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ÍCARO AGENOR FERREIRA DE OLIVEIRA

**Avaliação perfusional e de conectividade funcional cerebrais
em esquizofrenia por imagens por ressonância magnética**

Ribeirão Preto – SP

2017

<p>ÍCARO AGENOR FERREIRA DE OLIVEIRA</p>	<p>Avaliação perfusional e de conectividade funcional cerebrais em esquizofrenia por imagens por ressonância magnética</p>		<p>MESTRADO FFCLRP/USP 2017</p>
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Versão Corrigida

Dissertação apresentada à Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto da Universidade de São Paulo como parte das exigências para obtenção do título de Mestre em Ciências.

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Dedico este trabalho aos meus familiares. Aos meus pais pelo exemplo de honestidade, coragem e simplicidade. Aos meus irmãos pela ajuda e apoio em todos os momentos da minha vida e pelo exemplo de persistência em suas metas.

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A todos os pacientes e voluntários que participaram deste trabalho.

*“ANY DREAM IS ACHIEVABLE IF WE
HAVE THE COURAGE TO FIGHT FOR IT”*

WALT DISNEY

RESUMO

Oliveira, I. A. F. de. **Avaliação perfusional e de conectividade funcional cerebrais em esquizofrenia por imagens por ressonância magnética**. 2017. 95 f. Dissertação (Mestrado em Ciências na área de Física Aplicada à Medicina e Biologia) – Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto – SP, 2017.

A esquizofrenia é um transtorno psiquiátrico incapacitante que afeta estimadamente 1% da população mundial. Delírios, alucinações, desorganização de pensamento e prejuízo cognitivo são as principais marcas da Esquizofrenia. Fisiologicamente, além de anormalidades funcionais e estruturais, alterações na atividade neuronal são reportadas. Como a atividade neuronal possui uma relação direta com o fluxo sanguíneo cerebral (CBF, *Cerebral Blood Flow*), a técnica de Imagens por Ressonância Magnética, denominada Marcação dos Spins Arteriais (ASL, *Arterial Spin Labeling*), que permite a obtenção de mapa quantitativo de CBF, é uma ferramenta útil na avaliação funcional cerebral. Além disso, a ASL pode ser usada na avaliação da conectividade funcional, que é eficiente na investigação de rupturas funcionais entre as regiões do cérebro. Comparando com um grupo de sujeitos saudáveis, os pacientes com esquizofrenia, recrutados no Hospital das Clínicas de Ribeirão Preto (HCFMRP), apresentaram redução de CBF em regiões bilaterais do polo frontal e giro frontal superior, giro frontal medial direito, partes triangular e opercular do giro frontal inferior direito, divisão posterior do giro supramarginal esquerdo, divisão superior e inferior do córtex occipital lateral esquerdo e polo occipital. A conectividade funcional, avaliada por três diferentes métodos (baseado em semente, análise de componentes independentes e teoria dos grafos), se apresentou prejudicada em regiões envolvendo funções motoras, sensoriais e cognitivas dos pacientes. Portanto, utilizando uma técnica de imagem completamente não invasiva, foi possível observar déficits de CBF e alterações na organização funcional do cérebro de pacientes com esquizofrenia, relacionados com os sintomas e características da psicopatologia.

Palavras-chave: Perfusão cerebral. Conectividade funcional. Marcação dos Spins Arteriais. Imagem por ressonância magnética. Esquizofrenia.

ABSTRACT

Oliveira, I. A. F. de. **Assessment of cerebral perfusion and functional connectivity in schizophrenia using magnetic resonance imaging**. 2017. 95 f. Dissertation (Master in Sciences in Physics Applied to Medicine and Biology) – Faculdade de Filosofia, Ciências e Letras de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto – SP, 2017.

Schizophrenia is a disabling psychiatric disorder that affects around 1% of the population worldwide. Delusions, hallucinations, disorganized thought, and cognitive deficits are the main features of schizophrenia. Physiologically, in addition to functional and structural abnormalities, changes in neuronal activity are reported. Since the Cerebral Blood Flow (CBF) is directly related with neuronal activity, the Magnetic Resonance Imaging (MRI) technique called Arterial Spin Labeling (ASL), which allows the quantification of CBF, is a useful tool in brain functional evaluation. In addition, ASL can be used to assess functional connectivity, which is efficient in investigating functional impairment between regions of the brain. Patients with Schizophrenia, recruited at the Clinical Hospital (HCFMRP), presented a reduction of CBF in bilateral regions of the frontal pole and superior frontal gyrus, right medial frontal gyrus, triangular and opercular parts of the right inferior frontal gyrus, posterior division of left supramarginal gyrus, superior and inferior division of left lateral occipital cortex and occipital pole. Functional connectivity, assessed by three different methods (seed-based, independent component analysis and graph theory), was impaired in regions involving patients' motor, sensory and cognitive functions. Therefore, using a noninvasive imaging technique, it was possible to observe CBF deficits and alterations in the functional organization of the brain of schizophrenia patients, related to the symptoms and characteristics of the psychopathology.

Keywords: Cerebral perfusion. Functional connectivity. Arterial Spin Labeling. Magnetic Resonance Imaging. Schizophrenia.

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LIST OF ABBREVIATIONS

ACC	Anterior Cingulate Cortex
AG	Angular Gyrus
AI	Anterior Insula
aITG	Inferior Temporal Gyrus anterior division
aMTG	Middle Temporal Gyrus anterior division
aPaHC	Parahippocampal Gyrus anterior division
ASL	Arterial Spin Labeling
aSTG	Superior Temporal Gyrus anterior division
BOLD	Blood Oxygenation Level Dependent
CASL	Continuous ASL
CBF	Cerebral Blood Flow
CBV	Cerebral Blood Volume
CEN	Central Executive Network
CMRO ₂	Cerebral Metabolic Rate of Oxygen
CNR	Contrast-to-noise Ratio
CO	Central Opperculum Cortex
CSF	Cerebrospinal Fluid
DAN	Dorsal Attention Network
DLPFC	Dorsolateral Prefrontal Cortex
DMN	Default Mode Network
dmPFC	dorsomedial Prefrontal Cortex
DSC	Dice Similarity Coefficient
DSM-V	Diagnostic and Statistical Manual for Mental disorders
EPI	Echo Planar Imaging
FA	Flip Angle
FC	Functional Connectivity
FEF	Frotal Eye Field
FLAIR	Fluid Attenuated Inversion Recovery
fMRI	Functional Magnetic Resonance Imaging
FO	Frontal Opperculum Cortex
FOrb	Frontal Orbital Cortex
FOV	Field of View

FP	Frontal Pole
FPN	Fronto-Parietal Network
FWHM	Full Width at Half Maximum
GM	Gray Matter
HG	Herschl's Gyrus
ICA	Independent Component Analysis
ICC	Intracalcarine Cortex
IFGoper	Inferior Frontal Gyrus pars opercularis
IFGtri	Inferior Frontal Gyrus pars triangularis
iLOC	Latertal Occipital Gyrus inferior division
IPL	Inferior Parietal lobule
IPS	Intraparietal Sulcus
LPFC	Lateral Prefrontal Cortex
LSD	Lysergic Acid Diethylamide
LT	Labeling Time
MidFG	Middle Frontal Gyrus
MNI	Montreal Neurological Institute
mPFC	Medial prefrontal cortex
MRI	Magnetic Resonance Imaging
MT	Magnetization Transfer
MTT	Mean Transit Time
OFusG	Occipital Fusiform Gyrus
OP	Occipital Pole
PaCiG	Paracingulate Gyrus
PANSS	Positive and Negative Syndrome Scale
PASL	Pulsed ASL
PCA	Principal Component Analysis
pCASL	pseudocontinuous ASL
PCC	Posterior Cingulate Cortex
PET	Positron Emission Tomography
pITG	Inferior Temporal Gyrus posterior division
PLD	Post-labeling Delay
PO	Parietal Operculum
pPAHC	Parahippocampal Gyrus

PreCG	Precentral Gyrus
pSMG	Supramarginal Gyrus posterior division
pSTG	Superior Temporal Gyrus posterior division
PT	Planum Temporale
pTFUsc	Temporal Fusiform Cortex
RBN	Resting-state Brain Network
RF	Radiofrequency
ROI	Region of Interest
rPFC	rostral Prefrontal Cortex
RS-fMRI	Resting-state fMRI
SCC	Supracalcarine Cortex
SFG	Superior Frontal Gyrus
sLOC	Lateral Occipital Gyrus superior division
SMA	Supplementary Motor Area
SMG	Supramarginal Gyrus
SMN	Sensorimotor Network
SN	Salience Network
SNR	Signal-to-noise Ratio
SPECT	Single Photon Emission Computed Tomography
SPL	Superior Parietal Lobule
ST	Slice Time
TE	Time of Echo
TI	Time of Inversion
TOF	Time of Flight
toMTG	Temporal Gyrus tempooccipital part
TR	Time of Repetition
VN	Visual Network
WM	White Matter

LIST OF SYMBOLS

α	Label efficiency
λ	Partition coefficient blood-tissue of water
M_0	Equilibrium magnetization of blood
M_b	Longitudinal magnetization of tissue
M_b^0	Equilibrium Longitudinal magnetization of tissue
M_v	Longitudinal magnetization of the venous blood
M_A	Longitudinal magnetization of the arterial blood
M_A^0	Initial Longitudinal magnetization of the arterial blood
T_1	Longitudinal Relaxation time of blood
T_{1app}	Longitudinal Relaxation time of tissue
$M_{control}$	Magnetization of Control image
ΔM	Difference between Control and label image, perfusion weighted image
r	Pearson's coefficient correlation
$C_{x,y}$	Covariance from x and y variables
$S_{x,y}$	Standard deviation from x and y variables

SUMMARY

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1. SCHIZOPHRENIA

Schizophrenia is a severe brain disorder that literally means “split mind”; a representative term since the patient experiences symptoms that seem like a mind disruption. The Swiss psychiatrist Eugen Bleuler created the term “schizophrenia” in the beginning of the XX century. His great achievement was to distinguish individuals with auditory hallucinations, delusions, and disorganized thoughts from patients with dementia (STEFAN; TRAVIS; MURRAY, 2002).

Bleuler thought of schizophrenia in psychological rather than neuropathological terms, as the core of the illness, he believed, was a more generalized psychological deficit, characterized by loosening of language associations, deficits in attention, and incongruity of affect, ambivalence and autism. Despite knowing that there was a heterogeneity among schizophrenia patients, Bleuler was not able to subdivide them into groups (HECKERS, 2000; STEFAN; TRAVIS; MURRAY, 2002).

In 1959, the German psychiatrist Kurt Schneider set out a list of symptoms, which would lead to the diagnosis of schizophrenia, in the absence of organic brain disease (HECKERS, 2000; STEFAN; TRAVIS; MURRAY, 2002).

- Audible thoughts
- Voices heard arguing
- Voices heard commenting on one's actions
- The experience of influences playing on the body
- Thought withdrawal and other interferences with thought
- Delusional perception
- Feelings, impulses and acts experienced under influence of others

The peak periods of the disease onset are the adolescence and early adulthood, generally between 16 and 25 years for men, and between 25 and 30 years for women. In addition, about 1% of the world's population will develop schizophrenia during their lifetime.

1.1. SYMPTOMS

Once the disease is active, it is characterized by episodes in which the patient is unable to distinguish between real and unreal experiences. Delusions, hallucinations, disorganized thoughts and cognitive impairment are the main marks of this pathology. As many other disorders, the severity, duration, and frequency of symptoms can vary among patients. However, during the patient's lifetime, the incidence of severe psychotic symptoms often decreases, except in cases that the patient is not taking medication as prescribed, uses alcohol or illicit drugs, and suffers from stressful situations, when the symptoms tend to increase.

Schizophrenia symptoms are commonly separated into four different categories (WATKINS; ANDREWS, 2016). First, the positive symptoms include delusions; hallucinations, such as hearing voices; and false beliefs as bizarre or persecutory ideas that can lead to paranoid or distorted perception of reality. They are less frequent in schizophrenia patients, and generally respond well to medication.

Second, less responsive to medication, the negative symptoms are deficits of normal emotional responses or other processes, that include reduced ability to function, neglect of personal hygiene, lack of emotion, toneless voice, expressionless, inability to experience pleasure, inability to connect with family and friends, and lack of motivation.

Third, the disorganized symptoms are referred to the disorganized thinking and speech processes of the patients. They usually lose their train of thoughts, or the sentences have no meaning, being just random words and phrases.

Finally, the cognitive impairment include the problems with cognitive functions that tend to affect the patient's attention, executive functions and working memory. There are also deficits in language, processing speed and sensory processing (BRISCH, 2014). Some regions of the frontal lobe are known to be related to these cognitive functions, such as the dorsolateral prefrontal cortex (DLPFC), which is related to visual, verbal, central executive, episodic component and working memory disturbances in schizophrenia (BRISCH, 2014).

In addition, many patients have concomitant mood symptoms, including depression and anxiety, which may contribute to the 10% lifetime incidence of suicide. Notably, the suicide-related mortality is higher among subjects with a recent diagnosis of the disease (VENTRIGLIO et al., 2016).

1.2. CAUSES AND MECHANISMS

The causes of schizophrenia are still not completely understood. Scientists generally agree that it is a group of conditions rather than a simple disease, and therefore may have several causes (Figure 1.1). Chemical and structural brain differences may play a role in the disorder. In addition, genetic research suggests that while no gene is related to schizophrenia, several genes may cause a predisposition that can be triggered by certain life events (CANNON, 2015; GAEBEL; ZIELASEK, 2015). It is estimated that genetic risk markers are present in 10% of the cases of schizophrenia, but there is a clear tendency for familiar aggregation. However, the mode of transmission is not linear. Some recent studies point to a greater importance of genes related to immunity, which may be the basis of the neuro-immunological process involved with the disease.

In neurobiological terms, it is known that there are structural alterations of the neuronal organization that is the basis of the connections between brain regions, caused by the disruption of the nets during the pregnancy (result of hypoxia, maternal infection, stress or malnutrition) and changes in brain development, resulting in abnormal neural connections. However, it is not clear whether the modified neural networks observed in schizophrenia is the direct product of an aberrant brain development, or if it is an adaptation to other structural changes (CANNON, 2015; GAEBEL; ZIELASEK, 2015).

Environmental factors also play an important role in the disease's trigger, for example, drug abuse, such as ketamine, amphetamine, cocaine, LSD and cannabis. Psychoactive substance misuse both precedes and follows the onset of psychotic symptoms. Some patients report that they receive transient symptom relief and are using the drugs as a form of self-medication. However, it is known that abuse of certain drugs, cannabis, for instance, can increase the risk of schizophrenia (The Health Effects of Cannabis and Cannabinoids, 2017).

Other environmental risks are related to the place where children grow up. For example, in urbanized areas it is more common to find schizophrenia patients. In addition, there is evidence that some immigrant ethnic groups have a higher risk of developing psychotic disorders than native-born individuals, particularly if they live in a low ethnic density area or an area where there are fewer people of the same migrant group (VAN OS; KAPUR, 2009).

Many attempts have been made to explain the link between altered brain function and schizophrenia. One of the most discussed is the dopamine hypothesis, which attributes psychosis to the failure to interpret the misfiring of dopaminergic neurons. However, recent research has indicated that glutamate, GABA, acetylcholine, and serotonin alterations are also involved in the pathology of schizophrenia (CANNON, 2015), in addition to the immune system and the role of neuroinflammation (WATKINS; ANDREWS, 2016).

Schizophrenia is also associated with subtle differences in brain structures, such as ventricular enlargement, total brain volume decrease, and volume reduction of frontal, temporal and parietal regions (OTA et al., 2014). Moreover, because of the altered level of neuronal activity present in schizophrenia, brain functionality and perfusion have been extensively investigated, and Cerebral Blood Flow (CBF) alterations during resting state have been reported when compared with healthy age-matched subjects (ANDREASEN et al., 1997; KANAHARA et al., 2013). Previous studies using Single Photon Emission Computed Tomography (SPECT), Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI) showed significant reductions of regional CBF in frontal, parietal, and temporal regions of individuals with schizophrenia (OTA et al., 2014; PINKHAM et al., 2011; ZHU et al., 2015).

Figure 1.1: Scheme of the neurodevelopment of Schizophrenia.

Source: Author

1.3. DIAGNOSIS

The identification of delusions and hallucinations in psychosis is not a difficult task; however, their classification is not simple. Psychosis is not exclusive to schizophrenia and occurs in various diagnostic categories of psychotic disorders. The commonly used criteria (DSM-V, Diagnostic and Statistical Manual for Mental disorders – *Fifth Edition*) are based on duration, and severity ratings for positive, negative, mood and psychomotor symptoms, and cognitive impairments. It is particularly difficult to make the diagnose in teenagers who present some of the above symptoms, such as too much sleep, bad performance at school, or difficulties to relate with other people, which can be confused with typical problems of their age.

1.3.1. DSM-V CRITERIA

The criteria for schizophrenia classification are usually made using the DSM-V, published in 2013, by the American Psychiatric Association (GAEBEL; ZIELASEK,

2015; TANDON et al., 2013). The classification relies on the assessment of the clinical symptoms of schizophrenia, and the details are in Table 1.1.

Table 1.1: Summary of the DSM-V criteria.

<p>A. Two (or more) of the following, each present for a significant portion of time during 1 month period (or less if successfully treated)</p> <ol style="list-style-type: none"> 1. Delusions 2. Hallucinations 3. Disorganized speech (frequent derailment or incoherence) 4. Grossly disorganized or catatonic behavior 5. Negative symptoms (diminished emotional expression or avolition) <p>B. Social/occupational dysfunction</p> <p>C. Duration (6 months)</p> <p>D. Schizoaffective and Mood Disorder exclusion</p> <p>E. Substance/General Medical condition exclusion</p> <p>F. Relationship to pervasive developmental disorder</p>	
<p><u>Symptoms Specifiers</u></p> <ul style="list-style-type: none"> • Hallucinations • Delusions • Disorganized speech • Abnormal psychomotor behavior • Negative symptoms • Impaired cognition • Depression • Mania 	<p><u>Course Specifiers</u></p> <p>0 First episode, currently in acute episode</p> <p>1 First episode, currently in partial remission</p> <p>2 First episode, currently in full remission</p> <p>3 Multiple episodes, currently in acute episode</p> <p>4 Multiple episodes, currently in partial remission</p> <p>5 Multiple episodes, currently in full remission</p> <p>6 Continuous</p> <p>7 Unspecified</p>

Source: Gaebel et al., 2015

1.3.2. PANSS

The Positive and Negative Syndrome Scale (PANSS) is a medical scale used for measuring symptom severity of patients with schizophrenia (Appendix A). The PANSS is a relatively brief clinical interview, requiring 45 to 50 minutes to conduct. The patient

is rated from 1 to 7 on 30 different symptoms based on the interview as well as on reports of family members or primary care hospital workers (KAY; FISZBEIN; OPLER, 1987). It includes the Positive scale (7 items, minimum score = 7, maximum score = 49), Negative scale (7 items, minimum score = 7, maximum score = 49) and General Psychopathology scale (16 items, minimum score = 16, maximum score 112), resulting the PANSS total score (minimum = 30, maximum = 210).

1.4. DISEASE HANDLING

Although schizophrenia has no cure, many patients do well with minimal symptoms, and periods of recovery. The primary treatment is the use of antipsychotic medications, that can reduce symptoms and future relapses. A variety of antipsychotic medications is effective in reducing the psychotic symptoms present in the acute phase of the illness, and they help reduce the potential for future acute episodes.

In addition, psychotherapy is also used to reduce symptoms and enhance function, and other treatments aim to attenuate stress, support employment or improve social skills. Therapy and support can help people learn social skills, cope with stress, identify early warning signs of relapse and prolong periods of remission. It is also essential for the patient's well-being the support of the family, friends and mental health professionals.

2. ARTERIAL SPIN LABELING

The circulatory system is responsible for transporting oxygen and nutrients through the body up to the cells to produce energy. In the brain, the energy reserve is limited but its demand is high, leading to the necessity of a supply of oxygen and substrates through CBF. If the blood flow is interrupted, significant and permanent neuronal damage in the affected brain areas can occur within a few minutes. Thus, the cerebral perfusion has peculiar characteristics, such as cerebral vasoregulation, endothelial regulation and neurovascular coupling, to guarantee the integrity and functionality of brain tissue (IADECOLA; NEDERGAARD, 2007; PETERSON; WANG; BRITZ, 2011). In this context, the MRI technique known as Arterial Spin Labeling (ASL) appears as an important tool for quantitative measurements of CBF and evaluation of cerebral perfusion.

ASL is a non-invasive method that uses the water present on the arterial blood as a diffusible endogenous contrast. Unlike the majority of perfusion techniques that are qualitative, showing relative changes in CBF, Cerebral Blood Volume (CBV) and mean transit time (MTT), ASL can provide a quantitative measure of CBF (POLLOCK et al., 2009). Moreover, CBF maps present satisfactory temporal and spatial resolutions comparing to other methods, and ASL has been validated extensively against those other methods that use exogenous contrast agents, such as ^{15}O -PET (ALSOP et al., 2015; WINTERMARK et al., 2005; XU et al., 2011).

The fundamental concept of ASL is the manipulation of the longitudinal magnetization of the water present in the arterial blood so that it differs from the magnetization of the tissue (Figure 2.1). It is based on the labeling of the protons in the supplying vessels outside the imaging plane, which reach the parenchyma after a period of time known as Post-labeling Delay (PLD). A labeled image is then acquired. A control (unlabeled) image is also acquired (POLLOCK et al., 2009).

The subtraction of the labeled and control images eliminates the static tissue signal from the parenchyma. The remaining signal is a relative measure of the perfusion, resulting in a perfusion-weighted image, which is proportional to the local CBF. Unfortunately, the signal difference is just a fraction (1-2%) of the tissue signal and depends on many parameters such flow rate, T1 of the blood and tissue, and transit time for blood to travel from the labeling region to the imaging plane. Therefore,

multiple control and label images are acquired to ensure sufficient signal-to-noise ratio (SNR), and absolute quantitative CBF map can be obtained using the General Kinetic Model by Buxton (BUXTON et al., 1998; POLLOCK et al., 2009). Besides, the perfusion contrast also has a dependency with the label efficiency, which is related to the number of spins labeled in the labeling plane (DETRE et al., 2012).

Figure 2.1: General principle of arterial spin labeling.

Source: Author

There are two distinct and independent pulse sequence components in ASL approach, a preparation component, and an acquisition component (Figure 2.2). The preparation component labels the inflowing blood in different magnetic states for the control and label images. Once the blood has been labeled, the acquisition component acquires the data. The acquisition component typically uses a fast acquisition method such as Spiral or Echo Planar Imaging (EPI). The distinct and independent nature of the preparation and acquisition components has allowed researchers to choose the combination that best suits their specific applications (POLLOCK et al., 2009).

Figure 2.2: Arterial Spin Labeling scheme. CASL: continuous ASL; PASL: pulsed ASL; pCASL: pseudocontinuous ASL.

Source: Author

2.1. METHODS

There are three main types of ASL: continuous (CASL), pulsed (PASL), and pseudocontinuous (pCASL). They differ from each other on the preparation component (Figures 2.2 and 2.3), and were developed to attend the limitations and technical challenges encountered in previous implementations (ALSOP et al., 2015; POLLOCK et al., 2009).

Figure 2.3: Imaging and labeling planes for different ASL methods.

Source: Author

The first one to emerge was CASL, in which flowing spins were inverted using a long radiofrequency (RF) pulse in conjunction with magnetic field gradients applied in the direction of blood flow. This approach provides good labeling efficiency, and images with high SNR. However, the application of the long inversion pulse near the imaging plane may reduce the signal, because of the magnetization transfer (MT) phenomenon. The MT effect occurs via off-resonance water-macromolecular interactions and is entirely separated from the ASL signal changes caused by flow. Another problem present in CASL approach is the higher power deposition also because of the continuous RF pulse (PETERSEN et al., 2006). Some alternatives have been proposed for these two problems. One is the use of coil and hardware dedicated to labeling. Because of the small extent of this coil, the RF field produced is limited and does not physically reach the tissue of interest, so no MT effect is observed in the brain tissue (SILVA et al., 1995). However, the use of specific instrumentation makes it difficult to implement in a clinical or commercial environment. Therefore, because of the difficulty to implement on commercial scanners and the high power deposition that leads to significant tissue heating, CASL was largely abandoned in favor to the PASL method (ALSOP et al., 2015; BOROGOVAC; ASLLANI, 2012; POLLOCK et al., 2009).

The magnetization transfer and signal loss due to the transit time of blood between the labeling plane and the image plane observed when using CASL are minimized by labeling a relatively large volume of blood, in a region close to the slices

of interest, with a single and short RF pulse (5 to 20 ms). After the labeling, the volume of blood flows towards the tissue of interest during a period of time, called inversion time (TI), then the image is acquired. As advantages, PASL presents high label efficiency (95 - 98%) and easy clinical implementation since it does not require dedicated hardware for labeling (PETERSEN et al., 2006). Moreover, depends on how and where RF pulses are applied during the control stage, PASL can be divided into different approaches: EPISTAR, PICORE or FAIR (FERRÉ et al., 2013).

Because CASL and PASL were not used in the present study, just a brief description was present. However, detailed explanations of both methods can be found in previous reviews (ALSOP et al., 2015).

2.1.1. PSEUDOCONTINUOUS ASL

More recently a hybrid form has been developed, pCASL, that takes advantage of CASL superior contrast to noise ratio (CNR) and PASL high labeling efficiency and lower deposition without the need for long labeling pulses (BOROGOVAC; ASLLANI, 2012; FERRÉ et al., 2013; POLLOCK et al., 2009). pCASL uses a long series of short RF pulses in combination with a strong slice-selection gradient (DAI et al., 2008). Because the pulses are short compared to the arterial blood relaxation time, the disturbances caused by the pulse train are essentially the same as those caused by a single long pulse (WONG, 2013).

Dai and colleagues developed the most usual approach for pCASL in 2008. They used a train of short RF pulses (approximately 1 ms) and synchronized field gradients to invert blood spins flowing through the labeling plane. A selective Hanning pulse can be applied with equal spacing, and a similar steady state of CASL can be achieved with fewer technical restrictions (ALSOP et al., 2015; BOROGOVAC; ASLLANI, 2012; DAI et al., 2008; POLLOCK et al., 2009).

Recent studies have shown that pCASL provides data with high reproducibility even for long intervals between exams, and it can be enhanced by corrections for coil sensitivity and label efficiency. Moreover, this method allows consistent and reliable CBF quantification, with average values concordant with those obtained with other techniques, for example, ¹⁵O-PET (BOROGOVAC; ASLLANI, 2012; CHEN; WANG; DETRE, 2011; WU et al., 2014; XU et al., 2011).

Figure 2.4: The pCASL method consists in a train of RF pulses (exactly as in (B)), applied with the body coil in the labeling plane (red rectangle in A).

Source: Author

2.1.2. CBF QUANTIFICATION

The dependence between perfusion-weighted images and the CBF maps is primarily due to proton density, and T1 ratio of tissue and blood flowing. There is also a high dependence on the transit time of arterial blood. The general kinetic model of Buxton assumes a single compartment for the kinetic tracer to evaluate the difference of the longitudinal magnetization in the tissue due to the labeling of the blood (BUXTON et al., 1998).

The model combines single-compartment kinetics with the Bloch equations; the Bloch equation for the longitudinal magnetization is modified to include delivery and clearance terms proportional to the local flow.

$$\frac{dM_b(t)}{dt} = \frac{M_b^0 - M_b(t)}{T_1} + CBF(M_A(t) - M_v(t)) \quad (2.1)$$

Where M_b^0 is the equilibrium longitudinal magnetization of tissue; M_v and M_A are the time dependent longitudinal magnetizations of venous blood and arterial blood, respectively; and T_1 is the longitudinal relaxation time for blood. The only assumption that we must do is that the water distribution in the brain can be treated as a single well-mixed compartment. Because ASL uses a freely diffusible tracer, we can describe the longitudinal magnetization for venous blood as:

$$M_v(t) = \frac{M_b(t)}{\lambda} \quad (2.2)$$

The λ is the blood-brain partition coefficient for water. Considering the conservation of matter, the amount of arterial blood that arrives in the tissue is the same that leaves it through the venous blood. So,

$$M_A^0 = M_b^0 = \frac{M_b^0}{\lambda} \quad (2.3)$$

Where M_A is the initial arterial longitudinal magnetization, in a complete relaxation. Then, equation (2.1) can be re-written:

$$\frac{dM_b(t)}{dt} = \frac{M_b^0 - M_b(t)}{T_{1app}} - 2\alpha(t) \frac{CBF}{\lambda} (M_b^0) \quad (2.4)$$

T_1 is replaced by T_{1app} , which is the longitudinal relaxation time for the tissue considering the perfusion from the arterial blood through the tissue, and $\alpha(t)$ is the labeling efficiency of the arterial blood just after the RF pulse.

$$\frac{1}{T_{1app}} = \frac{1}{T_1} + \frac{CBF}{\lambda} \quad (2.5)$$

$$\alpha(t) = \frac{M_A^0 - M_A}{2M_A^0} \quad (2.6)$$

Considering the control acquisition, with no inversion or saturation of the magnetization:

$$M_A(t) = M_A^{(0)} \quad (2.7)$$

$$M(t = 0) = 0 \quad (2.8)$$

The Bloch equation can be written as:

$$M_{control}(t) = M_0(1 - e^{-t/T_{1app}}) \quad (2.9)$$

In the labeling condition, the magnetization of the arterial blood is inverted or saturated; moreover, the magnetization decays with T_1 . M_0 corresponds to the equilibrium magnetization of blood. So,

$$M_A(t) = M_0 \left(1 - e^{-t/T_{1app}} \right) \quad (2.10)$$

Therefore,

$$M(t) = M_0 \left(1 - e^{-t/T_{1app}} \right) - 2\alpha M_0 \frac{CBF}{\lambda} \frac{(e^{-t/T_{1app}} - e^{-t/T_1})}{\frac{1}{T_{1app}} - \frac{1}{T_1}} \quad (2.11)$$

$$\Delta M(t) = M_{control} - M(t) = 2\alpha M_0 \frac{CBF}{\lambda} \frac{(e^{-t/T_{1app}} - e^{-t/T_1})}{\left(\frac{1}{T_{1app}} - \frac{1}{T_1} \right)} \quad (2.12)$$

$$CBF = \Delta M(t) 2\alpha M_0 \frac{1}{\lambda} \frac{(e^{-t/T_{1app}} - e^{-t/T_1})}{\left(\frac{1}{T_{1app}} - \frac{1}{T_1} \right)} \quad (2.13)$$

For the pCASL sequence used in the present study:

$$CBF = \left\{ -\frac{2\alpha}{\lambda} \frac{M_0}{\Delta M} \left[\frac{\exp(-PLD/T_{1app}) - \exp(-(PLD+LT+ST)/T_1)}{1/T_1 - 1/T_{1app}} \right] \right\}^{-1} \quad (2.14)$$

ΔM is the magnetization difference between control and label images; LT is the labeling time; and ST is the slice time acquisition.

2.2. DRAWBACKS

Because ASL has an intrinsically low SNR, it is necessary to acquire multiple volumes of the brain, resulting in a long acquisition (around 6 minutes). Another drawback is the low temporal resolution; with repetition times (TR) of 2-4 seconds, each control/label pair is acquired every 4-8 seconds. For fast image acquisitions, EPI is

used in most of the ASL applications, which can introduce distortions in regions of high differences in magnetic susceptibility values (PETCHARUNPAISAN; RAMALHO; CASTILLO, 2010).

Recently, tridimensional (3D) acquisition sequences are being used to improve image quality, promoting higher SNR and reducing distortions (FERNÁNDEZ-SEARA et al., 2005). However, the temporal resolution is limited to TR that cannot be very low because of two factors: (1) after labeling, it is necessary to wait the blood travels to the imaging plane; (2) after image acquisition, it is important that there is sufficient time for the relaxation of the labeled spins, avoiding artifacts in the next set of slices to be acquired (PETCHARUNPAISAN; RAMALHO; CASTILLO, 2010).

Higher fields also show better results for ASL by increasing the signal and consequently the SNR. The T1 relaxation time is higher and this characteristic allows an amount of labeled spins within the tissue. However, higher fields bring more complications related to magnetic susceptibility and higher energy deposition (PETCHARUNPAISAN; RAMALHO; CASTILLO, 2010).

2.3.FUNCTIONAL ASL

Images obtained with ASL over time may reveal information about brain functionality either by a designed stimuli (task) or at rest (resting state), which has low signal intensity. It is possible to identify spontaneous low-frequency oscillations on CBF maps (BISWAL; VAN KYLEN; HYDE, 1997), as well as to determine network parameters (MELIE-GARCÍA; SANABRIA-DIAZ; SÁNCHEZ-CATASÚS, 2013), performing connectivity analyzes during rest or task, similar to those analysis commonly performed with BOLD-MRI (Blood Oxygenation Level Dependent) (KINDLER et al., 2015; LIANG et al., 2013; ZHU et al., 2013; ZOU et al., 2009).

Most functional MRI studies during resting state (rs-fMRI) have used BOLD contrast (VAN DIJK et al., 2010). Despite its success and widespread use, it has several drawbacks: (1) the non-specificity to the neuronal activity (many physiological and noise contribution to the contrast mechanism); (2) non-spatial specificity (contribution of draining veins to the BOLD contrast); (3) BOLD signal does not provide a direct and quantitative measure of brain function during the resting state, and cannot be used in isolation to derive neuronal metabolism metrics.

On the other hand, CBF is normally coupled to glucose metabolism and neuronal activity (CHEN; JANN; WANG, 2015), and compared to the BOLD signal, has potential advantages: (1) offers increased spatial specificity to neuronal activity (capillary/tissue origin of ASL signal); (2) has the capacity for absolute quantification of CBF (physiological parameter critical to the viability of the brain tissue); (3) it can be used to generate dynamic CBF and BOLD signal measurements simultaneously, allowing the computation of the cerebral metabolic rate of oxygen ($CMRO_2$), with a dual-echo acquisition(CHEN; JANN; WANG, 2015).

3. FUNCTIONAL CONNECTIVITY

To understand the concept of functional connectivity, we must reach first the definition of brain connectivity. It refers to a pattern of anatomical links (structural connectivity), of statistical dependences (functional connectivity) or causal interactions (effective connectivity) between distinct units within a nervous system. The units correspond to individual neurons, neuronal populations, or anatomically segregated brain regions. The connectivity pattern is formed by structural links such as synapses of fiber pathways, or it represents statistical or causal relationships measured as cross-correlations, coherence, or information flow. More important, neural activity is constrained by connectivity, which makes brain connectivity crucial to understand how neurons and neural networks process information (SPORNS, 2013).

Following the concept that Sporns brought about functional connectivity (FC), the definition stands for a statistical dependence among remote neurophysiological events, providing a characterization of the functional interactions between units or, in other words, a description about the observed correlations between units. Therefore, because of the neurophysiological nature of the FC, there are several applications in neuroscience and clinical environment. For example, one can study how FC and information integration relates to human behavior and how this organization may be altered in neurodegenerative diseases (VAN DEN HEUVEL; HULSHOFF POL, 2010a); obtain diagnostic and prognostic information; longitudinal studies and treatment effects; pre-operative mapping; and intervention targeting (FOX; GREICIUS, 2010).

Neuroimaging techniques are one of the most used methods to study FC, in special during resting state. These studies examine the level of coactivation between the functional time series of anatomically separated brain regions when the subject is at rest (VAN DEN HEUVEL; HULSHOFF POL, 2010a).

During the 20 years after fMRI development, the majority of the studies relied on task approaches. More recently, researchers started to measure and analyze FC between brain regions as the level of co-activation of spontaneous fluctuations recorded with the subject at rest (BISWAL et al., 1995; BISWAL; VAN KYLEN; HYDE, 1997; FOX; GREICIUS, 2010). In these resting-state experiments, participants are instructed

to relax and do not think about anything while BOLD images are being acquired. Biswal and colleagues conducted the first published study in 1995. They demonstrated that during resting state BOLD time series of the motor cortices of both hemispheres have a correlation between each other, which suggests an ongoing information processing and functional connectivity between these regions even with the subject at rest (BISWAL et al., 1995). The origin and neural basis of this spontaneous signal are not yet fully comprehended; however, our interest is in low-frequency oscillations ($\sim 0.01 - 0.1$ Hz), which have been observed in animal models and humans using different techniques (BISWAL et al., 1995; BISWAL; VAN KYLEN; HYDE, 1997; DE LUCA et al., 2006).

There are two methods to analyze FC: model-dependent and model-free. Biswal extracted a time series from a voxel (motor cortex) and correlated it with the time series obtained from the rest of the brain; this method is model-dependent and is called seed-voxel method. On the other hand, model-free methods include Independent Component Analysis (ICA), Principal Component Analysis (PCA), and clustering (VAN DEN HEUVEL; HULSHOFF POL, 2010a). In addition, there is another approach to better understand how units in the brain interact, based on a framework of a complex network, called Graph Theory (ONIAS et al., 2014).

Several resting state studies reported the existence of regions that are functionally linked, and are often referred to as resting-state brain networks (RBNs) (VAN DEN HEUVEL; HULSHOFF POL, 2010b). Most of these RBNs represent a functional network, overlapping regions that are known to share a common function (VAN DEN HEUVEL; HULSHOFF POL, 2010b). Figure 3.1 shows eight RBNs as a template.

Figure 3.1: Representation of the resting-state networks atlas (31 regions of interest combining in 8 networks) from FC toolbox.

Source: CONN – *functional connectivity toolbox v17*

3.1. SEED-BASED ANALYSIS

This model is known as the most straightforward to perform a connectivity study. The idea is to correlate the resting-state time series of a region of interest (ROI) or even a single voxel against the whole brain (ROI-to-Voxel, Voxel-to-Voxel) or against others ROIs (ROI-to-ROI) (Figure 3.2). In this context, the pre-defined ROI is called seed, which can be chosen based on the relation with specific tasks, disease or disorder (VAN DEN HEUVEL; HULSHOFF POL, 2010a). After choosing the seed, the next step is to measure the connectivity between the time series of the seed and another ROI or voxel, usually by calculating the Pearson's correlation:

$$r = \frac{C_{x,y}}{S_x S_y} \quad (3.1)$$

Which $C_{x,y}$ is the covariance or the variance from x and y variables, S_x and S_y are their standard deviation. The covariance is defined as a measure of how both variables change together. In our case, the variables are the time series. Then, a high level of correlation between both time series means that both regions have a high level

of functional connectivity. Therefore, expanding it for all voxels and their correlations with the seed, there is a seed-based FC map. Generally, a Z Fisher transformation is applied on the correlation coefficient; the purpose is to reach a normal distribution of the data, and then be able to perform a group analysis (WHITFIELD-GABRIELI; NIETO-CASTANON, 2012).

The advantage of using the seed approach is that you can select a specific ROI if you already know what your goal is. One study reported that using subject-specific ROIs the data is less susceptible to white matter (WM) and cerebrospinal fluid (CSF) noise, because the time series extracted are from functionally relevant regions for the that individual (SOHN et al., 2015).

Figure 3.2: Seed-based method (ROI-to-ROI) for functional connectivity analysis between left (L) and right (R) motor (MOT) areas. Adapted from Biswal experiment (VAN DIJK et al., 2010).

Source: VAN DIJK et al., 2010

3.2. INDEPENDENT COMPONENT ANALYSIS (ICA)

One disadvantage of the seed-based method is the need of a priori definition; this problem is partially overcome using the ICA method. ICA is a data-driven method

designed to search for a mixture of underlying sources that, in our context, can explain the resting-state patterns, looking for the existence of spatial sources of resting-state signals that are maximally independent from each other.

ICA is a group of methods for blind signal separation based on assumed statistical independence of source signals (MCKEOWN; HANSEN; SEJNOWSK, 2003). The divergent nature of the signals that contribute to fMRI recordings suggests that blind separation techniques can isolate these different sources (MCKEOWN; HANSEN; SEJNOWSK, 2003). Both spatial ICA and temporal ICA can be performed; however, for fMRI, spatial ICA is the most used approach. Spatial components are assumed to be statistically independent processes and the idea is to search for signals that are separated accordingly to the variance of the voxels to minimize mutual information between them (BROWN; YAMADA; SEJNOWSKI, 2001).

Typical algorithms for ICA use centering (subtract the mean to create a zero mean signal), whitening (usually with the eigenvalue decomposition), and dimensionality reduction. The most widespread and with higher-order statistical algorithms are the whitening, and it can be achieved using PCA. Well-known algorithms for ICA are the Infomax, FastICA, and Jade (MCKEOWN; HANSEN; SEJNOWSK, 2003).

Because the high dimensionality and high noise level of fMRI data, applying ICA on spatial or temporal dimension is likely to overfit the data and thus degrade the ICA estimation (LI; ADALI; CALHOUN, 2007). Therefore, the number of informative components is often assumed to be less than the spatial or temporal dimension of the fMRI data, and this step has important implications on the final result of ICA, being a drawback of the method (LI; ADALI; CALHOUN, 2007).

Table 3.1: Advantages and disadvantages of using seed-based and ICA approaches in rs-fMRI.

Method	Advantages	Disadvantages
Seed	Straightforward, simplicity	Totally dependent on the user defined ROI
ICA	Prior knowledge is not required	Prior informative component

Source: Author

On the other hand, in addition to not required a priori knowledge, ICA can be used to investigate multiple and simultaneous voxel-to-voxel interactions of distinct networks in the brain for individual or group analysis (MCKEOWN; HANSEN; SEJNOWSK, 2003; SMITHA et al., 2017).

3.3. GRAPH THEORY

Network or Graph is a mathematical representation of a real-world complex system using a collection of nodes (vertices) and edges (links) (Figure 3.3). In a social network, for example, people represent the nodes and the friendship between two of them is the link. In the context of RS-fMRI, nodes and edges represent the brain regions and their relationship (connectivity), respectively (ONIAS et al., 2014; RUBINOV; SPORNS, 2010; WANG, 2010). Neighbor or adjacent is the term used when two nodes are connected by a link. When a label or a weight is assigned to each link, it is called a weighted network. Otherwise, the network is called binary (or unweighted) (ONIAS et al., 2014). The common representation is the adjacency matrix (Figure 3.4).

Figure 3.3: Graph examples.

Source: Author

Figure 3.4: Example of a weighted adjacent matrix. As we can see we maintain the connectivity value.

Source: Author

Graph theory for FC analysis is an important tool and has the ability to show different aspects of the connectivity through several graph parameters, for example: node degree and strength; clustering coefficient; global and local efficiencies; and average path length (Table 3.2) (SMITHA et al., 2017). The degree and strength of a node, clustering coefficient and local efficiency are local properties, while global efficiency and path length are global properties of the graph.

Table 3.2: Topographic measures commonly used in fMRI studies and their definitions.

Measure	Definition
Degree	number of links connected to the node
Strength	sum of weights of links connected to the node
Clustering coefficient	local interconnection ability of the network
Global Efficiency	quantification of the exchange of information across the whole network
Local efficiency	characterization of how well information is exchanged by the neighbors when the node is removed
Path length	smallest number of edges required to connect one node to the other nodes in the network

Source: Rubinov and Sporns, 2010

4. MOTIVATION AND OBJECTIVES

Schizophrenia is a severe mental disorder, and in its most common form, schizophrenic individuals present themselves with paranoid delusions and auditory hallucination late in adolescence or early adulthood. A relevant issue on schizophrenia is the high rate of morbidity and mortality. A Finnish study in 2010 reported that 7% rate of suicide in schizophrenia, accounting 50% of all deaths by the age of 39 (ALARÄISÄNEN et al., 2009). Moreover, the life expectancy for those with serious mental illness can be reduced by 20 years. Therefore, it is of scientific interest and clinical relevance to investigate this disorder and try to add more information that may help its diagnosis and treatment.

Since schizophrenia is a neurodevelopmental disorder and patients can present several symptoms, including mood alterations and cognitive impairment, it is of interest to investigate their RBNs. In this context, ASL is a noninvasive tool that provides two information: quantitative regional CBF, and FC during resting-state. Therefore, the main objective of the present study was to assess resting-state FC and its relationship with regional CBF in schizophrenia patients using a pCASL method. Then, our specific objectives included:

- Evaluation of cerebral perfusion through quantitative CBF maps;
- Identification of resting-state brain networks using ICA and seed-based approaches;
- Evaluation of functional connectivity in schizophrenia using ICA, seed-based approaches and graph theory;
- Correlation between MRI results and the psychopathology of schizophrenia.

5. METHODOLOGY

5.1. SUBJECTS

Twenty-eight patients with schizophrenia and twenty-eight healthy age-matched subjects participated in the study. Patients were recruited at the Psychiatry Clinic of Medical School University Hospital – University of Sao Paulo (HCFMRP-USP) by a trained psychiatrist who applied the diagnostic criteria of the DSM-V. To be eligible for inclusion in the study, patients had to be between 18 and 45 years of age and have a diagnosis of schizophrenia with the presence of positive or negative symptoms, that is, score equal or greater than four in any item of the PANSS (Appendix A). All patients were in antipsychotic treatment when the MRI scans were performed.

Table 5.1: Demographic and clinical characteristics of the schizophrenia patients and control group.

Variables (Mean $\pm$ SD)	Schizophrenic patients	Healthy controls	p-value
Gender (female/male)	4/24	8/20	0.192 ^a
Age (years)	32.8 $\pm$ 7.8	31.1 $\pm$ 5.8	0.178 ^b
Duration of illness (years)	14.3 $\pm$ 8.3	-	-
Antipsychotic medication**			
CZP – Clozapine	23		
CLZ – Chlorpromazine	1		
OLZ – Olanzapine	3		
QTP – Quetiapina	1		
RSP – Risperidone	1		
SUL - Sulpirida	1		
PLP - Paliperidone	1		
PANSS			
Positive score	14.4 $\pm$ 6.4	-	-
Negative score	23.1 $\pm$ 8.2	-	-
Total score	69.4 $\pm$ 17.2	-	-

^aThe p-value was obtained by chi-square test

^bThe p-value was obtained by two sample t-test (Welch's test)

** There is one patients in treatment with CZP + RSP, one with CZP+SUL, one CZP+OLZ and one PLP+OLZ

Source: Author

For both groups, exclusion criteria included the presence of systemic medical illness such as cardiovascular disease, severe hypertension, kidney failure, liver disease, hypothyroidism, and epilepsy; any indication of neurovascular diseases (atherosclerotic, stroke or tumor); pregnancy; breastfeeding; drug and alcohol abuse; and presence of prosthesis incompatible with the magnetic resonance environment. The demographic and clinical characteristics of the two groups are described in Table 5.1.

The study was approved by the local ethics committee (Appendix B), and all volunteers gave their written consent to participate after being informed about the experimental procedures.

5.2. IMAGE ACQUISITION

MRI was performed in a 3T system (*Philips Achieva*, The Netherlands) using a 32-channel head coil for reception and a body coil for transmission. Comfortable foam pads and earplugs were used to minimize head motion and protect from scanner noise, respectively. Anatomical reference images were acquired using a 3D gradient-echo T1-weighted sequence with the following parameters: TR/TE = 7/3.2 ms, FA = 8°, matrix = 240 x 240, FOV = 240 x 240 mm², 160 1-mm slices.

Pseudocontinuous ASL images at resting-state were obtained using a 2D single-shot EPI sequence with the following parameter: TR/TE = 4000/14 ms, FA = 90°, FOV = 240 x 240 mm², matrix = 160 x 160, 20 5-mm slices, no gap; LT = 1650 ms, PLD = 1525 ms, 50 control/label pairs, total scan duration = 6 min and 48 s. All subjects were instructed to stay awake, think of nothing in particular and move as little as possible during the scan. For CBF quantification, a proton density (M_0) image was acquired separately with the same geometry and parameters of the pCASL sequence, but without labeling pulses.

In addition, other structural image (FLAIR) were acquired following the protocol used for clinical routine at the HCFMRP-USP, and used to check for brain lesion and vascular occlusion.

5.3. DATA PROCESSING

All data preprocessing was performed using Statistical Parametric Mapping (SPM12; <http://www.fil.ion.ucl.ac.uk/spm/software/spm12/>). For ASL images we used batch-based scripts available in ASLtbx (WANG et al., 2008) according to following steps: motion correction, coregistration to anatomical images, temporal filtering, spatial smoothing with an isotropic Gaussian kernel (FWHM = 4 mm for CBF quantification, and FWHM = 6 mm for CBF-FC analysis), CBF quantification, and normalization to MNI standard space (resolution: 2 x 2 x 2 mm³, matrix size: 79 x 95 x 79). The amended algorithm² was used for ASL image de-noising. Residual from motion correction and global signal were regressed out from the control/label ASL images (WANG, 2012) before CBF calculations. For quantification purposes, outlier CBF images were removed using the SCORE algorithm (DOLUI et al., 2016). Figure 5.1 shows the processing pipeline for both purposes (functional connectivity assessment and average CBF quantification).

Motion correction not only sought to eliminate the effects of accidental movements of the subjects, but also involuntary movements such as respiratory. Periodic involuntary movements can be highlighted by coregistration functions, in which certain volumes (from the subject itself) are taken by reference as being stationary. Therefore, there are always residual motion effects, due to the motion at each time point and to the relative motion between the control image and the label image within each pair (WANG, 2012).

To attenuate the noise in our data, we chose to do the head motion correction, followed by temporal filtering, spatial smoothing, and outlier cleaning in the quantitative CBF approach improving the SNR. Temporal filtering was performed using a high-pass Butterworth filter (cutoff frequency = 0.04 Hz) on control and label images to remove the low frequency part of the MR signal fluctuations before any further analysis. Using the filtering algorithm, we also removed residual motion and global fluctuations exactly as Ze Wang proposed to improve CBF quantification, reducing the zig-zag effect added by signal interpolation. Then, spatial smoothing was applied on control and label images to improve SNR before calculating perfusion by subtraction.

After CBF quantification, we used the SCORE algorithm to identify outlier volumes. This algorithm is based on spatial correlation, that is, a few corrupted volumes can cause a significant spatially constrained artifact. In this case, the individual volume in the time series that contributed most to the artifact on the mean CBF is the one that shows the highest spatial correlation with the mean. A mixture of the mean CBF map

and random noise in individual volumes represents non-outliers. While image averaging reduces the random noise in the resulting mean map, the artifacts present in the outliers would remain in the mean CBF map. Then the volumes having the highest spatial correlations with the intermediate mean CBF map are iteratively removed, followed by an update of the intermediate mean using the remaining mean CBF until no more outliers are detected (DOLUI et al., 2016).

Figure 5.1: Processing pipeline for both purposes: (A) functional connectivity assessment and (B) average CBF quantification.

Source: Author

5.4. CBF QUANTIFICATION

To quantify and assess resting CBF, perfusion images were obtained by sinc subtraction (Figure 5.1). Because the CBF fluctuations obtained from ASL methods contain a small fraction of BOLD effect, known as BOLD contamination (AGUIRRE et al., 2002), we used sinc subtraction to remove this contamination correcting for the temporal offset between label and control images (CHEN; JANN; WANG, 2015). It is done isolating the control or the label images from the total, doubling the temporal

resolution using sinc interpolation, and then resampling at the intermediate points in time. Equation 5.1 shows an example when isolating the control images.

$$[C_1, C_2, \dots, C_{\frac{n}{2}}] \rightarrow [C_{\frac{1}{2}}, C_1, C_{\frac{3}{2}}, \dots, C_{n-\frac{1}{2}}, C_{\frac{n}{2}}] \rightarrow [C_{\frac{1}{2}}, C_{\frac{3}{2}}, \dots, C_{n-\frac{1}{2}}] \quad (5.1)$$

Where C and n are, respectively, the control image and the dynamic number; and $n/2$ is the total number of control/label pairs. The result is an estimate of the control images if they were obtained at the same time as the label images (AGUIRRE et al., 2002).

Figure 5.2: Subtraction approaches to obtain perfusion-weighted images. Pairwise subtraction relies on the simple subtraction of the control image from the label image in the acquisition sequence.

Source: Author

For CBF quantification, we used General Kinect Model (BUXTON et al., 1998) (equation 2.14) with the following parameters: longitudinal relaxation time of blood =

1650 ms (at 3 T); labeling efficiency = 0.85; blood/tissue water partition coefficient = 0.9 g/mL (ALSOP et al., 2015); longitudinal relaxation time of tissue = 1020 for gray matter (GM) and 770 for WM, previously calculated from images acquired using a Look-Locker sequence (KARLSSON; NORDELL, 2000; LOOK; LOCKER, 1970). An average CBF map was obtained to assess regional differences between groups. Anatomical regions of interest (ROIs) were selected from the Harvard-Oxford atlas, excluding cerebellum and vermis areas not covered by our image acquisition.

5.5. FUNCTIONAL CONNECTIVITY

To assess FC from CBF maps, perfusion images were obtained by pairwise subtraction to improve the number of points of the time series (Figure 5.2). Then, CBF maps were calculated the same way as described in the previous section.

In the FC toolbox (version 17a) (WHITFIELD-GABRIELI; NIETO-CASTANON, 2012), CBF images were detrended and filtered with a low-pass filter ($f < 0.07$ Hz) (BOISSONEAULT et al., 2016; FERNÁNDEZ-SEARA et al., 2015; HAM et al., 2013). In addition, WM and CSF signals were removed by PCA with five components each (CONN default), using the CompCor algorithm (BEHZADI et al., 2007).

To obtain the RBNs from CBF maps, we first used group-level ICA with an *a priori* selection of 20 components, the fastICA algorithm for the estimation of spatially independent components, and GICA1 backprojection for individual subject-level spatial map estimation. The ICA group approach is the same as Calhoun published in 2001 (CALHOUN et al., 2001). Then, we assessed the spatial similarity of RBNs between groups (controls versus patients) calculating the Dice Similarity Coefficient (DSC) (DICE, 1945; JANN et al., 2015; ZHU et al., 2013).

We also performed a seed-based approach for FC analysis. Pearson's correlation was obtained between average filtered GM time series of anatomical and functional ROIs, selected from the Harvard-Oxford atlas and the FC toolbox, respectively. They totaled 136 ROIs used as seeds. Moreover, to confirm the frontal inefficiency in schizophrenia patients, an analysis using the dorsolateral prefrontal cortex (DLPFC; Figure 5.3) as seed was performed.

FC within some RBNs was also assessed: (1) Default Mode Network (DMN), comprising the medial prefrontal cortex (mPFC; 1, 55, -3), bilateral inferior parietal lobule (IPL; -39, -77, 33 left; 47, 67, 29 right) and posterior cingulate cortex (PCC; 1, -61, 38); (2) Salience Network (SN), formed by the anterior cingulate cortex (ACC; 0, 22, 35), bilateral anterior insula (AI; -44, 13, 11 left; 47, 14, 0 right), bilateral rostral prefrontal cortex (rPFC; -32, 45, 27 left; 32, 46, 27 right), and bilateral supramarginal gyrus (SMG; -60, -39, 31 left; 62, -35, 32 right); (3) Fronto-Parietal Network (FPN), formed by the bilateral lateral prefrontal cortex (LPFC; -43, 33, 28 left; 41, 38, 30 right), bilateral posterior parietal cortex (PPC; -46, -58, 49 left; 52, -52, 45 right); (4) Dorsal Attention Network (DAN), which includes the bilateral frontal eye field (FEF; -27, -9, 64 left; 30, -6, 64 right) and bilateral intraparietal sulcus (IPS; -39, -43, 52 left; 39, -42, 54 right); (5) Visual Network (VN), formed by the primary (2, -79, 12), ventral (0, -93, -4), and bilateral dorsal visual areas (-37, -79, 10 left; 38, -72, 13 right); and the (6) Sensorimotor Network (SMN), including the bilateral superior (0, -31, 67) and lateral motor cortices (-55, -12, 29 left; 56, -10, 29 right). The Central Executive Network (CEN) was also investigated through measures collected from the posterior parietal cortex (PPC), frontal eye fields, and part of the dorsomedial prefrontal cortex (dmPFC) (MULDERS et al., 2015). The numbers between parentheses correspond to coordinates x, y and z of the center of mass in MNI space.

Figure 5.3: Left (red) and right (blue) parts of the dorsolateral prefrontal cortex (DLPFC).

Source: Author

5.5.1. GRAPH ANALYSIS

The FC toolbox provides the Fisher's Z-transformation as a correlation matrix $Z(i,j)$, representing the level of functional connectivity between ROI i and ROI j . This matrix was thresholded selecting the 10% of the highest connections, regardless of the coefficient values (FORNITO et al., 2012), for each subject separately, resulting in an adjacency matrix, W , used to create individual brain graphs. Once the connectivity matrix was calculated for each subject, we were able to compute several properties of its organization. For each node, we calculated the degree, strength, clustering coefficient, path length, and global and local efficiencies using the *Brain Connectivity Toolbox* (RUBINOV; SPORNS, 2010). Figure 5.4 shows the steps with followed to perform the graph analysis.

Figure 5.4: Pipeline for graph analysis. 1 – Extraction of the time series (C) from CBF maps (B) within each anatomical ROI (node), obtained based on the Harvard-Oxford atlas (A). 2 – Calculation of functional connectivity correlation matrix (D) between any pair of nodes. 3 – Thresholding of the correlation matrix to obtain an association weighted matrix (E). 4 – Visualization of the association weighted matrix as graph (F).

Source: Author

5.6. STATISTICAL ANALYSIS

We used the software *R* to perform all the statistical analysis (R DEVELOPMENT CORE, 2008). Group differences were assessed at the ROI level using two-sample *t*-tests (Welch's *t*-test). FDR correction for multiple comparisons was used on mean CBF maps. For the analysis of FC, multiple comparisons were corrected for Sidak, adjusting correlated tests as though they were independent according to an effective number of independent tests (LI; JI, 2005).

6. RESULTS

A total of 28 schizophrenia patients and 28 healthy controls were included in the final analysis. Demographic and clinical characteristics were previously shown in Table 5.1. There was no difference between groups regarding age ($p = 0.178$) and gender ($p = 0.192$).

6.1. REGIONAL CBF

Figure 6.1 shows the average CBF maps of both subject groups. Compared with healthy controls, patients showed decreased CBF in the bilateral frontal poles (FP), bilateral superior frontal gyri (SFG), right middle frontal gyrus (MidFG), right inferior frontal gyrus pars triangularis (IFGtri), right inferior frontal gyrus pars opercularis (IFGoper), posterior division of the left supramarginal gyrus (pSMG), superior and inferior divisions of the left lateral occipital cortex (sLOC, iLOC), and bilateral occipital poles ($p < 0.05$, FDR-corrected). In contrast, patients with schizophrenia had increased CBF in the posterior division of the right parahippocampal gyrus (pPAHC) and right temporal fusiform cortex (pTFUsc), bilateral putamen and right hippocampus ($p < 0.05$, uncorrected for multiple comparisons).

Figure 6.1: Quantitative maps of CBF for (A) schizophrenia patients and (B) healthy controls. (L: left hemisphere, R: right hemisphere). (C) Regions with decreased CBF in patients.

Source: Author

6.2. FUNCTIONAL CONNECTIVITY

Using ICA and a selection of 20 independent components, we were able to obtain two RBNs for both groups (Figure 6.2): DMN, formed by the mPFC, PCC, and bilateral IPL; and CEN, including the bilateral LPFC and IPS. Both networks had reduced extension in patients compared to controls, and DSC between groups were 0.56 and 0.48 for the DMN and the CEN, respectively. Moreover, we found significantly decreased CBF in the mPFC ($p = 0.012$, FDR-corrected), right LPFC ($p = 0.030$), left IPS ($p = 0.038$), and right IPS ($p = 0.037$) of schizophrenia patients compared to healthy controls (Table 6.1).

Figure 6.2: Default Mode (DMN) and Central Executive (CEN) networks of schizophrenia patients (blue) and healthy subjects (red), showing differences in their spatial distribution.

Source: Author

Table 6.1: CBF values, in mL/100g/min, extracted from ICA masks of both groups.

Schizophrenic Patients					Healthy Controls			
DMN	IPL_L	ILP_R	PCC	mPFC	IPL_L	ILP_R	PCC	mPFC
	42 ± 12	51 ± 14	64 ± 17	35 ± 10*	46 ± 9	50 ± 9	64 ± 13	43 ± 10
CEN	LPFC_L	LPFC_R	IPS_L	IPS_R	LPFC_L	LPFC_R	IPS_L	IPS_R
	35 ± 10	40 ± 9 ⁺	39 ± 14 ⁺	40 ± 12 ⁺	35 ± 8	45 ± 10	45 ± 11	45 ± 11

Values are reported as mean ± standard deviation; L: left, R: right

* $p < 0.05$ (FDR-corrected); ⁺ $p < 0.05$ (uncorrected)

Source: Author

In ROI-based approach, we first analyze the 30 pre-select ROIs from each RBNs. We compared the connectivity between ROIs and also the strength within the specific RBNs. For the group comparison, the following results show decreased CBF connectivity in Schizophrenia patients:

- SN: ACC & right AI ($p = 0.037$), ACC & right rPFC ($p = 0.042$), left AI & left SMG ($p = 0.036$), left AI & right SMG ($p = 0.035$), right AI & left rPFC ($p = 0.012$), right AI & right rPFC ($p = 0.022$) and right AI & left SMG ($p = 0.014$). However, none survives a Sidak correction. The strength within SN was significantly different for all ROIs ($p < 0.016$, Sidak-corrected).
- Sensorimotor network: left lateral & superior ($p = 0.035$) and right lateral & superior ($p = 0.016$, Sidak-corrected). The strength within the sensorimotor network was significantly different for all ROIs ($p < 0.025$, Sidak-corrected).
- CEN: left FEF & right FEF ($p = 0.008$), left FEF & right IPS ($p = 0.02$), right FEF & left IPS ($p = 0.012$), right FEF & right IPS ($p = 0.016$), left FEF & left LPFC ($p = 0.0027$, Sidak-corrected), left FEF & right LPFC ($p = 0.014$), left IPS & right LPFC ($p = 0.036$) and left IPS & right LPFC ($p = 0.003$). The strength within the CEN was significantly different in left FEF ($p = 0.025$), right FEF ($p = 0.014$), left IPS ($p = 0.026$), left LPFC ($p = 0.021$), right LPFC ($p < 0.0125$, Sidak-corrected).

On the other hand, increased CBF connectivity in schizophrenia patients were observed for:

- DMN: left IpL & PCC ($p = 0.04$).

- SN: left rPFC & right rPFC ($p = 0.022$).
- CEN: left FEF & left PPC ($p = 0.049$).

In summary, schizophrenia patients had reduced FC between right lateral and superior motor regions ($p = 0.004$) and between the left FEF and left LPFC ($p = 0.001$). Moreover, all regions of the SN ($p < 0.02$) and the SMN ($p < 0.03$) in addition to the right LPFC ($p < 0.01$) showed decreased FC strength in patients compared to healthy controls. No increased connectivity between regions or their strengths were observed in patients when correcting for multiple comparisons.

Individual connectivity matrices involving all 103 anatomic nodes, interconnected by 10609 edges, showed an overall reduction of functional connectivity in patients compared to healthy controls, as exemplified in Figure 6.3. The distribution of connectivity weights showed fewer edges with high correlation values ($r > 0.4$) in schizophrenia patient (red curve), compared to healthy control (blue curve; Figure 6.3B). The adjacency matrices thresholded to match connection densities for both subjects are shown in Figure 6.3C. The mean cutoff value of the correlation coefficient was significantly reduced for the patient group ($r_{\text{mean}} = 0.44$) compared to healthy controls ($r_{\text{mean}} = 0.47$, $p = 0.004$).

Figure 6.3: (A) Representative functional connectivity matrices of a patient and a healthy control, involving 103 nodes (Anatomical atlas) interconnected by 10609 edges. (C) Distribution of correlation coefficients for matrices shown in A. (B) Connectivity matrices after thresholding to match connection densities, with lower minimum correlation coefficient for the patient compared to the healthy control.

Source: Author

Significant differences in degree, clustering coefficient, and local efficiency were also observed between groups (Figure 6.4). For global properties, only path length showed significant difference ($p=0.02$) (Figure 6.5). Regions with significant differences in at least three of the network properties and/or regional CBF included frontal, temporal and occipital regions, and also the putamen and thalamus.

Figure 6.4: Brain regions with significant differences of nodal properties (degree, strength, cluster coefficient and local efficiency) between patients and healthy controls.

Source: Author

Finally, decreased connectivity between the DLPFC and the left posterior central gyrus (PostCG) was observed in schizophrenia patients ($p = 0.002$, Sidak-corrected). Disregarding the correction for multiple comparisons, other regions also showed reduced connectivity with the DLPFC in patients compared to healthy controls, including the left precentral gyrus (PreCG), left supplementary motor area (SMA), right temporooccipital part of the middle temporal gyrus (toMTG), right pSMG, right angular gyrus (AG), and sLOC ($p < 0.05$, uncorrected).

Figure 6.5: No significant difference in global efficiency and significant difference in characteristic path length between groups.

Source: Author

7. DISCUSSION

We used a pCASL sequence to assess resting-state CBF and functional connectivity in schizophrenia patients and healthy age-matched controls. Patients showed decreased CBF in anatomical regions of frontal, parietal and occipital lobes, and in functional regions that constitute DMN and CEN. These findings are consistent with previous studies in schizophrenia using ASL (CUI et al., 2016; KINDLER et al., 2015; OTA et al., 2014; PINKHAM et al., 2011; SCHEEF et al., 2010; ZHU et al., 2015), Positron Emission Tomography (POTKIN et al., 2002) and Single-Photon Emission Computed Tomography (KANAHARA et al., 2009).

Regions of the frontal lobe with decreased perfusion in patients at rest are related to the regulation and processing of behavior and emotion, and with mentalizing (GREEN; HORAN; LEE, 2015; KOECHLIN; ODY; KOUNEIHHER, 2003; MILLER; COHEN, 2001). In particular, DLPFC plays an important role in executive control and working memory (CEASER, 2013; POTKIN et al., 2009). These results are consonant with frequent features of schizophrenia, such as difficulty of prioritizing, processing, and responding to information (SALGADO-PINEDA et al., 2007), and confirm the frontal inefficiency related to the pathology (CATANI et al., 2012; SHEFFIELD; BARCH, 2016). Other reported characteristics of schizophrenia are the impaired control of empathy towards other people and the impaired visual perception (BONFILS et al., 2016; BORA; GÖKÇEN; VEZNEVAROGLU, 2008; ZHU et al., 2015), which may be related to CBF deficits in SMG and occipital regions (BUTLER; SILVERSTEIN; DAKIN, 2008; OTA et al., 2014; SILANI et al., 2013), respectively.

Regarding the RBNs, both DMN and CEN obtained with ICA showed reduced extent and different spatial distribution in patients. This result may be related to the decreased ability of schizophrenia patients to engage in self-processing during non-goal-directed activities (PINKHAM et al., 2011), and also to the working memory deficits described in this population (CEASER, 2013; POTKIN et al., 2009), which supports the results obtained with the regional CBF analysis. Moreover, other regions showed impaired functional connectivity in patients. Decreased connectivity strengths of LPFC, and regions of the SMN and SN were observed in patients compared to healthy controls, indicating that the extent of local binding within these networks decreases in schizophrenia (WANG et al., 2014). Previous electroencephalography and fMRI results

suggest that LPFC supply feedback in a top-down manner to IPS and FEF, and the latter regions modulates activity in the visual cortex to bias sensory processing (LIU et al., 2016). However, there is a debate about whether the disconnections in sensorimotor areas seen in these patients are part of the pathological processes of schizophrenia or a side effect from antipsychotic medications (BERMAN et al., 2016; WALTHER; STRIK, 2012).

Graph theory analysis showed significant increase in characteristic path length of schizophrenia network, indicating less global integration; in addition to local hypo- and hyper-connectivity, as previously reported in functional MRI studies (FORNITO et al., 2012). This suggests that schizophrenia affects short-range connections and is related with the alteration of multiple systems (LIU; ZHUO; YU, 2016).

More specifically, bilateral paracingulate gyrus (PaCiG), ACC, and right FP presented decreased values of at least three local nodal properties. Previous studies reported that alterations in cingulate cortex of schizophrenia patients are related to psychotic symptoms, attentional deficits, difficulty in cognitive and emotional integration, and impairment in detecting sources of potential conflict and controlling to avoid errors (SCHEEF et al., 2010; SUTCLIFFE et al., 2016; YÜCEL et al., 2002; ZHU et al., 2015).

On the other hand, subcortical regions, such as thalamus and putamen, showed increased values of nodal degree, connectivity strength, clustering coefficient, and local efficiency. Alterations in thalamic connections were reported to be related to dysfunction in the coordination of mental activities and, therefore, to the psychotic symptoms of schizophrenia (SCHEEF et al., 2010). In addition, the putamen is also involved in cognitive and emotional functions, but its hyper-connectivity has been linked to the use of antipsychotic drugs (PINKHAM et al., 2011; ZHU et al., 2015).

In summary, our results showed reduced regional CBF and altered connectivity pattern with the presence of hypo- and hyper-connectivity in schizophrenia patients compared with healthy controls. Taking together, they suggest a diffuse dysregulation of blood flow distribution or possible compensatory changes to primary deficits in schizophrenia.

However, our study presents some limitations. We should mention that the 2D pCASL sequence has lower temporal resolution and SNR compared to BOLD EPI; however, its signal comes from the capillary bed, providing higher spatial specificity for neuronal activity, and CBF is more directly related to cerebral metabolism than BOLD

signal. Another issue is our sample size, which was relatively small for the nodal-level Graph Theory analysis because of the large number of links. Moreover, this analysis requires an appropriate choice of the Pearson's correlation coefficient. The approach used here considering the connectivity threshold as the highest 10% connections seems to be more reliable to assess nodal and global proprieties in cross-sectional studies in schizophrenia; however, for further studies should adopt variations of the correlation coefficients to investigate the dependency of the network proprieties.

8. CONCLUSION

Using the proposed methodology with a 2D pCASL method, we were able to quantify regional CBF and assess functional connectivity, highlighting abnormalities present in schizophrenia. More specifically, our conclusions include:

- Cerebral perfusion were successfully evaluated through quantitative CBF maps, and decreased CBF values were observed in anatomical regions of frontal, parietal and occipital lobes, and in functional regions that constitute DMN and CEN of schizophrenia patients compared to healthy controls.
- Two resting-state brain networks were identified using ICA, DMN and CEN, and both showed reduced extent and different spatial distribution in schizophrenia patients compared to healthy controls.
- Functional connectivity was successfully evaluated using three different approaches (ICA, seed-based and graph theory), and the presence of hypo- and hyper-connectivity in schizophrenia patients compared with healthy controls was observed.

Schizophrenia is a severe mental disorder, which affects many aspects of the patients' life. The impaired cognition and morbidity is a huge problem not only for the patients, but also their families. Prevention, early detection and efficient intervention are the goals of the studies to provide substantial improvements in the prognostic of the disease. Therefore, CBF may be used as a physiological marker of brain vulnerability in schizophrenia, and our methodology used as a complementary tool to assess functional abnormalities.

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APPENDIX A – POSITIVE AND NEGATIVE SYNDROME SCALE

(PANSS) RATING CRITERIA GENERAL RATING INSTRUCTIONS

Data gathered from this assessment procedure are applied to the PANSS ratings. Each of the 30 items is accompanied by a specific definition as well as detailed anchoring criteria for all seven rating points. These seven points represent increasing levels of psychopathology, as follows:

- 1- absent
- 2- minimal
- 3- mild
- 4- moderate
- 5- moderate severe
- 6- severe
- 7- extreme

In assigning ratings, one first considers whether an item is at all present, as judging by its definition. If the item is absent, it is scored 1, whereas if it is present one must determine its severity by reference to the particular criteria from the anchoring points. The highest applicable rating point is always assigned, even if the patient meets criteria for lower points as well. In judging the level of severity, the rater must utilise a holistic perspective in deciding which anchoring point best characterises the patient's functioning and rate accordingly, whether or not all elements of the description are observed.

The rating points of 2 to 7 correspond to incremental levels of symptom severity:

- A rating of 2 (minimal) denotes questionable or subtle or suspected pathology, or it also may allude to the extreme end of the normal range.
- A rating of 3 (mild) is indicative of a symptom whose presence is clearly established but not pronounced and interferes little in day-today functioning.
- A rating of 4 (moderate) characterises a symptom which, though representing a serious problem, either occurs only occasionally or intrudes on daily life only to a moderate extent.
- A rating of 5 (moderate severe) indicates marked manifestations that distinctly impact on one's functioning but are not all-consuming and usually can be contained at will.
- A rating of 6 (severe) represents gross pathology that is present very frequently, proves highly disruptive to one's life, and often calls for direct supervision.
- A rating of 7 (extreme) refers to the most serious level of psychopathology, whereby the manifestations drastically interfere in most or all major life functions, typically necessitating close supervision and assistance in many areas.

Each item is rated in consultation with the definitions and criteria provided in this manual. The ratings are rendered on the PANSS rating form overleaf by encircling the appropriate number following each dimension.

PANSS RATING FORM

		<u>abs: minimal</u>	<u>mild</u>	<u>moderate</u>	<u>moderate</u>	<u>severe</u>	<u>severe</u>	<u>extreme</u>
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P1	Delusions	1	2	3	4	5	6	7
P2	Conceptual disorganisation	1	2	3	4	5	6	7
P3	Hallucinatory behaviour	1	2	3	4	5	6	7
P4	Excitement	1	2	3	4	5	6	7
P5	Grandiosity	1	2	3	4	5	6	7
P6	Suspiciousness/persecution	1	2	3	4	5	6	7
P7	Hostility	1	2	3	4	5	6	7

N1	Blunted affect	1	2	3	4	5	6	7
N2	Emotional withdrawal	1	2	3	4	5	6	7
N3	Poor rapport	1	2	3	4	5	6	7
N4	Passive/apathetic social withdrawal	1	2	3	4	5	6	7
N5	Difficulty in abstract thinking	1	2	3	4	5	6	7
N6	Lack of spontaneity & flow of conversation	1	2	3	4	5	6	7
N7	Stereotyped thinking	1	2	3	4	5	6	7

G1	Somatic concern	1	2	3	4	5	6	7
G2	Anxiety	1	2	3	4	5	6	7
G3	Guilt feelings	1	2	3	4	5	6	7
G4	Tension	1	2	3	4	5	6	7
G5	Mannerisms & posturing	1	2	3	4	5	6	7
G6	Depression	1	2	3	4	5	6	7
G7	Motor retardation	1	2	3	4	5	6	7
G8	Uncooperativeness	1	2	3	4	5	6	7
G9	Unusual thought content	1	2	3	4	5	6	7
G10	Disorientation	1	2	3	4	5	6	7

G11	Poor attention	1	2	3	4	5	6	7
G12	Lack of judgement & insight	1	2	3	4	5	6	7
G13	Disturbance of volition	1	2	3	4	5	6	7
G14	Poor impulse control	1	2	3	4	5	6	7
G15	Preoccupation	1	2	3	4	5	6	7
G16	Active social avoidance	1	2	3	4	5	6	7

SCORING INSTRUCTIONS

Of the 30 items included in the PANSS, 7 constitute a **Positive Scale**, 7 a **Negative Scale**, and the remaining 16 a **General Psychopathology Scale**. The scores for these scales are arrived at by summation of ratings across component items. Therefore, the potential ranges are 7 to 49 for the Positive and Negative Scales, and 16 to 112 for the General Psychopathology Scale. In addition to these measures, a **Composite Scale** is scored by subtracting the negative score from the positive score. This yields a bipolar index that ranges from -42 to +42, which is essentially a difference score reflecting the degree of predominance of one syndrome in relation to the other.

POSITIVE SCALE (P)

P1. DELUSIONS - Beliefs which are unfounded, unrealistic and idiosyncratic.

Basis for rating - Thought content expressed in the interview and its influence on social relations and behaviour.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Presence of one or two delusions which are vague, uncrystallised and not tenaciously held. Delusions do not interfere with thinking, social relations or behaviour.
- 4 Moderate** - Presence of either a kaleidoscopic array of poorly formed, unstable delusions or a few well-formed delusions that occasionally interfere with thinking, social relations or behaviour.
- 5 Moderate Severe** - Presence of numerous well-formed delusions that are tenaciously held and occasionally interfere with thinking, social relations and behaviour.
- 6 Severe** - Presence of a stable set of delusions which are crystallised, possibly systematised, tenaciously held and clearly interfere with thinking, social relations and behaviour.
- 7 Extreme** - Presence of a stable set of delusions which are either highly systematised or very numerous, and which dominate major facets of the patient's life. This frequently results in inappropriate and irresponsible action, which may even jeopardise the safety of the patient or others.

- P2. CONCEPTUAL DISORGANISATION** - Disorganised process of thinking characterised by disruption of goal-directed sequencing, e.g. circumstantiality, loose associations, tangentiality, gross illogicality or thought block.
- Basis for rating** - Cognitive-verbal processes observed during the course of interview.
- 1 Absent** - Definition does not apply
 - 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
 - 3 Mild** - Thinking is circumstantial, tangential or paralogical. There is some difficulty in directing thoughts towards a goal, and some loosening of associations may be evidenced under pressure.
 - 4 Moderate** - Able to focus thoughts when communications are brief and structured, but becomes loose or irrelevant when dealing with more complex communications or when under minimal pressure.
 - 5 Moderate Severe** - Generally has difficulty in organising thoughts, as evidenced by frequent irrelevancies, disconnectedness or loosening of associations even when not under pressure.
 - 6 Severe** - Thinking is seriously derailed and internally inconsistent, resulting in gross irrelevancies and disruption of thought processes, which occur almost constantly.
 - 7 Extreme** - Thoughts are disrupted to the point where the patient is incoherent. There is marked loosening of associations, which result in total failure of communication, e.g. "word salad" or mutism.
- P3. HALLUCINATORY BEHAVIOUR** - Verbal report or behaviour indicating perceptions which are not generated by external stimuli. These may occur in the auditory, visual, olfactory or somatic realms. **Basis for rating** - Verbal report and physical manifestations during the course of interview as well as reports of behaviour by primary care workers or family.
- 1 Absent** - Definition does not apply
 - 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
 - 3 Mild** - One or two clearly formed but infrequent hallucinations, or else a number of vague abnormal perceptions which do not result in distortions of thinking or behaviour.
 - 4 Moderate** - Hallucinations occur frequently but not continuously, and the patient's thinking and behaviour are only affected to a minor extent.
 - 5 Moderate Severe** - Hallucinations occur frequently, may involve more than one sensory modality, and tend to distort thinking and/or disrupt behaviour. Patient may have a delusional interpretation of these experiences and respond to them emotionally and, on occasion, verbally as well.
 - 6 Severe** - Hallucinations are present almost continuously, causing major disruption of thinking and behaviour. Patient treats these as real perceptions, and functioning is impeded by frequent emotional and verbal responses to them.
 - 7 Extreme** - Patient is almost totally preoccupied with hallucinations, which virtually dominate thinking and behaviour. Hallucinations are provided a rigid delusional interpretation and provoke verbal and behavioural responses, including obedience to command hallucinations.

P4.	<p>EXCITEMENT - Hyperactivity as reflected in accelerated motor behaviour, heightened responsivity to stimuli, hypervigilance or excessive mood lability.</p> <p>Basis for rating - Behavioural manifestations during the course of interview as well as reports of behaviour by primary care workers or family.</p> <ol style="list-style-type: none"> 1 Absent - Definition does not apply 2 Minimal - Questionable pathology; may be at the upper extreme of normal limits 3 Mild - Tends to be slightly agitated, hypervigilant or mildly overaroused throughout the interview, but without distinct episodes of excitement or marked mood lability. Speech may be slightly pressured. 4 Moderate - Agitation or overarousal is clearly evident throughout the interview, affecting speech and general mobility, or episodic outbursts occur sporadically. 5 Moderate Severe - Significant hyperactivity or frequent outbursts of motor activity are observed, making it difficult for the patient to sit still for longer than several minutes at any given time. 6 Severe - Marked excitement dominates the interview, delimits attention, and to some extent affects personal functions such as eating or sleeping. 7 Extreme - marked excitement seriously interferes in eating and sleeping and makes interpersonal interactions virtually impossible. Acceleration of speech and motor activity may result in incoherence and exhaustion.
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P5.	<p>GRANDIOSITY - Exaggerated self-opinion and unrealistic convictions of superiority, including delusions of extraordinary abilities, wealth, knowledge, fame, power and moral righteousness. Basis for rating - Thought content expressed in the interview and its influence on behaviour.</p> <ol style="list-style-type: none"> 1 Absent - Definition does not apply 2 Minimal - Questionable pathology; may be at the upper extreme of normal limits 3 Mild - Some expansiveness or boastfulness is evident, but without clear-cut grandiose delusions. 4 Moderate - Feels distinctly and unrealistically superior to others. Some poorly formed delusions about special status or abilities may be present but are not acted upon. 5 Moderate Severe - Clear-cut delusions concerning remarkable abilities, status or power are expressed and influence attitude but not behaviour. 6 Severe - Clear-cut delusions of remarkable superiority involving more than one parameter (wealth, knowledge, fame, etc) are expressed, notably influence interactions and may be acted upon. 7 Extreme - Thinking, interactions and behaviour are dominated by multiple delusions of amazing ability, wealth, knowledge, fame, power and/or moral stature, which may take on a bizarre quality.
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P6. SUSPICIOUSNESS/PERSECUTION - Unrealistic or exaggerated ideas of persecution, as reflected in guardedness, ad distrustful attitude, suspicious hypervigilance or frank delusions that others mean harm.

Basis for rating – Thought content expressed in the interview and its influence on behaviour.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Presents a guarded or even openly distrustful attitude, but thoughts, interactions and behaviour are minimally affected.
- 4 Moderate** - Distrustfulness is clearly evident and intrudes on the interview and/or behaviour, but there is no evidence of persecutory delusions. Alternatively, there may be indication of loosely formed persecutory delusions, but these do not seem to affect the patient's attitude or interpersonal relations.
- 5 Moderate Severe** - Patient shows marked distrustfulness, leading to major disruption of interpersonal relations, or else there are clear-cut persecutory delusions that have limited impact on interpersonal relations and behaviour.
- 6 Severe** - Clear-cut pervasive delusions of persecution which may be systematised and significantly interfere in interpersonal relations.
- 7 Extreme** - A network of systematised persecutory delusions dominates the patient's thinking, social relations and behaviour.

P7. HOSTILITY - Verbal and nonverbal expressions of anger and resentment, including sarcasm, passive-aggressive behaviour, verbal abuse and assaultiveness.

Basis for rating – Interpersonal behaviour observed during the interview and reports by primary care workers or family.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Indirect or restrained communication of anger, such as sarcasm, disrespect, hostile expressions and occasional irritability.
- 4 Moderate** - Presents an overtly hostile attitude, showing frequent irritability and direct expression of anger or resentment.
- 5 Moderate Severe** - Patient is highly irritable and occasionally verbally abusive or threatening.
- 6 Severe** - Uncooperativeness and verbal abuse or threats notably influence the interview and seriously impact upon social relations. Patient may be violent and destructive but is not physically assaultive towards others.
- 7 Extreme** - Marked anger results in extreme uncooperativeness, precluding other interactions, or in episode(s) of physical assault towards others.

NEGATIVE SCALE (N)

- N1. BLUNTED AFFECT** - Diminished emotional responsiveness as characterised by a reduction in facial expression, modulation of feelings and communicative gestures. **Basis for rating** - Observation of physical manifestations of affective tone and emotional responsiveness during the course of the interview.
- 1 **Absent** - Definition does not apply
 - 2 **Minimal** - Questionable pathology; may be at the upper extreme of normal limits
 - 3 **Mild** - Changes in facial expression and communicative gestures seem to be stilted, forced, artificial or lacking in modulation.
 - 4 **Moderate** - Reduced range of facial expression and few expressive gestures result in a dull appearance
 - 5 **Moderate Severe** - Affect is generally 'flat' with only occasional changes in facial expression and a paucity of communicative gestures.
 - 6 **Severe** - Marked flatness and deficiency of emotions exhibited most of the time. There may be unmodulated extreme affective discharges, such as excitement, rage or inappropriate uncontrolled laughter.
 - 7 **Extreme** - Changes in facial expression and evidence of communicative gestures are virtually absent. Patient seems constantly to show a barren or 'wooden' expression.

- N2. EMOTIONAL WITHDRAWAL** - Lack of interest in, involvement with, and affective commitment to life's events.
- Basis for rating** - Reports of functioning from primary care workers or family and observation of interpersonal behaviour during the course of the interview.
- 1 **Absent** - Definition does not apply
 - 2 **Minimal** - Questionable pathology; may be at the upper extreme of normal limits
 - 3 **Mild** - Usually lack initiative and occasionally may show deficient interest in surrounding events.
 - 4 **Moderate** - Patient is generally distanced emotionally from the milieu and its challenges but, with encouragement, can be engaged.
 - 5 **Moderate Severe** - Patient is clearly detached emotionally from persons and events in the milieu, resisting all efforts at engagement. Patient appears distant, docile and purposeless but can be involved in communication at least briefly and tends to personal needs, sometimes with assistance.
 - 6 **Severe** - Marked deficiency of interest and emotional commitment results in limited conversation with others and frequent neglect of personal functions, for which the patient requires supervision.
 - 7 **Extreme** - Patient is almost totally withdrawn, uncommunicative and neglectful of personal needs as a result of profound lack of interest and emotional commitment.

N3. POOR RAPPORT - Lack of interpersonal empathy, openness in conversation and sense of closeness, interest or involvement with the interviewer. This is evidenced by interpersonal distancing and reduced verbal and nonverbal communication.

Basis for rating - Interpersonal behaviour during the course of the interview.

- 1 **Absent** - Definition does not apply
- 2 **Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 **Mild** - Conversation is characterised by a stilted, strained or artificial tone. It may lack emotional depth or tend to remain on an impersonal, intellectual plane.
- 4 **Moderate** - Patient typically is aloof, with interpersonal distance quite evident. Patient may answer questions mechanically, act bored, or express disinterest.
- 5 **Moderate Severe** - Disinvolvement is obvious and clearly impedes the productivity of the interview. Patient may tend to avoid eye or face contact.
- 6 **Severe** - Patient is highly indifferent, with marked interpersonal distance. Answers are perfunctory, and there is little nonverbal evidence of involvement. Eye and face contact are frequently avoided.
- 7 **Extreme** - Patient is totally uninvolved with the interviewer. Patient appears to be completely indifferent and consistently avoids verbal and nonverbal interactions during the interview.

N4. PASSIVE/APATHETIC SOCIAL WITHDRAWAL - Diminished interest and initiative in social interactions due to passivity, apathy, anergy or avolition. This leads to reduced interpersonal involvements and neglect of activities of daily living.

Basis for rating – Reports on social behaviour from primary care workers or family.

- 1 **Absent** - Definition does not apply
- 2 **Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 **Mild** - Shows occasional interest in social activities but poor initiative. Usually engages with others only when approached first by them.
- 4 **Moderate** – Passively goes along with most social activities but in a disinterested or mechanical way. Tends to recede into the background.
- 5 **Moderate Severe** - Passively participates in only a minority of activities and shows virtually no interest or initiative. Generally spends little time with others.
- 6 **Severe** - Tends to be apathetic and isolated, participating very rarely in social activities and occasionally neglecting personal needs. Has very few spontaneous social contacts.
- 7 **Extreme** – Profoundly apathetic, socially isolated and personally neglectful.

N5.	<p>DIFFICULTY IN ABSTRACT THINKING - Impairment in the use of the abstract-symbolic mode of thinking, as evidenced by difficulty in classification, forming generalisations and proceeding beyond concrete or egocentric thinking in problem-solving tasks.</p> <p>Basis for rating - Responses to questions on similarities and proverb interpretation, and use of concrete vs. abstract mode during the course of the interview.</p> <ol style="list-style-type: none"> 1 Absent - Definition does not apply 2 Minimal - Questionable pathology; may be at the upper extreme of normal limits 3 Mild - Tends to give literal or personalised interpretations to the more difficult proverbs and may have some problems with concepts that are fairly abstract or remotely related. 4 Moderate - Often utilises a concrete mode. Has difficulty with most proverbs and some categories. Tends to be distracted by functional aspects and salient features. 5 Moderate Severe - Deals primarily in a concrete mode, exhibiting difficulty with most proverbs and many categories. 6 Severe - Unable to grasp the abstract meaning of any proverbs or figurative expressions and can formulate classifications for only the most simple of similarities. Thinking is either vacuous or locked into functional aspects, salient features and idiosyncratic interpretations. 7 Extreme - Can use only concrete modes of thinking. Shows no comprehension of proverbs, common metaphors or similes, and simple categories. Even salient and functional attributes do not serve as a basis for classification. This rating may apply to those who cannot interact even minimally with the examiner due to marked cognitive impairment.
N6.	<p>LACK OF SPONTANEITY AND FLOW OF CONVERSATION - Reduction in the normal flow of communication associated with apathy, avolition, defensiveness or cognitive deficit. This is manifested by diminished fluidity and productivity of the verbal interactional process.</p> <p>Basis for rating - Cognitive-verbal processes observed during the course of interview.</p> <ol style="list-style-type: none"> 1 Absent - Definition does not apply 2 Minimal - Questionable pathology; may be at the upper extreme of normal limits 3 Mild - Conversation shows little initiative. Patient's answers tend to be brief and unembellished, requiring direct and leading questions by the interviewer. 4 Moderate - Conversation lacks free flow and appears uneven or halting. Leading questions are frequently needed to elicit adequate responses and proceed with conversation. 5 Moderate Severe - Patient shows a marked lack of spontaneity and openness, replying to the interviewer's questions with only one or two brief sentences. 6 Severe - Patient's responses are limited mainly to a few words or short phrases intended to avoid or curtail communication. (e.g. "I don't know", "I'm not at liberty to say"). Conversation is seriously impaired as a result and the interview is highly unproductive. 7 Extreme - Verbal output is restricted to, at most, an occasional utterance, making conversation not possible.

N7. STEREOTYPED THINKING - Decreased fluidity, spontaneity and flexibility of thinking, as evidenced in rigid, repetitious or barren thought content.

Basis for rating - Cognitive-verbal processes observed during the interview.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Some rigidity shown in attitude or beliefs. Patient may refuse to consider alternative positions or have difficulty in shifting from one idea to another.
- 4 Moderate** - Conversation revolves around a recurrent theme, resulting in difficulty in shifting to a new topic.
- 5 Moderate Severe** - Thinking is rigid and repetitious to the point that, despite the interviewer's efforts, conversation is limited to only two or three dominating topics.
- 6 Severe** - Uncontrolled repetition of demands, statements, ideas or questions which severely impairs conversation.
- 7 Extreme** - Thinking, behaviour and conversation are dominated by constant repetition of fixed ideas or limited phrases, leading to gross rigidity, inappropriateness and restrictiveness of patient's communication.

GENERAL PSYCHOPATHOLOGY SCALE (G)

G1. SOMATIC CONCERN - Physical complaints or beliefs about bodily illness or malfunctions. This may range from a vague sense of ill being to clear-cut delusions of catastrophic physical disease. **Basis for rating** - Thought content expressed in the interview.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Distinctly concerned about health or bodily malfunction, but there is no delusional conviction and overconcern can be allayed by reassurance.
- 4 Moderate** - Complains about poor health or bodily malfunction, but there is no delusional conviction, and overconcern can be allayed by reassurance.
- 5 Moderate Severe** - Patient expresses numerous or frequent complaints about physical illness or bodily malfunction, or else patient reveals one or two clear-cut delusions involving these themes but is not preoccupied by them.
- 6 Severe** - Patient is preoccupied by one or a few clear-cut delusions about physical disease or organic malfunction, but affect is not fully immersed in these themes, and thoughts can be diverted by the interviewer with some effort.
- 7 Extreme** - Numerous and frequently reported somatic delusions, or only a few somatic delusions of a catastrophic nature, which totally dominate the patient's affect or thinking.

G2. ANXIETY - Subjective experience of nervousness, worry, apprehension or restlessness, ranging from excessive concern about the present or future to feelings of panic.

Basis for rating - Verbal report during the course of interview and corresponding physical manifestations.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Expresses some worry, overconcern or subjective restlessness, but no somatic and behavioural consequences are reported or evidenced.
- 4 Moderate** - Patient reports distinct symptoms of nervousness, which are reflected in mild physical manifestations such as fine hand tremor and excessive perspiration.
- 5 Moderate Severe** - Patient reports serious problems of anxiety which have significant physical and behavioural consequences, such as marked tension, poor concentration, palpitations or impaired sleep.
- 6 Severe** - Subjective state of almost constant fear associated with phobias, marked restlessness or numerous somatic manifestations.
- 7 Extreme** - Patient's life is seriously disrupted by anxiety, which is present almost constantly and at times reaches panic proportion or is manifested in actual panic attacks.

G3. GUILT FEELINGS - Sense of remorse or self-blame for real or imagined misdeeds in the past.

Basis for rating - Verbal report of guilt feelings during the course of interview and the influence on attitudes and thoughts.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Questioning elicits a vague sense of guilt or self-blame for a minor incident, but the patient clearly is not overly concerned.
- 4 Moderate** - Patient expresses distinct concern over his responsibility for a real incident in his life but is not pre-occupied with it and attitude and behaviour are essentially unaffected.
- 5 Moderate Severe** - Patient expresses a strong sense of guilt associated with selfdeprecation or the belief that he deserves punishment. The guilt feelings may have a delusional basis, may be volunteered spontaneously, may be a source of preoccupation and/or depressed mood, and cannot be allayed readily by the interviewer.
- 6 Severe** - Strong ideas of guilt take on a delusional quality and lead to an attitude of hopelessness or worthlessness. The patient believes he should receive harsh sanctions as such punishment.
- 7 Extreme** - Patient's life is dominated by unshakable delusions of guilt, for which he feels deserving of drastic punishment, such as life imprisonment, torture, or death. There may be associated suicidal thoughts or attribution of others' problems to one's own past misdeeds.

G4. TENSION -Overt physical manifestations of fear, anxiety, and agitation, such as stiffness, tremor, profuse sweating and restlessness.

Basis for rating - Verbal report attesting to anxiety and thereupon the severity of physical manifestations of tension observed during the interview.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Posture and movements indicate slight apprehensiveness, such as minor rigidity, occasional restlessness, shifting of position, or fine rapid hand tremor.
- 4 Moderate** - A clearly nervous appearance emerges from various manifestations, such as fidgety behaviour, obvious hand tremor, excessive perspiration, or nervous mannerisms.
- 5 Moderate Severe** - Pronounced tension is evidenced by numerous manifestations, such as nervous shaking, profuse sweating and restlessness, but can conduct in the interview is not significantly affected.
- 6 Severe** - Pronounced tension to the point that interpersonal interactions are disrupted. The patient, for example, may be constantly fidgeting, unable to sit still for long, or show hyperventilation.
- 7 Extreme** - Marked tension is manifested by signs of panic or gross motor acceleration, such as rapid restless pacing and inability to remain seated for longer than a minute, which makes sustained conversation not possible.

G5. MANNERISMS AND POSTURING – Unnatural movements or posture as characterised be an awkward, stilted, disorganised, or bizarre appearance.

Basis for rating - Observation of physical manifestations during the course of interview as well as reports from primary care workers or family.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Slight awkwardness in movements or minor rigidity of posture
- 4 Moderate** – Movements are notably awkward or disjointed, or an unnatural posture is maintained for brief periods.
- 5 Moderate Severe** - Occasional bizarre rituals or contorted posture are observed, or an abnormal position is sustained for extended periods.
- 6 Severe** - Frequent repetition of bizarre rituals, mannerisms or stereotyped movements, or a contorted posture is sustained for extended periods.
- 7 Extreme** - Functioning is seriously impaired by virtually constant involvement in ritualistic, manneristic, or stereotyped movements or by an unnatural fixed posture which is sustained most of the time.

G6. DEPRESSION - Feelings of sadness, discouragement, helplessness and pessimism.

Basis for rating - Verbal report of depressed mood during the course of interview and its observed influence on attitude and behaviour.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Expresses some sadness or discouragement only on questioning, but there is no evidence of depression in general attitude or demeanor.
- 4 Moderate** - Distinct feelings of sadness or hopelessness, which may be spontaneously divulged, but depressed mood has no major impact on behaviour or social functioning and the patient usually can be cheered up.
- 5 Moderate Severe** - Distinctly depressed mood is associated with obvious sadness, pessimism, loss of social interest, psychomotor retardation and some interference in appetite and sleep. The patient cannot be easily cheered up.
- 6 Severe** - Markedly depressed mood is associated with sustained feelings of misery, occasional crying, hopelessness and worthlessness. In addition, there is major interference in appetite and or sleep as well as in normal motor and social functions, with possible signs of self-neglect.
- 7 Extreme** - Depressive feelings seriously interfere in most major functions. The manifestations include frequent crying, pronounced somatic symptoms, impaired concentration, psychomotor retardation, social disinterest, self neglect, possible depressive or nihilistic delusions and/or possible suicidal thoughts or action.

G7. MOTOR RETARDATION – Reduction in motor activity as reflected in slowing or lessening or movements and speech, diminished responsiveness of stimuli, and reduced body tone. **Basis for rating** - Manifestations during the course of interview as well as reports by primary care workers as well as family.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Slight but noticeable diminution in rate of movements and speech. Patient may be somewhat underproductive in conversation and gestures.
- 4 Moderate** - Patient is clearly slow in movements, and speech may be characterised by poor productivity including long response latency, extended pauses or slow pace.
- 5 Moderate Severe** – A marked reduction in motor activity renders communication highly unproductive or delimits functioning in social and occupational situations. Patient can usually be found sitting or lying down.
- 6 Severe** - Movements are extremely slow, resulting in a minimum of activity and speech. Essentially the day is spent sitting idly or lying down.
- 7 Extreme** - Patient is almost completely immobile and virtually unresponsive to external stimuli.

- G8. UNCOOPERATIVENESS** - Active refusal to comply with the will of significant others, including the interviewer, hospital staff or family, which may be associated with distrust, defensiveness, stubbornness, negativism, rejection of authority, hostility or belligerence. **Basis for rating** - Interpersonal behaviour observed during the course of the interview as well as reports by primary care workers or family.
- 1 Absent** - Definition does not apply
 - 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
 - 3 Mild** - Complies with an attitude of resentment, impatience, or sarcasm. May inoffensively object to sensitive probing during the interview.
 - 4 Moderate** - Occasional outright refusal to comply with normal social demands, such as making own bed, attending scheduled programmes, etc. The patient may project a hostile, defensive or negative attitude but usually can be worked with.
 - 5 Moderate Severe** - Patient frequently is in compliant with the demands of his milieu and may be characterised by other as an “outcast” or having “a serious attitude problem”. Uncooperativeness is reflected in obvious defensiveness or irritability with the interviewer and possible unwillingness to address many questions.
 - 6 Severe** - Patient is highly uncooperative, negativistic and possibly also belligerent. Refuses to comply with the most social demands and may be unwilling to initiate or conclude the full interview.
 - 7 Extreme** - Active resistance seriously impact on virtually all major areas of functioning. Patient may refuse to join in any social activities, tend to personal hygiene, converse with family or staff and participate even briefly in an interview.

- G9. UNUSUAL THOUGHT CONTENT** - Thinking characterised by strange, fantastic or bizarre ideas, ranging from those which are remote or atypical to those which are distorted, illogical and patently absurd. **Basis for rating** - Thought content expressed during the course of interview.
- 1 Absent** - Definition does not apply
 - 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
 - 3 Mild** - Thought content is somewhat peculiar, or idiosyncratic, or familiar ideas are framed in an odd context.
 - 4 Moderate** - Ideas are frequently distorted and occasionally seem quite bizarre.
 - 5 Moderate Severe** - Patient expresses many strange and fantastic thoughts, (e.g. Being the adopted son of a king, being an escapee from death row), or some which are patently absurd (e.g. Having hundreds of children, receiving radio messages from outer space from a tooth filling).
 - 6 Severe** - Patient expresses many illogical or absurd ideas or some which have a distinctly bizarre quality (e.g. having three heads, being a visitor from another planet).
 - 7 Extreme** - Thinking is replete with absurd, bizarre and grotesque ideas.

G10. DISORIENTATION - Lack of awareness of one's relationship to the milieu, including persons, place and time, which may be due to confusion or withdrawal. **Basis for rating** - Responses to interview questions on orientation.

- 1 **Absent** - Definition does not apply
- 2 **Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 **Mild** - General orientation is adequate but there is some difficulty with specifics. For example, patient knows his location but not the street address, knows hospital staff names but not their functions, knows the month but confuses the day of the week with an adjacent day, or errs in the date by more than two days. There may be narrowing of interest evidenced by familiarity with the immediate but not extended milieu, such as ability to identify staff but not the mayor, governor, or president.
- 4 **Moderate** - Only partial success in recognising persons, places and time. For example, patient knows he is in a hospital but not its name, knows the name of the city but not the borough or district, knows the name of his primary therapist but not many other direct care workers, knows the year or season but not sure of the month.
- 5 **Moderate Severe** - Considerable failure in recognising persons, place and time. Patient has only a vague notion of where he is and seems unfamiliar with most people in his milieu. He may identify the year correctly or nearly but not know the current month, day of week or even the season.
- 6 **Severe** - Marked failure in recognising persons, place and time. For example, patient has no knowledge of his whereabouts, confuses the date by more than one year, can name only one or two individuals in his current life.
- 7 **Extreme** - Patient appears completely disorientated with regard to persons, place and time. There is gross confusion or total ignorance about one's location, the current year and even the most familiar people, such as parents, spouse, friends and primary therapist.

G11. POOR ATTENTION - Failure in focused alertness manifested by poor concentration, distractibility from internal and external stimuli, and difficulty in harnessing, sustaining or shifting focus to new stimuli. **Basis for rating** – Manifestations during the course of interview.

- 1 **Absent** - Definition does not apply
- 2 **Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 **Mild** - Limited concentration evidenced by occasional vulnerability to distraction and faltering attention toward the end of the interview.
- 4 **Moderate** - Conversation is affected by the tendency to be easily distracted, difficulty in long sustaining concentration on a given topic, or problems in shifting attention to new topics.
- 5 **Moderate Severe** - Conversation is seriously hampered by poor concentration, distractibility, and difficulty in shifting focus appropriately..
- 6 **Severe** - Patient's attention can be harnessed for only brief moments or with great effort, due to marked distraction by internal or external stimuli.
- 7 **Extreme** - Attention is so disrupted that even brief conversation is not possible.

G12. LACK OF JUDGEMENT AND INSIGHT - Impaired awareness or understanding of one's own psychiatric condition and life situation. This is evidenced by failure to recognise past or present psychiatric illness or symptoms, denial of need for psychiatric hospitalisation or treatment, decisions characterised by poor anticipation or consequences, and unrealistic short-term and long-range planning. **Basis for rating** – Thought content expressed during the interview.

- 1 **Absent** - Definition does not apply
- 2 **Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 **Mild** - Recognises having a psychiatric disorder but clearly underestimates its seriousness, the implications for treatment, or the importance of taking measures to avoid relapse. Future planning may be poorly conceived.
- 4 **Moderate** - Patient shows only a vague or shallow recognition of illness. There may be fluctuations in acknowledgement of being ill or little awareness of major symptoms which are present, such as delusions, disorganised thinking, suspiciousness and social withdrawal. The patient may rationalise the need for treatment in terms of its relieving lesser symptoms, such as anxiety, tension and sleep difficulty.
- 5 **Moderate Severe** - Acknowledges past but not present psychiatric disorder. If challenged, the patient may concede the presence of some unrelated or insignificant symptoms, which tend to be explained away by gross misinterpretation or delusional thinking. The need for psychiatric treatment similarly goes unrecognised.
- 6 **Severe** - Patient denies ever having had a psychiatric disorder. He disavows the presence of any psychiatric symptoms in the past or present and, though compliant, denies the need for treatment and hospitalisation.
- 7 **Extreme** - Emphatic denial of past and present psychiatric illness. Current hospitalisation and treatment are given a delusional interpretation (e.g. as punishment for misdeeds, as persecution by tormentors, etc), and the patient thus refuse to cooperate with therapists, medication or other aspects of treatment.

G13. DISTURBANCE OF VOLITION – Disturbance in the wilful initiation, sustenance and control of one's thoughts, behaviour, movements and speech.

Basis for rating - Thought content and behaviour manifested in the course of interview.

- 1 **Absent** - Definition does not apply
- 2 **Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 **Mild** - There is evidence of some indecisiveness in conversation and thinking, which may impede verbal and cognitive processes to a minor extent.
- 4 **Moderate** - Patient is often ambivalent and shows clear difficulty in reaching decisions. Conversation may be marred by alteration in thinking, and in consequence, verbal and cognitive functioning are clearly impaired.
- 5 **Moderate Severe** - Disturbance of volition interferes in thinking as well as behaviour. Patient shows pronounced indecision that impedes the initiation and continuation of social and motor activities, and which also may be evidence in halting speech.
- 6 **Severe** - Disturbance of volition interferes in the execution of simple automatic motor functions, such as dressing or grooming, and markedly affects speech.
- 7 **Extreme** – Almost complete failure of volition is manifested by gross inhibition of movement and speech resulting in immobility and/or mutism.

G14. POOR IMPULSE CONTROL - Disordered regulation and control of action on inner urges, resulting in sudden, unmodulated, arbitrary or misdirected discharge of tension and emotions without concern about consequences.

Basis for rating – Behaviour during the course of interview and reported by primary care workers or family.

- 1 **Absent** - Definition does not apply
- 2 **Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 **Mild** - Patient tends to be easily angered and frustrated when facing stress or denied gratification but rarely acts on impulse.
- 4 **Moderate** - Patient gets angered and verbally abusive with minimal provocation. May be occasionally threatening, destructive, or have one or two episodes involving physical confrontation or a minor brawl.
- 5 **Moderate Severe** - Patient exhibits repeated impulsive episodes involving verbal abuse, destruction of property, or physical threats. There may be one or two episodes involving serious assault, for which the patient requires isolation, physical restraint, or p.r.n. sedation.
- 6 **Severe** - Patient frequently is impulsive aggressive, threatening, demanding, and destructive, without any apparent consideration of consequences. Shows assaultive behaviour and may also be sexually offensive and possibly respond behaviourally to hallucinatory commands.
- 7 **Extreme** - Patient exhibits homicidal, sexual assaults, repeated brutality, or self-destructive behaviour. Requires constant direct supervision or external constraints because of inability to control dangerous impulses.

G15. PREOCCUPATION - Absorption with internally generated thoughts and feelings and with autistic experiences to the detriment of reality orientation and adaptive behaviour. **Basis for rating** - Interpersonal behaviour observed during the course of interview.

- 1 **Absent** - Definition does not apply
- 2 **Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 **Mild** - Excessive involvement with personal needs or problems, such that conversation veers back to egocentric themes and there is diminished concern exhibited toward others.
- 4 **Moderate** - Patient occasionally appears self-absorbed, as if daydreaming or involved with internal experiences, which interferes with communication to a minor extent.
- 5 **Moderate Severe** - Patient often appears to be engaged in autistic experiences, as evidenced by behaviours that significantly intrude on social and communicational functions, such as the presence of a vacant stare, muttering or talking to oneself, or involvement with stereotyped motor patterns.
- 6 **Severe** - Marked preoccupation with autistic experiences, which seriously delimits concentration, ability to converse, and orientation to the milieu. The patient frequently may be observed smiling, laughing, muttering, talking, or shouting to himself.
- 7 **Extreme** - Gross absorption with autistic experiences, which profoundly affects all major realms of behaviour. The patient constantly may be responding verbally or behaviourally to hallucinations and show little awareness of other people or the external milieu.

G16. ACTIVE SOCIAL AVOIDANCE - Diminished social involvement associated with unwarranted fear, hostility, or distrust.

Basis for rating - Reports of social functioning primary care workers or family.

- 1 Absent** - Definition does not apply
- 2 Minimal** - Questionable pathology; may be at the upper extreme of normal limits
- 3 Mild** - Patient seems ill at ease in the presence of others of others and prefers to spend time alone, although he participates in social functions when required.
- 4 Moderate** - Patient begrudgingly attends all or most social activities but may needs to be persuaded or may terminate prematurely on account of anxiety, suspiciousness, or hostility.
- 5 Moderate Severe** - Patient fearfully or angrily keeps away from many social interactions despite others' efforts to engage him. Tends to spend unstructured time alone.
- 6 Severe** - Patient participates in very few social activities because of fear, hostility, or distrust. When approached, the patient shows a strong tendency to break off interactions, and generally he tends to isolate himself from others.
- 7 Extreme** - Patient cannot be engaged in social activities because of pronounced fears, hostility, or persecutory delusions. To the extent possible, he avoids all interactions and remains isolated from others.

APPENDIX B – ETHICS COMMITTEE APPROVAL

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Efeito da adição de mononitrato, um doador de óxido nítrico, ao tratamento habitual de pacientes com diagnóstico de esquizofrenia.

Pesquisador: Tiago Moraes Guimarães

Área Temática:

Versão: 2

CAAE: 31105914.4.0000.5440

Instituição Proponente: Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da USP -

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 773.290

Data da Relatório: 01/08/2014

Apresentação do Projeto:

Trata-se de projeto de pesquisa de Tiago Moraes Guimarães sob orientação do Prof. Dr. Jaime E. C. Hallak do Departamento de Neurociências e Ciências do Comportamento Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto-USP. Justificativa do estudo: a esquizofrenia é um transtorno crônico, que afeta cerca de 1% da população mundial. Podemos dividi-la em cinco dimensões sintomáticas: positiva, negativa, cognitiva, afetiva e de desorganização. A pedra angular do tratamento da esquizofrenia, na atualidade, é a farmacoterapia com antipsicóticos liséuquadores das receptores dopaminérgicos tipo 2. Essa terapêutica tem boa ação nos sintomas positivos, porém causas de impacto clínico nos sintomas negativos e cognitivos. Devido a isso, vários pesquisadores passaram a procurar outras vias que poderiam estar implicadas na fisiopatologia do transtorno, como a via do óxido nítrico. Um trabalho recente realizado por nosso grupo, evidenciou redução nos sintomas positivos, negativos e cognitivos em pacientes esquizofrênicos internados, após a administração de Nitroglicerato de Sódio, um doador do óxido nítrico. O fato de o Nitroglicerato de Sódio estar disponível apenas na forma intravenosa, necessitar ser aplicado em bomba de infusão e com monitorização hemodinâmica o torna difícil de ser utilizado na prática clínica diária em pacientes ambulatoriais. Devido a isso, optamos por testar o Mononitrato: droga segura,

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Objetivo da Pesquisa:

Segundo os pesquisadores:

1- Avaliar se a adição de mononitrato ao tratamento habitual de pacientes esquizofrênicos ambulatoriais apresenta benefícios nas dimensões positiva, negativa e cognitiva da esquizofrenia durante 30 dias de terapia.

2- Avaliar o impacto da terapia com o mononitrato nos exames de neuroimagem funcional.

Avaliação dos Riscos e Benefícios:

Segundo os pesquisadores:

-Riscos: os nitrovasodiladores são considerados seguros e livres de reações adversas graves, como já foi relatado anteriormente. Apesar disso, seu uso clínico é limitado pela redução de eficácia durante a administração continuada, resultando em ausência completa de seus efeitos hemodinâmicos após cerca de 24-48h de aplicação constante. Esse mecanismo de tolerância ainda não foi completamente elucidado (Marsh e Marsh, 2000; Fung, 2004). Nos pacientes com angina estável, a tolerância é evitada pela aplicação intermitente das drogas, por exemplo, a nitroglicerina dos adesivos transdérmicos de NTO (Abrams, 2002). Não existem relatos de anos permanentes à saúde com a terapia nitrovasodilatadora, revisão sistemática recente evidenciou cefaleia, tonturas e hipotensão postural como efeitos adversos comuns dos nitratos, esses efeitos costumam ser mais graves em doses mais altas. No caso dos adesivos transdérmicos podem ocorrer casos isolados de reação alérgica leve na região onde o adesivo é colocado (Wai, 2011). Um ensaio clínico de quatro meses de duração encontrou resultados semelhantes aos acima descritos (Ardissino, 1997). Em um estudo que avaliou o impacto do mononitrato de liberação prolongada, dose de 60mg, em indivíduos com hipertensão arterial estágio 1, não houve relatos de efeitos adversos graves e 3 de 12 pacientes que receberam a droga relataram cefaleia (Kaufman, 2010). Em um outro estudo, com 16 indivíduos saudáveis, utilizando mononitrato de liberação rápida na dose de 30mg, 3 vezes ao dia, 5 indivíduos abandonaram a pesquisa nas primeiras 24h: 1 por cefaleia, 3 por tonturas e 1 por náuseas (Christiansen, 2008). O maior risco durante o uso de qualquer nitrovasodilatador é a administração concomitante com os inibidores da fosfodiesterase 5, sendo o Sildenafil (Viagra) o mais famoso representante da classe. Os pacientes serão sempre lembrados dessa contra-indicação durante o estudo.

-Benefícios: se replicado, com o Mononitrato, o estudo realizado pelo nosso grupo com o

benéfico, utilizada há décadas para angina estável e disponível em várias vias de administração.

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nitroglicerato de sódio, será reforçada a hipótese que implica o sistema "NMDA-NO-OMPC" na etiologia da esquizofrenia e que sugere os doadores de óxido nítrico como novas fármacos no arsenal terapêutico dessa devastadora patologia. Seria, assim, inaugurada uma nova era no tratamento da esquizofrenia, onde uma classe de fármacos que não atua nos receptores dopaminérgicos teria lugar na terapêutica do transtorno. O mononitrato é uma

medicação de uso rotineiro na prática clínica diária do cardiologista, do clínico geral e do médico da família, está disponível em várias vias de administração, é barato, tem poucos efeitos colaterais e está no mercado há décadas. Todos esses fatores facilitariam sua aprovação para o tratamento de esquizofrenia caso os estudos sejam positivos.

Comentários e Considerações sobre a Pesquisa:

Serão avaliados 30 pacientes com esquizofrenia, segundo os critérios diagnósticos do DSM-V (Diagnostic and Statistical Manual of Mental Disorders – Fifth Edition), que estejam em acompanhamento psiquiátrico regular no Ambulatório de Psiquiatria do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da Universidade de São Paulo (HCFMRP-USP), mais especificamente no Ambulatório de Reabilitação Psicossocial (AREP) e no Ambulatório de Esquizofrenia (APOE). Os pacientes serão divididos em dois grupos de constituição equivalente emparelhados quanto à idade, sexo, escolaridade, tempo de transtorno, tempo de psicose sem tratamento, tempo de tratamento antipsicótico, tratamento antipsicótico atual (típico, atípico ou Clozapina) e síndrome clínica predominante (positiva ou negativa). Critérios de Inclusão: pacientes de ambos os sexos, com diagnóstico de esquizofrenia, em tratamento antipsicótico ambulatorial e com sintomas presentes nas dimensões positiva ou negativa (escala maior ou igual a quatro em qualquer item das escalas positiva ou negativa da PANSS - Positive and Negative Syndromes Scale) e idade entre 18 e 45 anos. Critérios de exclusão: presença de condição médica relevante (doenças cardiovasculares, hipertensão arterial grave, insuficiência renal, doença hepática, hipotireoidismo, hiponatremia, hipotensão ortostática, hipertensão intracraniana prévia, epilepsia, gravidez, amamentação). Aqui se inclui, também, pacientes que tenham sinais e sintomas sugestivos de hipertensão arterial fase como visão turva, tonturas, palidez e extremidades frias; história de hiperexcitabilidade aos nitratos orgânicos; diagnóstico de transtorno do uso de substâncias segundo os critérios do DSM-V, exceto álcool; pacientes com alteração no esquema antipsicótico nos últimos 30 dias e pacientes com critérios clínicos de internação integral. Quando os pacientes com diagnóstico de esquizofrenia forem a avaliação iniciamos ambulatoriais do HCFMRP-USP. Não feitos de rotina todas os exames necessários para identificação de uma condição médica geral relevante; anamnese detalhada (história clínica e exame físico) com informações colhidas do

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próprio paciente, de seus familiares e de seu prontuário médico; ressonância nuclear magnética; avaliação cardiológica (incluindo eletrocardiograma); função renal (ureia e creatinina); função hepática (TGO, TGP, bilirrubinas, albumina, TP); função tireoideana (TSH e T4 livre) e rastreio para drogas de abuso; teste de gravidez.

Procedimento: será um estudo placebo, controlado, duplo-cego, randomizado, cruzado. A população estudada consistirá de pacientes com diagnóstico de esquizofrenia em seguimento nos ambulatórios de psiquiatria do HCFMRP-USP. Os pacientes serão convidados, no dia de sua consulta, a participar do experimento. Após o consentimento para participação no estudo, os pacientes permanecerão em tratamento antipsicótico habitual, sem alterações por 60 dias, tempo de duração da pesquisa. Os pacientes serão randomizados em 2 grupos de 15 pessoas, um dos grupos irá iniciar com a droga ativa e assim permanecerá por 30 dias, enquanto o outro grupo irá iniciar com o placebo e assim permanecerá por 30 dias. Findado este prazo, os grupos farão a troca de droga ativa para placebo no grupo que iniciou com droga ativa e de placebo para droga ativa no grupo que iniciou com placebo. Caso haja reagudização do quadro com necessidade de alteração na dose ou no tipo de antipsicótico, o paciente será excluído do estudo. Caso tenha o efeito colateral mais comum do uso do MNT, que é a cefaleia, poderá ser utilizado o Paracetamol 500mg, até de 6/6h. Se a dor for de moderada a grave e não ceder com o analgésico, o paciente será retirado do estudo.

Os pacientes serão submetidos, durante a pesquisa, à aplicação da SCID, da PANSS, da UKU, da CGI e da MCCEB. A entrevista estruturada, SCID, será aplicada apenas uma vez, antes do início dos outros procedimentos, com o objetivo de confirmação diagnóstica. A escala psicopatológica, PANSS e as escalas UKU e CGI serão aplicadas antes do início da administração da MNT e, então, semanalmente, com a última aplicação sucedendo o último dia de administração do fármaco, após 60 dias. A avaliação cognitiva será realizada em três oportunidades, no dia que precede a primeira aplicação do fármaco, no dia da troca entre os grupos e no dia que sucede a última aplicação. Serão realizados exames de imagem (SOLD e ASL) por três vezes durante a pesquisa: no dia que antecede ao início da administração da droga ativa ou placebo, após o cruzamento dos grupos (primeiro primeiro dia) e após a última administração da droga ativa ou placebo (sexagésimo dia).

Droga: o mononitrato será utilizado na forma de cápsulas de liberação prolongada, via oral, contendo 50mg de sublingual ativa (Mononitrato Retard, Belducoff). O paciente será orientado a ingerir a medicação sem mastigar ou abir a cápsula, in água o café da manhã. O Mononitrato de liberação retard será utilizado como adjuvante no tratamento da esquizofrenia. Os fármacos de uso habitual não serão modificados. Dessa forma, saberemos a qual agente implicar quaisquer

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alterações clínicas que o paciente liver. Têm-se que, a dose inicial para Angina Pectoris é a de 30mg a 60mg e o incremento das doses se baseia na tolerabilidade e efetividade do fármaco, podendo chegar a 240mg em casos excepcionais. Efeitos adversos hemodinâmicos são raros, sendo os mais comuns cefaleia, náuseas e vômitos. Os nitrovasodilatadores são considerados seguros e livres de reações adversas graves, como já foi relatado anteriormente. Apesar disso, seu uso clínico é limitado pela redução de eficácia durante a administração continuada, resultando em ausência completa de seus efeitos hemodinâmicos após cerca de 24-48h de aplicação constante. Esse mecanismo de tolerância ainda não foi completamente elucidado(FUNO, 2004; MARSH, MARSH, 2000). Nos pacientes com angina estável, a tolerância é evitada pela aplicação intermitente das drogas, por exemplo, a retirada noturna dos adesivos transdérmicos de NTO ou uso uma vez ao dia da apresentação de liberação controlada do mononitrato. Não existem relatos de danos permanentes à saúde com a terapia nitrovasodilatadora, revisão sistemática recente evidenciou cefaleia, tonturas e hipotensão postural como efeitos adversos comuns dos nitratos, esse efeitos costumam ser mais graves em doses mais altas. No caso dos adesivos transdérmicos podem ocorrer casos isolados de reação alérgica leve na região onde o adesivo é colado(WEI et al., 2011). Um ensaio clínico de quatro meses de duração encontrou resultados semelhantes aos acima descritos (ARDISSINO et al., 1997). Em um estudo que avaliou o impacto do mononitrato de liberação prolongada, dose de 60mg, em indivíduos com hipertensão arterial estágio I, não houve relatos de efeitos adversos graves e 3 de 12 pacientes que receberam a droga, relataram cefaleia(KAUFMAN et al., 2010). Em um outro estudo, com 16 indivíduos saudáveis, utilizando mononitrato de liberação rápida na dose de 30mg, 3 vezes ao dia, 5 indivíduos abandonaram a pesquisa nas primeiras 24h; 1 por cefaleia, 3 por tonturas e 1 por náuseas(CHRISTIANSEN et al., 2008). O maior risco durante o uso de qualquer nitrovasodilatador é a administração concomitante com os inibidores da fosfodiesterase 5, sendo o Sildenafil (Viagra) o mais conhecido representante da classe. Os pacientes serão sempre lembrados dessa contra-indicação durante o estudo. No que diz respeito à avaliação dos riscos e benefícios da utilização da droga, no nosso estudo, essa escolha de pacientes portadores de um transtorno mental sem comorbidades clínicas é importante, pois se controlada essa possível interferência fica mais explícito que o efeito de melhora da sintomatologia psiquiátrica (se ocorrer) não terá sido por correção da condição comórbida. Placebo: a cápsula placebo será administrado da mesma forma e será idêntica ao da droga ativa. Os pacientes e o pesquisador não estarão avisados sobre que droga o paciente avaliado estará em

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uso. Em caso do surgimento de efeitos colaterais ou piora clínica do paciente, um dos médicos do ambulatório não envolvido com as avaliações, tomará conhecimento de todos os detalhes do problema para poder tomar a melhor decisão, o experimento poderá ser suspenso a qualquer momento. A segurança e o bem estar do paciente são prioridades. Todas essas avaliações serão realizadas pelo autor do projeto, que é médico-psiquiatra. As escalas de avaliação e os testes cognitivos foram selecionados para mensurar possíveis mudanças nos sintomas positivos, negativos e cognitivos dos pacientes após a administração da substância em estudo. O avaliador, T.M.O. (autor do projeto), foi treinado na aplicação da MCB por neuropsicólogo do Programa de Esquizofrenia da Universidade Federal de São Paulo – Faculdade Paulista de Medicina (PROCESQ – UNIFESP – EPM), grupo responsável pela tradução, validação e normatização da escala no Brasil. Já foi treinado na SCID como parte do programa de residência médica em psiquiatria do HCFMRP-USP. Para a aplicação da PANSS, URU e COI houve treinamento recebido por médico-psiquiatra doutorando do HCFMRP-USP e pesquisador do mesmo grupo. Implicações, segundo os pesquisadores: se replicado, com o Mononitrato, o estudo realizado pelo nosso grupo com o nitroglicerato de sódio, será reforçado a hipótese que implica o sistema "NMDA-NO-OMP" na etiologia da esquizofrenia e que sugere os doadores de óxido nítrico como novos fármacos no arsenal terapêutico dessa devastadora patologia. Além disso, novos dados poderão ser adquiridos em relação ao funcionamento cerebral na esquizofrenia. Seria, assim, inaugurada uma nova era no tratamento da esquizofrenia, onde uma classe de fármacos que não atua nos receptores dopaminérgicos teria lugar terapêutico do transtorno. O mononitrato é uma medicação de uso rotineiro na prática clínica diária do cardiologista, do clínico geral e do médico da família, está disponível em várias vias de administração, é barato, tem poucos efeitos colaterais e está no mercado há décadas. Todos esses fatores facilitarão sua aprovação para o tratamento da esquizofrenia caso os estudos sejam positivos.

Considerações sobre os Termos de apresentação obrigatória:

Foram representados o TCLE Versão 2.0 de 11/07/2014 e projeto de pesquisa de Versão 2.0 de 11/07/2014.

Recomendações:

Não há.

Conclusões ou Pendências e Lista de Inadéquações:

Diante do exposto e à luz da Resolução CNS 466/2012, o projeto de pesquisa versão 2.0 de

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11/07/2014, assim como o Termo de Consentimento Livre e Esclarecido versão 2.0 de 11/07/2014, podem ser enquadrados na categoria APROVADO.

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Considerações Finais a critério do CEP:

Projeto Aprovado: Tendo em vista a legislação vigente, devem ser encaminhados ao CEP, relatórios parciais anuais referentes ao andamento da pesquisa e relatório final ao término do trabalho. Qualquer modificação do projeto original deve ser apresentada a este CEP em nova versão, de forma objetiva e com justificativas, para nova apreciação.

RIBEIRÃO PRETO, 01 de Setembro de 2014

Assinado por:
MARCELA GOMARÊS VILLANOVA
(Coordenadora)

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