UNIVERSITY MEDICAL SCHOOL OF NAPLES “FEDERICO II”

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A pilot ^{18}(F)-Flutemetamol PET and neurocognitive assessment study in severe cognitive impaired schizophrenia patients

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Summary

Abstract

State of the art: Multiple lines of evidence suggest that schizophrenia (SZ) has neurodevelopmental origins (Rund, 2018). However, this theory alone cannot explain the numerous progressive neurodegenerative processes described in SZ that partially justify its heterogeneity, including the more severe difficult-to-treat condition known as treatment-resistant schizophrenia (TRS) (O. D. Howes et al., 2017). According to this view of a complex neurodevelopmental neurodegenerative progressive disorder, multiple pieces of evidence showed a prominent cognitive impairment in TRS patients compared to those who respond to treatment with antipsychotics (de Bartolomeis et al., 2013), reinforcing the theory that TRS may represent a categorically different illness to treatment-responsive schizophrenia (Gillespie, Samanaite, Mill, Egerton, & MacCabe, 2017). This project aims to investigate the cerebral β-amyloid (Aβ) accumulation as putative measures of neurodegeneration in patients affected by SZ with cognitive impairment, using ^{18}(F)-Flutemetamol Positron Emission Tomography (PET) to define molecular biomarkers leading to neuropathological progression (Rice & Bisdas, 2017).

Methods: TRS patients (n=12) underwent amyloid PET acquisition. All patients were recruited from the Department of Neuroscience, Unit of Treatment Resistant Psychosis, University of Naples "Federico II". The diagnosis was made according to Structured Interview for Diagnosis (SCID-I). Treatment resistance was assessed according to TRRIP (Treatment Response and Resistance in Psychosis) Working Group consensus guidelines (O. D. Howes et al., 2017). Inclusion criteria were age between 18 and 55 years; disease duration >2 years; no substantial medication switch or dose changes (6 months); no evidence of recent (3 months) worsening of psychotic symptoms. Exclusion criteria were macroscopic brain structural

anomalies or other major systemic, psychiatric, or neurological disorders, and substance use (6 months). Antipsychotic doses were transformed into chlorpromazine equivalents (Gardner, Murphy, O'Donnell, Centorrino, & Baldessarini, 2010). The clinical features were evaluated using the Positive and Negative Syndrome Scale (PANSS), the PANSS 5-factor model (van der Gaag et al., 2006), the Scale for the Assessment of Positive Symptoms (SAPS), the Scale for the Assessment of Negative Symptoms (SANS), and the Thought and Language Disorder (TALD) scale (Kircher et al., 2014). The cognitive symptoms were assessed using the Brief Assessment of Cognition in Schizophrenia (BACS) (Keefe et al., 2004), the Mini-Mental State Examination (MMSE), the Brief Intelligence Test (TIB), the Facial Emotion Identification Test (FEIT), and The Awareness of Social Inference Test (TASIT). Statistical procedures for clinical and demographic data were performed with the Statistical Package for the Social Sciences (SPSS) using a t-test ($p \leq 0.01$). TRS patients were assessed with the BACS and classified according to the severity of cognitive deficits into patients with Mild Cognitive Impairment (n=5) and Severe Cognitive Impairment (n=7). Patients performed amyloid PET acquisition at the Department of Advanced Biomedical Sciences, Nuclear Medicine, University of Naples "Federico II" to detect Aβ plaques after receiving an intravenous injection of 185 MBq of ^{18}(F)-Flutemetamol. Early images were acquired immediately after the injection (early-phase), and late images approximately 90 minutes post-injection (late-phase). PET images were reconstructed using iterative reconstruction Ordered Subset Expectation Maximization (OSEM) and corrected for attenuation with Computed Tomography (CT) images. Mild or Severe Cognitive Impairment was assessed with raw data adjusted according to the normative values of the Italian population (Anselmetti et al., 2008), (Galderisi et al., 2014). Corrected scores were fitted into a 4-point scale to collect equivalent scores, based on which patients were classified as having

Severe Cognitive Impairment (SCI, more than two cognitive domains with scores from 0 to 1) or Mild Cognitive Impairment (MCI, at least two cognitive domains with scores from 0 to 1 and the other domains with score ≥2) (Anselmetti et al., 2008). For TRS patients, the standardized uptake value region (SUVr) was calculated. SUVr expresses the ratio between the brain uptake of the amyloid tracer in the cerebral cortex and the uptake in the Cerebellum (i.e. a reference region not or less affected by disease). The visual analysis between the TRS patients with MCI and SCI in the early- and late-phase scanning was performed with SPM12 (Statistical Parametric Mapping) software, and the images were spatially normalized in the anatomical brain space MNI (Montreal Neurological Institute). The Pearson's correlation analysis between SUVr and the clinical scores was performed ($p \leq 0.01$) with Bonferroni's post hoc correction ($p \leq 0.003$). This study was approved and registered through the Ethical Committee for Clinical Studies of the University of "Naples Federico II" (Prot. CE n. 195/19, Substantive Amendment I).

Results: 1) Demographic, clinical, and cognitive features: TRS patients (males 8, females 4; mean age 36.25±8.82; disease duration 17.33±7.81; chlorpromazine equivalents 552.67±238.81; education years 13.25±1.36; age at onset 18.83±4) performed amyloid PET. Patients did not differ in all the demographic and clinical characteristics. Cognitive scores at BACS domains such as verbal fluency, processing speed, problem solving, the BACS composite t-score, and TIB scores were significantly lower in SCI patients compared to MCI patients ($p \leq 0.01$). ***2) Pet amyloid SUVr descriptive analysis in TRS patients with Mild and Severe Cognitive Impairment:*** TRS patients with MCI (n=5) and SCI (n=7) performed amyloid PET scans. Amyloid levels did not reach a deposition threshold that would be significant in TRS patients at the individual level. The average amyloid SUVr did not statistically differ between groups of TRS patients

with MCI and SCI. ***3) Visual analysis of the ^{18}F-Flutemetamol PET distribution in the TRS patient with Mild and Severe Cognitive Impairment:*** The early-phase images in both groups exhibit a similar pattern of reduced uptake in the frontal cortex. In the SCI group, visual analysis reveals a moderate reduction in radiotracer uptake in the posterior cortical regions (i.e., the parietal and temporal cortices). The late-phase images do not show increased uptake in the cerebral cortex in either group.

4) Pet amyloid SUVr correlation analysis with clinical scores in TRS patients: The correlation analysis between amyloid SUVr and clinical scores in TRS patients revealed significant negative correlations between the Left Parietal Cortex and PANSS POS scores ($p<0.003$, $r=0.597$) and 5-factor DIS scores ($p<0.002$, $r=0.645$). Moreover, amyloid SUVr in the Right Sensorimotor Cortex was negatively correlated with PANSS POS scores ($p<0.003$, $r=0.602$) and 5-factor EXC scores ($p<0.002$, $r=0.619$), indicating an inverse significant relationship between the degree of prodromal amyloid pathology and the severity of clinical symptoms in TRS patients. There was no significant correlation between cognitive deficits and cortical amyloid SUV region.

Conclusions: In conclusion, the results of this proof-of-concept amyloid PET imaging study did not reveal significant differences in regional cerebral cortical amyloid deposition between TRS patients with Mild and Severe Cognitive Impairment. Interestingly, however, the amyloid load was negatively correlated with clinical scores but not cognitive dysfunctions, suggesting a link between prodromal amyloid pathology and psychotic symptoms. These findings suggest that understanding the potential role of β-amyloid accumulation in TRS could be of interest for possible targeted therapeutic strategies and improved clinical outcomes in this challenging patient population. Future studies investigating longitudinal amyloid changes in TRS patients could provide further insights into this issue.

1. INTRODUCTION

Schizophrenia (SZ) is a severe mental disorder characterized by positive, negative, and cognitive symptoms. Cognitive impairment plays a key role in SZ, starting with Kraepelin's “dementia praecox”, which is described in both SZ and neurodegenerative diseases (Takamatsu et al., 2019) supporting the theory of SZ as a progressive neurodevelopmental disease. However, the neurodevelopmental origins of SZ (Rund, 2018) alone cannot explain the several processes related to progressive cognitive decline in SZ, described in the condition of treatment-resistant schizophrenia (TRS) (O. D. Howes et al., 2017), in which patients showed an early age at onset (Iasevoli et al., 2022) and a more significant cognitive impairment than those responding to treatment with antipsychotics (de Bartolomeis et al., 2013), (Gillespie et al., 2017). Studies show a significant cognitive decline after 13 years of disease in SZ patients with an early age at onset (Øie, Sundet, & Rund, 2010), with a rapid and progressive cognitive decline in older SZ patients (Loewenstein, Czaja, Bowie, & Harvey, 2012). In accordance with this finding, a population-based cohort study of SZ patients shows a higher risk of developing dementia symptoms in patients with SCZ, and this increased risk is not explained by dementia risk factors such as cardiovascular disease and diabetes (Ribe et al., 2015). Some evidence reported that while treatment with antipsychotics improves cognitive performance in patients with first-episode psychosis (Wang et al., 2013), long-term use may promote cognitive impairment in SZ patents (Husa et al., 2014). A recent ^{18}(F)-Fluorodeoxyglucose (FDG) Positron Emission Tomography (PET) study emphasized the presence of a progressive gradient of cerebral metabolic alteration in patients with SZ according to antipsychotics response, with reduced frontal metabolism specifically in TRS (Iasevoli et al., 2023). Neuroimaging studies suggest that cerebral metabolic reduction and Aβ accumulation may predict cognitive impairment and may be considered

biomarkers of molecular change leading to neuropathological progression (Chandra et al., 2019), while post-mortem studies in SZ found cortical neuritic plaques related to the severity of cognitive impairment (Rapp et al., 2010). In addition, a post-mortem study investigated the relationship between the levels of the β-peptide forms, Aβx-40 and Aβx-42, and cognitive impairment in patients with Alzheimer's disease (AD), elderly controls, and SZ patients with and without concomitant AD. The results revealed that AD patients had the highest levels of amyloid β-peptide, while SZ patients without AD had similar levels to elderly controls. Notably, SZ patients with AD showed significantly higher levels of Aβx-42 than those without AD, concluding that amyloid β-peptide is not responsible for cognitive deficits in SZ without AD (Religa et al., 2003). A recent systematic review evaluated the presence of neurodegenerative biomarkers in SZ patients measured in cerebrospinal fluid (CSF). The results reported significantly lower levels of Aβ42 compared to healthy controls but higher than those seen in AD patients (Wilson et al., 2024), suggesting that amyloid levels altered in SZ were not correlated with cognitive deficits as in AD.

A neuroimaging study demonstrated a common spatial pattern of abnormalities in both AD and SZ patients, suggesting that they might share similar pathophysiological mechanisms while they show a different age of onset (Douaud et al., 2014). Conversely, very early onset dementias and other neurodegenerative diseases with prominent behavioral disturbances can lead to misdiagnoses as SZ, particularly due to the young age of onset and the nature of the symptoms (Ducharme et al., 2020). In this frame, case studies reveal that conditions such as behavioral variant frontotemporal dementia, Wilson’s disease, early-onset AD, and others are frequently mistaken for SZ (Hugo & Ganguli, 2014). Differentiating between a primary psychiatric disorder and a neurodegenerative cause is essential, as the

implications for prognosis, treatment, and management differ significantly (Remschmidt & Theisen, 2005).

In the scientific literature, only one abstract from 2014 reports a neuroimaging study conducted in geriatric patients affected by chronic SZ (aged 55-75 years) that investigated amyloid burden in vivo, using Pittsburgh Compound-B (^{11}C-PIB) PET. This study aimed to explore the relationship between amyloid levels and cognitive impairment in late-onset SZ, reporting that overall levels were not significantly associated with cognitive performance, although some subjects exhibited amyloid deposits (Weldon, Boles Ponto, & Schultz, 2014). Our study differs from this single piece of evidence by focusing on TRS patients (aged 23-50 years) with early age at onset and prominent cognitive deficits reflecting a condition of MCI or SCI, not solely explained by cognitive symptoms ascribable to SZ.

To date, has been not found an association between Aβ levels and cognitive alteration in SZ, and the research gap in this area suggests investigating the neurodegeneration biomarkers in SZ.

1.1 An overview of Schizophrenia and Treatment Resistant Schizophrenia

Schizophrenia (SZ) is a severe psychiatric disorder that affects approximately 1% of the global population (Sullivan, Kendler, & Neale, 2003), with onset typically occurring in late adolescence or early adulthood (Häfner et al., 1994). Although it is widely accepted as a neurodevelopmental disorder resulting from abnormal prenatal and postnatal processes (DeLisi et al., 1997), the neurodevelopmental model alone does not fully explain the origins of SZ. Notably, this framework fails to explain why the age at onset of SZ occurs about a decade after the cortex reaches its peak thickness with reduced white matter integrity and an accelerated decline in chronic stages,

combining the disease trajectory with progressive neurodegeneration (Kochunov & Hong, 2014). Although in previous decades, the pathogenic hypotheses of SZ have successively focused on the dopaminergic system (Davis, Kahn, Ko, & Davidson, 1991), the glutamatergic system (Olney, Newcomer, & Farber, 1999), and multiple integrated neurotransmitter systems (O. D. Howes & Kapur, 2009), it is currently believed to be primarily a late-onset neurodevelopmental disorder (Niendam et al., 2018).

Antipsychotic medications represent the first-line treatment for SZ (Lieberman & First, 2018), reducing relapse rates in the short and medium term (Karson, Duffy, Eramo, Nylander, & Offord, 2016). Despite variability in receptor profiles, all antipsychotics share the ability to bind the dopamine D2 receptor, resulting in D2 receptor occupancy levels between 60% and 80%, as demonstrated by PET studies using ^{11}(C)-Raclopride, except for clozapine and quetiapine, which occupy between 20% and 50% (Seeman & Tallerico, 1999). The antipsychotic efficacy is associated with a D2 receptor occupancy of at least 65-72%, potentially up to 78%, while exceeding this threshold may lead to extrapyramidal side effects and hyperprolactinemia (Kapur, Zipursky, Jones, Remington, & Houle, 2000), (Iyo et al., 2013). Notably, clozapine's mechanism of action differs from other antipsychotics, exhibiting a transient weak binding to cortical D2 receptors rather than striatal ones, along with a greater affinity for D1 and D4 receptors (Ahlenius, 1999), (de Bartolomeis et al., 2022). However, the therapeutic effects of antipsychotics are limited to the reduction of positive symptoms, and evidence regarding the potential efficacy of antipsychotics on negative and cognitive symptoms remains controversial (Erhart, Marder, & Carpenter, 2006), (Leucht et al., 2009).

As previously described, antipsychotic medications are the pivotal treatment for schizophrenia; however, approximately one-third of patients do not respond effectively to these drugs (Lerner, Libov, Kotler, & Strous, 2004).

This clinical condition is known as 'Treatment Resistant Schizophrenia' (TRS). To date, the only approved antipsychotic with a regular indication for TRS is clozapine (Kane, 2017), which in a recent meta-analysis was found to be the antipsychotic with the greatest efficacy in the treatment of SZ (Leucht et al., 2009), (Stroup, Gerhard, Crystal, Huang, & Olfson, 2016). Despite this evidence of effectiveness, clozapine remains underused by clinicians (Rubio & Kane, 2020) due to the wide range of potentially life-threatening side effects such as agranulocytosis, and metabolic or cardiovascular disease (Henderson et al., 2000). The Treatment Response and Resistance In Psychosis (TRRIP) expert group released operational criteria for defining TRS (O. D. Howes et al., 2017). This condition requires a lack of response to two different antipsychotics administered for at least six weeks at dosages equivalent to 600 mg of chlorpromazine (Conley & Kelly, 2001). One of these should be a long-acting formulation for at least four months, with plasma levels monitored (O. D. Howes et al., 2017). The PANSS scale assesses the positive, negative, and general psychopathology, with scores set to at least 70 for the TRS patients (Leucht et al., 2006). Several studies have shown that TRS causes severe disability, which in terms of intensity and clinical-social consequences, such as hospitalizations (Elkis, 2007), poorer functioning (Iasevoli et al., 2016), a greater risk of suicide (Elkis & Buckley, 2016), are more significant than schizophrenia responsive to antipsychotic treatments with high disabling potential (Iasevoli et al., 2016).

1.2 Cognitive impairment in Schizophrenia

The cognitive model of SZ has highlighted the crucial role of dopaminergic signaling in the salience of stimuli. Dopaminergic dysregulation in SZ leads to an aberrant assignment of salience to stimuli, with the cognitive misinterpretation of these stimuli translated into psychotic symptoms (Heinz

& Schlagenhauf, 2010). Cognitive deficits have played a primary role in defining SZ, beginning with Kraepelin's description of "dementia praecox" (Rund, 1998). Alterations in cognitive function appear to precede approximately 2 to 5 years before the full-blown onset of SZ. During this prodromal phase, individuals may experience subtle changes in cognitive function, including difficulties in attention, memory, and executive functioning, which may not be immediately recognized as indicative of schizophrenia (McCutcheon, Keefe, & McGuire, 2023) (*Figure 1*).

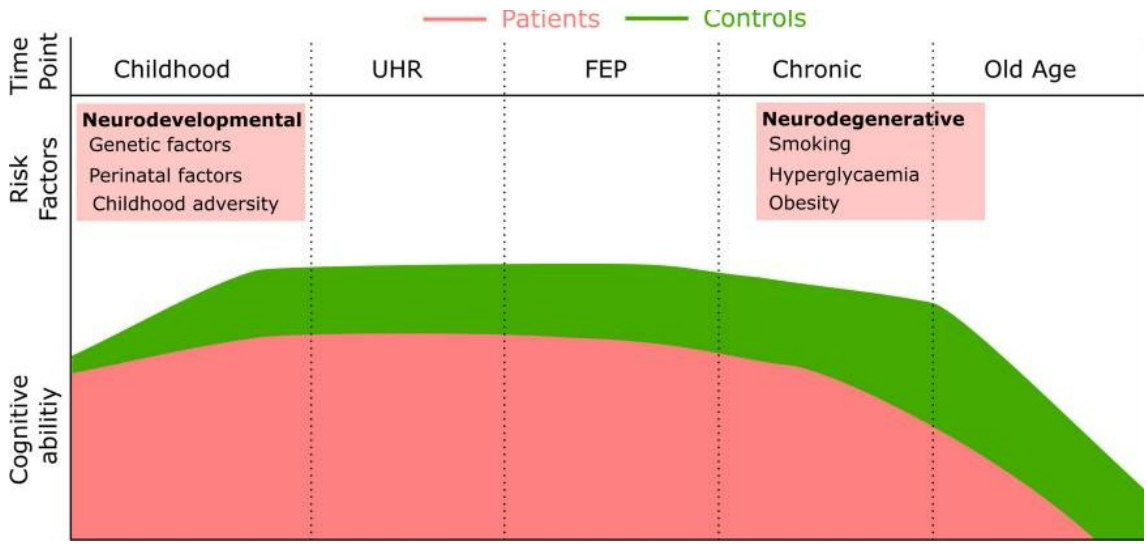


Figure 1. The decline in cognitive function that typically occurs in healthy individuals later in life is observed at an earlier age in those with schizophrenia. Source: *McCutcheon RA et al., 2023, Mol Psychiatry (McCutcheon et al., 2023).*

One of the most studied cognitive deficits in SZ is working memory (Kar & Jain, 2016), which is correlated with neurotransmitter dysfunctions in specific brain areas such as the left dorsolateral and medial prefrontal cortex (Van Snellenberg et al., 2016), (Brisch et al., 2014). Multiple neurotransmitters and brain circuits are implicated in cognitive dysfunction in SZ. Dopaminergic signaling plays a central role in the disorder (McCutcheon, Abi-Dargham, & Howes, 2019), with evidence of increased dopamine activity linked to positive symptoms and cognitive deficits

(Simpson, Kellendonk, & Kandel, 2010). While striatal hyperdopaminergia is associated with acute psychosis (O. Howes et al., 2011), cortical hypodopaminergia may contribute to cognitive impairments (Slifstein et al., 2015), (Frankle, Himes, Mason, Mathis, & Narendran, 2022). Cholinergic systems are essential for attention and memory (Hasselmo, 2006). Despite the acute administration of nicotine showing immediate cognitive benefits in SZ patients (Dondé et al., 2020), long-term use results in detrimental effects (Vermeulen et al., 2018). Muscarinic receptors also appear to influence cognitive functions (Carruthers, Gurvich, & Rossell, 2015), with neuroimaging studies indicating reduced receptor density in SZ patients (Scarr et al., 2009). Both nicotinic and muscarinic receptor systems influence GABAergic and glutamatergic signaling, thereby playing a role in maintaining the balance between excitatory and inhibitory (E/I) neurons within cortical microcircuits (Yi et al., 2014).

1.2.1 Neurodegenerative hypothesis of Schizophrenia

Cognitive functions whose impairment are key symptoms of SZ and AD (Douaud et al., 2014). The overlap of symptoms between SCZ and AD further supports the notion of shared pathophysiological mechanisms despite differing ages of onset, with psychotic symptoms existing in about 50% of patients with AD (P. S. Murray, Kumar, Demichele-Sweet, & Sweet, 2014). The neurodegenerative hypothesis of SZ, first proposed by Kraepelin in 1896, suggests that the disorder is characterized by a progressive deterioration of symptoms, particularly negative and cognitive ones (R. M. Murray, 1994). Approximately 60% of SZ patients experience a decline in these symptoms over time (McGlashan, 1984b), (McGlashan, 1984a)). While cognitive symptoms remain stable during mid-life, early-onset patients may show cognitive decline after several years, indicating a

potential neuroprogressive aspect of the illness (Øie et al., 2010). Notably, patients with TRS show an early onset of the disorder, which is considered a predictor for this condition (Iasevoli et al., 2022) and may increase the risk of cognitive decline. In elderly patients, significant cognitive decline can occur rapidly after the age of 65 (Rapp et al., 2010), particularly affecting processing speed and verbal memory (Loewenstein et al., 2012). Neuroimaging studies reveal progressive anatomical changes in SZ patients, including accelerated reductions in white matter integrity (Kochunov et al., 2013). In this frame, TRS patients exhibit impaired neuroimaging and cognitive measures from the onset of the illness, suggesting that these alterations may serve as a biological signature of treatment resistance (Matrone et al., 2022). Evidence indicates that neuroanatomical changes and cognitive deficits occur early in the disease trajectory (Matrone et al., 2022). In summary, the evidence indicates that SZ may be associated with neurodegenerative processes akin to those seen in AD, which challenges current neurodevelopmental theories. A more thorough understanding of these mechanisms could offer insights into the cognitive decline observed in older individuals with SZ and guide the development of more effective treatment approaches. Additionally, the early onset of TRS serves as a predictor for this condition, suggesting that these patients are at a higher risk for significant cognitive decline, underscoring the need for targeted interventions to address cognitive deficits in this population.

1.3 Functional Neuroimaging in Treatment Resistant Schizophrenia

Neuroimaging techniques have enhanced the understanding of the biological mechanisms underlying SZ, particularly the potential pathogenic implications of dopaminergic dysfunction associated with the disorder. Using tracers that assess dopamine synthesis capacity, release, and receptor binding, neuroimaging studies observed that baseline dopamine levels

increased in the striatum of SZ patients (Fusar-Poli & Meyer-Lindenberg, 2013a), (Fusar-Poli & Meyer-Lindenberg, 2013b). Despite the significance of TRS, few neuroimaging studies have focused on this patient population (Nakajima et al., 2015). Among the limited studies available, presynaptic dopaminergic activity was analyzed in TRS patients, non-responsive schizophrenia (non-TRS) patients, and healthy controls (HC) using ^{18}(F)DOPA PET imaging. The findings revealed that dopamine synthesis capacity was lower in TRS patients and HC compared to non-TRS patients, suggesting that non-TRS patients exhibited increased dopamine synthesis capacity in the striatum, a phenomenon not observed in TRS patients or HC. This result may explain the pharmacological response of non-TRS patients to antipsychotics that primarily block the D2 receptor. Additionally, this underscores why clozapine, which has minimal binding potential on D2 receptors, is considered the gold standard pharmacological treatment for TRS (Demjaha, Murray, McGuire, Kapur, & Howes, 2012). It has been hypothesized that the lack of response to other D2-blocking antipsychotics in TRS may be mainly due to the involvement of other neurotransmitter systems, particularly the glutamatergic system. This dysfunction could make this population of schizophrenia patients less responsive to dopaminergic modulation induced by antipsychotic drugs (Demjaha et al., 2014), (O. D. Howes & Kapur, 2014), (E. Kim et al., 2017).

Studies on cerebral metabolism using PET have identified a metabolic reduction in the frontal lobe and hypermetabolism in the striatum among patients with TRS (Potkin et al., 1994). Previous neuroimaging studies have observed hypoperfusion in the prefrontal cortex (PFC) and relative hyperperfusion in the basal ganglia (BG) among TRS patients (Molina Rodríguez et al., 1997). Conversely, other studies found functional alterations in patients treated with clozapine, leading to hypoperfusion in both the PFC and the BG, along with hyperperfusion in the occipital,

cingulate, and insular cortex (Nakajima et al., 2015), (Molina et al., 2005), (Molina et al., 2008), suggesting that brain functional alterations could be modified by clozapine treatment. However, contrasting results have emerged from studies investigating clozapine's effects on metabolic patterns in TRS patients (Molina Rodríguez et al., 1996), (Rodríguez et al., 1997), (Ertugrul et al., 2009). The results from these prior studies have limited applicability for optimizing therapeutic strategies and thoroughly defining the clinical and molecular characteristics of treatment resistance in SZ. Antipsychotics may lead to a decrease in frontal activity, which is more pronounced in TRS patients receiving clozapine (Holcomb et al., 1996), (Cohen et al., 1997), (Lahti, Holcomb, Weiler, Medoff, & Tamminga, 2003), (Molina et al., 2005). However, it remains uncertain whether these metabolic changes associated with clozapine are unique to this medication or if they also apply to other atypical antipsychotics (Buchsbaum et al., 2009). Notably, the results of functional neuroimaging studies are influenced by various factors, including the patient's psychopathology, the disease state at the time of assessment, the stimulation during acquisition, and the pharmacological treatment administered at the time of the examination (Miller et al., 1997), (Chakos et al., 1994).

Recent findings showed that TRS patients exhibit a higher level of cognitive dysfunctions and neurological soft signs compared to non-TRS patients (de Bartolomeis et al., 2018). Functional neuroimaging study using ^{18}(F)-FDG PET to compare brain metabolism between TRS and non-TRS patients suggests the presence of potential biological markers differing between the two groups that gradually alter the brain metabolism with progressive more severe aberrant functional brain network, associated with the resistance to antipsychotics (Iasevoli et al., 2023). Evidence demonstrated that hypometabolism in the parietal cortex correlates with worse clinical outcomes in TRS patients, suggesting significant involvement of parietal

lobe dysfunction in TRS neurobiology (Iasevoli et al., 2023). Specifically, this altered brain metabolic pattern may stem from a generalized disconnection of neural circuits, with predominant disorganization symptoms in TRS serving as a proxy for cognitive dysfunction (Barone et al., 2022) and leading to more severe cognitive impairment. To date, the cerebral metabolic alteration in TRS has not yet been assessed in relation to significant cognitive impairment.

2. AIM OF THE STUDY

2.1 Study design

The study is observational, encompassing both prospective and retrospective components. The prospective analysis was performed on patients enrolled following informed consent, who subsequently underwent cognitive testing, psychopathological assessment scales, and functional imaging as part of the routine clinical-psychopathological diagnostic procedures intended to tailor therapeutic interventions at our Unit. The retrospective evaluation involved extracting previously collected data from medical records of individuals admitted at the Department of Neuroscience, Unit of Psychiatry and Psychology of the University of Naples "Federico II", either as an inpatient or a Day-Hospital patient. Careful consideration was given to matching subjects across the different groups in a 1:1 ratio based on age and socio-cultural status to mitigate the potential for selection bias.

The objective of this research project is to analyze and categorize individuals with TRS based on the operational criteria established by the TRRIP Working Group (O. D. Howes et al., 2017) and cognitive disorders such as MCI according to the latest recommendations of the Italian consensus group (Boccardi et al., 2020). Participants underwent psychopathological assessment scales, neurocognitive evaluation, and functional imaging investigations, including brain Magnetic Resonance Imaging (MRI) and amyloid (Aβ) PET, in order to 1) highlight distinct patterns of Aβ accumulation relative to TRS patients; and 2) correlate the Aβ patterns of different brain areas with the outcomes of psychopathological and neurocognitive assessment scale scores. The psychopathological and clinical assessment scales used: Positive and Negative Syndrome Scale (PANSS); Thought And Language Dysfunction Scale (TALD); Scale for the

Assessment of Positive Symptoms; Scale for the Assessment of Negative Symptoms (SAPS and SANS).

The neurocognitive tests employed: Mini-Mental State Examination (MMSE); Brief Assessment of Cognition in Schizophrenia (BACS); the Brief Intelligence Test (TIB); the Awareness of Social Inference Test (TASIT); and Facial Emotion Identification Test (FEIT).

2.2 Study Sample

The study was conducted at the Department of Neuroscience, Unit of Psychiatry and Psychology of the University of Naples "Federico II", where 12 subjects from the Psychiatry Pharmacoresistance Outpatient Clinic were deemed eligible. All patients underwent detailed medical and psychiatric evaluations to refine their diagnoses and personalize pharmacological treatment as much as possible, aiming to increase response rates and reduce relapses, recurrences, and chronicity. This comprehensive assessment utilized modern internationally recognized standardized testing tools for psychiatric evaluation. Alongside these assessments, in accordance with ministerial guidelines, patients were invited to undergo Aβ PET scan at the Department of Advanced Biomedical Sciences, Nuclear Medicine Service of the University of Naples "Federico II".

The patient TRS group consisted of 4 females and 8 males (n=12) aged between 23 and 50 years. All data collected anonymously were stored in a database at the at the Department of Neuroscience, Unit of Psychiatry and Psychology of the University of Naples "Federico II". The populations studied were grouped to maintain as much homogeneity as possible regarding age and duration of illness.

Patients defined as TRS showed:

1. Failure to respond to treatment with at least two different antipsychotics within the five years preceding the study, along with the absence of clear symptom remission during the prior five years;
2. Current symptomatic condition (identified by PANSS score > 70 or PANSS positive symptom subscale score > 25);
3. Persistence of symptoms despite further adequate attempts with antipsychotic medication at our clinic;

Patients whose lack of response to antipsychotic treatments is attributed to non-pharmacodynamic factors, considered as pseudo-TRS, were excluded. These factors include poor compliance, substance abuse, drug-drug interactions with concomitant therapies, organic pathologies, and severely stressful or traumatic social conditions.

Enrolled patients will be diagnosed with TRS condition and divided into two subgroups based on the BACS assessment and classified according to the severity of cognitive deficits into Mild Cognitive Impairment (n=5) and Severe Cognitive Impairment (n=7) patients. Mild or Severe Cognitive Impairment was assessed with raw data adjusted according to the normative values of the Italian population (Anselmetti et al., 2008), (Galderisi et al., 2014). Corrected scores were fitted into a 4-point scale to collect equivalent scores, based on which patients were classified as having Severe Cognitive Impairment (SCI, more than two cognitive domains with scores from 0 to 1) or Mild Cognitive Impairment (MCI, at least two cognitive domains with scores from 0 to 1 and the other domains with score ≥2) (Anselmetti et al., 2008).

2.3 Inclusion Criteria

- Diagnosis of Schizophrenia according to DSM-5 criteria;
- Severe disorders in two or more cognitive performances whose dysfunction have been described in schizophrenia (a score of 0 or 1 on

at least two cognitive tasks), requiring differential diagnosis from cognitive dysfunction of organic origin; however, the cognitive dysfunction must not be severe enough to constitute a general cognitive impairment (MMSE score must exceed 24) and should not impair the patient's ability to provide valid informed consent;

- Age between 18 and 55 years;
- Approval of participation through written ratification of informed consent;
- The presence of at least one caregiver, deemed reliable by the experimenters;
- Have performed MRI and amyloid PET as part of the diagnostic-therapeutic procedure for the underlying pathology.

2.4 Exclusion Criteria

- Age over 55 or under 18;
- Neurological pathologies of significant clinical entity;
- Confirmed diagnosis of Intellectual Disability, Dementia of any type, or other neurocognitive disorders with significant alteration of cognitive functions. (e.g., Korsakoff syndrome; amnestic syndrome);
- Substance dependence;
- Substance use/abuse within the past six months prior to screening;
- Duration of illness less than two years;
- Pregnancy or breastfeeding;
- Severe or unstable organic pathologies: cardiovascular, endocrine, metabolic, diabetes;
- Inability to express consent;
- Enrollment in interventional clinical trial (within three months prior to screening);

- Presence of autoimmune diseases (e.g., Systemic Lupus Erythematosus; Behcet's disease).

2.5 Methods – Demographic, clinical, and cognitive characteristics

The study involved a diagnostic evaluation in accordance with DSM-5-TR criteria and the completion of a demographic clinical form containing data related to age, education, age at onset, course, and duration of the illness, substance use and/or abuse, and pharmacological treatments received. Particular emphasis was placed on the number and dosages of antipsychotics taken in the past and those currently prescribed at the time of recruitment to accurately determine the presence or absence of a treatment-resistant condition. The dosages of antipsychotics were converted into chlorpromazine equivalents (Gardner et al., 2010). Additionally, the presence of other classes of psychotropic medications included in each patient's pharmacotherapy was duly recorded. A comprehensive medical examination was conducted, including a detailed neurological assessment and personal medical history review, to identify any non-modifiable exclusion criteria. All available information sources were utilized for the completion of the forms: patients, family members, clinical records, and mental health professionals. Statistical procedures for Demographic, clinical, and cognitive data were performed with the Statistical Package for the Social Sciences (SPSS) using a t-test ($p \leq 0.01$).

2.5.1 Psychopathological and cognitive assessment tools

The psychopathological and clinical rating scales administered were as follows:

• The Positive and Negative Syndrome Scale (PANSS) is designed to explore and quantify psychotic symptoms, consists of 30 items categorized into three clusters (positive symptoms, negative symptoms, and general

psychopathology), and is administered following a 30–40-minute formal semi-structured interview;

• The five-factor model developed from individual items of the PANSS describes five psychopathological dimensions involved in schizophrenia (van der Gaag et al., 2006): POS refers to positive symptoms, which include various items such as delusions, hallucinatory behavior, and suspiciousness; NEG encompasses negative symptoms, including emotional withdrawal, apathy, and motor retardation; DIS evaluates items related to disorganization, such as disorientation and conceptual disorganization; EXC represents the domain of excitement, including tension and psychomotor agitation; and finally, EMO pertains to emotional distress, with items such as worry and depressed mood. The scoring was calculated based on the total score for specific items related to each psychopathological dimension as follows: POS: P1 (delusions) + P3 (hallucinatory behavior) + G9 (unusual thought content) + P6 (suspiciousness/persecution) + P5 (grandiosity) + G1 (somatic concern) + G12 (loss of judgment and insight) + G16 (active social withdrawal) - N5 (difficulty in abstract thinking); NEG: N6 (lack of spontaneity and fluency in conversation) + N1 (affective flattening) + N2 (emotional withdrawal) + N4 (passive/apathic social withdrawal) + G7 (motor retardation) + N3 (insufficient rapport) + G16 (active social withdrawal) + G8 (non-cooperativeness) + G13 (volitional disturbances) - P2 (conceptual disorganization); DIS: N7 (stereotyped thinking) + G11 (attentional poverty) + G10 (disorientation) + P2 (conceptual disorganization) + N5 (difficulty in abstract thinking) + G5 (mannerisms) + G12 (loss of judgment and insight) + G13 (volitional disturbances) + G15 (worry) + G9 (unusual thought content); EXC: G14 (poor impulse control) + P4 (excitement) + P7 (hostility) + G8 (non-cooperativeness) + P5 (grandiosity) + N3 (insufficient rapport) + G4 (tension) + G16 (active social withdrawal); EMO: G2 (anxiety) + G6 (depression) + G3 (feelings of guilt)

+ G4 (tension) + P6 (suspiciousness/persecution) + G1 (somatic concern) + G15 (worry) + G16 (active social withdrawal);

• The Scale for the Assessment of Positive Symptoms and the Scale for the Assessment of Negative Symptoms (SAPS and SANS) are used for evaluating positive and negative symptoms, respectively. The SAPS consists of 34 items divided into four domains (Hallucinations, Delusions, Behavioral Abnormalities, and Positive Formal Thought Disorders), whereas the SANS comprises 25 items categorized into five domains (Affective Flattening, Alogia, Abulia-Apathy, Anhedonia-Asociality, and Attention). For both scales, each item is rated on a scale from 0 (absence of symptom) to 5 (severe symptom), and a global severity score is obtained for each of the respective domains;

• The Thought And Language Dysfunction Scale (TALD) is an operational tool for evaluating language and thought disorganization, encompassing both objectively observable and subjectively reported aspects. It comprises an initial section of open-ended questions followed by a semi-structured interview consisting of 30 items, each rated on a scale from 0 (absent symptom) to 4 (severe symptom).

Neurocognitive functions were investigated using specific tests:

• The Mini-Mental State Examination (MMSE) is used to assess disturbances in intellectual functioning and detect cognitive impairment. It is commonly employed as a screening tool in the evaluation of individuals with dementia or MCI. The test comprises 30 items distributed across seven distinct cognitive domains: temporal orientation, spatial orientation, verbal memory, attention and calculation, recall, language, and constructive praxis;

• The Brief Intelligence Test (TIB), measures the correlation between general intelligence and reading ability. It consists of a quick reading assessment of 54 words with regular and irregular stress patterns, useful for estimating intelligence quotient (IQ) in normal subjects. The scale evaluates premorbid

IQ in cases of dementia, schizophrenia, and traumatic brain injury due to its resilience against cognitive deterioration.

• The Brief Assessment of Cognition in Schizophrenia (BACS), a battery specifically designed for patients with schizophrenia, assessing various cognitive domains:

i. Verbal Memory is measured through a list-learning task consisting of 5 recall trials from a list of 15 words;
ii. Working Memory is evaluated through the repetition of random sequences of numbers, increasing in difficulty, which the patient must repeat to the examiner in ascending order;
iii. Verbal Fluency is assessed through a semantic fluency trial, in which the patient is asked to generate as many words as possible within a specific category (e.g., “animals”) in 60 seconds, and a letter fluency trial, where the patient must generate as many words as possible beginning with a designated letter (e.g., “N”) within the same time frame;
iv. Processing Speed is measured using a Symbol Coding test;
v. Problem Solving is evaluated using the "Tower of London" test, which involves a task initiated by a stimulus image. The patient is presented with two images simultaneously, each depicting colored balls arranged on an abacus, and must determine the minimum number of moves required to transform the arrangement in the first image to match the second.

Metacognitive functions were investigated through:

• The Awareness of Social Inference Test (TASIT) is an audiovisual tool for assessing social cognition, composed of three parts. The first part focuses on emotion recognition, while the second and third parts evaluate the ability to comprehend both literal and non-literal (e.g., sarcastic) meanings of phrases spoken by actors in short sketches, as well as the patient’s ability to express

judgments and opinions regarding the feelings, intentions, and beliefs of the observed characters.

• The Facial Emotion Identification Test (FEIT) is used to assess the ability to identify and discriminate emotions. The patient views black-and-white photographs of human faces displaying various emotions (happiness, sadness, anger, surprise, fear, disgust, or neutrality) on a computer screen and is required to identify the corresponding emotion for each image.

2.6 Methods – PET acquisition for the detection of β amyloid plaques

Patients will undergo a PET study to investigate β-amyloid plaques after receiving an intravenous administration of 185 MBq of ^{18}(F)-Flutemetamol. Initial images will be obtained immediately following the radiopharmaceutical injection, while subsequent images will be captured approximately 90 minutes later. The duration of image acquisition will be approximately 10-20 minutes for the initial images and approximately 20 minutes for the delayed images. PET images will be acquired in 3D mode using a whole-body PET/CT scanner (PHILIPS IngenuityTF 64) with an axial field of view (FOV) of 18 cm, providing 90 sections with a thickness of 2 mm and an axial and transaxial resolution (FWHM) of 4.7 and 4.8 mm, respectively. The obtained images will be reconstructed using iterative reconstruction (Ordered Subset Expectation Maximization, OSEM) and corrected for attenuation using CT images.

2.6.1 CortexID analysis of Standardized Uptake Value region (SUVr) based on β-amyloid tracer

CortexID Suite software divides the cerebral cortex into reference regions and provides a statistical assessment of the differences between groups of healthy controls and the patient analyzed. The voxel-based statistical evaluation was performed through three-dimensional stereotactic surface

projection (3D SSP), along with a Z-score assessment to create a 3D model. The Standardized Uptake Value (SUV) measures the amount of tracer that accumulates in tissues. PET scanners are designed to measure the *in vivo* radioactivity concentration [*kBq/ml*]. Crucial factors are considered, such as the amount of injected tracer and the size differences between patients. The SUV is calculated as:

$$SUV = \frac{r}{ID_{WB}}$$

where *ID* [*kBq*] is the injected tracer amount corrected for decay, and *WB* [g] is the patient's body weight, serving as a surrogate for tracer distribution. The SUV is a dimensionless value assuming that 1 ml of tissue weighs 1 mg. Moreover, the intensity of the values is normalized using brain regions that are less affected by the disease. The choice of reference region is influenced by the type of tracer used, for the 18(F)-Flutemetamol tracer was used the Cerebellar Cortex. The regional normalization process is expressed as:

$$SUV_n = \frac{SUV}{SUV_{region}}$$

where *SUVregion* refers to the mean intensity of tracer uptake measured in a specific reference region of the brain that is considered less affected by the disease. This reference region serves as a baseline for comparison against other regions where amyloid deposition or other pathological changes may occur. The choice of the reference region is crucial and is typically one that is expected to have minimal involvement in the disease process. For example, when using the ^{18}(F)-Flutemetamol tracer for amyloid imaging, the

cerebellar cortex is often selected as the reference region because it generally shows less amyloid accumulation compared to other cortical areas.

2.6.2 SPM 12 visual analysis of the ^{18}(F)-Flutemetamol PET brain distribution

The analysis of the ^{18}F-Flutemetamol PET images was performed using SPM12 software (Wellcome Department of Cognitive Neurology, London, UK; http://www.fil.ion.ucl.ac.uk/spm). The images were spatially normalized in the anatomical brain space MNI (Montreal Neurological Institute). The visual analysis of the PET images obtained in the TRS patient groups with MCI and SCI were evaluated based on the early-phase and late-phase of the acquisition related to the distribution of the amyloid tracer at different anatomical brain levels. Early-phase images reflect cerebral perfusion and/or metabolism, whereas late-phase images indicate amyloid burden.

2.6.3 Amyloid SUVr data TRS group correlation analysis

The amyloid SUVr data analysis considered sixteen brain regions, including Cerebellar whole (reference region), Right and Left Prefrontal Cortices, Anterior Cingulate Cortices, Precuneus Posterior Cingulate Cortices, Parietal Cortices, Occipital Cortices, Sensorimotor Cortices, Mesial Temporal and Lateral Temporal Cortices. Statistical procedures for SUVr data were performed with SPSS using a t-test ($p \leq 0.01$) with Bonferroni post hoc correction analysis ($p \leq 0.003$). The correlation graphs were performed with Microsoft Excel functions.

2.6.4 Amyloid SUVr data individual analysis with the Compound Local Score and Compound Global Score

The Compound Local Score (CmpLS) is a metric that combines several measurements (like tracer uptake, binding affinity, or target density) for a specific brain region of interest (ROI) to give a single composite score. This procedure is implemented in the DORIAN s.r.l. software (https://www.dorian-tech.com). This score helps clinicians or researchers assess the likelihood of pathological changes in that region relative to a healthy control population. The CmpLS helps prioritize ROI in brain imaging studies, particularly neurodegenerative disease research, as a useful tool for identifying the region's most likely affected by conditions like AD. The CmpLS is calculated by integrating different neuroimaging metrics for a given ROI, such as 1) SUVr, measuring amyloid tracer uptake in PET imaging; 2) ELBA (Effectively Linear Binding Affinity), reflecting binding efficacy in the early-phase of amyloid PET acquisition (Chincarini et al., 2016); 3) TDr (Target Density Ratio), resulting in the late-phase of amyloid PET scan relative to the density of specific targets, like proteins, within the ROI (Chincarini et al., 2020). These metrics are individually compared to cutoff values, indicating the normal or abnormal range. The CmpLS aggregates these measurements into a single value, often by applying statistical or machine-learning models. This composite score can weigh each metric based on its significant values. Finally, the CmpLS reflects the likelihood that a particular ROI is affected by an amyloid pathological process. The Compound Global Score indicator provides information on the likelihood of an amyloid PET scan belonging to a positive or negative population based on several neuroimaging metrics SUVr, ELBA, and TDr, returning a number that falls on a composite score global probability scale. The scale shows different multipliers representing an individual's probability of belonging to the negative (green), borderline (yellow), or positive (orange and red) population.

3. RESULTS

3.1 Descriptive statistics for the demographic, clinical and cognitive characteristics of the sample

In this section were analyzed the demographic characteristics of the sample for the five selected variables: age, chlorpromazine equivalent (CPZ eq), duration of illness, age at onset, and education (*Table 1*). Clinical variables are reported as follows: PANSS POS, PANSS NEG, PANSS GEN, PANSS Tot, 5-Factor domains scores (POS, NEG, DIS, EXC, EMO), SAPS Tot, SANS Tot, and TALD (*Table 2*). Moreover, cognitive variables are reported as follows: BACS cognitive domains scores (Verbal Memory, Working Memory, Motor Coordination, Verbal Fluency, Processing Speed, Problem Solving, Composite t-score), MMSE, TIB, TASIT, and FEIT (*Table 3*). For each of these variables, we considered the sample size, the sample mean, and the standard deviation.

Variables	N	Minimum	Maximum	Mean±DS
Age	12	23	50	36.25±8.82
Cpz eq	12	225	900	552.67±238.81
Duration	12	6	28	17.33±7.81
Age at onset	12	13	26	18.83±4.00
Education	12	10	16	13.25±1.36

Table 1. Demographic characteristics of the sample.

Variables	N	Minimum	Maximum	Mean±DS
PANSS POS	12	11	38	21.08±7.39
PANSS NEG	12	15	38	23.67±6.93
PANSS GEN	12	33	73	47.00±11.49
PANSS Tot	12	65	149	91.75±24.43
5-Factor POS	12	16	38	22.75±6.61
5-Factor NEG	12	14	41	24.92±7.55

5-Factor DIS	12	22	55	31.75±9.81
5-Factor EXC	12	14	29	21.08±4.91
5-Factor EMO	12	16	44	25.17±7.79
SAPS Tot	12	31	65	45.08±12.84
SANS Tot	12	27	57	40.92±10.44
TALD	12	6	73	31.3±21.36

Table 2. Clinical characteristics of the sample.

Variables	N	Minimum	Maximum	Mean±DS
Verbal Memory score	12	5.93	50.46	31.54±14.82
Working Memory score	12	0.30	24.14	13.90±6.74
Motor Coordination score	12	15.75	64.36	34.85±15.61
Verbal Fluency score	12	17.17	71.79	37.84±18.80
Processing Speed score	12	0.56	58.78	33.38±17.49
Problem Solving score	12	-0.08	22.08	10.94±8.78
Composite t-score	12	0	59	25.58±21.03
MMSE	12	21	30	27.58±2.94
TIB	12	99.1	117.18	108.9±6.22
TASIT	12	0	128	40.92±45.75
FEIT	12	60	89.1	75.44±7.45

Table 3. Cognitive characteristics of the sample.

3.1.1 Test of differences between averages of the groups (t-test)

The sample (n=12) is then divided into two groups of TRS patients: the first with MCI (n=5) and the second with SCI (n=7). At this point, it is interesting to check whether the differences between the sample averages are significant, i.e., whether they also reflect real differences in the population or whether they are due to random chance. The t-test answers this question.

The t-test tests the *null hypothesis* that the means of two independent populations are equal:

$H_0: \mu_1 = \mu_2 \Leftrightarrow \mu_1 - \mu_2 = 0$

Against the *alternative hypothesis*:

$H_1: \mu_1 \neq \mu_2 \Leftrightarrow \mu_1 - \mu_2 \neq 0$

It is not possible to reject the null hypothesis of equal group means if the t-statistic value is close to 1 and the p-value is greater than the chosen significance level. Conversely, high values of the t-statistic with p-values lower than the chosen significance level indicate that the *null hypothesis* should be rejected, as the statistic values are consistent with the *alternative hypothesis* (i.e., the means are not homogeneous). The chosen significance level is set at 99%, meaning that the p-value must be less than 0.01 for the *null hypothesis* to be rejected.

3.1.2 Assumptions underlying the t-test

Before applying a hypothesis test, it is always necessary to assess whether the underlying assumptions of the test can be reasonably met. The basic assumptions of the t-test are essentially three: the observations must be random and independent of each other; the dependent variable should be normally distributed; and the variances of the two groups must be homogeneous. The assumption of homogeneity of variances ($\sigma_1^2 = \sigma_2^2$) can be ignored if the two samples have equal sizes; in this case, the t-test does not distort the results significantly, and the t-distribution can be used without issues.

3.2 Results of the t-test for demographic characteristics between groups

From the results in *Table 4*, it can be seen that with regard to age, chlorpromazine equivalent, duration of illness, age of onset and education the averages between groups are likely to be equal.

	MCI/SCI	N	Mean±DS	p-value≤0.01
Age	MCI	5	32.60±10.11	0.24
	SCI	7	38.86±7.45	
Cpz eq	MCI	5	464.23±229.69	0.30
	SCI	7	615.84±241.26	
Duration	MCI	5	14.00±8.69	0.23
	SCI	7	19.71±6.75	
Age at onset	MCI	5	18.60±4.39	0.87
	SCI	7	19.00±4.04	
Education	MCI	5	13.80±1.30	0.25
	SCI	7	12.86±1.35	

Table 4. t-test for the demographic characteristics of the sample.

3.3 Results of the t-test for clinical characteristics between groups

The scores of the PANSS sub-scales (PANSS POS, PANSS NEG, PANSS GEN, PANSS Tot), the scores in the domains of the PANSS five-factor model (POS, NEG, DIS, EXC, EMO), the scores on the SAPS Tot and SANS Tot scales and the score on the TALD scale were analyzed in the two groups of TRS patients (MCI; SCI). Table 5 reveals that the averages between the two groups are almost identical for all variables considered.

	MCI/SCI	N	Mean±DS	p-value≤0.01
PANSS POS	MCI	5	16.20±3.56	0.047
	SCI	7	24.57±7.59	
PANSS NEG	MCI	5	21.20±5.26	0.32
	SCI	7	25.43±7.81	
PANSS GEN	MCI	5	41.40±5.03	0.16
	SCI	7	51.00±13.43	
PANSS Tot	MCI	5	78.80±10.38	0.13

	SCI	7	101.00±27.98	
5-Factor POS	MCI	5	18.60±2.51	0.061
	SCI	7	25.71±7.16	
5-Factor NEG	MCI	5	24.60±6.19	0.91
	SCI	7	25.14±8.88	
5-Factor DIS	MCI	5	24.20±2.17	0.015
	SCI	7	37.14±9.58	
5-Factor EXC	MCI	5	19.00±4.18	0.23
	SCI	7	22.57±5.13	
5-Factor EMO	MCI	5	23.20±4.09	0.49
	SCI	7	26.57±9.73	
SAPS Tot	MCI	5	39.60±8.33	0.23
	SCI	7	49.00±14.60	
SANS Tot	MCI	5	37.80±8.41	0.41
	SCI	7	43.14±11.78	
TALD	MCI	5	19±8.83	0.089
	SCI	7	38.29±23.75	

Table 5. t-test for the clinical characteristics of the sample.

3.4 Results of the t-test for cognitive characteristics between groups

The results in *Table 6* showed that for Verbal Memory, Working Memory, Motor Coordination, and MMSE scores, the means between the groups are likely equal. In contrast, regarding Verbal Fluency, Processing Speed, Problem Solving scores, the cognitive Composite t-score, and TIB scores the SCI group shows a higher mean than the MCI group: this difference is statistically significant as the p-value is less than the chosen confidence threshold of 0.01. Therefore, we decide to reject the null hypothesis in favor of the alternative hypothesis.

	MCI/SCI	N	Mean±DS	p-value≤0.01
Verbal Memory score	MCI	5	41.98±8.18	0.031
	SCI	7	24.08±14.23	

Working Memory score	MCI	5	18.81±3.77	0.024
	SCI	7	10.39±6.27	
Motor Coordination score	MCI	5	47.01±16.06	0.013
	SCI	7	26.16±7.95	
Verbal Fluency score	MCI	5	57.20±11.68	**≤0.001**
	SCI	7	24.01±4.64	
Processing speed score	MCI	5	49.13±7.99	**0.002**
	SCI	7	22.14±12.82	
Problem Solving score	MCI	5	19.98±2.27	**≤0.001**
	SCI	7	4.47±4.57	
Composite t-score	MCI	5	48.00±8.63	**≤0.001**
	SCI	7	9.57±6.55	
MMSE	MCI	5	29.60±0.55	0.037
	SCI	7	26.14±3.13	
TIB	MCI	5	114.21±2.18	**0.005**
	SCI	7	105.11±5.25	
TASIT	MCI	5	66.20±49.54	0.1
	SCI	7	22.86±35.9	
FEIT	MCI	5	75.64±11.32	0.94
	SCI	7	75.30±4	

Table 6. t-test for the cognitive characteristics of the sample.

3.5 Descriptive statistics for the amyloid SUVr of the sample

In this section were analyzed the amyloid SUVr of the sample for the sixteen brain regions: Cerebellar whole (reference region), Right and Left Prefrontal Cortices, Anterior Cingulate Cortices, Precuneus Posterior Cingulate Cortices, Parietal Cortices, Occipital Cortices, Sensorimotor Cortices, Mesial Temporal and Lateral Temporal Cortices (*Table 7*). For each of these regions, we considered the sample size, the sample mean, and the standard deviation.

Variables	N	Minimum	Maximum	Mean±DS
SUVr Prefrontal Right	12	0.7	1.11	0.87±0.11
SUVr Prefrontal Left	12	0.66	1.05	0.85±0.10
SUVr Anterior Cingulate Right	12	0.56	0.97	0.83±0.11
SUVr Anterior Cingulate Left	12	0.68	1.05	0.90±0.13
SUVr Precuneus Posterior Cingulate Right	12	0.82	1.12	0.96±0.07
SUVr Precuneus Posterior Cingulate Left	12	0.91	1.35	1.04±0.11
SUVr Parietal Right	12	0.78	1.17	1.01±0.11
SUVr Parietal Left	12	0.70	1.08	0.95±0.10
SUVr Temporal Lateral Right	12	0.96	1.23	1.11±0.08
SUVr Temporal Lateral Left	12	0.97	1.19	1.06±0.07
SUVr Occipital Right	12	1.01	1.28	1.11±0.07
SUVr Occipital Left	12	0.99	1.32	1.10±0.09
SUVr Sensorimotor Right	12	0.69	1.21	0.98±0.14
SUVr Sensorimotor Left	12	0.68	1.20	0.95±0.12
SUVr Temporal Mesial Right	12	0.97	1.16	1.06±0.06
SUVr Temporal Mesial Left	12	0.15	1.15	0.97±0.26
SUVr Cerebellar whole	12	0.84	1.13	0.96±0.08

Table 7. Descriptive analysis of the semi-quantitative estimation of brain amyloid accumulation using the Standardized Uptake Value region (SUVr) in the different brain cortex regions of the sample.

3.6 Results of the t-test for PET amyloid SUVr between groups

From the results in *Table 8*, it can be seen that with regard to amyloid SUVr, the averages between groups are likely to be equal.

	MCI/SCI	N	Mean±DS	p-value≤0.01
SUVr Prefrontal Right	MCI	5	0.94±0.10	0.113
	SCI	7	0.83±0.10	
SUVr Prefrontal Left	MCI	5	0.91±0.09	0.102
	SCI	7	0.81±0.09	
SUVr Anterior Cingulate Right	MCI	5	0.88±0.08	0.180
	SCI	7	0.79±0.12	
SUVr Anterior Cingulate Left	MCI	5	0.97±0.06	0.121
	SCI	7	0.86±0.15	
SUVr Precuneus Posterior Cingulate Right	MCI	5	0.99±0.08	0.445
	SCI	7	0.95±0.07	
SUVr Precuneus Posterior Cingulate Left	MCI	5	1.08±0.15	0.310
	SCI	7	1.01±0.07	
SUVr Parietal Right	MCI	5	1.07±0.06	0.118
	SCI	7	0.96±0.12	
SUVr Parietal Left	MCI	5	0.99±0.06	0.211
	SCI	7	0.91±0.12	
SUVr Temporal Lateral Right	MCI	5	1.15±0.05	0.113
	SCI	7	1.07±0.09	
SUVr Temporal Lateral Left	MCI	5	1.09±0.05	0.221
	SCI	7	1.04±0.07	
SUVr Occipital Right	MCI	5	1.14±0.09	0.211
	SCI	7	1.08±0.05	
SUVr Occipital Left	MCI	5	1.14±0.11	0.199
	SCI	7	1.07±0.06	
SUVr Sensorimotor Right	MCI	5	1.04±0.13	0.233
	SCI	7	0.93±0.14	
SUVr Sensorimotor Left	MCI	5	1.00±0.11	0.271
	SCI	7	0.91±0.13	
SUVr Temporal Mesial Right	MCI	5	1.07±0.07	0.765
	SCI	7	1.06±0.06	
SUVr Temporal Mesial Left	MCI	5	0.88±0.41	0.413
	SCI	7	1.04±0.06	
SUVr Cerebellar whole	MCI	5	1.01±0.06	0.087
	SCI	7	0.92±0.08	

Table 8. t-test for the SUVr between the groups.

3.7 Visual analysis of the amyloid PET images

This section reports the global visual analysis of the 18F-Flutemetamol PET images obtained in the TRS patient groups with MCI and SCI, showing the early- and late-phase of amyloid tracer distribution at different anatomical brain levels (*Figure 2*). The early-phase images, reflecting cerebral perfusion and/or metabolism, exhibit a similar pattern of reduced uptake in the frontal cortex in both groups. In the SCI group, visual analysis also reveals a moderate reduction in radiotracer uptake in the posterior cortical regions (i.e., the parietal and temporal cortices). The late-phase images, which capture the amyloid burden, do not show increased uptake in the cerebral cortex in either group.

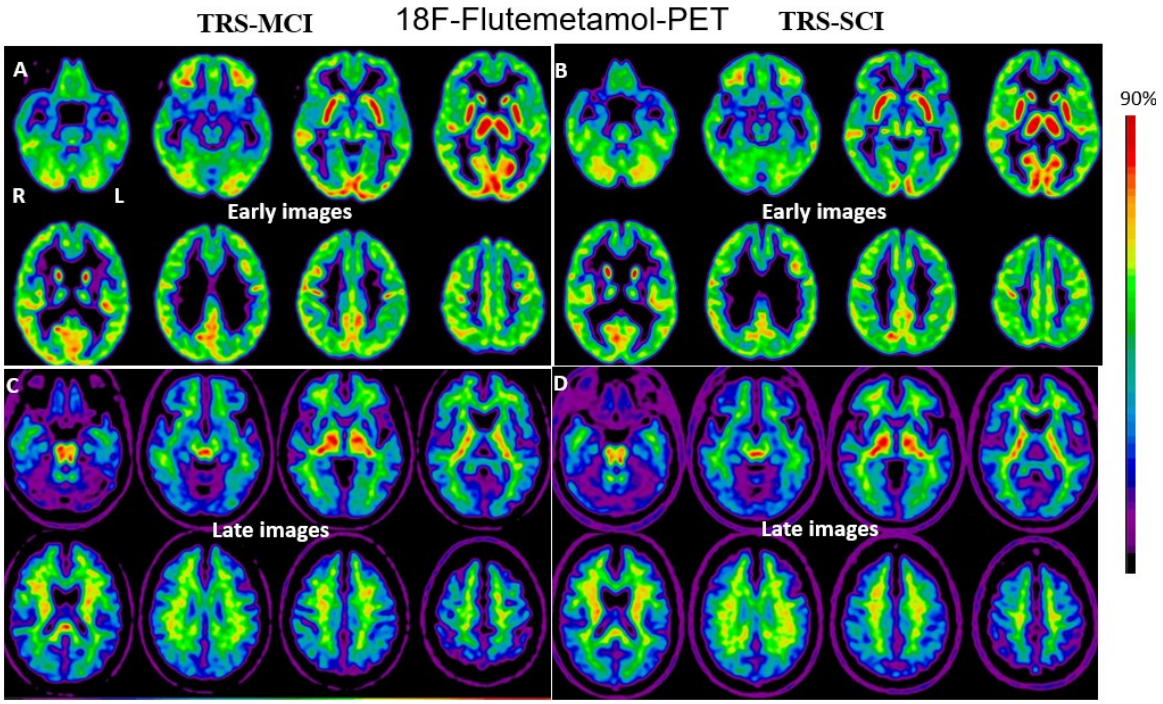


Figure 2. Averaged early-phase (A, B) and late-phase (C, D) images of ^{18}F-Flutemetamol uptake at different cerebral anatomical levels in TRS patients with MCI and SCI. The images were spatially normalized to the MNI anatomical space using SPM12. R=right, L=left.

3.8 Correlation analysis for PET amyloid SUVr with the clinical variables in TRS patients

In this study, we correlate SUVr values with clinical variables. By analyzing the data, we would identify any significant correlations that could provide insights into TRS patient outcomes and neuroimaging data. To quantify the strength and direction of these relationships, we calculated the Pearson correlation coefficient. The *r* correlation coefficient ranges from -1 to 1, where *r=1* indicates a perfect positive correlation, *r=−1* indicates a perfect negative correlation, and *r=0* signifies no correlation, using the formula:

$$r = \frac{n(\Sigma xy) - (\Sigma x)(\Sigma y)}{\sqrt{[n\Sigma x^2 - (\Sigma x)^2][n\Sigma y^2 - (\Sigma y)^2]}}$$

We analyzed our data set, where *n* represents the number of pairs of scores, *x* denotes the values of SUVr, and *y* represents the values of clinical variables. The summations correspond to their respective calculations. To ensure the robustness of our correlation analysis, we conducted a post hoc Bonferroni test with a significance level set at p≤0.003. The Bonferroni correction adjusts the significance level to control for multiple comparisons by dividing the original alpha level α by the number of tests *n*. The formula used is:

$$Bonferroni\ Correction = \frac{\alpha}{n}$$

For instance, if our original significance level was 0.05 and we conducted sixteen tests, our adjusted significance level would be:

$$\frac{0.05}{16} = 0.003$$

This adjustment helps prevent Type I errors (false positives) when multiple hypothesis tests are performed simultaneously. Through this comprehensive analysis, we aimed to uncover significant patterns that could enhance our understanding of how SUVr values relate to clinical variables.

3.8.1 Results of correlation analysis between PET amyloid SUVr in the Left Parietal Cortex with PANSS POS and 5-factor DIS scores

The analysis returned a negative correlation between amyloid tracer SUVr value in the Left Parietal Cortex and PANSS POS scores ($p<0.003$, $r=0.597$) and 5-factor DIS scores ($p<0.002$, $r=0.645$). (*Table 9, Figure 3).*

Correlation			
		PANSS POS	**5-factor DIS**
SUVr Parietal Left	Pearson	-0.773**	-0.804**
	Sign.	**0.003**	**0.002**
	N	12	12

Table 9. Negative correlation between amyloid SUVr in the Left Parietal Cortex with PANSS POS and 5-factor DIS scores.

[**. The correlation is significant at the 0.01 level (two-tailed); post-hoc Bonferroni correction $p\leq0.003$.]

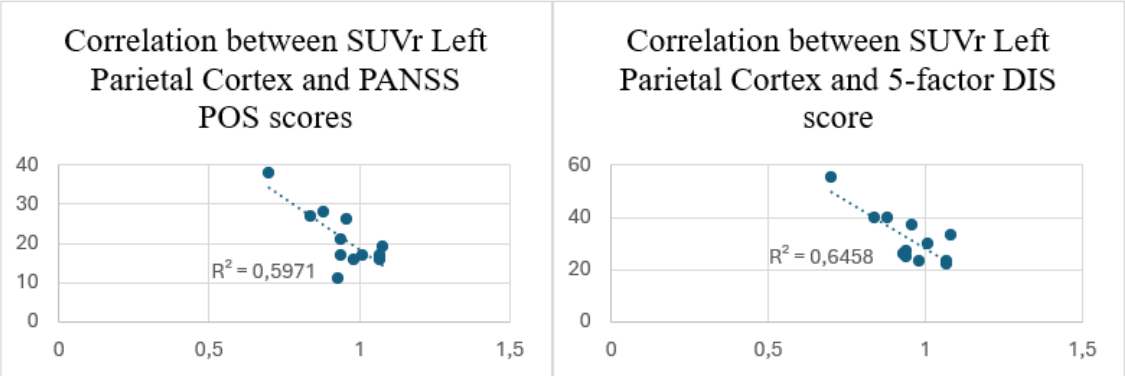


Figure 3. Negative correlation between amyloid SUVr in the Left Parietal Cortex with PANSS POS and 5-factor DIS scores.

3.8.2 Results of correlation analysis between PET amyloid SUVr in the Right Sensorimotor Cortex with PANSS POS and 5-factor EXC scores

The correlation analysis showed a negative correlation between amyloid SUVr in the Right Sensorimotor Cortex with PANSS POS scores ($p<0.003$, $r=0.602$) and 5-factor EXC scores ($p<0.002$, $r=0.619$) (*Table 10, Figure 4*).

Correlation			
		PANSS POS	**5-factor EXC**
SUVr Sensorimotor Right	Pearson	-0.776**	-0.787**
	Sign.	**0.003**	**0.002**
	N	12	12

Table 10. Negative correlation between amyloid SUVr in the Right Sensorimotor Cortex with PANSS POS and 5-factor EXC scores.
[**. The correlation is significant at the 0.01 level (two-tailed); post-hoc Bonferroni correction $p\leq0.003$.]

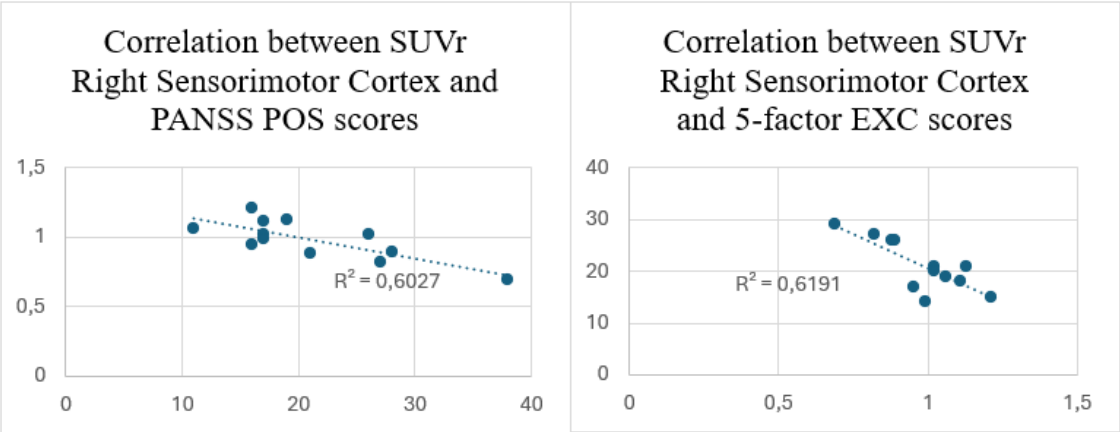


Figure 4. Negative correlation between amyloid SUVr in the Right Sensorimotor Cortex with PANSS POS and 5-factor EXC scores.

3.9 Results of amyloid SUVr data individual analysis with the Compound Local Score and Compound Global Score

We also performed an individual analysis in all TRS patients using the Compound Local Score that provides regional cortical scores and cut-off of SUVr, ELBA (early-phase of amyloid PET acquisition), and TDr (late-phase of amyloid PET acquisition), as shown in *Figure 5*. In only one patient, reported in *Figure 5,* the SUVr values were above the cut-off in the Left and

Right Frontal, Occipital, Lateral Temporal, Precuneus + Post Cingulate, and Left Posterior Parietal while the ELBA, and TDr were below the cut-off in all regions.

Values table.

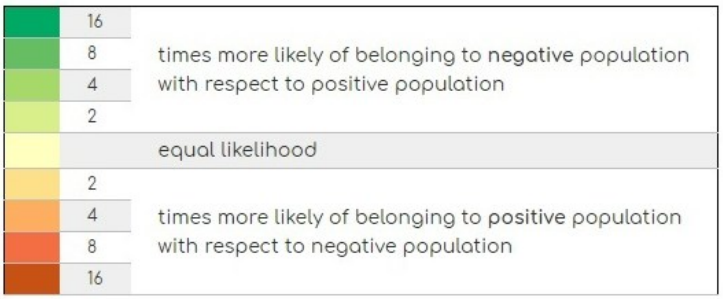
	ROI	SUVr		ELBA		TDr		CmpLS	
		Score	Cut-off	Score	Cut-off	Score	Cut-off	Order	Visual
Left	Frontal	1.28	1.15	0.67	0.8	0.35	0.54	4th	●
	Occipital	1.31	1.2	0.66	0.71	0.37	0.47	2nd	●
	Posterior Parietal	1.25	1.24	0.62	0.81	0.35	0.55	10th	●
	Lateral Temporal	1.23	1.18	0.67	0.73	0.31	0.53	6th	●
	Precuneus + Post Cingulate	1.39	1.32	0.83	0.9	0.37	0.58	9th	●
Right	Frontal	1.31	1.23	0.68	0.81	0.37	0.57	5th	●
	Occipital	1.23	1.12	0.65	0.68	0.38	0.46	1st	●
	Posterior Parietal	1.21	1.29	0.6	0.78	0.35	0.52	8th	●
	Lateral Temporal	1.24	1.14	0.7	0.76	0.34	0.49	3rd	●
	Precuneus + Post Cingulate	1.34	1.25	0.78	0.83	0.38	0.53	7th	●

CmpLS is around (●), below (●) or above (●) its cut-off

Figure 5. The compound local score (CmpLS) individual TRS patient analysis of the brain ROIs with the different neuroimaging metrics: SUVr (Standardized Uptake Value region), ELBA (likely representing binding affinity or effectiveness), and TDr (likely related to target density).

Each ROI was listed in a ranking position (e.g., 1st, 2nd) based on its likelihood or significance, potentially indicating the risk level of each region in terms of positive or negative findings. The dots indicator with different colors indicates the red signals above cutoffs, with a higher likelihood of abnormality; the gray dots with scores around cutoffs, near normal or borderline condition; and the green marks with scores below cutoffs, generally pointing to a lower likelihood of abnormality. The key findings of *Figure 4* include the Right Occipital Region as the most impacted in the amyloid pathology when the 3 methods were combined. This chart provides a quick reference to identify which brain areas show potential prodromal abnormalities to further investigate in a longitudinal study adopted for the patient affected by TRS condition.

Additionally, *Figure 6* shows a Compound Global Score indicator and related information on the probability that the amyloid PET scan data belongs to a positive or negative population according to neuroimaging metrics (SUVr, ELBA, and TDr).



Global cortical scores.

SUVR	CUTOFF SUVR	ELBA	CUTOFF ELBA	TDR	CUTOFF TDR
1.27	1.18	0.68	0.83	0.36	0.53

Figure 6. The Compound Global Score indicator and related information on the probability that a given amyloid PET brain scan or measurement result belongs to a positive or negative population according to different neuroimaging metrics SUVr (Standardized Uptake Value region), ELBA (likely representing binding affinity or effectiveness), and TDr (likely related to target density).

Specifically, the considered TRS patient presents an indicator value of 0.45 on the global composite score scale. The colored scale shows different multipliers representing an individual's probability of belonging to either the negative or positive population: green (16, 8, 4, 2 times more likely to belong to the negative population); yellow (equal probability), there is a 1:1 ratio,

i.e., the probability of belonging to the negative population decreases; orange and red (2, 4, 8, 16 times more likely to belong to the positive population. These colors indicate an increasing probability of belonging to the positive population associated with a higher risk of developing a neurodegenerative disease. Since the Compound Global Score of 0.45 is on the yellow side, it falls within the range of subjects more likely to belong to the positive population. However, mainly because the ELBA and TDR scores are below the cutoffs and the SUVr, although slightly elevated, the Compound Global Score of 0.45 is not conclusive to indicate a high risk.

4. DISCUSSION AND CONCLUSIONS

4.1 Discussion

The rationale behind this study was to investigate the accumulation of amyloid in patients with schizophrenia, particularly those with TRS, due to the prominent cognitive symptoms observed in these individuals (de Bartolomeis et al., 2013), which configure a condition of Mild or Severe Cognitive Impairment. The Cognitive Impairment observed in our patients mirrors the cognitive deficits observed in the early symptoms of dementia, suggesting a potential overlap of underlying neurobiological mechanisms (Douaud et al., 2014). Notably, neuroimaging evidence indicates that amyloid accumulation may precede cognitive decline by several years (Wolk et al., 2018), (Jansen et al., 2015), highlighting the relevance of studying this biomarker in younger patients with TRS.

The results of this study provide valuable insights into the neurobiological underpinnings of TRS and its relationship with cognitive impairment. Despite the lack of significant differences in global amyloid deposition between TRS patients with Mild and Severe Cognitive Impairment, the observed negative correlations between amyloid SUVr in specific brain

regions, such as the Left Parietal Cortex and Right Sensorimotor Cortex, and clinical symptom scores suggest a complex interplay between amyloid pathology and psychotic symptoms.

The negative correlation between amyloid subthreshold accumulation in the Left Parietal Cortex and its potential effects on psychotic and disorganization symptoms suggests several hypotheses. First, disruption in the Parietal Cortex may impair sensory integration, relevant to the coherent perception of self and environment. This impairment could lead to the development of delusional beliefs, emphasizing the integrative role of the parietal lobe and its vulnerability to amyloid deposition in aging (Aizenstein et al., 2008), (Rentz et al., 2010), affecting the brain's ability to produce accurate perceptions, potentially reducing the occurrence of psychotic symptoms (H. H. Kim et al., 2024). Additionally, aberrant neural activity within the Parietal Cortex may disrupt connections with other brain regions involved in psychotic symptoms, such as the temporal cortex and frontal areas. This interruption could result in a reduction of positive symptoms associated with schizophrenia (Imabayashi et al., 2022). Selective damage to frontal-temporal-limbic circuits, where the parietal cortex acts as an integrative hub, may also contribute to psychotic symptomatology (Chung et al., 2022). Conversely, a compensatory mechanism at the early stages of amyloid deposition could trigger balancing mechanisms that suppress excessive neural activity associated with psychosis. This compensatory effect may enhance cognitive performance and mitigate disorganized thoughts and behaviors (Oh, Steffener, Razlighi, Habeck, & Stern, 2016), (Grothe et al., 2017). Neuroplasticity mechanisms during early amyloid deposition could temporarily increase the brain's adaptation to cognitive demands, thus improving the management of disorganized thoughts (Collij et al., 2021). Additionally, amyloid plaques are known to be associated with neuroinflammation, which can alter brain function and potentially lead to a

decrease in the expression of psychotic symptoms (Bakhtiari et al., 2024). Finally, the progression of the disease also plays a critical role; while early amyloid deposition might lead to compensatory mechanisms that reduce positive symptoms, later stages are often characterized by pronounced cognitive decline and worsening negative symptoms (H. H. Kim et al., 2024). The negative correlation between amyloid subthreshold accumulation in the Right Sensorimotor Cortex and psychotic and excitement symptoms presents a compelling avenue for understanding its impact on behavioral symptoms, particularly in the context of TRS. A plausible explanation is that the Sensorimotor Cortex regulates motor responses and behaviors, with a diminished reactivity to stimuli contributing to a reduction in overall behavioral excitation (Bilgel et al., 2018). The decreased responsiveness could lead patients to respond inadequately to situations that would typically elicit an excitatory or psychotic response (Angelopoulou, Bougea, Hatzimanolis, Scarmeas, & Papageorgiou, 2024). Such changes may reflect a broader adaptation strategy employed by the brain and a progression to both cognitive decline and negative symptoms. Moreover, we need to consider the influence of early amyloid deposition on neurotransmitter circuits, particularly those related to dopamine (Hampel et al., 2021), (Ren et al., 2018) linked to psychotic symptoms and relevant to schizophrenia. Other implications of the prodromal amyloid deposition in brain regions affecting the cholinergic system warrant attention. The cholinergic system is crucial for cognitive function and is known to be compromised in various forms of dementia (Hampel et al., 2018). Disruption of cholinergic signaling may lead to impaired dopaminergic control, which is central to the pathophysiology of schizophrenia (Nunes, Addy, Conn, & Foster, 2024). The interplay between cholinergic dysfunction and dopaminergic dysregulation could further exacerbate cognitive impairments in TRS patients. The potential therapeutic role of cholinergic drugs, particularly

muscarinic agonists like xanomeline, emerges as a promising avenue for treating TRS. Xanomeline has shown efficacy in enhancing cognitive function and reducing psychotic symptoms by targeting cholinergic receptors, thus addressing both cognitive deficits and dopaminergic dysregulation (Kidambi, Elsayed, & El-Mallakh, 2023), (Peng et al., 2024). Additionally, we may consider the possible activation of compensatory mechanisms within the brain regions that help mitigate behavioral symptoms. The brain's efforts to maintain homeostatic balance in response to prodromal amyloid accumulation in the Sensorimotor Cortex might explain why some patients exhibit reduced manifestations of psychosis despite underlying neurodegenerative changes (Couch, Ashford, Zhang, & Prina, 2023). While previous research has linked amyloid accumulation to cognitive decline in neurodegenerative disorders (Chapleau, Iaccarino, Soleimani-Meigooni, & Rabinovici, 2022), this study suggests that other factors may contribute to cognitive deficits in TRS.

4.2 Limitations

The study has several limitations concerning the sample. Regarding sample size, the limitation lies in the small number of participants enrolled, due to strict inclusion criteria aimed at creating groups as homogeneous as possible in terms of age, disease duration and chlorpromazine equivalents. In addition, the lack of compliance with scanning neuroimaging techniques, firstly MRI and then amyloid PET with early- and late-phase, led to several dropouts (N=4), as well as exclusions due to brain malformations detected on MRI (N=1). The number of subjects should be increased in future studies, as the current sample size did not allow for more refined statistical analyses. At the same time, this is the first study to assess the presence of amyloid deposits in patients with SZ and would benefit from a longitudinal design to evaluate the progression of accumulation over time. Another limitation is the

absence of a young control group, such as our pull of recruited SZ patients (aged 23-50 years). To partially overcome this limitation, we relied on databases of maps of healthy controls aged 45-80 years used by the OSEM analysis software.

4.3 Conclusion

In conclusion, these findings underscore the importance of considering both neurodevelopmental and neurodegenerative perspectives in schizophrenia. Future longitudinal study designs should assess how amyloid levels and cognitive function may evolve during the TRS patient's disease trajectory. Our findings highlight the need for further exploration into the role of early amyloid deposition and possible cholinergic dysfunction in TRS. Understanding these mechanisms could pave the way for innovative treatment strategies that target both cognitive and psychotic symptoms, ultimately improving the quality of life for affected individuals. While this study did not find conclusive evidence supporting a direct link between β-amyloid accumulation and cognitive impairment in TRS, it opens avenues for further exploration into the neurobiological mechanisms underlying this complex condition. Understanding these mechanisms is crucial for developing targeted therapeutic strategies that address both psychotic symptoms and cognitive deficits in individuals affected by TRS.

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