UNIVERSIDADE DE LISBOA

Faculdade de Medicina

Clinical profile in schizophrenia spectrum: relation with unconjugated bilirubin (UCB) and other psychophysiological features − a longitudinal exploratory study.

João Carlos Pereira Gama Marques

Orientadora:

Professora Doutora Sílvia Raquel Soares Ouakinin

Tese especialmente elaborada para a obtenção do grau de Doutor em Ciências Biomédicas, especialidade de Neurociências.

2020

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2020

The opinions expressed in this publication are the sole responsibility of the author.

I shall pass this way but once;

Any good that I can do or any kindness I can show to any human being;

Let me do it now.

Let me not defer nor neglect it,

For I shall not pass this way again.

Étienne de Grellet du Mabillier (1773−1855)

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CONTENTS

ABSTRACT

Objective: Our objective was to assess unconjugated bilirubin (UCB) as a biomarker candidate for schizophrenia and schizoaffective spectrums disorder and its clinical severity. For that purpose we prepared two different studies: a five year transversal restrospective study, and a one year longitudinal prospective study.

Methods: For our five years transversal restrospetive study we searched for an eventual correlation between UCB mean levels, duration of psychiatric admission, and diagnosis in 255 individuals, including 56 healthy controls and 199 acute patients (namely 44 with schizophrenia, 99 with schizoaffective disorder and 56 with bipolar disorder). For our one year longitudinal prospective study 88 psychotic patients completed a first assessment during relapse at ward admission, half with schizophrenia half with schizoaffective disorder. Forty−four acute bipolar patients were used as controls. In a twelve−month follow−up we collected longitudinal protocol (laboratory, psychopathological and psychosocial data) from 60 of those patients, half with schizophrenia half with schizoaffective disorder.

Results: In our five year transversal retrospective study we found a statistically significant difference between UCB mean levels of patients with schizophrenia (0.41 mg/dL) versus patients with schizoaffective (0.34 mg/dL) ($p<0.03$), bipolar disorders (0.28mg/dL) ($p<0.001$) and healthy controls (0.28)($p<0.001$) using Bonferroni multiple comparison test. We also found a statistically significant difference ($p<0.04$) between UCB mean levels of patients with schizoaffective disorder (0.34 mg/dL) and bipolar disorder (0.28 mg/dL). Regarding average admissions duration for the five−year period, diagnosis and UCB level, when applying a linear regression ANOVA model, with average admissions duration as a dependent variable we found statistically significant difference between schizophrenia (29 days) versus bipolar (16 days) ($p<0.001$) but not with patients with schizoaffective disorder. In our one year longitudinal prospective study during acute psychotic episode (N=88) we found: a statistically significant difference (ANOVA; $p=0.002$), confirmed after post−hoc Bonferroni multiple comparisons between between UCB mean levels of schizophrenia (N=44) and both schizoaffective (N=44) ($p=0.05$) and bipolar controls (N=44) ($p=0.05$); a positive

Pearson's correlation (r=0.314) between UCB mean levels and Personal and Social Performance item d) "disturbing and aggressive behavior"; and a positive correlation (R^2=0.223), with statistically significance (p=0.008), between UCB mean levels and mean length of stay at the psychiatric ward in schizoaffective patients who completed full protocol (N=30). During partial remission (N=60) we found: a statistically ANOVA significant difference (p=0.006), confirmed after post−hoc Bonferroni multiple comparisons between between UCB mean levels of schizophrenia (N=30) and schizoaffective (N=30) (p=0.05); plus a negative Pearson's correlation (r=−0.399) between UCB mean levels and Positive And Negative Syndrome Scale item G7 "psychomotor retardation". Comparing first and second assessments, with paired samples T−test, we found a statistically significant difference (p=0.034) in UCB mean levels only among patients with schizoaffective disorder (N=30).

Conclusion: there is an interesting potential in the research of UCB as a biological marker for schizophrenia and schizoaffective spectrums disorders, for both relapse and remission episodes of these syndromes.

Keywords: unconjugated bilirubin, schizophrenia, schizoaffective, psychosis.

SUMÁRIO

Objectivo: O nosso objectivo consistiu em avaliar a bilirrubina não conjugada (*UCB*) como candidato a biomarcador no espectro e na gravidade da esquizofrenia e perturbação esquizoafectiva. Para esse fim preparámos dois estudos: um estudo transversal restrospetivo de cinco anos, e um estudo longitudinal prospectivo com um ano de duração.

Métodos: No nosso estudo transversal retrospetivo de 5 anos procurámos correlação entre os níveis médios de UCB, duração média de internamento e o diagnóstico em 255 indivíduos, incluindo 56 controlos saudáveis e 199 doentes (nomeadamente 44 com esquizofrenia, 99 com perturbação esquizoafectiva e 56 com perturbação bipolar). No nosso estudo longitudinal prospetivo 88 doentes psicóticos completaram a primeira avaliação durante internamento psiquiátrico em contexto de episódio psicótico agudo, metade com esquizofrenia, metade com perturbação esquizoafectiva. Nesta fase 44 doentes com perturbação bipolar, foram usados como controlos. Aos doze meses de seguimento completámos o protocolo longitudinal (dados laboratoriais, psicopatológicos e psicossociais) de 60 doentes, metade com esquizofrenia, metade com perturbação esquizoafectiva.

Resultados: No nosso estudo transversal retrospetivo de 5 anos encontrámos diferença estatisticamente significativa, após teste de multicomparações de Bonferroni, entre os níveis médios de *UCB* dos doentes com esquizofrenia (0.41 mg/dL) e os doentes com perturbação esquizoafectiva (0.34 mg/dL) ($p<0.03$), os doentes com perturbação bipolar (0.28mg/dL) ($p<0.001$) e também os controlos saudáveis (0.28) ($p<0.001$). Encontrámos ainda diferença estatisticamente significativa entre os níveis médios de *UCB* de doentes com perturbação esquizoafectiva (0.34 mg/dL) e perturbação bipolar (0.28 mg/dL) ($p<0.04$). Relativamente à duração média de internamentos durante o período dos 5 anos, ao diagnóstico e ao nível de UCB, quando aplicámos um modelo de regressão linear ANOVA, com a duração média dos internamentos como variável dependente, encontrámos uma diferença estatisticamente significativa ($p<0.001$) entre os doentes com esquizofrenia (29 dias) e os doentes com perturbação bipolar (16 dias) mas não com os doentes com perturbação esquizoafectiva. No nosso estudo longitudinal

prospetivo de 1 ano, encontrámos, aquando da primeira avaliação durante o episódio psicótico agudo (N=88): uma diferença estatisticamente significativa (ANOVA; p=0.002), confirmada após teste de multicomparações de Bonferroni, entre doentes com esquizofrenia (N=44), quer com os doentes com perturbação esquizoafectiva (N=44) (p=0.05), quer com os controlos com perturbação bipolar (N=44) (p=0.05). Encontrámos também uma correlação positiva (Pearson's r=0.314) entre os valores médios de UCB e o item d) *"disturbing and aggressive behavior"* da escala de *Personal and Social Performance*; e ainda uma correlação positiva (R^2=0.223), com significância estatística (p=0.008), entre os níveis médios de *UCB* e a duração média de internamento psiquiátrico dos doentes com perturbação esquizoafectiva que completaram o protocolo (N=30). Durante a remissão parcial, aquando da segunda avaliação (N=60), encontrámos: uma diferença estatisticamente significativa (ANOVA; p=0.006), confirmada após teste de multicomparações de Bonferroni (p=0.05) entre os doentes com esquizofrenia (N=30) e perturbação esquizoafectiva (N=30); bem como uma correlação negativa (Pearson's r=−0.399) entre os níveis médios de *UCB* e o item G7 "*psychomotor retardation*" da *Positive And Negative Syndrome Scale*. Ao compararmos a primeira com a segunda avaliação (teste *T Student* para amostras emparelhadas) encontrámos uma diferença estatisticamente significativa (p=0.034) nos valores médios apenas entre doentes com perturbação esquizoafectiva (N=30).

Conclusão: existe um interessante potencial na investigação da *UCB* como biomarcador das psicoses e respectiva gravidade, quer no espectro da esquizofrenia, quer no da perturbação esquizoafectiva, seja na recaída ou na remissão.

Palavras−Chave: bilirrubina não conjugada, esquizofrenia, esquizoafectiva, psicose.

INTRODUCTION

THEORETICAL BACKGROUND

For the theoretical background of our thesis we included one epistemological perspective about the schizophrenia disorders spectrum plus a review regarding the correlation between UCB and schizophrenia.

Then we present a chapter that tries to summarize the epistemological and historical background for a broader schizophrenia spectrum, going from schizophrenia to bipolar disorder, with schizoaffective disorder right in between. We believe that without this schizophrenia – schizoaffective – bipolar spectra concept in our minds all our efforts will keep failing one of the hardest quests: the never−ending search for biomarkers in schizophrenia and related disorders.

Afterwards we included an already published review, supervised by us, becoming the master thesis of one of our students, and fully focused on the most important research findings contributing to the hypothesis of UCB as a biomarker for schizophrenia disorders. Many previous attempts have been made on the search for biomarkers in schizophrenia, but without significant success to our date. Mostly because of various kind of problems that have emerged in the last decades regarding the nosology and etiopathogeny of the psychotic disorders of the schizophrenia spectrum. In the last twenty years some studies have been published suggesting UCB as possible biomarker for schizophrenia spectrum disorders. On one hand it seems that UCB may have some utility for the categorical distinction with bipolar disorder, while on the other hand it seems that UCB may also become a valuable dimensional indicator for acute schizophrenic psychotic episodes.

The methods section consisted on the adaptation of one perspective article published for the dissemination of our study protocol.

The results section will be a compilation of two different manuscripts: one already published article with five years of retrospective data and another, yet unpublished, fully original paper with twelve months of prospective data. All of them exploring correlations between UCB mean levels and schizophrenia variables in human subjects.

Schizophrenia − schizoaffective − bipolar spectra: an epistemological perspective

Many authors have written regarding and discussing about the conceptual history of schizophrenia (Garrabé 1992, Berrios et al 2003). One could assume that, before modern age, schizophrenia patients would be included under the unspecific terms as melancholia or mania, terms already in use in classic age, depending if the predominant psychomotor symptoms were retardation or agitation (Zilborg 1941). But in modern age this epopee started with the first description of *démence*, a deteriorating psychological condition (Pinel 1801), and *démence précoce*, nothing more than a progressive, heritable decline in function in young people (Morel 1852). Later the concept of *dementia praecox* was adapted (Pick 1891) and developed as an ingeniously and consistent nosological entity, described as a chronic, deteriorating psychotic disorder characterized by rapid cognitive disintegration, usually beginning in the late teens or early adulthood (Kraepelin 1896). Although these words could be considered French and Latin translations of the same term, they were not quite true synonymous as concepts. There was, indeed, an abyss separating *démence précoce* from *dementia praecox* (Minkowski 1925). Nevertheless *dementia praecox* was the starting point for what would be the first ever use of the term, and concept for schizophrenia, describing a group of diseases that cause changes in thought processes and behavior in humans as well as difficulties relating to the world (Bleuler 1911).

Throughout our readings we collected some more outstanding citations that clearly show how the scientific community has raised, in the last half century, so many different opinions regarding this fascinating topic. Thus, schizophrenia has been labeled by various authors in many diverse manners such as: the graveyard of neuropathologists (Plum 1972), nothing more than a myth with a strong genetic component (Kety 1974); existing since the birth of humanity (Strömgren 1982), a scientific delusion (Boyle 1990), representing nothing than a different way of being human (Jenner et al 1993) or the price mankind pays nature for language use (Crow 1997).

The schizophrenia – schizoaffective – bipolar meta–spectrum.

For decades that clinicians and researchers have been thinking and writing about the spectrum of schizophrenia disorders. Indeed both Kraepelin and Bleuler believed in schizophrenia as a spectrum, both in a clinical (individual) and hereditary (family) continuum, from just some exquisite personality traits to unquestionable chronic and debilitating psychosis (Weller 1987). Other authors would put the schizophrenia spectrum disorders on different levels of continuum: developmental, psychofunctional, existential and genetic (Reich 1975).

An intermediate entity between schizophrenia and bipolar affective disorder is often the so called schizoaffective disorder. Patients with these schizoaffective psychoses are less likely to achieve a full remission than patients with bipolar disorders, who also present a much smaller prevalence of residual state between episodes (Angst et al 1980). Considerable debate have surrounded the inclusion of schizoaffective disorder in psychiatric nosology: some authors believe that schizoaffective disorder may be a variant of schizophrenia in which mood symptoms are unusually prominent but not unusual in type; while others believe it is a condition that may instead reflect a severe form of bipolar disorder in which episode−related psychotic symptoms fail to remit completely between mood episodes. Alternatively, schizoaffective disorder could correspond to the co−occurrence of two relatively common psychiatric illnesses: schizophrenia and bipolar disorder, or even represent a third and different type of psychosis, completely autonomous from the bipolar and schizophrenia categories (Abram 2008). On the other hand schizoaffective disorder could be an interform created by a combination of some, but not all, of the unknown aetiological factors responsible for schizophrenia and some, but not all, of the equally unknown factors responsible for bipolar disorder; or last but not least, some combination of the above described five theories (Kendell 1986). Schizoaffective disorder diagnosis is, indeed, an authentic conundrum in the clinical setting (Wilson et al 2014).

Some authors suggest a genetic susceptibility continuum spanning across schizophrenia and bipolar disorder and including, at its two extreme ends, unique genetic factors associated with each disorder and a middle zone of overlap, occupied by schizoaffective disorder and containing shared genetic factors (Owen et al 2007). Several twin analysis are consistent with genetic influences on schizoaffective episodes being entirely shared

with genetic influences on schizophrenic and manic episodes, while association studies suggest the possibility of some relatively specific genetic influences on broadly defined schizoaffective disorder (Cardno and Owen 2014).

More recently influent researchers wrote about the slow death of the concept of schizophrenia and the painful birth of the psychosis spectrum (Guloksuz and van Os 2018). Soon there were many voices rebutting this idea: some claimed that the concept of schizophrenia is still alive (Kahn 2018); some argued that, although clearly not well and with limited validity, the concept of schizophrenia has a continued utility (Zoghbi and Lieberman 2018); and others almost shouted that the half−alive concept of schizophrenia is still better than the spectrum of everything (Bora 2018).

After reading this insightful discussion we had to agree that schizophrenia is part of a spectrum, but it does not represent the extreme of a normally distributed trait (Curtis 2017), and shall be kept as a useful concept (Lawrie 2016) both for researchers, clinicians, patients, families and society. Indeed, we do not believe in a psychotic spectrum of everything but instead in a meta−spectrum composed by three different spectra: bipolar disorders spectrum, the schizophrenias spectrum, and the schizoaffective disorders spectrum. Each of those spectra would include different nosological independent entities, from personality disorder to chronic full−blown psychosis.

The bipolar disorders spectrum

To understand the complexity of the concepts include in bipolar disorders spectrum we point some relevant aspects about the classification and description of these.

- Classic bipolar disorder (Kleist 1911), previously known as manic depressive insanity, nowadays known as bipolar I (Akiskal and Pinto 1999), with manic or mixed episode, with or without major depressive episodes, not forgetting the renewed interest in the new mixed features specifier for episodes of mood disorders (Stahl et al, 2017; Vázquez et al, 2018).
- Other bipolar disorders in all its other modern subtypes (Akiskal and Pinto 1999): I ½ protracted hypomania without depression; II depressive and hypomanic episodes;

II ½ depressive episodes with cyclothymic temperament; III depressive episodes with antidepressant−induced hypomania; III ½ bipolar disorder with substance abuse; IV depressive episodes with hyperthymic temperament; V depression with mixed hypomania; VI bipolarity in the setting of dementia.

- Hyperthymic and cyclothymic temperaments (Akiskal and Malya 1987).

The schizophrenias spectrum

Also, for the clarification of concepts in schizophrenia spectrum, we highlight that:

- Schizoid and schizothypal personality disorders (Bolinskey et al 2017) and all its equivalents are cited in all previous versions of the World Health Organization's (WHO) International Classification of Diseases (ICD), such as latent schizophrenia, borderline schizophrenia, prodromal schizophrenia, prepsychotic schizophrenia, pseudoneurotic schizophrenia and pseudopsychopathic schizophrenia are at the milder extreme of this spectrum.
- Paranoid personality disorder, delusional disorder (previously known as paranoia) and paraphrenia (Ravindran et al 1999) are included in the spectrum.
- Schizophrenia in all old subtypes: simple, hebephrenic or disorganized, paranoid, residual, undifferentiated, catatonic (McGlashan and Fenton 1991), and even other obscure clinical scenarios, more common in European eastern countries such as cenesthopathic schizophrenia (Basov 1980) or sluggish schizophrenia (Jargin 2011) would constitute the more extreme severe conditions at the other end of our spectrum proposal.

The schizoaffective disorders spectrum

Our contribution for this meta−spectrum would be the proposal of a new spectrum for schizoaffective disorders that would include:

- Schizoaffective patients included in the original description (Kasanin 1933) plus the newer descriptions such as schizomania (Carloni and Spadoni 1959, van Os et al 1995) and schizobipolar (Stahl 2013).

- Cycloid psychosis on its classic forms: hyperkinetic−akinetic motility psychosis; excited−inhibited confusion psychosis; and anxiety−happiness psychosis (Leonhard 1979, Zaudig 1990, Pfuhlmann 2004, Jabs 2007, van der Kerkhof et al 2016).
- Borderline personality disorder patients, who may have brief psychotic episodes (but not enough for schizophrenia diagnosis) independently of important mood changes (but also not enough for bipolar disorder diagnosis) (Zimmerman 2013, Slotema et al 2018). We assume this idea as the most controversial of our proposal as psychotic symptoms in borderline personality disorder continue to be poorly understood and further research should try to ascertain the relationships between hallucinations and delusions on one hand and the processing of trauma, emotion regulation, distress tolerance and interpersonal sensitivity on the other (D' Agostino et al 2019).

With this short epistemological perspective we want to raise awareness, among clinicians but also among researchers, regarding the importance of the spectrum concept for the understanding and studying of idiopathic psychoses (previously known as nonorganic psychoses), especially regarding the authentic conundrum of schizoaffective disorder.

In the American Psychiatry Association's (APA) Diagnostic and Statistical Manual of Mental Disorders, fifth edition (DSM 5), the diagnosis of schizoaffective disorder can be made only if full mood disorder episodes have been present for the majority of the total active and residual course of illness, from the onset of psychotic symptoms up until the current diagnosis. In earlier DSM versions, the boundary between schizophrenia and schizoaffective disorder was only qualitatively defined, leading to poor reliability. This change will provide a clearer separation between schizophrenia with mood symptoms from schizoaffective disorder and will most likely reduce rates of diagnosis of schizoaffective disorder while increasing the stability of this diagnosis once made (Malaspina et al, 2013).

On the other hand, clinicians all around the world have shown small improvements in accurately diagnosing vignettes, using the World Health Organization's International Classification of Diseases, 11th edition (ICD 11). It seems that the discrepancy in diagnose schizoaffective disorder is related primarily to the presence of mood symptoms and discrepancies about whether those symptoms are more consistent with

schizoaffective disorder or a mood disorder diagnosis. Continuing to identify ways to more accurately capture this clinical picture will be important in the future as well as systematic efforts to educate clinicians about differential diagnosis (Peterson et al, 2019)

As a small contribute for this theoretical reflection we would like to present here an historical chronology for the schizophrenia–schizoaffective–bipolar spectra (Table 1) plus a tridimensional model for these spectra (Figure 1): the first axis for categories (affective versus nonaffective psychoses), the second axis for dimensions (personality versus full blown psychosis), and a third axis for biomarkers (remission versus relapse).

We believe that without the schizophrenia–schizoaffective–bipolar spectra concept in our minds all our efforts will keep failing one the hardest quest: searching for biomarkers in schizophrenia and related disorders.

Generally, a biomarker can be developed for three main purposes: diagnostic (to classify as having a disease); prognostic (to make predictions on who will develop a disease); or theranostics (to predict an individual's disease response to a particular treatment) (Weickert et al 2013). Unfortunately, there are still no validated and reliable biomarkers for schizophrenia in clinical use (Weickert et al 2013, Perkovic et al 2017). The fact that schizophrenia is on heterogenic disorder with multiple probable causes with distinct biological mechanisms means that attempting to find a single biomarker or group of biomarkers that would coincide with all cases of schizophrenia is unlikely (Weickert et al 2013). We do not have, indeed, strong biological markers that could help disentangle this heterogeneity and be useful in clinical practice (Dazzan 2014).

In an attempt to change this, efforts have been made to find biomarkers for schizophrenia (Hallak et al 2015). Though many potential biomarkers have been studied, some of the most promising and potentially clinically relevant include neuroimaging biomarkers (Goldsmith et al 2018). Since neuroimaging techniques results are inconclusive, a search, in recent years, for blood−based biomarkers for schizophrenia has been conducted (Lai et al 2016). The most frequently used blood−based biomarkers candidates in schizophrenia have been divided into six categories: brain−derived neurotrophic factor; immune function; epigenetics / genetics;

transcriptome / proteome studies; neurochemistry; and oxidative stress response (Lai et al 2016).

As any biomarker in medicine a biomarker for schizophrenia has to have clinical utility and this will take time, money, and coordinated research to develop (Weickert et al 2013). We emphasize other research groups' efforts in the study of UCB as a biomarker in schizophrenia, all around the world, in prestigious institutions such as the Milan Center for Neuroscience (Bentivegna et al 2019) or the Yale School of Medicine (Pradeep et al 2019). There is indeed an impending necessity for further research in this area and we are aware of those efforts (Gama Marques 2020).

Table 1: Epistemology of the schizophrenia − schizoaffective − bipolar spectra.

<table>
<tr><th>Author Date</th><th colspan="3">Better Prognosis Spectra</th><th colspan="2">Worse Prognosis Spectra</th></tr>
<tr><td>Pinel 1801</td><td colspan="2">Mania</td><td>Melancholia</td><td colspan="2" rowspan="2">Démence</td></tr>
<tr><td>Falret 1853</td><td colspan="3" rowspan="2">Folie Circulaire</td></tr>
<tr><td>Morel 1860</td><td colspan="2">Démence Précoce</td></tr>
<tr><td>Kraepelin 1896</td><td rowspan="4">Manic Depressive Insanity</td><td colspan="3">Dementia Praecox</td></tr>
<tr><td>Bleuler 1911</td><td colspan="3" rowspan="3">Schizophrenias</td></tr>
<tr><td>Kasanin 1933</td><td colspan="2">Schizoaffective Disorder</td></tr>
<tr><td>Leonhard 1957</td><td colspan="2">Cycloid Psychosis</td></tr>
<tr><td>ICD 9 1979</td><td rowspan="5">Bipolar Disorders</td><td colspan="2">Schizoaffective</td></tr>
<tr><td>DSM IV 1994</td><td colspan="3" rowspan="4">Schizoaffective Disorders</td></tr>
<tr><td>ICD 10 1994</td></tr>
<tr><td>DSM 5 2013</td></tr>
<tr><td>ICD11 2018</td></tr>
</table>

ICD: International Classification of Diseases; DSM: Diagnostic and Statistical Manual of Mental Disorders

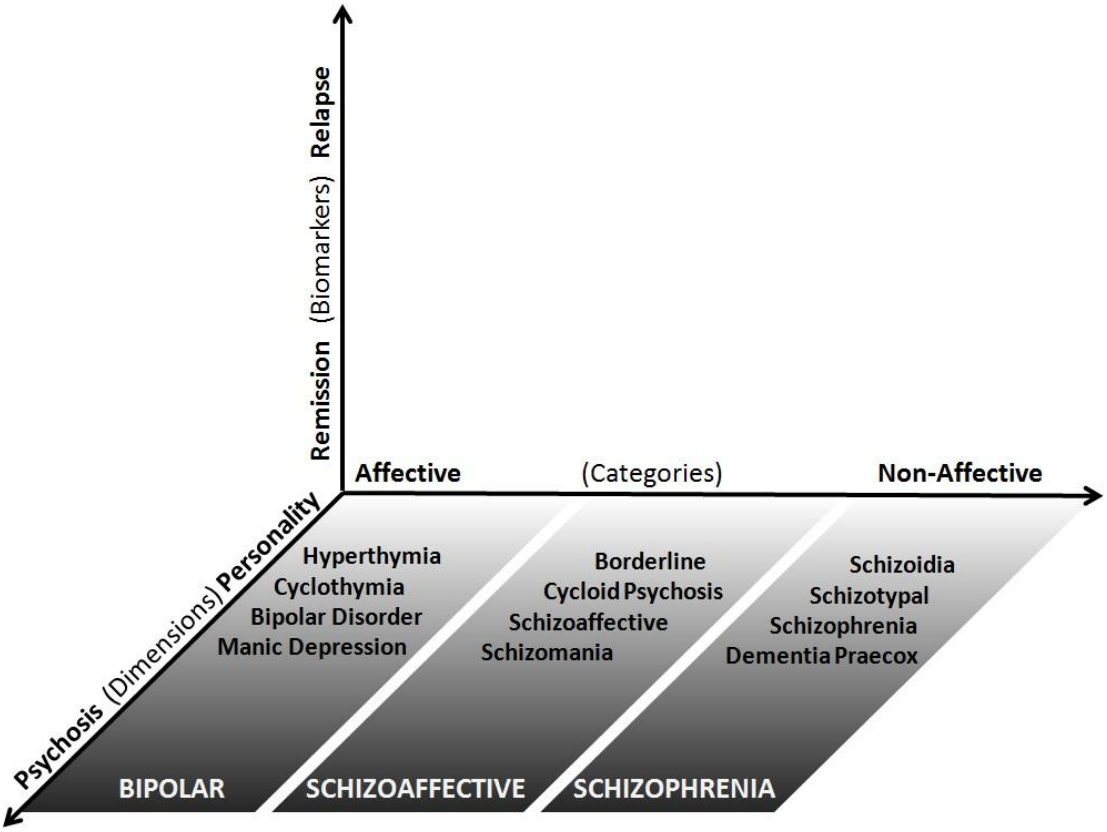


Figure 1: The Schizophrenia − Schizoaffective − Bipolar spectra.

Unconjugated bilirubin and schizophrenia: a review.

Schizophrenia (SZ) is an incredibly complex syndrome, a group of signs and symptoms of unknown etiology mainly defined by observed signs of psychosis; in its most common form, it presents itself with paranoid delusions and auditory hallucinations late in adolescence or in early adulthood (Insel 2010) . Currently available pharmacological treatments have limited efficacy, and there we have seen little substantial improvement since the advent of the first antipsychotics in the 1950s (Hallak et al 2015).

Unconjugated bilirubin is the water−insoluble fraction of total bilirubin in the serum. After it is bound to albumin and transported to the liver, it processed by the hepatic 1A1 isoform of uridinediphosphate−glucuronosyltransferase (UGT1A1) and becomes conjugated bilirubin (direct bilirubin), which can in turn be excreted into the bile (Hirschfields et al 2006). In practice, unconjugated bilirubin is calculated through the difference between the directly measurable levels of serum total bilirubin (TB) and conjugated bilirubin (CB); for this reason, unconjugated bilirubin (UCB) is also sometimes called indirect bilirubin. Some studies have found a possible correlation between UCB levels and an increased incidence of schizophrenia (Miyaoka et al 2000, Miyaoka et al 2008, Semnani e al 2010, Radhakrishnan et al 2011,). UCB levels may have both direct and indirect toxic effects for the Central Nervous System (CNS) (Brites et al 2012), with animal models (Gunn rat) presenting schizophrenia−like behavior (Hayashida et al 2009). Therefore, the measurement of serum concentration of UCB has been proposed as a possible biomarker for the management of schizophrenia (Gama−Marques et al 2017).

While UCB concentrations below 100 nM are able to protect the CNS against oxidative damage (Doré et al 1999), moderate and high levels preferentially cause early necrosis (Hankø et 2008). Neurons are more susceptible to UCB−induced damage than astrocytes (Brito et al 2008) as neurons commonly show higher levels of Reactive Oxygen Species (ROS), protein oxidation, and lipid peroxidation upon UCB exposure than astrocytes (Conforti et al 2007). Interaction of UCB causes increased polarity and fluidity (Rodrigues et al 2002), disrupting the neuronal membrane structure. This is accompanied by reduced activity of Mg2+ −ATPase aminophospholipid translocase (flippase) and Na + , K + −ATPase, together with enhanced intracellular levels of Ca2+, effects which can lead to cell destruction (Khan et al 2011). Developing neurons are

particularly susceptible to UCB interaction, with neurogenesis impairment, neuritic atrophy, and cell death by both necrosis and apoptosis (Brito et al 2002, Falcão et al 2007). The contact results in sudden and lasting decrease in neuronal arborization (Fernandes et al 2009), and cells stays vulnerable to a secondary toxic stimulus (Falcão et al 2007). UCB also causes synaptotoxicity which has been related with potentiation of inhibitory synaptic transmission (Shi et al 2006) and with impairment of the induction of long term potentiation (Chang et al 2009). N−methyl−d−aspartate (NMDA) receptor mediated excitotoxic mechanism is another pathway leading to UCB−induced neuronal injury (Bellefontaine et al 2011). UCB was demonstrated to raise the extracellular concentration of glutamate, by enhancing its release mainly from immature neurons and overstimulating NMDA receptors (Falcão et al 2006). UCB also causes mitochondrial swelling (Solá et al 2002), Bax translocation and activation of caspase−3 (Rodrigues et al 2002). The survival and proper functioning of neurons is ensured by the large number of glial cells (Streit et al 2002), which include oligodendrocytes (OLGs), astrocytes and microglial cells. Therefore, induced glial damage must be considered an important event in Central Nervous System (CNS) injury by UCB. Astrocytes are the most abundant type of glia (Nair et al 2008) and have multiple homeostatic properties (Orellana et al 2008). UCB causes a rapid increase in the extracellular glutamate content (Fernandes et al 2004), mainly in immature astrocytes (Falcão et al 2005) that appears to result from the inhibition of its uptake by UCB (Silva et al 2001) and also from enhanced reactive astrocyte secretion (Falcão et al 2006); excessive exposure to glutamate causes neuronal injury, and this excitotoxicity can be harmful to neurons and OLGs (Brites 2012). Elevated concentrations of UCB can cause the secretion of Tumor Necrosis Factor Alfa (TNF−α) and Interleukin 1 Beta (IL−1β) by astrocytes (Fernandes et al 2004); it can also cause inhibition of cell endocytosis (Silva et al 2001) and upregulation of both TNF−α receptor (TNFR) 1 and IL−1β receptor (IL−1R)1 (Fernandes et al 2011). UCB also has deleterious effects on OLGs. Moderate perinatal systemic inflammation is related to deficient OLGs precursors' maturation, with diminished density of myelinating, therefore causing long−lasting effects (Favrais et al 2012). Under these conditions, vulnerable microglia may modify their functional capabilities and become more susceptible to injury or aging (Harry et al 2012). UCB also compromises the mechanism of myelinogenesis (Brites 2012). The microglia, which accounts for around 12% of total glial population (Ladeby et al 2005), were shown to be involved in neurogenesis, postlesional tissue repair and

synaptic stripping (Graeber et al 2011). They play a major role in synaptic pruning during postnatal development (Paolicelli et al 2011). The first reaction of microglia against UCB insult is an increase in phagocytosis (Brites 2012). Microglial cells can be activated or overactivated, depending on the stimuli, consequently becoming neurotoxic (Carson et al 2007); the cause of increased microglial activation is still unclear, but oxidative stress appears to have a central role (Floyd et al 2002). The most marked effect of UCB on microglia was shown to be elevated levels of IL−1β, which was related to loss of neuronal integrity (Depino et al 2005); these cells also release the cytotoxic molecule glutamate when exposed to UCB (Gordo et al 2006). Additionally, microglia contributes to the generation of NO, an important component in microglia−induced neuronal death (Graber et al 2012). Finally, these cells undergo cytoplasmic fragmentation and nuclear condensation when exposed to UCB for longer periods (Silva et al 2010), thus thwarting any beneficial effect they might have in the protection against neuronal damage. Neuroinflammation plays a role in UCB−induced neuronal damage as well. UCB inhibits the function of polymorphonuclear leukocytes and lymphocytes (Brites 2012) and stimulates the release of proinflammatory cytokines, reactive oxygen species (ROS) ROS and nitric oxide (NO), which can disrupt nerve terminals and impair synaptic activity (Haustein et al 2010). Neurons previously exposed to UCB have demonstrated an increased vulnerability to a subsequent inflammatory injury with lipopolysaccharide or TNF−α (Falcão et al 2007), implying that the priming of neurons by UCB may cause long−term neurological dysfunction. A recent study showed that UCB can induce pyroptosis, a highly inflammatory form of cell death, in cultured rat cortical astrocytes (Feng et al 2018). It reported that UCB caused in vitro activation of caspase−1, known as an important mediator of pyroptosis (Bergsbaken et al 2010), which was in turn involved in plasma membrane rupture and loss of cell viability.

In this paper, we review the relation between UCB and schizophrenia. We used the Preferred Reporting Items for Systematic Reviews and Meta−Analyses (PRISMA) available at http://www.prisma−statement.org/. Since the available literature on the subject of the relationship between UCB and schizophrenia (SZ) isn't overly vast, we considered as potentially eligible for this article any relevant studies published in English, regardless of date of publication, sample size, outcomes, comparators or length of follow−up. The studies which considered the role of UCB in CNS inflammation in

vivo and in vitro and/or the correlation between the levels of UCB or its oxidative metabolite biopyrrin and schizophrenia were included. The studies which only screened its samples for TB were not included, in order to keep this article focused. PubMed and Cochrane Library databases were used for the search as well as ClinicalTrials.gov. The search on the platforms was last done on 26/02/2018. The terms included were the truncated terms (bilirubin*[All fields] AND schizo*[All fields]). The research yielded 75 results from PubMed and 15 from Cochrane Library; no results came up in ClinicalTrials.gov. After the inclusion of one extra paper (found in the bibliography of the above−mentioned articles) and the exclusion of case reports/duplicates/off−topic articles, 13 articles remained for inclusion in the review. Figure 2 resumes the articles selection process for review.

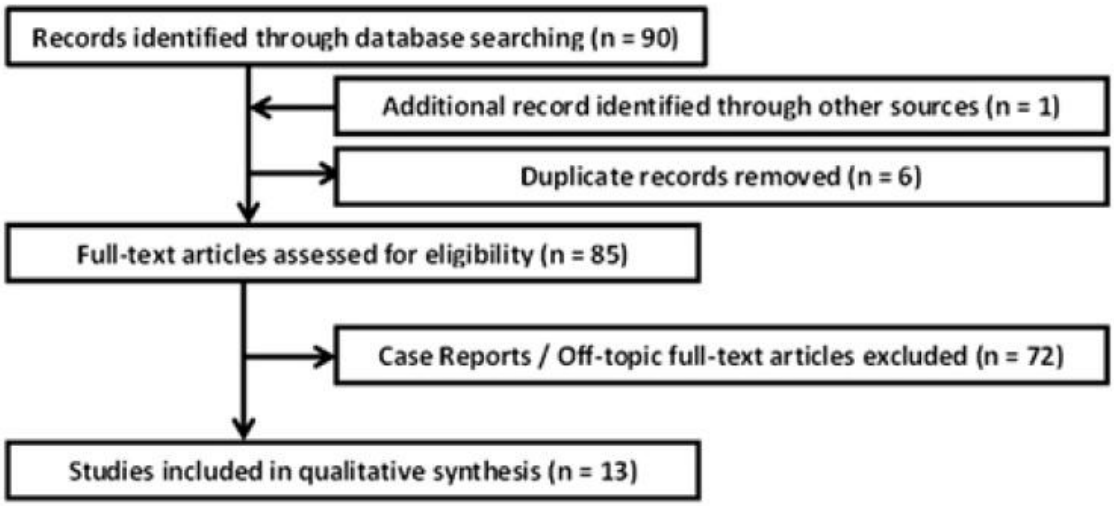


Figure 2. Articles selection process for PRISMA review.

We found 3 studies reporting high levels of UCB in 26 Gunn rats, in vivo models of schizophrenia, and 10 studies reporting high levels of UCB in 473 human patients with schizophrenia.

High levels of unconjugated bilirubin in vivo models of schizophrenia

In the year of 1944, Gunn reported (Gunn 1944) a new type of mutant rats showing unconjugated hyperbilirubinemia and jaundice. The Gunn rats have since been used in several previous studies as the classic animal model of UCB encephalopathy (Rice et al 2008). The homozygotic recessive jaundiced (j/j) Gunn rat have a congenital deficiency of the bilirubin liver conjugating enzyme UGT1A1, and consistently develops acute CNS dysfunction and kernicterus when given sulfadimethoxine (Rose et al 1979) or phenylhydrazine (Rice et al 2008). Hayashida et al. (Hayashida et al 2009) analyzed the behavioral changes induced by high serum UCB in the Gunn rat and considered the possibility of using it as new in vivo models for schizophrenia (Carpenter et al 2008). The animals used in the study were male hyperbillirubinemic j/j Gunn rats (with severe high levels of UCB), nonjaundiced +/j male Gunn rats (with non−severe high levels of UCB) and male Wistar rats. Several behavioral studies were carried out, in which certain specific actions (such as stretching, grooming, sniffing, and defecation, among others) and social interactions were counted during a specific amount of time in controlled environments (open field, home cage and new cage). In order to simulate environmental stress, the rats were exposed to 20 ms burst of white noise at 120 dB (pulse test) and 20 ms burst of white noise of 70 dB (70 prepulse + pulse test) followed by a 20 ms white noise burst at 120 dB or 20 ms white noise burst at 80 dB (80 prepulse + pulse test) followed by a 20 ms white noise burst at 120 dB. Start response was measured using a startle reflex measurement system. Both Gunn j/j and +/j rats exhibited hyperlocomotion, a higher frequency of sniffing, and a lower frequency of defecation. These stereotypical behaviors (hyperlocomotion, continuous sniffing, licking, and gnawing) are thought to be animal equivalents of positive symptoms of schizophrenia (Nabeshima et al 1983). In the social interaction tests, frequently observed behaviors of Gunn j/j and +/j rats were attacking. Additionally, j/j rats showed impaired prepulse inhibition; hypersensitivity to psychostimulants is suspected to be one of the features in SZ, and patients with SZ often perform poorly in prepulse

inhibition tests (Braff et al 1990) . In a follow−up study (Liaury et al 2012), Liaury et al. set out to examine the effects of UCB in the morphology of the microglial cells of Gunn rats. In it, the hippocampus Dentate Gyrus (DG) of 10 Gunn rats was compared with those of 10 Wistar rats. Microglial cells were marked with antibodies through immunohistochemistry; rabbit anti ionized calcium binding adaptor molecule 1 (Iba1) and mouse antiCD11b were used. With light and confocal microscopy, Iba1−marked cells were found to have elongated cell somata with fewer branches but thicker processes in the hilus, subgranular zone (SGZ), granular layer (GL), and molecular layer (ML) of the hippocampus dentate gyrus. This type of morphology is known as activated morphology, a term that describes proliferating cells that demonstrate changes in their immunophenotype but have not undergone transformation into brain macrophages. Such a transformation can be stimulated by neuronal death but not by sublethal neuronal injury (Streit et al 1988). The ML cells showed small rod−shaped somata with numerous thin and highly ramified processes. This kind of morphology is known as ramified/resting morphology and is similar to the morphology found in Wistar rats. After counting the Iba1−labeled cell numbers, no significant difference between Gunn and Wistar rats in the estimated numbers of Iba1−labeled cells in all areas of the DG was found. Using a technique of electron microscopy, activated Iba1−labeled cells were shown to have an elongated cell body and enlarged cytoplasm. An organelle−rich cytoplasm that includes the Golgi apparatus, lysosomes, rough endoplasmic reticulum and some small vesicles containing low−density material was also observed. Next, the expression of a marker that increases during microglia activation, CD11b (integrin aM) (Sierra et al 2010), was examined. Microglial cells in Gunn rats were reported to express high levels of CD11b immunoreactivity, while microglial cells in Wistar rats did not show significant CD11b expression. Statistical analysis showed that areas of CD11b expression were significantly greater in Gunn rats than in Wistar rats in the hilus ($p<0.005$), SGZ ($p<0.001$), and GL ($p<0.005$). There was no significant difference of CD11b expression in the ML between Gunn rats and Wistar rats.

We have discussed previously the activation of microglial cells and its role in CNS toxicity. This mechanism is a probable indicator of neurotoxicity and oxidative stress in the hippocampus. Indeed, significant reduction of hippocampal volume has been a frequent finding in both post−mortem and in vivo brain imaging studies (Shenton et al 2001, Velakoulis et al 2006); it has also been suggested that SZ pathology could begin

with excitotoxic damage to the hippocampus (Schobel et al 2013). Tsuchie et al. published a study (Tsuchie et al 2013) which focused on the potential effects of antipsychotic therapy on the behavioral abnormalities of Gunn rats mentioned above. In this study, Gunn rats and Winstar rats (serving as the control group) were given 0.1 mg/kg risperidone, 0.4 mg/kg aripiprazole, 0.2 mg/kg haloperidol or saline as control; the drugs were orally administered one day before the open−field and prepulse−inhibition tests and were continued for 14 days for the social interaction test. Six of each rat type (12 in total), were used in each medication group. Rats from each medication group were tested for their horizontal (ambulation) and vertical (rearing) locomotor activity in a novel environment (open−field test), their level and quality of social interaction when paired together in a single cage, and their startle response prepulse inhibition (with no stimulus, or 120 dB pulse of 20 ms duration, or 120 dB pulse preceded 100 ms by a 70 dB or 80 dB pulses of 20 ms in duration). In the open−field test, there was a significant difference between the Gunn Control (GC) group (10,595.4 ±964.3) and the Wistar rat control group (WC) (7988.0 ±1164.8) in the time spent in ambulation ($p<0.05$). The period of ambulation for the Gunn rat risperidone group (GR) (7341.8 ±1930.6) was significantly lower than that of the GC ($p<0.05$), and although there was no significant difference between the Gunn Haloperidol (GH) group (8740.7 ±1493.5) and the Gunn Aripiprazole (GA) group (9371.3 ±1869.5) and GC, there was a tendency for the activity of the GH and GA groups to be reduced. The activity of the Wistar rat medication groups (WM) was slightly decreased, but there was no significant difference between the WC and Wistar Medication (WR) groups (WR: 6812.5 ±1812.8; WH: 6563.2 ±900.4; WA: 6487.3 ±4056.4); there was also no substantial difference between the WC and Gunn rat medication groups. In rearing frequency, there was a significant difference between the GC (217.5 ±27.3) and WC (109.2 ±34.4) groups ($p<0.01$). The frequency of rearing by the Gunn rat medication groups (GR: 159.5 ±45.5; GH: 141.8 ±28.4; GA: 149.3 ±46.2) was significantly lower than those of the GC ($p<0.01$). There was no significant difference between the WC and Wistar rat medication groups (WR: 97.8 ±35.2; WH: 70.2 ±30.6; WA: 100.7 ±55.3). The purpose of the open−field test was to measure behavioral changes when the animals were put in new environments. Exploratory activities increase when rats feel anxiety or fear or have problems of adaptation in new environments. SZ patients have a lower capability of adapting to their surroundings when placed in a foreign environment, and a subset of patients exhibits psychomotor

agitation; thus, increased locomotor activity might mimic this psychomotor agitation seen in humans with SZ (Powell et al 2006). Indeed, the activity of the GC group was increased compared with that of the WC group. Additionally, when the Gunn rats were given antipsychotics, their rearing activity was significantly decreased, implying a therapeutic effect of the drugs tested in the Gunn rat's hyperactivity and ability to adapt. In the social interaction test, Gunn rats (49.0 ±15.9) exhibited significantly fewer social behaviors than the Wistar rats (236.2 ±37.8) ($p<0.01$) and also exhibited more antisocial behaviors (227.8 ±60.3) than the Wistar rats (8.8 ±8.9) ($p<0.01$). The social behaviors of the Gunn rat medication groups were increased (GR: 94.5 ±38.2; GH: 74.8 ±30.5; GA: 93.2 ±38.0), and although the improvement in the Gunn rat social behavior after treatment with antipsychotics was not significant, the medication still tended to have a positive effect. The anti−social behaviors of the Gunn rat with medication groups were improved (GR: 187.8 ±60.3; GH: 180.7 ±37.9; GA: 157.0 ±39.1), and there was a significant difference between Gunn rat control group and medicated groups ($p<0.01$). The purpose of this test was to measure the decrease of motivation and social behaviors in rats, mimicking the behavior frequently seen in SZ patients. Antipsychotic drugs significantly improved Gunn rat's anti−social behaviors. In the prepulse inhibition (PPI) test there was a significant difference between GC (13.2 ±21.2) and WC (40.3 ±28.9) ($p<0.01$) on the 70 dB prepulse test; although there was no significant difference between the GC and Gunn rat medication groups (GR: 20.3 ±27.0; GH: 25.0 ±9.2; GA: 24.2 ±22.2), the Gunn rat PPI deficit tended to be ameliorated by the administered antipsychotics. In the 80 dB prepulse there was a significant difference between GC (43.7 ±13.9) and WC (77.1 ±8.2) ($p<0.01$), while there was a tendency for the PPI deficit of the Gunn rat medication groups to be inhibited (GR: 54.5 ±21.6; GH: 51.7 ±19.7; GA: 47.2 ±8.9). This study thus shows that antipsychotic medications affect Gunn rat behaviors in a similar way to SZ behaviors. The findings of this and of the previously mentioned studies suggest that the Gunn rat represents a promising new model for schizophrenia, and that this model might help further SZ investigation and the role of UCB in its pathogenesis.

High levels of unconjugated bilirubin in humans with schizophrenia

A considerable number of published studies have demonstrated an increase in serum total bilirubin in patients with schizophrenia, but few have specified the correlation between the unconjugated form of the protein and SZ.

In 1991, Muller et al. demonstrated a significantly higher incidence of hyperbilirubinemia in schizophrenics than patients suffering from other disorders (Muller et al 1991), being one if not the first study to propose a relationship between the molecule and the illness.

Considering the specific correlation between UCB and SZ, there were few but interesting studies published. In 2000, Miyaoka et al. published a study (Miyaoka et al 2000) tackling the association between Gilbert's Syndrome (GS), a disease which courses with high plasma unconjugated bilirubin, and SZ. In the previous study, the plasma bilirubin concentration was collected from a sample of 290 patients admitted to the Department of Psychiatry at the Shimane Medical University, and the TB and CB levels were subsequently determined through the bilirubin oxidase method. UCB was calculated on the basis of the difference between both parameters. The patients were subdivided in 3 groups based on their diagnosis (ICD 10): schizophrenia (97 patients), affective psychosis (145 patients) and neurosis/personality disorders (48 patients). A total of 9% of all patients and 20.6% of schizophrenics were found to have elevated plasma bilirubin levels; the normal prevalence of GS in the general population is of 2.4−7.0%. All schizophrenics with hyperbilirubinemia had an increased UCB, whereas CB was within the normal range. Statistically significant differences were found between patients with schizophrenia and affective psychosis ($\chi 2=18.8$, $p < .0001$), between those with schizophrenia and mania ($\chi 2=3.991$, $p<0.0457$), between those with schizophrenia and psychotic depression ($\chi 2=15.66$, $p<0.0001$), and between those with schizophrenia and neurosis/personality disorders ($\chi 2=5.543$, $p<0.0186$). The mean standard deviation (SD) plasma bilirubin level of schizophrenic patients with hyperbilirubinemia was 2.05 ±0.15 mg/dL. During hospitalization, hyperbilirubinemia decreased in 80% (N=16), fluctuated in 20% (N=4), and increased in 0% (N=0) of the 20 patients receiving antipsychotic treatment. Of the schizophrenic patients with hyperbilirubinemia, 45% (N=9) had been drug−free for at least 6 to 8 weeks prior to their admission. Statistically, the schizophrenic patients with hyperbilirubinemia

showed a significant decrease in plasma bilirubin concentration during the course of the neuroleptic treatment. Next, the subgroup of SZ patients with hyperbilirubinemia had their psychiatric symptoms evaluated with the Positive and Negative Syndrome Scale (PANSS). Positive PANSS scores differed significantly between schizophrenic patients with and without hyperbilirubinemia ($t=8.057$, $p<0.0001$). Similarly, a significant difference was found between schizophrenic patients with and without hyperbilirubinemia on the general scores of the PANSS. However, no difference was found between SZ subjects with and without hyperbilirubinemia on the negative subscale. It's important to note that this study did not have a healthy or non−psychiatric control group, which increases the risk of bias.

In 2008, Japanese authors published a more in−depth study (Miyaoka et al 2008). In it, the team expanded their research on the same sample group using several imaging techniques and testing for clinical features through different scales. It again showed an increase in positive and Positive And Negative Syndrome Scale (PANSS) general scores in schizophrenia patients with idiopathic increased plasma UCB or GS during the acute phase of the psychotic episodes, when compared to those without GS. It also didn't find a significant difference in negative scores between patients with and without GS in the acute phase. In the stable phases, however, it showed a relevant increase in all PANSS subscales scores in schizophrenics with GS compared to those without GS. Studying the brain morphology of all SZ patients via brain computed tomography (CT) scans, the same paper reported a diffuse enlargement of almost all internal and external components of cerebrospinal fluid (CSF) spaces in schizophrenia patients with GS relative to schizophrenia patients without elevated UCB and controls. The study also did a fluid−attenuated inversion−recovery magnetic resonance (FLAIR MR) analysis: axial 5−mm−thick FLAIR MR Images from schizophrenia patients with GS (n=18) and schizophrenia patients without GS (n=18), all diagnosed according to Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM IV) criteria, were compared with age− and sex−matched non−psychiatric controls (n=18); signal intensities in the hippocampus, caudate, thalamus, anterior cingulated gyrus, and insula were graded relative to cortical signal intensity in the frontal lobe. Compared to both schizophrenia patients without GS and normal controls, the schizophrenia patients with GS showed significantly increased signal intensities in almost all regions studied, suggesting specific changes in the fronto−temporal cortex, limbic system, and basal

ganglia. Lastly, in order to confirm this metabolic alteration, levels of N−acetylaspartate (NAA), choline (Cho), and myoinositol (ml) in the hippocampus, basal ganglia, Anterior Cingulate Gyrus (ACC), and insula of the cerebellum of schizophrenic patients with GS were analyzed through proton magnetic resonance spectroscopy (1H−MRS). According to the paper, the major finding was that SZ with GS, compared to both schizophrenics without GS and normal controls, showed significant decreases of NAA/Creatine (Cr) and ml/Cr in the hippocampus. In basal ganglia, the schizophrenics with GS, compared to normal controls, showed significant decreases of NAA/Cr and ml/Cr, and compared to schizophrenics without GS, showed significant decrease of ml/Cr. In the ACC, patients with schizophrenia and GS showed significant decreases in NAA/Cr, Cho/Cr, and ml/Cr compared healthy subjects and compared with schizophrenia without GS. In the Insular cortex, patients with schizophrenia and GS showed significant decrease in NAA/Cr, Cho/Cr, and ml/Cr compared with healthy subjects and compared with patients with schizophrenia without GS. Patients without GS also showed significant decreases in NAA/Cr, and Cho/Cr, and ml/Cr compared with healthy subjects. Assuming that NAA is a neuron number and/or viability marker, a decrease of NAA/Cr with schizophrenics with GS might suggest the effect of UCB on the structure and/or function of the hippocampus, basal ganglia, ACC, and Insular cortex. And a decrease of ml/Cr, as a glial marker and/or viability marker, in schizophrenics with GS might suggest the effect of UCB on the glial structure and/or function of the hippocampus.

In 2010 Semnani et al. have published a study (Semnani et al 2010) in which 162 patients with SZ, 155 patients with Bipolar Disorder (BD) type I and 95 patients with cardiac disease had their serum levels of TB and CB measured both at admission and at discharge, after receiving treatment. The initial mean TB level of SZ patients was in the normal range but significantly higher than BD patients and the same parameter was higher in BD patients than in the cardiac disease patients. All schizophrenic patients received antipsychotic medications, including haloperidol (100 patients), risperidone (53 patients), and clozapine (9 patients). 57 of BD patients also underwent antipsychotic therapy: (haloperidol, 42 patients), perphenazine (1 patient), and risperidone (14 patients). The mean UCB level was decreased in all three groups at discharge (P0.05, R2=0.042, F(2.137)=2.9, p=0.05; β=0.004, t(137)=0.042, p>0.05, R2=0.039, F(2.137)=2.79, p>0.05, respectively) and UCB levels (β=0.06, t(137)=0.680, p>0.05,

R2=0.039, F(2.137)=2.78, p>0.05; β=0.01, t(137)=0.116, p>0.05, R2=0.036, F(2.137)=2.55, p>0.05, respectively). On comparing the paranoid SZ and non−paranoid SZ groups, the mean serum TB and UCB (in mg/dl) were significantly higher in the paranoid SZ (mean TB=0.60 ±0.43, t(69)=2.08, p=0.03; mean UCB=0.44 ±0.43, t(69)=1.95, p=0.05) and non−paranoid SZ groups (mean TB=0.80 ±0.49, t(69)=2.76, p=0.03; mean UCB=0.65 ±0.43, t(69)=2.99, p=0.004) compared with the BD group (mean TB=0.47±0.07, mean UCB=0.32±0.23), but there was no significant difference between the paranoid and non−paranoid SZ groups (mean TB=0.60±0.43 vs 0.8±0.48, t(69)=1.07, p=0.29; mean UCB=0.44 ±0.43 vs 0.65 ±0.43, t(69)=1.11, p=0.26). As in the aforementioned research, there was no healthy or non−psychiatric control group in this study.

A 2017 study (Gama Marques 2017) set out to evaluate whether high yet within normal range UCB levels could be considered a potential biomarker in the differentiation among acute psychotic patients with SZ, schizoaffective disorder and bipolar disorder (BD). Two hundred and four acute patients (50 with Z, 69 with schizoaffective disorder and 85 with BD) and 55 healthy controls were included. SZ patients presented higher UCB mean values (0.39 mg/dL, ±0.16 mg/dL), schizoaffective patients presented intermediate values for UCB mean values (0.36 mg/dL, ±0.13 mg/dL), and BD presented lower values for UCB mean values (0.29 mg/dL, ±0.13 mg/dL). A one−way Analysis of Variance (ANOVA) test was used to demonstrate a statistically significant difference ($p \leq 0.0001$) between mean values of UCB. On the post−hoc Bonferroni multiple comparison tests there was statistically significant difference between patients with SZ and BD ($p \leq 0.0001$) as well between schizoaffective and BD ($p \leq 0.01$). Both patients with SZ ($p \leq 0.0001$) and schizoaffective disorder ($p \leq 0.01$) showed statistically significant difference when compared with controls. A univariate corrected ANOVA model with age and gender as covariates was employed to test for possible bias; no influence was found.

The reaction of bilirubin with ROS produces many kinds of biopyrrins, also known as bilirubin oxidative metabolites (BOMs). Yamaguchi et al. determined, in 1994, the chemical structures of two biopyrrins (biotripyrin−a and biotripyrin−b) purified from the urine of healthy volunteers (Yamaguchi et al 1994). Yamaguchi et al. reported in a later study (Yamaguchi et al 2002) that emotional stimuli are associated with an

increase in the BOMs in human urine, and thus could be useful markers of psychological stress. Following this logic, some studies have analyzed the urinary secretion of BOM's in SZ patients in order to examine the antioxidative activity of bilirubin in this group. Miyaoka et al. (Miayaoka et al 2005), in a study in which 15 acute SZ patients and 10 patients with depression (both groups diagnosed according to DSM IV) were compared with controls of 48 men and 48 women, found that mean levels of urinary BOMs in patients with schizophrenia and depression were significantly higher (11.3 ±7.1 and 4.74 ±1.1 μmol/g creatinine) than in healthy controls (2.5±1.3 μmol/g creatinine). Moreover, the mean level of urinary BOMs in schizophrenic patients was significant higher than that in the depressed patients, and the level of urinary BOM's showed correlations with Brief Psychiatric Rating Scale (BPRS) scores in SZ patients ($r=0.671$, $p<0.0001$).

Yasukawa et al. (Yasukawa et al 2007) published a similar study, in which 15 SZ patients (diagnosed with DSM IV criteria) and 100 healthy controls (50 men and 50 women) had their urinary levels of BOMs analyzed. They reported significantly higher urinary concentrations of BOMs in patients with diagnoses of schizophrenia on admission (15.9 ±8.2 μmol/g creatinine) compared to controls (2.2 ±1.3 μmol/g creatinine; $p=0.0164$). Response to treatment was associated with significant decreases in the concentration of BOMs.

Miyaoka et al. (Miyaoka et al 2015) published a paper in which the urinary BOMs of 29 SZ patients (DSM IV criteria) were compared with those of 30 healthy controls. The mean levels of urinary BOMs in patients with schizophrenia and normal controls were 1.51 ±0.74 and 1.11 ±0.54 μmol/g creatinine, respectively; the mean levels of urinary BOMs in patients with schizophrenia were significantly higher than those in the control subjects ($p=0.019$). The level of urinary BOMs showed a correlation with the duration of illness ($r=-0.419$, $p=0.024$) and with BPRS scores ($r=0.583$, $p=0.001$). The main findings of our review were chronologically gathered in Table 1.

Table 2 – Some of the main positive findings of the review suggesting a positive correlation between UCB levels and schizophrenia.

Authors (year)	Sample	UCB or BOMs mean levels in schizophrenia patients	After treatment	Clinical
Miyaoka et al. (2000)	N=290 humans	High UCB.	Decreased UCB.	Positive correlation with Positive symptoms on PANSS scores.
Miyaoka et al. (2005)	N=15 humans	High BOMs.	-	Positive correlations with BPRS scores.
Yasukawa et al. (2007)	N=15 humans	High BOMs.	Decreased BOMs.	-
Miyaoka et al. (2008)	N=290 humans	High UCB.	-	Positive correlation with PANSS scores during acute phases.
Hayashida et al. (2009)	N=10 Gunn rats	High UCB.	-	Positive correlation with impaired PPI and stereotypical behaviors.
Semnani et al. (2010)	N=162 humans	Normal UCB, but higher vs. bipolar patients.	Decreased UCB.	Positive correlation with PANSS scores.
Radhakrishnan et al. (2011)	N=71 humans	Normal UCB, but higher vs. bipolar patients.	No significant change.	-
Liaury et al. (2012)	N=10 Gunn rats	High UCB.	-	Positive correlation with Iba1, CD11b marked hippocampus cells.
Tsuchie et al. (2013)	N=6 Gunn rats	High UCB.	-	Improvement in hyperactivity and social behaviors after treatment.
Miyaoka et al. (2015)	N=29 humans	High BOMs.	-	Positive correlation with BPRS scores.
Gama-Marques et al. (2017)	N=50 humans	Normal UCB, but higher vs. bipolar patients.	-	-

BOMs = Bilirubin Oxidative Metabolites (urine); BPRS = Brief Psychiatric Rating Scale; PANSS = Positive And Negative Syndrome Scale; PPI = Prepulse Inhibition test; UCB = Unconjugated Bilirubin (plasma).

The topic of the correlation between unconjugated bilirubin and the incidence of schizophrenia cannot be said to be a completely new one; many studies have been made in order to elucidate this very specific aspect of a particularly complex disorder. The interest of this research lies mainly in the possibility of finding an objective, measurable and potentially influenceable parameter which could help complement the still somewhat subjective approach to the diagnosis, management and even prevention of schizophrenia. If this possibility turned out to be true, it would imbue medical professionals with a powerful weapon against SZ. Many studies have found a statistical relationship to exist between UCB and SZ. However, this link remains vague, as it is so far unclear if there is a solid causal connection and, if present, which is the precedent event. It is also uncertain whether there is a direct or inverse proportionality between the serum levels of UCB and the incidence of SZ. Some studies have reported an increased incidence of SZ with higher levels of UCB (Miyaoka et al 2000, Miyaoka et al 2008, Semnani et al 2010, Radhakrishnan et al 2011, Gama Marques et al 2017, Gama Marques et al 2019, Pradeep et al 2019), while others have reported the opposite: an increase in SZ among individuals with lower UCB levels (Yao et al, 2000, Vitek et al 2010). Some studies showed a reduction in plasma TB levels with treatment (Miyaoka et al 2000, Semnani et al 2010), and some reported a correlation between symptomatic scales and plasma UCB concentrations (Miyaoka et al 2000, Miyaoka et al 2008, Semnani et al 2010). However, some studies reported an absence of significant effect of antipsychotic therapy on TB levels (Bach et al 2010, Radhakrishnan et al 2011). This could thus point to a non−linear, complex link between UCB and the pathophysiology of SZ, a hypothesis which certainly does not appear farfetched when one considers the complicated role of UCB in the antioxidant and inflammatory responses, as well as the multifactorial nature of SZ. As shown by various articles cited in this review, UCB has been linked to in vitro and in vivo neurotoxicity. It appears to have direct and indirect nefarious effects towards most (if not all) cell types in the CNS. The threshold above which UCB begins to lose its beneficial antioxidant effects on CSN cells appears to be rather small (Doré et al 1999), so that even small imbalances in the regulation of this molecule could potentially change its positive properties to deleterious ones. In both study groups – the studies which report a direct correlation between increased UCB levels and SZ incidence, and those which report an inverse correlation – the crux of the matter appears to be a dysfunction in the inflammatory mechanisms in the brain. When the concentration of plasma UCB is too high, it appears to directly cause

neuroinflammation, ROS production and cell apoptosis; when it is too low, however, it might impair the body's antioxidative defenses and result also in increased inflammation and increased ROS levels. Therefore, UCB could be both the cause and the effect of the alterations seen in the schizophrenic patient.

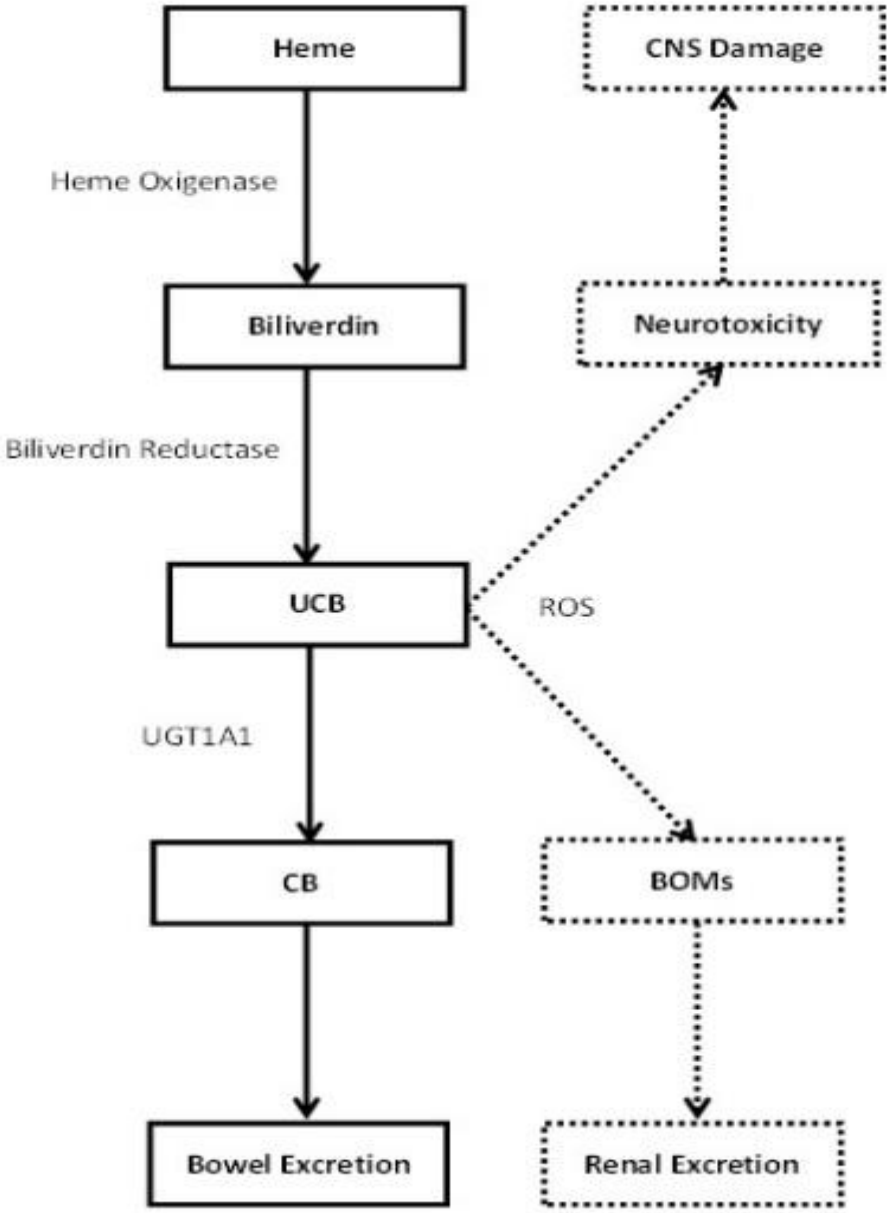
There currently are two main models used to explain the possible causes of schizophrenia. The neurodevelopmental model posits that SZ is the consequence of an anomaly in the developmental stages of the brain which leads to problems in acquiring cognitive skills (Bora et al 2014), a process that starts long before the onset of clinical symptoms (Rapoport et al 2012). The neurodegenerative model hypothesizes that the dysfunctional development is progressive and persists after the onset of the illness, resulting in structural changes in the brain; it involves a cytopathological process with loss of neurons and synapses and subsequent gliosis (Rund 2018). Additionally, some studies support the theory that the psychotic episode is, in itself, neurotoxic and contributes to the structural changes seen after the onset of the symptoms (Rund 2014). It appears plausible that the pathophysiology of SZ could be explained by a combination of these models; it could be seen as an early neurodevelopmental aberration exacerbated by a limited ongoing neurodegenerative process and/or by recurring neurotoxic psychotic episodes (Buoli et al 2017). UCB could potentially play different roles in these hypotheses. As previously stated, neurons previously exposed to elevated concentrations of UCB become more susceptible to further inflammatory damage and death by apoptosis, which could point to an early priming leading to faults later in life. A subsequent decrease in the cortical cytoarchitecture of mice previously exposed to high levels of UCB was also reported (Falcão et al 2007). Since neuritic breakdown is believed to underlie the clinical and cognitive symptoms of schizophrenia (Gilmore et al 2004) and a decrease in cortical areas of the brains of SZ patients has been reported in the past (Haukvik et al 2013), this could signify that the progressive impairment of neuronal development by UCB might explain the proposed link between schizophrenia and hyperbilirubinemia. As shown above, UCB can also directly cause an inflammation reaction, and could be one of the main culprits of the neurodegeneration model. The lower UCB levels, reported by some studies, could indicate a chronic proinflammatory state caused by increased ROS and depletion of the antioxidant potential, thus leading to the progressive loss of neurons. However, some studies have shown a decrease of UCB levels in patients with previously high plasma UCB with the initiation of antipsychotic

treatment (Miyaoka et al 2000, Semnani et al 2010). The fluctuation of UCB concentrations – be it an increase or a decrease – could explain some of the mechanisms involved in the neurotoxicity of psychotic episodes. The study published by Radhakrishnan et al. (Radhakrishnan et al 2010) has the particularity of making a distinction between different types of SZ patients, comparing the UCB levels of paranoid schizophrenia patients with those of non−paranoid schizophrenia patients; however, it didn't find statistically relevant differences between the two groups. The studies focused on the fluctuations of biopyrrin concentration are also potentially elucidating. As we have stated, biopyrrins are the product of the oxidation of UCB by ROS. We have also discussed how an elevation in UCB levels can independently cause the production of ROS. This could mean the existence of a buffer system of sorts between UCB and ROS: a surplus of UCB causes inflammation and ROS production, which in turn oxidizes UCB, decreasing its plasma concentration (Figure 2). Biopyrrins were found to be correlated with rating scores (Miyaoka et al 2005, Miyaoka et al 2015), response to treatment (Yasukawa et al 2007) and duration of disorder (Miyaoka et al 2015). The variation in the oxidative metabolism of bilirubin logically indicates the differences in the antioxidant activity of the molecule. However, this could either mean an increased recruitment of healthy antioxidant defenses or the result of compensation by the UCB ROS buffer system previously discussed. In order to clarify this point, future studies should consider comparing mean plasma UCB levels with mean urinary excretion of biopyrrins in acute and chronic episodes.

Also noteworthy are the comparisons between the bilirubin levels of schizophrenic patients and those of patients with bipolar disorder and schizoaffective disorder. Several studies showed higher UCB levels in schizophrenic patients when compared to bipolar ones (Semnani et al 2010, Radhakrishnan et al 2010, Gama Marques et al 2017, Pradeep et al 2019), and higher UCB levels among BD patients when compared to controls (Semnani et al 2010). The studies by Gama Marques et al. also showed higher UCB levels in patients with schizoaffective disorder when compared to BD (Gama Marques et al 2017, Gama Marques et al 2019). This could just be the translation of greater subjective stress among SZ patients. It could, nonetheless, be the evidence of a similar pathophysiological background between the diseases.

The DSM 5 criteria already consider the schizoaffective disorder as part of the schizophrenia spectrum. Additionally, some studies have reported a significant overlap in phenomenological, biological and genetic characteristics between SZ and BD (Potash 2006, Potash et al 2009) suggesting that they could also represent different points in the same continuous pathological spectrum (Keshavan et al 2012).

Figure 3 − UCB physiologic (left) and pathologic (right) metabolic pathways.



As we have seen, the evidence supporting the existence of a correlation between UCB and SZ is considerable. The vast majority of studies included in this review have managed to report a statistically relevant link between the two variables, thus making it possible for us to affirm that it seems very likely that UCB is in some way implicated in the pathology of SZ. However, there existed a discrepancy in the nature of this relation, with some studies claiming that SZ was correlated with higher levels of UCB and some claiming that SZ was linked with lower levels of the molecule. We didn't see clear reasons why one hypothesis might be more probable than the other, be it biases, statistical errors, bad sample selection or others. Considering the complexity of SZ, we have conjectured that this association might be multi−factorial and non−linear, with UCB and the pathophysiology of SZ being mutually influenced by each other. In this more holistic hypothesis, SZ is viewed simultaneously as the cause and the effect of fluctuations in UCB levels and vice−versa, creating a vicious cycle that would perpetuate the symptoms of SZ. However, as of yet no studies have pointed unequivocally towards this answer. Maybe, as the research on the mechanisms and causes underlying SZ advances, we will better understand the role of UCB in this disease. In spite of the present lack of clarity, we believe there is a great potential in the research of UCB as a biological marker for management of SZ.

UCB is likely implicated in many different stages of the progression of the illness. It could be responsible for making one's nervous system more susceptible to neurodevelopmental faults, consequently qualifying its fluctuations as a promising risk factor. It seems to be linked with the acute phases of the disease, thus making it eligible as a possible tool for early diagnosis and subsequent follow−up. Finally, it appears to be intimately related to the subjective stress and the intensity of symptoms, therefore being a propitious guide for treatment selection or even a possible target for neuroprotective, antioxidant and anti−inflammatory adjunctive therapies for bilirubin−induced neurological dysfunction (Kapitulnik et al 2012) in schizophrenia and spectrum related disorders. We truly believe there will be, in a near future, interesting opportunities for research in this fascinating topic (Gama Marques et al 2018).

PERSONAL CONTRIBUTE FOR THE RESEARCH

After dealing, at the emergency room, with a particular psychotic patient, with high levels of UCB, we started thinking that might be interesting looking back to psychotic patient's laboratory records at our hospital. This work would result in various poster and oral communications presentations, in different scientific conferences, all around the world: Athens 2014, Lisbon 2015, Toronto 2015, Tokyo 2016, and Oxford 2016.

After publishing the aforementioned case report in a journal dedicated to clinical neurosciences (Gama Marques 2017), we would manage to publish the preliminary results of our retrospective studies, in a psychiatric journal (Gama Marques et al 2017).

Our next step would be the creation of a longitudinal study research protocol for the study UCB as a possible biomarker candidate in the schizophrenia psychosis spectrum. We were particulary interested, not only in the classic distinction between schizophrenia and bipolar disorder patients, but also in a possible difference among patients with schizophrenia and schizoaffective disorder. That effort would become our first perspective paper (Gama Marques and Arantes−Gonçalves 2018).

Besides our clinical work we start, in an academic setting, supervising some master students. One of those would result in a review dedicated to the potential value of UCB for the diagnosis in the spectrum of schizophrenia disorders (Pommerening Dornelles et al 2019).

In the meanwhile, we published the full report on our retrospective data, through a journal dedicated to international clinical psychiatry. That would be another step in our hypothesis direction: UCB might be a promising candidate biomarker, somehow useful for the clinical distinction of patients with schizophrenia from those with schizoaffective disorder (Gama Marques et al 2019).

We felt that our findings deserved a conceptual framework, in order to clarify clinical and basic neurosciences important questions, such as finding biomarkers for schizophrenia spectrum related disorders. That was our goal with our latest paper, an

epistemological perspective on the schizophrenia – schizoaffective – bipolar spectrum. (Gama Marques and Ouakinin 2019).

Although our research group contribution did replicate data from other human studies from different parts of the world we were particularly interested in adding new perspectives looking for the relation between UCB at a basic level and the clinical expression of the disorders in a translational approach.

With that objective in mind we worked in our first longitudinal protocol. That included the assessment of UCB mean levels at two different occasions: during the psychiatric admission due to psychotic relapse, and at the outpatient follow−up appointment, twelve months after. For this particular study, besides classic psychopathological evaluation, we also included other important clinical variables such as psychossocial function and neuropsychological tests. We believe that our work opened already new avenues for future research:

1. Gama Marques J, Ouakinin S. Schizophrenia − Schizoaffective − Bipolar disorder spectra: an epistemological perspective. CNS Spectr. 2019 Oct 28:1−5. doi: 10.1017/S1092852919001408. Impact Factor 3.940, Quartile Q1.
2. Gama Marques J, Arantes−Gonçalves F. A perspective on a possible relation between the psychopathology of the Schizophrenia/Schizoaffective spectrum and Unconjugated Bilirubin: a longitudinal protocol study. Front Psychiatry. 2018 Apr 23; 9:146. doi: 10.3389/fpsyt.2018.00146. Impact Factor 3.161, Quartile Q1.
3. Gama Marques J, Pedro I, Ouakinin S. Unconjugated bilirubin and acute psychosis: a five years retrospective observational and controlled study in patients with schizophrenia, schizoaffective and bipolar disorders. Int J Psychiatry Clin Pract. 2019 Nov; 23(4):281−285. doi: 10.1080/13651501.2019.1638940. Impact Factor 1.821, Quartile Q2.
4. Gama Marques J, Ouakinin S. Clinical profile in schizophrenia spectrum: relation with unconjugated bilirubin and other psychophysiological features − a longitudinal exploratory study. CNS Spectrums. CNS Spectr. 2019 Dec 19:1−8. doi: 10.1017/S1092852919001639. Impact Factor 3.940, Quartile Q1.

OBJECTIVES

Unconjugated bilirubin and acute psychosis: a five years retrospective observational and controlled study in patients with schizophrenia, schizoaffective and bipolar disorders.

Our objective is to assess UCB levels as a possible biomarker for acute psychotic patients in the distinction among those with schizophrenia, schizoaffective and bipolar disorder.

Our hypothesis is that UCB levels will be higher in patients with schizophrenia (ICD10, WHO, F20.X code) than in patients with bipolar disorder (ICD10, WHO, F31.X code) and that patients with schizoaffective disorder (ICD10, WHO, F25.X code) will have intermediate values between the other two groups.

Clinical profile in schizophrenia and schizoaffective spectrum: relation with unconjugated bilirubin in a prospective and controlled study with psychopathological and psychosocial variables.

In this study our main objective is to assess patients suffering from acute psychosis, trying to understand if UCB mean levels may have any potential interest as a biomarker in both categorical nosological axis, e.g. schizophrenia (ICD 10, WHO, F20.X code) versus schizoaffective disorder (ICD 10, WHO, F25.X code) and a severity axis, e.g. relapse versus partial remission.

Our first hypothesis (primary objective) is to test if UCB levels will be higher in patients with schizophrenia than in patients with schizoaffective disorder.

Our second hypothesis (secondary objective) is to test if UCB levels will be higher during relapse (at ward admission) versus partial remission (at outpatient appointment), after follow−up, independently of the diagnosis of schizophrenia or schizoaffective disorder.

METHODS

Unconjugated bilirubin and acute psychosis: a five years retrospective observational and controlled study in patients with schizophrenia, schizoaffective and bipolar disorders.

For this study we used data collected during 5 years (from the beginning of 2011 until the end of 2015). At our psychiatric hospital all clinical files are easily accessible through a software interface exclusively used by physicians and blood sample analyses are usually taken on entrance to everyone admitted in our acute patient's wards. All blood samples were taken in fasting individuals, with Monovette® vacuum system and Serum Gel Z/4.9mL Sarstedt® tubes. Serum was obtained by centrifugation and maintained in ambient temperature, and readily processed with ABX Pentra 400® Horiba Medical equipment. The total and conjugated bilirubin measurement was based on the diazotized 2,4−dichloroaniline method. Laboratory internal quality was assured by ABX Pentra Multical® and ABX Pentra N Controlo® calibrators. External quality of laboratory results quality was evaluated by Ricardo Jorge's National Institute of Public Health. First we excluded all individuals with abnormal high values for TB, Aspartate transaminase (AST), Alanine transaminase (ALT), Gamma−glutamyltransferase (GGT), Lactate Dehydrogenase (LDH) or serologic signs of viral hepatitis type B (VHB) and\or viral hepatitis type C (VHC). We also excluded all patients with positive alcohol/drugs on blood/urine test. Secondly we checked all individuals' clinical files, searching for psychiatric admissions and ICD10 psychiatric diagnosis obtained through routine clinical observation. We excluded all chronic patients admitted in long term rehabilitation wards. We also excluded patients with ICD10 diagnosis different than schizophrenia (not ICD 10, WHO, F20.X code), schizoaffective (not ICD 10, WHO, F25.X code) or bipolar disorder (not ICD 10, WHO, F31.X code). Individuals without known psychiatric diagnosis were our control group composed of healthy persons (most of them workers at our hospital) who did routine lab tests through occupational medicine services. Thirdly we calculated the UCB value as the subtraction difference between TB and CB of remaining patients. Simultaneously we calculated the average duration of admission, as a ratio of total number of days during admissions dividing by

the total number of admissions. Our final sample was composed by 4 different groups: SZ patients (ICD10 WHO F20.X code), schizoaffective patients (ICD10 WHO F25.X code), BD patients (ICD 10 WHO F31.X code) and general population healthy controls. Statistical analyses, Kolmogorov−Smirnov, post−hoc Bonferroni multiple comparison, one way and univariate corrected ANOVA were done using Statistical Package for the Social Sciences (SPSS) version 25.

Clinical profile in schizophrenia and schizoaffective spectrum: relation with unconjugated bilirubin in a prospective and controlled study with psychopathological and psychosocial variables.

Our study was an observational longitudinal study, with two assessments in one year time−span (from thre beginning of 2016 until the beginning of 2017). The first assessment occurred during relapse of psychosis at the psychiatric ward admission and the second assessment during partial remission of psychosis at the outpatient appointment.

Our inclusion criteria looked for all patients with acute psychosis under the diagnosis of SZ (WHO ICD10, F20.X code) or schizoaffective disorder (WHO ICD10, F25.X code), with age between 18 and 65 years old, admitted to our psychiatric ward.

Exclusion criteria were hepatic, hemolytic or cholestasis related condition detected through blood sample analyses, as well as any important changes in AST, ALT, GGT and LDH values or positive tests for HVB or HVC infection. Substance related psychosis like cannabinoids, amphetamines, cocaine and heroin and psychoactive substances use was detected through urine sample analyses, for both assessments, yielding respective exclusion from study. Organic central nervous system disorder related psychosis was excluded in routine single computed tomography (CT) brain scan and/or routine repeated electroencephalography (EEG). As controls, we used admitted patients with acute bipolar disorder (WHO ICD10, F31.X code) paired for age and sex. All patients included were able to understand and sign informed consent. Scientific and Ethical approval was obtained at local boards, and investigation was developed according to the Declaration of Helsinki.

Sociodemographic and clinical variables collected for our patients were age, gender, occupation, education years, smoking pack−year, use of contraceptive pill, body mass index, number of affected kin or Family Psychiatric History (FPH), years of duration of psychiatric disorder, years of Duration of Untreated Psychosis (DUP), days of Mean Length of Stay (MLS) at psychiatric ward and psychiatric medication, e.g. Chlorpromazine Equivalents (CPZE).

Biochemistry study included calculated UCB serum levels (UCB=TB – CB). Fasting blood samples were collected after a restful night and before breakfast, for both assessments, using vacuum S−Monovette® Serum Gel Z/4.9 ml (Sarstedt AG&Co) and our analytic method was 2,4−dicloroaniline photometry. Our laboratory used hardware system ABX Pentra 400® (Horiba Group) and software system was SISLAB® (Glintt).

Psychopathological instruments used were Clinical Global Impression (CGI) for general clinical severity and PANSS (Kay et al 1987) for psychosis severity. The CGI measures symptom severity and it is widely used to measure treatment response and the efficacy of treatments in studies of patients with mental disorders. It is a 7−point scale that requires the clinician to rate the severity of the patient's illness at the time of assessment, relative to the clinician's past experience with patients who have the same diagnosis, with possible ratings from 1 “Normal, not at all ill” up to 7 “Among the most extremely ill patients”. The PANSS is a medical scale used for measuring symptom severity of patients with schizophrenia. It is widely used in the study of antipsychotic therapy and it is usually accepted as the golden standard that all assessments of psychotic behavioral disorders should follow. The patient is rated from 1 “absent” to 7 “extreme” on 30 different symptoms based on the interview as well as reports of caretakers; therefore its total score ranges from 30 to 210.

Psychosocial instruments used were Global Assessment of Functioning (GAF) (Endicott et al 1976) and Personal and Social Performance (PSP) (Morosini et al 2000) for social functioning assessment. GAF is a numeric scale used by mental health technicians to rate subjectively the social and occupational functioning of an individual, from 1 “severely impaired” to 100 “extremely high functioning”. The PSP is a 100 points rating scale, with ratings being based mainly on the assessment of patient's functioning in four main areas: (a) “socially useful activities”; (b) “personal and social relationships”; (c) “self−care”; and (d) “disturbing and aggressive behaviors”, on a six−point scale from “absent” to “very severe”.

All scales used in our protocol were translated and validated for Portuguese language (Lima et al 2007, Higuchi et al 2014, Brissos et al 2012).

For the schizophrenia and schizoaffective patients groups, all quantitative variables were summarized through descriptive statistics. This analysis included

socio−demographics characterization as well as the previously described biological, psychopathological and psychosocial variables.

Statistical analyses with Kolmogorov−Smirnov, post−hoc Bonferroni multiple comparison, Student's T−test, one way and univariate corrected ANOVA and linear regression models were done using Statistical Package for the Social Sciences (SPSS) version 25.

Our complete protocol was discussed with international reviewers and is freely available online (Gama Marques and Arantes−Gonçalves 2018). Patient data collection is summarized in Figure 4.

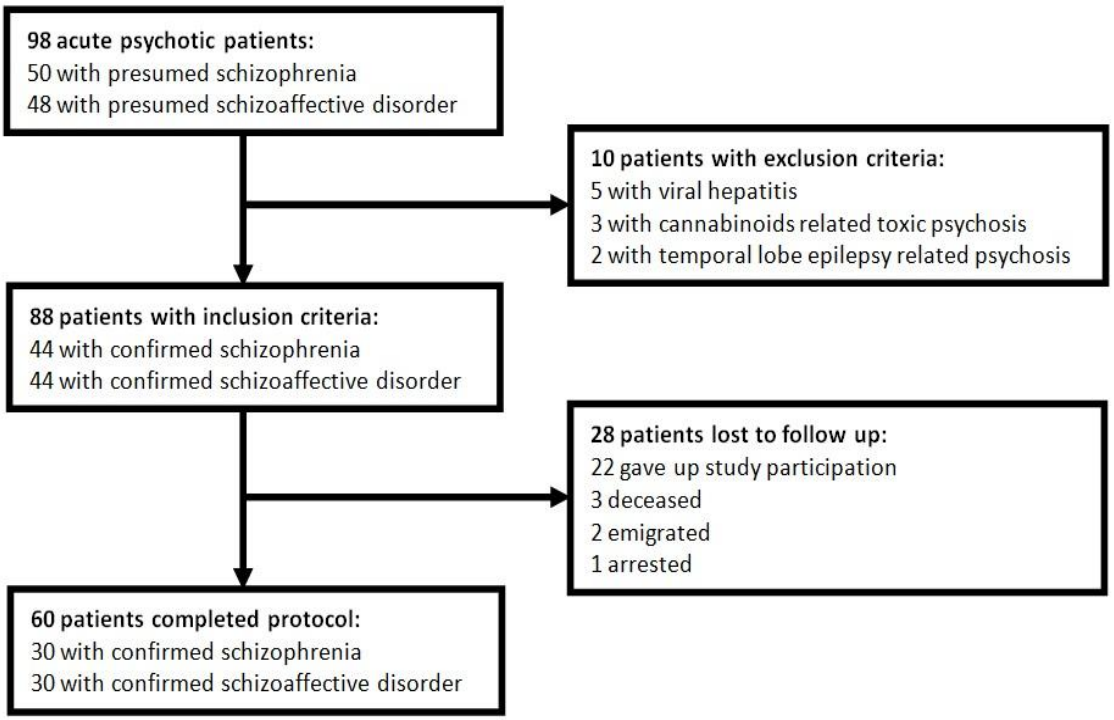


Figure 4 – UCB mean levels prospective study: patient data collection.

RESULTS

Unconjugated bilirubin and acute psychosis: a five years retrospective observational and controlled study in patients with schizophrenia, schizoaffective and bipolar disorders.

We were able to include 255 individuals, including 56 healthy controls and 199 acute patients (namely 44 with SZ, 99 with schizoaffective disorder and 56 with BD).

Regarding gender characteristics of our sample 42% were males. The mean age was 46.6 years old (±11.8 years old), ranging from 21 to 80 years old. The mean number for average duration of admission in the psychiatric ward for acute patients was 17.1 days (±18.2 days), ranging from 1 to 116 days. The individuals in the control group were excluded from this analysis.

Schizophrenia patients presented lower mean age (41 years old), higher prevalence for male gender (62%), higher UCB mean values (0.41mg/dL, ±0.15 mg/dL) and higher average admission duration (29 days). Schizoaffective patients presented intermediate values for mean age (48 years old), male gender (52%), UCB mean values (0.34mg/dL, ±0.12 mg/dL) and average admission duration (22 days). Bipolar patients presented the higher mean age (56 years old), lower prevalence for male gender (25%) as well as lower values for UCB mean values (0.28 mg/dL, ±0.12 mg/dL) and average admission duration (16 days). Figure 5 presents the difference between UCB mean levels among groups.

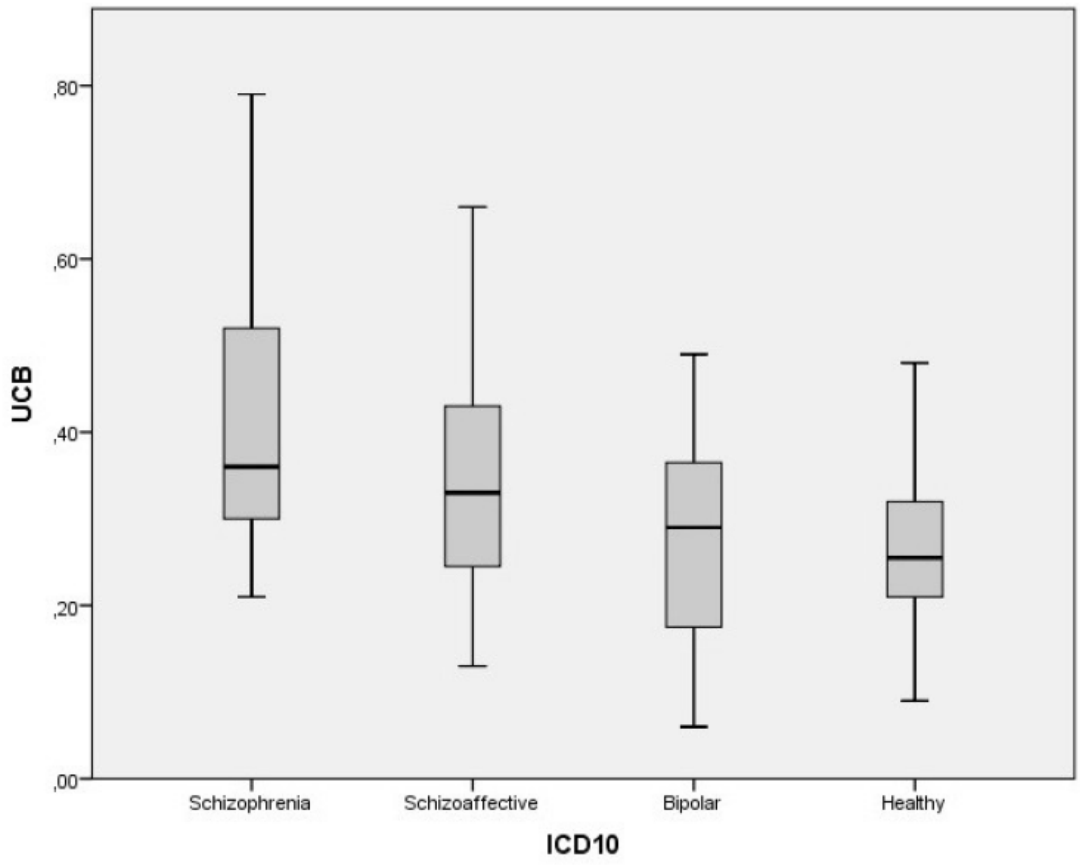


Figure 5 – UCB mean levels in groups of patients and healthy controls.

Regarding UCB mean values, age and diagnosis: first we applied one sample Kolmogorov−Smirnov test to confirm normal distribution for age and UCB in the four groups (schizophrenia, schizoaffective, bipolar and controls). Then we applied one way ANOVA test to look for a statistically significant difference ($p \leq 0.0001$) between the mean values of UCB across the four groups. On the post−hoc Bonferroni multiple comparison test there was statistically significant difference in schizophrenia versus bipolar ($p<0.001$) and schizoaffective ($p<0.03$) as well in schizoaffective versus bipolar ($p<0.04$). There was no differences found between bipolar and control. Regarding a plausible bias on these results we applied a univariate corrected ANOVA model with age ($p=0.6$) and gender ($p=0.2$) as covariates, and found no influence. On Table 3 we represent our study's main findings.

Table 3 – UCB mean levels (mg/dL) and average admissions duration.

ICD10 Diagnosis	N	Man (%)	Woman (%)	Mean Age (Years)	Mean UCB (mg/dL)	Average Admissions Duration (days)
Schizophrenia	44	62	38	41	0.41[a,b]	29[a]
Schizoaffective	99	52	48	46	0.34[b,c]	22
Bipolar	56	25	75	56	0.28[a,c]	16[a]
Control	56	23	77	56	0.28	–

[a]p<0.001 [b]p<0.03 [c]p<0.04

Regarding average admissions duration for the five year period and diagnosis: when applying a linear regression ANOVA model, with average admissions duration as a dependent variable we found statistically significant difference between schizophrenia versus bipolar ($p<0.001$) but there no statistically significant difference when comparing the average admissions duration between the other groups (e.g. schizoaffective versus bipolar, and schizoaffective versus schizophrenia).

Clinical profile in schizophrenia and schizoaffective spectrum: relation with unconjugated bilirubin in a prospective and controlled study with psychopathological and psychosocial variables.

First assessment (relapse at ward admission)

We had an initial number of 98 patients in our study: 48 with presumptive diagnosis of schizophrenia and 50 with presumptive diagnosis of schizoaffective disorder. We lost 10 patients because of exclusion criteria: 5 had at least one blood sample positive for HVB and/or HVC infection; 3 had at least one urine sample positive for cannabinoids; and 2 had at least one EEG exam suggesting temporal lobe epilepsy. These excluded patients received new appropriate diagnosis and treatment and were referred to specific outpatient consultations after discharge from admission, namely gastroenterology, addiction and neurology services, respectively. The remaining 88 patients had no changes at all on their first blood and urine samples, EEG exam and CT brain scan, so they were included in our study for follow up.

At the end of the first assessment (relapse; at ward admission) we included 88 patients (44 with confirmed diagnosed of schizoaffective disorder and 44 with confirmed diagnosis of schizophrenia). Although there was a higher prevalence of male patients in the group of patients with schizophrenia versus the group of patients with schizoaffective disorder, there was no statistically significant difference between groups. Other variables like age, education, family psychiatric history, smoking pack years and duration of untreated psychosis also didn't show statistically significant differences between the two groups. No female patient was taking oral anti−conception pill during our study. General demographic data for the 88 patients that completed our first assessment is resumed in Table 4.

Table 4: General Demographic Data (first assessment).

	N	Male	Age (years)	Education (years)	Single (%)	Retired (%)	FPH (kin)	SPY (years)	DUP (years)
Schizophrenia	44	61%	43.1±9.6	10.7±4.9	84	36	1±1	12.7±12.6	3.3±5.1
Schizoaffective	44	39%	44.4±9.7	11.2±3.9	68	52	2±1	13.0±13.4	4.1±7.3

FPH=Family Psychiatric History; SPY=Smoking Pack Years; DUP=Duration of Untreated Psychosis.

Regarding all clinical variables at first assessment (relapse; at ward admission) we did not find any statistically significant differences between patients with schizophrenia (N=44) and schizoaffective disorder (N=44). Patients with schizophrenia showed at admission higher mean PANSS values and also higher mean CPZE values, versus schizoaffective patients, but the difference had no statistical meaning. General clinical data for the 88 patients that completed our first assessment is resumed in Table 5.

Table 5: Clinical Data (first assessment).

	N	CGI	PANSS	PSP	GAF	CPZE
Schizophrenia	44	4.9±1.1	91.0±7.5	25.7±5.4	26.9±5.5	823±566
Schizoaffective	44	5.0±0.9	87.1±11.7	30.6±9.1	31.0±8.1	655±455

CGI=Clinical Global Impression; PANSS=Positive And Negative Syndrome Scale; PSP=Personal and Social Performance; GAF=Global Assessment of Functioning; CPZE=Chlorpromazine Equivalent.

Regarding these 88 patients who completed the first assessment we compared (ANOVA) the mean UCB levels between our two groups (44 patients with schizophrenia and 44 patients with schizoaffective disorder) versus our control group (44 patients with bipolar disorder) and found a statistically significant difference (p=0.002). After performing Bonferroni post−hoc multiple comparisons we found statistically significant differences between the 44 patients from the group with schizophrenia and the other two groups, namely the 44 patients from the group with schizoaffective disorder (p=0.05) and the 44 patients from the control group with bipolar disorder (p=0.05). We found no statistically significant difference between the three groups for other variables such as sex, age or mean length of stay at the psychiatric ward (Table 6).

Table 6: Mean UCB mean levels comparisons ANOVA (first assessment).

	N	Male	Age (Years)	MLS (Days)	UCB (mg/dL)
Schizophrenia	44	61%	43.1±9.6	22.4±21.1	0.38±0.18[ab]
Schizoaffective	44	38%	44.4±9.7	20.5±14.8	0.30±0.13[a]
Bipolar	44	31%	47.4±11.0	17.2±14.0	0.27±0.13[b]

MLS=Mean Length of Stay; UCB=Unconjugated Bilirubin; [a]p=0.05; [b]p=0.05

Regarding correlations between psychopathological, psychosocial scales and laboratory data on patients who completed the first assessment (N=88), we only found a positive correlation (Pearson's r=0.314; p=0.01) between UCB mean levels and PSP item (d) "disturbing and aggressive behaviors". When looking for linear regression models for

UCB and all other variables, separately among the two groups of patients (schizophrenia and schizoaffective disorder) we were able to find a positive correlation (R^2=0.223), with statistically significance (p=0.008), between the UCB mean level at first assessment (admission) in patients with schizoaffective disorder who completed full protocol (N=30) and mean length of stay (Figure 6).

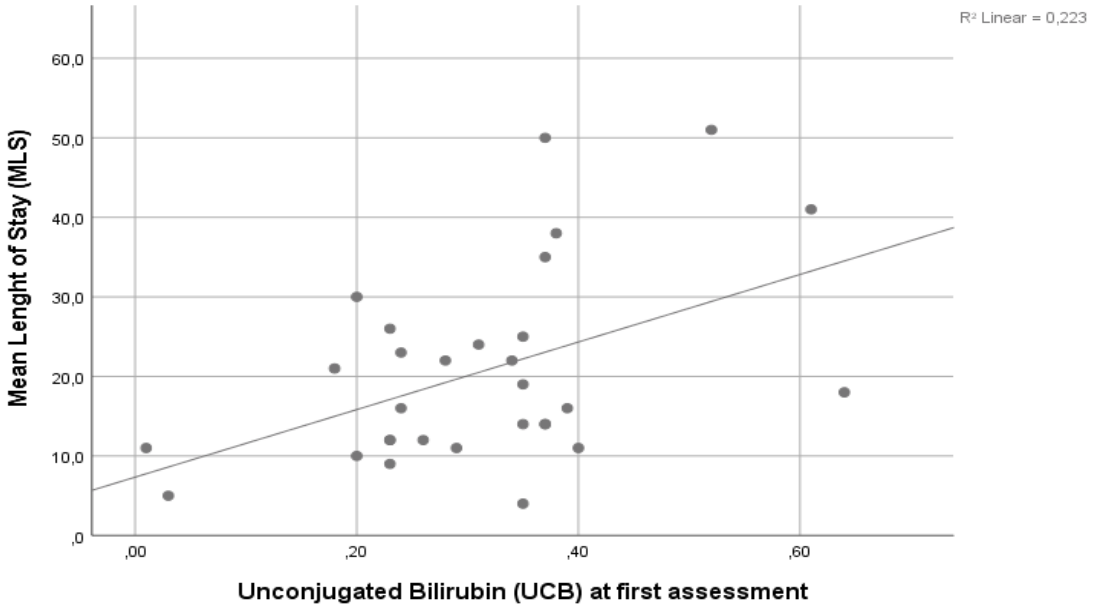


Figure 6 – Linear Regression: UCB at first assessment and MLS in schizoaffective disorder (p=0.008).

Second assessment (partial remission at outpatient appointment).

From the 88 patients initially included in our study (44 with diagnosis of schizophrenia and 44 with diagnosis of schizoaffective disorder) we lost 28 patients: 22 quit the study before doing the second assessment (outpatient appointment); 3 died (stroke, acute renal failure and suicide by hanging); 2 traveled to live in another country and 1 was arrested for criminal behavior. The remaining 60 patients had no changes at all on at least two blood and two urine samples, two EEG exams and one CT brain scan so they were included on the final phase of data analysis.

Although there was a higher prevalence of male patients in the group of patients with schizophrenia versus the group of patients with schizoaffective disorder, there was no statistically significant difference. Other variables like age, education, Family Psychiatric History (FPH), Smoking Pack Years (SPY) and Duration of Untreated Psychosis (DUP) didn't show statistically significant difference between the two groups. The Median Follow-up (MFU) time for all sample (N=60) was 398±164 days; namely 392±159 days for patients with schizophrenia (N=30) and 405±172 days for patients with schizoaffective disorder (N=30), without statistically significant difference. Although we found a male gender prevalence among patients with schizophrenia there was no statistical significant difference when compared with patients with schizoaffective disorder. General demographic data for the patients that completed full protocol (both first and second assessment) versus patients who were lost to follow−up, is resumed in Table 7.

Table 7: General Data (second assessment).

	N	Male	Age (years)	Education (years)	Single (%)	Retired (%)	FPH (kin)	SPY (years)	DUP (years)	MFU (days)
Schizophrenia	30	63%	43.8±9.5	10.0±5.2	87	37	1±1	14.9±13.1	2.9±4.9	392±159
Schizoaffective	30	30%	45.5±9.0	11.3±3.8	70	57	2±1	12.9±13.1	3.8±6.2	405±172
Lost Patients	28	63%	41.8±9.7	11.5±3.9	54	50	2±1	12.8±14.8	2.7±4.8	−

FPH=Family Psychiatric History; SPY=Smoking Pack Years; DUP=Duration of Untreated Psychosis; MFU=Median Follow−up

We found clinical improvement between the first assessment (relapse at ward admission) and second assessment (partial remission at outpatient appointment) for psychopathological (CGI and PANSS) and psychosocial scales (GAF and PSP). We found a statistically significant difference ($p<0.0001$) between the first assessment

(relapse at ward admission) and second assessment (relapse at ward admission) on both groups (schizophrenia, N=30); and schizoaffective disorder, N=30) for psychopathological (CGI and PANSS) and psychosocial scales (GAF and PSP). Both groups of patients (schizophrenia and schizoaffective disorder) showed a statistically significant clinical (CGI and PANSS) and functional (PSP and GAF) improvement from first assessment during admission until the second admission at outpatient appointment. Nevertheless there were no statistically significant differences among patients with different diagnosis (schizophrenia vs. schizoaffective disorder). General clinical data for the patients that completed full protocol, both first and second assessment, is resumed (Table 8).

Table 8: General Clinical Data, Paired Samples T−test (first and second assessments)

	N	CGI1	CGI2	PANSS1	PANSS2	PSP1	PSP2	GAF1	GAF2
Schizophrenia	30	4.9±1.1[a]	3.4±1.0[a]	91.0±7.5[b]	80.6±8.6[b]	25.7±5.4[c]	53.8±13.9[c]	26.9±5.5[d]	49.6±12.9[d]
Schizoaffective	30	5.0±0.9[e]	3.5±1.1[e]	87.1±11.7[f]	79.6±8.3[f]	30.6±9.1[g]	51.2±19.6[g]	31.0±8.1[h]	49.8±18.6[h]

CGI=Clinical Global Impression; PANSS=Positive And Negative Syndrome Scale; PSP=Personal and Social Performance; GAF=Global Assessment of Functioning; [abcdefgh] p<0.0001

Regarding these 60 patients who finished our full protocol we compared (ANOVA) the mean UCB levels at the first assessment between the two groups (schizophrenia, N=30; and schizoaffective disorder, N=30) and the control group (bipolar disorder, N=30) and found a statistically significant difference (p=0.006). After performing a Bonferroni post−hoc multiple comparison, we found statistically significant difference between the first assessment (relapse at ward admission) of patients with schizophrenia (N=30) and the unique assessment (relapse at ward admission) of patients with bipolar disorder (N=30) (p=0.05).

Regarding these 60 patients who finished our full evaluation protocol we compared the mean UCB levels at the second assessment (outpatient appointment) between the two groups (schizophrenia, N=30; and schizoaffective disorder, N=30; ANOVA) and found a statistically significant difference (p=0.006). After performing a Bonferroni post−hoc multiple comparison we found statistically significant difference between the second assessment (partial remission at outpatient appointment) of the 30 patients with schizophrenia and the second assessment (partial remission at outpatient appointment) of the 30 patients with schizoaffective disorder (p=0.05). Performing a paired samples T−test for these 60 patients from the two groups who completed the full protocol, we

only found a statistically significant difference between the UCB mean levels at the first assessment (relapse at ward admission) and the UCB means levels at the second assessment (partial remission at outpatient appointment) in the group of 30 patients with schizoaffective disorder (p=0.034).

Table 9: Mean UCB mean levels comparisons ANOVA and Paired Samples T−test (first and second assessment).

	N	Male	Age (years)	MLS (days)	Stay (days)	UCB1 (mg/dL)	UCB2 (mg/dL)
Schizophrenia	30	63%	43.8±9.5	23.3±24.5	37.0±27.9	0.38±0.19[a]	0.34±0.16[b]
Schizoaffective	30	30%	45.5±9.0	20.5±12.2	35.1±23.7	0.31±0.14[c]	0.25±0.10[bc]
Bipolar Control	30	46%	49.3±11.2	17.2±15.2	–	0.25±0.14[a]	–

MLS=Mean Length of Stay; UCB=Unconjugated Bilirubin; [a]p=0.05; [b]p=0.05; [c]p=0.034

Looking at correlations between psychopathological, psychosocial scales and laboratory data on patients who completed the second assessment (N=60), we only found a negative correlation between UCB mean levels and PANSS item G7 "psychomotor retardation" (Pearson's r=−0.399; p=0.002).

Laboratory data is resumed for bilirubins (Table 10), liver function (Table 11) and metabolic syndrome (Table 12). There were no statistically significant differences for all these variables, except for the already mentioned UCB mean levels between first (relapse at ward admission) and second assessment (partial remission at outpatient appointment), among patients with schizoaffective disorder.

Table 10: Bilirubin Laboratory Data, Paired Samples T−test. (first and second assessment).

	N	UCB1 (mg/dL)	UCB2 (mg/dL)	CB1 (mg/dL)	CB2 (mg/dL)	TB1 (mg/dL)	TB2 (mg/dL)
Schizophrenia	30	0.38±0.19	0.34±0.16	0.16±0.06	0.18±0.06	0.54±0.23	0.52±0.21
Schizoaffective	30	0.31±0.14[a]	0.25±0.10[a]	0.14±0.06	0.17±0.05	0.44±0.17	0.42±0.12

UCB=Unconjugated Bilirubin; CB=Conjugated Bilirubin; TB=Total Bilirubin; [a] p=0.034

Table 11: Liver Function Laboratory Data, Paired Samples T−test. (first and second assessment).

	N	ALT1 (mg/dL)	ALT2 (mg/dL)	AST1 (mg/dL)	AST2 (mg/dL)	GGT1 (mg/dL)	GGT2 (mg/dL)
Schizophrenia	30	21.2±9.0	26.2±20.2	23.1±10.5	21.8±10.8	22.0±7.7	24.93±16.64
Schizoaffective	30	24.1±14.7	26.8±16.4	24.1±13.5	22.3±10.7	47.6±124.9	50.73±91.42

ALT=Alanine Transaminase; AST=Aspartate Transaminase; GGT=Gamma−Glutamyl Transferase.

Table 12: Metabolic Laboratory Data, Paired Samples T−test. (first and second assessment).

	N	BMI (Kg/m^2)	Col1 (mg/dL)	Col2 (mg/dL)	TAG1 (mg/dL)	TAG2 (mg/dL)	BGL1 (mg/dL)	BGL2 (mg/dL)
Schizophrenia	30	24.7±5.8	194.0±33.6	202.9±41.9	114.5±48.2	142.9±91.2	96.3±13.5	100.4±19.8
Schizoaffective	30	26.3±5.6	196.8±34.3	203.3±35.2	127.5±65.7	191.3±196.2	99.5±20.1	105.4±33.0

BMI=Body Mass Index; Col=Cholesterol; TAG=Triacylglyceride; BGL=Blood Glucose Level.

Regarding other laboratory parameters (Table 13), we only found a statistically significant difference (p=0.033) between Creatine Kinase (CK) mean levels in patients with schizophrenia with higher levels at first assessment (relapse at ward admission) 213.0 ±297.2 mg/dL versus second assessment (partial remission at outpatient appointment) 106.5 ±74.4mg/dL.

Table 13: Other laboratory data, Paired Samples T−test (first and second assessment).

	N	CPR1	CPR2	LDH1	LDH2	CK1	CK2
Schizophrenia	30	0.53±0.60	0.54±0.82	184.3±41.7	172.3±29.2	213.0±297.2[a]	106.5±74.4[a]
Schizoaffective	30	0.38±0.49	0.44±0.60	192.8±42.3	194.3±57.1	253.3±467.1	194.1±381.6

CPR=C−Reactive Protein; LDH=Lactate Dehydrogenase; CK=Creatine Kinase. [a]p=0.033

Using paired samples Student's T−test for comparison between first and second assessment we found no statistically significant difference regarding PANSS positive symptoms, both in patients with schizophrenia and schizoaffective disorder (Table 14).

On the other hand we found some statistically significant decrease regarding PANSS negative symptoms, when comparing the first assessment versus second assessment in patients with schizophrenia namely for itens N5 "difficulty in abstract thinking" (p=0.007), N6 "lack of spontaneity and flow of conversation" (p=0.03), N7 "stereotyped thinking" (p=0.002), and NTotal (p=0.01) (Table 15).

We found some statistically significant decrease regarding PANSS negative symptoms, when comparing the first assessment versus second assessment in patients with schizoaffective disorder namely for itens N3 "poor rapport" (p=0.009), N5 "difficulty in abstract thinking" (p=0.011), and N7 "stereotyped thinking" (p=0.003) (Table 15).

We found some statistically significant decrease regarding PANSS general symptoms, when comparing the first assessment versus second assessment in patients with schizophrenia namely for item G1 "somatic concern" (p=0.01) (Table 16), G9 "unusual

thought content" (p=0.0001), G10 "disorientation" (p=0.002), G11 "poor attention" (p=0.004), G12 "lack of judgment and insight" (p=0.003) and G15 "preoccupation" (p=0.001) (Table 17).

We also found some statistically significant decrease regarding PANSS general symptoms, when comparing the first assessment versus second assessment in patients with schizoaffective disorder for item G3 "guilt feelings" (p=0.005) (Table 16), G9 "unusual thought content" (p<0.0001), G12 "lack of judgment and insight" (p=0.011), and G15 "preoccupation" (p=0.016) (Table 17).

Using paired samples Student's T−test for comparison between first and second assessment we found no statistically significant difference regarding most of the neuropsychological tests.

Nevertheless we found some statistically significant increase regarding TMTA (p=0.09), TMTB (p=0.009) (Table 18), MOCA item l "language" (p=0.043), and MOCA item o "orientation" (p=0.006) (Table 19), plus a statistically significant decrease in MOCA item n "naming" (p=0.09) (Table 19) in patients with schizophrenia.

Finally, we found some statistically significant increase regarding WMS item bds "backward digit span" (p=0.03) (Table 18) and MOCA item l "language" (p=0.026) (Table 19) in patients with schizoaffective disorder.

Table 14: PANSS' Positive Symptoms Scale Data (Paired Samples T−Test)

	N	P1_1	P1_2	P2_1	P2_2	P3_1	P3_2	P4_1	P4_2	P5_1	P5_2	P6_1	P6_2	P7_1	P7_2	Ptotal1	Ptotal2
Schizophrenia	30	5.1±1.2	3.5±1.2	3.0±0.9	2.4±0.6	3.8±1.3	2.7±0.9	2.3±0.8	2.3±0.5	3.5±1.3	2.6±0.9	4.3±1.4	3.3±1.0	2.2±0.6	2.2±0.5	24.3±3.8	19.0±3.8
Schizoaffective	30	5.0±1.3	3.1±1.4	3.0±1.0	2.6±0.8	3.5±1.5	2.5±0.8	2.5±0.9	2.7±0.8	3.6±1.3	2.7±0.9	4.3±1.4	3.0±1.0	2.2±0.9	2.4±0.6	24.1±5.2	19.0±3.9

Table 15: PANSS' Negative Symptoms Scale Data (Paired Samples T−Test)

	N	N1_1	N1_2	N2_1	N2_2	N3_1	N3_2	N4_1	N4_2	N5_1	N5_2	N6_1	N6_2	N7_1	N7_2	Ntotal1	Ntotal2
Schizophrenia	30	3.4±1.0	3.0±0.9	2.9±0.7	3.0±0.4	2.8±0.6	2.8±0.5	2.9±0.7	3.0±0.6	3.8±1.1[a]	3.3±1.1[a]	2.5±0.7[b]	2.4±0.6[b]	2.9±0.7[c]	2.4±0.5[c]	21.2±3.2[d]	19.9±2.9[d]
Schizoaffective	30	2.9±0.8	3.0±0.7	2.6±0.7	2.8±0.6	2.5±0.6[e]	2.9±0.6[e]	2.7±0.6	2.8±0.5	3.4±1.2[f]	2.8±0.9[f]	2.3±0.8	2.4±0.6	2.8±0.6[g]	2.3±0.5[g]	19.1±3.3	19.1±2.7

[a]p=0.007; [b]p=0.03; [c]p=0.002; [d]p=0.01; [e]p=0.009; [f]p=0.011 ; [g]p=0.003

Table 16: PANSS' General Symptoms Scale Data, Part 1 (Paired Samples T−Test)

	N	G1_1	G1_2	G2_1	G2_2	G3_1	G3_2	G4_1	G4_2	G5_1	G5_2	G6_1	G6_2	G7_1	G7_2	G8_1	G8_2
Schizophrenia	30	3.2±1.0[a]	2.8±0.8[a]	2.4±0.7	2.4±0.6	2.5±0.8	2.3±0.7	2.5±0.6	2.6±0.7	2.2±0.7	2.5±0.7	2.4±0.8	2.4±0.6	2.5±0.7	2.5±0.6	2.3±0.9	2.1±0.4
Schizoaffective	30	2.9±0.9	2.9±0.5	2.8±1.1	2.5±0.7	2.7±1.0[b]	2.3±0.5[b]	2.5±0.9	2.5±0.7	2.0±0.6	2.3±0.5	2.3±0.8	2.3±0.6	2.2±0.8	2.5±0.7	2.3±1.1	2.2±0.6

[a]p=0.01; [b]p=0.005

Table 17: PANSS' General Symptoms Scale Data, Part 2 (Paired Samples T−Test)

	N	G9_1	G9_2	G10_1	G10_2	G11_1	G11_2	G12_1	G12_2	G13_1	G13_2	G14_1	G14_2	G15_1	G15_2	G16_1	G16_2	Gtotal_1	Gtotal_2
Schizophrenia	30	4.4±1.1[a]	3.5±1.2[a]	3.0±1.1[b]	2.5±0.7[b]	3.1±0.8[c]	2.7±0.6[c]	4.7±1.3[d]	3.8±1.2[d]	2.1±0.4	2.1±0.3	2.2±0.5	2.1±0.3	3.2±0.7[e]	2.6±0.7[e]	2.8±0.8	2.8±0.8	45.5±4.3	41.8±4.5
Schizoaffective	30	4.1±1.4[f]	3.1±1.1[f]	2.7±1.1	2.6±0.9	2.9±1.3	2.9±0.7	4.2±1.5[g]	3.5±1.0[g]	2.0±0.3	2.2±0.6	2.5±1.0	2.5±0.7	3.1±0.8[h]	2.7±0.7[h]	2.5±0.7	2.5±0.6	43.8±6.1	41.5±4.2

[a]p=0.0001; [b]p=0.002; [c]p=0.004; [d]p=0.003; [e]p=0.001; [f]p<0.0001; [g]p=0.011; [h]p=0.016

Table 18: Neuropsychological Scales Data, Part 1 (Paired Samples T−Test)

	N	WMSfds1	WMSf±2	WMSbds1	WMSbds2	TMTA1	TMTA2	TMTB1	TMTB2
Schizophrenia	30	6.6±1.5	6.7±1.9	3.5±1.3	3.4±0.9	82.3±53.8[a]	72.3±39.7[a]	183.2±93.1[b]	153.4±77.1[b]
Schizoaffective	30	6.5±1.2	6.9±1.2	3.1±1.0[c]	3.4±1.1[c]	92.1±62.7	80.3±47.9	168.0±85.2	155.6±83.8

WMS=Wechsler Memory Scale; TMT=Trail Making Test; [a]p=0.09; [b]p=0.009; [c]p=0.03

Table 19: Neuropsychological Scales Data, Part 2 (Paired Samples T−Test)

	N	MOCAve1	MOCAve2	MOCAn1	MOCAn2	MOCAat1	MOCAl1	MOCAl2	MOCAab1	MOCAab2	MOCAdm1	MOCAdm2	MOCAo1	MOCAo2	MOCAtotal1	MOCAtotal2
Schizophrenia	30	3.3±1.0	3.3±1.0	2.0±0.8[a]	1.9±0.6[a]	4.0±1.5	2.1±0.8[b]	2.3±0.7[b]	1.4±0.7	1.5±0.7	0.7±1.1	0.4±1.1	4.5±1.3[c]	5.4±1.3[c]	18.2±5.3	18.8±4.9
Schizoaffective	30	3.3±1.3	3.3±1.1	1.8±0.7	2.1±0.7	4.1±1.9	2.0±0.8[d]	2.4±0.8[d]	1.5±0.6	1.4±0.7	0.7±1.1	0.6±1.2	5.0±1.1	5.3±1.1	18.4±5.4	19.2±5.2

MOCA=Montreal Cognitive Assessment; [a]p=0.09; [b]p=0.043; [c]p=0.006; [d]p=0.026

DISCUSSION

Unconjugated bilirubin and acute psychosis: a five years retrospective observational and controlled study in patients with schizophrenia, schizoaffective and bipolar disorders.

UCB mean levels are clearly higher in patients with acute psychotic episodes of the schizophrenia and schizoaffective spectrum, when compared with bipolar patients and healthy controls. This UCB mean results were statistically independent from age, gender and correlated with the average duration of admission. We are not aware of previous studies reporting neurotoxic effect of normal level UCB. Thus our findings suggesting a possible correlation between clinical profile and UCB levels could be of interest as they open new possibilities of neurotoxicity research, even in patients with UCB normal levels. Our study cannot determine a causal relationship between higher level of UCB and psychosis. Indeed UCB level may be cause or consequence of a psychotic state. This is another one of the highlights of our study that can be used for further and future investigations. Our results are similar to those already described before (Semnani et al 2010, Radhakrishnan et al 2011, Gama−Marques et al 2017, Pradeep et al 2019) where patients with schizophrenia had significantly higher UCB levels than bipolar patients, although all patients of our sub−groups were in normal range for (unconjugated, conjugated and total bilirubin values).

Other important aspect of our results is that acute schizoaffective patients stand right in between the patients with schizophrenia and the patients with bipolar disorder. Schizoaffective patients seem to be, not only clinically speaking, but also regarding this specific biomarker candidate, somewhere between patients with schizophrenia and patients with bipolar disorder.

This data reinforces the theory that schizoaffective disorder, might be an intermediate clinical entity, so often misunderstood but somewhere in between the schizophrenic and the bipolar spectra, both in phenotypic and physiopathologic aspects.

Some limitations of our methodology include the non−longitudinal design without randomization of sample, as well the absence of individual pairing with the healthy controls group. We are also aware about the high proportion of schizoaffective patients in our sample which may represent a result of instability in clinical diagnosis, an important bias in this kind of retrospective studies. For instance, it would have been interesting to see individual standardized assessment (e.g. CGI or PANSS) or whether bipolar patients with a manic episode showed differences from other patients. Unfortunately we didn't have the opportunity to check some social reasons for each patient admission nor exclude social factors that may have influenced the duration of each admission. Last but not least our study didn't control for medications influence on the assessed variables.

Although our study is the first one suggesting a possible positive correlation between higher yet normal UCB mean levels and acute admission duration of psychotic patients with schizophrenia, schizoaffective and bipolar disorders, we are aware that more and better clinical studies are necessary to clarify these findings.

Indeed we cannot conclude that normal level of UCB can be neurotoxic and lead to acute psychosis, our work suggests a possible relation between UCB mean levels and psychosis severity. Thus we believe that UCB mean levels in normal range, deserves further investigation in psychotic patients or animal models of psychosis. Our work also opens a possibility for UCB mean levels as a predictor on both psychosis type diagnosis (schizophrenia vs. schizoaffective vs. bipolar) and psychosis severity (duration of admission). However, more powerful studies shall be further conducted to clarify the relation between UCB levels and acute psychotic states. In our future studies we intend to study the longitudinal correlation of UCB mean levels in psychotic patients with a standard assessment protocol that shall include neuroimaging, neurophysiology, psychopathological, neuropsychological and psychosocial variables. These variables are quite important for the diagnosis and prognosis of the patients and this kind of work might become quite relevant for a better understanding of the etiology of psychotic disorders of the schizophrenia and schizoaffective spectrum (Gama Marques and Arantes−Gonçalves 2018).

Clinical profile in schizophrenia and schizoaffective spectrum: relation with unconjugated bilirubin in a prospective and controlled study with psychopathological and psychosocial variables.

Our study results suggest that both of our experimental hypotheses might be true. Firstly, we confirmed that UCB mean levels are statistically significantly higher (p=0.05) in relapsing psychotic patients with schizophrenia (N=44; 0.38±0.18 mg/dL) than in relapsing psychotic patients with schizoaffective disorder (N=44; 0.30±0.13 mg/dL). This outcome gives us some insight on the possible role of UCB as a biomarker for categorical distinction between schizophrenia and schizoaffective disorder, during an acute psychotic state, as described in our previous works. We also confirmed that UCB mean levels are statistically significantly higher (p=0.05) in remitting patients with schizophrenia (N=30; 0.34 ±0.16 mg/dL) than in remitting patients with schizoaffective disorder (N=30; 0.25 ±0.10 mg/dL). This outcome gives us some insight on the possible role of UCB as a biomarker for categorical distinction between schizophrenia and schizoaffective disorder, during the partial remission of an acute psychotic state, and that is, to our knowledge, the first time this kind of data is published. Secondly, we confirmed that UCB mean levels are higher in patients with schizophrenia (N=30) on relapse (0.38±0.19 mg/dL) versus partial remission (0.34±0.16 mg/dL). We hipothezize that the absence of any statistically significant difference, for schizophrenia patients, may be simply due to the small sample size. Nevertheless we confirmed that UCB mean levels are statistically significantly higher (p=0.034) in patients with schizoaffective disorder (N=30) on relapse (0.31 ±0.14 mg/dL) than in partial remission (0.25 ±0.10 mg/dL), and that is, to our knowledge the first time this kind of data is published. This outcome gives us some insight on the possible role of UCB as a biomarker for a dimensional distinction in schizoaffective disorder.

Besides our main objective we were also able to notice new interesting findings. We found, for the first time, through a linear regression model applied separately for patients with schizophrenia and patients with schizoaffective disorder, a statistically significant (p=0.008) positive correlation (R^2=0.223) between UCB mean levels at admission and Mean Length of Stay (MLS) in patients with schizoaffective disorder (N=30) suggesting that UCB may have a potential role as a biomarker for admission duration, which is widely accepted as a indirect indicator of psychosis severity. Our

study was the first one to date, to our knowledge, looking for a correlation between UCB mean levels and psychosocial performance (GAF and PSP). Although we did not found any correlation between UCB mean levels and GAF scale, we were able to describe, for the first time, a positive Pearson's correlation (r=0.314) with significant statistically meaning (p=0.01), between UCB mean level of relapsing patients (N=88) at the first assessment (admission) and one of the four items of PSP, the PSP item (d) "disturbing and aggressive behaviors". Last but not least we have also found a new negative Pearson's correlation (r=−0.399), with significant statistically meaning (p=0.002), between the UCB mean level of remitting patients (N=60) at the second assessment (outpatient appointment) and one of the thirty items on PANSS item G7 "psychomotor retardation".

Interestingly enough, these two outcomes bring new insight to a new role of UCB level as a eventual biomarker for psychomotor agitation, both in the categorical spectrum (schizophrenia and schizoaffective disorder) and the severity spectrum (relapse at admission and partial remission at outpatient appointment). One could say that psychomotor agitation could be causing changes in UCB levels, but that hypothesis is clearly excluded as we found no Pearson's correlation between hemolysis biomarkers (e.g. LDH) and rhabdomyolysis biomarkers (e.g. CK). The statistically significant decrease (p=0.03) of CK levels in patients with schizophrenia (N=30) from the first assessment (213.0 ±297.2 mg/dL) to the second assessment (106.5 ±74.4 mg/dL) seem to be an independent epiphenomenon, probably secondary to the expected clinical improvement on psychomotor agitation but not contributing at all to the already mentioned Pearson's correlations.

Nevertheless we acknowledge other issues that should be discussed in all subjects included in our study: it would be important to have genetic tests in order to exclude GS; CSF analysis to exclude encephalitis; a more specific EEG analysis; more sensitive and specific brain scans, such as FLAIR MRI or even 1H−MRS for a better comparison of our data with other previous studies.

Although our study suggests a possible role of higher, yet normal, UCB mean levels as a risk factor for psychotic relapse, we are much aware that more and better clinical studies are necessary to clarify these findings.

UCB serum level as a good biomarker candidate with diagnostic, nosological and categorical potential, for distinction between groups of patients with different psychotic disorders (schizophrenia or schizoaffective disorder)

All our results suggest that UCB serum levels may be a useful biomarker in the distinction not only between patients with schizophrenia and bipolar disorder, but also between patients with schizoaffective disorder. This seems to be true independently of the phase of the disorder, with higher levels being characteristic of relapse and lower levels typical of remission.

In our retrospective five−year study we confirmed that UCB mean levels are clearly higher in patients with acute psychotic episodes of the schizophrenia and schizoaffective spectrum, when compared with bipolar patients and healthy controls. Our results are similar to those already described before, although all patients of our sub−groups were in normal range for (unconjugated, conjugated and total) bilirubin values. Other important aspect of our results is that acute schizoaffective patients stand right in between the patients with schizophrenia and the patients with bipolar disorder. This data reinforces the theory that schizoaffective disorder, might be an intermediate clinical entity, so often misunderstood but somewhere in between the schizophrenic and the bipolar spectra, both in phenotypic and physiopathologic aspects.

In our prospective twelve−month study, we verified that UCB mean levels were statistically significantly higher in relapsing psychotic patients with schizophrenia than in relapsing psychotic patients with schizoaffective disorder. We also confirmed that UCB mean levels were statistically significantly higher in remitting patients with schizophrenia than in remitting patients with schizoaffective disorder. This outcome gives us some insight on the possible role of UCB as a biomarker for categorical distinction between schizophrenia and schizoaffective disorder, not only during relapse but also during the partial remission of an acute psychotic state.

UCB serum level as a good biomarker candidate with psychopathological, psychosocial and dimensional potential for severity among groups of patients with the same psychotic disorder (schizophrenia and schizoaffective disorder)

Our results also suggest that UCB serum levels may be a useful biomarker in the distinction not only between patients with schizophrenia and schizoaffective disorder, but also in the distinction between remission and relapse among both of these groups.

In our prospective twelve−month study we confirmed that UCB mean levels are higher in patients with schizophrenia on relapse versus partial remission. We believe we failed to find a statistically significant difference for schizophrenia patients just because of our small sample size. Nevertheless we confirmed that UCB mean levels are statistically significantly higher in patients with schizoaffective disorder on relapse versus partial remission, and that is, to our knowledge the first time this kind of data is published. This outcome gives us some insight on the possible role of UCB as a biomarker for a dimensional distinction in schizoaffective disorder. We also found, through a linear regression model applied separately for patients with schizophrenia and patients with schizoaffective disorder, a statistically significant positive correlation between UCB mean levels at admission and Mean Length of Stay in patients with schizoaffective disorder suggesting that UCB may have a potential role as a biomarker for admission duration.

We were also able to describe, for the first time, a positive correlation with significant statistically meaning, between UCB mean level of relapsing patients at the first assessment (admission) and the PSP item (d) “disturbing and aggressive behaviors”. Last but not least we have also found a new negative correlation, with significant statistically meaning, between the UCB mean level of remitting patients at the second assessment (outpatient appointment) and PANSS item G7 “psychomotor retardation”.

Symptomatology of schizophrenia is heterogeneous, there is not any pathognomonic symptom and the diagnosis is difficult, since it is based on subjective information, instead of markers (Garcia−Alvarez et al 2016). Given the heterogeneity of symptoms in patients with schizophrenia and current treatment limitations, biomarkers may play an important role in diagnosis, subtype stratification, and the assessment of treatment response (Goldsmith et al 2017).

Identifying biomarkers that can be used as diagnostics or predictors of treatment response (theranostics) in people with schizophrenia will be an important step towards being able to provide personalized treatment (Lay et al 2016). Nevertheless understanding the biological process and progression of schizophrenia is the first step to developing novel approaches and interventions; therefore research on new biomarkers is extremely important when the goal is an early diagnosis and precise theranostics (Rodrigues−Amorim et al 2017). The combination of different markers, or complex multi−marker panels, might help in the discrimination of patients with different underlying pathologies and in the better classification of the more homogenous groups (Perkovic et al 2017).

Schizophrenia is considered as a syndrome comprised by several disease phenotypes, covering a range of underlying pathologies, and one of these disease mechanisms seems to involve immune dysregulation and neuroinflammation (Kroken et al 2018). Schizophrenia is hypothesized to be a syndrome with different underlying pathological processes and the same is true for inflammation, which has various stages and processes, ranging from acute to chronic and can also be an autoimmune process (Müller 2018). An increasing number of clinical, epidemiological, and experimental studies have shown links between schizophrenia and inflammatory conditions (Feigenson et al 2014). The evidence to date, coupled with advances in immunology and genetics has afforded the field an unparalleled opportunity to investigate the hypothesis that a subset of patients with schizophrenia may manifest an immunophenotype, toward new potential diagnostics and therapeutics to reduce risk, alleviate symptoms, and improve quality of life in both at−risk populations and patients with established schizophrenia (Miller and Goldsmith 2017).

Although there is no clear evidence to support widespread clinical use of inflammation as a biomarker in schizophrenia, future studies in this area show promise towards a

greater understanding of the etiopathophysiology of this heterogeneous syndrome, towards new potential diagnostics and therapeutics to reduce risk, alleviate symptoms and improve quality of life, in both at−risk and established patient populations (Miller and Goldsmith 2019).

The vulnerability−stress−inflammation model may help to explain the role of inflammation in schizophrenia because stress can increase pro−inflammatory cytokines and may even contribute to a chronic pro−inflammatory state (Müller 2018). Chance findings often lead to later robust scientific discovery (Buckley 2019). A patient with treatment−refractory schizophrenia went into remission of his psychosis contemporaneously with the bone marrow treatment he received for leukemia (Miyaoka 2017). His remission of psychosis was considered to be the result of immune cellular therapy; provocative indeed, but only time will tell whether immune dysfunction is a primary mechanism or a secondary consequence in the neurobiology of schizophrenia (Buckley 2019).

All the already cited authors (Feigenson et al 2014, Lay et al 2016, Garcia−Alvarez et al 2016, Miller and Goldsmith 2017, Goldsmith et al 2017, Perkovic et al 2017, Rodrigues−Amorim et al 2017, Kroken et al 2018, Müller 2018, Miller and Goldsmith 2019, Buckley 2019) who wrote reviews regarding the importance of neuroinflammation in schizophrenia have not included UCB in their long lists of potential neuroinflammatory biomarkers. We hope that in the near future UCB will be brought to the attention of other groups of clinical researchers as we believe it is a lost piece on the fascinating neuroinflammatory jigsaw puzzle of schizophrenia.

General Limitations of our studies

Unfortunately we had a high rate of patients lost to follow−up. On one hand we had some patients with psychosis secondary to drug abuse (e.g. cannabis) or organic conditions (e.g. temporal lobe epilepsy) that couldn't meet criteria for schizophrenia nor schizoaffective disorder and had to be excluded from the study. On the other hand we had too many patients not coming to the second assessment, as it turned out to be very hard to convince psychotic patients (even in remission) to come to the psychiatric hospital, to see the physician just for research purposes.

We are also aware of other problems that should be discussed. It would be important to have genetic tests to exclude GS; cerebrospinal fluid analysis to exclude encephalitis; a more specific electroencephalography analysis; or even a more sensitive and specific brain scan with functional magnetic resonance imaging. We hope better studies in the future will address these issues.

We would like to highlight that in neuropsychological evaluation (we were able to complete 120 neuropsychological assessments), we did not find any correlation with enough statistical significance between those results and the UCB levels.

Last but not least, our study suggests a possible role of higher, yet normal, UCB mean levels as a risk factor for psychotic relapse, but we must recognize that we were not able to test or calculate neither specificity nor sensitivity of UCB as a biomarker for psychosis.

Research Perspectives for the future

Unconjugated bilirubin seems to be a serious candidate for biomarker in the schizophrenia – schizoaffective – bipolar spectra. We believe that further similar studies with bigger samples will have more statistical power, yielding possibilities for replication of our results and help to clarify our findings.

There is indeed some potential for other researchers to explore UCB as a biomarker for psychosis and further studies shall focus on the search for significant statistically correlations between UCB mean levels in psychotic patients and other kind of variables such as genetics, neuroimaging, neurophysiology and neuropsychology. These variables are quite important for the diagnosis and prognosis of the patients and this kind of work might become quite relevant for a better understanding of the etiology of psychotic disorders of the schizophrenia and schizoaffective spectrum.

Another opportunity for possible research is the search for new therapeutic targets, as it is widely accepted that the classic treatment of schizophrenia and other psychotic disorder has changed very little in the last half of the century. New biological and disease−modifying therapies are urgently needed in order to increase the treatment chances of all patients that have been taking antipsychotics (a class of medication that is already on its third generation), many times, unfortunately, without achieving the desired full clinical remission, social recovery and/or gratifying quality of life.

CONCLUSION

The results presented seem to support a role of UCB in the etiopathogeny of schizophrenia spectrum disorder and add novel information also regarding the schizoaffective disorder spectrum. According to these results UCB may have a possible role as a biomarker for categorical distinction between schizophrenia and schizoaffective disorder, especially during an acute psychotic state. Also they point to interesting dimensional correlations between the levels of this biomarker candidate and important clinical aspects, such as psychopathological (e.g. psychomotor retardation) and psychossocial (e.g. duration of psychiatric admission or disturbing and aggressive behaviors), thus yielding some predicting value potential.

Despite the limitations already discussed we would like to propose a quite simple tridimensional model (Figure 7) that might be useful for studying the role of other biomarkers candidates in psychotic spectrum disorders. This model includes a categorical axis from affective to non−affective psychosis; a dimensional axis from personality to psychosis; and a biomarker axis from remission to relapse.

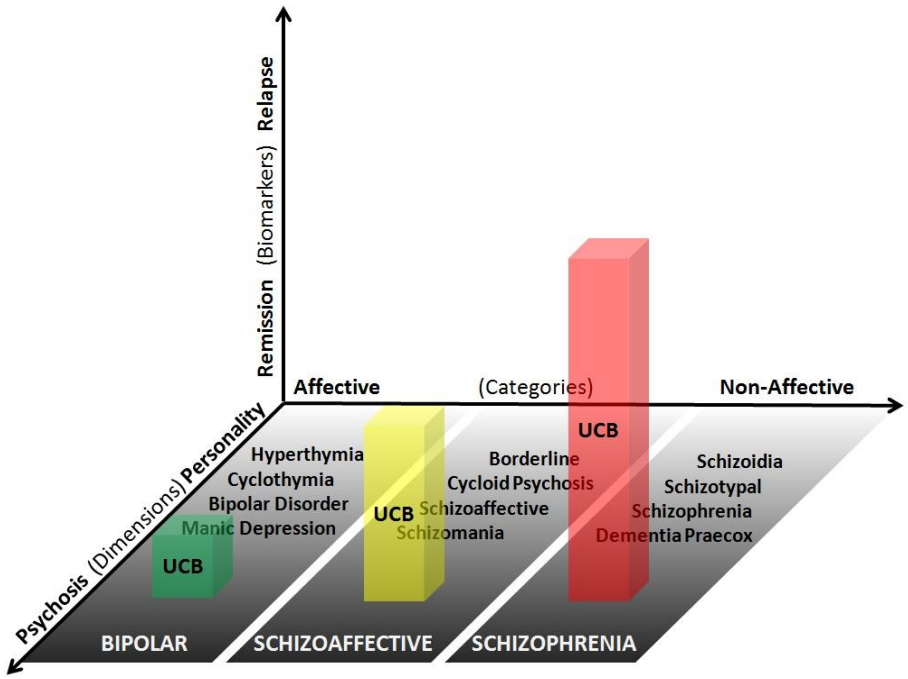


Figure 7 – UCB as biomarker in the psychotic spectra tridimensional model.

LIST OF ABBREVIATIONS

1H−MRS Proton Magnetic Resonance Spectroscopy

ACC Anterior Cingulate Gyrus

ALT Alanine Transaminase

ANOVA Analysis of Variance

APA American Psychiatry Association

AST Aspartate Transaminase

BD Bipolar Disorder

BGL Blood Glucose Level

BMI Body Mass Index

BOMs Bilirubin Oxidative Metabolites

BPRS Brief Psychiatric Rating Scale

CB Conjugated Bilirubin

CGI Clinical Global Impression

Cho Choline

CK Creatine Kinase

CNS Central Nervous System

Col Cholesterol

CPR C Reactive Protein

CPZE Chlorpromazine Equivalent

Cr Creatine

CSF Cerebrospinal Fluid

CT Computed Tomography

dB Decibel

DG Dentate Gyrus

DSM IV Diagnostic and Statistical Manual of mental disorders, fourth edition

DSM 5 Diagnostic and Statistical Manual of mental disorders, fifth edition

DUP Duration of Untreated Psychosis

EEG Electroencephalography

FLAIR MR Fluid Attenuated Inversion Recovery Magnetic Resonance

FPH Family Psychiatric History

GA Gunn rat Aripiprazole group

GAF Global Assessment of Functioning

GC Gunn Control

GGT Gamma−glutamyltransferase

GH Gunn rat Haloperidol group

GL Granular Layer

GR Gunn rat Risperidone group

GS Gilbert’s Syndrome

Iba1 Ionized Calcium Binding Adaptor Molecule 1

ICD 9 International Classification of Diseases, ninth edition

ICD 10 International Classification of Diseases, tenth edition

ICD 11 International Classification of Diseases, eleventh edition

IL1R Interleukin 1 Beta Receptor

IL1β Interleukin 1 Beta

MFU Median Follow Up

ML Molecular Layer

ml Myoinositol

MLS Mean Length of Stay

ms Milliseconds

NAA N Acetylaspartate

NMDA N Methyl D Aspartate

NO Nitric Oxide

OLGs Oligodendrocytes

PANSS Positive And Negative Syndrome Scale

PhD Philosophy Doctor

PPI Prepulse Inhibition

PRISMA Preferred Reporting Items for Systematic reviews and Meta−Analyses

PSP Personal and Social Performance

ROS Reactive Oxygen Species

SD Standard Deviation

SGZ Subgranular Zone

SPSS Statistical Package for the Social Sciences

SPY Smoking Pack Years

SZ Schizophrenia

TAG Triacylglyceride

TB Total Bilirubin

TNFR1 Tumor Necrosis Factor Alfa Receptor 1

TNFα Tumor Necrosis Factor Alfa

UCB Unconjugated Bilirubin

UGT1A1 Uridinediphosphate Glucuronosyltransferase 1A1

VHB Viral Hepatitis type B

VHC Viral Hepatitis type C

WC Wistar rat Control group

WHO World Health Organization

WM Wistar rat Medication group

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