Author’s response to reviews

Title: Clozapine and desmethylclozapine: correlation with neutrophils and leucocytes counting in Mexican patients with schizophrenia

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Reviewer reports:

Emilio Fernández-Egea (Reviewer 1): Dear Editor in chief of BMC

Many thanks for asking me to review this manuscript. I apology for the delay. This work is a cross-sectional analysis of 41 clozapine treated patients. They found an significant correlation between clozapine (and norclozapine) plasma levels and the white cells counts. Author suggest that this could help preventing episodes of fatal agranulocytosis. I think the study has few major issues to address before considering this conclusions as firm.

Issues:

1- It is unclear the goal of the study. If the goal is to help detecting agranulocytosis, perhaps is simpler to check white cells rather than checking clozapine plasma levels as risk factor? If author are suggesting high plasma levels could predict future agranulocytosis, then a longitudinal study is needed.

OUR ANSWER
Properly, the design of our study is a repeated cross-sectional study, as the measurements were obtained at different times after CLZ administration in different patients. Even when it was not a longitudinal study, this design allowed us to perform an evaluation the impact of the time on cells’ counting. This is now stated in the methods section.

2- Poor control of potential confounders:

a. Patients are at different stages of the clozapine treatment (some within first month of treatment, other after three years). For instance, it is know a decrease in white cells counts after 8 weeks on treatment.

OUR ANSWER

The study was designed to know the possible impact of time as a variable in the multiple linear regression. In fact, the effect of CLZ and DMC are already corrected by time taking the medication, as they were included as covariates. We have added a text in the results section to emphasize this effect.

b. There is no information regarding other medications. This is relevant as might influence plasma levels and white cells counts.

ANSWER.

Thanks for your suggestion, we have added a table (table 3) with the information about concomitant medications.

3- Intro: I found it perhaps excessively long and not really focused on the study goals. I would benefit of greater concision, use of better terms (extrapyramidal disorder or symptoms?) and precision (TRS is considered after one or two antipsychotic resistance?)

OUR ANSWER:

Introduction was shortened and the terms corrected, as suggested by the reviewer.

4- Discussion:

a. The limitation section should be extended.

Our Answer: We added a paragraph to the methods section to clarify the limitations of the study.

b. Clozapine metabolism is mainly through 1A2.
In this regard, the metabolic transformation of CLZ to produce reactive oxygen species and nitrenium ion, is performed by NADPH oxidase/myeloperoxidase system, and CYP3A4, CYP2D6 and CYP1A2 mainly [19]

OUR ANSWER: We added the missing CYP1A2 to the text.

Anssi Solismaa (Reviewer 2): Authors present an interesting study investigating the correlation between clozapine/n-desmethylclozapine and neutrophil/neutrophil counts and examining the possibility of using drug concentrations as biomarkers for safer use of clozapine and possibly preventing agranulocytosis.

Background:

-The definition of TRS has varied widely in the literature. A recent consensus guideline (Howes et al. 2017) suggested a definition that includes that at least two antipsychotics have been tried, not one as is written here.

OUR ANSWER: Sorry, this was a mistake, now corrected.

“Treatment-resistant schizophrenia (TRS) is defined in clinical guidelines as a situation in which a significant improvement in psychopathology has not been demonstrated despite two or more different antipsychotic treatment trials, each with adequate dose and duration [3].”

-It cannot be said that clozapine causes lower side effects than other antipsychotics, constipation, sedation, metabolic adverse effects, sialorrhea for example are common, only the extrapyramidal adverse effects are lower

OUR ANSWER: You are right, we changed the sentence to:

“The CLZ causes lower extrapyramidal symptoms from the side effects caused by neuroleptic drugs, mainly tardive dyskinesia [6]”

-The stated incidence of agranulocytosis seems high (1 to 2%), Honigfeld et al. 1998 report 0.38% incidence with 0.012% mortality. Agranulocytosis should not be confused with neutropenia.

ANSWER: Again, you are right, we changed the text, as follows:

“However, the use of CLZ has been associated to agranulocytosis reported in 0.38% (995 cases) of treated patients, 12 deaths attributed to complications of agranulocytosis [7]”
Authors refer that some studies have shown that CLZ and DMC may be related to the neutrophil counts in patients. More information about these studies is needed as they are essential background information on the subject, the references are missing.

Our answer: We added one more reference and a new paragraph at the end of the discussion section, regarding the relationship between neutrophils counts and CLZ and DMC metabolism, as suggested by the reviewer.

Methods:

-The purpose of reanalyzing the samples after 90 days is unclear and not mentioned in the aims of the study

OUR ANSWER: This re-analysis was performed only during the validation of the method, in order to know if samples are enough stable to be maintained frozen over that time period. We deleted the table in order to clarify the text, as the validation of the analytical method is not the aim of the study.

Results:

-Tables 2 and 3 should be clarified. What does the nominal concentration mean? Abbreviations CV and RE should be opened. A correlation graph between CLZ plasma levels and neutrophils/leukocytes could be informative.

OUR ANSWER: We deleted table 3. Figures showing the relationships between neutrophils counts and CLZ, DMC and time taking CLZ are now figures 2 and 3.

-Author states that the time taking the drug was associated with neutrophil and leukocyte count, these results should be opened and discussed, how and why did the taking time affect the neutrophil/leukocyte count?

OUR ANSWER: Time taking CLZ is also a proxy of the cumulative dose of the drug administered to each patient. This is now stated in the methods section.

Discussion:

-The magnitude of variance in neutrophil/leukocyte levels in relation to CLZ concentrations should be addressed.

OUR ANSWER: The multiple regression analyses applied to data explain 45 and 46 % of the variance of neutrophils and leucocyte countings. Therefore, most of the variability of both countings can be explained by two variables: plasma CLZ and time taking CLZ, both, in turn,
related to CLZ dosing and metabolism. We added a new paragraph in the results section remarking those percentages of variance explanation.

-The found correlation is interesting and it supports the idea of CLZ being toxic to neutrophils. The authors suggest that drug monitoring of CLZ may help in detecting a decrease in neutrophil count, but I do not see how this would help in practise, as the neutrophil counts are regularly monitored in any case, and CLZ/DMC concentrations are monitored mainly for achieving optimal drug response, for drug safety (avoiding adverse effects, especially seizures which are dose/concentration dependent) and for assessing adherence to clozapine treatment.

OUR ANSWER: Thank you for the comment. We modified the discussion section as suggested by the reviewer. The drug monitoring of CLZ/DMC concentrations are monitored mainly for achieving optimal drug response, for drug safety and to assess compliance to clozapine treatment. This is now stated in the discussion section.

-Sample size is relatively low, and the study might have benefited from multiple concentration measurements on the same dose on different days.

OUR ANSWER: Yes, that is a more fruitful approach, but it is also technically more difficult to perform, as patients may refuse to participate in a longitudinal study that should last for months in order to observe changes in cells’ countings. We are now starting a second study, with more patients, including other markers of CLZ toxicity.

Takashi Kanbayashi (Reviewer 3): Clozapine is a useful compound for the treatment resistant schizophrenia.

In this study, authors measured plasma clozapine and N-desmethyl clozapine and found the correlation between neutrophils and leucocytes. Compared to animal study, plasma clozapine had better correlations between neutrophils and leucocytes.

This study is important for the detecting these side effects and monitoring them.

However, the authors should mention about several studies that did not find the similar results (plasma concentrations were not correlated side effects) and discuss about the reasons.

OUR ANSWER: We have added 2 references to studies not finding a correlation between plasma CLZ and cells ‘counting, as suggested by the reviewer. We also added a paragraph to the discussion section, as follows:

“Two factors may explain the differences between the results presented here and previous studies exploring the subject: 1) Methodological differences, as some studies are case reports of patients with agranulocytosis or longitudinal short-term studies. 2) Obvious differences in populations’ genetics and a higher percentage of smokers…”
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