Official Title: Aspirin as Adjuvant Therapy in Young Psychotic Patients
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Study Protocol and Statistical Analysis Plan

Effect of adjuvant aspirin therapy on neurological soft signs and PANSS in young psychotic patients

A randomized, interventional, double-blind, placebo-controlled clinical trial

Protocol name:	Aspirin2016.2
Date:	25 October 2016.
Donor:	Stanley Medical Research Institute, 8401 Connecticut Avenue, Suite 200, Chevy Chase, MD 20815, SAD
Sponsor:	Clinic for Mental Diseases "Dr Laza Lazarević", Višegradska 26, 11000 Belgrade, Serbia
Principal Investigator, Grant Holder:	MD Dragana Pavićević, psychiatrist
Co-investigators:	MD/PhD Slavica Đukić Dejanović, neuropsychiatrist MD Đorđe Ćurčić, psychiatrist MD Nebojša Živković, psychiatrist

Background:

The clinical trial team would conduct a randomized, interventional, double-blind, placebo-controlled study in two parallel groups of patients who were neuroleptically naive or previously minimally medicated (who did not take antipsychotics during the previous 6 months) with the illness duration of up to seven years. Patients of both sexes, ages 18 to 28, would participate in the trial, in accordance with the tenth revision of the International Classification of Diseases (ICD10), they would meet the diagnostic criteria for the illnesses ranging from F 20 to F 29. After meeting the inclusion and exclusion criteria and having signed an informed consent form, the patients would be randomized into two groups:

Experimental group – EG: antipsychotic + Aspirin

and

Control group – CG: antipsychotic + placebo.

The EG patients would be administered 1000 mg aspirin pro die with gastric protection pantoprazole 40 mg pro die, both medicines in two doses during 6 weeks of hospital treatment at Clinic form Mental Disorders "Dr Laza Lazarevic". The trial would last for 18 months and would include the total of 100 to 120 patients.

Objective and hypotheses:

The objective of the trial would be to determine the effect of adjuvant aspirin therapy on neurological soft signs, positive and negative syndrome in schizophrenia, cognition, inflammatory factors and cytokine profile.

Working hypotheses:

1. Changes in PANSS and MoCA scores as well as inflammation factors in both patient groups, more in the experimental group.
2. Changes in the cytokine profile in both patient groups.
3. In case of significant change of SNS and cytokine profile in the experimental group - the results would support the cytokine hypothesis. If there were no significant changes in the SNS in the experimental group, the results would support the SNS as a trait of schizophrenia.

Clinical Trial Design:

A randomized, interventional, double-blind, placebo-controlled clinical trial in two parallel groups of patients who were neuroleptically naive or previously minimally medicated (who did not take antipsychotics during the previous 6 months) with the illness duration of up to seven years. Patients of both sexes, ages 18 to 28, would participate in the trial, in accordance

with the tenth revision of the International Classification of Diseases, would meet the diagnostic criteria for the illnesses ranging from F20 to F29. Upon fulfilment of inclusion and exclusion criteria and signed Informed consent form, they would be randomized in two groups: experimental (EG) and control group (CG). Patients belonging to the control group would be administered antipsychotic therapy and placebo, whereas patients in the experimental group would be administered regular antipsychotic therapy and aspirin. EG patients would be administered 1000 mg aspirin pro die with gastric protection- pantoprazole 40 mg pro die, both medicines in two doses in duration of 6 weeks.

A software application specialized in the randomization of subjects for clinical trials - ***Sealed Envelope*** - will be used for randomization. The obtained list will be in possession of one co-investigator and only he or she will know which group the patient belongs to. Only upon completion of the trial, after taking the serum samples from the last patient, and cytokine profile analysis (cytokine profile analysis would be performed at the end of the trial, after the serum sample from the last patient was taken at both test times) would the patient's belonging to the EG or CG group be revealed.

The co-investigator in charge of randomization would give out the medicine boxes and the medicines would all look identical - packed in the same capsules for overcapsulation by the company **Capsugel**. Placebo tablets would be manufactured by Galenika A.D. The patient would be administered Bayer's Aspirin tbl. á 500 mg and Nolpaza tbl. á 20 mg produced by Krka-Farma D.O.O.

Three visits are planned. The first visit after admission to the Clinic. If the patient fulfils the inclusion criteria and none of the exclusion criteria (anamnestic data, somatic and psychiatric examination), and after the Informed consent form has been signed, PANSS scale test and blood sample would be taken for laboratory analysis. Laboratory analysis would be complete blood count (RBC, WBC, PTC, Hgb, Hct), sedimentation, C reactive protein, blood biochemical analysis (glycaemia, cholesterol, triglycerides, transaminases, CK, bilirubin, uric acid, creatinine, urea), and electrolytes (Na, K). Th1, Th2 and Th17 cytokines would be subject to the immunological assays. Each patient would be given an identification number (from one upwards) and the tubes with serum for the cytokine profile would be labelled with the same number, in terms of x/y, where *x* is the patient's identification number and *y*- may be "1" or "2" ("a" or "b"), where "1" or "a" would mean the first blood draw (at the beginning of the trial) and the number "2" or "b" - the second blood draw (at the very end of the trial).

After calming down of the acute signs of psychosis, between the third and fifth day of hospitalization, the second visit would be made when testing of the neurological soft signs would be performed according the Heidelberg Soft Neurological Signs Scale, the MoCA Cognitive Assessment Scale, and then the patient would be randomized to an experimental or control group if he/she meets the inclusion and no-exclusion criteria, if there are no somatic contraindications (with a stable ECG), and if he/she still wishes to participate in the trial.

The staff of the ward where the patient would be accommodated would be given two boxes of medicines labelled "Clinical Trial Medication 1" and "Clinical Trial Medication 2" that would be administered to patients – one medicine from each box, in the morning and in the evening.

After 6 weeks from the second visit (+/- 3 days), the third visit would be made when the patient would be examined, the PANSS, MoCA and Heidelberg scales testing repeated, and the blood sample taken again for laboratory analyses - inflammatory factors and cytokine profiles.

Control laboratory analyses would be performed on each patient on days 14^{th} and 28^{th} from the second visit. If patients received high dose valproic acid in therapy, valproatemia control would be performed, and if they received antidepressants from the SSRI group, bleeding time would be controlled.

Serums administered to patients would be transported in a cold chain to the Centre for Molecular Medicine and Stem Cell Research at the Faculty of Medical Sciences, University of Kragujevac, where they would be stored at -70°C until analyses were performed.

Criteria for Trial Termination:

The criteria for trial termination for individual subjects, parts of the trial or the whole trial would be defined as follows: the subject may voluntarily leave the trial at any moment. In the event that side effects of adjuvant aspirin therapy occur, the patient would be excluded from the trial. Occurrence of bleeding from the nose or gums, bruising, bleeding in the GIT or brain, and confusion, anaphylactic reactions or acute renal failure would imply the immediate exclusion of subjects from the trial. If there is an increase in valproatemia (in patients taking valproic acid in therapy) - the dose of the medicine would be adjusted. In case of significant deviation in the laboratory analyses - the subject would be excluded from the trial. The entire trial would be discontinued by the emergence of a large number of complicated side effects, as well as by compromising drug storage.

Selection and inclusion of subjects:

- **Inclusion criteria:**
  - Age 18 to 28
  - Diagnostic categories from F 20 to F 29
  - Illness duration ≤ 7 years

- **Exclusion criteria:**
  - PAS abuse
  - Primary cognitive impairment
  - Contraindications and special caution for acetylsalicylic acid and pantoprazole:
    - Hypersensitivity to acetylsalicylic acid and other NSAIDs (possible cross-reaction)
    - Gastric ulcer and/or duodenum ulcer, gastritis
    - Pregnancy
    - Haemophilia, bleeding disorders
    - Gout

- Asthma, COPD, NSAID-induced bronchospasm
- Angioedema, urticaria
- Haemolytic anaemia
- Use of warfarin, methotrexate
- Diabetes
- Decreased liver and / or kidney function
- Heart failure
- Surgical/dental intervention
- Interactions with specific psycho-pharmaceuticals
- Sensitivity to pantoprazole

Statistical analysis plan:

Statistical data analysis will be done in the programme IBM SPSS Statistics v.20.

Continuous variables will be displayed descriptively using the minimum and maximum values, mean values and standard deviation. Categorical variables will be presented descriptively using absolute and relative frequency as well as graphically using a pie chart.

Kolmogorov-Smirnov or Shapiro-Wilk test, depending on sample size, will be used to check the normality of data distribution, as well as graphical method of using histogram. If the data follow the normal distribution, parametric tests will be used for the statistical analysis of the data; otherwise the corresponding non-parametric tests will be used.

For the analysis of significant continuous variables over time, the Paired Samples t Test will be used if the data follow a normal distribution or the Wilcoxon test if the data do not follow the normal distribution. Significant results will be presented graphically using a bar chart or boxplot.

For the analysis of significant difference of continuous variables between the control and experimental groups, the Independent Samples t Test will be used if the data follow a normal distribution or the Mann-Whitney test if the data do not follow the normal distribution. Significant results will be presented graphically using a bar chart or boxplot.

For the analysis of the dependence between two continuous variables, the value of Pearson's or Spirman's correlation coefficient will be interpreted. In the case of strong correlations, the dependence will be shown both by the regression equation and graphically using the scatterplot.

For the analysis of the dependence between the two categorical variables, a Chi-squared test, that is, a Fisher's exact-dependency test will be used. The results will be presented graphically using a bar cluster graph.

The results will be considered statistically significant if the p value is less than 0.05.

SUBJECT INFORMED CONSENT

Effect of adjuvant aspirin therapy on Soft Neurological Signs and PANSS
in young psychotic patients
A double-blind, randomized, interventional clinical trial
(Second version, 11/07/2016)

We invite you to participate in our study to examine the impact of additional aspirin therapy on mental illnesses' symptoms and signs. If you agree to participate in the trial while taking your regular psychiatric treatment you would receive *aspirin* and *pantoprazole* or placebo (depending on randomization). Aspirin is a drug from the group of the so-called *antirheumatics* – they soothe pain and inflammation, and pantoprazole is administered to protect the gastric mucosa during aspirin therapy.

You need to understand why this study is conducted, what is demanded of you, and take the time to carefully read the following information to decide whether or not you want to participate in it. It is also advisable to ask if something is not clear to you or if you need further clarification.

The aim of the study is to determine whether additional aspirin therapy will result in a faster and essential improvement of mental illness - signs and symptoms of the illness.

If you agree to participate in this study/trial, you are expected to take the prescribed therapy regularly and to fill-in the tests (it will take approximately 90 minutes) at the beginning and end of the trial. Also, you would have been taken blood sample from your vein twice for laboratory tests - at the beginning and at the end of the trial. A 5 ml sample of venous blood sample would be sent (subject to randomization) to determine additional biological markers (analysis) in the USA at the Stanley Neurobiology Laboratory at Johns Hopkins University.

As with any medicines, there are contraindications related to the use **of Aspirin and pantoprazole**, as well as possible side effects that you should consider.

- *Aspirin should not be used by person who are allergic to **NSAIL** (salicylate, ibuprofen, naproxen).*
- *Particular attention should be paid to patients suffering from **asthma**, **gastric ulcer**, mild **diabetes**, **gastritis**, **haemophilia**, persons suffering from kidney diseases, hyperuricemia or **gout**.*
- *Even in people without these difficulties, there is still a risk of internal bleeding in the gastrointestinal system if aspirin is taken after consuming **alcohol** or **warfarin**; it can cause haemolytic anaemia in people with G6PD genetic disorder. It increases the risk of Reye's syndrome.*
- Hypersensitivity to pantoprazole.

You need to inform the investigator of any previous and present illnesses and treatment.

If you choose to participate, the data collected during the trial (personal and medical data) would be recorded and stored in the NIMH database (National Institute of Mental Health Data Archive, USA) and published in accordance with applicable personal data protection regulations by persons conducting trial and other investigators who may access the database. A description of this clinical trial will be available http://www.ClinicalTrials.gov, in accordance with the US law. This website will not include information by which you may be identified. At best, the website will include a summary of the results. You may search the website at any time.

Your participation will not entail additional costs on your part, nor will you receive material compensation for participation.

Your full compliance with the investigator's requests and instructions is expected. Information will be made available during the trial that may affect your willingness to continue participating within a reasonable time.

Participation in a study/trial is voluntary and you can terminate it at any time, without any sanction and without losing any benefit to which you are entitled.

TO BE FILLED-IN BY A SUBJECT by putting the sign ×:

☐ I have read and understood this information and the subject informed consent form.

☐ I was given the opportunity to ask additional questions; I am satisfied with the answers I obtained.

☐ I hereby authorize investigators to submit the trial data and results to the NIMH database and to the website http://www.ClinicalTrials.gov, and publish the results in accordance with applicable personal data protection regulations.

☐ I confirm that I have received a copy of the signed document.

☐ I am aware that my participation is entirely voluntary and confirm that I am aware of the terms of participation.

☐ I fully understand that signing of this document also means accepting all views stated in the Subject Informed Consent Form with full notice.

Subject:

Name and Surname	*Date*	*Signature*

Investigator:

Name and Surname	*Date*	*Signature*