J Neurol (2013) 260:2831–2835
DOI 10.1007/s00415-013-7074-0

ORIGINAL COMMUNICATION

Seroprevalence of anti-*N*-methyl-D-aspartate receptor antibodies in women with ovarian teratoma

Mandy Mangler · Isabel Trebesch de Perez · Bianca Teegen · Winfried Stöcker · Harald Prüss · Andreas Meisel · Achim Schneider · Jekaterina Vasiljeva · Dorothee Speiser

Received: 12 July 2013 / Revised: 6 August 2013 / Accepted: 7 August 2013 / Published online: 24 August 2013

Abstract Recently antibodies against neuronal receptors have been identified as cause of a new type of encephalitis. The anti-*N*-methyl-D-aspartate receptor (anti-NMDA-R) encephalitis is the prototype of these disorders. Patients have a high incidence of teratomata. Removal of teratoma is considered the essential treatment of anti-NMDA-R encephalitis. Here, we aimed to investigate whether neurologically asymptomatic individuals suffering from ovarian teratomata may have positive anti-NMDA-R antibodies to be detected by an established assay. Over a time period of 15 months, all patients suffering from ovarian teratomata without neurological symptoms were included in this prospective study. Twenty consecutive patients were pair matched to patients with other benign ovarian disease and healthy controls. Preoperatively, patients had a gynaecological examination, transvaginal ultrasound, neurological examination and determination of anti-NMDA-R antibodies. None of the patients or controls presented with neurological symptoms. All tumours could be removed completely by laparoscopy. Anti-NMDA-R antibodies were absent in the group of patients with teratomata as well as in patients with benign ovarian tumours and healthy controls. Testing for anti-NMDA-R antibodies revealed negative findings in well-characterised patients with ovarian teratomata lacking neurological symptoms. Our data support the current clinical practice that a systematic screening for anti-NMDA-R antibodies in teratoma patients is not indicated.

Keywords Anti-*N*-methyl-D-aspartate receptor antibodies · Ovarian teratoma · NMDA-receptor-encephalitis · Screening for anti-*N*-methyl-D-aspartate receptor autoantibodies

Introduction

In 2007, female encephalitis patients with pathogenic autoantibodies targeting the *N*-methyl-D-aspartate receptor (NMDA-R) have been identified for the first time [1]. Over the last years hundreds of patients with anti-NMDA-R encephalitis have been reported. In up to 60 % of these patients underlying ovarian teratoma has been diagnosed [2–5]. Anti-NMDA-R encephalitis is still an underdiagnosed disease and thought to be the most common non-infectious encephalitis that can occur in both patients with and without tumours [6]. Although potentially reversible, the disorder is often severe in course. Mortality rates are up to 25 % [7]. The precise pathogenesis is unknown and the subject of intense research. It predominantly occurs in young women leading not only to neurological symptoms but also to severe psychiatric alterations [8]. The disease

Electronic supplementary material The online version of this article (doi:10.1007/s00415-013-7074-0) contains supplementary material, which is available to authorised users.

M. Mangler (✉) · I. Trebesch de Perez · A. Schneider · J. Vasiljeva · D. Speiser
Department of Gynecology, Charitéplatz 1, Charité Campus Mitte, 10117 Berlin, Germany
e-mail: mandy.mangler@charite.de

B. Teegen · W. Stöcker
Institute for Experimental Immunology, Affiliated to Euroimmun AG, Lübeck, Germany

H. Prüss · A. Meisel
Department of Neurology and Experimental Neurology, Charité Campus Mitte, 10117 Berlin, Germany

appears in stereotypical stages [5, 9–11], including with prodromal symptoms such as headache, fever, nausea, vomiting, diarrhoea, or upper respiratory-tract symptoms. Altered levels of consciousness, psychotic symptoms and memory deficits commonly develop into a stupor-like phase partly accompanied by dyskinesias and seizures [12–14]. Autonomic dysregulation affecting body temperature, respiration and circulation control causes life-threatening conditions [6, 11, 15].

Since in up to 59 % of cases of anti-NMDA-R encephalitis in adults ovarian teratomas have been identified, an association between both conditions has been presumed [9]. Presumably as part of an anti-tumour response or after immune system stimulation in the context of an infection, autoimmune antibodies against the neuronal NMDA receptor are produced, which are ectopically expressed in ectodermic teratoma tissue derived from totipotent stem cells in the embryonic phase [2].

Early removal of the teratomata is a mainstay of treatment in anti-NMDA-R encephalitis and thought to be an essential step for neurological recovery [4, 5]. Therapeutic options developed have been continuously re-evaluated. At detection of a tumour, the operative removal is indicated. Prophylactic removal of the ovaries has been beneficial in selected cases even if no ovarian tumours were found [16]. Beyond teratoma removal, immunosuppressive treatment such as steroids, intravenous immunoglobulins, plasmapheresis, cyclophosphamide or rituximab have been established as therapy standards in anti-NMDA-R encephalitis despite missing evidence from clinical trials [17, 18].

Although teratomas are commonly identified in NMDA-R encephalitis, it is unknown how frequent NMDA-R antibodies are detected in serum samples of female patients with teratomata or other ovarian pathologies. Our study aimed at identifying the rate of anti-NMDA-R autoantibody-positive teratoma patients without neurological deficits to answer the question of whether antibody testing should be recommended in this group of patients.

Patients and methods

Between October 2011 and December 2012, thirty-five women with benign ovarian disease were prospectively enrolled in the study at the Department of Gynecology and Gynecologic Oncology Campus Benjamin Franklin and Campus Mitte of the Charité Berlin. Ethical approval of the ethic commission of the Charité was obtained. All women gave their informed consent to participation. Patients were included if benign ovarian tumours were assumed by clinical examination or preoperative diagnostical tools, such as transvaginal ultrasound. We enrolled all women who suffered from ovarian teratomata during the study period. We matched the patients to individuals with other ovarian pathologies such as cysts or benign tumours and also to a healthy control group.

Directly prior to the operation, patients underwent a transvaginal examination, gynaecological ultrasound and blood testing. All patients were interviewed and examined by consultants for previous and current neurological and gynaecological symptoms and history. Examiners responsible for anti-NMDA-R antibody testing were blinded for disease groups. Serum samples from all participants were collected and tested for the presence of NMDAR antibodies and a large panel of further anti-neuronal antibodies as follows: Biochip mosaics (Euroimmun, Lübeck, Germany) contained frozen tissue sections (rat hippocampus, monkey cerebellum) and recombinant cell substrates, each expressing a different neural antigen (NMDAR-NR1a, NMDAR-NR1a/NR2b, AMPAR-GluR1/GluR2, DPPX-IF1, DPPX-IF2, GABAR-B1/B2, LGI1, CASPR2, GLRA1b, GRM1, GRM5, MOG, Tr/DNER, AQP4, GAD65, GAD67, ZIC, ARHGAP26, Yo, amphiphysin, Hu, Ri, Ma1, Ma2, CV2, Sox-1, recoverin). Samples were applied to the reaction fields of a reagent tray. Pipetting volume was 60 µl per field. Serum samples were diluted starting with 1:10 in PBS-Tween.

Positive and negative controls were included with every test procedure and incubation times were standardised. Slides carrying the mosaics were incubated with patient's serum at serial dilutions for 30 min at room temperature, washed with PBS-Tween and immersed in PBS-Tween for 5 min. Bound antibodies were detected by fluorescein isothiocyanate (FITC)-labelled goat anti-human IgG (Euroimmun, Lübeck, Germany) for 30 min at room temperature. Slides were washed again with a flush of PBS-Tween and then immersed in PBS-Tween for 5 min. Drops of glycerol (approximately 20 µl per field) were placed onto a cover glass and the Biochip slides were embedded in this mounting medium simultaneously and examined by fluorescence microscopy.

Samples were classified as positive or negative based on the intensity of surface immunofluorescence of transfected cells in direct comparison with non-transfected cells and control samples. Endpoint titres refer to the last dilution showing a measurable degree of fluorescence, with 1:10 being the cut-off for positivity (Fig. 1).

The biochip has been validated and established in previous studies [14, 19–21].

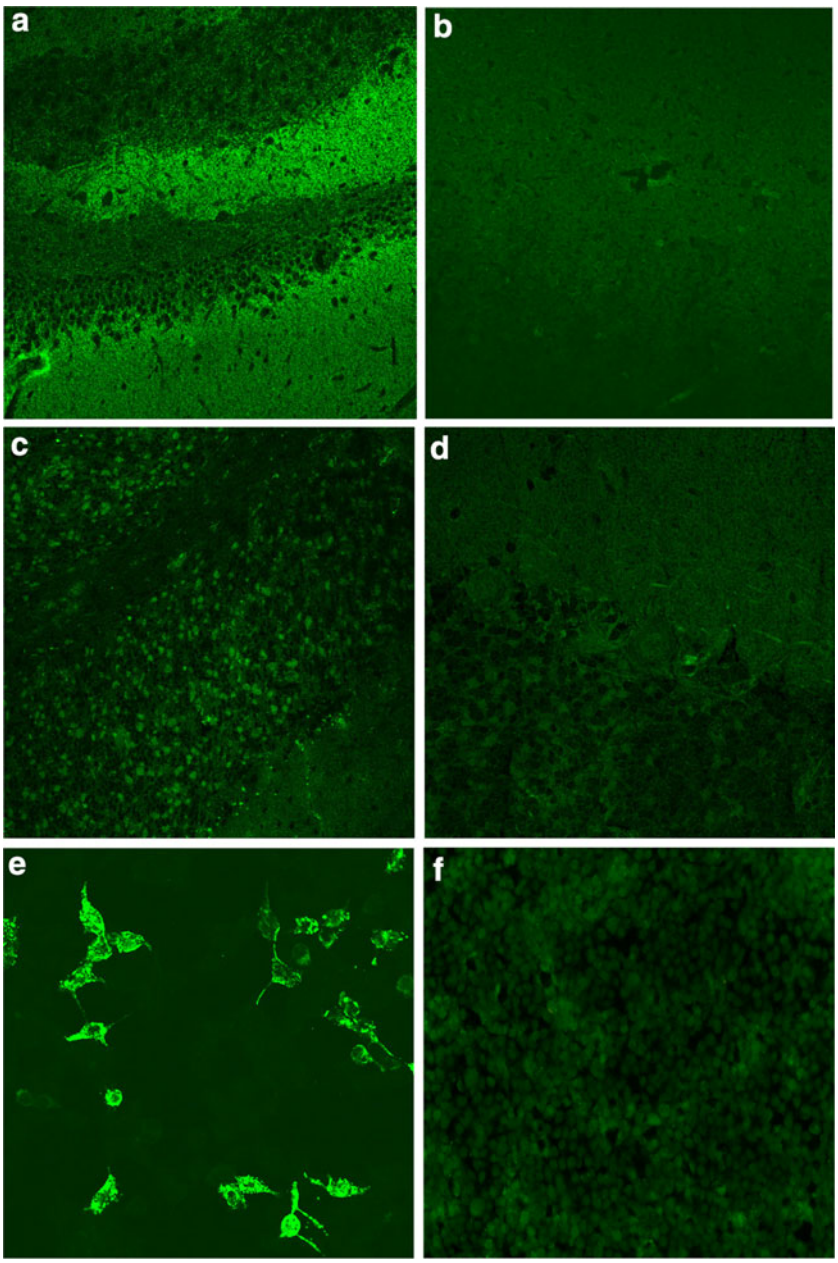

Fig. 1 Detection of antibodies to NMDA-R using indirect immunofluorescence assays. Patient serum was applied to rat hippocampus (**a**, **b**), monkey cerebellum (**c**, **d**), HEK293 cells transfected with NMDA-R (**e**) and **f** control-transfected cells. Bound antibodies were detected with a FITC-labelled secondary anti-human IgG antibody. **a**, **c**, **e** NMDA-positive patient's antibodies bound specifically to rat hippocampus, monkey cerebellum and HEK293 cells expressing NMDA-R, but **f** not to control-transfected HEK293 cells. **b**, **f** NMDA-negative sample on rat hippocampus and monkey cerebellum

Results

Anti-NMDA-receptor antibodies of 20 women with ovarian teratomata, 11 patients with benign ovarian tumours and four healthy controls were analysed. The women were between 25 and 45 years old (mean 30 years). The neurological examinations before the operation showed no pathological clinical findings in any of the participants. All patients except for healthy controls presented with symptoms due to the ovarian mass. Twenty patients suffering from teratomata had a mean tumour size of 4.5 m (2–12 cm) (supp Fig. 1).

The 11 women with benign ovarian tumours who were matched by age and tumour size to the group with teratomata had a mean tumour size of 5.5 cm (3–14 cm). None of the patients and neither individual in the control group presented any neurological symptoms. All tumours could be removed completely by laparoscopy. No complications occurred in any of the patients. In 20 patients teratomas were confirmed histopathologically (supp Fig. 2).

IgG antibodies against NMDA receptors could neither be detected in women with teratomata nor in patients with other ovarian pathologies or in healthy controls. In one patient with benign ovarian cystadenoma, antibodies

against GAD65 were detected. Although the specimen showed a strong reaction with the cytoplasm of Purkinje cells, testing for common cytoplasmic antigens of these cerebellar cells, e.g. ARHGAP26, Yo, Tr/DNER, remains negative. The pattern of immunofluorescence was reminiscent as Purkinje cell cytoplasmic antibody type 2 (PCA-2). The patient did not show any neurological symptoms, in particular no signs of ataxia. All patients were followed up for a median of 13 months (6–20 months). No neurological symptoms occurred in the time of follow-up.

Discussion

Anti-NMDA-R encephalitis is a disorder with a strong association to ovarian teratomata [1]. Although the disorder is thought to be mediated by antibodies targeting the NR1 subunit of the NMDA receptor, their pathogenesis is not fully understood [1, 6]. The aim of our study was to analyse whether anti-NMDA-R antibodies are present in female patients with teratomata or other ovarian pathologies also in the absence of neurological abnormalities. The main finding of our study is that none of the patients with teratomata or other ovarian tumours had anti-NMDA-R autoantibodies.

Most teratomata contain neuronal tissue with NMDA-R expression in the parietal cell membrane deriving from the ectoderm, which is one tessera in association with NMDA-R encephalitis [22]. Nervous tissue in teratomata could be observed in all cases of NMDAR-encephalitis with neoplasms analysed by Dalmau et al [7, 9]. NMDA-R expression in oocytes of ovaries of healthy controls could help decipher the pathogenesis of NMDA-R encephalitis in patients without neoplasms [2]. However, the mere presence of NMDA-R expression in neoplasms or ovaries likely does not explain how antibody production is triggered [7, 9]. Often patients with anti-NMDA-R encephalitis are reported to have suffered from fever and other flu-like symptoms before the onset of the encephalitis, suggesting that the immunological process may be triggered by an infection, e.g. by boosting immune responses fostering antibody production and/or increasing the permeability of the blood–brain barrier. Conversely, the absence of such immunostimulatory events in our patients with teratomata might explain the absence of detectable levels of anti-NMDA-R antibodies. Our patients were symptomless regarding neurologically distinctive features. Thus, screening for anti-NMDA-R antibodies in patients with teratomata seems only indicated in the case of neurological symptoms or if prodromi of anti-NMDA-R encephalitis have been identified.

Detection of anti-NMDA-R autoantibodies in patients with anti-NMDA-R encephalitis has been routinely performed in both serum and CSF samples. However, antibody levels are often higher in CSF than in serum, and after successful treatment patients can lose anti-NMDA-R autoantibodies in serum but retain them in the CSF [6, 9]. Our study lacks data from CSF, which implies that we could potentially have overlooked a minority of positive cases. However, for obvious ethical reasons we did not obtain CSF samples from the patients and controls.

Patients with prodromal signs or symptoms of encephalitis should be checked for teratoma immediately, since quick removal of these tumours could improve the situation of these patients immensely. Laparoscopic removal is strongly recommended in patients with benign ovarian teratoma. There is no need for laparotomy in almost any size of the tumour. There is no clear data supporting a prophylactic complete removal of the ovaries. In some patients adnexectomy improved the symptoms of the encephalitis [14, 19]. Nevertheless, preservation of ovarian tissue is strongly recommended, as a complete ovariectomy increases the risk for cardiovascular disease and osteoporosis dramatically. Considering fertility issues one should preserve as much of the ovarian tissue as possible or offer cryoconservation if removal is necessary. In any case, a careful examination for teratoma even intraoperatively is mandatory in these young patients, also to possibly spare complete ovarian removal. Until now, positive IgG NMDAR antibodies have not been reported in asymptomatic patients. Thus, IgG NMDAR antibodies are considered highly specific for anti-NMDAR encephalitis in previous studies [23–26].

Summary

The association between ovarian teratomata and anti-NMDA-R antibodies leading to NMDA-R encephalitis is well established [27]. Conversely, our study suggests that measurement of anti-NMDA-R antibodies in neurologically asymptomatic patients with ovarian teratomata or tumours is futile. Systematic antibody screening will likely not be helpful in identifying patients that could develop neurological symptoms or benefit from additional therapy. These findings match with previous data obtained by questionnaire demonstrating that neuropsychiatric symptoms are not more frequent in patients with teratomata than without [28]. Moreover, our data support previous studies that positive findings in anti-NMDA-R antibody screening are specific for patients suffering from neuropsychiatric symptoms [29].

Acknowledgments The authors thank Dr. Julia Lange very much for providing the ultrasound picture of a teratoma. We would also like to thank Barbara Ingold-Heppner very much for kindly preparing the pathological figure.

Conflicts of interest The authors declare that they have no conflict of interest.

References

1. Dalmau J, Tüzün E, Wu HY, Masjuan J, Rossi JE, Voloschin A, Baehring JM, Shimazaki H, Koide R, King D, Mason W, Sansing LH, Dichter MA, Rosenfeld MR, Lynch DR (2007) Paraneoplastic anti-*N*-methyl-D-aspartate receptor encephalitis associated with ovarian teratoma. Ann Neurol 61(1):25–36
2. Tachibana N, Shirakawa T, Ishii K, Takahashi Y, Tanaka K, Arima K, Yoshida T, Ikeda S (2010) Expression of various glutamate receptors including *N*-methyl-D-aspartate receptor (NMDAR) in an ovarian teratoma removed from a young woman with anti-NMDAR encephalitis. Inter Med 49:2167–2173
3. Tüzün E, Zhou L, Baehring JM, Bannykh S, Rosenfeld MR, Dalmau J (2009) Evidence for antibody-mediated pathogenesis in anti-NMDAR encephalitis associated with ovarian teratoma. Acta Neuropathol 118(6):737–743
4. Uchino A, Iizuka T, Urano Y, Arai M, Hara A, Hamada J, Hirose R, Dalmau J, Mochizuki H (2011) Pseudo-piano playing motions and nocturnal hypoventilation in anti-NMDA receptor encephalitis: response to prompt tumor removal and immunotherapy. Intern Med 50(6):627–630
5. Iizuka T, Sakai F, Ide T, Monzen T, Yoshii S, Iigaya M, Suzuki K, Lynch DR, Suzuki N, Hata T, Dalmau J (2008) Anti-NMDA receptor encephalitis in Japan: long-term outcome without tumor removal. Neurology 70(7):504–511
6. Dalmau J, Lancaster E, Martinez-Hernandez E, Rosenfeld MR, Balice-Gordon R (2011) Clinical experience and laboratory investigations in patients with anti-NMDAR encephalitis. Lancet Neurol 10(1):63–74 Review
7. Tumbi A, Gilani A, Scarff JR, Kaur G, Lippmann S (2011) Anti-*N*-methyl-D encephalitis. Innov Clin Neurosci 8(9):24–25
8. Ryan SA, Costello DJ, Cassidy EM, Brown G, Harrington HJ, Markx S (2013) Anti-NMDA receptor encephalitis: a cause of acute psychosis and catatonia. J Psychiatr Pract 19(2):157–161. doi:10.1097/01.pra.0000428562.86705.cd
9. Dalmau J, Gleichman AJ, Hughes EG, Rossi JE, Peng X, Lai M, Dessain SK, Rosenfeld MR, Balice-Gordon R, Lynch DR (2008) Anti-NMDA-receptor encephalitis: case series and analysis of the effects of antibodies. Lancet Neurol 7(12):1091–1098
10. Iizuka T, Yoshii S, Kan S, Hamada J, Dalmau J, Sakai F, Mochizuki H (2010) Reversible brain atrophy in anti-NMDA receptor encephalitis: a long-term observational study. J Neurol 257(10):1686–1691. doi:10.1007/s00415-010-5604-6
11. Sansing LH, Tüzün E, Ko MW, Baccon J, Lynch DR, Dalmau J (2007) A patient with encephalitis associated with NMDA receptor antibodies. Nat Clin Pract Neurol 3(5):291–296
12. Spiegel DR, Zaki N (2013) A case of new onset psychosis in a young woman with minimal response to risperidone, ultimately diagnosed with *N*-methyl-D-aspartate receptor encephalitis. Clin Schizophr Relat Psychoses:1–20. (Epub ahead of print)
13. Dericioglu N, Vural A, Acar P, Agayeva N, Ismailova V, Kurne A, Saka E, Arsava EM, Topcuoglu MA (2013) Antiepileptic treatment for anti-NMDA receptor encephalitis: the need for video-EEG monitoring. Epileptic Disord 15(2):166–170. doi:10.1684/epd.2013.0566
14. Wandinger KP, Saschenbrecker S, Stoecker W, Dalmau J (2011) Anti-NMDA-receptor encephalitis: a severe, multistage, treatable disorder presenting with psychosis. J Neuroimmunol 231(1–2):86–91
15. Prüss H, Dalmau J, Arolt V, Wandinger K-P (2010) Anti-NMDA-Rezeptor-Enzephalitis. Ein interdisziplinäres Krankheitsbild. Nervenarzt 81:396–408
16. Boeck AL, Logemann F, Krauß T, Hussein K, Bültmann E, Trebst C, Stangel M (2013) Ovarectomy despite negative imaging in anti-NMDA receptor encephalitis: effective even late. Case Rep Neurol Med 2013:843192. doi:10.1155/2013/843192
17. Batra R, Pang Y, Friedman MT (2012) Therapeutic plasma exchange in anti-*N*-methyl-D-aspartate-receptor (anti-NMDA-R) encephalitis associated with benign ovarian teratoma. J Clin Apher 27(4):227–228. doi:10.1002/jca.21212 No abstract available
18. Kitada M, Suzuki H, Ichihashi J, Mitsui Y, Tanaka K, Kusunoki S (2011) Dramatic improvement in two cases of anti-NMDA receptor encephalitis after immunomodulating therapy. Rinsho Shinkeigaku 51(9):683–687 Japanese
19. Peery HE, Day GS, Dunn S, Fritzler MJ, Prüss H, De Souza C, Doja A, Mossman K, Resch L, Xia C, Sakic B, Belbeck L, Foster WG (2012) Anti-NMDA receptor encephalitis. The disorder, the diagnosis and the immunobiology. Autoimmun Rev 11:863–872
20. Wandinger KP, Klingbeil C, Gneiss C, Waters P, Dalmau J, Saschenbrecker S, Borowski K, Deisenhammer F, Vincent A, Probst C, Stöcker W () New serological markers for the differential diagnosis of autoimmune limbic encephalitis. Laboratoriumsmedizin. Band 0, Heft 0, Seiten, ISSN (online) 1439-0477, ISSN (print) 0342-3026. doi:10.1515/jlm.2011.059et
21. Stöcker W, Saschenbrecker S, Rentzsch K, Komorowski L, Probst C (2013) Autoantikörperdiagnostik in der Neurologie mittels nativer und rekombinanter Antigensubstrate. Der Nervenarzt 84(4):471–476
22. Martinez-Hernandez E, Horvath J, Shiloh-Malawsky Y, Sangha N, Martinez-Lage M, Dalmau J (2011) Analysis of complement and plasma cells in the brain of patients with anti-NMDAR encephalitis. Neurology 77:589–593
23. Titulaer MJ, Kayser MS, Dalmau J (2013) Prevalence and treatment of anti-NMDA receptor encephalitis—authors' reply. Lancet Neurol 12:425–426
24. Irani SR, Bera K, Waters P et al (2010) *N*-methyl-D-aspartate antibody encephalitis: temporal progression of clinical and paraclinical observations in a predominantly non-paraneoplastic disorder of both sexes. Brain 133:1655–1667
25. Steiner J, Walter M, Glanz W et al (2013) Increased prevalence of diverse *N*-methyl-D-aspartate glutamate receptor antibodies in patients with an initial diagnosis of schizophrenia: specific relevance of IgG NR1a antibodies for distinction from *N*-methyl-D-aspartate glutamate receptor encephalitis. JAMA psychiatry 70(3):271–278. doi:10.1001/2013.jamapsychiatry.86
26. Prüss H, Finke C, Höltje M et al (2012) *N*-methyl-D-aspartate receptor antibodies in herpes simplex encephalitis. Ann Neurol 72(6):902–911. doi:10.1002/ana.23689
27. Prüss H, Dalmau J, Harms L, Höltje M, Ahnert-Hilger G, Borowski K, Stoecker W, Wandinger KP (2010) Retrospective analysis of NMDA receptor antibodies in encephalitis of unknown origin. Neurology 75(19):1735–1739
28. Ella Wotzke Dissertation an der Rheinischen Friedrich-Wilhelms-Universität Bonn "Häufigkeit und Phänotyp neurologischer und psychiatrischer Symptome bei Patientinnen mit ovariellen Teratomen: Eine Fragebogenstudie". http://hss.ulb.uni-bonn.de/2011/2449/2449.pdf
29. Niehusmann P, Dalmau J, Rudlowski C, Vincent A, Elger CE, Rossi JE, Bien C (2009) Diagnostic value of *N*-methyl-D-aspartate receptor antibodies in women with new-onset epilepsy. Arch Neurol 66(4):458–464. doi:10.1001/archneurol.2009.5