

A BLOOD BIOLOGICAL TEST IN RECENT ONSET SCHIZOPHRENIA

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SUMMARY. A study was done of blood samples of 10 schizophrenic patients of recent onset (less than 1 year of evolution) of the illness without treatment and in critical period of the disease and 10 controls volunteers of the nurse personal of the Psychiatric Hospital of Havana without antecedents of psychiatric or neurological illness, matched with similar ages between 17 and 35 years old and equal socio-economic conditions.

In this additional study, we have observed similar alterations in platelets to those observed in patient with more time of evolution and also in critical state so that the possible influence of neuroleptic treatment is discarded like a factor related to platelet morphological alterations. Therefore the platelet morphological alterations observed that can contribute to the knowledge of the etiology of the illness can also constitutes a biological diagnostic test (endophenotypic marker) when allowing to differ by means of an analysis of blood a patient of a normal person.

Key words: schizophrenia, platelets, diagnostic biological test.

INTRODUCTION.

A biological diagnostic test doesn't exist in schizophrenia. However it is known that the prognosis of the illness only is modified in a favorable sense for an early treatment in the prodromic phase or in the period of duration of untreated psychosis (DUP) that is the time from manifestation of the first psychotic symptoms to initiation of adequate treatment (1-20) for what the absence of a biological test that allows to discriminate a patient of another psychiatric pathology in that stage of the illness doesn't allow the installation of an early treatment. A biological test would be also not only useful for the diagnosis but rather it could be applied for the investigation of the illness in any field that includes among other, the therapeutic assays, epidemiologic studies and in any investigation where it is necessary to

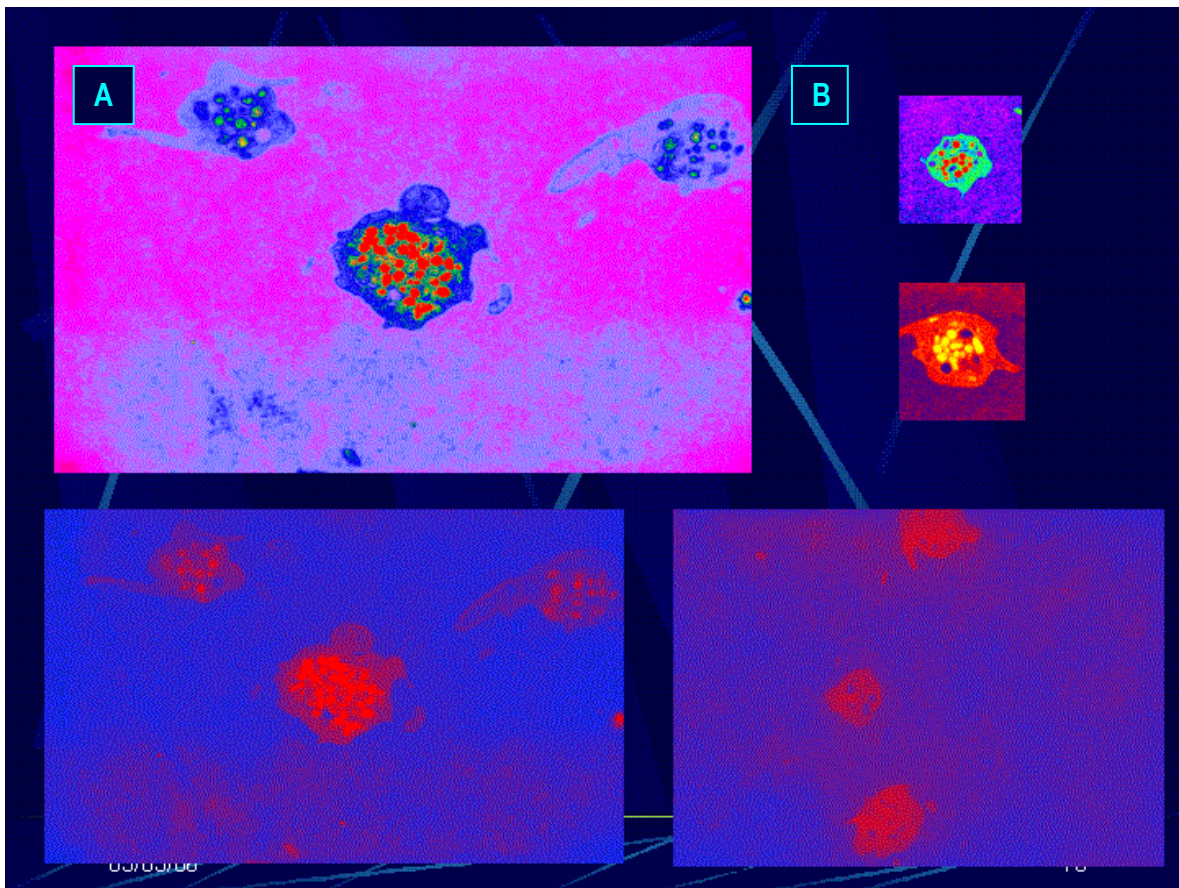
differentiate a patient of a control. In this study the observation of platelet morphological alterations in young schizophrenic patients -in relation to size, forms and specific characteristics- during the critical period of the illness and the absence of these alterations in controls made us consider the use of this observation as a biological test that allowed us to differ patients of healthy controls and therefore of diagnostic value.

MATERIAL AND METHOD.

Samples of venous blood (3ml) was drawn from a peripheral vein of a total of 10 schizophrenic patients of the Psychiatric Hospital of Havana [PHH] with DSM-IV diagnostic criteria and different clinical forms (21) and 10 controls of the personal nurse of the PHH without antecedents of psychiatric or neurological conditions. The samples were obtained by means of informed consent, according to the ethical approaches for research and with the approval of the Committee of Ethics of the Institution. All the samples were obtained in test tubes with EDTA type potassium anticoagulant. The ages of all the patients and controls were between 15 and 35 years. Both sexes were represented in a proportional form. To each patient and control was carried out a questionnaire to obtain data related with name, age, date of birth, family antecedents of psychosis; in the case of the patients: psychiatric symptoms present, time of evolution of the illness, age, sex, race, received treatment, as well as frequency and duration of the crises. The blood samples were centrifuged at 1.000 rev/min during 10 minutes; obtained the platelet-rich plasma it was centrifuged later on at 3.000 rev/min during 10 minutes. Then they were studied by means of the following ultramicroscopic techniques: fixation in buffer phosphate containing 3% glutaraldehyde (Millonig) 0,15M pH 7,4, during one hour at 4 °C and washed with buffer, later on. Section of the samples and washed during the night in buffer at 4 °C. Then fixation with 2% osmium tetroxide in buffer phosphate during one hour at 4 °C and washed twice with buffer solution by five minutes every time at the same temperature. The dehydration was made in 50 and 70% alcohol, during five minutes in each phase, at the same temperature, and in absolute alcohol in three occasions, 10 minutes in each occasion, at ambient temperature. The inclusion was carried out in araldite.

RESULTS.

In platelets of schizophrenic patients morphological alterations were observed consistent in: a) increase of the size, in occasions up to three times the size observed in control platelets. b) alterations in the form, acquiring rare forms with increase in the longitude of the filopodias and deformity of the same ones with increase of the traverse diameter. c) vacuolation with formation of big vacuoles inside of and outside of the platelets. Presence of spherical and hexagonal particles inside the big vacuoles as well as remains of membranes e) Increase of microtubules with formation of bundles of the same ones. f) increase in the glycogen concentration occupying great part of the platelet and inside the big vacuoles. g) diminished electrondensity. Fig. 1 These results were in agreement with the cytopathologic diagnosis valuation scale already mentioned in previous works. Table. The punctuations above 11 corresponded to pathological punctuations.



The platelet alterations observed in this additional study of schizophrenic illness allowed us to identify these alterations as a result of the illness and not as a result of the population's ethnic form of the illness.

A. Platelets
B. Platelets
Electron micrographs

DISCUSSION.

The results obtained in the present work can also have not only practical application for the positive diagnosis, but also for epidemiologic studies in high risk populations of families with a great genetics load, in investigations of another nature where it is necessary to distinguish patient of controls, in legal medicine as a possible skill test and in the control of the therapeutic effect to different pharmacos that includes the use of antiviral agents given the obtained results that guide toward this etiology, among other aspects of practical application. The morphological alterations [already reported in previous studies] [22] observed in platelets of schizophrenic patients consisted in increased size, alterations in the form, glycogen increment, abnormal vacuolation within and outside the platelet, increase of microtubules, the presence of particles with a morphology similar to virus [hexagonal particles] and decreased of the platelet electron density that is reported in the present study. In the immunoelectron microscopic studies using the same antiherpes antibodies we have obtained results that can relate these findings with those observed in the CNS. These results allowed us to establish a cytopathological diagnosis valuation scale [23]. The punctuations above 11 corresponded to pathological punctuations. When a preliminary double blind computerized analysis was carried out of only two of the parameters to evaluate of the scale, this analysis allowed to distinguish among controls and patient in 100 percent of the cases and evaluated controls (24). The sensibility and specificity of the test after having finished the study were above 95% of the cases and controls studied. The relationship with the gold pattern, the clinical diagnosis, was evident, but its biggest importance resides in its possible application in the early diagnosis of the illness that like we have referred it is the only factor that can modify the prognosis of schizophrenia. Unfortunately the diagnosis is made in the habitual clinical practice when the psychotic symptoms are already present. The non relationship observed with the schizophrenia type, the time of evolution of the illness, the age, sex, the color of the skin or the ethnic characteristics of the studied populations [25] guide toward the consideration that it is an unique nosological entity in which a common etiology should be kept in mind. In the peripheral blood we observed particles in occasions with similar characteristics to those observed in the SNC. In other occasions, they were observed inside the external vacuoles coming from the platelets. In this case they appeared as empty particles most of the times, with a hexagonal form and related to membrane structures. When immuno-

electron microscopic techniques were carried out a platelet labeling was observed. The observation of spherical and hexagonal particles especially within the vacuoles and their connection with the cellular membranes look also like the alterations found by us in post-mortem studies of brain samples of young schizophrenic patients, some of them in critical stage of the illness [26]. We have observed in membranes and particles a positive reaction when using antibodies to herpes simplex hominis type I. Recent studies have demonstrated a relationship of this virus with schizophrenia when being observed an increase of antibodies titles against this virus in the blood of schizophrenic patients with cognitive dysfunctions [27]. A virus-platelet-nervous system interaction can play an important role in the physiopathology of the illness and should be reason of later studies since the morphological alterations can be related to functional alterations acting over the central nervous system.

Although this test was carried out in patient of recent onset of the illness its diagnostic value could influence the prognosis since the results obtained by other researchers related to the study of duration of the not treated psychosis [DUP] and the prognosis, they reported the following observations:

1. "The duration of untreated psychosis (DUP) is a concept of importance in schizophrenia research from the perspective of secondary prevention. Although findings to date are mixed, several studies have demonstrated an inverse association between the duration of treatment delay and a variety of clinical and psychosocial outcomes" [28].
2. "Duration of untreated psychosis (DUP) is the time from manifestation of the first psychotic symptom to initiation of adequate treatment. It has been postulated that a longer DUP leads to a poorer prognosis. If so, outcome might be improved through earlier detection and treatment" [29].
3. "First-episode schizophrenia (FE-SZP) presents a diagnostic challenge because of symptomatic overlap between the various causes of psychosis" [30].

4. “The antipsychotic treatment response of first-episode schizophrenics is better than chronic multipisode patients and suggest that specific pathobiologic markers reflect pathophysiologic processes that mediate antipsychotic treatment response” [31].

Table. Schizophrenia. Cytopathological Diagnosis. Valuation Scale.

Points	0	1	2	3	
Platelets Form	Normal form Rounded, smooth and regular borders	Filopodia slightly increased in number	Filopodia moderately increased in number with slight deformation	Filopodia increased in number with moderate deformation (“elephant feet” and or ballooning)	Filopodia increased “elephant feet” and or ballooning
Platelets Size	Normal size, 2-4 micr.	Platelets slightly increased in size, less than 10%	Platelets moderately increased in size, 15%	Platelets very increased in size (**) 30%	Platelets very increased in size
External vacuoles (***) Form and size	Normal	Irrregular borders, slightly increased in size, less than 5 per field	Irrregular borders, slightly increased in size, 5 to 10 per field	Smooth borders, slightly increased in size, 10 to 15 per field	Very increased (****) with glycogen granules
Internal vacuoles. Form and size	Normal (small and few)	Few, slightly increased in size	Few, moderately increased in size	Moderately increased in size, surrounded by glycogen in isolated occasion	Very increased in size surrounded by glycogen with particles within
Glycogen	Few uniformly distributed	Slightly increased. Uniformly distributed	Moderately increased. Uniformly distributed occasionally	Moderately increased. Concentred	Very increased. Concentred, prolonged, around vacuoles
Microtubules	Absent	Present	Slightly increased in number	Moderately increased in number	Very increased in number
Hexagonal particles within the external vacuoles	Absent	Some. Atypical	Some. Atypical	Some. Atypical	Present. Typical.

* “Elephant feet”: Term which we describe the deformity that appears on the surface of platelets and filopodia

** Two to three times larger than normal average

*** Those which appear free in the plasma or outside of the platelets but related to them

**** Two to five times larger than those which appear slightly increased in size

Result: 0-11

normal > 11 pathological

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