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Longitudinal Assessment of brain-derived neurotrophic factor in Sardinian Psychotic patients (LABSP): a study protocol for a prospective observational study

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Complete List of Authors:	Primavera, Diego; Universita degli Studi di Cagliari, Medical Science and Public Health Manchia, Mirko; Dalhousie University, Pharmacology; Universita degli Studi di Cagliari, Medical Science and Public Health Deriu, Luca; Universita degli Studi di Cagliari, Medical Science and Public Health Tusconi, Massimo; Universita degli Studi di Cagliari, Medical Science and Public Health Collu, Roberto; Universita degli Studi di Cagliari, Biomedical Sciences Scherma, Maria; Universita degli Studi di Cagliari, Biomedical Sciences Fadda, Paola; Universita degli Studi di Cagliari, Biomedical Sciences Fratta, Walter; Universita degli Studi di Cagliari, Biomedical Sciences Carpiniello, Bernardo; Universita degli Studi di Cagliari, Medical Science and Public Health
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3 **Longitudinal Assessment of brain-derived neurotrophic factor in Sardinian Psychotic**
4 **patients (LABSP): a study protocol for a prospective observational study**
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7 Diego Primavera¹, Mirko Manchia^{1,2}, Luca Deriu¹, Massimo Tusconi¹, Roberto Collu³, Maria
8 Scherma³, Paola Fadda^{3,4}, Walter Fratta^{3,4}, Bernardo Carpiniello¹
9

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11
12 ¹Section of Psychiatry, Department of Public Health, Clinical and Molecular Medicine,
13 University of Cagliari, Cagliari, Italy. ²Department of Pharmacology, Dalhousie University,
14 Halifax, Nova Scotia, Canada. ³Department of Biomedical Sciences, Division of Neuroscience
15 and Clinical Pharmacology, University of Cagliari, Cagliari, Italy. ⁴Centre of Excellence
16
17 “Neurobiology of Dependence”, University of Cagliari, Cagliari, Italy.
18
19

20
21
22 *Correspondence to:
23

24
25 Professor Bernardo Carpiniello, MD
26

27
28
29 Section of Psychiatry, Department of Public Health, Clinical and Molecular Medicine,
30 University of Cagliari, Cagliari, Italy.
31

32
33 Via Liguria, 13
34

35
36 09127 Cagliari, Italy
37

38
39 Tel: +3907041518; Fax:+39070480083; E-mail: bcarpini@iol.it.
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ABSTRACT

Introduction: Brain-derived neurotrophic factor (BDNF), the most widely distributed neurotrophin in the central nervous system (CNS), plays a crucial role in neurodevelopment, synaptic plasticity, and neuronal function and survival. BDNF plays a crucial role in mood disorder and it has also been implicated in psychotic disorders.

Methods and analysis: We propose to study of a cohort of SCZ and SAD patients, with the aim of disentangling the relationship between peripheral BDNF serum levels and changes of psychopathology, cognition, and drug treatments. Patients were recruited at the Section of Psychiatry of the Department of Public Health, Clinical and Molecular Medicine of the University of Cagliari, Cagliari, Italy. We collect BDNF serum levels as well as socio-demographic, psychopathological and neurocognitive measures. Structured, semi-structured, and self-rating assessment tools, such as the Positive and Negative Syndrome Scale (PANSS), and the Premorbid Adjustment Scale (PAS) for psychopathological measures, the Brief Assessment of Cognition in Schizophrenia (BACS) for cognitive function, and the World Health Organization-Quality of Life (WHO-QOL) and the Subjective Well-Being Under Neuroleptics (SWN) for social and personal functioning will be used.

Ethics and dissemination: This study protocol was approved by the University of Cagliari Health Agency Ethics Committee (NP2016/5491). The study will be conducted in accordance with the principles of good clinical practice, in the Declaration of Helsinki in compliance with the regulations. Participation will be voluntary and written informed consent will be obtained for each participant upon entry into the study.

Strengths and limitations of this study

- This is a longitudinal prospective study in a sample of psychotic patients with accurate clinical characterization
- Data obtained from this study will clarify whether changes in brain-derived neurotrophic factor (BDNF) serum levels are correlated with longitudinal variation of psychopathology
- This study will assess whether psychotropic treatment mediates the relationship between BDNF serum levels and psychopathology
- Sampling will be performed in a tertiary clinic specialized in treatment of psychotic patients, possibly limiting ecological validity of the study.
- The sample size might not be sufficient to detect effect sizes of small magnitude.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is the most widely distributed neurotrophin in the central nervous system (CNS),¹ being expressed in almost all the cortical areas, as well as in several spinal cord regions.² There is substantial evidence that BDNF plays a crucial role during brain development, as well as in the process of differentiation, synaptogenesis and neuronal plasticity.³⁻⁶ BDNF was the second neurotrophic factor to be characterized after the nerve growth factor (NGF).⁷ Similarly to other neurotrophins, BDNF is synthesized in a pre-pro form consisting of 47 amino acids.² Extracellular BDNF appears to have two forms: pro-BDNF and mature BDNF,^{2,8} which are formed after cleavage of the precursor protein prepro-BDNF.^{8,9} To exert its molecular effects in the adult CNS, mature BDNF interacts with its high affinity receptor, the tropomyosin-related kinase receptor type B (TRKB), that, similarly to BDNF, is widely distributed in the adult CNS.² BDNF induces dimerization of TRKB with kinase activation and autophosphorylation of tyrosine residues, with subsequent activation of several adaptor proteins, which, in turn leads to phosphorylation of phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase - extracellular signal-regulated kinase (MEK-ERK), phospholipase C γ 1 (PLC γ 1) and cyclic AMP-responsive element-binding protein (CREB), all downstream pathways regulating neurite outgrowth, cell survival, cellular differentiation and synaptic plasticity.^{2,10}

It is known that BDNF freely crosses the blood-brain barrier.¹¹ In keeping with this observation, it has been shown that levels of BDNF in serum are strongly correlated with CNS concentrations.¹² Consequently, peripheral serum levels of BDNF might be a reliable biomarker of disease status for several severe psychiatric disorders, such as major depressive disorder (MDD), bipolar disorder (BD) and schizophrenia (SCZ),¹³ all conditions

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3 that appear to share a dysregulation of BDNF, and of its related molecular pathways, as a
4
5 key pathophysiological feature.¹⁴
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8 A decline in serum BDNF levels has been consistently identified in chronic and medicated
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10 SCZ patients,¹⁵⁻²⁰ as well as in first episode and medication naïve SCZ patients,²¹⁻²⁵
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12 compared to unaffected subjects. Conversely, other studies showed increased BDNF serum
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14 levels in patients with SCZ,^{26 27} or have not been able to demonstrate differences in serum
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16 BDNF concentrations between drug-free or drug-naïve patients and healthy controls.^{28 29}

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18 This discrepancy may depend on the clinical heterogeneity of the samples studied (i.e.
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20 different stage of illness, subtype of schizophrenia, and effect of medication), as well as on
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22 the different assessment tools employed. However, following a recent quantitative meta-
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24 analytical estimate by Fernandes et al.,³⁰ there is general consensus that SCZ is associated
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26 with a moderate decrease of serum and plasma BDNF levels compared to healthy controls.
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28 This entails that decreased serum BDNF levels might be a marker of illness status in SCZ (i.e.
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30 disease biomarker). There is a lack of knowledge, however, on the temporal manifestation
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32 of this decline. The decrease of peripheral BDNF could be constant, with pre-morbid levels
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34 roughly similar to those of unaffected individuals, linearly declining during the course of SCZ.
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36 Conversely, BDNF peripheral levels might fluctuate in association with acute
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38 psychopathological phases of the disorder. On top of this, BDNF peripheral levels might vary
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40 as a result of drug treatments typically used in major psychoses, such as antipsychotics. To
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42 date, however, the study of the relationship between BDNF serum levels and drug
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44 treatment in SCZ and SAD patients remains inconclusive. There is extensive pre-clinical
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46 evidence suggesting that typical antipsychotics might decrease BDNF expression whereas
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48 atypical antipsychotics, with exception of risperidone, could increase BDNF expression.³¹
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52 Data on clozapine, for instance, show that treated chronic SCZ patients have higher BDNF
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3 levels than patients treated with typical neuroleptics^{16 18} or with risperidone.¹⁶ Other
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5 studies, however, found no effect of antipsychotic treatment on the levels of serum BDNF.²³
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8 ^{32 33} Taken together, these data appear contradictory, making difficult to infer conclusions
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10 about the effects of antipsychotic treatment on serum BDNF levels. Meta-analytical findings,
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12 however, point to small but significant increase of serum BDNF levels under antipsychotic
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14 treatment, although a class-specific effect has not been investigated.³⁰

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17 One final introductory remark concerns the link between BDNF and cognitive function in
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19 SCZ. Indeed, cognitive disruption seems to represent the core symptomatology that
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21 distinguishes SCZ patients from those affected by other psychoses. Cognitive deficits are
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23 related to social integration, to social problem solving and to the acquisition of various
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25 skills,³⁴ and are considered the most powerful predictors of functional outcome in patients
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27 with SCZ.³⁵ Cognitively impaired chronic SCZ patients appear to show low serum BDNF
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29 levels.³⁶ A recent meta analysis confirmed indeed this observation,³⁷ showing that higher
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31 levels of BDNF peripheral expression correlated with better cognitive performances in
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33 reasoning and problem solving areas.

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38 The apparent gap in the knowledge about the longitudinal variation of BDNF serum levels,
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40 and its relationship with clinical and treatment variables in major psychoses, needs to be
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42 filled by additional clinical research. Here we present the design and methodology of a
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44 prospective observational study that takes advantage of a well-characterized population of
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46 psychotic patients carefully monitored at an academic community health centre.
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Aims of the current study

Our study has the following primary and secondary aims:

Primary aims:

1. Explore the longitudinal relationship between peripheral serum BDNF levels and variation in psychopathology, as well as in drug treatment, in a cohort of patients with major psychosis recruited at different stages of the illness.
2. Assess whether peripheral serum BDNF levels might predict variation of cognitive function.

Secondary aims:

1. Study whether patients' baseline characteristics prior to enrolment, namely duration of illness, duration of untreated psychosis (DUP), psychiatric and somatic comorbidity, age at onset, gender, family history of psychiatric disorders, premorbid adjustment, and the type of illness course might moderate the longitudinal relationship between peripheral serum BDNF and psychopathology.
3. Study whether measures of peripheral serum BDNF levels correlated with baseline measure of social functioning.
4. Analyze the relationship of peripheral BDNF levels with tolerability of antipsychotic treatment, as expressed by the presence of extrapyramidal symptoms (EPS).

METHODS

Study design

Longitudinal Assessment of BDNF in Sardinian Psychotic patients (LABSP) is an 18-month observational prospective cohort study. The cohort will be recruited from patients followed-up at the community mental health centre of the Psychiatry Research Unit of the,

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3 Department of Public Health, Clinical and Molecular Medicine of the University of Cagliari.
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5 Blood samples from recruited patients will be taken at baseline (T_0), and at three
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7 consecutive time points: 6 months (T_1), 12 months (T_2), and 18 months (T_3) (Figure 1). The
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9 temporal fluctuations of psychopathology, as well as the assessment of pharmacotherapy,
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11 measures of cognitive function, and manifestation of treatment-related side effects (i.e.
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13 EPS) will be also evaluated at each time point. The observational prospective design permits
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15 to disentangle how the variables might influence the outcome under study. Thus, it should
16
17 be possible to infer causality and assess whether changes in peripheral serum levels BDNF
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19 predate or, alternatively, are a consequence of psychopathological changes and/or drug
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21 treatment. As we will study the trajectory of peripheral serum BDNF levels over time, the
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23 recruited cohort will serve as its own control. We also expect to obtain a more accurate
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25 estimation of the patterns of association between BDNF serum levels and all the
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27 independent clinical variables under consideration.
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36 **Participants and recruitment**

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38 The recruitment of the sample will be based on a two-step process. In the first step, all
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40 patients followed-up at the community mental health centre of the University of Cagliari,
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42 Cagliari, Italy with a diagnosis of SCZ or SAD according to the Diagnostic and Statistical
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44 Manual of Mental Disorders-IV-Text Revision (DSM-IV-TR)³⁸ will be identified in the medical
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46 record database. The electronic search will be performed using lower (18) and upper (65)
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48 age limits, with no constraint regarding the presence of comorbid psychiatric disorders.
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50 After eligible patients will be identified in the database, the diagnosis of SCZ or SAD will be
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52 confirmed using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders, Patient
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54 Edition (SCID-I/P)³⁹ administered by trained mental-health professionals (psychiatry
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3 residents or senior clinical staff). Written, informed consent will be obtained from all eligible
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5 participants. Participation to the study will be voluntary and patients will be able to
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7 withdraw consent at any point with no disadvantage to their treatments. A psychiatric
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9 assessment will establish that patients' ability to consent is not compromised by their
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11 psychopathological status.
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14 15 16 17 **Inclusion and exclusion criteria**

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19 Inclusion criteria are: 1) age between 18 and 65 years; 2) diagnosis of SCZ or SAD according
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21 to DSM-IV-TR; and 3) stability during the six months before recruitment. Exclusion criteria
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23 are: 1) refusal to provide consent; 2) presence of acute psychopathological symptoms; 3)
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25 presence of illness-related cognitive impairment of such severity that affects their ability to
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27 cooperate; 4) presence of major unstable medical illness; 5) severe mental retardation.
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33 34 **Sample size and power calculation**

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36 Our main null hypotheses are that there is no difference between mean BDNF serum levels
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38 at different time points in relation to: 1) the longitudinal psychopathological changes of SCZ
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40 and SAD patients; 2) drug treatment; and 3) measures of cognitive functioning. We based
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42 our power analysis on previous findings of BDNF serum levels longitudinal variation in a
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44 small cohort of SCZ patients.²² A sample size of 53 individuals would be sufficient to obtain a
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46 90% statistical power to detect significant difference at $\alpha = 0.05$ between mean serum BDNF
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48 levels at each time point, considering an attrition rate for time point of 5%. This analysis has
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50 been performed using repeated measures and sample size (RMASS) software.⁴⁰
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Measures and materials

Socio-demographic variables, personal and family history data

Socio-demographic data will be collected using the Association for Methodology and Documentation in Psychiatry (AMDP)⁴¹ assessment tool. Briefly, we will gather information on gender, age, level of education, marital status, offspring, economic status, and profession. Further, we will collect information on previous and current substance and/or alcohol abuse, and smoking status, the latter being quantified also as number of cigarettes smoked per day. Information on family history of any psychiatric disorders will be collected through direct interview of the participant and at least one first-degree relative or significant other, as well as through an accurate review of available medical records.

Psychopathological measures

The main psychopathological measures will be collected through the AMDP syndrome scale,⁴¹ which has shown to be valid in discriminating various forms of psychoses, as well as in providing a detailed psychopathological description of the subjects under study.⁴² As previously reported, patients' diagnosis will be confirmed using the SCID-I/P.³⁹ Furthermore, all recruited patients will undergo assessment with the Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II)⁴³ to detect the presence of comorbid personality disorders. Information on age of onset, age at first intervention (pharmacological and/or psychotherapeutic and/or psychosocial) will be based on retrospective information collected through direct interview of the patient, and at least one relative or significant other, as well as through an accurate review of available medical records. This approach should minimize the impact of recall bias on the assessment of key clinical measures.

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3 We will also estimate DUP, the interval between the age of onset of full-blown psychotic
4 symptoms and first antipsychotic treatment, using the Psychopathological Onset Latency
5 and Treatment Questionnaire.⁴⁴ Indeed a longer DUP appears to be related to a worse
6 short-⁴⁵ and long-term clinical outcome^{46 47} in SCZ individuals, as well as with lower BDNF
7 serum levels,⁴⁸ although the latter finding has been not replicated in subsequent studies.⁴⁹

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14 Other measures of illness severity, such as number of hospital admissions and type of
15 clinical course will be collected for the analysis.

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Psychopathological assessment will include the Positive and Negative Symptom Scale for
Schizophrenia (PANSS),⁵⁰ and the Clinical Global Impression Scale for Schizophrenia (CGI-
SCH).⁵¹ The latter measure is a valid assessment tool for monitoring longitudinal
psychopathological symptoms in routine clinical practice.⁵² Moreover, we will assess
premorbid dysfunction, a well established prognostic marker in SCZ patients, using the
Premorbid Adjustment Scale (PAS).⁵³ All these psychopathological measures will be analyzed
in relation to BDNF serum levels.

Social functioning and quality of life measures

Evaluation of social functioning will be carried out with the Personal and Social Performance
Scale (PSP),⁵⁴ which showed good reliability in SCZ patients.⁵⁵ Further, assessment of quality
of life (QOL) will be performed using the World Health Organization Quality-Bref (WHOQOL)
version which has shown good validity and reliability in variety of psychiatric illnesses
including SCZ.^{56 57} Finally, the standardized assessment of subjective well-being and will be
made with the Subjective Wellbeing under Neuroleptics-Short Version (SWN-S).⁵⁸

Cognitive measures

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3 The cognitive performance will be examined in a standardized way using the Brief
4 Assessment of Cognition in Schizophrenia Scale (BACS).⁵⁹ It takes approximately half an hour
5 to administer in healthy controls, and includes brief assessments of four of the seven
6 neurocognitive domains designated as important by the Measurement and Treatment
7 Research to Improve Cognition in Schizophrenia (MATRICS) battery: reasoning and problem
8 solving, processing speed, verbal memory, and working memory. The composite score was
9 shown to have high test–retest reliability in SCZ patients.⁵⁹ Moreover, the BACS appears to
10 assess reliably measures of functional capacity.⁶⁰ The BACS will permit a rapid assessment of
11 cognitive function at different time points, allowing correlation of these measures with
12 peripheral serum BDNF levels. Indeed, several studies have shown that peripheral BDNF
13 levels might be biomarkers of cognitive function in schizophrenia.⁶¹⁻⁶⁴

31 Assessment of side effects

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33 We will monitor the onset of antipsychotics related side effects using the Dosage and
34 Treatment Emergent Symptoms Scale (DOTES),⁶⁵ and the Extrapyrimal Symptoms Rating
35 scale (ESRS).⁶⁶ Of note, one study showed that SCZ patients with tardive dyskinesia (TD) had
36 lower plasma BDNF levels than those without TD.⁶⁷

46 Biometric, metabolic, and cardiovascular measures

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48 There is growing evidence for a role of BDNF in appetite and weight regulation as well as in
49 eating disorders, possibly via its interaction with the serotonin system. BDNF is expressed in
50 brain regions including the hypothalamus, where its infusion in rats has been shown to
51 increase serotonin turnover, and suppress appetite.⁶⁸ Conversely, mice with only one copy
52 of the BDNF gene displayed increased food intake and weight, with decreased serotonin
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3 metabolite to serotonin ratio.⁶⁹ Conditional ablation of the BDNF gene also leads to similar
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5 hyperphagic and obese phenotypes.⁷⁰ BDNF may also regulate weight and food intake via its
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7 regulation of the dopamine system.^{71 72} Antagonism on dopamine receptors 3 (DRD3) was
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9 shown to alter attentional bias to food cues in low-restrained obese or overweight
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11 individuals.⁷³ This study suggests that BDNF may be a key factor in the regulation of eating
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13 behavior upstream of dopamine and serotonin systems and BDNF gene variants may play a
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15 role in antipsychotic-induced weight gain. Based on these findings, we will evaluate the
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17 following clinical parameters: weight, waist circumference, body mass index (BMI), liver and
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19 kidney function panel, serum glucose, glycosylated haemoglobin, lipid profile, prolactin
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21 (PRL), and creatine phosphokinase (CPK). The cardiovascular function will be monitored by
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23 electrocardiogram (ECG), with evaluation of QTc interval.
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31 **Assessment of BDNF serum levels**

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33 BDNF serum will be evaluated using human BDNF ELISA Kit (Booster Immunoleader, Cat. N.
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35 EK0307), for quantitative detection in cell culture supernatants, serum and plasma. This kit
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37 is based on a standard sandwich enzyme-linked immune-sorbent assay technology for
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39 specific quantifications of natural and recombinant human BDNF with a high sensitivity (<
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41 2pg/ml). After blood sampling, serum will be allowed to clot in a serum separator tube for
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43 about 4 hours at room temperature. After that it will be centrifuged at approximately 1000
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45 X g for 15 minutes. Supernatant serum samples will be collected in small aliquot and stored
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47 immediately at -20°C for future analysis. Then, samples will be processed according to kit
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49 protocol and instructions. Optical density absorbance of each sample will be read with a
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51 450nm filter in a microplate reader (Thermo Scientific Multiskan FC) within 30 minutes after
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3 the final step of the kit procedure. Data obtained will be analyzed using the Thermo
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5 Scientific SkanIt Software 3.0 for Multiskan FC.
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10 **Data analysis plan**

11 Mixed-effects linear regression models (MLRM) will be used to analyze longitudinal data.^{74 75}

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13 Specifically, we will regress independent variables (both categorical and continuous) on
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15 BDNF serum levels (dependent variable). Indeed, MLRM allow to model individual change
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17 cross time and appear to be more flexible in terms of repeated measures, particularly when
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19 the number of observations per subject is not the same over time.^{74 75} Further, these models
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21 allow generalization of non-normally distributed data. Missing data for independent
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23 variables will be dealt with imputation. All data will be analyzed using “lme4” package
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25 implemented in R.⁷⁶
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34 **DISCUSSION**

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36 Schizophrenia (SCZ) is characterized by three distinct, but to a certain extent intertwined,
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38 clusters of symptoms: positive, negative and cognitive symptoms. These three clusters of
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40 symptoms present with a varying degree of severity and with a specific prevalence
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42 depending on the stage of the illness.⁷⁷ Indeed, patients with stable diagnoses of SCZ and
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44 SAD present often incomplete remissions (particularly from negative symptoms) and at
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46 times recurrences or relapses of positive symptoms, mainly delusions and hallucinations.⁷⁸
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49 Cognitive function, however, appears to decline steadily over the course of the illness,
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51 although most of the magnitude of the differences in cognition between SCZ patients and
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53 unaffected subjects appears to be modulated by age.⁷⁸
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3 One key aspect in diagnostically stable SCZ and SAD patients is being able to predict the
4 manifestation of these psychopathological exacerbations. Certainly, given that fluctuations
5 appear to be more prominent for positive symptoms and to a lesser extent for negative
6 ones, rather than for cognition, researcher have focused on the identification of
7 prodromal signs of relapses/recurrences using, for instance, home telemonitoring of
8 symptoms change via mobile phone-based platforms.^{79 80} This approach has proven
9 effective in reducing the number of relapses, and consequent hospitalizations, in psychotic
10 individuals.^{79 80}

11
12 Our prospective study, LABSP, expands on this evidence aiming at monitoring not only the
13 clinical signs of SCZ patients over an 18-month period, but, more importantly, assessing
14 the predictive power of a well-established biomarker of illness status in SCZ (BDNF serum
15 levels), on the clinical trajectory of SCZ. We expect to find correlation of BDNF levels with
16 longitudinal variation of psychopathology, particularly concerning positive symptoms. This
17 prospective approach, to our knowledge, has not been proposed previously in the
18 literature, and might offer novel data on serum BDNF as a biomarker of
19 psychopathological changes in accurately monitored SCZ patients. If such a correlation will
20 be identified, the joint analysis of clinical and BDNF data might explain a larger proportion
21 of psychopathological change that clinical data alone.

22
23 One additional point of novelty concerns the analysis of the impact of psychiatric
24 comorbidity on BDNF levels. Indeed, SCZ is not rarely associated with other axis I and II
25 disorders that typically affect negatively the course and outcome of the disorder.⁸¹
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27 However, to our knowledge the relationship between serum BDNF levels in SCZ and
28 psychiatric comorbidities has not been tested in previous work. Although this analysis

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3 remains a secondary aim of our study, the sample size should allow an adequately powered
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5 post-hoc stratification of our prospective data to test this relationship.
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8 The wealth of clinical data we are planning to collect with LABSP (at the moment of writing,
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10 the research protocol is currently under review at the local Ethics Committee) will also allow
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12 testing the moderating influence of relevant clinical variables, such as for instance, family
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14 history of SCZ. In fact, it is plausible that recruited participants with a positive family history
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16 of SCZ, will have lower baseline BDNF levels compared to those without a family history, and
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18 a different pattern of association between serum BDNF levels and the various clinical
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20 independent variables.
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26 **CONCLUSIONS**

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28 In summary, LABSP will allow the prospective assessment of BDNF serum levels, as well as
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30 of key clinical and treatment-related measures, in a relatively large cohort of SCZ and SAD
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32 patients followed-up in a naturalistic setting. This work should provide useful information
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34 on the causal relationship between BDNF serum levels and psychopathological changes
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36 over time. Our data might show whether a change in BDNF peripheral levels might be
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38 predictive of the psychopathological trajectory, and if validated in independent samples,
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40 might point to a role of BDNF as a biomarker of clinical trajectory in SCZ.
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48 **Contributors**

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50 DP has contributed to the design of the study, and drafted the first version of the
51
52 manuscript. BC conceived the study, led the study team, and critically revised the
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54 manuscript. MM has contributed to the assessment protocol, the design of the study, and
55
56 the drafting of the manuscript. LD and MT have contributed to the study design and
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3 assessments. MS and RC have contributed to BDNF serum levels assessments and laboratory
4
5 procedures. PF and WF have designed the experimental procedures for BDNF assessment.
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8 All authors have read and approved the final version of the manuscript.
9

10 11 12 **Data sharing statement**

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14 The data of this study will only be shared between the authors and the University of
15
16 Cagliari Health Agency.
17

18 19 20 21 **Competing interests**

22
23 The authors have no competing interests relevant to this study.
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28 29 **Ethics approval**

30
31 This study protocol was approved by the University of Cagliari Health Agency Ethics
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33 Committee (NP2016/5491).
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38 39 **Funding**

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41 None.
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Table 1. Assessment protocol in the Longitudinal Assessment of BDNF in Sardinian Psychotic patients (LABSP)

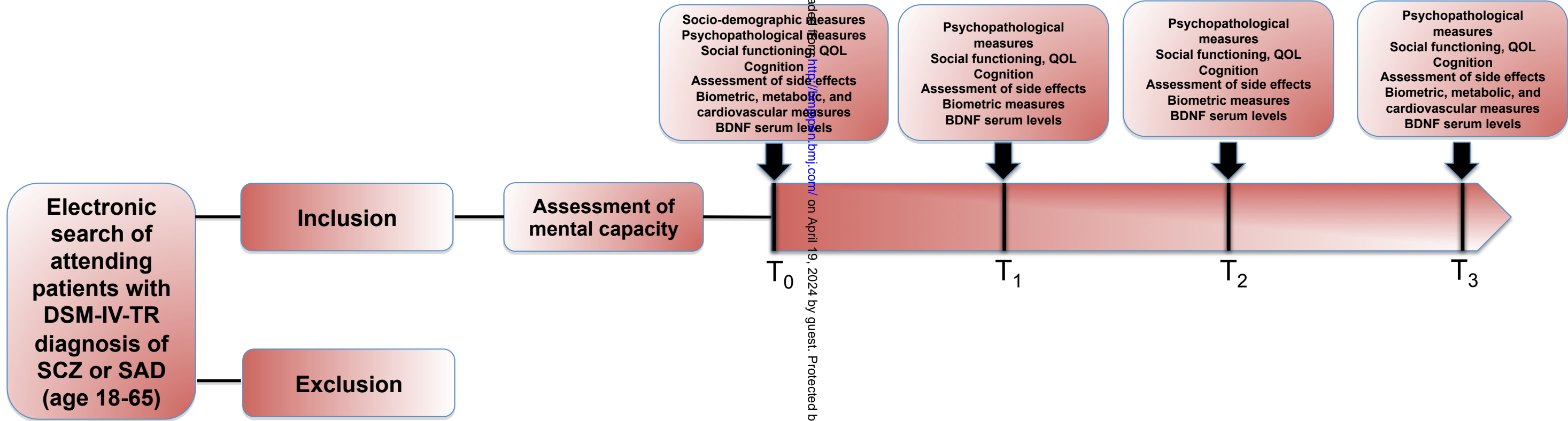
Measure	Time points			
	T ₀ (enrolment)	T ₁ (6 months)	T ₂ (12 months)	T ₃ (18 months)
BDNF serum levels	✓	✓	✓	✓
Descriptive variables				
Demographic data	✓			
Socio-economic data	✓			
Clinical history	✓			
Family history	✓			
Psychopathology				
SCID-I/P	✓			
SCID-II	✓			
PANSS	✓	✓	✓	✓
CGI-SCH	✓	✓	✓	✓
POLTQ	✓			
PAS	✓	✓	✓	✓
Social functioning and quality of life				
PSP	✓	✓	✓	✓
SWN-S	✓	✓	✓	✓
WHOQOL-Bref	✓	✓	✓	✓
Cognition				
BACS	✓	✓	✓	✓
Assessment of side effects				
DOTES	✓	✓	✓	✓
ESRS	✓	✓	✓	✓
Biometric, metabolic, and cardiovascular measures				
Lipid profile	✓			✓
Kidney panel	✓			✓
Liver panel	✓			✓
Electrolytes	✓			✓
Weight, height, waist circumference, BMI	✓	✓	✓	✓
Glucose	✓			✓
Glycosylated haemoglobin	✓			✓
PRL	✓			✓
CPK	✓			✓
ECG	✓			✓

BDNF: brain-derived neurotrophic factor; SCID-I/P: Structured Clinical Interview for DSM IV Axis I Disorders; SCID-II: Structured Clinical Interview for DSM IV Axis II Disorders; PANSS: Positive And Negative Syndrome Scale; CGI-SCH: Clinical Global Impression – Schizophrenia Scale; POLTQ: Psychopathological Onset Latency and Treatment Questionnaire; PAS: Premorbid Adjustment Scale; PSP: Personal and Social Performance scale; SWN-S: Subjective Well-Being Under Neuroleptics Scale; WHOQOL-Bref: World Health Organization Quality Of Life – Bref; BACS: Brief Assessment Cognition Schizophrenia; DOTES: Dosage Record and Treatment Emergent Symptom Scale; ESRS: Extrapiramidal Symptom Rating Scale; BMI: Body Mass Index; PRL: Prolactine; CPK: Creatine phosphokinase; ECG: Electrocardiogram.

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BMJ Open

Longitudinal Assessment of brain-derived neurotrophic factor in Sardinian Psychotic patients (LABSP): a study protocol for a prospective observational study

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Secondary Subject Heading:	Pharmacology and therapeutics, Diagnostics
Keywords:	Schizophrenia & psychotic disorders < PSYCHIATRY, Antipsychotics, BDNF, Prospective study

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Manuscripts

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3 **Longitudinal Assessment of brain-derived neurotrophic factor in Sardinian Psychotic**
4 **patients (LABSP): a study protocol for a prospective observational study**
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7 Diego Primavera¹, Mirko Manchia^{1,2}, Luca Deriu¹, Massimo Tusconi¹, Roberto Collu³, Maria
8 Scherma³, Paola Fadda^{3,4}, Walter Fratta^{3,4}, Bernardo Carpiniello¹
9

10
11
12 ¹Section of Psychiatry, Department of Medical Science and Public Health, University of
13 Cagliari, Cagliari, Italy. ²Department of Pharmacology, Dalhousie University, Halifax, Nova
14 Scotia, Canada. ³Department of Biomedical Sciences, Division of Neuroscience and Clinical
15 Pharmacology, University of Cagliari, Cagliari, Italy. ⁴Centre of Excellence "Neurobiology of
16 Dependence", University of Cagliari, Cagliari, Italy.
17
18
19

20 *Correspondence to:
21

22 Professor Bernardo Carpiniello, MD
23

24 Section of Psychiatry, Department of Public Health, Clinical and Molecular Medicine,
25 University of Cagliari, Cagliari, Italy.
26

27 Via Liguria, 13
28

29 09127 Cagliari, Italy
30

31 Tel: +3907041518; Fax:+39070480083; E-mail: bcarpini@iol.it.
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ABSTRACT

Introduction: Brain-derived neurotrophic factor (BDNF) plays a crucial role in neurodevelopment, synaptic plasticity, and neuronal function and survival. Serum and plasma BDNF levels are moderately, but consistently, decreased in patients with schizophrenia (SCZ) compared to healthy controls. There is a lack of knowledge, however, on the temporal manifestation of this decline. Clinical, illness-course, and treatment factors might influence the variation of BDNF serum levels in psychotic patients. In this context, we propose a longitudinal study of a cohort of SCZ and SAD Sardinian patients with the aim of disentangling the relationship between peripheral BDNF serum levels and changes of psychopathology, cognition, and drug treatments.

Methods and analysis: Longitudinal Assessment of BDNF in Sardinian Psychotic patients (LABSP) is a 24-month observational prospective cohort study. Schizophrenic and schizoaffective disorder (SAD) patients will be recruited at the Psychiatry Research Unit of the Department of Medical Science and Public Health, University of Cagliari, and University of Cagliari Health Agency, Cagliari, Italy. We will collect BDNF serum levels as well as socio-demographic, psychopathological, and neurocognitive measures. Structured, semi-structured, and self-rating assessment tools, such as the Positive and Negative Syndrome Scale (PANSS) for psychopathological measures, and the Brief Assessment of Cognition in Schizophrenia (BACS) for cognitive function will be used.

Ethics and dissemination: This study protocol was approved by the University of Cagliari Health Agency Ethics Committee (NP2016/5491). The study will be conducted in accordance with the principles of good clinical practice, in the Declaration of Helsinki in compliance with the regulations. Participation will be voluntary and written informed

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3 consent will be obtained for each participant upon entry into the study. We plan to
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5 disseminate the results of our study through conference presentations and publication in
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7 international peer-reviewed journals. Access to raw data will be available in anonymized
8
9 form upon request to the corresponding author.
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Strengths and limitations of this study

- This is a prospective cohort study of psychotic patients aiming to clarify whether longitudinal changes in brain-derived neurotrophic factor (BDNF) serum levels are correlated with psychopathological, cognitive, and treatment factors.
- The secondary outcomes of this study will offer insight on the impact of psychiatric comorbidities on longitudinal trajectory of BDNF in psychotic patients
- Sampling will be performed in a tertiary clinic specialized in treatment of psychotic patients, possibly limiting ecological validity of the study results.
- The sample size, based on previous work on longitudinal change of BDNF over time, might not achieve sufficient statistical power to detect association signals with small effect sizes between clinical predictors and BDNF levels.
- Notwithstanding the prospective design, clinical (illness duration, duration of untreated psychosis), and treatment history prior to study entry might impact on findings.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is the most widely distributed neurotrophin in the central nervous system (CNS),¹ being expressed in almost all the cortical areas, as well as in several spinal cord regions.² There is substantial evidence that BDNF plays a crucial role during brain development, as well as in the process of differentiation, synaptogenesis and neuronal plasticity.³⁻⁶ BDNF was the second neurotrophic factor to be characterized after the nerve growth factor (NGF).⁷ Similarly to other neurotrophins, BDNF is synthesized in a pre-pro form consisting of 47 amino acids.² Extracellular BDNF appears to have two forms: pro-BDNF and mature BDNF,^{2, 8} which are formed after cleavage of the precursor protein prepro-BDNF.^{8, 9} Mature BDNF interacts with its high affinity receptor, the tropomyosin-related kinase receptor type B (TRKB), to exert its molecular effects in the adult CNS.² BDNF induces dimerization of TRKB with kinase activation and autophosphorylation of tyrosine residues, with subsequent activation of several adaptor proteins, which, in turn leads to phosphorylation of phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase - extracellular signal-regulated kinase (MEK-ERK), phospholipase C γ 1 (PLC γ 1) and cyclic AMP-responsive element-binding protein (CREB), all downstream pathways regulating neurite outgrowth, cell survival, cellular differentiation and synaptic plasticity.^{2, 10} There is evidence that the cleaved prodomain of BDNF might be, by itself, an active biological modulator.^{11, 12} BDNF and its cleaved pro-peptide have been found in large vesicles located in the presynaptic terminals of excitatory neurons in the adult hippocampus of mice.¹¹ Interestingly, the Val66Met polymorphism of BDNF, which is located in the prodomain genomic region, alters substantially the prodomain structure.¹² Specifically, Met66 BDNF prodomain induces modulation of neuronal morphology through acute cone retraction.¹²

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3 It is known that BDNF freely crosses the blood-brain barrier.¹³ In keeping with this
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5 observation, levels of BDNF in serum are correlated with CNS concentrations.¹⁴ Altered
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7 peripheral serum levels of BDNF have been shown to be a reliable biomarker for severe
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9 psychiatric disorders, including schizophrenia (SCZ).¹⁵ This corresponds to the dysregulation
10
11 of BDNF, and of its related molecular pathways, observed in affected individuals.¹⁶
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15 A decline in serum BDNF levels has been consistently identified in chronic and medicated
16
17 SCZ patients,¹⁷⁻²² as well as in first episode and medication naïve SCZ patients,²³⁻²⁷
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19 compared to unaffected subjects. Conversely, other studies showed increased BDNF serum
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21 levels in patients with SCZ,^{28 29} or have not been able to demonstrate differences in serum
22
23 BDNF concentrations between drug-free or drug-naïve patients and healthy controls.^{30 31}
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27 This discrepancy may depend on the clinical heterogeneity of the samples studied (i.e.
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29 different stage of illness, subtype of schizophrenia, and effect of medication), as well as on
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31 the different assessment tools employed. However, a recent quantitative meta-analytical
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33 estimate by Fernandes et al.,³² confirmed that SCZ is associated with a moderate decrease
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35 of serum and plasma BDNF levels compared to healthy controls. This entails that decreased
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37 serum BDNF levels might be a marker of illness status in SCZ (i.e. disease biomarker). There
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39 is a lack of knowledge, however, on the temporal manifestation of this decline. The
40
41 decrease of peripheral BDNF could be constant, with pre-morbid levels roughly similar to
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43 those of unaffected individuals, linearly declining during the course of SCZ. Conversely,
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45 BDNF peripheral levels might fluctuate in association with acute psychopathological phases
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47 of the disorder. On top of this, BDNF peripheral levels might vary as a result of drug
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49 treatments typically used in major psychoses, such as antipsychotics. To date, however, the
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51 study of the relationship between BDNF serum levels and drug treatment in SCZ and SAD
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53 patients remains inconclusive. There is extensive pre-clinical evidence suggesting that
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3 typical antipsychotics might decrease BDNF expression whereas atypical antipsychotics, with
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5 the exception of risperidone, could increase BDNF expression.³³ Clozapine-treated chronic
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7 SCZ patients have higher BDNF levels than patients treated with typical neuroleptics^{18 20} or
8
9 with risperidone.¹⁸ Other studies, however, found no effect of antipsychotic treatment on
10
11 the levels of serum BDNF.^{25 34 35} Taken together, these data appear contradictory, making
12
13 difficult to infer conclusions about the effects of antipsychotic treatment on serum BDNF
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15 levels. Meta-analytical findings, however, point to a small but significant increase of serum
16
17 BDNF levels under antipsychotic treatment, although a class-specific effect has not been
18
19 investigated.³²

20
21 One final introductory remark concerns the link between BDNF and cognitive function in
22
23 SCZ. Indeed, cognitive disruption seems to represent the core symptomatology that
24
25 distinguishes SCZ patients from those affected by other psychoses. Cognitive deficits are
26
27 related to social integration, to social problem solving and to the acquisition of various
28
29 skills,³⁶ and are considered the most powerful predictors of functional outcome in patients
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31 with SCZ.³⁷ Cognitively impaired chronic SCZ patients appear to show low serum BDNF
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33 levels.³⁸ A recent meta analysis confirmed this observation,³⁹ showing that higher levels of
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35 BDNF peripheral expression correlated with better cognitive performances in reasoning and
36
37 problem solving areas.

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39 The apparent gap in the knowledge about the longitudinal variation of BDNF serum levels,
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41 and its relationship with clinical and treatment variables in major psychoses, needs to be
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43 filled by additional clinical research. Here we present the design and methodology of a
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45 prospective observational study that takes advantage of a well-characterized population of
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47 psychotic patients carefully monitored at an academic community health centre.
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Aims of the current study

Our study has the following primary and secondary aims:

Primary aims:

1. Explore the longitudinal relationship between peripheral serum BDNF levels and variation in psychopathology in a cohort of patients with major psychosis recruited at different stages of the illness.
2. Assess whether variation of cognitive function correlates with peripheral BDNF serum levels.
3. Establish whether drug treatment impacts on the relationship between psychopathology and cognition with BDNF serum levels

Secondary aims:

4. Study whether patients' baseline characteristics prior to enrolment, namely duration of illness, duration of untreated psychosis (DUP), psychiatric and somatic comorbidity, age at onset, gender, family history of psychiatric disorders, premorbid adjustment, and the type of illness course moderate the longitudinal relationship between peripheral serum BDNF and psychopathology.
5. Study whether measures of peripheral serum BDNF levels correlate with baseline measure of social functioning.
6. Analyze the relationship of peripheral BDNF levels with tolerability of antipsychotic treatment, as expressed by the presence of extrapyramidal symptoms (EPS).

METHODS

Study design

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3 Longitudinal Assessment of BDNF in Sardinian Psychotic patients (LABSP) is a 24-month
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5 observational prospective cohort study. The sample will be recruited from patients
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7 followed-up at the community mental health centre of the Psychiatry Research Unit of the,
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9 Department of Medical Science and Public Health, of the University of Cagliari and
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11 University of Cagliari Health Agency, Cagliari, Italy. Blood samples from recruited patients
12
13 will be taken at baseline (T_0), and at three consecutive time points: 6 months (T_1), 12
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15 months (T_2), and 18 months (T_3) (Figure 1). The temporal fluctuations of psychopathology,
16
17 as well as the assessment of pharmacotherapy, measures of cognitive function, and
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19 manifestation of treatment-related side effects (i.e. EPS) will be also evaluated at each time
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21 point (Table 1). The observational prospective design permits to disentangle how the
22
23 variables might influence the outcome under study. Thus, we will assess whether changes in
24
25 peripheral serum levels BDNF predate or, alternatively, are a consequence of
26
27 psychopathological changes and/or drug treatment. As we will study the trajectory of
28
29 peripheral serum BDNF levels over time, the recruited cohort will serve as its own control.
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31 We also expect to obtain a more accurate estimation of the patterns of association between
32
33 BDNF serum levels and all the independent clinical variables under consideration.
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43 **Participants and recruitment**

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45 The recruitment of the sample will be based on a two-step process. In the first step, all
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47 patients followed-up at the community mental health centre of the University of Cagliari
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49 Health Agency, Cagliari, Italy with a diagnosis of SCZ or SAD according to the Diagnostic and
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51 Statistical Manual of Mental Disorders-IV-Text Revision (DSM-IV-TR)⁴⁰ will be identified in
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53 the medical record database. The electronic search will be performed using lower (18) and
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55 upper (65) age limits, with no constraint regarding the presence of comorbid psychiatric
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3 disorders. After eligible patients will be identified in the database, the diagnosis of SCZ or
4
5 SAD will be confirmed using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders,
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7 Patient Edition (SCID-I/P)⁴¹ administered by trained mental-health professionals (psychiatry
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9 residents or senior clinical staff).

14 **Inclusion and exclusion criteria**

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17 Inclusion criteria are: 1) age between 18 and 65 years; 2) diagnosis of SCZ or SAD according
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19 to DSM-IV-TR; and 3) stability during the six months before recruitment. Exclusion criteria
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21 are: 1) refusal to provide consent; 2) presence of acute psychopathological symptoms; 3)
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23 presence of illness-related cognitive impairment of such severity that affects their ability to
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25 cooperate; 4) presence of major unstable medical illness; 5) severe mental retardation; 6)
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27 major neurological disorder or previous head injury; 7) current drug and alcohol
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29 dependence.
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36 **Sample size and power calculation**

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38 Our main null hypotheses are that there is no difference between mean BDNF serum levels
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40 at different time points in relation to: 1) the longitudinal psychopathological changes of SCZ
41
42 and SAD patients; 2) measures of cognitive functioning; and 3) drug treatment. We based
43
44 our power analysis on previous findings of BDNF serum levels longitudinal variation in a
45
46 small cohort (n = 21) of first-episode SCZ patients.²⁴ Specifically, Palomino et al. performed a
47
48 1-year prospective assessment of BDNF finding an absolute mean difference between
49
50 baseline and the last time point of 1.65.²⁴ Thus, a sample size of 59 individuals would be
51
52 sufficient to obtain a 90% statistical power to detect significant difference at $\alpha = 0.05$
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54 between mean serum BDNF levels of this magnitude at each time point, considering an
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3 attrition rate for time point of 5%. Power analysis has been performed using repeated
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5 measures and sample size (RMASS) software.⁴²
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10 **Measures and materials**

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12 Socio-demographic variables, personal and family history data

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14 Socio-demographic data will be collected using the Association for Methodology and
15 Documentation in Psychiatry (AMDP)⁴³ assessment tool. Briefly, we will gather information
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17 on gender, age, level of education, marital status, offspring, economic status, and
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19 occupation. Further, we will collect data on previous and current substance and/or alcohol
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21 abuse, and smoking status. The latter will be quantified also as number of cigarettes smoked
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23 per day. Information on family history of any psychiatric disorders will be collected through
24
25 direct interview of the participant and at least one first-degree relative or significant other,
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27 as well as through an accurate review of available medical records. We will gather
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29 information on the stage of menstrual cycle whenever available. Finally, we will collect data
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31 on the presence of a previous history of stressful life events as well as monitor longitudinally
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33 their eventual manifestation.
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43 Psychopathological measures

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45 The main psychopathological measures will be collected through the AMDP syndrome
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47 scale,⁴³ which has shown to be valid in discriminating various forms of psychoses, as well as
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49 in providing a detailed psychopathological description of the subjects under study.⁴⁴ As
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51 previously reported, patients' diagnosis will be confirmed using the SCID-I/P.⁴¹ Furthermore,
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53 all recruited patients will undergo assessment with the Structured Clinical Interview for
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55 DSM-IV Axis II Personality Disorders (SCID-II)⁴⁵ to detect the presence of comorbid
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3 personality disorders. Information on age of onset, age at first intervention
4 (pharmacological and/or psychotherapeutic and/or psychosocial) will be based on
5 retrospective information collected through direct interview of the patient, and at least one
6 relative or significant other, as well as through an accurate review of available medical
7 records. This approach should minimize the impact of recall bias on the assessment of key
8 clinical measures.
9

10 We will also estimate DUP, the interval between the age of onset of full-blown psychotic
11 symptoms and first antipsychotic treatment, using the Psychopathological Onset Latency
12 and Treatment Questionnaire.⁴⁶ Indeed a longer DUP appears to be related to a worse
13 short-⁴⁷ and long-term clinical outcome^{48 49} in SCZ individuals, as well as with lower BDNF
14 serum levels,⁵⁰ although the latter finding has been not replicated in subsequent studies.⁵¹

15 Other measures of illness severity, such as number of hospital admissions and type of
16 clinical course will be collected for the analysis.

17 Psychopathological assessment will include the Positive and Negative Symptom Scale for
18 Schizophrenia (PANSS),⁵² and the Clinical Global Impression Scale for Schizophrenia (CGI-
19 SCH).⁵³ The latter measure is a valid assessment tool for monitoring longitudinal
20 psychopathological symptoms in routine clinical practice.⁵⁴ Moreover, we will assess
21 premorbid dysfunction, a well established prognostic marker in SCZ patients, using the
22 Premorbid Adjustment Scale (PAS).⁵⁵ All these psychopathological measures will be analyzed
23 in relation to BDNF serum levels.
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51 Social functioning and quality of life measures

52 Evaluation of social functioning will be carried out with the Personal and Social Performance
53 Scale (PSP),⁵⁶ which showed good reliability in SCZ patients.⁵⁷ Further, assessment of quality
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3 of life (QOL) will be performed using the World Health Organization Quality-Bref (WHOQOL)
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5 version which has shown good validity and reliability in variety of psychiatric illnesses
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7 including SCZ.^{58 59} Finally, we will collect data using the Subjective Wellbeing under
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9 Neuroleptics-Short Version (SWN-S).⁶⁰
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12 13 14 15 Cognitive measures

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17 The cognitive performance will be examined in a standardized way using the Brief
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19 Assessment of Cognition in Schizophrenia Scale (BACS).⁶¹ It takes approximately half an hour
20
21 to administer in healthy controls, and includes brief assessments of four of the seven
22
23 neurocognitive domains designated as important by the Measurement and Treatment
24
25 Research to Improve Cognition in Schizophrenia (MATRICS) battery: reasoning and problem
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27 solving, processing speed, verbal memory, and working memory. The composite score has
28
29 been shown to have high test–retest reliability in SCZ patients.⁶¹ Moreover, the BACS
30
31 appears to assess reliably measures of functional capacity.⁶² The BACS will permit a rapid
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33 assessment of cognitive function at different time points, allowing correlation of these
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35 measures with peripheral serum BDNF levels. Indeed, several studies have shown that
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37 peripheral serum BDNF level might be a biomarker of cognitive function in schizophrenia.⁶³⁻
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50 51 52 53 54 55 56 57 58 59 60 Assessment of side effects

60 We will monitor the onset of antipsychotics related side effects using the Dosage and
Treatment Emergent Symptoms Scale (DOTES),⁶⁷ and the Extrapyrimal Symptoms Rating
scale (ESRS).⁶⁸ Of note, one study showed that SCZ patients with tardive dyskinesia (TD) had
lower plasma BDNF levels than those without TD.⁶⁹

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5 Biometric, metabolic, and cardiovascular measures
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8 There is growing evidence for a role of BDNF in appetite and weight regulation as well as in
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10 eating disorders, possibly via its interaction with the serotonin system. BDNF is expressed in
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12 brain regions including the hypothalamus, where its infusion in rats has been shown to
13
14 increase serotonin turnover, and suppress appetite.⁷⁰ Conversely, mice with only one copy
15
16 of the BDNF gene displayed increased food intake and weight, with decreased serotonin
17
18 metabolite to serotonin ratio.⁷¹ Conditional ablation of the BDNF gene also leads to similar
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20 hyperphagic and obese phenotypes.⁷² BDNF may also regulate weight and food intake via its
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22 regulation of the dopamine system.^{73 74} Antagonism on dopamine receptors 3 (DRD3) was
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24 shown to alter attentional bias to food cues in low-restrained obese or overweight
25
26 individuals.⁷⁵ This study suggests that BDNF may be a key factor in the regulation of eating
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28 behavior upstream of dopamine and serotonin systems and BDNF gene variants may play a
29
30 role in antipsychotic-induced weight gain. Based on these findings, we will evaluate the
31
32 following clinical parameters: weight, waist circumference, body mass index (BMI), liver and
33
34 kidney function panel, serum glucose, glycosylated haemoglobin, lipid profile, prolactin
35
36 (PRL), and creatine phosphokinase (CPK). The cardiovascular function will be monitored by
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38 electrocardiogram (ECG), with evaluation of QTc interval. Finally, we will collect information
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40 on exercise as this can impact on BDNF levels.⁷⁶
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50 **Sampling and assessment of BDNF serum levels**

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52 Blood samples for each patient will be taken at the same time of the day (between 8:00 and 10:00
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54 AM). BDNF serum will be evaluated using BDNF ELISA Kit (Booster Immunoleader, Cat. N°
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56 EK0307) for quantitative detection of human BDNF in cell culture supernatants, serum and
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3 plasma. This kit is based on a standard sandwich enzyme-linked immune-sorbent assay
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5 technology for specific quantifications of natural and recombinant human BDNF with a high
6
7 sensitivity (< 2 pg/mL) and with no detectable cross-reactivity with other relevant proteins.
8
9 After blood sampling, serum will be allowed to clot in a serum separator tube for about 4
10
11 hours at room temperature. After that it will be centrifuged at approximately 1000 X g for
12
13 15 min. Supernatant serum samples will be collected in small aliquot and stored
14
15 immediately at -20°C for future analysis. Then, samples will be processed according to kit
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17 protocol and instructions. Optical density absorbance of each sample will be read with a
18
19 450nm filter in a microplate reader (Thermo Scientific Multiskan FC) within 30 minutes after
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21 the final step of the kit procedure. Data obtained will be analysed using the Thermo
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23 Scientific SkanIt Software 3.0 for Multiskan FC.
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32 **Data analysis plan**

33 Mixed-effects linear regression models (MLRM) will be used to analyze longitudinal data.^{77 78}
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35 Specifically, we will regress independent variables (both categorical and continuous) on
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37 BDNF serum levels (dependent variable). Mixed-effects linear regression models allow to
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39 model individual change over time and appear to be more flexible in terms of repeated
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41 measures, particularly when the number of observations per subject is not the same at each
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43 time point.^{77 78} Further, these models allow generalization of non-normally distributed data
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45 for independent variables. Our analysis plan will consist of the following steps. First, we will
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47 perform a visual inspection of mean BDNF serum levels at each time point using boxplots.
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49 This will allow to check for normality of BDNF serum levels at each time point as well as to
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51 identify outliers. Secondly, MLRM will be analyzed in order to assess the longitudinal
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53 variation of BDNF levels while correcting for age and sex. Finally, independent variables will
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3 be regressed on BDNF serum levels. Covariates will be added to significant models to
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5 account for possible intercorrelations. All data will be analyzed using “lme4” package
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7 implemented in R.⁷⁹ Missing data for independent variables will be dealt with the
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9 “na.action” function implemented in R. The statistical significance of identified MLRM will
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11 be calculated using the “multcomp” R package. Finally, graphical representation of MLRM
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13 will be obtained with R packages “sjPlot” e “sjmisc”.

14 15 16 17 18 19 20 **Ethics and dissemination**

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22 This study protocol was approved by the University of Cagliari Health Agency Ethics
23
24 Committee (NP2016/5491). The study will be conducted in accordance with the principles of
25
26 good clinical practice, in the Declaration of Helsinki in compliance with the regulations.
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28 Written, informed consent will be obtained from all eligible participants. Participation to the
29
30 study will be voluntary and patients will be able to withdraw consent at any point with no
31
32 disadvantage to their treatments. A psychiatric assessment will establish that patients’
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34 ability to consent is not compromised by their psychopathological status.

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36 We plan to disseminate the results of our study through conference presentations and
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38 publication in international peer-reviewed journals. Access to raw data will be available in
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40 anonymized form upon request to the corresponding author.
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48 49 **DISCUSSION**

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51 Schizophrenia is characterized by three distinct, but to a certain extent intertwined,
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53 clusters of symptoms: positive, negative, and cognitive. Each one of these clusters
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55 presents with a varying degree of severity and with a specific prevalence depending on the
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3 stage of the illness.⁸⁰ Patients with stable diagnoses of SCZ and SAD have often incomplete
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5 remissions (particularly from negative symptoms), and not rarely recurrences or relapses
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7 of positive symptoms, mainly delusions and hallucinations.⁸¹ Cognitive function, however,
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9 appears to decline steadily over the course of the illness, although most of the magnitude
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11 of the differences in cognition between SCZ patients and unaffected subjects appears to
12
13 be modulated by age.⁸¹
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16
17 One key aspect in diagnostically stable SCZ and SAD patients is being able to predict the
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19 manifestation of these psychopathological exacerbations. Certainly, given that fluctuations
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21 appear to be more prominent for positive symptoms and to a lesser extent for negative
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23 ones, rather than for cognition, researcher have focused on the identification of
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25 prodromal signs of relapses/recurrences using, for instance, home telemonitoring of
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27 symptoms change via mobile phone-based platforms.^{82 83} This approach has proven
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29 effective in reducing the number of relapses, and consequent hospitalizations, in psychotic
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31 individuals.^{82 83}
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37 Our prospective study, LABSP, expands on this evidence aiming at monitoring not only the
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39 clinical signs of SCZ patients over an 24-month period, but, more importantly, assessing
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41 the predictive power of a well-established biomarker of illness status in SCZ (BDNF serum
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43 levels), on the clinical trajectory of SCZ. We expect to find correlation of BDNF levels with
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45 longitudinal variation of psychopathology, particularly concerning positive symptoms. This
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47 prospective approach, to our knowledge, has not been proposed previously in the
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49 literature, and might offer novel data on serum BDNF as a biomarker of
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51 psychopathological changes in accurately monitored SCZ patients. If such a correlation will
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3 be identified, the joint analysis of clinical and BDNF data might explain a larger proportion
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5 of psychopathological change that clinical data alone.
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8 One additional point of novelty concerns the analysis of the impact of psychiatric
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10 comorbidity on BDNF levels. Indeed, SCZ is not rarely associated with other axis I and II
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12 disorders that typically affect negatively the course and outcome of the disorder.⁸⁴
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14 However, to our knowledge the relationship between serum BDNF levels in SCZ and
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16 psychiatric comorbidities has not been tested in previous work. Although this analysis
17
18 remains a secondary aim of our study, the sample size should allow an adequately powered
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20 post-hoc stratification of our prospective data to test this relationship.
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24 The wealth of clinical data we are planning to collect with LABSP will also allow testing the
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26 moderating influence of relevant clinical variables, such as for instance, family history of
27
28 SCZ. In fact, it is plausible that recruited participants with a positive family history of SCZ,
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30 will have lower baseline BDNF levels compared to those without a family history, and a
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32 different pattern of association between serum BDNF levels and the various clinical
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34 independent variables.
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41 **Limitations**

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43 Our study has some limitations inherent to its design. First, we will study a population
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45 recruited in a tertiary clinic specialized in treatment of psychotic patients, possibly limiting
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47 ecological validity (i.e. generalizability) of the results. Second, our power analysis is based on
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49 previous longitudinal estimates of 1.65 mean variation of serum BDNF levels over a 1-year
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51 follow-up. Although a sample size of 59 might be sufficient to detect a longitudinal variation
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53 of this magnitude in BDNF serum levels we might not be able to detect association signals of
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55 small effect size between clinical, treatment, and cognitive variables and BDNF. Thus, we
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3 plan to extend our recruitment well beyond the number provided by our power analysis.
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5 Finally, notwithstanding the prospective design, clinical (illness duration, duration of
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7 untreated psychosis), and treatment history prior to study entry might impact on findings.
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10 11 12 **CONCLUSIONS**

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14 In summary, LABSP will allow the prospective assessment of BDNF serum levels, as well as
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16 of key clinical and treatment-related measures, in a relatively large cohort of SCZ and SAD
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18 patients followed-up in a naturalistic setting. This work will provide useful information on
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20 the relationship between BDNF serum levels and psychopathological changes over time.
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22 Changes in BDNF peripheral levels might be predictive of the psychopathological trajectory
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24 during the 24-month follow-up, and if validated in independent samples, might point to a
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26 role of BDNF as a biomarker of clinical trajectory in psychotic patients.
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34 **Contributors**

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36 DP has contributed to the design of the study, and drafted the first version of the
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38 manuscript. BC conceived the study, led the study team, and critically revised the
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40 manuscript. MM has contributed to the assessment protocol, the design of the study, and
41
42 the drafting of the manuscript. LD and MT have contributed to the study design and
43
44 assessments. MS and RC have contributed to BDNF serum levels assessments and laboratory
45
46 procedures. PF and WF have designed the experimental procedures for BDNF assessment.
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48 All authors have read and approved the final version of the manuscript.
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55 **Data sharing statement**

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3 Access to raw data will be available in anonymized form upon request to the
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5 corresponding author.
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10 **Competing interests**

11
12 The authors have no competing interests relevant to this study.
13
14

15 **Ethics approval**

16
17 This study protocol was approved by the University of Cagliari Health Agency Ethics
18
19 Committee (NP2016/5491).
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23

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25
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27
28 of Cagliari (BC).
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Table 1. Assessment protocol in the Longitudinal Assessment of BDNF in Sardinian Psychotic patients (LABSP)

Measure	Time points				
	T ₀ (enrolment)	T ₁ (6 months)	T ₂ (12 months)	T ₃ (18 months)	T ₄ (24 months)
BDNF serum levels	✓	✓	✓	✓	✓
Descriptive variables					
Demographic data	✓				
Socio-economic data	✓				
Clinical history	✓				
Family history	✓				
Psychopathology					
SCID-I/P	✓				
SCID-II	✓				
PANSS	✓	✓	✓	✓	✓
CGI-SCH	✓	✓	✓	✓	✓
POLTQ	✓				
PAS	✓	✓	✓	✓	✓
Social functioning and quality of life					
PSP	✓	✓	✓	✓	✓
SWN-S	✓	✓	✓	✓	✓
WHOQOL-Bref	✓	✓	✓	✓	✓
Cognition					
BACS	✓	✓	✓	✓	✓
Assessment of side effects					
DOTES	✓	✓	✓	✓	✓
ESRS	✓	✓	✓	✓	✓
Biometric, metabolic, and cardiovascular measures					
Lipid profile	✓				✓
Kidney panel	✓				✓
Liver panel	✓				✓
Electrolytes	✓				✓
Weight, height, waist circumference, BMI	✓	✓	✓	✓	✓
Glucose	✓				✓
Glycosylated haemoglobin	✓				✓
PRL	✓				✓
CPK	✓				✓
ECG	✓				✓

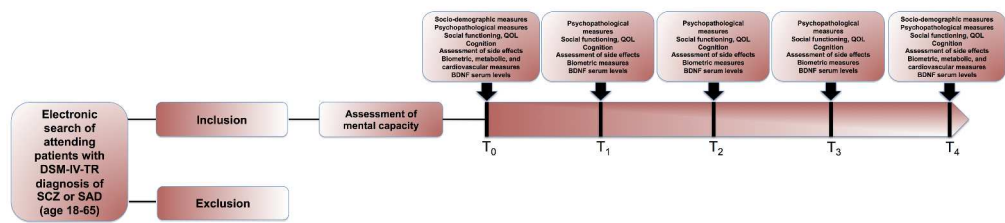
BDNF: brain-derived neurotrophic factor; SCID-I/P: Structured Clinical Interview for DSM IV Axis I Disorders; SCID-II: Structured Clinical Interview for DSM IV Axis II Disorders; PANSS: Positive And Negative Syndrome Scale; CGI-SCH: Clinical Global Impression – Schizophrenia Scale; POLTQ: Psychopathological Onset Latency and Treatment Questionnaire; PAS: Premorbid Adjustment Scale; PSP: Personal and Social Performance scale; SWN-S: Subjective Well-Being Under Neuroleptics Scale; WHOQOL-Bref: World Health Organization Quality Of Life – Bref; BACS: Brief Assessment Cognition Schizophrenia; DOTES: Dosage Record and Treatment Emergent Symptom Scale; ESRS: Extrapiramidal Symptom Rating Scale; BMI: Body Mass Index; PRL: Prolactine; CPK: Creatine phosphokinase; ECG: Electrocardiogram.

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Figure legend

Flowchart of recruitment and assessment procedures for the Longitudinal Assessment of brain-derived neurotrophic factor in Sardinian Psychotic patients (LABSP) study.

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Flowchart of recruitment and assessment procedures for the Longitudinal Assessment of brain-derived neurotrophic factor in Sardinian Psychotic patients (LABSP) study.

489x149mm (300 x 300 DPI)

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BMJ Open

Longitudinal Assessment of brain-derived neurotrophic factor in Sardinian Psychotic patients (LABSP): a study protocol for a prospective observational study

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Primary Subject Heading:	Mental health
Secondary Subject Heading:	Pharmacology and therapeutics, Diagnostics
Keywords:	Schizophrenia & psychotic disorders < PSYCHIATRY, Antipsychotics, BDNF, Prospective study

SCHOLARONE™
Manuscripts

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3 **Longitudinal Assessment of brain-derived neurotrophic factor in Sardinian Psychotic**
4 **patients (LABSP): a study protocol for a prospective observational study**
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7 Diego Primavera¹, Mirko Manchia^{1,2}, Luca Deriu¹, Massimo Tusconi¹, Roberto Collu³, Maria
8 Scherma³, Paola Fadda^{3,4}, Walter Fratta^{3,4}, Bernardo Carpiniello¹
9

10
11
12 ¹Section of Psychiatry, Department of Medical Science and Public Health, University of
13 Cagliari, Cagliari, Italy. ²Department of Pharmacology, Dalhousie University, Halifax, Nova
14 Scotia, Canada. ³Department of Biomedical Sciences, Division of Neuroscience and Clinical
15 Pharmacology, University of Cagliari, Cagliari, Italy. ⁴Centre of Excellence "Neurobiology of
16 Dependence", University of Cagliari, Cagliari, Italy.
17
18
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20
21
22
23

24 *Correspondence to:

25 Professor Bernardo Carpiniello, MD
26

27
28
29 Section of Psychiatry, Department of Medical Science and Public Health, University of
30 Cagliari, Cagliari, Italy.
31
32

33 Via Liguria, 13
34

35 09127 Cagliari, Italy
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37
38 Tel: +3907041518; Fax:+39070480083; E-mail: bcarpini@iol.it.
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ABSTRACT

Introduction: Brain-derived neurotrophic factor (BDNF) plays a crucial role in neurodevelopment, synaptic plasticity, and neuronal function and survival. Serum and plasma BDNF levels are moderately, but consistently, decreased in patients with schizophrenia (SCZ) compared to healthy controls. There is a lack of knowledge, however, on the temporal manifestation of this decline. Clinical, illness-course, and treatment factors might influence the variation of BDNF serum levels in psychotic patients. In this context, we propose a longitudinal study of a cohort of SCZ and SAD Sardinian patients with the aim of disentangling the relationship between peripheral BDNF serum levels and changes of psychopathology, cognition, and drug treatments.

Methods and analysis: Longitudinal Assessment of BDNF in Sardinian Psychotic patients (LABSP) is a 24-month observational prospective cohort study. Schizophrenic and schizoaffective disorder (SAD) patients will be recruited at the Psychiatry Research Unit of the Department of Medical Science and Public Health, University of Cagliari, and University of Cagliari Health Agency, Cagliari, Italy. We will collect BDNF serum levels as well as socio-demographic, psychopathological, and neurocognitive measures. Structured, semi-structured, and self-rating assessment tools, such as the Positive and Negative Syndrome Scale (PANSS) for psychopathological measures, and the Brief Assessment of Cognition in Schizophrenia (BACS) for cognitive function will be used.

Ethics and dissemination: This study protocol was approved by the University of Cagliari Health Agency Ethics Committee (NP2016/5491). The study will be conducted in accordance with the principles of good clinical practice, in the Declaration of Helsinki in compliance with the regulations. Participation will be voluntary and written informed

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3 consent will be obtained for each participant upon entry into the study. We plan to
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5 disseminate the results of our study through conference presentations and publication in
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7 international peer-reviewed journals. Access to raw data will be available in anonymized
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9 form upon request to the corresponding author.
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Strengths and limitations of this study

- This is a prospective cohort study of psychotic patients aiming to clarify whether longitudinal changes in brain-derived neurotrophic factor (BDNF) serum levels are correlated with psychopathological, cognitive, and treatment factors.
- The secondary outcomes of this study will offer insight on the impact of psychiatric comorbidities on longitudinal trajectory of BDNF in psychotic patients
- Sampling will be performed in a tertiary clinic specialized in treatment of psychotic patients, possibly limiting ecological validity of the study results.
- The sample size, based on previous work on longitudinal change of BDNF over time, might not achieve sufficient statistical power to detect association signals with small effect sizes between clinical predictors and BDNF levels.
- Notwithstanding the prospective design, clinical (illness duration, duration of untreated psychosis), and treatment history prior to study entry might impact on findings.

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is the most widely distributed neurotrophin in the central nervous system (CNS),¹ being expressed in almost all the cortical areas, as well as in several spinal cord regions.² There is substantial evidence that BDNF plays a crucial role during brain development, as well as in the process of differentiation, synaptogenesis and neuronal plasticity.³⁻⁶ BDNF was the second neurotrophic factor to be characterized after the nerve growth factor (NGF).⁷ Similarly to other neurotrophins, BDNF is synthesized in a pre-pro form consisting of 47 amino acids.² Extracellular BDNF appears to have two forms: pro-BDNF and mature BDNF,^{2, 8} which are formed after cleavage of the precursor protein prepro-BDNF.^{8, 9} Mature BDNF interacts with its high affinity receptor, the tropomyosin-related kinase receptor type B (TRKB), to exert its molecular effects in the adult CNS.² BDNF induces dimerization of TRKB with kinase activation and autophosphorylation of tyrosine residues, with subsequent activation of several adaptor proteins, which, in turn leads to phosphorylation of phosphoinositide 3-kinase (PI3K), mitogen-activated protein kinase - extracellular signal-regulated kinase (MEK-ERK), phospholipase C γ 1 (PLC γ 1) and cyclic AMP-responsive element-binding protein (CREB), all downstream pathways regulating neurite outgrowth, cell survival, cellular differentiation and synaptic plasticity.^{2, 10} There is evidence that the cleaved prodomain of BDNF might be, by itself, an active biological modulator.^{11, 12} BDNF and its cleaved pro-peptide have been found in large vesicles located in the presynaptic terminals of excitatory neurons in the adult hippocampus of mice.¹¹ Interestingly, the Val66Met polymorphism of BDNF, which is located in the prodomain genomic region, alters substantially the prodomain structure.¹² Specifically, Met66 BDNF prodomain induces modulation of neuronal morphology through acute cone retraction.¹²

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3 It is known that BDNF freely crosses the blood-brain barrier.¹³ In keeping with this
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5 observation, levels of BDNF in serum are correlated with CNS concentrations.¹⁴ Altered
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7 peripheral serum levels of BDNF have been shown to be a reliable biomarker for severe
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9 psychiatric disorders, including schizophrenia (SCZ).¹⁵ This corresponds to the dysregulation
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11 of BDNF, and of its related molecular pathways, observed in affected individuals.¹⁶
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15 A decline in serum BDNF levels has been consistently identified in chronic and medicated
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17 SCZ patients,¹⁷⁻²² as well as in first episode and medication naïve SCZ patients,²³⁻²⁷
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19 compared to unaffected subjects. Conversely, other studies showed increased BDNF serum
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21 levels in patients with SCZ,^{28 29} or have not been able to demonstrate differences in serum
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23 BDNF concentrations between drug-free or drug-naïve patients and healthy controls.^{30 31}
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27 This discrepancy may depend on the clinical heterogeneity of the samples studied (i.e.
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29 different stage of illness, subtype of schizophrenia, and effect of medication), as well as on
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31 the different assessment tools employed. However, a recent quantitative meta-analytical
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33 estimate by Fernandes et al.,³² confirmed that SCZ is associated with a moderate decrease
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35 of serum and plasma BDNF levels compared to healthy controls. This entails that decreased
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37 serum BDNF levels might be a marker of illness status in SCZ (i.e. disease biomarker). There
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39 is a lack of knowledge, however, on the temporal manifestation of this decline. The
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41 decrease of peripheral BDNF could be constant, with pre-morbid levels roughly similar to
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43 those of unaffected individuals, linearly declining during the course of SCZ. Conversely,
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45 BDNF peripheral levels might fluctuate in association with acute psychopathological phases
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47 of the disorder. On top of this, BDNF peripheral levels might vary as a result of drug
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49 treatments typically used in major psychoses, such as antipsychotics. To date, however, the
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51 study of the relationship between BDNF serum levels and drug treatment in SCZ and SAD
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53 patients remains inconclusive. There is extensive pre-clinical evidence suggesting that
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3 typical antipsychotics might decrease BDNF expression whereas atypical antipsychotics, with
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5 the exception of risperidone, could increase BDNF expression.³³ Clozapine-treated chronic
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7 SCZ patients have higher BDNF levels than patients treated with typical neuroleptics^{18 20} or
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9 with risperidone.¹⁸ Other studies, however, found no effect of antipsychotic treatment on
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11 the levels of serum BDNF.^{25 34 35} Taken together, these data appear contradictory, making
12
13 difficult to infer conclusions about the effects of antipsychotic treatment on serum BDNF
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15 levels. Meta-analytical findings, however, point to a small but significant increase of serum
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17 BDNF levels under antipsychotic treatment, although a class-specific effect has not been
18
19 investigated.³²

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21 One final introductory remark concerns the link between BDNF and cognitive function in
22
23 SCZ. Indeed, cognitive disruption seems to represent the core symptomatology that
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25 distinguishes SCZ patients from those affected by other psychoses. Cognitive deficits are
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27 related to social integration, to social problem solving and to the acquisition of various
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29 skills,³⁶ and are considered the most powerful predictors of functional outcome in patients
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31 with SCZ.³⁷ Cognitively impaired chronic SCZ patients appear to show low serum BDNF
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33 levels.³⁸ A recent meta analysis confirmed this observation,³⁹ showing that higher levels of
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35 BDNF peripheral expression correlated with better cognitive performances in reasoning and
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37 problem solving areas.

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39 The apparent gap in the knowledge about the longitudinal variation of BDNF serum levels,
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41 and its relationship with clinical and treatment variables in major psychoses, needs to be
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43 filled by additional clinical research. Here we present the design and methodology of a
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45 prospective observational study that takes advantage of a well-characterized population of
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47 psychotic patients carefully monitored at an academic community health centre.
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Aims of the current study

Our study has the following primary and secondary aims:

Primary aims:

1. Explore the longitudinal relationship between peripheral serum BDNF levels and variation in psychopathology in a cohort of patients with major psychosis recruited at different stages of the illness.
2. Assess whether variation of cognitive function correlates with peripheral BDNF serum levels.
3. Establish whether drug treatment impacts on the relationship between psychopathology and cognition with BDNF serum levels

Secondary aims:

4. Study whether patients' baseline characteristics prior to enrolment, namely duration of illness, duration of untreated psychosis (DUP), psychiatric and somatic comorbidity, age at onset, gender, family history of psychiatric disorders, premorbid adjustment, and the type of illness course moderate the longitudinal relationship between peripheral serum BDNF and psychopathology.
5. Study whether measures of peripheral serum BDNF levels correlate with baseline measure of social functioning.
6. Analyze the relationship of peripheral BDNF levels with tolerability of antipsychotic treatment, as expressed by the presence of extrapyramidal symptoms (EPS).

METHODS

Study design

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3 Longitudinal Assessment of BDNF in Sardinian Psychotic patients (LABSP) is a 24-month
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5 observational prospective cohort study. The sample will be recruited from patients
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7 followed-up and treated at the community mental health centre of the Psychiatry Research
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9 Unit of the Department of Medical Science and Public Health, University of Cagliari and
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11 University of Cagliari Health Agency, Cagliari, Italy. Blood samples from recruited patients
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13 will be taken at baseline (T_0), and at three consecutive time points: 6 months (T_1), 12
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15 months (T_2), and 18 months (T_3) (Figure 1). The temporal fluctuations of psychopathology,
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17 as well as the assessment of pharmacotherapy, measures of cognitive function, and
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19 manifestation of treatment-related side effects (i.e. EPS) will be also evaluated at each time
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21 point (Table 1). The observational prospective design permits to disentangle how the
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23 variables might influence the outcome under study. Thus, we will assess whether changes in
24
25 peripheral serum levels BDNF predate or, alternatively, are a consequence of
26
27 psychopathological changes and/or drug treatment. As we will study the trajectory of
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29 peripheral serum BDNF levels over time, the recruited cohort will serve as its own control.
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31 We also expect to obtain a more accurate estimation of the patterns of association between
32
33 BDNF serum levels and all the independent clinical variables under consideration.
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43 **Participants and recruitment**

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45 The recruitment of the sample will be based on a two-step process. In the first step, all
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47 patients followed-up at the community mental health centre of the University of Cagliari
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49 Health Agency, Cagliari, Italy with a diagnosis of SCZ or SAD according to the Diagnostic and
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51 Statistical Manual of Mental Disorders-IV-Text Revision (DSM-IV-TR)⁴⁰ will be identified in
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53 the medical record database. The electronic search will be performed using lower (18) and
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55 upper (65) age limits, with no constraint regarding the presence of comorbid psychiatric
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3 disorders. After eligible patients will be identified in the database, the diagnosis of SCZ or
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5 SAD will be confirmed using the Structured Clinical Interview for DSM-IV-TR Axis I Disorders,
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7 Patient Edition (SCID-I/P)⁴¹ administered by trained mental-health professionals (psychiatry
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9 residents or senior clinical staff). Further, we will collect detailed information on
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11 pharmacological treatment regime. Given the characteristics of the patient population
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13 followed-up at our community mental health centre, we anticipate that our sample will not
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15 be comprised of drug-naïve patients and will be on a pharmacological treatment regime
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17 mainly based on antipsychotics.
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24 **Inclusion and exclusion criteria**

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26 Inclusion criteria are: 1) age between 18 and 65 years; 2) diagnosis of SCZ or SAD according
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28 to DSM-IV-TR; and 3) stability during the six months before recruitment. Exclusion criteria
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30 are: 1) refusal to provide consent; 2) presence of acute psychopathological symptoms; 3)
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32 presence of illness-related cognitive impairment of such severity that affects their ability to
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34 cooperate; 4) presence of major unstable medical illness; 5) severe mental retardation; 6)
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36 major neurological disorder or previous head injury; 7) current drug and alcohol
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38 dependence.
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46 **Sample size and power calculation**

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48 Our main null hypotheses are that there is no difference between mean BDNF serum levels
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50 at different time points in relation to: 1) the longitudinal psychopathological changes of SCZ
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52 and SAD patients; 2) measures of cognitive functioning; and 3) drug treatment. We based
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54 our power analysis on previous findings of BDNF serum levels longitudinal variation in a
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56 small cohort (n = 21) of first-episode SCZ patients.²⁴ Specifically, Palomino et al. performed a
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3 1-year prospective assessment of BDNF finding an absolute mean difference between
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5 baseline and the last time point of 1.65.²⁴ Thus, a sample size of 59 individuals would be
6
7 sufficient to obtain a 90% statistical power to detect significant difference at $\alpha = 0.05$
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9 between mean serum BDNF levels of this magnitude at each time point, considering an
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11 attrition rate for time point of 5%. Power analysis has been performed using repeated
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13 measures and sample size (RMASS) software.⁴²
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20 **Measures and materials**

21 Socio-demographic variables, personal and family history data

22 Socio-demographic data will be collected using the Association for Methodology and
23
24 Documentation in Psychiatry (AMDP)⁴³ assessment tool. Briefly, we will gather information
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26 on gender, age, level of education, marital status, offspring, economic status, and
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28 occupation. Further, we will collect data on previous and current substance and/or alcohol
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30 abuse, and smoking status. The latter will be quantified also as number of cigarettes smoked
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32 per day. Information on family history of any psychiatric disorders will be collected through
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34 direct interview of the participant and at least one first-degree relative or significant other,
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36 as well as through an accurate review of available medical records. We will gather
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38 information on the stage of menstrual cycle whenever available. Finally, the systematic
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40 review of clinical record will allow us to retrospectively collect data on the presence of a
41
42 previous history of stressful life events (SLE), including childhood maltreatment. We will also
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44 monitor longitudinally the eventual manifestation of SLE.
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55 Psychopathological measures
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3 The main psychopathological measures will be collected through the AMDP syndrome
4 scale,⁴³ which has shown to be valid in discriminating various forms of psychoses, as well as
5 in providing a detailed psychopathological description of the subjects under study.⁴⁴ As
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10 previously reported, patients' diagnosis will be confirmed using the SCID-I/P.⁴¹ Furthermore,
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13 all recruited patients will undergo assessment with the Structured Clinical Interview for
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The main psychopathological measures will be collected through the AMDP syndrome scale,⁴³ which has shown to be valid in discriminating various forms of psychoses, as well as in providing a detailed psychopathological description of the subjects under study.⁴⁴ As previously reported, patients' diagnosis will be confirmed using the SCID-I/P.⁴¹ Furthermore, all recruited patients will undergo assessment with the Structured Clinical Interview for DSM-IV Axis II Personality Disorders (SCID-II)⁴⁵ to detect the presence of comorbid personality disorders. Information on age of onset, age at first intervention (pharmacological and/or psychotherapeutic and/or psychosocial) will be based on retrospective information collected through direct interview of the patient, and at least one relative or significant other, as well as through an accurate review of available medical records. This approach should minimize the impact of recall bias on the assessment of key clinical measures.

We will also estimate DUP, the interval between the age of onset of full-blown psychotic symptoms and first antipsychotic treatment, using the Psychopathological Onset Latency and Treatment Questionnaire.⁴⁶ Indeed a longer DUP appears to be related to a worse short-⁴⁷ and long-term clinical outcome^{48 49} in SCZ individuals, as well as with lower BDNF serum levels,⁵⁰ although the latter finding has been not replicated in subsequent studies.⁵¹ Other measures of illness severity, such as number of hospital admissions and type of clinical course will be collected for the analysis.

Psychopathological assessment will include the Positive and Negative Symptom Scale for Schizophrenia (PANSS),⁵² and the Clinical Global Impression Scale for Schizophrenia (CGI-SCH).⁵³ The latter measure is a valid assessment tool for monitoring longitudinal psychopathological symptoms in routine clinical practice.⁵⁴ Moreover, we will assess premorbid dysfunction, a well established prognostic marker in SCZ patients, using the

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2
3 Premorbid Adjustment Scale (PAS).⁵⁵ All these psychopathological measures will be analyzed
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5 in relation to BDNF serum levels.
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10 Social functioning and quality of life measures

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12 Evaluation of social functioning will be carried out with the Personal and Social Performance
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14 Scale (PSP),⁵⁶ which showed good reliability in SCZ patients.⁵⁷ Further, assessment of quality
15
16 of life (QOL) will be performed using the World Health Organization Quality-Bref (WHOQOL)
17
18 version which has shown good validity and reliability in variety of psychiatric illnesses
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20 including SCZ.^{58 59} Finally, we will collect data using the Subjective Wellbeing under
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22 Neuroleptics-Short Version (SWN-S).⁶⁰
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29 Cognitive measures

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31 The cognitive performance will be examined in a standardized way using the Brief
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33 Assessment of Cognition in Schizophrenia Scale (BACS).⁶¹ It takes approximately half an hour
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35 to administer in healthy controls, and includes brief assessments of four of the seven
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37 neurocognitive domains designated as important by the Measurement and Treatment
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39 Research to Improve Cognition in Schizophrenia (MATRICS) battery: reasoning and problem
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41 solving, processing speed, verbal memory, and working memory. The composite score has
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43 been shown to have high test–retest reliability in SCZ patients.⁶¹ Moreover, the BACS
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45 appears to assess reliably measures of functional capacity.⁶² The BACS will permit a rapid
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47 assessment of cognitive function at different time points, allowing correlation of these
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49 measures with peripheral serum BDNF levels. Indeed, several studies have shown that
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51 peripheral serum BDNF level might be a biomarker of cognitive function in schizophrenia.⁶³⁻
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Assessment of side effects

We will monitor the onset of antipsychotics related side effects using the Dosage and Treatment Emergent Symptoms Scale (DOTES),⁶⁷ and the Extrapyramidal Symptoms Rating scale (ESRS).⁶⁸ Of note, one study showed that SCZ patients with tardive dyskinesia (TD) had lower plasma BDNF levels than those without TD.⁶⁹ Data on antipsychotics dosages will be collected and converted to chlorpromazine equivalents for data analysis.

Biometric, metabolic, and cardiovascular measures

There is growing evidence for a role of BDNF in appetite and weight regulation as well as in eating disorders, possibly via its interaction with the serotonin system. BDNF is expressed in brain regions including the hypothalamus, where its infusion in rats has been shown to increase serotonin turnover, and suppress appetite.⁷⁰ Conversely, mice with only one copy of the BDNF gene displayed increased food intake and weight, with decreased serotonin metabolite to serotonin ratio.⁷¹ Conditional ablation of the BDNF gene also leads to similar hyperphagic and obese phenotypes.⁷² BDNF may also regulate weight and food intake via its regulation of the dopamine system.^{73 74} Antagonism on dopamine receptors 3 (DRD3) was shown to alter attentional bias to food cues in low-restrained obese or overweight individuals.⁷⁵ This study suggests that BDNF may be a key factor in the regulation of eating behavior upstream of dopamine and serotonin systems and BDNF gene variants may play a role in antipsychotic-induced weight gain. Based on these findings, we will evaluate the following clinical parameters: weight, waist circumference, body mass index (BMI), liver and kidney function panel, serum glucose, glycosylated haemoglobin, lipid profile, prolactin (PRL), and creatine phosphokinase (CPK). The cardiovascular function will be monitored by

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3 electrocardiogram (ECG), with evaluation of QTc interval. Finally, we will collect information
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5 on exercise as this can impact on BDNF levels.⁷⁶
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10 **Sampling and assessment of BDNF serum levels**

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12 Blood samples for each patient will be taken at the same time of the day (between 8:00 and
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14 10:00 AM). BDNF serum will be evaluated using BDNF ELISA Kit (Booster Immunoleader, Cat.
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16 N° EK0307) for quantitative detection of human BDNF in cell culture supernatants, serum
17
18 and plasma. This kit is based on a standard sandwich enzyme-linked immune-sorbent assay
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20 technology for specific quantifications of natural and recombinant human BDNF with a high
21
22 sensitivity (< 2 pg/mL) and with no detectable cross-reactivity with other relevant proteins.
23
24 After blood sampling, serum will be allowed to clot in a serum separator tube for about 4
25
26 hours at room temperature. After that it will be centrifuged at approximately 1000 X g for
27
28 15 min. Supernatant serum samples will be collected in small aliquot and stored
29
30 immediately at -20°C for future analysis. Then, samples will be processed according to kit
31
32 protocol and instructions. Optical density absorbance of each sample will be read with a
33
34 450nm filter in a microplate reader (Thermo Scientific Multiskan FC) within 30 minutes after
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36 the final step of the kit procedure. Data obtained will be analysed using the Thermo
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38 Scientific SkanIt Software 3.0 for Multiskan FC.
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48 **Data analysis plan**

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50 Mixed-effects linear regression models (MLRM) will be used to analyze longitudinal data.^{77 78}
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52 Specifically, we will regress independent variables (both categorical and continuous) on
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54 BDNF serum levels (dependent variable). Mixed-effects linear regression models allow to
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56 model individual change over time and appear to be more flexible in terms of repeated
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3 measures, particularly when the number of observations per subject is not the same at each
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5 time point.^{77 78} Further, these models allow generalization of non-normally distributed data
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7 for independent variables. Our analysis plan will consist of the following steps. First, we will
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9 perform a visual inspection of mean BDNF serum levels at each time point using boxplots.
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11 This will allow checking for normality of BDNF serum levels at each time point as well as to
12
13 identify outliers. Secondly, MLRM will be analyzed in order to assess the longitudinal
14
15 variation of BDNF levels while correcting for age and sex. Finally, independent variables will
16
17 be regressed on BDNF serum levels. Covariates will be added to significant models to
18
19 account for possible intercorrelations. All data will be analyzed using “lme4” package
20
21 implemented in R.⁷⁹ Missing data for independent variables will be dealt with the
22
23 “na.action” function implemented in R. The statistical significance of identified MLRM will
24
25 be calculated using the “multcomp” R package. Finally, graphical representation of MLRM
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27 will be obtained with R packages “sjPlot” e “sjmisc”.

28 29 30 31 32 33 34 35 36 37 **Ethics and dissemination**

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39 This study protocol was approved by the University of Cagliari Health Agency Ethics
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41 Committee (NP2016/5491). The study will be conducted in accordance with the principles of
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43 good clinical practice, in the Declaration of Helsinki in compliance with the regulations.
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45 Written, informed consent will be obtained from all eligible participants. Participation to the
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47 study will be voluntary and patients will be able to withdraw consent at any point with no
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49 disadvantage to their treatments. A psychiatric assessment will establish that patients’
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51 ability to consent is not compromised by their psychopathological status.
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3 We plan to disseminate the results of our study through conference presentations and
4 publication in international peer-reviewed journals. Access to raw data will be available in
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7 anonymized form upon request to the corresponding author.
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10 11 12 13 **DISCUSSION**

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15 Schizophrenia is characterized by three distinct, but to a certain extent intertwined,
16 clusters of symptoms: positive, negative, and cognitive. Each one of these clusters
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18 presents with a varying degree of severity and with a specific prevalence depending on the
19
20 stage of the illness.⁸⁰ Patients with stable diagnoses of SCZ and SAD have often incomplete
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22 remissions (particularly from negative symptoms), and not rarely recurrences or relapses
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24 of positive symptoms, mainly delusions and hallucinations.⁸¹ Cognitive function, however,
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26 appears to decline steadily over the course of the illness, although most of the magnitude
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28 of the differences in cognition between SCZ patients and unaffected subjects appears to
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30 be modulated by age.⁸¹
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38 One key aspect in diagnostically stable SCZ and SAD patients is being able to predict the
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40 manifestation of these psychopathological exacerbations. Certainly, given that fluctuations
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42 appear to be more prominent for positive symptoms and to a lesser extent for negative
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44 ones, rather than for cognition, researcher have focused on the identification of
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46 prodromal signs of relapses/recurrences using, for instance, home telemonitoring of
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48 symptoms change via mobile phone-based platforms.^{82 83} This approach has proven
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50 effective in reducing the number of relapses, and consequent hospitalizations, in psychotic
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52 individuals.^{82 83}
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3 Our prospective study, LABSP, expands on this evidence aiming at monitoring not only the
4 clinical signs of SCZ patients over an 24-month period, but, more importantly, assessing
5 the predictive power of a well-established biomarker of illness status in SCZ (BDNF serum
6 levels), on the clinical trajectory of SCZ. We expect to find correlation of BDNF levels with
7 longitudinal variation of psychopathology, particularly concerning positive symptoms. This
8 prospective approach, to our knowledge, has not been proposed previously in the
9 literature, and might offer novel data on serum BDNF as a biomarker of
10 psychopathological changes in accurately monitored SCZ patients. If such a correlation will
11 be identified, the joint analysis of clinical and BDNF data might explain a larger proportion
12 of psychopathological change that clinical data alone.
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27 One additional point of novelty concerns the analysis of the impact of psychiatric
28 comorbidity on BDNF levels. Indeed, SCZ is not rarely associated with other axis I and II
29 disorders that typically affect negatively the course and outcome of the disorder.⁸⁴
30 However, to our knowledge the relationship between serum BDNF levels in SCZ and
31 psychiatric comorbidities has not been tested in previous work. Although this analysis
32 remains a secondary aim of our study, the sample size should allow an adequately powered
33 post-hoc stratification of our prospective data to test this relationship.
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The wealth of clinical data we are planning to collect with LABSP will also allow testing the moderating influence of relevant clinical variables, such as for instance, family history of SCZ. In fact, it is plausible that recruited participants with a positive family history of SCZ, will have lower baseline BDNF levels compared to those without a family history, and a different pattern of association between serum BDNF levels and the various clinical independent variables.

Limitations

Our study has some limitations inherent to its design. First, we will study a population recruited in a tertiary clinic specialized in treatment of psychotic patients, possibly limiting ecological validity (i.e. generalizability) of the results. Second, our power analysis is based on previous longitudinal estimates of 1.65 mean variation of serum BDNF levels over a 1-year follow-up. Although a sample size of 59 might be sufficient to detect a longitudinal variation of this magnitude in BDNF serum levels we might not be able to detect association signals of small effect size between clinical, treatment, and cognitive variables and BDNF. Thus, we plan to extend our recruitment well beyond the number provided by our power analysis. Finally, notwithstanding the prospective design, clinical (illness duration, duration of untreated psychosis), and treatment history prior to study entry might impact on findings.

CONCLUSIONS

In summary, LABSP will allow the prospective assessment of BDNF serum levels, as well as of key clinical and treatment-related measures, in a relatively large cohort of SCZ and SAD patients followed-up in a naturalistic setting. This work will provide useful information on the relationship between BDNF serum levels and psychopathological changes over time. Changes in BDNF peripheral levels might be predictive of the psychopathological trajectory during the 24-month follow-up, and if validated in independent samples, might point to a role of BDNF as a biomarker of clinical trajectory in psychotic patients.

Contributors

DP has contributed to the design of the study, and drafted the first version of the manuscript. BC conceived the study, led the study team, and critically revised the

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3 manuscript. MM has contributed to the assessment protocol, the design of the study, and
4
5 the drafting of the manuscript. LD and MT have contributed to the study design and
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7 assessments. MS and RC have contributed to BDNF serum levels assessments and laboratory
8
9 procedures. PF and WF have designed the experimental procedures for BDNF assessment.
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11 All authors have read and approved the final version of the manuscript.
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14 15 16 17 **Data sharing statement**

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19 Access to raw data will be available in anonymized form upon request to the
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21 corresponding author.
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24 25 26 27 **Competing interests**

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29 The authors have no competing interests relevant to this study.
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32 33 34 **Ethics approval**

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36 This study protocol was approved by the University of Cagliari Health Agency Ethics
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38 Committee (NP2016/5491).
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44
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Table 1. Assessment protocol in the Longitudinal Assessment of BDNF in Sardinian Psychotic**patients (LABSP)**

Measure	Time points				
	T ₀ (enrolment)	T ₁ (6 months)	T ₂ (12 months)	T ₃ (18 months)	T ₄ (24 months)
BDNF serum levels	✓	✓	✓	✓	✓
Descriptive variables					
Demographic data	✓				
Socio-economic data	✓				
Clinical history	✓				
Family history	✓				
Psychopathology					
SCID-I/P	✓				
SCID-II	✓				
PANSS	✓	✓	✓	✓	✓
CGI-SCH	✓	✓	✓	✓	✓
POLTQ	✓				
PAS	✓	✓	✓	✓	✓
Social functioning and quality of life					
PSP	✓	✓	✓	✓	✓
SWN-S	✓	✓	✓	✓	✓
WHOQOL-Bref	✓	✓	✓	✓	✓
Cognition					
BACS	✓	✓	✓	✓	✓
Assessment of side effects					
DOTES	✓	✓	✓	✓	✓
ESRS	✓	✓	✓	✓	✓
Biometric, metabolic, and cardiovascular measures					
Lipid profile	✓				✓
Kidney panel	✓				✓
Liver panel	✓				✓
Electrolytes	✓				✓
Weight, height, waist circumference, BMI	✓	✓	✓	✓	✓
Glucose	✓				✓
Glycosylated haemoglobin	✓				✓
PRL	✓				✓
CPK	✓				✓
ECG	✓				✓

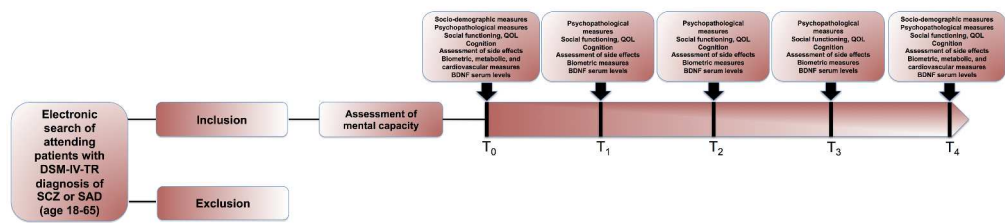
BDNF: brain-derived neurotrophic factor; SCID-I/P: Structured Clinical Interview for DSM IV Axis I Disorders; SCID-II: Structured Clinical Interview for DSM IV Axis II Disorders; PANSS: Positive And Negative Syndrome Scale; CGI-SCH: Clinical Global Impression – Schizophrenia Scale; POLTQ: Psychopathological Onset Latency and Treatment Questionnaire; PAS: Premorbid Adjustment Scale; PSP: Personal and Social Performance scale; SWN-S: Subjective Well-Being Under Neuroleptics Scale; WHOQOL-Bref: World Health Organization Quality Of Life – Bref; BACS: Brief Assessment Cognition Schizophrenia; DOTES: Dosage Record and Treatment Emergent Symptom Scale; ESRS: Extrapiramidal Symptom Rating Scale; BMI: Body Mass Index; PRL: Prolactine; CPK: Creatine phosphokinase; ECG: Electrocardiogram.

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Figure legend

Flowchart of recruitment and assessment procedures for the Longitudinal Assessment of brain-derived neurotrophic factor in Sardinian Psychotic patients (LABSP) study.

For peer review only



Flowchart of recruitment and assessment procedures for the Longitudinal Assessment of brain-derived neurotrophic factor in Sardinian Psychotic patients (LABSP) study.

489x149mm (300 x 300 DPI)

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