Metabolic Abnormalities and Spectroscopy Biochemical Cerebral Compounds Modifications in Children and Adolescents with Antipsychotic Treatment

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We approach an integrated, multidisciplinary, innovative research-action model in children and adolescents with psychosis. Our main focus was: to investigate the biochemical cerebral compounds and metabolites (NAA-N-acetylaspartate, GABA-Gama-Aminobutyric Acid, Asp-Aspartate, CR-Creatine, Gln-Glutamine, GPC-Glicerophosphocholine, PC-Phosphocholine, PCr-Phosphocreatine, Tau-Taurine, N-MDA-N-Metyl-D-Aspartate, Serine, Glicine, Cho-Choline); the neuroimagistic and neurobiological markers and the metabolic abnormalities in correlation with the molecular pharmacogenetic testing in psychoses, treated with antipsychotic medication; the dynamic evaluation of the clinical evolution for the studied groups in correlation with specific biochemical, metabolic, neurobiological and neuroimagistic variables and markers. Our research was conducted in the period 2010-2016 on 85 patients, children and adolescents with psychosis (42 took treatment after pharmacogenetic testing, 43 without). Also, the patients were evaluated through magnetic resonance (MR) spectroscopy at baseline and after pharmacotherapy. The efficacy of the chosen therapy in correlation with the pharmacogenetic testing was evaluated through the mean change in the Positive and Negative Syndrome Scale (PANSS) total scores, in the Clinical Global Impression Severity (CGI-S/I), Clinical Global Assessment of Functioning (CGAS) and through the change registered for the relevant biochemical, metabolic, neurobiological markers and MR spectroscopy metabolites, from baseline till endpoint in different timepoints. Our results, showed statistically significant differences of the clinical scores between the studied groups. Our research was a proof, that the biochemical brain metabolites register in psychoses modified values in the MR Spectroscopy, the administration of antipsychotics can determine metabolic abnormalities (changed lipid profiles, high insulin and blood sugar levels, weight gain, obesity). But on the other side, if the antipsychotic treatment is chosen properly according to the pharmacogenetic profile of the patient, then the biochemical metabolites obtained through the MR Spectroscopy, register improvement of the values correlated with the good clinical evolution.

Keywords: biochemical metabolites, metabolic abnormalities, antipsychotics, NAA-N-acetylaspartate, GABA-Gama-Aminobutyric Acid, N-MDA

A modern approach in the management and follow-up of psychoses implies a multidisciplinary view and imposes integrative correlations between the biochemical, clinical, neurobiological, metabolic, neuroimagistic, molecular and pharmacogenetic markers [1-9]. The new perspectives in the field of biochemistry, neuroimagistics and pharmacogenetics give us the opportunity to make some connections between the clinical features, the biochemical, neurobiological and neuroimagistic markers and the further clinical evolution and prognostic in psychoses [10-16].

When implementing a treatment, it is important, first of all, to identify some biochemical, neurobiological, neuroimagistic vulnerability markers, so that we know, which treatment, should be applied [17-19].

Taking the specific biochemical brain metabolites' modifications (NAA-N-acetylaspartate, GABA-Gama-Aminobutyric Acid, Asp-Aspartate, CR-Creatine, Gln-Glutamine, GPC-Glicerophosphocholine, PC-Phosphocholine, PCr-Phosphocreatine, Tau-Taurine, N-MDA-N-Metyl-D-Aspartate, Serine, Glicine, Cho-Choline) and neuroimagistic vulnerability markers into account, helps us to engage the proper treatment [4, 6, 7]. Also, these biochemical, neuroimagistic markers are helpful in quantifying the medication response, the clinical evolution and they also could have prognostic significance concerning the remission and relapses in psychoses [17, 20-27].

The antipsychotic treatment can bring with it also some severe metabolic abnormalities-abnormally changed lipid profiles, high insulin blood levels, high blood sugar levels, prediabetes states, diabetes mellitus, obesity, gastro-esophagial reflux, metabolic syndrome, hypertension, in the patients taking it, especially in the pediatric population, these adverse effects, having high impact [16, 18, 28-33].

The treatment of election in the management of psychosis should be chosen in correlation with the biochemical, neurobiological, pharmacogenetic, neuroimagistic and clinical profile of the target patients. When choosing the suitable pharmacotherapy, the pharmacogenetic markers should be analyzed carefully, because through the pharmacogenetic testing, the effects of the genetic variations-polymorphisms on the medication response, safety, tolerability and efficacy, are investigated [1, 3, 34-37]. In our present research, we will capture the clinical (somatic, metabolic, psychiatric) evolution of the patients taking antipsychotics, correlated with the evaluation of the biochemical and neuroimagistic markers, especially through RM Spectroscopy and with the pharmacogenetic testing [4, 14, 16, 18, 25]. The main objectives of our study were: to investigate in what manner the antipsychotic treatment could determine metabolic

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abnormalities and dysfunctional biochemical brain metabolites's modifications, especially when it is chosen without a proper correlation with the pharmacogenetic profile of the psychotic patients [1, 14, 34].

Experimental part

Material and methods

The present research was performed between the years 2010 and 2016, in the University Hospital for Child and Adolescent Psychiatry and Neurology and the Center of Genomics, Discipline of Genetics of the Victor Babes University of Medicine and Pharmacy. We recruited patients, children and adolescents with psychosis

Our actual study is focusing especially on biochemical, metabolic, neurobiological, neuroimagistic, respectively clinical aspects and on specific pharmacogenetic correlations.

The study samples consisted of 85 patients, children and adolescents with psychosis. The patients included in the study were aged between 12 and 20 years (median age 15.78±4).

We obtained for each patient the informed assent and the informed consent from the parents/legal guardians. Our study was done in accordance with the Ethical Committee regulations of the University of Medicine and Pharmacy Victor Babes Timisoara, with the ICH-GCP (Good Clinical Practice) regulations and guidelines.

Our study sample was divided in 2 groups: from the 85 children with psychosis - 42 took treatment after pharmacogenetics testing and 43 without the pharmacogenetic testing before the treatment election.

Biochemical metabolites' and neuroimagistic investigations (MR Spectroscopy)

For the correlation of clinical data with the cerebral biochemical, neurobiological changes we performed the neuroimagistic investigations.

The patients have been evaluated through MR Spectroscopy at baseline and after the chosen pharmacotherapy with or without pharmacogenetic testing before. Through the MR Spectroscopy, we investigated key aspects of the cerebral function and metabolism. We quantified the following neurmetabolites: NAA-N-acetylaspartate, GABA-Gama-Aminobutyric Acid, Asp-Aspartate, CR-Creatine, Gln-Glutamine, GPC-Glicerophosphocholine, PC-Phosphocholine, PCr-Phosphocreatine, Tau-Taurine, N-MDA-N-Metyl-D-Aspartate, Serine, Glicine, Cho-Choline.

We used the MR Spectroscopy Software Package for the MR spectral quantification, which automatically calculates a matrix of the correlation quotients and of concentrations of the cerebral biochemical metabolites.

The efficacy of the chosen therapy in correlation with the pharmacogenetic testing, has been evaluated through the modification of the applied clinical scales total scores and through the change registered for the significant biochemical and neurobiologic markers and neuro-metabolites, from the initial values till endpoint. So that, we evaluated the efficacy of the chosen pharmacotherapy in correlation with the pharmacogenetic testing and the variation of the biochemical cerebral metabolites, quantified through the MR Spectroscopy, through the change of the mean total scores of the scales from baseline till endpoint.

Metabolic abnormalities investigations

We investigated the metabolic parameters – lipid profiles (blood cholesterol levels, triglycerides), insulin blood levels, blood sugar levels, blood pressure, BMI (body mass index) change, weight gain. For every patient, it was performed a clinical examination, a set of hematological, biochemical, lipid, coagulation analyses and ECG, especially with focus on the QTc complex prolongation. Blood pressure, heart rate and vital signs were also monitored. Also glucose blood levels were monitored. Glucose (molecular formula: $C_6H_{12}O_6$) is a mono-sachharide existing in nature only as D-isomer form.

During the antipsychotic treatment is also important to monitor the renal function. We also evaluated the renal function through the serum urea, creatinine and uric acid. Urea (CH_4N_2O) is an organic compound with a carbonyl (C=O) functional group linked to two NH_2 groups. Creatinine- 2-Amino-1-methyl-5H-imidazol-4-one, an important indicator of renal function.

Uric acid (7,9-Dihydro-1H-purine-2,6,8(3H)-trione)-a diprotic aromatic acid- is a product of purine nucleotides metabolism, leading to metabolic abnormalities in case of high values.

The lipid profiles of the patients were assessed through the high-density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, total cholesterol and triglycerides.

HDL is the smallest and most dense of lipoprotein particles and plays an anti-atherogenic role by removing the fat molecules from cells. The non-HDL cholesterol is considered to cause atheroma and it is a good predictor of cardiovascular events.

LDL has a larger diameter than normal cholesterol and represents a high cardiovascular risk.

Cholesterol (=(3beta)-cholest-5-en-3-ol), an organic molecule with 256 stereo-isomers biosynthesized by all cells, being a crucial component of cell membranes and a precursor for steroid hormones, vitamin D and bile acids.

Triglycerides are esters derived from the combination of glycerol and three fatty acids (RCO_2H, $R'CO_2H$ and $R"CO_2H$), according to the formula:

$$HOCH_2CH(OH)CH_2OH + RCO_2H + R'CO_2H + R"CO_2H = \\ = RCO_2CH_2CH(O_2CR')CH_2CO_2R" + 3H_2O$$

Clinical evaluation of the patients

In order to analyze the clinical evolution of the patients in each group, we applied the following instruments and scales: PANSS-Positive and Negative Syndromes Scale, CGI-S/I-Clinical Global Impression of Severity and Improvement, CGAS-Clinical Global Assessment of Functioning.

Pharmacogenetic Testing

The pharmacogenetic testing was done through the genotyping - SNP – Single Nucleotide Polymorphisms, through RT-PCR (Poly-Chain Reaction), after the DNA prelevation. The genotypes of the allelic variants CYP* have been determined through the specific allelic fluorescence measurement, using the software for allelic discrimination. The identification of the alleles CYP2D6 *3, *4, *5, *41, responsible for the medication metabolizing types, was significant. Genomic DNA was extracted from EDTA blood using QIAamp DNA Mini Kit (Qiagen, Germany). The CYP2D6 genotyping was performed, so that the laboratory staff was blinded to the patients' data. CYP2D6 *3, *4, *5, *41 allele identification was performed by using TaqMan Drug Metabolism Genotyping Assay for Allelic Discrimination CYP2D6* and TaqMan® PCR Master Mix (Applied Biosystems) according to the protocol provided by the producer. Genotypes were determined by measuring allele-specific fluorescence using the software for allelic discrimination (Applied Biosystems).

Statistical analysis

All analyses were carried out using SPSS software (version 17.0, Chicago, IL, USA) and Microsoft Excel. For

comparing the clinical scales scores (PANSS, CGI-S/I, CGAS) and also the MR Spectroscopy biochemical brain metabolites values at different time points, the Friedman non-parametric test for pair values was used. For comparing the clinical response, evolution between the groups - (who benefited of pharmacogenetic testing in choosing the proper medication) = G1 (42 psychotic patients) + (without pharmacogenetic testing) = G2 (43 psychotic patients), the Mann-Whitney non-parametric test was applied. For comparing the mean total clinical scales scores and also the MR Spectroscopy biochemical brain metabolites values at two different time points and in each 2 with 2 different timepoints, the nonparametric test Wilcoxon signed Ranks was used.

Results and discussions

We obtained significant results through our present research. We identified some relevant metabolic abnormalities - hypercholesterolemia, high triglycerides, high blood insulin levels, high blood sugar levels, BMI -Body Mass Index increase, weight gain, also modified urea and creatinine values and prolonged ECG QTc complexes, especially in the group of patients, who were treated with antipsychotics (atypical and typical), without pharmacogenetic testing done before. For the group G1=42 patients with psychosis, where the pharmacogenetic testing was applied, pharmacogenetic polymorphisms at the level of CYP450 enzymes and so we observed in our studied samples the WT-Wild Type or normal type metabolizer, the patients who had SNP (Single Nucleotid Polymorphism), who need in the clinical practice, the adjustment of the doses of the administered pharmacotherapy, as well as careful choosing of the medication and the WT/SNP= mixt type, who encounter also some difficulties in this area (fig. 1).

In the group, where the pharmacogenetic testing was not performed (G2=43 patients with psychosis), the medication has been assigned according to the clinical symptoms but not to the personalized, pharmacogenetic profile of the patients. So that, in the group without pharmacogenetic testing, highly modified and abnormal lipid profiles, weight gain, BMI increase and abnormal metabolic values were found, in comparison with the group with testing (table1 and table 2).

For the patients from group 2, without pharmacogenetic testing, the increase of the insulin values from baseline until 18 months is statistically significant (α=0.001), meaning that the patients in this group, because the antipsychotic treatment was not adapted to their genotype, were most prone and exposed to the adverse effects – increased insulin values or even hyperinsulinism, with high morbidity consequences.

The increase of the BMI values from baseline until 18 months is also statistically significant, with a threshold of significance α=0.001. The increase The BMI increase was

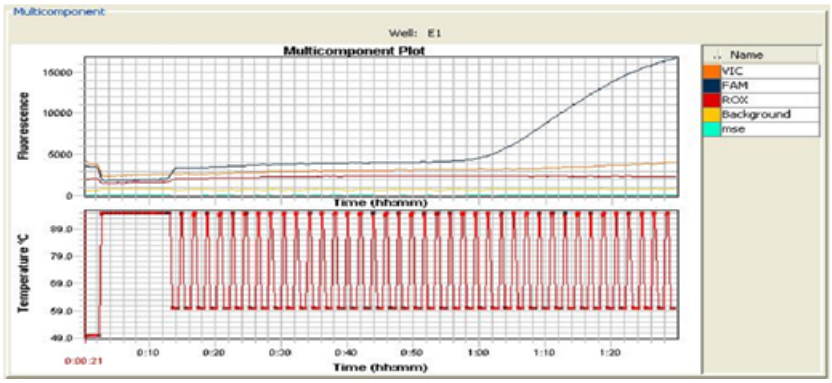

Fig. 1. Results of the pharmacogenetic testing for the patient group with CYP450 SNP's (Single Nucleotide Polymorphism)

Table 1

COMPARISONS BETWEEN THE DIFFERENT TIMEPOINTS FOR THE GROUP 2, WITHOUT PHARMACOGENETIC TESTING

		BMI				Insulin values			
Timepoint	N	Mean	Std. Deviation	Min.	Max.	Mean	Std. Deviation	Min.	Max.
BASELINE	43	21.28	2.92	16.00	28.00	4.40	2.11	4.4	12.6
3 Months	43	22.41	2.78	17.30	28.70	11.35	1.46	5.6	17.8
6 Months	43	26.39	1.99	21.70	29.90	16.55	3.56	7.9	24.9
1 Year	43	28.45	1.93	22.80	32.00	26.69	1.30	24.9	28.9
18 Months	43	30.38	2.14	25.30	36.00	28.56	1.98	25.3	30.7

Table 2

COMPARISONS BETWEEN THE DIFFERENT TIMEPOINTS FOR THE GROUP 1, WITH PHARMACOGENETIC TESTING

		BMI				Insulin values			
Time	N	Mean	Std. Deviation	Min.	Max.	Mean	Std. Deviation	Min.	Max.
BASELINE	42	20.27	2.44	17.00	29.00	5.59	3.53	4.4	17.7
3 Months	42	21.39	2.58	17.30	29.30	798	4.12	4.9	18.3
6 Months	42	22.54	2.67	18.00	29.90	11.31	3.76	4.8	20.5
1 Year	42	22.67	2.75	19.10	30.90	12.37	10.26	4.9	81.4
18 Months	42	22.99	3.13	20.32	36.00	12.99	3.94	4.9	19.9

Table 3

COMPARISONS CONCERNING THE BIOCHEMISTRY AND LIPID MARKERS DATA BETWEEN THE GROUP 1, WITH PHARMACOGENETIC TESTING AND G2 WITHOUT

	G1 – with pharmacogenetic testing		G2-without	
Time After 6 months	Mean	Std. Deviation	Mean	Std. Deviation
Glycaemia	96.27	45.49	139.59	58.053
Cholesterol	140.34	42.53	183.71	50.676
HDL	60.67	26 .95	40.37	21.26
LDL	100.06	32.87	149.98	34.59

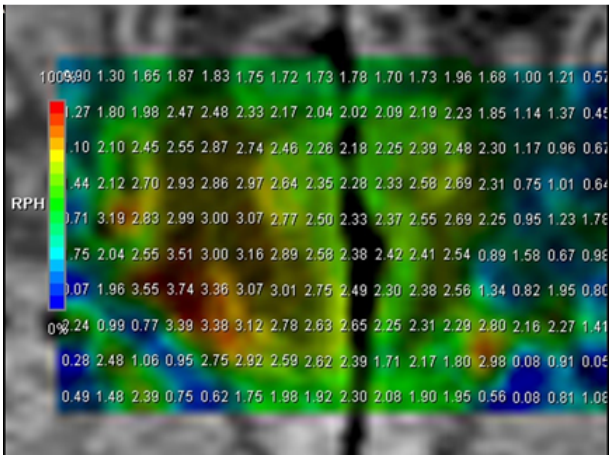

Fig. 2. RM Spectroscopy matrix quantifying the concentrations of brain metabolites captured

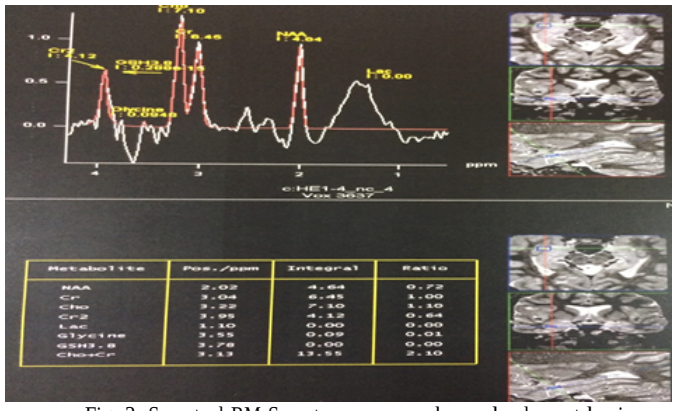

Fig. 3. Spectral RM Spectroscopy peaks and relevant brain metabolites concentrations/ ratios in the studied groups

much higher than for the group 1, meaning that the patients from group 2 were much prone and exposed to adverse effects, expressed through weight gain and BMI increase.

Concerning the patients from group 1, the comparisons between the different timepoints are summarized in table 2.

The increase of the BMI values from baseline until 18 months is not statistically significant. We obtained the threshold of significance $\alpha = 0.05$ for the comparison baseline - 18 months. The BMI increase was much lower than for patients from group 2, meaning that the patients from group 1 were not so prone and exposed to adverse effects, expressed through weight gain, because their antipsychotic treatment has been chosen adapted to their pharmacogenetic profile.

We also obtained significant results concerning the biochemistry data, the lipid markers (HDL-high-density lipoproteins, LDL-serum low-density lipoprotein levels, triglycerides) and inflammation comparative data between the 2 groups – G1 with pharmacogenetic and G2 without pharmacogenetic testing. So, we observed hypercholesterolemia, high triglycerides and blood glycaemia levels especially in G2, the patients with antipsychotic treatment, chosen without pharmacogenetic testing (table 3).

We obtained interesting results, when comparing the study samples (with and without pharmacogenetic testing), concerning the variation of the cerebral biochemical metabolites values of the MR Spectroscopy.

Through the MR Spectroscopy, we found modified values and concentrations of the biochemical cerebral metabolites for the group of patients with psychosis:

-very high: *GABA* values, especially in the prefrontal cortex, *Glutamate* values especially in the frontal cortex, identifying brain lesions.

-very low *NAA* (N-Acetylaspartate) and *NAAG* (N-Acetylaspartylglutamate) values.

We also observed high values for the: *Glutamat/Glutamin, Lactat/NAA, Glutamat/Cr, Cho/Cr, NAA/Cr, NAA/Cho* report (fig. 2).

We also obtained interesting results concerning the MR Spectroscopy quantified metabolites and their variation from baseline till endpoint (fig. 3).

So that, we observed the *normalization* of the brain biochemical metabolites - the decrease of Glutamat and GABA and the increase of NAA and NAAG and the normalization/decrease of the pathologic values of the metabolites' reports after the treatment with correctly chosen antipsychotic medication in the group, who benefited of prior pharmacogenetic testing. We also made some correlations concerning the biochemical neurometabolites' pathways and the treatment response – the patients who had good clinical response, showed also the normalization of the metabolites' levels identified through the MR Spectroscopy. The obtained results proved, that the patients, who took medication chosen after the prior pharmacogenetic testing, registered the improvement of the Spectroscopy biochemical metabolites, as a positive response to the chosen pharmacotherapy. Our study is especially valuable in the light of a multidisciplinary approach, implying complex correlations between the biochemical, clinical, metabolic, neurobiological, pharmacogenetic and neuroimagistic markers [1, 2, 4-6].

The most relevant vulnerability markers in psychosis were: NAA, NAAG, GABA and Glutamate [36, 37]. The NAA, which has a neurotrophic role, was very low for the psychotic patients. On this fact relies the value of some treatments, which have a neuroprotective, neurotrophic role, because they prevent the decrease of NAA in the brain [4]. The Glutamate, being a brain metabolite with significant role in the neurotransmission, has very high values in psychotic patients. The glutamatergic pathways are implied in the cognition and memory processes and the excessive concentrations of Glutamate in the brain

are neurotoxic. On this principle relies the efficacy of some treatments and of the Lithium, as neurostabilizers, which decrease the brain Glutamate values [4, 14, 18]. Some of the neurometabolic, neurochemical, neurobiological, neuroimagistic modifications persisted even after the clinical remission of the psychotic patients, as significant vulnerability markers. The therapeutic conduct must be ethical, personalized, guided in function of some relevant markers [36-39]. Also the aspects concerning the potential adverse events, quality of life and vulnerabilities, must be carefully approached [10-12]. The risk and resilience factors must be known and evaluated and the the therapeutic approach must be ethical with the lowest number of adverse events encountered [28, 38-44]. The obtained results revealed important and useful correlations between the several factors and domains.

Conclusions

Antipsychotics are very useful, when properly and carefully administered, but can bring into the forefront some relevant metabolic adverse events and abnormalities, when chosen without pharmacogenetic testing before.

The pharmacogenetic testing, the fingerprinting of the biochemical, neurobiological and spectroscopic, MR Spectroscopy markers, represent strongly predictive factors of the clinical evolution in child psychoses after the administration of antipsychotic medication.

The evaluation of biochemical, metabolic, neurobiological and neuroimagistic markers in psychotic patients, proved the high clinical utility of an integrative, multidisciplinary approach.

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