PEER REVIEW HISTORY

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ARTICLE DETAILS

<table>
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<tr>
<th>TITLE (PROVISIONAL)</th>
<th>Longitudinal Assessment of brain-derived neurotrophic factor in Sardinian Psychotic patients (LABSP): a study protocol for a prospective observational study</th>
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<tr>
<td>AUTHORS</td>
<td>Primavera, Diego; Manchia, Mirko; Deriu, Luca; Tusconi, Massimo; Collu, Roberto; Scherma, Maria; Fadda, Paola; Fratta, Walter; Carpiniello, Bernardo</td>
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VERSION 1 - REVIEW

<table>
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<tr>
<th>REVIEWER</th>
<th>Michael Notaras</th>
<th>Center for Neurogenetics, Brain &amp; Mind Research Institute, Weill Cornell Medical College, Cornell University; United States of America</th>
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<td>REVIEW RETURNED</td>
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GENERAL COMMENTS

The manuscript submitted by the authors is suitable for publication, following revision that addresses the feedback of the reviewers. In my opinion, further information regarding the statistical analysis (and power analysis) and funding sources (listed as 'none' at the end of the manuscript). Further, an explicit discussion on the limitations is required, as is whether any supplementary reporting is necessary per the BMJ Open reviewer guidelines (this appears to be okay, but requires verification from the authors). Language is acceptable but can be improved with further revision. Overall, this article requires minor, but important, revision. Otherwise, the article is scientifically useful to the schizophrenia, BDNF and psychiatry disciplines, and will be welcomed by the BMJ Open readership. See below for detailed comments, which should be forwarded to the authors along with this comment.

In the manuscript submitted to BMJ Open by Primavera et al., a protocol for a prospective study is described which seeks to quantify longitudinal changes in BDNF expression within the periphery of Sardinian psychotic patients. Relevant clinical characteristics will also be examined over the course of the study period, and these variables will be contrasted against fluctuations in BDNF concentrations. Overall, this study will be of value to the conjoint BDNF-schizophrenia literature, where it has been difficult to disentangle an effect of BDNF within this disorder. This prospective study protocol would likely be of interest to consumers of BMJ Open as well as the broader psychiatry community. Barring only minor
comments, and perhaps following another round of revisions that addresses the feedback of reviewers, the manuscript would be well suited for publication.

**Experimental Considerations:**

1) The power analysis lacks a commentary on the size of effect that is predicted to be observable with a cohort of just \( n = 53 \). While the authors note that they will be sufficiently powered to detect a difference with this number of participants, it is unclear what this magnitude of difference may be. Given that the authors concede themselves in their introduction that fluctuations in peripheral BDNF concentrations in schizophrenia may only be small, it is important to establish whether this study protocol will be sufficiently powered to detect small effect sizes. Therefore, the power analysis presented here should be expanded so that it can be determined 1) what effect size can be detected with an \( n = 53 \) and 2) whether small effects can be detected with a sample size this small. Likewise, additional information regarding statistical analysis would be useful as this is a criterion set by *BMJ Open* in the assessment guidelines provided to reviewers.

Finally, funding is listed as “none”. This is unclear – will the funding be provided by the host medical center? This is also a criterion of *BMJ Open* emphasized in the reviewers materials and should also be revisited in further detail, as should whether any supplementary reporting is necessary (e.g. trial registration; funding details; CONSORT, STROBE or PRISMA checklist)?

2) Quantifying BDNF can be difficult, for multiple reasons. This is compounded by the fact that many BDNF ELISA kits have variable sensitivity and accuracy. Consequently, it becomes important to establish whether the BDNF ELISA kit selected by the authors is able to 1) accurately detect BDNF independent of other neurotrophins (i.e., is there is low cross-reactivity?), 2) establish whether the kit detects total BDNF concentrations or just mature BDNF, and 3) whether the kit has adequate sensitivity to detect BDNF in human serum. Do the authors have any preliminary data, or prior studies, where these considerations are addressed? If any of this information is available from the manufacturer, it would also be worth including such in the methods of the manuscript. Lastly, it would be interesting to also assay proBDNF concentrations if financially feasible given several papers in 2016 which have reported potential mismatches in proBDNF and
total BDNF expression in mood disorder cases. Longitudinal assessments of proBDNF in schizophrenia patients may also yield important data that may correlate with clinical features of this disorder.

3) A final recommendation would be to include further discussion on when BDNF concentrations will be collected, and what covariates will be recorded or controlled for. BDNF expression follows a diurnal cycle, can be modulated by estrogens and can be suppressed long-term due to a history of stress/stressful life events. Exercise as well as dietary factors can also modulate BDNF expression. To be as thorough as possible, I would suggest that BDNF samples are taken at the same time of day (approx., if possible, such as AM or PM), stage of menstrual cycle recorded (if possible) and data on a history of stress/stressful life events collected as a covariate. Some commentary on these factors in this prospective protocol, or any other information on how potential covariates and confounds will be avoided, would benefit the design of this study.

**Other Considerations:**

1) In the introduction, it is stated that extracellular BDNF has two forms. However, recent advances in the neurotrophin field have implicated that the BDNF prodomain (the sequence cleaved in the conversion from pro to mature BDNF) is also likely to be biologically active. There are some experimental papers on this topic, but more information can be found in some recent reviews if needed. Brief amendment of this discussion is thus required.

2) The manuscript should undergo another phase of grammatical inspection so to ensure proper syntax and orthography are adapted throughout the manuscript. Of note, there is inconsistent use of tenses and plurality throughout the manuscript. Amending these small errors will avoid ambiguity as well as polysemous text, increasing the clarity of the manuscript. Another writing-related comment is that many of the sentences in this manuscript are long and complex, which interrupts the flow of the paper. Specific examples of this can be found on Pg. 4 (sentences on lines 24 and 29), as well as on Pg. 8 (line 15). Truncating these sentences, or splitting them into more than 1 sentence, would benefit the paper. Further proofing for typographical errors should also be conducted (e.g. Pg. 15 on line 44).

3) On Pg. 4, lines 45-50, it is noted that “strong” correlations
between serum and brain BDNF concentrations have been previously observed and reported. However, the study cited to support this statement only reported weak-to-moderate correlations ($r^2 = 0.40$). I therefore believe that the word “strong” should be subsequently removed. The following sentence in this paragraph is also long/fragmented, and should be revised.

4) On page 8, the sentence beginning on line 17 states that the prospective observational protocol being reported in the manuscript will permit assessments of “causality”. Given the design of this study, I do not believe that direct causality can be established as fluctuations in BDNF expression or clinical symptoms may be caused by uncontrolled covariates that influence both factors concomitantly but independently. In an observational study such as this, any such effects cannot be ruled out. Revising this text would therefore benefit the paper.

5) Lastly, in a prospective study as this, it is important to establish the boundaries of this study so that clear and reasoned conclusions can be drawn but not overgeneralized. I would therefore suggest including a concise limitations paragraph in the discussion so to directly acknowledge such.

Overall, the protocol submitted to *BMJ Open* by Primavera et al. is suitable for publication in this journal, but should be revised pending the feedback of reviewers.

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**GENERAL COMMENTS**

In my opinion a study period of 18 months is too short to really thoroughly address the questions posed. In a prospective longitudinal study of this nature it would be important to allow sufficient time for the subjects to experience periods when they are symptomatic, in recovery and an adequate period of remission/stability and also for changes in treatment regime to be made, stabilised and evaluated for individual subjects within the study. One aim of the study is to assess the association with serum BDNF levels and changes in negative symptoms and cognitive impairment in subjects. Changes in which occur over a much more protracted time period. It is in my view unlikely that 18 months will be sufficiently long to study these changes in a large enough proportion of the sample.

Exclusion criteria should include major neurological disorder or previous head injury and current drug and alcohol dependence particularly given that cognitive tests are part of the methodology of the study.

It would be useful to include a test of premorbid IQ in the initial
There was no discussion of the limitations of this study.

VERSION 1 – AUTHOR RESPONSE

Reviewer #1

Q1) Further information regarding the statistical analysis (and power analysis) and funding sources (listed as "none" at the end of the manuscript).

R1) As recommended, we added further details on statistical analysis (please see Q4) and funding sources. Concerning the latter, we added the following sentence to the revised version of the manuscript (page 20, line 13): “This study is funded through the grant “Fondo integrativo per la ricerca” of the University of Cagliari (BC).”

Q2) Further, an explicit discussion on the limitations is required, as is whether any supplementary reporting is necessary per the BMJ Open reviewer guidelines (this appears to be okay, but requires verification from the authors).

R2) A limitation section has been added to the Discussion section in the revised version of the manuscript (page 18, line 17).

Q3) Language is acceptable but can be improved with further revision.

R3) We thoroughly revised and streamlined the manuscript to facilitate readability.

Q4) The power analysis lacks a commentary on the size of effect that is predicted to be observable with a cohort of just n = 53. While the authors note that they will be sufficiently powered to detect a difference with this number of participants, it is unclear what this magnitude of difference may be. Given that the authors concede themselves in their introduction that fluctuations in peripheral BDNF concentrations in schizophrenia may only be small, it is important to establish whether this study protocol will be sufficiently powered to detect small effect sizes. Therefore, the power analysis presented here should be expanded so that it can be determined 1) what effect size can be detected with an n = 53 and 2) whether small effects can be detected with a sample size this small. Likewise, additional information regarding statistical analysis would be useful as this is a criterion set by BMJ Open in the assessment guidelines provided to reviewers. Finally, funding is listed as “none”. This is unclear – will the funding be provided by the host medical center? This is also a criterion of BMJ Open emphasized in the reviewers materials and should also be revisited in further detail, as should whether any supplementary reporting is necessary (e.g. trial registration; funding details; CONSORT, STROBE or PRISMA checklist)?

R4) The section on power analysis has been now corrected and expanded. Specifically, we recalculated the sample size needed to achieve 90% statistical power to detect a mean difference in serum BDNF levels of 1.65. Modifications are as follows (page 10, line 18): “We based our power analysis on previous findings of BDNF serum levels longitudinal variation in a small cohort (n = 21) of first-episode SCZ patients.22 Specifically, Palomino et al, performed a 1-year prospective assessment of BDNF finding an absolute mean difference between baseline and the last time point of 1.65.22 Thus, a sample size of 59 individuals would be sufficient to obtain a 90% statistical power to detect significant difference at α = 0.05 between mean serum BDNF levels of this magnitude at each time point, considering an attrition rate for time point of 5%. This analysis has been performed using repeated measures and sample size (RMASS) software.40"

We described in more detail the statistical analysis as follows (page 15, line 14): “Mixed-effects linear regression models (MLRM) will be used to analyze longitudinal data.74 75 Specifically, we will regress independent variables (both categorical and continuous) on BDNF serum levels (dependent variable). Mixed-effects linear regression models allow to model individual change over time and appear to be more flexible in terms of repeated measures, particularly when the number of
observations per subject is not the same at each time point. Further, these models allow generalization of non-normally distributed data for independent variables. Our analysis plan will consist of the following steps. First, we will perform a visual inspection of mean BDNF serum levels at each time point using boxplots. This will allow to check for normality of BDNF serum levels at each time point as well as to identify outliers. Secondly, MLRM will be analyzed in order to assess the longitudinal variation of BDNF levels while correcting for age and sex. Finally, independent variables will be regressed on BDNF serum levels. Covariates will be added to significant models to account for possible intercorrelations. All data will be analyzed using “lme4” package implemented in R. Missing data for independent variables will be dealt with the “na.action” function implemented in R. The statistical significance of identified MLRM will be calculated using the “multcomp” R package. Finally, graphical representation of MLRM will be obtained with R packages “sJPlot” e “sjmisc”.

We added funding information in the appropriate section (please see R1).

Q5) Quantifying BDNF can be difficult, for multiple reasons. This is compounded by the fact that many BDNF ELISA kits have variable sensitivity and accuracy. Consequently, it becomes important to establish whether the BDNF ELISA kit selected by the authors is able to 1) accurately detect BDNF independent of other neurotrophins (i.e., is there is low cross-reactivity?), 2) establish whether the kit detects total BDNF concentrations or just mature BDNF, and 3) whether the kit has adequate sensitivity to detect BDNF in human serum. Do the authors have any preliminary data, or prior studies, where these considerations are addressed? If any of this information is available from the manufacturer, it would also be worth including such in the methods of the manuscript. Lastly, it would be interesting to also assay proBDNF concentrations if financially feasible given several papers in 2016 which have reported potential mismatches in proBDNF and total BDNF expression in mood disorder cases. Longitudinal assessments of proBDNF in schizophrenia patients may also yield important data that may correlate with clinical features of this disorder.

R5) We thank the reviewer for highlighting these important issues. We have now rewritten the methods description regarding the serum measurements of BDNF including all the information requested. We have now specified, from the manufacture, the following points: the absence of cross-reactivity with other proteins, the specific quantification of natural and recombinant human BDNF in serum samples and the kit sensitivity (< 2 pg/mL).

We revised, as requested by the reviewer, the Methods/Assessment of BDNF serum levels section as follow (page 14, line 21): “BDNF serum will be evaluated using BDNF ELISA Kit (Booster Immunoleader, Cat. N° EK0307) for quantitative detection of human BDNF in cell culture supernates, serum and plasma. This kit is based on a standard sandwich enzyme-linked immune-sorbent assay technology for specific quantifications of natural and recombinant human BDNF with a high sensitivity (< 2 pg/mL) and with no detectable cross-reactivity with other relevant proteins. After blood sampling, serum will be allowed to clot in a serum separator tube for about 4 hours at room temperature. After that it will be centrifuged at approximately 1000 X g for 15 min. Supernatant serum samples will be collected in small aliquot and stored immediately at -20°C for future analysis. Then, samples will be processed according to kit protocol and instructions. Optical density absorbance of each sample will be read with a 450nm filter in a microplate reader (Thermo Scientific Multiskan FC) within 30 minutes after the final step of the kit procedure. Data obtained will be analyzed using the Thermo Scientific SkanIt Software 3.0 for Multiskan FC.”

Concerning proBDNF, we agree with the reviewer that it would be of importance to study the longitudinal variation of this marker in our prospective cohort. However, such analysis would sensibly increase the costs of the project and as of now it is not financially feasible. We plan, however, to store aliquots of serum from sampled patients in order to perform new sets of analysis once new funding will be secured.

Q6) A final recommendation would be to include further discussion on when BDNF concentrations will be collected, and what covariates will be recorded or controlled for. BDNF expression follows a diurnal cycle, can be modulated by estrogens and can be suppressed long-term due to a history of
stress/stressful life events. Exercise as well as dietary factors can also modulate BDNF expression. To be as thorough as possible, I would suggest that BDNF samples are taken at the same time of day (approx., if possible, such as AM or PM), stage of menstrual cycle recorded (if possible) and data on a history of stress/stressful life events collected as a covariate. Some commentary on these factors in this prospective protocol, or any other information on how potential covariates and confounds will be avoided, would benefit the design of this study.

R6) These are points well taken. We added the following sentences to clarify these methodological aspects. Concerning the time of sampling we added (page 14, line 22): “Blood samples for each patient will be taken at the same time of the day (between 8:00 and 10:00 AM).” Further, we added (page 11, line 13): “We will gather information on the stage of menstrual cycle whenever available.” Concerning life events, we added (page 11, line 14): “Finally, we will collect data on the presence of a previous history of stressful life events as well as monitor longitudinally their eventual manifestation.”

Q7) In the introduction, it is stated that extracellular BDNF has two forms. However, recent advances in the neurotrophin field have implicated that the BDNF prodomain (the sequence cleaved in the conversion from pro to mature BDNF) is also likely to be biologically active. There are some experimental papers on this topic, but more information can be found in some recent reviews if needed. Brief amendment of this discussion is thus required.

R7) The Introduction has been amended as follows (page 5, line 17): “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”

Q8) The manuscript should undergo another phase of grammatical inspection so to ensure proper syntax and orthography are adapted throughout the manuscript. Of note, there is inconsistent use of tenses and plurality throughout the manuscript. Amending these small errors will avoid ambiguity as well as polysemous text, increasing the clarity of the manuscript. Another writing-related comment is that many of the sentences in this manuscript are long and complex, which interrupts the flow of the paper. Specific examples of this can be found on Pg. 4 (sentences on lines 24 and 29), as well as on Pg. 8 (line 15). Truncating these sentences, or splitting them into more than 1 sentence, would benefit the paper. Further proofing for typographical errors should also be conducted (e.g. Pg. 15 on line 44).

R8) We have thoroughly revised the paper according to the reviewer indications.

Q9) On Pg. 4, lines 45-50, it is noted that “strong” correlations between serum and brain BDNF concentrations have been previously observed and reported. However, the study cited to support this statement only reported weak-to-moderate correlations ($r^2 = 0.40\sim$). I therefore believe that the word “strong” should be subsequently removed. The following sentence in this paragraph is also long/fragmented, and should be revised.

R9) We modified the manuscript following the reviewer suggestion (page 6, line 1): “It is known that BDNF freely crosses the blood-brain barrier.13 In keeping with this observation, levels of BDNF in serum are correlated with CNS concentrations.14 Altered peripheral serum levels of BDNF have been shown to be a reliable biomarker for severe psychiatric disorders, including schizophrenia (SCZ).15 This corresponds to the dysregulation of BDNF, and of its related molecular pathways, observed in affected individuals.16”

Q10) On page 8, the sentence beginning on line 17 states that the prospective observational protocol being reported in the manuscript will permit assessments of “causality”. Given the design of this study, I do not believe that direct causality can be established as fluctuations in BDNF expression or clinical symptoms may be caused by uncontrolled covariates that influence both factors concomitantly but
independently. In an observational study such as this, any such effects cannot be ruled out. Revising this text would therefore benefit the paper.

R10) We agree with the reviewer and we removed any reference to causality in the manuscript.

Q11) Lastly, in a prospective study as this, it is important to establish the boundaries of this study so that clear and reasoned conclusions can be drawn but not over-generalized. I would therefore suggest including a concise limitations paragraph in the discussion so to directly acknowledge such.

R11) A limitation section has been added at the end of the Discussion (page 18, line 17): “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.”

Reviewer #2

Q1) In my opinion a study period of 18 months is too short to really thoroughly address the questions posed. In a prospective longitudinal study of this nature it would be important to allow sufficient time for the subjects to experience periods when they are symptomatic, in recovery and an adequate period of remission/stability and also for changes in treatment regime to be made, stabilised and evaluated for individual subjects within the study. One aim of the study is to assess the association with serum BDNF levels and changes in negative symptoms and cognitive impairment in subjects, changes in which occur over a much more protracted time period. It is in my view unlikely that 18 months will be sufficiently long to study these changes in a large enough proportion of the sample.

R1) This point is well taken. We are aware that longitudinal studies in schizophrenia typically last well beyond 18 months, with some cohorts followed-up for decades. However, our aim was to identify trajectories of change in BDNF levels over 18 months also in relation to psychopathological and cognitive variables. To our knowledge, most of the studies have measured BDNF levels over follow-up period of 1 year. For instance, the meta-analysis of Fernandes et al. (2014 supplementary information) shows that clinical studies exploring the effect of drug treatment (mainly antipsychotics) on BDNF had a maximum of 52 weeks of length of follow-up. Further, the meta-analysis of Szöke et al. (2008) shows that the mean time between test and retest in longitudinal studies on cognition in SCZ was 12 months, with a median of 4 months. In planning this study we needed a trade off between robustness of the design and feasibility. On the basis of the previous literature and following the suggestion of the reviewer we extended the follow-up to 24 months. Figure and Table have been modified accordingly.

References

Q2) Exclusion criteria should include major neurological disorder or previous head injury and current drug and alcohol dependence particularly given that cognitive tests are part of the methodology of the
study.
R2) We thank the reviewer for this observation. We modified the exclusion criteria as follows (page 10, line 11): “Exclusion criteria are: 1) refusal to provide consent; 2) presence of acute psychopathological symptoms; 3) presence of illness-related cognitive impairment of such severity that affects their ability to cooperate; 4) presence of major unstable medical illness; 5) severe mental retardation; 6) major neurological disorder or previous head injury; 7) current drug and alcohol dependence.”

Q3) It would be useful to include a test of premorbid IQ in the initial assessments.
R3) We thank the reviewer for this comment. The assessment of IQ is a standard procedure in our community mental health center. Therefore, patients who will be asked to participate in LABSP will have already IQ evaluation before study entry.

Q4) There was no discussion of the limitations of this study.
R4) A limitation section has been added to the revised version of the manuscript (page 18, line 17): “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.”
will result in detectable alterations in serum BDNF given that they've discussed that the effects on serum BDNF can be small and how will they account for different anti-psychotic drugs having differential effects on serum BDNF in an individual?  
3) will there be anti-psychotic naive subjects included in the sample to assess whether the introduction of anti-psychotic medication alters serum BDNF from an untreated baseline and if so how many are anticipated?  
4) Is there an assumption that 6 months of stability in psychopathological symptoms will result in stabilisation of serum BDNF levels at at baseline level so that as stated the subjects can act at their own control? What is the evidence for this and is this equivalent to a healthy control?  
5) Will the study sample contain individuals with chronic schizophrenia who have ongoing positive symptoms who are on a stable treatment regime and how do the authors anticipate this affecting the results?  
6) It seems there is potential for huge variability within the sample, will this be controlled for or adjusted for?

**VERSION 2 – AUTHOR RESPONSE**

Reviewer #2

Q1) Can the authors clarify how they are going to assess for adverse life events and does this include childhood maltreatment which is known to alter BDNF levels?  
R1) Clinical data on stressful life events, including childhood maltreatment, will be assessed through systematic review of clinical records. We added the following statement: “Finally, the systematic review of clinical record will allow us to retrospectively collect data on the presence of a previous history of stressful life events (SLE), including childhood maltreatment. We will also monitor longitudinally the eventual manifestation of SLE.”

Q2) Can the authors further define their anticipated study cohort. They say that subjects will be recruited from "patients followed-up at the community mental health centre of the University of Cagliari Health Agency, Cagliari, Italy with a diagnosis of SCZ or SAD". This would suggest that all subjects will have a pre-existing diagnosis of schizophrenia or schizoaffective disorder and will probably be on some form of psychotropic (including anti-psychotic) medication - This will affect serum BDNF levels and presumably there will be variation in pharmacological treatment regimes between subjects.  
R2) The reviewer is correct. Patients participating in the study will all have a pre-existing diagnosis as well as an ongoing psychopharmacological treatment regime, mainly based on antipsychotics. This has been now clarified in the "Methods, Participants and Recruitments" section: "Given the characteristics of the patient population followed-up at our community mental health center, we anticipate that our sample will not be comprised of drug-naïve patients and will be on a pharmacological treatment regime, mainly based on antipsychotics."  
We are fully aware that psychopharmacological treatment will affect serum BDNF levels. However, as already stated in our protocol, we will include pharmacological treatment as an independent variable in our analysis of longitudinal variation of BDNF levels. The naturalistic design of our study should not only be seen as a limitation, but rather as an added value. That is the analysis of the longitudinal trajectory of serum BD in relation to the clinical and treatment variables in real-life clinical settings will help understanding whether BDNF levels might serve as illness course biomarker. This could not be achieved in drug-naïve patients or in randomized clinical trials that are not representative of what is typically observed in clinical practice.
Q3) They state that the subjects will act as their own controls - do they hypothesise that changes in dose or anti-psychotic treatment regime will result in detectable alterations in serum BDNF given that they’ve discussed that the effects on serum BDNF can be small and how will they account for different anti-psychotic drugs having differential effects on serum BDNF in an individual?

R3) Changes in the dose of pharmacotherapy will likely happen in concomitance with psychopathological alterations. Given the available literature (Fernandes et al., 2015), it is likely that the earlier factor will be the main responsible of the possibly observable changes in BDNF levels. As detailed in the Data analysis plan, we will have a sample size with statistical power to detect associations of moderate effect size, a magnitude that we expect to observe in relation to treatment and BDNF serum levels fluctuations. Concerning the differential effects of antipsychotic drugs on serum BDNF there are no data suggesting a class-specific effect (i.e. typical versus atypical) (see for instance Green et al., 2011). Nevertheless, we will test the differential effect of antipsychotic class in our data analysis as it has been already described in the Data analysis plan. We agree that the dosage of antipsychotics can impact on BDNF serum levels (Green et al., 2011). This variable will be analyzed in our models as chlorpromazine equivalents. We added this in “Methods, Assessment of side effects” section: “Data on antipsychotics dosages will be collected and converted to chlorpromazine equivalents for data analysis.”

Q4) Will there be anti-psychotic naive subjects included in the sample to assess whether the introduction of anti-psychotic medication alters serum BDNF from an untreated baseline and if so how many are anticipated?

R4) There will be no drug-naïve patients included in the study, as this is not a population of individuals monitored in our community mental-health center. This has been clarified in the text. Please see R2.

Q5) Is there an assumption that 6 months of stability in psychopathological symptoms will result in stabilisation of serum BDNF levels at baseline so that as stated the subjects can act at their own control? What is the evidence for this and is this equivalent to a healthy control?

R5) We are not assuming that 6 months of stability in psychopathological symptoms will result in stabilization of serum BDNF levels. Simply, since BDNF levels are influenced by many factors, and, most significantly, by antipsychotic treatments (Fernandes et al., 2015) the absence of psychopathological fluctuations should correspond to a stable treatment regime and ultimately in a decreased variability in serum BDNF.

Q6) Will the study sample contain individuals with chronic schizophrenia who have ongoing positive symptoms who are on a stable treatment regime and how do the authors anticipate this affecting the results?

R6) We do not anticipate to have chronic schizophrenic patients with ongoing positive symptoms as recruited patients will have to be clinically stable (See Inclusion and Exclusion criteria).

Q7) It seems there is potential for huge variability within the sample, will this be controlled for or adjusted for?

R7) We certainly expect variability as it would be in any naturalistic study. However, we would like to stress that all the patients will be recruited only at one clinical center, ensuring uniformity of assessment and reliability of follow-up measures. In addition, covariates will be included in mixed-effects linear regression models, which, given the not negligible sample size, should be quite robust in handling variability.

Reference