ORIGINAL ARTICLE

Borna disease virus circulating immunocomplex positivity and psychopathology in psychiatric patients in the Czech Republic

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Abstract

OBJECTIVES: Borna disease virus (BDV) is an RNA virus belonging to the family Bornaviridae. BDV is a neurotropic virus that causes changes in mood, behaviour and cognition. Patients with psychiatric disorders have a higher incidence of BDV positivity than healthy individuals.

METHODS: We examined the seropositivity of BDV circulating immunocomplexes (CIC) in psychiatric patients and healthy individuals (blood donors). We examined 39 psychiatric inpatients for the presence of BDV CIC in the serum by ELISA on day 0, 28 and 56. During the same period psychopathology was measured using psychiatric scales (CGI, CGI-I, MADRS, SDS, PANSS). This is the first such study performed in the Czech Republic.

RESULTS: BDV CIC positivity was detected in 66.7% of psychiatric patients (26/39) on day 0, in 53.9% (14/26) on day 28 and in 52.9% on day 56 (9/17). The control group was 22.2% (28/126) positive. The incidence of BDV CIC was significantly higher in psychiatric patients than in healthy individuals (p=0.001). The significantly higher level of BDV CIC was associated with the higher severity of psychopathology in comparison with patients with mild or moderate psychopathology (p=0.03). We did not find any association between BDV CIC positivity and other characteristics (age, diagnosis, family, personal history, the history of infectious diseases, contact with animals).

CONCLUSION: In our study psychiatric patients had significantly higher levels of BDV CIC than the control group. The highest levels of BDV CIC were detected in patients with more severe psychopathology.

Authors' contributions: SR designed the study protocol, obtained the approval of the local ethical committee, wrote the manuscript, performed the clinical evaluation of psychiatric patients, performed the evaluation of psychiatric patients with psychiatric scales and anamnestic questionnaires and blood sampling for BDV CIC examination. **LJ** designed the study protocol, wrote the manuscript, performed the clinical evaluation of psychiatric patients, the evaluation of psychiatric patients with psychiatric scales and anamnestic questionnaires. **HK** performed the analysis (laboratory tests) of blood samples for BDV CIC positivity from psychiatric patients and from blood donors.

List of abbreviations:

Ag – antigen
Ab – antibody
BDV – Borna Disease Virus
CGI – Clinical Global Impression
CGI-I – Clinical Global Impression-Improvement
CIC – circulating immunocomplexes
CNS – central nervous system
EIA – enzyme immunoassay
ICD-10 – International Statistical Classification of Diseases
IFA – immunofluorescence assay
MADRS – Montgomery-Asberg Depression Rating Scale
PANSS – Positive and Negative Syndrome Scale
RNA – ribonucleic acid
SDS – Zung Self-Rating Depression Scale

INTRODUCTION

Borna disease virus (BDV) is an RNA virus that belongs to the family Bornaviridae, order Mononegavirales. BDV is a neurotropic virus with a high affinity to the limbic system. BDV infects warm-blooded animals including humans and birds.

The clinical symptoms of infected animals range from asymptomatic, through mild symptomatology (cognitive impairment), neurological and behavioural changes to lethal course (meningoencephalitis non-purulenta) (Dietrich *et al.* 1998; Durrwald & Ludwig 1997; Ludwig & Bode 2000).

BDV is transmitted by infected saliva or other secretions through the nasal mucosa. BDV spreads intraaxonally and trans-synaptically towards olphactoric structures and then to the limbic system. BDV causes persistent infection of the central nervous system (CNS) (Solbrig *et al.* 2003). The activation of persistent BDV infection is associated with several clinical symptoms such as meningoencephalitis, and neurological, behavioural and mood disturbances. This activation is due to immune changes, stress and other factors (Durrwald & Ludwig 1997; Ludwig & Bode 2000).

BDV influences the CNS directly (by binding of viral proteins with neurotransmitter receptors) Dietrich *et al.* 1998; Solbrig *et al.* 2003; Stitz *et al.* 1998; Volmer *et al.* 2007) and indirectly (by immune response and inflammatory reactions). As a result, BDV infection is associated with psychiatric disorders in humans, especially affective disorders and schizophrenia (Dietrich *et al.* 1998). Viral antigens (Ag), antibodies (Ab), circulating immunocomplexes (CIC) and viral RNA have been detected in human tissues (plasma, serum, brain tissue, cerebrospinal fluid). A higher prevalence of BDV infection was found in psychiatric and immunocompromised patients (Cotto *et al.* 2003).

After activation of persistent BDV infection it is possible to detect Ag. In the second phase of acute viremia Ag binds with Ab and forms CIC. Ag and CIC positivity are associated with acute infection and viremia. Ab positivity means that the organism has been in contact with BDV but does not necessarily imply active BDV infection.

BDV positivity in psychiatric patients ranges from negative to highly positive (about 90%). These differences can be caused by using various laboratory methods, biological materials, diagnosis of patients and various regions.

BDV Ab were first detected in psychiatric patients in 1985 using an IFA method (immunofluorescence assay). Positivity was detected in 1 to 4% of patients, and in 20% of acutely depressed patients (Bode *et al.* 1996; Bode & Ludwig 2003; Rott *et al.* 1985). Higher BDV positivity based on the detection of viral Ag and CIC has been found in psychiatric patients (between 50 and 90% positivity) compared to healthy individuals (between 20 and 30%) (Bode & Ludwig 2003; Bode *et al.* 2001). Several studies failed to detect BDV in psychiatric patients, and did not confirm the higher positivity (Chalmers *et al.* 2005; Fukuda *et al.* 2001; Iwata *et al.* 1998; Tsuji *et al.* 2000). Furthermore, a pilot study carried out in the Czech Republic between 2001 and 2003 found 26% BDV CIC in psychiatric inpatients and 22% in healthy individuals (Rackova & Beran 2003).

Here we report the results of a study to detect BDV CIC positivity in psychiatric patients. This is the first such study performed in the Czech Republic. Our aim was to determine the relationship between BDV infection and other factors (psychiatric diagnosis, age, gender, the duration and number of episodes of psychiatric disorder and others). We expected to detect higher BDV CIC positivity in psychiatric patients and to find an association between BDV CIC positivity and the phase of psychiatric disorder. We compared our results with a control group of healthy individuals (blood donors).

MATERIAL AND METHODS

Study population

We have examined two groups: psychiatric patients and a control group of healthy subjects (blood donors).

Psychiatric patients

Patients who were hospitalised in the Psychiatric Department of the University Hospital in Pilsen, Czech Republic from 2006 to 2008 were included in this study. These patients had to fulfill the following criteria: 1) psychiatric patients hospitalised in the psychiatric department with diagnosis of affective disorders (depressive and bipolar disorders) or psychotic disorders (acute psychotic disorders, schizophrenia and schizoaffective disorders) according to ICD-10 (International Statistical Classification of Diseases); 2) aged 18 years or older and 3) patients from whom informed consent was obtained. We included 39 patients (13 males, 26 females) aged from 22 to 61 years (average age 42.59 +/- 10.86 years), 29 of which had a diagnosis of affective disorders (26 with depressive and three bipolar disorders) and ten with psychotic disorders.

Table 1. The detection of BDV CIC positivity on day 0, 28 and 56

Day	Total number (No.)	No. of BDV CIC positive (%)	No. of males	No. of BDV positive males	No. of females	No. of BDV positive females
Day 0	39	26 (66.67%)	13	5	26	14
Day 28	26	14 (53.85%)	11	4	15	10
Day 56	17	9 (52.94%)	10	4	7	5

Control population

As the control group of healthy individuals we examined blood donors from the blood bank of the Central Military Hospital in Prague, Czech Republic. Inclusion criteria for blood donors were: 1) individuals aged 18 years or older; 2) those who agreed to participate in this study and gave informed consent. We included 126 blood donors (97 males and 29 females) aged from 25 to 65 years (average age 40.31 +/- 11.32 years).

Laboratory tests

Patients were examined for BDV positivity in the serum. Sera were determined for specific circulating immunocomplexes by EIA (enzyme immuno assay) - CIC assay using a washing apparatus (MRW Dynex) and ELISA reader (MUREX MRX). This method was developed by Bode and Ludwig and uses specific monoclonal antibodies to trap the antigen part of the CIC (Bode *et al.* 2001). The antibody part of the CIC is visualised by enzyme reaction (alkaline phosphatase). The levels of BDV CIC were scored as: 0 negative, + low positivity, ++ mean positivity, +++ and ++++ high positivity.

Psychiatric scales

Standard psychiatric scales were used to measure and evaluate psychopathology and its severity in all patients: CGI (Clinical Global Impression) and CGI-I (Clinical Global Impression Improvement) for all patients; MADRS (Montgomery-Asberg Depression Rating Scale), SDS (Zung Self-Rating Depression Scale) for patients with the diagnosis of depression; and PANSS (Positive and Negative Syndrome Scale) for patients with psychotic disorders.

Study design

All patients were examined for serum BDV CIC positivity on day 0 (the day of admission to the psychiatric department), on day 28 and 56. At the same time psychopathology was measured using psychiatric scales.

Anamnestic data (somatic disorders, the history of infectious diseases, allergy…), psychiatric family history, and the presence of infection at the time of blood sampling were recorded for all participants. Psychiatric diagnosis according to ICD-10, the number of episodes of psychiatric disorder and contact with animals (breeding, farming, pets) were also recorded. Data were obtained directly from the patients and from medical examination records.

Ethical aspects

The study was approved by the ethical committee of the University Hospital Medical Faculty, Charles University in Pilsen, Czech Republic (registration number 303/2001). The purpose and the procedures of the study were explained to all participants. Written informed consent was obtained.

RESULTS

We examined 39 psychiatric patients on day 0 (13 males, 26 females), 26 patients on day 28 (11 males, 15 females) and 17 patients on day 56 (10 males, 7 females).

Age differences between the group of psychiatric patients and blood donors were not statistically significant (p=0.16, the Wilcoxon rank-sum test). The groups differed significantly in gender but no association between BDV CIC positivity and gender was demonstrated.

Serum BDV CIC positivity indicates the presence of BDV and its activity (the period of viremia). BDV CIC positivity was detected in 66.7% of psychiatric patients (26/39) on day 0, in 53.9% (14/26) on day 28 and in 52.9% on day 56 (9/17) (Table 1). BDV CIC positivity was found in 22.2% (28/126) of the control group (blood donors). BDV CIC positivity was significantly higher in psychiatric patients than in blood donors (p=0.004, contingency tables, the chi-square test). Higher levels of BDV CIC (++ and +++) were found in psychiatric patients in comparison with blood donors (p=0.01, the Fisher exact test, contingency tables).

Unlike the level of BDV CIC positivity, BDV CIC positivity was not associated with the psychopathology (day 0 p=0.513, day 28 p=0.232, day 56 p=0.707, contingency tables, the chi-square test). However, higher levels of BDV CIC in the serum were significantly associated with higher severity of psychiatric disorder measured by the CGI. Patients with GGI 5–6 (markedly or severely ill) had higher levels of BDV CIC than patients with CGI 1–4 (normal, borderline, mildly or moderately ill) (p=0.03, the Fisher exact test) at the beginning of hospitalisation (on day 0) but not on day 28 (p=0.079, contingency tables, the chi-square test) or day 56 (p=0.825, contingency tables, the chi-square test).

Table 2. The association of BDV CIC positivity and other anamnestic and demographic characteristics

Characteristics	Day	*p*	Statistical methods
Gender	Day 0	0.428	contingency tables, chi-square test
	Day 28	0.242	contingency tables, chi-square test
	Day 56	0.602	contingency tables, chi-square test
Age	Day 0	0.472	Spearman test
		0.49	Kendall test
	Day 28	0.104	Spearman test
		0.123	Kendall test
	Day 56	0.491	Spearman test
		0.51	Kendall test
Diagnosis	Day 0	0.765	contingency tables, chi-square test
	Day 28	0.822	contingency tables, chi-square test
	Day 56	0.12	contingency tables, chi-square test
Psychiatric family history	Day 0	0.232	contingency tables, chi-square test
	Day 28	0.731	contingency tables, chi-square test
	Day 56	0.333	contingency tables
Infection in the time of blood sampling	Day 0	0.981	Spearman test
		0.981	Kendall test
		0.838	contingency tables, chi-square test
	Day 28	0.51	Spearman test
		0.513	Kendall test
		0.429	contingency tables, chi-square test
	Day 56	0.803	Spearman test
		0.805	Kendall test
		0.718	contingency tables, chi-square test
Number of episodes of psychiatric disorder	Day 0	0.916	Spearman test
		0.932	Kendall test
	Day 28	0.517	Spearman test
		0.544	Kendall test
	Day 56	0.164	Spearman test
		0.138	Kendall test
Contact with animals	Day 0	0.505	contingency tables, chi-square test
	Day 28	0.398	contingency tables, chi-square test
	Day 56	0.376	contingency tables, chi-square test

Other characteristics and BDV CIC positivity

We obtained data about family and personal history, history of infectious diseases, and infection at the time of blood sampling, and data about psychiatric disorders (diagnosis, duration, number of episodes) and contact with animals from all participants. We also examined the possible relationship with age and gender. However, we did not find any significant association between BDV CIC positivity and other characteristics (Table 2).

DISCUSSION

The purpose of this study was to examine BDV CIC positivity in psychiatric patients, in comparison with a control group of healthy individuals. A secondary aim was to determine the association between BDV CIC positivity and psychopathology and their changes during two months of observation.

We detected BDV CIC positivity, which indicates the presence of BDV in the organism and the acute phase of infection (viremia). The EIA CIC assay was used to

test for BDV infection due to its high sensitivity and specificity (Bode, 2008; Bode & Ludwig 2003; Bode *et al.* 2001). Complete detection of the acute phase of BDV infection requires the detection of viral Ag. We did not have this method at our disposal, which could explain the lower positivity of acute BDV infection in our study in comparison with results from German psychiatric inpatients (Bode & Ludwig 2003; Bode *et al.* 2001).

BDV CIC positivity in our study was significantly higher in psychiatric patients than in blood donors. The same results were obtained in several other studies (Bode & Ludwig 2003; Bode *et al.* 2001; Rybakowski *et al.* 2001; Taieb *et al.* 2001; Vahlenkamp *et al.* 2000).

Significantly higher levels of BDV CIC were found in psychiatric patients in comparison with blood donors. The higher levels of BDV CIC were associated with more severe psychopathology at the beginning of hospitalisation but not on day 28 and 56. This finding is consistent with German studies that reported increased BDV CIC and Ag in severely depressed patients in comparison with moderately depressed patients and outpatients (Bode & Ludwig 2003; Bode *et al.* 2001).

Several studies have reported higher BDV positivity in younger individuals, especially in children. BDV infection can affect the development of brain structures (Patti *et al.* 2008; Scholbach & Bode 2008). We did not find any association between age and gender in our study population.

There was no interaction between psychiatric family history and BDV CIC positivity. It seems that genetically predisposed patients are not at higher risk of BDV infection.

There was no connection with personal history (somatic disorders), the history of infectious diseases and infection at the time of blood sampling. In some studies patients with other infections or immune changes had higher BDV positivity (Cotto *et al.* 2003).

One route of BDV infection transmission is contact with animals. Some studies show higher BDV positivity in people who work with animals (farming) (Weisman *et al.* 1994). There was no connection between BDV CIC positivity and contact with animals in our study.

CONCLUSIONS

We detected higher BDV CIC positivity in psychiatric inpatients than in the control group of blood donors. BDV CIC positivity did not significantly change over time (on day 0, 28 and 56) but a significantly higher level of BDV CIC was found in patients with the worst psychopathology. We have not established any association between BDV CIC positivity and other characteristics. Further studies are required to measure BDV CIC and detect Ag in psychiatric inpatients, addicted patients and other psychopathologies.

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REFERENCES

1 Bode L (2008). Human Borna virus infection – towards a valid diagnostic system. APMIS. **116**(Suppl. 124): 21–39.
2 Bode L, Durrwald R, Rantam FA, Ferszt R, Ludwig H (1996). First isolates of infectious human Borna disease virus from patients with mood disorders. Mol Psychiatry. **1**: 200–212.
3 Bode L, Ludwig H (2003). Borna disease virus infection, a human mental-health risk. Clin Microbiol Rev. **16** (3): 534–545.
4 Bode L, Reckwald P, Severus WE, Stoyloff R, Ferszt R, Dietrich DE, Ludwig H (2001). Borna disease virus-specific circulating immune complexes, antigenemia, and free antibodies – the key marker triplet determining infection and prevailing in severe mood disorders. Mol Psychiatry. **6**(4): 481–491.
5 Chalmers RM, Thomas DR, Salmon RL (2005). Borna disease virus and the evidence for human pathogenicity: a systematic review. Q J Med. **98**: 255–274.
6 Cotto E, Neau D, Cransac-Neau M, Auriacombe M, Pellegrin JL, Ragnaud JM, Fillet AM, Belnard M, Fleury H, Lafon ME (2003). Borna disease virus RNA in immunocompromised patients in southwestern France. J Clin Microbiol. **41**(12): 5577–5581.
7 Dietrich DE, Schedlowski M, Bode L, Ludwig H, Emrich HM (1998). A viro-psycho-immunological disease-model of a subtype affective disorder. Pharmacopsychiatry. **31**: 77–82.
8 Durrwald R, Ludwig H (1997). Borna disease virus (BDV), a (zoonotic?) worldwide pathogen. A review of the history of the disease and the virus infection with comprehensive bibliography. Journal of Veterinary Medicine. **44**(3): 147–184.
9 Fukuda K, Takahashi K, Iwata Y, Mori N, Gonda K, Ogawa T, Osonoe K, Sato M, Ogata S, Horimoto T, Sawada T, Tashiro M, Yamaguchi K, Niwa S, Shigeta S (2001). Immunological and PCR analyses for Borna disease virus in psychiatric patients and blood donors in Japan. J Clin Microbiol. **39**(2): 419–429.
10 Iwata Y, Takahashi K, Peng X, Fukuda K, Ohno K, Ogawa T, Gonda K, Mori N, Niwa S, Shigeta S (1998). Detection and sequence analysis of Borna disease virus p24 RNA from peripheral blood mononuclear cells of patients with mood disorders or schizophrenia and of blood donors. J Virol. **72**(12): 10044–10049.
11 Ludwig H, Bode L (2000). Borna disease virus: new aspects on infection, disease, diagnosis and epidemiology. Rev Sci Tech Off Int Epiz. **19**(1): 259–288.
12 Patti AM, Vulcano A, Candelori E, Donfrancesco R, Ludwig H, Bode L (2008). Borna disease virus infection in Italian children. A potential risk for the developing brain? APMIS. **116**(Suppl. 124): 70–73.
13 Patti AM, Vulcano A, Candelori E, Travali S (2008). Serological evidence of Borna disease virus infection in children, cats and horses in Sicily (Italy). APMIS. **116**(Suppl. 124): 77–79.
14 Rackova S, Beran J (2003). Borna disease virus-its influence on the onset and course of mental disorders. Psychiatrie. **7**(2): 149–150.
15 Rott R, Herzog S, Fleischer B, Winokur A, Amsterdam J, Dyson W, Koprowski H (1985). Detection of serum antibodies to Borna disease virus in patients with psychiatric disorders. Science. **228**(4700): 755–756.

16 Rybakowski F, Sawada T, Yamaguchi K (2001). Borna disease virus-reactive antibodies and recent-onset psychiatric disorders. European Psychiatry. **16**(3): 191–192.
17 Scholbach T, Bode L (2008). Borna disease virus infection in young children. APMIS. **116**(Suppl. 124): 83–88.
18 Solbrig M, Koob V, George F (2003). Neuropharmacological sequelae of persistent CNS viral infections: lessons from Borna disease virus. Pharmacol Biochem Behav. **74**(4): 777–787.
19 Stitz L, Noske K, Planz O, Furrer E, Lipkin WI, Bilzer T (1998). A functional role for neutralizing antibodies in Borna disease: influence of virus tropism outside the central nervous system. J Virol. **72** (11): 8884–8892.
20 Taieb O, Baleyte JM, Mazet P, Fillet AM (2001). Borna disease virus and psychiatry. European Psychiatry.**16**(1): 3–10.
21 Tsuji K, Toyomasu K, Imamura Y, Maeda H, Toyoda T (2000). No association of Borna disease virus with psychiatric disorders among patients in northern Kyushu, Japan. J Med Virol. **61**(3): 336–340.
22 Vahlenkamp TW, Enbergs HK, Muller H (2000). Experimental and natural Borna disease virus infections: presence of viral RNA in cells of the peripheral blood. Vet Microbiol. **76**(3): 229–244.
23 Volmer R, Prat ChMA, Masson G, Garenne A, Gonzales-Dunia D (2007). Borna disease virus infection impairs synaptic plasticity. J Virol. **81**(16): 8833–8837.
24 Weisman Y, Huminer D, Malkinson M, Meir R, Kliche S, Lipkin WI, Pitlik S (1994). Borna disease virus antibodies among workers exposed to infected ostriches. Lancet. **344**(8931): 1232–1233.