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Blind Verification of Elevated Platelet Autoantibodies in Serum of Schizophrenic Patients – Part I: Young Subjects

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Key Words
Schizophrenia · Diagnosis · Blood test · Platelets · Autoantibodies · Young subjects

Abstract
Background: It has been hypothesized that the etiology of schizophrenia, in a distinct group of patients, originates from an autoimmune reaction against platelets. Previous open screenings have recorded significantly higher blood titers of platelet-associated autoantibodies (PAA) in schizophrenic patients as compared to normal healthy subjects. In addition, young schizophrenic patients at the early stages of their disorder displayed higher PAA titers than older patients with a longer duration of the disorder. A blood test based on these observations was proposed. ***Aim:*** To verify by a blind test a significant difference in PAA between young schizophrenic patients and matched healthy control subjects, for the validation of a blood test for schizophrenia. ***Methods:*** A total of 36 young schizophrenic patients in an active psychotic state, aged 13–20 years (mean ± SD: 16.2 ± 2.1 years) with an average PANSS score of 115.6 ± 14.5 and illness duration of 9.5 ± 9.4 months, were examined. The control group consisted of 49 healthy young subjects between the ages of 13 and 21 years (16.2 ± 2.2 years). The blood titers of PAA were evaluated blindly 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 1.3 OD units was defined as positive. ***Results:*** The PAA titers of schizophrenia patients (1.6 ± 0.4 OD units, range: 0.7–2.3 OD units) were significantly higher than those of the control group (1.0 ± 0.4 OD units, range: 0.4–1.8 OD units; $p < 0.0001$). In 61% of the young schizophrenic patients (22 out of the 36 patients), a positive result (OD >1.3 units) was recorded. In the control group, only 12.2% (6 of the 49 subjects) displayed a positive result ($p < 0.0001$). ***Conclusions:*** These findings support further assessment of PAA titers as a potential biomarker for patients with clinical signs and symptoms of schizophrenia.

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Introduction

Schizophrenia is a severe neuropsychiatric disorder which affects 1% of the population worldwide [1–3]. The etiology of schizophrenia is still obscure, which imposes a serious obstacle in the development of specific treatments or objective tests. Distinct features of immune dysfunctions associated with autoimmune processes have been implicated in some forms of schizophrenia [4]. These include an altered risk of developing certain autoimmune diseases and the apparent involvement of genes known to affect the immune response repertoire [5, 6]. Most of the recorded immune dysregulations in schizophrenia overlap with central pathophysiological mechanisms as well as clinical manifestations of the disorder [4–6].

Our autoimmune hypothesis for schizophrenia implies that the etiology of a distinct group of patients in this disorder originates from a peripheral autoimmune reaction against platelets [7–9]. Accordingly, specific 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 infectious insult [10] with an ensuing psychotic eruption [8, 11]. Pervious open screenings [7–9, 12], as well as the following single-blind controlled study, have shown significantly higher blood titers of PAA in schizophrenia patients as compared to matched normal healthy subjects. Furthermore, young schizophrenic patients in the early stages of their disorder displayed higher PAA titers than older patients with a longer duration of the disorder [12].

It is important to note that the titers of these PAA were not influenced by any of the antipsychotic medications, except for clozapine which was found to reduce PAA titers [11, 12].

A correlation between age or stage of the illness and elevated levels of blood PAA is of a great importance for understanding this putative arm in the etiology of schizophrenia and providing a basis for a diagnostic blood test. In the following blind study (blind for the laboratory), we have demonstrated a significantly elevated level of PAA in children and adolescents suffering from schizophrenia, which adds support for a possible future application of this measure as a laboratory-based diagnostic tool.

Methods

Participants

The study participants were young schizophrenic patients (age range: 13–20 years) admitted to the Ness-Ziona Mental Health Center while in an acute psychotic state, with a duration of the disorder not longer than 24 months. Inclusion criteria required that the participating patients met the DSM-IV diagnostic criteria of schizophrenia, according to the Structured Clinical Interview for Axis-I DSM-IV Disorders – Patient Version [13] and scored a minimal of 60 on the Positive and Negative Symptom Scale (PANSS) [14]. All patients had to have normal routine laboratory tests. Patients with an unstable physical medical condition, past or current medical or neurological illness, past or current alcohol or any other substance abuse, or current major routine laboratory abnormalities were excluded from the study. In addition, patients who had been treated with any non-psychotropic medication or with clozapine were not included.

The control group consisted of normal healthy volunteers aged 13–21 years with a normal medical history, who did not suffer from any chronic or current psychological or physical disturbances, and were not being treated with any medication. All the subjects, both schizophrenic and healthy subjects, underwent an assessment for physical diseases using a special questionnaire and a physical examination.

The details of the study were fully explained to all potential participants and to the parents of the minors prior to study commencement. All participants and the parents of the minors signed a written informed consent. The study protocol was approved by the Ness-Ziona and Beer Yaakov Hospital Institutional Review Board.

Laboratory Procedure

Twenty milliliters of venous blood was drawn from each of the participants, and then encoded and transferred blindly to the laboratory at the Weizmann Institute (M.Sh. and M.Sc.). Serum samples obtained from the blood samples were isolated within 12 h and then stored at –20°C until use. Titers of PAA were evaluated by a previously described ELISA test [12] that was optimized to a highly reproducible test. In this test, an immobilized dimmer of a peptide epitope from a platelet antigen [12] 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 pre-evaluation to set an empirical cutoff for OD, below and above which the test was registered as negative or positive, respectively. This cutoff value was pre-evaluated with a battery of stored serum samples of registered normal subjects and schizophrenic patients, and was defined as 1.3 OD units. It should be noted that in this method, the magnitude of the linear OD scale can be reduced or increased along selected conditions that are most convenient with no effect on the results.

The level of serum antibodies against a specific epitope is expected to be spread over a wide range of binding capacity. Diagnostic monitoring of circulating antibodies therefore requires pre-evaluation of sensitivity, i.e. the probability of positive detection, versus specificity, i.e. the probability of negative detection in the control group. A recently published method for the diagnosis of multiple sclerosis by antibody monitoring provides a good example [15]. The cutoff value for our test was pre-evaluated with a battery of stored serum samples of registered normal subjects and

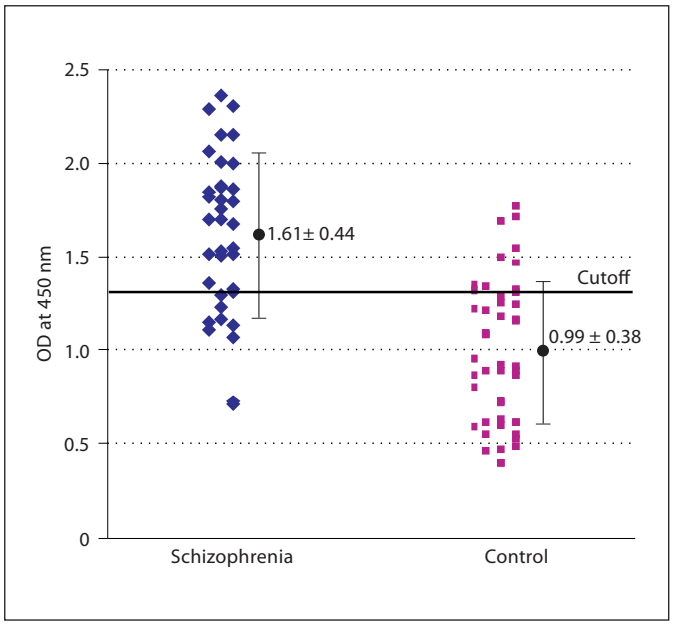

Fig. 1. Recorded PAA in the sera of young schizophrenic patients (n = 36) and control subjects (n = 49).

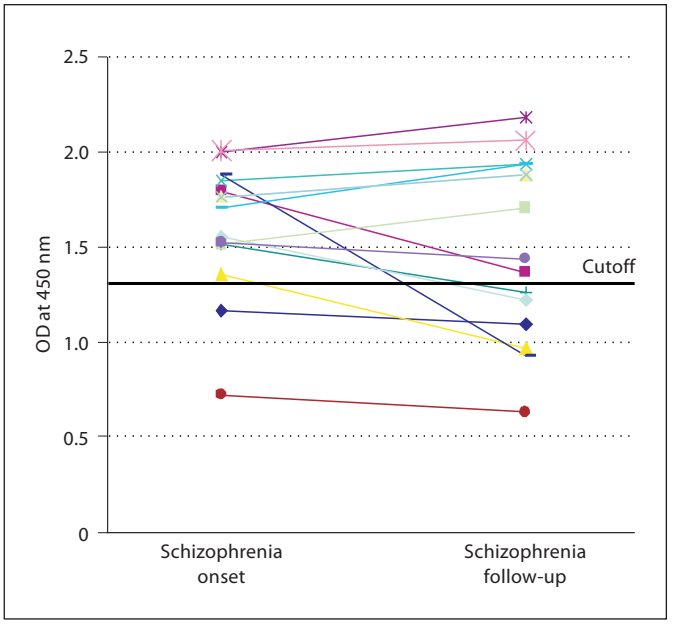

Fig. 2. PAA levels in young schizophrenic patients at onset and after 6–12 months of hospitalization.

schizophrenic patients, and was selected as 1.3 OD units. For this cutoff value, the sensitivity in our registered samples was 63%, while the percentage of negative recordings in the registered normal samples, i.e. the specificity, was 88%.

The exact records and coding of the examined subjects were filed (Y.S.) and kept undisclosed until all laboratory results were collected. Thereafter, the coding and the ELISA results, together with the rest of the clinical information, were processed for final statistical analysis.

Statistical Analysis

Blood titers of PAA of the schizophrenic 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. Averaged values were expressed as means ± SD.

Results

A group of 36 young schizophrenic patients (23 males and 13 females) in the age range of 13–20 years (16.2 ± 2.1 years) participated in the study. The disease duration of the patients was 9.5 ± 9.4 months, and their mean number of hospitalizations was 1.2 ± 0.4 (first hospitalization for 30 out of the 36 patients).

The control group consisted of 49 healthy young subjects (19 males and 30 females) between the ages of 13 and 21 years (mean: 16.2 ± 2.2 years). The age range and average age of both groups were similar (two-tailed unpaired t test: $p > 1.0$; d.f. = 83, t = 2.0).

The schizophrenia patients were in an active psychotic state at the time of examination, with a relatively high average PANSS score (115.6 ± 14.5), of which the score for positive psychotic symptoms was 28.9 ± 5.5 and the score for negative psychotic symptoms was 30.4 ± 8.1. Most of the patients were defined by the Severity of Illness Scale from the Clinical Global Impression Scale as suffering from a severe disturbance on a scale ranging from 4 (moderately ill) to 7 (extremely ill), with an average score of 5.8 ± 0.7 (markedly to severely ill).

All patients had been free of neuroleptic treatments and any other medications for at least 3 weeks at the time of hospitalization. The assessment of blood levels of PAA was performed on the first days of hospitalization in order to minimize the possible effects of medications.

A summary of the results is presented in figure 1. As shown, the titers of PAA of schizophrenia patients averaged 1.61 ± 0.44 OD units (range: 0.7–2.3 OD units), while the titers of PAA of healthy subjects were 0.99 ± 0.38 OD units (range: 0.4–1.8 OD units). The average ti-

ters of PAA in the group of schizophrenia patients were significantly higher than in the control group ($p < 0.0001$; two-tailed unpaired t test: $t = 6.95$, d.f. = 83).

In the group of schizophrenia patients, 22 out of the 36 patients (61.1%) displayed a positive test, while in the control group, only 6 of the 49 subjects (12.2%) displayed a positive test. The comparison of the 2 groups by Fisher's exact test yielded a highly significant difference of $p < 0.0001$ (OR = 11.26, 95% CI: 3.80–33.36). There were no statistical differences in the rate of positive tests between females and males, either in the patient group (10/3 vs. 12/11; Fisher's exact test, $p = 0.18$) or in the control group (5/25 vs. 1/18; Fisher's exact test, $p = 0.38$).

In 15 patients, PAA recordings were carried out immediately after the onset of the first psychotic episode and 6–12 months later. The results of these follow-up records are presented in figure 2. As shown, in 7 patients the PAA level increased after hospitalization, while in 8 it decreased. The correlation of these opposite trends with type of treatment and with the overall psychological state may turn out to be an important follow-up tool, for which further investigations are planned.

Discussion

It has been previously documented that the serum of schizophrenic patients is enriched with PAA [7–9]. The main objective of this study was to verify by a blind test the validity of the above findings for a potential laboratory-based biomarker for young subjects with clinical signs and symptoms of schizophrenia.

In this study, an analogous blind test with adult subjects was performed [16]. An overall summary of the results of this study is presented in figure 1. It shows a clear difference in average PAA levels between the patients (1.61 OD units) and control group (0.99 OD units). In addition, the recorded values indicate that a high rate of young schizophrenic patients were positive for PAA in the early stage of their disorder (61.1%), as compared to the healthy control group (12.2%).

PAA is probably a mixed population of antibodies, some of which are induced by drug treatments other than neuroleptics [17, 18] and may be non-pathogenic and unrelated to the etiology or the course of the disease. It is plausible, however, that in schizophrenia a specific PAA subgroup prevails, which makes up the elevated amounts recorded in our test. It may be suggested that this subgroup of PAA is the one which has been proposed to participate in the etiology of the disease by cross-reacting with brain tissue [8, 11]. High titers of PAA may thus indicate an active autoimmune process that contributes to the schizophrenic disorder. Patients with such high PAA titers may represent some forms of autoimmune pathophysiology, and may be considered for immunosuppressive therapy in addition to their regular antipsychotic treatments [5, 19]. In our study, we tried to minimize scoring of autoantibodies induced by neuroleptic medications [20–22] by including untreated patients or patients who had received a very short duration of treatment. Moreover, our previous experience with PAA testing clearly indicated that the blood titers of PAA are not influenced by any antipsychotic medication, except clozapine [11, 12].

The highly significant difference in the OD scoring between the patients and the healthy subjects presented in this blind study clearly supports the further assessment of PAA titers as a potential biomarker for schizophrenia in young patients. This novel laboratory approach could also be applied in the monitoring of young individuals at high risk of developing schizophrenia by correlating behavioral or mental deteriorations with increases in PAA.

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