

High Circulatory Titer of Platelet-Associated Autoantibodies in Childhood Onset Schizophrenia and Its Diagnostic Implications

Tanya Ebert^{a, b} Mila Schechtman^c Anka Ram^{a, b} Ira Kosov^{a, b}
Abraham Weizman^{b, d, e} Meir Shinitzky^c Baruch Spivak^{a, b}

^aBeer Yaakov – Ness-Ziona Mental Health Center, Beer Yaakov, ^bDepartment of Psychiatry, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, ^cDepartment of Biological Chemistry, Weizmann Institute of Science, Rehovot, and ^dGeha Mental Health Center and ^eLaboratory of Biological Psychiatry, Felsenstein Medical Research Center, Petah Tikva, Israel

Key Words

Schizophrenia · Childhood onset schizophrenia · Blood test · Biomarker · Platelet associated autoantibody · Immune system

Abstract

Background: It has been suggested that the etiology of schizophrenia, in a distinct group of patients, originates from an autoimmune reaction against platelets. Previous studies have demonstrated significantly higher blood titers of platelet-associated autoantibodies (PAA) in adult schizophrenia patients as compared to normal healthy subjects. In addition, young adult schizophrenia patients at their early stages of the disorder displayed higher PAA titers than older patients with longer duration of the disorder. **Aim:** To assess the blood titers of PAA in children with schizophrenia as compared to matched control subjects without psychotic disorders, as a possible diagnostic parameter. **Methods:** Twenty-nine children with DSM-IV schizophrenia in the active psychotic state, with an age range of 6–12 years (mean $\pm$ SD: 9.6 ± 1.5 years), with average Positive and Negative Syndrome Scale scores of 108 ± 19.2 , were assessed. The control

group consisted of 25 children with DSM-IV conduct disorder in a similar age range of 5–12 years (mean $\pm$ SD: 9.5 ± 1.6 years). The blood titers of PAA were evaluated using an optimized ELISA test, expressed by a linear optical density (OD) scale. The blood samples of all participants were tested anonymously and were scored under a code number. A test recording above 1.4 OD units was predefined as positive. **Results:** The titers of PAA of children with schizophrenia (1.9 ± 0.5 OD units, range: 0.7–2.44 units) were significantly ($p < 0.00001$) higher than those of the control group (1.0 ± 0.4 OD units, range: 0.45–2.28 units). In 83% of the children with schizophrenia (24 out of the 29 patients) a positive test, i.e. OD > 1.4 , was detected. In contrast, in the control group, only 12% (3 of the 25 subjects) displayed a positive test, $p < 0.00001$. **Conclusions:** High titers of PAA in children with schizophrenia as compared with nonpsychotic controls may indicate an active autoimmune process in the early onset of schizophrenia. The PAA level may therefore provide a supportive diagnostic biomarker for childhood schizophrenia.

Copyright © 2013 S. Karger AG, Basel

T.E. and M.S. contributed equally to this work.

Introduction

Distinct features of immune dysfunctions associated with autoimmune processes have been implicated in some forms of schizophrenia [1]. Most of the reported immune dysregulations in schizophrenia overlap with central pathophysiological mechanisms, as well as with clinical manifestations of the disorder [1–3]. It has been suggested that in a distinct group of patients, psychotic symptoms may originate from an autoimmune reaction against platelets [4–6]. Accordingly, platelet-associated autoantibodies (PAA), which can cross-react with brain tissue, penetrate the blood-brain barrier as a result of a traumatic physical, emotional or inflammatory insult [7] with an ensuing psychotic eruption [5, 8]. Previous studies have demonstrated significantly higher blood titers of PAA in adult schizophrenia patients as compared to normal healthy subjects [4–6]. In addition, young schizophrenia patients at their early stages of the disorder displayed higher PAA titers than older patients with longer duration of the disorder [9–11].

Childhood onset schizophrenia (COS) is a very rare and severe form of schizophrenia characterized by an onset of psychotic symptoms by the age of 12 years or earlier (very-early-onset schizophrenia) [12, 13]. The frequency of COS is reported to be less than 1 case in 10,000–30,000 children. There is an extreme paucity of immunological studies in COS [14, 15] and blood titers of PAA in this group of patients have not yet been investigated.

The aim of the present study was to determine the blood levels of PAA in children with schizophrenia in comparison with matched children without psychotic disorders and to evaluate their diagnostic potential.

Methods

Subjects

The study participants were children with schizophrenia, with an age range of 6–12 years, admitted to the Ness-Ziona Mental Health Center while in the acute psychotic state. Inclusion criteria required that the participating patients met the DSM-IV diagnosis criteria of schizophrenia, according to the Structured Clinical Interview for Axis-I DSM-IV Disorders – Patient Version [16] and exhibited a minimal score of 60 on the Positive and Negative Syndrome Scale [17]. All patients had to have a normal routine laboratory test. Patients with unstable physical-medical conditions, past or current medical or neurological illness, or current major routine laboratory abnormalities were excluded from the study. In addition, treatment-resistant patients under treatment with clozapine were also excluded. The control group consisted of children with DSM-IV conduct dis-

order in the age range of 5–12 years. All tested subjects of both groups underwent an assessment of physical diseases, using a special questionnaire and a physical examination. The study was approved by the Institutional Review Board and all parents of the minors signed a written informed consent for participating in the study.

Laboratory Procedure

A procedure outlined in our previous publications [9–11] was used. Twenty milliliters of venous blood were drawn from each of the participants and then evaluated at the laboratory at the Weizmann Institute. Serum samples were isolated within 12 h and then stored at -20°C until use. Titers of PAA were evaluated by a previously described ELISA procedure [9] which was optimized to a highly reproducible test (to be published). In this test an immobilized dimer of a peptide epitope from a specific platelet antigen [9] provided the binder of circulating PAA, followed by an enzymatic color release proportional to the level of bound PAA expressed in units of optical density, OD. The color intensity scale required a preevaluation of an empirical cutoff OD, below and above which the test was registered as negative or positive, respectively. This cutoff value was preevaluated with a battery of stored serum samples of registered normal subjects and schizophrenia patients and was defined as 1.4 OD units.

Statistical Analysis

Blood titers of PAA of the schizophrenia patients were compared with PAA titers of the control subjects, using a two-tailed unpaired Student's *t* test. Categorical data were analyzed using Fisher's exact test. All values are expressed as means $\pm$ SD.

Results

A group of 29 children with schizophrenia with an age range of 6–12 years (mean $\pm$ SD: 9.6 ± 1.5 years) participated in the study. The control group consisted of 25 children with an age range of 5–12 years (mean $\pm$ SD: 9.5 ± 1.6 years). The children with schizophrenia were in the active psychotic state at the time of examination, with average Positive and Negative Syndrome Scale scores of 108 ± 19.2 of which the score for positive psychotic symptoms was 27 ± 5.3 and the score for negative psychotic symptoms was 24.8 ± 7.5 . Most of the patients were defined by the Clinical Global Impression Scale as suffering from severe disturbance, with scores ranging from 4 (moderately ill) to 7 (extremely ill), with an average score of 5.8 ± 0.8 (markedly to severely ill). All children of the control group were diagnosed with conduct disorder according to the DSM-IV diagnostic criteria.

The titers of PAA of children with schizophrenia were 1.9 ± 0.5 OD units (mean $\pm$ SD; range: 0.7–2.44 units), while the titers of the control group were 1.0 ± 0.4 OD

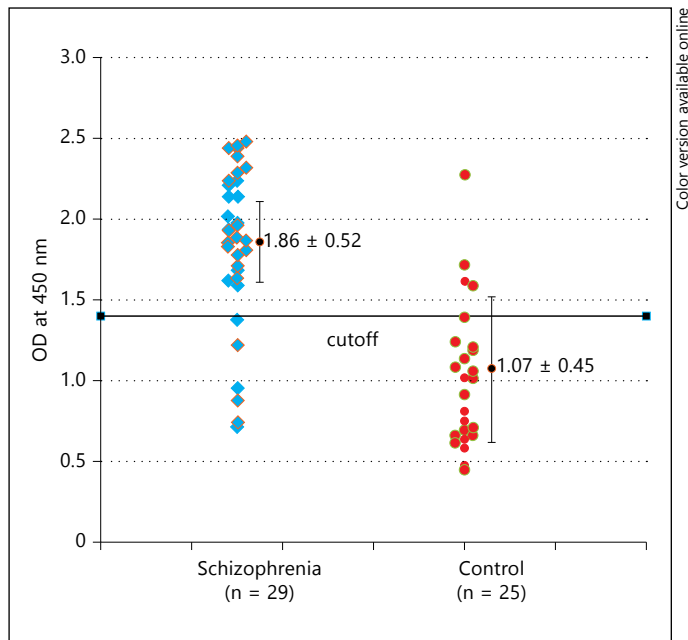


Fig. 1. Summary of the OD recordings scored for children aged 6–12 years diagnosed with schizophrenia as compared to the recorded values for control children (children suffering from conduct disorder). The cutoff value is presented by a thick black line.

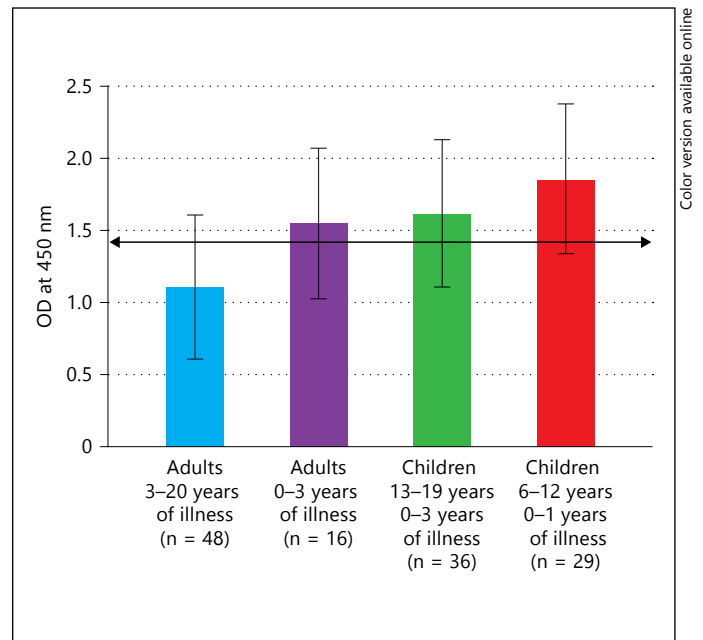


Fig. 2. A comparative summary of the ranges of serum PAA levels, presented as OD values, recorded in 4 separate groups of patients with schizophrenia [10, 11; this study]. The experimental cutoff value of OD = 1.4 is presented by the horizontal black line. The vertical bars represent the range of the recorded OD values.

units (mean $\pm$ SD; range: 0.45–2.28 units). The average titers of PAA in children with schizophrenia were significantly ($p < 0.00001$) higher (almost 2-fold) than those of the control group. In the group of children with schizophrenia 24 out of the 29 patients (83%) displayed a positive test (i.e. OD > 1.4), i.e. test sensitivity = 83%. In contrast, in the control group, only 3 of the 25 subjects (12%) displayed a positive test, i.e. test specificity = 88%. Comparison of the two groups by Fisher's exact test yielded a highly significant difference between the groups: $p < 0.000001$. The results are summarized graphically in figure 1.

Discussion

PAA is probably a mixed population of autoantibodies, some of which being natural constituents of normal blood sera. It is plausible that in schizophrenia a specific PAA subgroup prevails, contributing the excess amount recorded in our COS patients. It might be suggested that such a subgroup of PAA is involved in the pathophysiology of the disease by cross-reacting with brain tissue [5,

8]. High titers of PAA may thus indicate an active autoimmune process which contributes to the overt neurodevelopmental impairments, at the very early onset of schizophrenia.

It has been previously reported that the serum of schizophrenia patients is enriched with PAA [4, 6, 10, 11] and an inverse correlation has been detected between the age of patients and the level of PAA titers [10, 11]. The main objective of the present study was to further investigate the validity of the above findings in children with schizophrenia. We found significantly higher PAA serum levels in children with schizophrenia as compared to the control group of children with conduct disorder. In addition, a very high rate (83%) of positive tests for blood PAA levels was demonstrated in children with schizophrenia at their very early stage of the disorder as compared to children without psychotic spectrum disorder, where only 12% displayed a positive test for blood PAA levels. In comparison to our previous studies in adult and adolescent patients [10, 11], we found in this study even a higher level of positive tests for PAA blood levels in children with COS expressed by sensitivity of 83% and specificity of 88%. Figure 2 presents a compari-

son of the results of the present study with the results obtained by this method in our previous studies [10, 11]. It clearly indicates that PAA evaluation could serve as an important diagnostic parameter for the possibility of eventual onset of schizophrenia in children with prodromal symptoms.

It appears that high serum titers of PAA in children with schizophrenia as compared with nonpsychotic controls may indicate an active autoimmune process in the early onset of schizophrenia, and thus circulatory PAA levels may provide a supportive diagnostic biomarker for childhood schizophrenia.

References

- 1 Jones AL, Mowry BJ, Pender MP, Greer JM: Immune dysregulation and self-reactivity in schizophrenia: do some cases of schizophrenia have an autoimmune basis? *Immunol Cell Biol* 2005;83:9–17.
- 2 Knight JG, Menkes DB, Highton J, Adams DD: Rationale for a trial of immunosuppressive therapy in acute schizophrenia. *Mol Psychiatry* 2007;12:424–431.
- 3 Strous RD, Shoenfeld Y: Schizophrenia, autoimmunity and immune system dysregulation: a comprehensive model updated and revisited. *J Autoimmunity* 2006;27:71–80.
- 4 Shinitzky M, Deckmann M, Kessler A, Sirota P, Rabbs A, Elizur A: Platelet autoantibodies in dementia and schizophrenia – possible implication for mental disorders. *Ann NY Acad Sci* 1991;621:205–217.
- 5 Kessler A, Shinitzky M: Platelets from schizophrenic patients bear autoimmune antibodies that inhibit dopamine uptake. *Psychobiology* 1993;21:299–306.
- 6 Deckmann M, Shinitzky M, Leykin I, Cheng D, Guy J, Sirota P: Humoral and cellular response against autologous platelets. *Ital J Psych Behav Sci* 1996;6:29–34.
- 7 Schwarz MJ, Ackenheil M, Riedel M, Muller N: Blood-cerebrospinal fluid barrier impairment as indicator for an immune process in schizophrenia. *Neurosci Lett* 1998;253:201–203.
- 8 Shinitzky M, Leykin I, Deckmann M: Autoimmunity against platelets in schizophrenia; in Shoenfeld Y (ed): *The Decade of Autoimmunity*. Amsterdam, Elsevier, 1999, pp 277–284.
- 9 Deckmann M, Mamillapalli R, Schechtman M, Shinitzky M: A conformational epitope which detects autoantibodies from schizophrenic patients. *Clin Chim Acta* 2002;322:91–98.
- 10 Spivak B, Schechtman M, Blumensohn R, Schonherz-Pine Y, Yoran-Hegesh R, Deckmann M, Mayer R, Weizman A, Shinitzky M: Blind verification of elevated platelet autoantibodies in serum of schizophrenic patients. I. Young subjects. *Neuropsychobiology* 2009;60:44–48.
- 11 Spivak B, Schechtman M, Schonherz-Pine Y, Blumensohn R, Mostovoy S, Amital D, Deckmann M, Weizman A, Shinitzky M: Blind verification of elevated platelet autoantibodies in serum of schizophrenic patients. II. Adult subjects. *Neuropsychobiology* 2009;60:49–54.
- 12 Remschmidt HE, Schulz E, Martin M, Warnke A, Trott G: Childhood-onset schizophrenia: history of the concept and recent studies. *Schizophrenia Bull* 1994;20:727–745.
- 13 Gordon CT, Frazier JA, McKenna K, Giedd J, Zametkin A, Zahn T, Hommer D, Hong W, Kaysen D, Albus KE, Rapoport JL: Childhood-onset schizophrenia: an NIMH study in progress. *Schizophrenia Bull* 1994;20:697–712.
- 14 Mittleman BB, Castellanos FX, Jacobsen LK, Rapoport JL, Swedo SE, Shearer GM: Cerebrospinal fluid cytokines in pediatric neuropsychiatric disease. *J Immunol* 1997;159:2294–2299.
- 15 Jacobsen LK, Mittleman BB, Kumra S, Lenane MC, Barracchini KC, Adams S, Simonis T, Lee PR, Long RT, Sharp W, Sidransky E, Ginns EI, Rapoport JL: HLA antigens in childhood onset schizophrenia. *Psychiatry Res* 1998;78:123–132.
- 16 First MB, Spitzer RL, Gibbon M: *Structured Clinical Interview for Axis I DSM-IV Disorders, Patient Version (SCID-I/P), Version 2*. New York, New York State Psychiatric Institute, Biometrics Research, 1995.
- 17 Kay S, Fiszbein A, Opler LA: The positive and negative syndrome scale for schizophrenia. *Schizophrenia Bull* 1987;13:261–276.

Copyright: S. Karger AG, Basel 2013. Reproduced with the permission of S. Karger AG, Basel. Further reproduction or distribution (electronic or otherwise) is prohibited without permission from the copyright holder.