brain tissue sections (rat hippocampus, rat cerebellum, monkey cerebellum) and a battery of fixed recombinant Human Embryonic Kidney (HEK) 293 cells each expressing a different neuronal antigen. The biochip mosaics were incubated with different dilutions of patients' serum, washed, and then incubated with fluorescein-labelled anti-human immunoglobulin (IgG, IgM, or IgA). The results were determined by microscopic evaluation of the fluorescence pattern.

Results for intracellular antigens were confirmed by immunoblot assays (Euroline) as it is recommended to determine autoantibodies using two unrelated methods in the serological diagnostics of paraneoplastic syndromes (51). Immunoblotting uses antigen-antibody specific reactions for identification of a specific protein target. Specific

target antigens are coated on membranes and incubated with patient serum. If specific antibodies are present in the patient sample, they bind to the membrane-bound antigens. Then, an enzyme-labelled antibody is added, which binds to the specific antibodies and catalyzes a color reaction leading to the formation of a dark line at the respective antigen position. The intensity of the band is proportional to the antibody concentration in the sample (51, 52). Euroline assays are multiparameter line blots, which enable the generation of combined antibody profiles on one test strip (51).

2.3 Cognitive assessment, patient-reported outcome measures and neurological examination

A carefully selected subset of 102 patients underwent detailed neuropsychological assessment and a complete neurological examination after exclusion of patients with possible confounding factors for cognitive impairment as defined by the exclusion criteria: age >80 years, non-fluent in German language, history of prior severe neurological or psychiatric diseases, radiographic evidence of brain metastasis, consumption of psychotropic medication. Detailed demographic and clinical data for this subset of patients is provided in Table 2.

Table 2. Demographics of all tested patients and comparison of antibody-positive vs. antibody-negative patients.

	All patients *(%)* (n=102)	Ab- (%) (n=84)	Ab+ (%) (n=18)	p
Age (years)				
Median	67.0	67.0	67.0	0.338[†]
Range	47-79	47-79	59-78	
T category of primary tumor				
Unknown	0	0	0	
T1	1 *(1.0)*	1 *(1.2)*	0	0.895[x]
T2	61 *(59.8)*	50 *(59.5)*	11 *(61.1)*	
T3	40 *(39.2)*	33 *(39.3)*	7 *(38.9)*	
N category				
Unknown	14 *(13.7)*	10 *(11.9)*	4 *(22.2)*	
N0	73 *(71.6)*	62 *(73.8)*	11 *(61.1)*	0.463[x]
N1	15 *(14.7)*	12 *(14.3)*	3 *(16.7)*	
M category				
Unknown/Metastasis possible	50 *(49.0)*	42 *(50.0)*	8 *(44.4)*	
M0	44 *(43.1)*	35 *(41.7)*	9 *(50.0)*	0.787[x]

M1	8 (7.8)	7 (8.3)	1 (5.6)	
M1a (non-regional lymph nodes)	1 (1.0)	1 (1.2)	0	1.000‡
Lymph node metastases	3 (2.9)	3 (3.6)	0	1.000‡
M1b (bone)	6 (5.9)	6 (7.1)	0	0.582‡
Bone metastases	6 (5.9)	6 (7.1)	0	0.582‡
M1c (other sites with or without bone disease)	1 (1.0)	0	1 (5.6)	0.192‡
Lung	0	0	0	
Liver	1 (1.0)	0	1 (5.6)	0.192‡
Multiple metastases	2 (2.0)	2 (2.4)	0	1.000‡
Pretreatment serum PSA level (ng/ml)				
Mean	13.5	14.0	11.1	0.700†
Median	8.0	8.0	9.0	
Range	0.01-159.8	0.01-159.8	0.92-25.1	
Gleason score				
3+3=6	7 (6.9)	6 (7.1)	1 (5.6)	
3+4=7	44 (43.1)	33 (39.3)	11 (61.1)	
4+3=7	26 (25.5)	23 (27.4)	3 (16.7)	0.528ˣ
4+4=8	5 (4.9)	4 (4.8)	1 (5.6)	
4+5=9	2 (19.6)	18 (21.4)	2 (11.1)	
5+5=10	0	0	0	
Grade group				
Grade group 1 (GS ≤ 6)	7 (6.9)	6 (7.1)	1 (5.6)	
Grade group 2 (GS 3+4=7)	44 (43.1)	33 (39.3)	11 (61.1)	
Grade group 3 (GS 4+3=7)	26 (25.5)	23 (27.4)	3 (16.7)	0.528ˣ
Grade group 4 (GS 8)	5 (4.9)	4 (4.8)	1 (5.6)	
Grade group 5 (GS 9 or 10)	2 (19.6)	18 (21.4)	2 (11.1)	
AJCC prognostic stage group (Eighth Edition 2017)/UICC stage				
I	2 (2.0)	2 (2.4)	0	
II	41 (40.2)	35 (41.7)	6 (33.3)	
IIA	0	0	0	
IIB	24 (23.5)	20 (23.8)	4 (22.2)	
IIC	17 (16.7)	15 (17.9)	2 (11.1)	
III	24 (23.5)	21 (25.0)	3 (16.7)	
IIIA	2 (2.0)	1 (1.2)	1 (5.6)	0.502ˣ
IIIB	12 (11.8)	10 (11.9)	2 (11.1)	
IIIC	10 (9.8)	10 (11.9)	0	
IV	19 (18.6)	15 (17.9)	4 (22.2)	
IVA	11 (10.8)	8 (9.5)	3 (16.7)	
IVB	8 (7.8)	7 (8.3)	1 (5.6)	

X	16 (15.7)	11 (13.1)	5 (27.8)	

L category (infiltration of lymphatic vessels)

Unknown	5 (4.9)	5 (6.0)	0	
L0	83 (81.4)	69 (82.1)	14 (77.8)	0.324^x
L1	14 (13.7)	10 (11.9)	4 (22.2)	

V category (infiltration into vein)

Unknown	5 (4.9)	5 (6.0)	0	
V0	93 (91.2)	77 (91.7)	16 (88.9)	0.138^x
V1	4 (3.9)	2 (2.4)	2 (11.1)	

Perineural invasion

Yes	77 (75.5)	65 (77.4)	12 (66.7)	
No	22 (21.6)	17 (20.2)	5 (27.8)	0.570^x
Unknown	3 (2.9)	2 (2.4)	1 (5.6)	

Lymphocytic infiltration of primary site

Yes	35 (34.3)	29 (34.5)	6 (33.3)	
No	46 (45.1)	35 (41.7)	11 (61.1)	0.161^x
Unknown	21 (20.6)	20 (23.8)	1 (5.6)	

Prostate cancer treatment

Surgical treatment: Radical prostatectomy	98 (96.1)	80 (95.2)	18 (100.0)	1.000‡
Resection margin R0	52 (51.0)	43 (51.2)	9 (50.0)	
Resection margin R1	44 (43.1)	35 (41.7)	9 (50.0)	0.676^x
Resection margin RX	2 (2.0)	2 (2.4)	0	
Radiation therapy: External beam radiation therapy or brachytherapy	5 (4.9)	3 (3.6)	2 (11.1)	0.604‡
Androgen deprivation therapy	10 (9.8)	8 (9.5)	2 (11.1)	1.000‡
Current ADT	9 (8.8)	7 (8.3)	2 (11.1)	0.657‡
Chemotherapy	0	0	0	
Antibody therapy	2 (2.0)	2 (2.4)	0	1.000‡

Other current/prior malignancy in history

Yes	18 (17.6)	16 (19.0)	2 (11.1)	0.733^x
Hematological malignancies (CLL, lymphoma)	2 (2.0)	2 (2.4)	0	
CLL	0	0	0	
Lymphoma	2 (2.0)	2 (2.4)	0	
Gastrointestinal cancer	4 (3.9)	3 (3.6)	1 (5.6)	
Esophageal squamous cell carcinoma	0	0	0	
Colon cancer	2 (2.0)	2 (2.4)	0	
Pancreas cancer	1 (1.0)	1 (1.2)	0	

Rectum carcinoma	1 (1.0)	0	1 (5.6)	
Stomach cancer	0	1 (1.2)	0	
Urological cancer	3 (2.9)	3 (3.6)	0	
Bladder carcinoma	1 (1.0)	5 (3.3) +	0	
Renal cell carcinoma	1 (1.0)	1 (1.2)	0	
Testicular cancer	1 (1.0)	1 (1.2)	0	0.457ˣ
Skin cancer	5 (4.9)	5 (6.0)	0	
Basal cell carcinoma	4 (3.9) +	4 (4.8) +	0	
Malignant melanoma	2 (2.0) +	2 (2.4) +	0	
Parotid gland carcinoma	1 (1.0)	1 (1.2)	0	
Thyroid carcinoma	0	0	0	
CUP	1 (1.0)	0	1 (5.6)	
Medical history				
Arterial hypertension	63 (61.8)	53 (63.1)	10 (55.6)	0.550ˣ
Diabetes mellitus type 2	18 (17.6)	13 (15.5)	5 (27.8)	0.309‡
Urologic and nephrological disease	24 (23.5)	16 (19.0)	8 (44.4)	0.032‡
Cardiovascular disease	24 (23.5)	19 (22.6)	5 (27.8)	0.760‡
Pulmonary disease	10 (9.8)	10 (11.9)	0	0.202‡
Gastroenterological disease	21 (20.6)	15 (17.9)	6 (33.3)	0.196‡
Neurological disease *	16 (15.7)	12 (14.3)	4 (22.2)	0.475‡
Psychiatric disease §	3 (2.9)	3 (3.6)	0	1.000‡
Autoimmune diseaese	3 (2.9)	3 (3.6)	0	1.000‡
Rheumatic disease	4 (3.9)	4 (4.8)	0	1.000‡
Thyroid disease	11 (10.8)	8 (9.5)	3 (16.7)	0.405‡

χ: Chi-square test; ‡: Fisher's exact test; †: Mann-Whitney U test; +: multiple prior malignancies; *: includes (multiple diseases possible): intervertebral disc disorder with radiculopathy/myelopathy (7), spinal stenosis (2), cranial neuritis due to Lyme disease (1), former cluster headache syndrome (1), former/current migraine (2), pituitary adenoma (1), TIA (2). §: includes: former depression (2), former panic disorder (1)

Cognitive function was assessed using a detailed neuropsychological battery of standardized tests covering the domains of verbal memory, visuospatial memory, short-term working memory, attention, executive function, semantic fluency, fluid intelligence, and crystallized intelligence.

The following neuropsychological tests were performed: the Verbal Learning Memory Test (VLMT) for the assessment of verbal memory (53), the Rey Osterrieth Complex Figure (ROCF) for the assessment of visuospatial memory (54), digit span forward and backward (Wechsler Memory Scale-Revised, WMS-R) for the assessment of short-term working memory (55), the tonic/phasic alertness and divided attention task from

the computerized test battery for attention assessment (TAP) for the assessment of attention (56), the TAP Go/NoGo task and Stroop test for the assessment of executive function (57), the Regensburger word fluency test for the assessment of semantic fluency (58), the subtest 3 of the Leistungsprüfsystem (LPS, German equivalent to Raven's Progressive Matrices) for the assessment of fluid intelligence (59) and finally the "Mehrfachwahl-Wortschatz-Intelligenztest-A" (MWT-A, the German equivalent to the National adult reading test (NART)) for the assessment of crystallized intelligence (60).

For between-group comparisons of neuropsychological test results, the International Cognition and Cancer Task Force (ICCTF) recommends the use of both disease-specific and healthy controls including both local controls and published normative data (61). In this study, antibody-negative prostate cancer patients represented the disease-specific control group. Published normative means and standard deviation of the respective neuropsychological tests served as healthy control data. A supplementary group of local healthy controls was not included.

Following the recommended ICCTF criteria (61), cognitive impairment was defined by at least one test score two standard deviations (SDs) below the normative control group. The frequency of overall cognitive impairment as well as deficits in individual cognitive domains and subtests were assessed. A domain deficit was defined by one test score from the cognitive domain >2SDs below the normative control group. Z-scores were calculated for comparison of cognitive performance across all cognitive tests and domains, and for calculation of a composite cognitive score. Z-transformation was performed using the group mean and the standard deviation of antibody-negative patients as reference group. The composite cognitive score represents the averaged mean of obtained z-values from 22 subtests.

Quality of life was assessed using the Short Form 12 Health Survey (SF-12) for physical and mental health status. Fatigue was evaluated with the FACIT Fatigue Scale (Version 4) and the Beck Depression Inventory-Fast Screen (BDI-FS) was used to screen for depression.

Neurological examination included a complete assessment of cranial nerves, motor function, sensory function, coordination, and gait.

2.4 Statistical analysis

Continuous data (neuropsychological test scores) were compared by independent t-tests for normally distributed data and by Mann-Whitney U tests for non-normally distributed data. Between-group comparison of categorical data (demographic characteristics, cognitive impairment) was performed using Pearson's Chi-square test or Fisher's exact test. Further predictors for cognitive impairment were analyzed by correlation analysis, multiple linear regression and logistic regression depending on the type of data. All statistical tests were two-tailed and the cut-off for significance was set at p-values <0.05. Adjusted p-values were reported for t-tests when Levene's test for homogeneity of variance was significant. Statistical analysis was performed using IBM SPSS Statistics for Windows, Version 25®.

3. Results

3.1 Neuronal autoantibodies

Neuronal autoantibodies were detected in 34 of 187 prostate cancer patients (18.2%). The majority of antibodies were directed against neuronal surface antigens (27/187 [14.4%]), most frequently against the NMDA receptor (26/187 [13.9%]). Antibodies directed against synaptic and intracellular epitopes were identified in 10 of 187 patients (5.3%). Most of the antibodies targeting the NMDA receptor were of the IgA (11/187 [6.4%]) and IgM isotype (15/187 [8.0%]), all other antibodies were of the IgG isotype.

A carefully selected subgroup of 102 patients underwent detailed neuropsychological testing. In this subgroup, 18 of 102 patients were antibody-positive (17.6%). In 15 patients (14.7%), the antibodies were directed against surface antigens, mostly against the NMDA receptor; in four patients (3.9%), the antibodies were directed against synaptic and intracellular epitopes.

A detailed overview of the identified neuronal antibodies is provided in Table 3.

Table 3. Neuronal autoantibodies in prostate cancer patients.

	All prostate cancer patients (n=187)		Patients included in statistical analysis of neuropsychological results (n=102)	
	No.*	Percent*	No.**	Percent**
Antibody-positive	**34**	**18.2%**	**18**	**17.6%**
One antibody only	25	13.4%	14	13.7%
Combination of two antibodies†	7	3.7%	2	2.0%
Combination of three antibodies‡	2	1.1%	2	2.0%
Surface antigens	**27**	**14.4%**	**15**	**14.7%**
NMDAR	26	13.9%	15	14.7%
NMDAR IgM†‡	*15*	*8.0%*	*9*	*8.8%*
NMDAR IgA†‡	*11*	*5.9%*	*6*	*5.9%*
NMDAR IgG	*4*	*2.1%*	*2*	*2.0%*
pre-GLRA 1b†	2	1.1%	1	1.0%
MOG*‡	1	0.5%	1	1.0%
Synaptic intracellular antigens	**4**	**2.1%**	**3**	**2.9%**
GAD 65‡	2	1.1%	1	1.0%
GAD 67‡	1	0.5%	1	1.0%
Homer3	1	0.5%	1	1.0%
Amphiphysin	1	0.5%	1	1.0%
Intracellular non-synaptic antigens	**6**	**3.2%**	**1**	**1.0%**
Ma2	2	1.1%	0	0
Yo†	1	0.5%	0	0
Hu†	1	0.5%	0	0
ZIC4	1	0.5%	1	1.0%
ARHGAP26†	1	0.5%	0	0
ITPR1†	1	0.5%	0	0

* Numbers do not add up to 100% due to antibody combinations. † Combinations of two antibodies include NMDAR IgM + IgA (n=3), NMDAR IgM + Hu, NMDAR IgM + ITPR1, NMDAR IgA + pre-GLRA1b, Yo + ARHGAP26. ‡ Combinations of three antibodies include NMDAR IgM + IgA + MOG, and NMDAR IgA + GAD65 + GAD67. **Numbers do not add up to 100% due to antibody combinations. † Combination of two antibodies includes NMDAR IgA + pre-GLRA1b, NMDAR IgM + IgA. ‡ Combinations of three antibodies include MOG + NMDAR IgA + NMDAR IgM and GAD65 + GAD67 + NMDAR IgA.

3.2 Cognitive test results

3.2.1 Cognitive impairment in prostate cancer patients

According to ICCTF criteria, 48% (49/102) of all prostate cancer patients that underwent neuropsychological testing showed cognitive impairment. A detailed analysis of individual cognitive domains revealed a verbal memory deficit in 14.7% (15/102), a visuospatial memory deficit in 1% (1/102), a short-term memory deficit in 3.9% (4/102), an attention deficit in 21.6% (22/102) and an executive function deficit in 22.5% (23/102) of prostate cancer patients.

3.2.3 Association between neuronal autoantibodies and cognitive function

In a detailed analysis of individual cognitive domains, we found that antibody-positive patients were significantly more likely to have a verbal memory deficit compared to antibody-negative patients (6/18 [33.3%] vs. 9/84 [10.7%]; p=0.014) and the odds of having a memory deficit was significantly higher in antibody-positive compared to antibody-negative patients (OR=4.2 [95%CI: 1.3-13.8]; p=0.014).

Table 4. Memory deficits in antibody-positive vs. antibody-negative patients: Verbal Learning Memory Test.

1 test score >2 SD below normative control group	Ab+ patients *(N, %)*	Ab- patients *(N, %)*	Odds ratio (OR); 95% CI; p
Sum score	0	1/84 *(1.2%)*	1.000
Trial 1 - Immediate verbal memory	1/18 *(5.6%)*	2/84 *(2.4%)*	1.2; 0.2-28.1; 0.445
Trial 5 - Best verbal learning	0	1/84 *(1.2%)*	1.000
Trial 6 - Susceptibility to interference	4/18 *(22.2%)*	3/84 *(3.6%)*	7.7; 1.6-38.2; **0.017**
Trial 7 - Delayed recall	3/18 *(16.7%)*	2/84 *(2.4%)*	8.2; 1.3-53.3; **0.037**
Verbal recognition	3/18 *(16.7%)*	3/84 *(3.6%)*	5.2; 1.0-28.3; 0.072
Memory domain deficit	6/18 *(33.3%)*	9/84 *(10.7%)*	4.2; 1.3-13.8; **0.014**

As previous research has shown an association between IgA and IgM NMDA receptor antibodies and cognitive impairment and dementia, an independent analysis of this subgroup was performed. Patients with IgA/IgM NMDA receptor antibodies, similarly showed a significantly higher frequency of memory deficits in comparison to antibody-

negative patients (6/13 [46.2%] vs. 9/84 [10.7%]; OR=7.1 [95%CI: 2.0-26.0]; p=0.001). Memory deficits were also more frequent in both individual subgroups of patients with NMDA receptor antibodies of either the IgA isotype (3/6 [50.0%] vs. 9/84 [10.7%]; OR=8.3 [95%CI:1.5-47.6]; p=0.029) or the IgM isotype (4/9 [44.4%] vs. 9/84 [10.7%]; OR=6.7 [95%CI:1.5-29.4]; p=0.020) compared to antibody-negative patients.

Table 5. Memory deficits in NMDAR-Ab+ IgA/IgM vs. Ab-, NMDAR-Ab+ IgA vs. Ab- and NMDAR-Ab+ IgM vs. Ab- patients: Verbal Learning Memory Test.

1 test score >2 SD below normative control group	NMDAR-Ab IgA/IgM (N, %)	Odds ratio (OR); 95% CI; p	NMDAR-Ab IgA (N, %)	Odds ratio (OR); 95% CI; p	NMDAR-Ab IgM (N, %)	Odds ratio (OR); 95% CI; p
Sum score	0	/; /; 1.000	0	/; /; 1.000	0	/; /; 1.000
Trial 1 - Immediate verbal memory	1/13 (7.7%)	3.4; 0.3-40.6; 0.354	0	/; /; 1.000	1/9 (11.1%)	5.1; 0.4-63.0; 0.266
Trial 5 - Best verbal learning	0	/; 1.000	0	/; /; 1.000	0	/; 1.000
Trial 6 - Susceptibility to interference	4/13 (30.8%)	12.0; 2.3-62.3; **0.006**	2/6 (33.3%)	13.5; 1.7-105.0; **0.034**	2/9 (22.2%)	7.7; 1.1-54.1; 0.072
Trial 7 - Delayed recall	3/13 (23.1%)	12.3; 1.8-82.7; **0.016**	2/6 (33.3%)	20.5; 2.3-185.4; **0.021**	2/9 (22.2%)	11.7; 1.4-96.3; **0.045**
Verbal recognition	3/13 (23.1%)	7.8; 1.4-44.0; 0.033	2/6 (33.3%)	13.0; 1.7-101.2; 0.036	2/9 (22.2%)	7.4; 1.1-52.2; 0.076
Memory domain deficit	6/13 (46.2%)	7.1; 2.0-26.0; **0.001**	3/6 (50.0%)	8.3; 1.5-47.6; **0.029**	4/9 (44.4%)	6.7; 1.5-29.4; **0.020**

Furthermore, on a test level, patients with IgA NMDA receptor antibodies showed reduced performance in verbal and visuospatial memory tasks compared to antibody-negative patients: VLMT trial 4 (9.8 vs. 12.2 words, p=0.042), trial 6 (7.7 vs. 10.6 words, p=0.025), trial 7 (7.5 vs. 10.5 words, p=0.034) and recognition (8.5 vs. 12.2 words, p=0.027); ROCF immediate recall (14.0 vs. 20.3 points, p=0.014) and ROCF late recall (13.0 vs. 20.2 points, p=0.005) (Table 6, Figures 2 and 3).

Table 6. Neuropsychological test results in NMDAR-Ab+ IgA vs. Ab- patients: Verbal Learning Memory Test and Rey Osterrieth Complex Figure.

Neuropsychological results (n=102)	Ab- (SD) (n=84)	NMDAR-Ab IgA+ (SD) (n=6)	d	p
Verbal memory (VLMT)				
Sum score (words)	52.4 *(8.8)*	45.7 *(10.3)*	0.74	0.172*
Trial 1 - Immediate memory (words)	6.7 *(1.9)*	6.0 *(2.4)*	0.35	0.440†
Trial 3 (words)	11.2 *(2.2)*	9.7 *(1.8)*	0.70	0.096†
Trial 4 (words)	12.2 *(2.1)*	9.8 *(3.0)*	1.05	**0.042†**
Trial 5 - Best learning (words)	12.8 *(2.0)*	11.3 *(2.3)*	0.73	0.102†
Trial 6 - Susceptibility to interference (words)	10.6 *(3.0)*	7.7 *(2.7)*	0.98	**0.025†**
Trial 7 - Delayed recall (words)	10.5 *(3.1)*	7.5 *(3.1)*	0.97	**0.034†**
Recognition (after 30 min) (words)	12.2 *(3.1)*	8.5 *(5.1)*	1.05	**0.027†**
Visuospatial memory (ROCF)				
Early recall (points)	20.3 *(6.2)*	14.0 *(4.5)*	1.06	**0.016***
Delayed recall (points)	20.2 *(6.0)*	13.0 *(3.8)*	1.27	**0.004***

* normal distribution: T-test, † non-normal distribution: Mann-Whitney U test

T-tests were adjusted when Levene's test for inhomogeneity of variance was significant; d: effect size (Cohen's d)
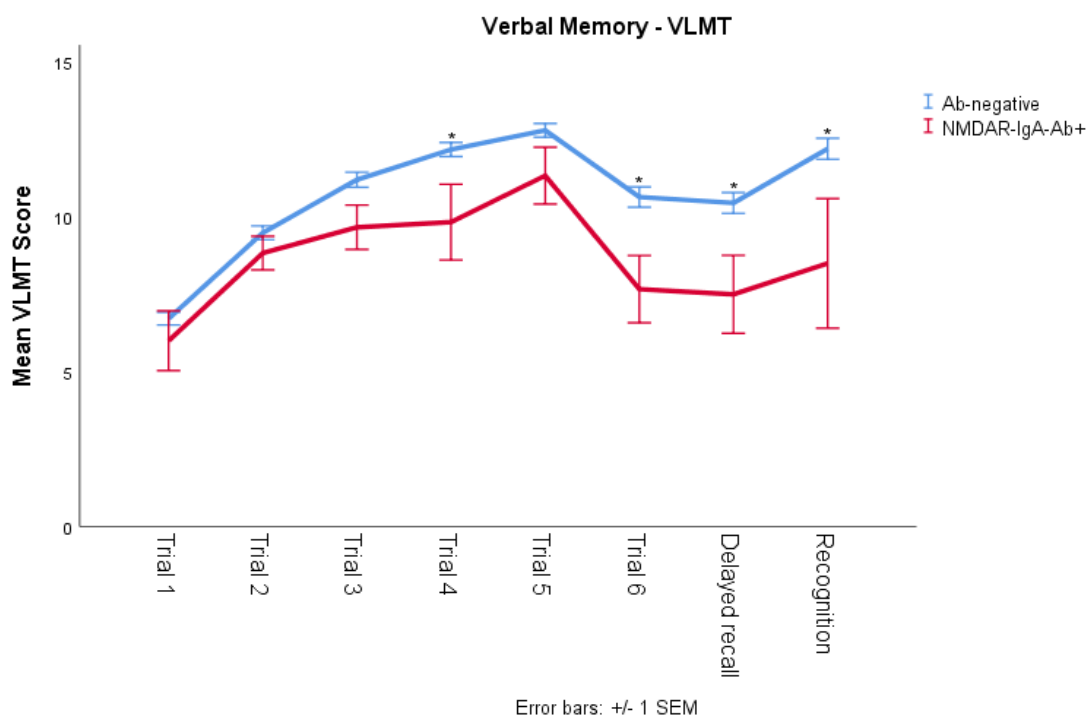

Figure 2. Verbal memory test scores

NMDAR IgA Ab+ patients showed reduced performance in all trials of the VLMT compared to Ab- patients indicating reduced verbal memory. The difference in test scores was significant for trial 4, trial 6, trial 7 and recognition. Error bars: +1 SEM, * p<0.05, ab: antibody, VLMT: Verbal Learning Memory Test.
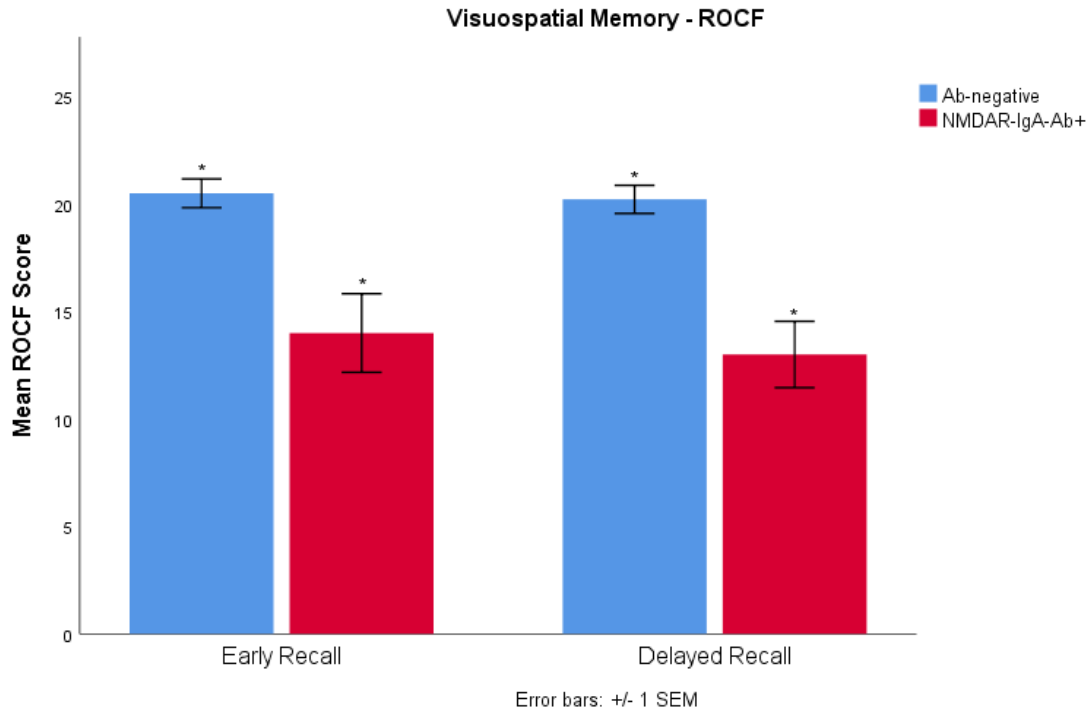
Visuospatial Memory - ROCF

Figure 3. Visuospatial memory test scores
NMDAR IgA Ab+ patients had significantly lower scores compared to Ab- patients in the immediate recall and late recall subtests of the ROCF, indicating reduced visuospatial memory. Error bars: +1 SEM, * p<0.05, ab: antibody, ROCF: Rey Osterrieth Complex Figure.

Finally, test raw scores were standardized to z-scores in order to compare cognitive performance across all cognitive tests and domains and to calculate a composite cognitive score. Z-score transformation confirmed a significantly reduced memory performance in the IgA NMDA receptor antibody subgroup (Figure 4). Patients with IgA NMDA receptor antibodies scored worse in the majority of subtests with significantly lower test scores in trials 6, 7 and recognition of the VLMT as well as in the early and late recall of the ROCF (z-values: VLMT trial 6: -0.99±0.89 vs. 0.00±1.00, t=2.36, d=88, p=0.020; VLMT trial 7: -0.96±0.89 vs. 0.00±1.00, t=2.27, d=88, p=0.026; VLMT recognition: -1.19±1.65 vs. 0.00±1.00, t=2.70, d=88, p=0.008; ROCF early recall: -1.02±0.72 vs. 0.00±1.00, t=2.45, d=87, p=0.016; ROCF late recall: -1.21±0.0.63 vs. -0.00±1.00, t=2.92, d=86, p=0.004). This subgroup also showed a lower composite cognitive score indicating reduced performance in overall cognitive function although this result did not reach the significance level (z-score -0.43±0.44 vs. 0.00±0.54; p=0.064).
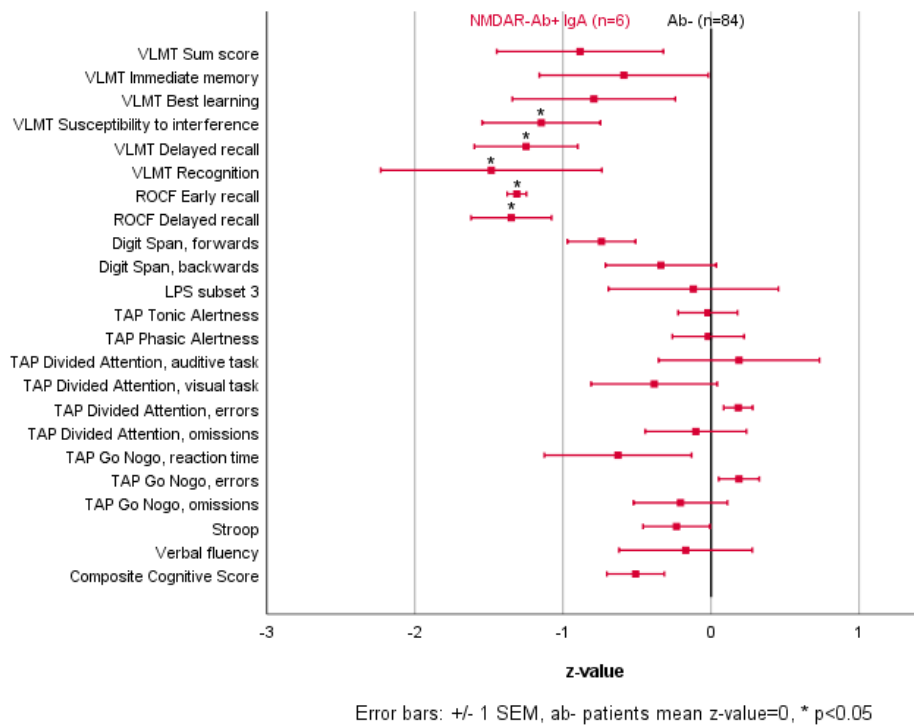
Figure 4. Z-scores and composite cognitive score in patients with NMDAR IgA antibodies
Group comparison between NMDAR IgA Ab+ and Ab- patient's neuropsychological results. Z-transformation of neuropsychological raw data with Ab- patients as reference group: From all test results Ab- patient's group mean was subtracted and then divided by Ab+ patient's SD to set Ab- patient's mean test results to 0 and their SD to 1. Test scales with lower values representing a better performance were multiplied by -1 so that lower values always stand for lower performance. The composite cognitive score was calculated by averaging the obtained z-values from the 22 subtests for every patient.
Significant group differences between cognitive subtests are marked with an asterix (* p<0.05).
NMDAR IgA Ab+ patients scored worse in the majority of subtests with significantly lower test scores in trials 6, 7 and recognition of the VLMT as well as in the ROCF early and late recall. The NMDAR IgA ab+ patients also showed a lower composite cognitive score, however the value did not meet the significance value of p<0.05.
Error bars: ±1 standard error of the mean (SEM); ab: antibody, VLMT: Verbal Learning Memory Test, ROCF: Rey Osterrieth Complex Figure

In order to rule out relevant differences in potential confounding factors for cognitive impairment, a detailed comparison of the examined groups and subgroups was performed. Detailed results are provided in Table 7. Importantly, age and premorbid intelligence level showed no significant differences between antibody-positive and antibody-negative patients or between patients with IgA and IgM NMDA receptor antibodies and antibody-negative patients. The antibody-positive group even had a higher level of education compared to antibody-negative patients (16.7 years vs. 14.6 years; p=0.024).

Table 7. Comparison of mean age, years of education, intelligence level and quality of life scores: antibody-positive vs. antibody-negative patients.

Mean (n=102)	Ab- (SD) (n=84)	Ab+ (SD) (n=18)	d	p
Age (years)	66.1 (7.8)	68.3 (6.2)	0.29	0.338†
Median	67.0 (7.6)	67.0 (6.2)		
Years of education	14.6 (3.2)	16.7 (3.5)	0.64	**0.024†**
Median	14.0 (3.2)	17.0 (3.6)		
MWT-A (words)	31.4 (3.1)	30.9 (2.6)	-0.17	0.273†
IQ	120.7 (13.1)	116.3 (13.6)	-0.33	0.197*
Quality of life				
SF-12 physical health (score)	48.0 (7.6)	46.9 (9.8)	-0.14	0.972†
SF-12 mental health (score)	53.5 (8.2)	55.1 (5.5)	0.21	0.604†
BDI-FS (points)	1.1 (1.4)	1.2 (1.4)	0.07	0.703†
FACIT-Fatigue (points)	41.4 (9.6)	41.7 (7.4)	0.03	0.742†

Table 8. Comparison of mean age, years of education, intelligence level and quality of life scores: IgA NMDAR-Ab+ vs. Ab- patients.

Mean (n=102)	NMDAR-Ab IgA/IgM+ (SD) (n=13)	d	p	NMDAR-Ab IgA+ (SD) (n=6)	d	p
Age (years)	66.7 (5.3)	0.08	0.970†	65.7 (6.9)	-0.05	0.740†
Median	67.0 (5.4)			65.0 (6.9)		
Years of education	15.9 (3.0)	0.41	0.117†	16.5 (3.1)	0.60	0.159†
Median	16.0 (3.1)			17.5 (3.1)		
MWT-A (words)	31.3 (2.2)	-0.03	0.582†	30.8 (2.1)	-0.20	0.282†
IQ	116.9 (14.3)	-0.29	0.338*	116.5 (10.8)	-0.32	0.442*
Quality of life						
SF-12 physical health (score)	47.5 (9.8)	-0.06	0.808†	48.8 (8.3)	0.11	0.796†
SF-12 mental health (score)	55.0 (4.2)	0.20	0.890†	56.5 (3.0)	0.38	0.571†
BDI-FS (points)	1.4 (1.6)	0.21	0.488†	1.3 (2.0)	0.14	0.993†
FACIT-Fatigue (points)	42.9 (7.2)	0.16	0.861†	42.8 (8.5)	0.15	0.839†

* normal distribution: T-test, † non-normal distribution: Mann-Whitney U test

3.2.3 Association between cognitive impairment and androgen deprivation therapy

Recent research has shown that hormonal therapy in prostate cancer patients may be associated with cognitive impairment as a potential side effect. In our patient cohort that underwent neuropsychological testing, 10 patients had a history of androgen deprivation therapy (10/102 [9.8%]), in the subgroup of patients with IgA/IgM NMDA receptor antibodies two patients had a history of ADT (2/13 [15.4%]) and in the subgroup of patients with IgA NMDA receptor antibodies one patient had a history of ADT (1/6 [16.7%]). To evaluate the effects of androgen deprivation therapy (ADT) in

our patient cohort, we first compared cognitive function between patients with a history of ADT and hormone therapy naïve patients. While patients with a history of ADT showed significantly reduced performance in the domain of visuospatial memory (ROCF early recall: ADT+ vs. ADT-: 1.3 vs. 20.7 points; p=0.009; ROCF late recall: ADT+ vs. ADT-: 14.7 vs. 20.7 points; p=0.003), they did not present an increased risk for a memory deficit as defined by the ICCTF criteria (Table 9).

Table 9. Memory deficits in ADT+ vs. ADT- patients: Verbal Learning Memory Test and Rey Osterrieth Complex Figure.

1 test score >2 SD below normative control group	ADT+ patients (N, %)	ADT- patients (N, %)	Odds ratio (OR); 95% CI; p
Sum score	0	1/92 (1.1%)	/; /; 1.000
Trial 1 - Immediate verbal memory	0	3/92 (3.3%)	/; /; 1.000
Trial 5 - Best verbal learning	0	1/92 (1.1%)	/; /; 1.000
Trial 6 - Susceptibility to interference	2/10 (20.0%)	5/92 (5.4%)	4.4; 0.7-26.1; 0.139
Trial 7 - Delayed recall	1/10 (10.0%)	4/92 (4.3%)	2.4; 0.2-24.3; 0.410
Verbal recognition	1/10 (10.0%)	5/92 (5.4%)	1.9; 0.2-17.8; 0.481
Verbal memory domain deficit	2/10 (20.0%)	13/92 (14.1%)	1.5; 0.3-8.0; 0.639
Early recall	0	1/92 (1.1%)	/; /; 1.000
Delayed recall	0	0	/; /; /
Visuospatial memory domain deficit	0	1/92 (1.1%)	/; /; 1.000

To exclude ADT as a potential confounder of the observed association between neuronal autoantibodies and cognitive impairment, multiple analyses were performed. First, we assessed a potential association between androgen deprivation therapy and neuronal antibody seroprevalence. The prevalence of neuronal autoantibodies, both antibodies targeting the NMDA receptor and antibodies targeting intracellular antigens, were similar between patients with a history of ADT compared to patients without ADT (Table 10).

Table 10. Neuronal autoantibodies in ADT+ vs. ADT- patients.

Neuronal antibodies	ADT+ patients (N, %)	ADT- patients (N, %)	Odds ratio (OR); 95% CI; p
Antibody-positive	2/10 (20.0%)	16/92 (17.4%)	1.2; 0.2-6.1; 1.000
NMDAR IgA/IgM	2/10 (20.0%)	11/92 (12.0%)	1.8; 0.3-9.8; 0.612
NMDAR IgM	1/10 (10.0%)	8/92 (8.7%)	1.2; 0.1-10.4; 1.000
NMDAR IgA	1/10 (10.0%)	5/92 (5.4%)	1.9; 0.2-18.4; 0.470
NMDAR IgG	0	2/92 (2.2%)	/; /; 1.000
Intracellular antibodies	1/10 (10.0%)	3/92 (3.3%)	3.3; 0.3-35.1; 0.342

Second, exclusion of patients with a history of ADT from the analyses did not alter the significant association identified between neuronal autoantibodies and memory deficits in prostate cancer patients: deficits in the cognitive domain of memory remained significantly more frequent in antibody-positive patients compared to antibody-negative patients (5/16 [31.3%] vs. 8/76 [10.5%]; OR=3.9; 95%CI:1.1-14.0; p=0.046). Similar results were observed in the subgroup of patients with IgA/IgM NMDA receptor antibodies (5/11 [46.2%] vs. 8/76 [10.5%]; OR=7.1; 95%CI:1.8-28.6; p=0.010) and in the subgroup of patients with IgA NMDA receptor antibodies (3/5 [60.0%] vs. 8/76 [10.5%]; OR=12.8; 95%CI:1.8-88.1; p=0.016). Furthermore, when comparing individual test results, patients with IgA receptor antibodies remained significantly more likely to present verbal and visuospatial memory deficits compared to antibody-negative patients (VLMT trial 6: 7.4 vs. 10.7 words; p=0.024; VLMT trial 7: 7.4 vs. 10.5 words, p=0.049; VLMT recognition: 8.0 vs. 12.2 words; p=0.037; ROCF early recall: 14.6 vs. 20.8 points; p=0.030; ROCF late recall: 13.5 vs. 20.8 points; p=0.008).

Third, a logistic regression model including IgA NMDA receptor antibody seroprevalence, ADT, age, distant metastasis, and years of education as predictor variables confirmed IgA NMDA receptor antibodies, but not ADT, as an independent predictor of a verbal memory deficit (Table 11). The logistic regression model was statistically significant (χ^2=14.523, p=0.013), explained 42.3% (Nagelkercke R^2) of the variance in memory deficits and correctly classified 86.5% of cases. In a similar model to evaluate IgA/IgM NMDA receptor antibodies as a predictor variable of a verbal memory deficit, corresponding results were found (Table 12). The logistic regression model was statistically significant (χ^2=16.940, p=0.005), explained 48.2% (Nagelkercke R^2) of the variance in memory deficits and correctly classified 88.5% of cases.

Table 11. Logistic regression with verbal memory deficit (> 2 SD below normative control group) as dependent variable (NMDAR-IgA antibodies).

	Coefficient β	SE β	Wald	p	Exp (B) (95% CI)
Constant	-2.525	5.493	0.211	0.646	0.080
NMDAR-Ab+ (IgA)	4.033	1.522	7.021	**0.008**	56.414 (2.857-1113.917)
ADT	1.908	1.412	1.825	0.177	6.737 (0.423-107.266)
Age	0.082	0.077	1.141	0.285	1.085 (0.934-1.261)
Years of education	-0.404	0.197	4.220	**0.040**	0.667 (0.454-0.982)
Distant metastasis	-2.078	1.973	1.109	0.292	0.125 (0.003-5.985)

A logistic regression was performed to ascertain the effects of NMDAR-IgA antibodies, androgen deprivation therapy, age, years of education and distant metastasis on the likelihood that patients have a verbal memory deficit. The logistic regression model was statistically significant χ^2=14.523, p=0.013. The model explained 42.3% (Nagelkercke R^2) of the variance in memory deficits and correctly classified 86.5% of cases. Patients with IgA antibodies against the NMDA receptor were approximately 56 times more likely to exhibit a memory deficit. A lower level of education was also significantly associated with an increased likelihood of exhibiting a memory deficit. Androgen deprivation therapy, increasing age and distant metastasis were not significant predictors.

Table 12. Logistic regression with verbal memory deficit (> 2 SD below normative control group) as dependent variable (NMDAR-IgA/IgM antibodies).

	Coefficient β	SE β	Wald	p	Exp (B) (95% CI)
Constant	-3.452	6.353	0.295	0.587	0.032
NMDAR-Ab+ (IgA/IgM)	4.361	1.571	7.712	**0.005**	78.369 (3.608-1702.059)
ADT	0.403	1.858	0.047	0.828	1.497 (0.039-57.072)
Age	0.103	0.087	1.406	0.236	1.108 (0.935-1.314)
Years of education	-0.457	0.209	4.794	**0.029**	0.633 (0.421-0.953)
Distant metastasis	-0.695	2.184	0.101	0.750	0.499 (0.007-36.075)

A logistic regression was performed to ascertain the effects of NMDAR-IgA/IgM antibodies, androgen deprivation therapy, age, years of education and distant metastasis on the likelihood that patients have a verbal memory deficit. The logistic regression model was statistically significant χ^2=16.940, p=0.005. The model explained 48.2% (Nagelkercke R^2) of the variance in memory deficits and correctly classified 88.5% of cases. Patients with IgA/IgM antibodies against the NMDA receptor were approximately 78 times more likely to exhibit a memory deficit. A lower level of education was also significantly associated with an increased likelihood of exhibiting a memory deficit. Androgen deprivation therapy, increasing age and distant metastasis were not significant predictors.

Furthermore, multiple linear regression models confirmed IgA NMDA receptor antibodies as an independent predictor of worse verbal memory test scores (VLMT 6, VLMT 7), worse performance in visuospatial memory tasks (ROCF early recall, ROCF late recall) and worse composite cognitive z-score. While androgen deprivation therapy did not significantly predict verbal memory test scores or composite cognitive z-score, it was also a significant predictor of worse test scores in the ROCF test, suggesting a separate negative effect of ADT on visuospatial memory (Tables 13, 13, 14, 15 and 17).

Table 13. Multiple regression – predictors for VLMT Trial 6.

	B (95% CI)	SE B	β	p
Constant	18.702 (13.706-23.695)	2.517		0.000
NMDAR-Ab+ (IgA)	-2.941 (-5.299-(-0.582))	1.189	-0.231	**0.015**
Age	-0.122 (-0.197-(-0.047))	0.038	-0.307	**0.002**
ADT	-0.357 (-2.256-1.542)	0.957	-0.035	0.710

Multiple regression with VLMT Trial 6 score as dependent variable. IgA antibodies against the NMDA receptor and increasing age significantly predicted reduced test scores in VLMT trial 6 (susceptibility to interference), androgen deprivation therapy was not a significant predictor. F=5.765, p=0.001, R^2=0.150.

Table 14. Multiple regression – predictors for VLMT Trial 7 (Delayed Recall).

	B (95% CI)	SE B	β	p
Constant	21.413 (16.416-26.411)	2.518		0.000
NMDAR-Ab+ (IgA)	-2.979 (-5.339-(-0.618))	1.189	-0.226	**0.014**
Age	-0.166 (-0.241-(-0.091))	0.038	-0.402	**0.000**
ADT	-0.052 (-0.054-0.957)	0.958	-0.005	0.957

Multiple regression with VLMT trial 7 score as dependent variable. IgA antibodies against the NMDA receptor and increasing age significantly predicted reduced test scores in VLMT trial 7 (delayed recall), androgen deprivation therapy was not a significant predictor. F=8.625, p=0.000, R^2=0.209.

Table 15. Multiple regression – predictors for ROCF Early Recall.

	B (95% CI)	SE B	β	p
Constant	35.626 ((25.396-45.855)	5.154		**0.000**
NMDAR-Ab+ (IgA)	-6.468 (-11.294-(-0.1643)	2.431	-0.244	**0.009**
Age	-0.220 (-0.374-(-0.067))	0.078	-0.265	**0.005**
ADT	-4.094 (-7.982-(-0.206)	1.959	-0.195	**0.039**

Multiple regression with ROCF Early recall score as dependent variable. IgA antibodies against the NMDA receptor, increasing age and androgen deprivation therapy significantly predicted reduced test scores in ROCF Early recall. F=7.551, p=0.000, R^2=0.189.

Table 16. Multiple regression – predictors for ROCF Delayed Recall.

	B (95% CI)	SE B	β	p
Constant	38.373 (28.954-47.791)	4.745		**0.000**
NMDAR-Ab+ (IgA)	-7.453 (-11.891-(-3.016))	2.236	-0.292	**0.001**
Age	-0.262 (-0.403-(-0.120))	0.071	-0.327	**0.000**
ADT	-4.368 (-7.944-(-0.793)	1.801	-0.216	**0.017**

Multiple regression with ROCF Delayed recall score as dependent variable. IgA antibodies against the NMDA receptor, increasing age and androgen deprivation therapy significantly predicted reduced test scores in ROCF Delayed recall. F=11.674, p=0.000, Final R^2=0.267.

Table 17. Multiple regression – predictors for composite cognitive score.

	B (95% CI)	SE B	β	p
Constant	2.073 (1.234-2.912)	0.423		0.000
NMDAR-Ab+ (IgA)	-0.455 (-0.851-(-0.059))	0.200	-0.202	**0.025**
Age	-0.031 (-0.044-(-0.018))	0.006	-0.439	**0.000**
ADT	-0.046 (-0.283-0.778)	0.161	-0.026	0.778

Multiple regression with composite cognitive score as dependent variable. IgA antibodies against the NMDA receptor and increasing age significantly predicted a reduced cognitive composite score, androgen deprivation therapy was not a significant predictor. F=10.016, p=0.000, R^2=0.235.

In summary, while androgen deprivation therapy was shown to have an additional negative effect on visuospatial memory in this study, models with adjustment for ADT as a potential confounder did confirm the observed association between NMDA receptor autoantibodies and memory impairment.

3.3 Patient-reported outcome measures

As quality of life plays a critical role in the care of cancer patients, we evaluated whether the presence of neuronal autoantibodies and the associated memory impairment coincided with reduced mental health, physical health, fatigue and depression. Comparison of quality of life between antibody-positive and antibody-negative patients as well as between the IgA/IgM NMDA receptor antibody subgroup and antibody-negative patients revealed no significant differences in physical health, mental health and depression (Tables 7 and 8).

3.4 Neurological examination

The only significant difference detected upon neurological examination was a significantly higher frequency of limb ataxia in antibody-positive versus antibody-negative patients (6/18 [33.3%] vs. 9/84 [10.7%]; p=0.024). Neurological examination revealed no differences when comparing the subgroup of patients with IgA and IgM NMDA receptor antibodies or the individual subgroup of patients with IgA NMDA receptor antibodies to antibody-negative patients (Table 18).

Table 18. Neurological Examination.

Neurological Deficit	All (%) (n=102)	Ab- (%)(n=84)	Ab+ (%)(n=18)	p	NMDAR-Ab IgA/IgM+ (%)(n=13)	p	NMDAR-Ab IgA+ (%)(n=6)	p
CN II pathology *	2 (2.0)	2 (2.4)	0	1.000‡	0	1.000‡	0	1.000‡
Pathological oculomotor function §	13 (12.7)	13 (15.5)	0	0.117‡	0	0.205‡	0	0.587‡
Trigeminal hypoesthesia/dysaesthesia	0	0	0	/	0	/	0	/
Facial weakness	0	0	0	/	0	/	0	/
Hypacusis uni-/bilateral	30 (29.4)	22 (26.2)	8 (44.4)	0.156‡	5 (38.5)	0.506‡	3 (50.0)	0.342‡
Cranial nerve pathology	7 (6.9)	7 (8.3)	0	0.348‡	0	0.589‡	0	1.000‡
Motor function								
Reduced muscle strength/weakness	1 (1.0)	1 (1.2)	0	1.000‡	0	1.000‡	0	1.000‡
Abnormal muscle tone (spasticity/rigidity)	9 (8.8)	7 (8.3)	2 (11.1)	0.657‡	2 (15.4)	0.346‡	1 (16.7)	0.438‡
Asymmetric reflexes	29 (28.4)	24 (28.6)	5 (27.8)	1.000‡	5 (38.5)	0.521‡	3 (50.0)	0.359‡
Positive Babinski sign	1 (1.0)	0	1 (5.6)	0.180‡	0	/	0	/
Sensory function								
Sensory disturbances (hypoesthesia, dysaesthesia)	9 (8.8)	8 (9.5)	1 (5.6)	1.000‡	0	0.593‡	0	1.000‡
Hypopallesthesia	37 (36.3)	29 (34.5)	8 (44.4)	0.427ˣ	6 (46.2)	0.537‡	3 (50.0)	0.662‡
Absent Achilles tendon reflex	21 (20.6)	16 (19.0)	5 (27.8)	0.520‡	3 (23.1)	0.715‡	1 (16.7)	1.000‡
Polyneuropathic symptoms $	44 (43.1)	35 (41.7)	9 (50.0)	0.517ˣ	6 (46.2)	0.761ˣ	3 (50.0)	0.694‡
Coordination								
Limb ataxia	15 (14.7)	9 (10.7)	6 (33.3)	**0.024‡**	4 (30.8)	0.070‡	1 (16.7)	0.517‡
Gait ataxia	13 (12.7)	10 (11.9)	3 (16.7)	0.450‡	2 (15.4)	0.673‡	1 (16.7)	0.572‡
Positive Romberg's test	3 (2.9)	3 (3.6)	0	1.000‡	0	1.000‡	0	1.000‡
Cerebellar symptoms †	27 (26.5)	20 (23.8)	7 (38.9)	0.127‡	5 (38.5)	0.306‡	2 (33.3)	0.653‡

* includes: impaired visual field, pupillary abnormalities

§ includes: diploplia, eye deviation, impaired convergence, nystagmus, saccaded pursuit movements

$ includes: hypopallesthesia, hypoesthesia, absent Achilles tendon reflex

† includes: dysmetria, dysdiadochokinesia, gait ataxia, positive Rhomberg's test

3.5 Clinical and tumor characteristics associated with antibody seroprevalence

When investigating the relationship of clinical and tumor-associated factors with neuronal autoantibody seroprevalence we found that antibody-positive patients had a significantly higher prevalence of organ metastasis (4/34 [11.8%] vs. 1/153 [0.7%]; p=0.004) compared to antibody-negative patients (Table1). In addition, IgA NMDA receptor antibody-positive patients showed a significantly higher prevalence of lymphatic vessel infiltration (4/11 [36.4%] vs. 16/153 [10.5%]; p=0.032) compared to antibody-negative patients (Table 19).

Table 19. Demographics of all patients and comparison of IgA NMDAR antibody-positive vs. antibody-negative patients.

	All patients *(%)* (n=187)	Ab- (%) (n=153)	NMDAR-Ab IgA+ (%) (n=11)	p
Age (years)				
Mean	66.9	66.7	64.4	0.230[†]
Median	67.0	67.0	63.0	
Range	47-88	47-88	57-78	
T category of primary tumor				
Unknown	7 *(3.7)*	5 *(3.3)*	0	
T1	2 *(1.1)*	2 *(1.3)*	0	0.725[x]
T2	103 *(55.1)*	86 *(56.2)*	5 *(45.5)*	
T3	75 *(40.1)*	60 *(39.2)*	6 *(54.5)*	
N category				
Unknown	37 *(19.8)*	30 *(19.6)*	2 *(18.2)*	
N0	121 *(64.7)*	100 *(65.4)*	7 *(63.6)*	0.960[x]
N1	29 *(15.5)*	23 *(15.0)*	2 *(18.2)*	
M category				
Unknown/Metastasis possible	20 *(10.7)*	16 *(10.5)*	2 *(18.2)*	
M0	150 *(80.2)*	124 *(81.0)*	8 *(72.7)*	0.722[x]
M1	17 *(9.1)*	13 *(8.5)*	1 *(9.1)*	
M1a (non-regional lymph nodes)	1 *(0.5)*	1 *(0.7)*	0	1.000[‡]
Lymph node metastases	5 *(2.7)*	4 *(2.6)*	0	1.000[‡]
M1b (bone)	11 *(5.9)*	11 *(7.2)*	0	1.000[‡]
Bone metastases	13 *(7.0)*	11 *(7.2)*	0	1.000[‡]
M1c (other sites with or without bone disease)	5 *(2.7)*	1 *(0.7)*	1 *(9.1)*	0.120[‡]
Lung	2 *(1.1)*	1 *(0.7)*	0	1.000[‡]
Liver	3 *(1.6)*	0	1 *(9.1)*	0.062[‡]
Multiple metastases	5 *(2.7)*	3 *(2.0)*	0	1.000[‡]
Pretreatment serum PSA level (ng/ml)				
Mean	15.8	14.4	11.9	0.459[†]
Median	8.4	8.1	10.8	
Range	0.01-159.8	0.01-159.8	0.92-25.1	
Gleason score				

3+3=6	13 (7.0)	11 (7.2)	0	
3+4=7	71 (38.0)	54 (35.3)	5 (45.5)	
4+3=7	50 (26.7)	44 (28.8)	2 (18.2)	
4+4=8	9 (4.8)	7 (4.6)	0	0.796ˣ
4+5=9	40 (21.4)	34 (22.2)	4 (36.4)	
5+5=10	1 (0.5)	1 (0.7)	0	
Unknown	3 (1.6)	2 (1.3)	0	
Grade group				
Grade group 1 (GS ≤ 6)	13 (7.1)	11 (7.3)	0	
Grade group 2 (GS 3+4=7)	71 (38.6)	54 (35.8)	5 (45.5)	
Grade group 3 (GS 4+3=7)	50 (27.2)	44 (29.1)	2 (18.2)	0.711ˣ
Grade group 4 (GS 8)	9 (4.9)	7 (4.6)	0	
Grade group 5 (GS 9 or 10)	41 (22.3)	35 (23.2)	4 (36.4)	
AJCC prognostic stage group (Eighth Edition 2017)/UICC stage				
I	5 (2.7)	4 (2.6)	0	
II	60 (32.1)	50 (32.7)	3 (27.3)	
IIA	2 (1.1)	2 (1.3)	0	
IIB	37 (19.8)	29 (19.0)	2 (18.2)	
IIC	21 (11.2)	19 (12.4)	1 (9.1)	
III	40 (21.4)	33 (21.6)	2 (18.2)	
IIIA	2 (1.1)	1 (0.7)	0	0.997ˣ
IIIB	25 (13.4)	20 (13.1)	1 (9.1)	
IIIC	13 (7.0)	12 (7.8)	1 (9.1)	
IV	36 (19.3)	28 (18.3)	3 (27.3)	
IVA	19 (10.2)	15 (9.8)	2 (18.2)	
IVB	17 (9.1)	13 (8.5)	1 (9.1)	
X	46 (24.6)	38 (24.8)	3 (27.3)	
L category (infiltration of lymphatic vessels)				
Unknown	15 (8.0)	11 (7.2)	0	
L0	149 (79.7)	126 (82.4)	7 (63.6)	0.032ˣ
L1	23 (12.3)	16 (10.5)	4 (36.4)	
V category (infiltration into vein)				
Unknown	16 (8.6)	12 (7.8)	0	
V0	164 (87.7)	136 (88.9)	9 (81.8)	**0.044ˣ**
V1	7 (3.7)	5 (3.3)	2 (18.2)	
Perineural invasion				
Yes	139 (74.3)	118 (77.1)	5 (45.5)	
No	38 (20.3)	28 (18.3)	5 (45.5)	0.062ˣ
Unknown	10 (5.3)	7 (4.6)	1 (9.1)	
Lymphocytic infiltration of primary site				
Yes	63 (33.7)	52 (34.0)	2 (18.2)	
No	71 (38.0)	55 (35.9)	7 (63.6)	0.187ˣ
Unknown	53 (28.3)	46 (30.1)	2 (18.2)	
Prostate cancer treatment				
Surgical treatment: Radical prostatectomy	174 (93.0)	144 (94.1)	11 (100.0)	1.000‡
Resection margin R0	104 (55.6)	87 (56.9)	4 (36.4)	0.201ˣ

Resection margin R1	66 *(35.3)*	53 *(34.6)*	7 *(63.6)*	
Resection margin RX	4 *(2.1)*	4 *(2.6)*	0	
Radiation therapy: External beam radiation therapy or brachytherapy	9 *(4.8)*	6 *(3.9)*	1 *(9.1)*	0.834‡
Androgen deprivation therapy	23 *(12.3)*	16 *(10.5)*	2 *(18.2)*	0.345‡
Current ADT	19 *(10.2)*	14 *(9.2)*	1 *(9.1)*	1.000‡
Chemotherapy	1 *(0.5)*	0	0	1.000‡
Antibody therapy	1 *(0.5)*	1 *(0.7)*	0	1.000‡
Other current/prior malignancy in history				
Yes	29 *(15.5)*	24 *(15.7)*	3 *(27.3)*	0.392‡
Hematological malignancies (CLL, lymphoma)	3 *(1.6)*	2 *(1.3)*	1 *(9.1)*	
CLL	1 *(0.5)*	0	1 *(9.1)*	
Lymphoma	2 *(1.1)*	2 *(1.3)*	0	
Gastrointestinal cancer	6 *(3.2)*	5 *(3.3)*	0	
Esophageal squamous cell carcinoma	1 *(0.5)*+	1 *(0.7)*	0	
Colon cancer	2 *(1.1)*	2 *(1.3)*	0	
Pancreas cancer	1 *(0.5)*	1 *(0.7)*	0	
Rectum carcinoma	1 *(0.5)*	0	0	
Stomach cancer	1 *(0.5)*	1 *(0.7)*	0	**0.007‡**
Urological cancer	9 *(4.8)*	8 *(5.2)*	1 *(9.1)*	
Bladder carcinoma	5 *(2.7)*+	5 *(3.3)* +	0	
Renal cell carcinoma	1 *(0.5)*	1 *(0.7)*	0	
Testicular cancer	3 *(1.6)*	2 *(1.3)*	1 *(9.1)*	
Skin cancer	8 *(4.3)*	8 *(5.2)*	0	
Basal cell carcinoma	7 *(3.7)* +	7 *(4.8)*	0	
Malignant melanoma	3 *(1.6)* +	3 *(2.0)*	0	
Parotid gland carcinoma	1 *(0.5)*	1 *(0.7)*	0	
Thyroid carcinoma	1 *(0.5)*	0	1 *(9.1)*	
CUP	1 *(0.5)*	0	0	
Medical history				
Arterial hypertension	105 *(56.1)*	89 *(58.2)*	5 *(45.5)*	0.531‡
Diabetes mellitus type 2	27 *(14.4)*	20 *(13.1)*	2 *(18.2)*	0.660‡
Urologic and nephrological disease	48 *(25.7)*	37 *(24.2)*	1 *(9.1)*	0.460‡
Cardiovascular disease	49 *(26.2)*	40 *(26.1)*	1 *(9.1)*	0.294‡
Pulmonary disease	16 *(8.6)*	14 *(9.2)*	0	0.601‡
Gastroenterological disease	39 *(20.9)*	32 *(20.9)*	2 *(18.2)*	1.000‡
Neurological disease *	34 *(18.2)*	27 *(17.6)*	2 *(18.2)*	1.000‡
Psychiatric disease §	12 *(6.4)*	10 *(6.5)*	2 *(18.2)*	0.186‡
Autoimmune diseaese	7 *(3.7)*	5 *(3.3)*	1 *(9.1)*	0.345‡
Rheumatic disease	5 *(2.7)*	5 *(3.3)*	0	1.000‡
Thyroid disease	17 *(9.1)*	13 *(8.5)*	3 *(27.3)*	0.078‡

χ: Chi-square test; ‡: Fisher's exact-test; †: Mann-Whitney U test; +: multiple prior malignancies; *: includes (multiple diseases possible): stroke (9 patients), TIA (3), mild cognitive impairment (1), cerebral hemorrhage (2), former hypoxic ischemic encephalopathy (1), pituitary adenoma (1), former/current cluster headache syndrome (2), former/current migraine (3), multiple sclerosis (1), meningoradiculoneuritis due to Lyme disease (2), intervertebral disc disorder with radiculopathy/myelopathy (9), spinal stenosis (3), polyneuropathy (2), essential tremor (2). §: includes: former/current depression (10), former panic disorder (1), burnout (1)

4. Discussion

In this study, neuronal antibodies were found in a high percentage of prostate cancer patients. The majority of detected antibodies were directed against the NMDA receptor and were of the IgA and IgM isotype. Seroprevalence of these antibodies was associated with memory impairment affecting verbal and visuospatial memory. An adjustment for androgen deprivation therapy confirmed these results.

4.1 NMDA receptor antibodies and cognitive impairment

Neuronal autoantibodies were detected in approximately 20% of prostate cancer patients. In about 14% of patients, the antibodies were directed against the NMDA receptor and were predominantly of the IgA and IgM isotypes. This is in line with the retrospective study by Finke et al. that identified neuronal autoantibodies in approximately 30% of the 23 included prostate cancer patients, most frequently IgA and IgM NMDA receptor antibodies, identified in 26% of cases (49). The slightly higher antibody prevalence may be explained by the fact that the 300 included cancer patients presented neurological comorbidities as a possible independent pathogenic factor for neuronal antibody formation.

This study observed that the presence of these neuronal autoantibodies, especially IgA/IgM NMDA receptor antibodies, were associated with memory deficits in prostate cancer patients. These deficits specifically affected the domains of verbal and visuospatial memory. On a pathophysiological level, these results are supported by the fact that the NMDA receptor plays a central part in synaptic plasticity and memory function. In several in vitro and in vivo mouse models, it was shown that while blocking the NMDA receptor in the mouse brain impairs synaptic plasticity and compromises learning and memory, enhancement of the NMDA receptor function can improve memory in adult mice (62-64). Specifically, the NR2 subunit of the NMDA receptor is responsible for the property of the NMDA receptor channels and level of synaptic plasticity (62). Genetic enhancement of the NMDA receptor function by breading of transgenic mice with overexpression of the NR2B subunit led to enhanced synaptic plasticity and superior learning and memory function as well as maintenance of function in ageing mice (65, 66).

The association between memory impairment and IgA/IgM NMDA receptor antibodies is further corroborated by recent studies that identified IgA/IgM NMDA receptor antibodies

in a subset of dementia patients (24) and demonstrated an association with slow cognitive decline (23). Moreover, the results of the previous retrospective study by Finke et al., suggested an association between IgA/IgM NMDA receptor antibodies and cognitive deficits in cancer patients including a subset of patients with prostate cancer. Cognitive deficits were detected in 21% of antibody-positive cancer patients compared to 7% of antibody-negative patients and in 27% of patients with neuronal cell-surface antibodies compared to 5% of patients with neuronal intracellular antibodies. The analysis of the individual NMDA receptor antibody subgroups revealed cognitive deficits in 31% of cancer patients with antibodies of the IgA isotype, in 24% of cancer patients with the IgM isotype and in 26% of cancer patients with both IgA and IgM NMDA receptor antibodies (49). In the present study, the prevalence of verbal memory deficits was 33% in antibody-positive patients, 50% in the IgA NMDA receptor antibody subgroup, 44% in the IgM NMDA receptor antibody subgroup and 46% in the IgA/IgM subgroup. These differences can be explained by the fact that this study was limited to prostate cancer patients and applied detailed cognitive testing with rigorous application of ICCTF criteria, while the previous retrospective study included over 10 different cancer entities and was based solely on cognitive screening tools. Hence, these results cannot be compared directly. In a recent prospective study on melanoma patients, the robust association between IgA/IgM NMDAR antibodies and cognitive impairment was confirmed (50). Approximately 40% of melanoma patients showed cognitive impairment with threefold higher odds in antibody-positive (57%) compared to antibody-negative patients (30%). Besides impairment of overall cognitive performance, antibody-positive melanoma patients showed deficits in the domains of memory, attention, and executive function.

While current research supports an increased prevalence of NMDA receptor antibodies in cancer patients and in patients with neuropsychiatric disorders, their prevalence in the general population remains unclear. In 2013, Steiner et al. reported a seroprevalence of NMDA receptor antibodies of 0.4% in healthy controls compared to 2.9-38% in various neuropsychiatric disorders (schizophrenia, major depression and borderline personality disorder) (67). Doss et al. found a significantly higher seroprevalence of NMDA receptor antibodies in dementia patients compared to healthy controls (16.1% vs. 2.8%) (24). In contrast, Dahm et al. and Hammer et al. identified NMDA receptor antibodies in approximately 10% of healthy individuals, comparable with the seroprevalence in patients with neuropsychiatric disease (68, 69). In an updated analysis performed by Steiner et

al., NMDA receptor antibodies were detected in 7% of healthy control individuals, which was attributed to the improved sensitivity of new assays (70). Further research is needed to identify the true seroprevalence and effect of NMDA receptor antibodies in healthy individuals. To date, the interplay of blood-brain barrier integrity and immunological activity are suspected to be crucial for the development of central nervous system pathology and psychiatric disease (69).

4.2 Molecular mechanisms of NMDA receptor antibody formation and antibody effects

Neuronal autoantibodies are thought to be produced as an immune response against tumor epitopes (15). Interestingly, around 40% of prostate cancer samples were found to express NMDA receptors and their activation was shown to be involved in the proliferation of tumor cells of the prostate (71). This suggests a potential trigger mechanism of an immune response against the NMDA receptor in prostate cancer patients, which could account for the high prevalence of neuronal NMDA receptor antibodies identified in this study. As a potential endogenous anti-tumor response to reduce tumor cell proliferation, future studies should assess whether the presence of these antibodies correlates with an improved oncological outcome.

Distant metastases and lymphatic vessel infiltration were identified as predisposing factors for neuronal autoantibody seroprevalence in prostate cancer. This supports the theory that increased invasive tumor properties and lymphogenic and hematological metastasis, characteristic of advanced disease, lead to increased tumor antigen presentation and immune cross-reaction against tumor antigens.

As the NMDA receptor plays a central role in memory (62), its inactivation by binding of NMDA receptor autoantibodies could in turn explain the high prevalence of memory deficits observed in prostate cancer patients. Some molecular studies have shown that IgM and IgA NMDA receptor antibodies can reduce the density of NMDA receptor and other synaptic proteins in a titer-dependent manner and can induce a profound decrease of NMDAR-mediated currents (23) as well as causing internalization of the NMDA receptor and reduction of glutamate currents (72). In contrast, Hara et al. did not detect a change in the levels of synaptic and extrasynaptic NMDA receptor expression in the presence of IgA and IgM antibodies (73). These conflicting results are most likely

explained by the differences in study design. While the study by Prüss et al. included seven patients with IgA antibodies with slow cognitive decline (23), the study by Hara et al. included three patients with IgM antibodies with stroke and only one patient with IgA antibodies with dementia (73). This suggests that the alteration of NMDA receptor expression and function may specifically play a pathogenic role in the induction of neuronal autoantibody induced cognitive impairment associated with IgA NMDA receptor antibodies.

In the study by Finke et al., the effect of neuronal autoantibodies was shown to be associated with the integrity of the blood-brain barrier (49). A more dysfunctional blood-brain barrier, defined by a higher albumin cerebrospinal fluid (CSF)/serum ratio, in antibody-positive patients with cognitive deficits compared to patients with other neurological deficits and healthy controls, led to the hypothesis that cognitive deficits arise in patients where neuronal autoantibodies can access brain antigens by means of barrier disruption. The formation of antibodies targeting brain-restricted antigens after recognition of neuronal antigens via a dysfunctional blood-brain barrier was suggested as a further pathophysiological mechanism. This is supported by further evidence from a study by Hammer et al. suggesting that the clinical significance of NMDA receptor antibodies depends on the integrity of the blood-brain barrier (69).

In search of potential therapeutic approaches, recent studies have shown that patients with cognitive deficits and other neurological syndromes associated with neuronal surface antibodies including IgA/IgM NMDAR receptor antibodies have responded well to immunotherapy (23, 24). This raises the question of existence of potentially treatable immune-mediated cognitive impairment and calls for investigation of response to immunomodulatory treatment.

4.3 Androgen deprivation therapy, cognitive impairment and dementia in prostate cancer patients

In view of the continuously improving survival rates and increasing number of older long-term survivors, cancer-related cognitive impairment is a complication of increasing importance in prostate cancer patients. Indeed, almost half of all prostate cancer patients presented cognitive impairment. So far, cognitive function in prostate cancer has primarily been studied in the context of androgen deprivation therapy. Recent evidence supports

a pathogenic role of ADT in cognitive decline by means of interference with binding of androgens to brain androgen receptors (40). However, the ultimate impact of ADT on cognitive function in prostate cancer patients remains controversial (74). Several studies demonstrated a correlation between ADT and cognitive decline, mainly affecting visuomotor function (75), verbal memory (76), visuospatial memory and executive functions (77-79). However, these effects were mostly limited to the duration of treatment and resolved afterwards. In contrast, other studies did not show worsening of cognitive function under ADT over a treatment period of up to 36 months (80). In the current prospective, cross-sectional study, patients with androgen deprivation therapy did not show a significantly increased risk for memory deficits as defined by ICCTF criteria. Furthermore, ADT was not a significant predictor for overall cognitive impairment or for verbal and visuospatial memory deficits in multiple linear and logistic regression analyses. However, patients receiving ADT did show significantly worse scores in the spatial memory test (ROCF, early and late recall), supporting the evidence that visuospatial functions are particularly sensitive to androgen deprivation. In line with the study by Alibhai et al., we applied the ICCTF criteria for definition of cognitive impairment and our study cohort had a high level of education, potentially explaining similar results (80). As noted by Mohile et al., many prostate cancer patients present with cognitive impairment before initiation of ADT, supporting the hypothesis of additional pathogenic factors of cognitive impairment (81).

As demonstrated, the data of this current study suggests that IgA/IgM NMDA receptor antibodies represent such a pathogenic factor associated with cognitive impairment in prostate cancer patients. As this study was not designed to evaluate the association between ADT and cognitive impairment and the number of patients treated with ADT was low, conclusions regarding the potential role of ADT in accelerating cognitive decline in patients at high risk due to reduced cognitive reserve are very limited.

Recent evidence also suggests a relationship between low androgen levels and the development of dementia and Alzheimer disease. Ramsden et al. showed that gonadectomy led to increased levels of β-amyloid protein in the mouse brain, which reversed after supplementation of dihydrotestosterone (82). Raber et al. showed that androgen deprivation in rodents expressing the apolipoprotein E4 allele, a known human risk factor for Alzheimer disease, was associated with impaired cognitive function (83). However, given the many challenges and limitations faced in the prospective assessment

of the association of dementia with ADT, the results from the studies conducted to date remain inconclusive. While some studies presented evidence in favor of such an association (84, 85), other studies, including an analysis of over 1.2 million prostate cancer patients, did not confirm an increased risk for Alzheimer's disease or dementia (86-88).

4.4 Neuronal intracellular autoantibodies and classic paraneoplastic disorders

In this study, neuronal autoantibodies directed against intracellular epitopes were only detected in 5% of all prostate cancer patients and in 4% of the subgroup that underwent neuropsychological and neurological examination. Antibody targets included the synaptic intracellular antigens GAD 65, GAD 67, Homer3 and Amphiphysin as well as the intracellular non-synaptic antigens Ma2, Yo, Hu, ZIC4, ARHGAP26 and ITPR1. None of the examined patients presented manifest classic paraneoplastic syndromes. This is in line with previous studies that reported the rare occurrence of paraneoplastic syndromes in prostate cancer (44). One patient with antibodies directed against the intracellular antigen ZIC4 showed hypopallesthesia and reduced reflexes of the distal extremities in the neurological examination. As peripheral neuropathy has been reported as one of the most common PND in prostate cancer (43), this may have been a subclinical manifestation of PND. Therefore, it is possible that the seroprevalence of these antibodies and their effects are higher than expected in asymptomatic patients and that increased autoantibody testing could increase the detection of subclinical paraneoplastic disorders, thus potentially improving early cancer detection and survival. As anti-Hu antibodies are the most common onconeural antibody reported in prostate cancer patients and are typically associated with brainstem and cerebellar syndromes (44), the examination of a larger patient cohort may have led to the detection of cases of this classic PND.

In contrast, in SCLC, the most common cancer associated with PND, paraneoplastic neurological disorders have been reported in up to 10% of patients (89). The seroprevalence of antineuronal antibodies is estimated at around 90% in patients with manifest PND, with the intracellular antigens SOX2, VGCC or HuD identified as the most frequent antibody targets. In SCLC patients without paraneoplastic disorders, neuronal antibody prevalence is estimated to lie under 30%. Neuronal antibodies directed against cell-surface proteins have shown to be less prevalent in SCLC.

4.5 New biomarkers in prostate cancer

The lack of sufficient specificity and sensitivity of PSA in the detection of prostate cancer has led to the search for better biomarkers for early, non-invasive diagnosis. Tumor-associated autoantibodies, resulting from a humoral immune response against tumor-associated antigens in the process of tumorigenesis, have been proposed as promising early biomarkers (90). In 2008, Taylor et al. showed that serum humoral response profiles can be used in the detection and distinction between prostate cancer and benign prostatic hyperplasia (91). A significantly higher frequency of autoantibodies to p90 and p62 was reported in prostate cancer patients compared to patients with benign prostatic hyperplasia and healthy individuals, and a panel of six selected tumor-associated antibodies was proposed as a diagnostic marker with high sensitivity and specificity (92). Liu et al. showed that the combination of p90 and p62 with two further proteins (IMP1 and Koc/IMP3) as a mini-array of four tumor-associated autoantibodies, further increased the antibody frequency in comparison to healthy controls (70% vs. 10.6%) (93). Ummanni et al. identified anti-PRDX6 and anti-ANXA11 autoantibodies in the serum of prostate cancer patients in contrast to healthy individuals. In combination with existing markers such a PSA, these autoantibodies were suggested as a further potential marker for the non-invasive diagnosis of prostate cancer (94). Xie et al. showed that the combination of a characteristic autoantibody signature with PSA improved the diagnostic value of PSA alone in distinguishing prostate cancer from non-malignant cases (95). However, to date, no tumor-associated antibodies have shown sufficient sensitivity and specificity for the implementation into the standard screening and therapeutic monitoring guidelines of prostate cancer. Due to important role of the NMDA receptor in the proliferation of prostate cancer cells (71) and the high seroprevalence of NMDA receptor autoantibodies detected in prostate cancer patients, the NMDA receptor could potentially contribute to an improved screening method for prostate cancer.

4.6 Limitations

Limitations of the current study include the lack of follow-up data on neuronal autoantibody seroprevalence, cognitive function, clinical outcome and survival. Therefore, longitudinal studies are necessary to evaluate the effects of cancer treatment and disease activity on antibody seroprevalence and cognitive impairment in order to

provide a more detailed insight on long-term clinical effects in antibody-positive cancer patients.

Due to the small sample size, especially in the subgroup analyses, further studies in larger patient cohorts are necessary to validate the observed results. However, we postulate that the heterogeneous study population across all stages prevented overrepresentation of possible characteristic features associated with a specific study population as a potential confounding factor for the results.

Furthermore, the high level of education of the patients undergoing neuropsychological examination may have reduced the strength of the relationship observed between neuronal autoantibodies and memory impairment in prostate cancer patients, thus the prevalence of memory deficits could potentially have been higher, had the study been conducted in a population of reduced cognitive reserve.

Although no association was found between androgen deprivation therapy and cognitive impairment, this study was not designed to evaluate the correlation between ADT and cognitive impairment and the number of patients receiving ADT was low with heterogeneous treatment modalities and duration. Therefore, a prospective study designed to specifically evaluate cognitive impairment in patients with neuronal autoantibodies and hormone treatment is necessary.

To date, it is recommended to include both CSF and serum for neuronal antibody testing as sensitivity of antibody detection has shown to be higher in CSF than in serum (27, 28). In a study examining paired serum and CSF samples of patients with anti-NMDA receptor encephalitis, 14% of patients had detectable CSF antibodies while serum testing was negative (96). The concentration of CSF antibodies also seemed to correlate better with the clinical course than the antibody concentration in the serum (96). In this study, neuronal antibody screening was only performed in serum. Based on the observational nature of the study, it was ethically not justified to perform a lumbar puncture for collection of a CSF sample in patients without manifest clinical neurological symptoms. Therefore, cases with isolated CSF antibodies could have gone undetected. However, in the study by Finke et al., neuronal antibodies were almost exclusively detected in the serum but not in the cerebrospinal fluid of cancer patients (24.5% vs. 1.2%) (49). This suggests that paraneoplastic cognitive deficits may be associated with isolated serum antibodies, making them sufficient for detection and diagnosis of this novel syndrome.

4.7 Future research

Recognition of cognitive impairment induced by neuronal autoantibodies as a novel paraneoplastic disorder should encourage the elaboration of current diagnostic criteria to improve detection rates and facilitate between-study comparison. Larger study populations are required to evaluate the effects of other less prevalent neuronal surface antibodies and identify the corresponding disorders. Furthermore, antibody screening could increase early detection of subclinical paraneoplastic syndromes leading to an improvement of successful treatment and oncological outcome.

Taking into consideration that antibody type and associated paraneoplastic syndromes vary strongly in different cancers, further prospective studies on each individual cancer entity are necessary to identify their characteristic features. More studies on a molecular level may help to identify characteristic antigens expressed in both different tumor tissues and the nervous system (e.g. NMDA receptor in prostate cancer cells (71)), responsible for immune cross-reaction and formation of pathogenic antibodies leading to the characteristic neurological disorders. These antibodies could serve as potential biomarkers for early cancer detection and disease activity, thus being integrated into screening and cancer follow-up programs. This is of particular interest in the current era of liquid biopsy.

4.8 Conclusion

In summary, this study shows that a large group of prostate cancer patients presents neuronal autoantibodies, mainly cell surface antibodies of the IgA and IgM isotypes directed against the NMDA receptor. Importantly, these antibodies were associated with an increased risk for memory deficits, specifically affecting verbal and visuospatial memory. An adjustment for androgen deprivation therapy confirmed these results.

As an independent risk factor for memory impairment, these neuronal antibodies represent a potential pathogenic factor in cancer-related cognitive impairment. Future studies should investigate the underlying pathophysiological mechanisms of this neuronal autoimmune response and determine whether cognitive deficits in antibody-positive prostate cancer patients respond to immunotherapy.

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6. Eidesstattliche Versicherung

„Ich, Kimberley Louise Farmer, versichere an Eides statt durch meine eigenhändige Unterschrift, dass ich die vorgelegte Dissertation mit dem Thema: „Neuronal autoantibodies associated with memory impairment in prostate cancer patients" / „Neuronale Antikörper und assoziierte Gedächtnisstörungen in Prostatakarzinom-Patienten" selbstständig und ohne nicht offengelegte Hilfe Dritter verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel genutzt habe.

Alle Stellen, die wörtlich oder dem Sinne nach auf Publikationen oder Vorträgen anderer Autoren/innen beruhen, sind als solche in korrekter Zitierung kenntlich gemacht. Die Abschnitte zu Methodik (insbesondere praktische Arbeiten, Laborbestimmungen, statistische Aufarbeitung) und Resultaten (insbesondere Abbildungen, Graphiken und Tabellen) werden von mir verantwortet.

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.

Meine Anteile an etwaigen Publikationen zu dieser Dissertation entsprechen denen, die in der untenstehenden gemeinsamen Erklärung mit dem/der Erstbetreuer/in, angegeben sind. Für sämtliche im Rahmen der Dissertation entstandenen Publikationen wurden die Richtlinien des ICMJE (International Committee of Medical Journal Editors; www.icmje.og) zur Autorenschaft eingehalten. Ich erkläre ferner, dass ich mich zur Einhaltung der Satzung der Charité – Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis verpflichte.

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."

Datum   Unterschrift

7. Lebenslauf

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

8. Publikationsliste

1. Dobos G, <u>Farmer K</u>, Gutzmer R, Kiecker F, Ulrich C. Malignes Melanom −
    Früherkennung, Diagnostik und Nachsorge. Der Onkologe. 2018;24(6):453-63.

2. Kiecker F, Poch G, <u>Farmer K</u>. Läsionale Therapieoptionen beim maligen
    Melanom. Der Onkologe. 2018;24(6):464-71.

3. Bartels F, Strönisch T, <u>Farmer K</u>, Rentzsch K, Kiecker F, Finke C. Neuronal
    autoantibodies associated with cognitive impairment in melanoma patients.
    Ann Oncol. 2019;30(5):823-9.

4. Trager MH, <u>Farmer K</u>, Ulrich C, Basset-Seguin N, Herms F, Geskin LJ, Bouaziz
    JD, Lebb C, de Masson A, Bagot M, Dobos G. Actinic cheilitis: a systematic
    review of treatment options. Journal of the European Academy of Dermatology
    and Venereology: JEADV. 2021;35(4):815-23.

9. Danksagung