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Article type : Systematic Review or Meta-analysis

Impact of smoking behavior on clozapine blood levels – a systematic review and meta-analysis

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Word Count Abstract: 249
Word Count Manuscript (main text): 2709
Tables: 1
Figures: 2

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This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ACPS.13228

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Abstract

Objective: Tobacco smoking significantly impacts clozapine blood levels and has substantial implications on individual efficacy and safety outcomes. By investigating differences in clozapine blood levels in smoking and non-smoking patients on clozapine, we aim to provide guidance for clinicians how to adjust clozapine levels for patients on clozapine who change their smoking habits.

Methods: We conducted a meta-analysis on clozapine blood levels, norclozapine levels, norclozapine/clozapine ratios and concentration to dose (C/D) ratios in smokers and non-smokers on clozapine. Data were meta-analysed using a random-effects model with sensitivity analyses on dose, ethnic origin and study quality.

Results: Data from 23 studies were included in this meta-analysis with 21 investigating differences between clozapine blood levels of smokers and non-smokers. In total, data from 7125 samples were included for the primary outcome (clozapine blood levels in ng/ml) in this meta-analysis. A meta-analysis of all between-subject studies (N=16) found that clozapine blood levels were significantly lower in smokers compared to non-smokers (Standard Mean Difference (SMD) -0.39, 95% confidence interval (CI) -0.55 to -0.22, $p<0.001$, $I^2=80\%$). With regard to the secondary outcome, C/D ratios (N=16 studies) were significantly lower in the smoker-group (n=645) compared to the non-smoker-group (n=813) (SMD -0.70, 95%CI -0.84 to -0.56, $p<0.00001$, $I^2=17\%$).

Conclusion: Smoking behavior and any change in smoking behavior is associated with a substantial effect on clozapine blood levels. Reductions of clozapine dose of 30% are recommended when a patient on clozapine stops smoking. Reductions should be informed by clozapine steady-state trough levels and a close clinical risk-benefit evaluation.

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Key Words: schizophrenia, clozapine, meta-analysis, smoking;

Summations:

- Smoking behavior significantly reduces clozapine blood levels
- When a patient stops smoking, reductions of clozapine dose of 30% are recommended

Considerations:

- Besides smoking behavior, other factors impacting clozapine blood levels (e.g. age, gender, comedication with clinically relevant CYP1A2-interaction, caffeine intake, Cytochrome P-polymorphisms, ethnic origin) should be accounted for in future studies and, more importantly, measurement of clozapine blood levels has to be implemented in future studies to provide further evidence for a safe and efficacious use of clozapine

1. Introduction

Clozapine is the most effective antipsychotic agent for treatment-resistant schizophrenia (TRS) (1, 2). Clozapine is one of the few antipsychotics where therapeutic level monitoring is used, with levels above the maximum threshold (1000 ng/ml) being associated with an increased risk of seizures (3). In clinical practice, pseudo-resistance to clozapine may occur as a result of blood levels below the minimum threshold definition (350 ng/ml) and there is an association between clozapine blood levels and response (4). Cytochrome P450 (CYP) 1A2 (CYP1A2) is the major clozapine metabolic enzyme and is responsible for approximately 70% of clozapine's metabolism (5). Clozapine is metabolized to its primary metabolite norclozapine (5). In this regard, polycyclic aromatic hydrocarbons generated by tobacco smoking induce the activity of CYP1A2 (6) which leads to increased clozapine metabolism.

Rates of smoking is up to five times higher among people with schizophrenia compared to the general population, with smoking rates among people with schizophrenia as high as 60% (7). A daily consumption of 7-12 cigarettes may be sufficient for maximum induction of clozapine metabolism (8). Beginning smoking is therefore a clinically relevant risk for relapse and inadequate response to clozapine treatment (9) and smoking cessation among clozapine users can induce severe clozapine intoxication (10). Ethnicity (Asian heritage) (11, 12), gender (13), age

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(13-15), CYP-polymorphisms (16), caffeine (17), and comedication with clinically relevant CYP1A2-interaction (9) can also influence clozapine blood levels through the CYP-450 system. In this context, e.g. people of Asian heritage are presumed to need a lesser clozapine dose compared to Caucasian or American populations (12, 18) which might be due to a relatively reduced CYP1A2 activity (11). On a similar note, the clinical relevance of CYP1A2 can be observed in smoking patients after transition to electronic cigarettes, where the termination of CYP1A2 induction also induces a clinically relevant increase in clozapine blood levels (19).

Concentration to dose (C/D) ratio is a measure of clozapine clearance and higher ratios - indicating lower clearance - are associated with females, non-smokers, Asians, genetic poor metabolizers, CYP-inhibitors, obesity, inflammation and possibly with renal impairment and pregnancy (18), whereas lower ratios indicate lack of adherence or are associated with males, smokers, non-Asians, and CYP-inducers (18).

In summary, there is a lack of clarity in the available literature as to how great an influence changes in smoking habit can have on clozapine blood levels. To guide clinical care of people treated with clozapine, and to assist in averting sub- or supra-therapeutic clozapine levels and their associated deleterious effects, we conducted a systematic review and meta-analysis of the impact on changes in smoking habit on clozapine blood levels among people on clozapine. Our primary outcome of interest was impact on clozapine blood levels, with secondary outcomes of impact on clozapine to C/D ratios, norclozapine/clozapine ratios, and norclozapine levels. We planned sensitivity analyses and meta-regression analyses on other factors which influence clozapine metabolism including ethnicity, age and gender, to assist in clarifying the role of potential confounders, if any.

Subtitle:

To investigate the impact of smoking behavior on clozapine blood levels and clozapine concentration to dose ratios in order to provide guidance for clinicians how to manage patients on clozapine who smoke or stop smoking.

Data Availability Statement:

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The data that support the findings of this study are available from the corresponding author upon reasonable request.

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2. Methods

The methods are based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (20). This study was registered with PROSPERO (registration number CRD42020185579), an international database of prospectively registered systematic reviews.

Searches

Systematic searches were conducted of publications indexed in PubMed, EMBASE and PsycINFO using the search terms (level OR levels OR concentration OR concentrations OR ratio OR ratios) AND (blood OR serum OR plasma) AND (clozapin* OR clozaril OR zaponex OR denzapin* OR clopine OR norclozapine OR desmethylclozapine). The abstracts and titles of articles identified through electronic searches were independently screened by two reviewers (EW, LM). Publications in all languages were considered for inclusion.

Inclusion criteria

Studies were included if they reported information on mean (and SD) of clozapine blood levels OR C/D ratios for people with psychiatric disorders who were smoking and not smoking in cohort studies, case-control studies or randomized or non-randomized controlled trials.

Exclusion criteria

Case reports and case series were excluded.

Assessment of reporting strength

As all studies suitable for inclusion were observational studies, we used a modified Newcastle-Ottawa Quality Assessment Scale (21) for assessment of quality. The maximum score was 5 and studies with score of at least 3 were rated as high-quality studies. We considered the following domains: representativeness of the sample, sample size, comparability between smokers and non-smokers, ascertainment of clozapine blood levels (outcome) and quality of descriptive statistics

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(quality scores are provided in Supplement A Table 2, modified Newcastle-Ottawa Quality Assessment Scale is provided in Supplement B).

Data extraction

Two reviewers (EW, LM) independently extracted the data into an electronic spreadsheet and disagreements were resolved by joint examination of the papers. The following data were extracted:

1. Sample size of subjects on clozapine (smokers and non-smokers)
2. Mean (and SD) clozapine blood levels in both groups (ng/ml)
3. Mean (and SD) clozapine dose in both groups (in mg/day)
4. Type of comparison (within-subject or between-subject comparisons between the two groups)

The following characteristics of each study were recorded where possible and if available, both for male and female participants in both groups separately:

1. Mean age of subjects in both groups
2. Ratio male:female in both groups
3. Clozapine blood levels and clozapine doses for male and female participants
4. Whether data collection was prospective or retrospective
5. Inpatient or outpatient status of subjects
6. Amount of smoking in the smoking group
7. Concentration to dose (C/D) ratio in both groups
8. Norclozapine levels in both groups
9. Norclozapine to clozapine ratios in both groups

Data synthesis and analysis

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The primary outcome was the clozapine blood level (in ng/ml) in the smoker and non-smoker group. Where adequate quantitative data was not reported, corresponding authors were contacted to provide means and SDs. Where confidence intervals were reported, these were converted to SD using the Cochrane Handbook formula (17). Included studies were divided into between-subject studies and within-subject studies. Between-subject studies compared data from subjects on clozapine divided into smokers and non-smokers, whereas within-subject studies investigated effects within the same individuals as smokers and then non-smokers. Meta-analyses were conducted using RevMan (Version 5.3) and meta-regression analyses were conducted using Comprehensive Meta-Analysis (Version 3.3). We assessed heterogeneity using the I^2 statistic, a measure that does not depend on the number of studies in the meta-analysis and hence has greater power to detect heterogeneity when the number of studies is small. I^2 provides an estimate of the percentage of variability due to heterogeneity rather than chance alone. An estimate of 50% or greater indicates possible heterogeneity, and scores of 75–100% indicate considerable heterogeneity. Given the observational nature of primary studies and expected high rates of heterogeneity, a random effects model was used for all the analyses. A significance level of $\alpha < 0.05$ was applied for all analyses.

Subgroup and sensitivity analyses

Subgroup analyses were undertaken for between-subject studies for high-quality (as defined by ≥3 out of 5 points on the modified Newcastle Ottawa Scale) vs. low-quality studies, male populations, female populations and studies with people from Asian origin vs. studies with people not from Asian origin. Meta-regression analyses were performed for between-subject studies for clozapine blood levels and the moderators 1) difference in clozapine dose between smokers and non-smokers 3) proportion of male smokers and 4) proportion of male non-smokers 5) age (smokers) and 6) age (non-smokers).

Publication bias

Where meta-analyses included at least 10 studies, publication bias was tested using funnel plot asymmetry with Kendall's Tau, where low p-values suggest publication bias. A threshold of 10 for

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funnel plots was chosen in accordance with the recommendations from the Cochrane Handbook (22).

3. Results

In total, 7600 articles were independently screened on title/abstract level and 113 articles on full-text level by two reviewers. A total of 90 articles were excluded at full text review (see Supplement A Table 1 for list of excluded studies with reasons), with 23 articles (8, 16, 23-43) included in the meta-analysis (see Fig. 1, List of included studies see Table 1). Twenty-one articles (8, 16, 23-31, 34-43) investigated differences between smokers and non-smokers as between-subject analyses and two studies (32, 33) compared differences as within-subject analyses change of blood levels among smokers after institutional smoking ban). In total, data from 7125 samples were included for the primary outcome (clozapine blood levels in ng/ml) in this meta-analysis. Of these, 4925 were from smokers and 2185 from non-smokers (N=21, between-subject analyses) and 15 were smokers and subsequent non-smokers (N=2, within-subject analyses). 18 out of 23 studies were performed exclusively in schizophrenia-spectrum populations, with the remaining five additionally including (a small amount of) patients with bipolar disorder (26, 33), unspecified psychiatric disorders (38, 43) and psychiatric disorders except organic disorders (27).

Between-subject analyses

a) Clozapine blood levels

16 studies (8, 23, 25-31, 34, 35, 37-39, 41, 42) reported on blood levels for samples of smokers (n=4925) and non-smokers (n=2185). Overall, study quality was moderate, with 6/16 rated as high-quality (23, 26, 31, 35, 39, 41) and 10 as low/moderate-quality according to modified Newcastle-Ottawa Rating Scale (see Supplementary File B). Four studies were retrospective (23, 26, 27, 41) and 12 prospective (8, 25, 28-31, 34, 37-39, 42). All except three studies (26, 27, 38) were exclusively covering people with schizophrenia-spectrum disorders. From these 16 studies, four (28-30, 41) were restricted to people of Asian origin.

A meta-analysis of all between-subject studies (N=16) found that clozapine blood levels were statistically significantly lower by more than a third in smokers compared to non-smokers

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(Standard Mean Difference (SMD) -0.39, 95% confidence interval (CI) -0.55 to -0.22, p<0.00001, I^2=80%) (see Figure 2). On sensitivity analyses, when restricted to the six high-quality studies (n=4393 samples in the smoker-group and n=1583 samples in the non-smoker group), results remained significant (SMD -0.29, 95%CI -0.51 to -0.07, p<0.009) (see Supplement A Figure 1). Neither the difference between clozapine dose for smokers or non-smokers (Q=0.04, df=1, p=0. 848) nor age of smokers or non-smokers significantly impacted the effect size in a meta-regression (Q=0.36, df=2, p=0.834).

Nevertheless, a higher proportion of male participants among smokers and non-smokers had increased the effect size (Q=18, df=2, p=0.0001). Scatterplots for moderator variables are displayed in the Supplement A Fig. 19-25. There was no evidence of publication bias (τ=-0.05, p=0.77, see Supplement A Table 3).

When restricted to the five studies conducted among people with Asian origin, results were not significant (SMD -0.43, 95% CI -0.97 to 0.11, p=0.12). However, there was one outlier study (41) from Asia where levels in the smoker-group were reported to be higher than in the non-smoker group (see Supplement A Fig. 2). When this study was removed (N=4 studies, 75 smokers vs. 127 non-smokers), results were significant (SMD -0.63, 95%CI -1.05 to -0.21, p=0.003). When the analysis was restricted to non-Asian studies, results were also significant (SMD -0.39, 95%CI -0.58 to -0.21, p<0.0001) (see Supplement A Fig. 3).

There was insufficient relevant data to conduct sensitivity analysis or meta-regression for gender.

b) clozapine dose

Sixteen studies reported on associated clozapine doses (in mg per day) for samples of smokers (n=4925) and non-smokers (n=2185). Doses were significantly higher in the smoker-group compared to the non-smoker group (SMD 0.22, 95%CI 0.07 to 0.36, p<0.003) with high between-sample heterogeneity (p<0.00001, I^2=71%) (see Supplement A Fig. 4). When analyses were restricted to the six high-quality studies, results were not significant (SMD 0.14, 95%CI -0.03 to 0.31, p=0. 11) between samples of smokers (n=4393) and non-smokers (n=1583) (see Supplement A Fig. 5). When restricted to studies among people with Asian origin (N=4), doses were significantly higher in smokers (n=125) compared to non-smokers (n=218) (SMD 0.33, 95%CI 0.01 to 0.65, p=0.04) (see Supplement A Fig. 6). When restricted to non-Asian studies, results

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were similar for associated doses between samples of smokers (n=4800) and non-smokers (n=1967) (SMD 0.18, 95%CI 0.03 to 0.33, p=0.02) (see Supplement A Fig. 7). There was not enough data for additional analyses (e.g. male vs. female comparisons).

c) norclozapine/clozapine ratio

Five studies (23, 24, 28, 30, 35) reported on norclozapine/clozapine ratios for samples of smokers (n=4232) and non-smokers (n=1460), with no significant difference in norclozapine/clozapine ratios between the two groups (SMD -0.00, 95%CI -2.04 to 2.03, p=1.00) (see Supplement A Fig. 8). There was not enough data for additional analyses.

d) norclozapine blood levels

Seven studies (23, 28-30, 34, 35, 38) reported on norclozapine blood levels for samples of smokers (n=4321) and non-smokers (n=1643). There was no significant difference between the two groups (SMD -0.08, 95%CI -0.19 to 0.04, p=0.18) with low/moderate heterogeneity (p=0.12, I^2=39%) (see Supplement A Fig. 9). When restricted to five non-Asian studies, results remained non-significant between samples of smokers (n=4263) and non-smokers (n=1512) (SMD -0.05, 95%CI -0.16 to 0.07, p=0.44) (see Supplement A Fig. 10). There was not enough data for additional analyses.

e) C/D ratio

Eight studies (8, 16, 23, 24, 27, 36, 40, 43) reported on C/D ratios for smokers (n=645) and non-smokers (n=813). C/D ratios were statistically significantly lower in the smoker-group (SMD -0.70, 95%CI -0.84 to -0.56, p<0.00001) with low between-sample heterogeneity (p=0.28, I^2=17%) (see Supplement A Fig. 11). In a sensitivity analysis restricted to four high-quality samples, results remained significant between smokers (n=140) and non-smokers (n=349) (SMD -0.58, 95%CI -0.83 to -0.34, p<0.00001) (see Supplement A Fig. 12). In a sensitivity analysis restricted to five samples of people from Asian origin from one study (36) with n=138 smokers and n=432 non-smokers, results remained significant (SMD -0.59, 95%CI -0.79 to -0.39, p<0.00001) with low between-sample heterogeneity (p=0.39, I^2=2%) (see Supplement A Fig. 13). When restricted to

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seven non-Asian studies, results remained significant between smokers (n=507) and non-smokers (n=381) (SMD -0.78, 95%CI -0.97 to -0.59, p<0.00001) (see Supplement A Fig. 14). There was not enough data for additional analyses (e.g. male vs. female comparisons).

Within-subject analyses

Clozapine blood levels

A meta-analysis of two within-subject studies (32, 33) found significantly increased levels after transition from smoking status (n=15) to non-smoking status (n=15) (SMD -0.84, 95%CI -1.60 to -0.08, p=0.03) (see Supplement A Fig. 15). There was insufficient data for further analyses.

4. Discussion

This meta-analysis from a total of N=23 studies comprising more than 7000 subjects with psychiatric disorders represents the most comprehensive analysis on the relationship of clozapine levels/ratios and smoking to date. We found that clozapine blood levels are reduced by around a third in smokers compared to non-smokers. Our analysis is the first to comprehensively combine and quantify published data on the impact of smoking on clozapine levels. For patients on clozapine who smoke and subsequently quit smoking, dosages should be decreased by 30% and clozapine blood level analyses should be performed. It is important to note that the CYP1A2 activity decrease may be a gradual process over the first three to four days after smoking cessation (44). Conversely, if patients start to smoke clozapine blood levels may fall by 30% resulting in the need to increase the dose and to monitor blood levels. In this regard clinical signs of clozapine underdosing, such as anxiety, restlessness and sleep disturbances must be monitored in patients who commence smoking.

In our analyses clozapine blood levels were significantly lower in smokers. However, there may be unaccounted for confounding factors. Co-occurring caffeine intake might have increased clozapine blood levels due to CYP1A2 inhibition as previously observed in the literature (17). Smoking and caffeine use may co-occur among people with schizophrenia (45). Thus, differences in blood levels between smokers and non-smokers might have been underestimated since none of the included studies controlled for caffeine consumption. We were not able to conduct sensitivity

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analyses for caffeine as this data was not reported. We were only able to include information on other reported relevant CYP-450 interacting comedication.

C/D ratios were significantly lower in smokers in our analyses and our results remained significant when analyses were restricted to high-quality studies or studies from Asian origin. This allows for adjustment for certain factors associated with CYP activity such as Asian genetic heritage. However, impact of other factors associated with influence on C/D ratios (e.g. gender, poor metabolizer, obesity, inflammation) were not able to be controlled for in our analyses. Furthermore, we were able to undertake meta-regressions examining the impact of dose, age and gender on clozapine blood levels between smokers and non-smokers. Our findings regarding the influence of gender on clozapine blood levels with smoking should be treated with caution, since only three studies reported blood levels (33, 35, 39), one reported C/D ratios (36) between smokers and non-smokers for male and female populations separately and one study only included only one gender (41). In one of the studies which disaggregated data by gender and smoking status, only 14% of men were non-smokers (35), which may have skewed the results. Between-subject analyses may be biased since the included studies, with the exception of Lu et al. (30), were not fixed-dose studies and doses in smokers might have been increased by the treating clinicians. Thus, the true difference induced by smoking might be bigger than estimated, as suggested by within-subject analyses based on limited subject numbers. Furthermore, one study from Rostami et al. reported samples of smokers and non-smokers, and thus various samples might be derived from the same patients increasing the risk of bias.

Our analyses had a high degree of heterogeneity, and as such our results should be viewed with caution. We were able to conduct sensitivity analyses and meta-regression on dose, gender, ethnicity and age. There was insufficient data to permit meta-regression for comedications, smoking quantity, genetics for fast metabolizer, caffeine use or body weight, since none of the included studies reported these confounders for smokers and non-smokers homogenously allowing for meta-analyses. Nevertheless, comedication was considered as an item in our Assessment of reporting strength and thus high-quality studies excluded clinically relevant CYP1A2 interacting comedication. Furthermore, seven out of our included studies were retrospective analyses which may be more prone to bias than prospective approaches, especially since data may not be available on medication adherence and ascertainment of smoking behavior is less certain. We attempted to address this through our sensitivity analysis of study quality.

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Ours is the largest meta-analysis on clozapine blood levels and C/D ratios from a total of four studies (46). In contrast to the work from Tsuda et al., we included studies of patients of Asian heritage, assessed risk of bias and conducted sensitivity analyses and meta-regression analyses, if possible. Based on their meta-analysis on C/D ratios, Tsuda et al. estimated that if 200 and 400mg per day of clozapine would be administered to smokers, about 100 and 200mg per day, respectively, should be administered to non-smokers, based on a SMD C/D ratio of 1.1 (46). In our analyses, SMD of C/D ratio between smokers and non-smokers (0.7 ng/ml per mg/day) was lower than in the one from Tsuda et al. suggesting an estimated 30% reduction of dose after a patient stops smoking.

Conclusion

Our meta-analysis confirms that smoking behavior and any change in smoking behavior is associated with substantial clinical implications for patients on clozapine and extends the current knowledge by providing an evidence-based quantification of these effects. According to our analyses, reductions of clozapine dose by 30% are recommended when a patient on clozapine stops smoking. Nevertheless, reductions have to be performed with TDM of clozapine steady-state trough levels and a clinical risk-benefit evaluation since high variability between individuals (95% CI in the range of -0.55 to -0.22 in our analyses) has to be expected. Dose reductions should be combined with instruction of patient and nurses (for signs of intoxication and relapse) and a close monitoring of clozapine blood levels.

Acknowledgements: None.

Contributors: EW, DS, AH, and PF conceptualized the present analysis. EW and LM conducted the literature search with validation by DS and AH. The analyses were conducted by AH and DS.

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EW and DS wrote the first draft of the manuscript. All authors have contributed to editing subsequent drafts and have approved the final manuscript.

Funding: None.

Disclosures: E. Wagner reports no conflicts of interest. L. McMahon reports no conflict of interest. P. Falkai was honorary speaker for Janssen-Cilag, Astra-Zeneca, Eli Lilly, Bristol Myers-Squibb, Lundbeck, Pfizer, Bayer Vital, SmithKline Beecham, Wyeth, and Essex. During the last 5 years he was a member of the advisory boards of Janssen-Cilag, AstraZeneca, Eli Lilly, and Lundbeck. Presently, he is a member of the advisory boards of Richter Pharma, Abbot and Otsuka. A. Hasan has been invited to scientific meetings by Lundbeck, Janssen-Cilag, and Pfizer, and he received paid speakerships from Desitin, Janssen-Cilag, Otsuka and Lundbeck. He was member of Roche, Otsuka, Lundbeck and Janssen-Cilag advisory boards. D. Siskind reports no conflict of interest.

References

1. SISKIND D, MCCARTNEY L, GOLDSCHLAGER R, KISELY S. Clozapine v. first-and second-generation antipsychotics in treatment-refractory schizophrenia: systematic review and meta-analysis. The British Journal of Psychiatry. 2016;209:385-92.

2. LAND R, SISKIND D, MCARDLE P, KISELY S, WINCKEL K, HOLLINGWORTH SA. The impact of clozapine on hospital use: a systematic review and meta-analysis. Acta Psychiatrica Scandinavica. 2017;135:296-309.

3. VARMA S, BISHARA D, BESAG FM, TAYLOR D. Clozapine-related EEG changes and seizures: dose and plasma-level relationships. Therapeutic advances in psychopharmacology. 2011 Apr;1:47-66.

4. HOWES OD, MCCUTCHEON R, AGID O, et al. Treatment-Resistant Schizophrenia: Treatment Response and Resistance in Psychosis (TRRIP) Working Group Consensus Guidelines on Diagnosis and Terminology. The American journal of psychiatry. 2017 Mar 1;174:216-29.

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5. BERTILSSON L, CARRILLO JA, DAHL ML, et al. Clozapine disposition covaries with CYP1A2 activity determined by a caffeine test. British journal of clinical pharmacology. 1994 Nov;38:471-3.

6. HUKKANEN J, JACOB P, 3RD, PENG M, DEMPSEY D, BENOWITZ NL. Effect of nicotine on cytochrome P450 1A2 activity. British journal of clinical pharmacology. 2011 Nov;72:836-8.

7. DE LEON J, DIAZ FJ. A meta-analysis of worldwide studies demonstrates an association between schizophrenia and tobacco smoking behaviors. Schizophrenia research. 2005 Jul 15;76:135-57.

8. HASLEMO T, EIKESETH PH, TANUM L, MOLDEN E, REFSUM H. The effect of variable cigarette consumption on the interaction with clozapine and olanzapine. Eur J Clin Pharmacol. 2006 Dec;62:1049-53.

9. RAJKUMAR AP, POONKUZHALI B, KURUVILLA A, JACOB M, JACOB KS. Clinical predictors of serum clozapine levels in patients with treatment-resistant schizophrenia. International clinical psychopharmacology. 2013 Jan;28:50-6.

10. CORMAC I, BROWN A, CREASEY S, FERRITER M, HUCKSTEP B. A retrospective evaluation of the impact of total smoking cessation on psychiatric inpatients taking clozapine. Acta Psychiatr Scand. 2010 May;121:393-7.

11. GHOTBI R, CHRISTENSEN M, ROH HK, INGELMAN-SUNDBERG M, AKLILLU E, BERTILSSON L. Comparisons of CYP1A2 genetic polymorphisms, enzyme activity and the genotype-phenotype relationship in Swedes and Koreans. Eur J Clin Pharmacol. 2007 Jun;63:537-46.

12. SUHAS S, KUMAR V, DAMODHARAN D, et al. Do Indian patients with schizophrenia need half the recommended clozapine dose to achieve therapeutic serum level? An exploratory study. Schizophrenia research. 2020 Jun 6.

13. LANE HY, CHANG YC, CHANG WH, LIN SK, TSENG YT, JANN MW. Effects of gender and age on plasma levels of clozapine and its metabolites: analyzed by critical statistics. The Journal of clinical psychiatry. 1999 Jan;60:36-40.

14. ULRICH S, BAUMANN B, WOLF R, et al. Therapeutic drug monitoring of clozapine and relapse--a retrospective study of routine clinical data. International journal of clinical pharmacology and therapeutics. 2003 Jan;41:3-13.

15. BOWSKILL S, COUCHMAN L, MACCABE JH, FLANAGAN RJ. Plasma clozapine and norclozapine in relation to prescribed dose and other factors in patients aged 65 years and over: data from a therapeutic drug monitoring service, 1996-2010. Human psychopharmacology. 2012 May;27:277-83.

16. VAN DER WEIDE J, STEIJNS LS, VAN WEELDEN MJ. The effect of smoking and cytochrome P450 CYP1A2 genetic polymorphism on clozapine clearance and dose requirement. Pharmacogenetics. Mar;13:169-72.

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17. RAASKA K, RAITASUO V, LAITILA J, NEUVONEN PJ. Effect of caffeine-containing versus decaffeinated coffee on serum clozapine concentrations in hospitalised patients. Basic & clinical pharmacology & toxicology. 2004 Jan;94:13-8.

18. DE LEON J, RUAN CJ, SCHORETSANITIS G, DE LAS CUEVAS C. A Rational Use of Clozapine Based on Adverse Drug Reactions, Pharmacokinetics, and Clinical Pharmacopsychology. Psychotherapy and psychosomatics. 2020 Apr 14:1-15.

19. KHORASSANI F, KAUFMAN M, LOPEZ LV. Supatherapeutic Serum Clozapine Concentration After Transition From Traditional to Electronic Cigarettes. Journal of clinical psychopharmacology. 2018 Aug;38:391-2.

20. MOHER D, LIBERATI A, TETZLAFF J, ALTMAN DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. Journal of clinical epidemiology. 2009 Oct;62:1006-12.

21. STANG A. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. European journal of epidemiology. 2010 Sep;25:603-5.

22. HIGGINS JPT TJ, CHANDLER J, CUMPSTON M, LI T, PAGE MJ, WELCH VA (EDITORS). Cochrane Handbook for Systematic Reviews of Interventions version 6.0 (updated July 2019). Cochrane; 2011.

23. AUGUSTIN M, SCHORETSANITIS G, PFEIFER P, GRUNDER G, LIEBE C, PAULZEN M. Effect of fluvoxamine augmentation and smoking on clozapine serum concentrations. Schizophrenia research. 2019 Aug;210:143-8.

24. DETTLING M, SACHSE C, BROCKMOLLER J, et al. Long-term therapeutic drug monitoring of clozapine and metabolites in psychiatric in- and outpatients. Psychopharmacology (Berl). 2000 Sep;152:80-6.

25. GONZALEZ-ESQUIVEL DF, CASTRO N, RAMIREZ-BERMUDEZ J, et al. Plasma levels of clozapine and norclozapine in Mexican schizophrenia patients. Arzneimittelforschung. 2011;61:335-9.

26. HARING C, FLEISCHHACKER W, SCHETT P, HUMPEL C, BARNAS C, SARIA A. Influence of patient-related variables on clozapine plasma levels. The American journal of psychiatry. 1990;147:1471-5.

27. KUZIN M, SCHORETSANITIS G, HAEN E, et al. The Effects of Co-prescription of Pantoprazole on the Clozapine Metabolism. Pharmacopsychiatry. 2020 Mar;53:65-70.

28. LEE S-T, RYU S, NAM HJ, LEE S-Y, HONG KS. Determination of pharmacokinetic properties of clozapine and norclozapine in Korean schizophrenia patients. International clinical psychopharmacology. 2009;24:139-44.

29. LIN SK, SU SF, PAN CH. Higher plasma drug concentration in clozapine-treated schizophrenic patients with side effects of obsessive/compulsive symptoms. Ther Drug Monit. 2006 Jun;28:303-7.

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30. LU ML, LANE HY, CHEN KP, JANN MW, SU MH, CHANG WH. Fluvoxamine reduces the clozapine dosage needed in refractory schizophrenic patients. The Journal of clinical psychiatry. 2000 Aug;61:594-9.

31. MAYEROVA M, USTOHAL L, JARKOVSKY J, PIVNICKA J, KASPAREK T, CESKOVA E. Influence of dose, gender, and cigarette smoking on clozapine plasma concentrations. Neuropsychiatr Dis Treat. 2018;14:1535-43.

32. MEYER JM. Individual changes in clozapine levels after smoking cessation: results and a predictive model. Journal of clinical psychopharmacology. 2002 Dec;21:569-74.

33. MURAYAMA-SUNG L, AHMED I, GOEBERT D, ALAIMALO E, SUNG H. The impact of hospital smoking ban on clozapine and norclozapine levels. Journal of clinical psychopharmacology. 2011 Feb;31:124-6.

34. OLMOS I, IBARRA M, VAZQUEZ M, MALDONADO C, FAGIOLINO P, GIACHETTO G. Population Pharmacokinetics of Clozapine and Norclozapine and Switchability Assessment between Brands in Uruguayan Patients with Schizophrenia. Biomed Res Int. 2019;2019:3163502.

35. ROSTAMI-HODJEGAN A, AMIN AM, SPENCER EP, LENNARD MS, TUCKER GT, FLANAGAN RJ. Influence of dose, cigarette smoking, age, sex, and metabolic activity on plasma clozapine concentrations: a predictive model and nomograms to aid clozapine dose adjustment and to assess compliance in individual patients. Journal of clinical psychopharmacology. 2004 Feb;24:70-8.

36. RUAN C-J, WANG C-Y, TANG Y-L, et al. Exploring the prevalence of clozapine phenotypic poor metabolizers in 4 Asian samples: They ranged between 2% and 13%. Journal of Clinical Psychopharmacology. 2019 2019;39:644-8.

37. SALAZAR-PEREYRA A, TOME ISR, RAMIREZ-BERMUDEZ J, CASTRO-ROMAN R, CASTRO-TORRES NN, JUNG-COOK H. Monitoring of plasma clozapine concentrations in patients with schizophrenia. [Spanish]. Archivos de Neurociencias. 2011 January-March;16:4-7.

38. SCHERF-CLAVEL M, SAMANSKI L, HOMMERS LG, DECKERT J, MENKE A, UNTERECKER S. Analysis of smoking behavior on the pharmacokinetics of antidepressants and antipsychotics: evidence for the role of alternative pathways apart from CYP1A2. International clinical psychopharmacology. 2019 Mar;34:93-100.

39. SEPPALA NH, LEINONEN EV, LEHTONEN ML, KIVISTO KT. Clozapine serum concentrations are lower in smoking than in non-smoking schizophrenic patients. Pharmacol Toxicol. 1999 Nov;85:244-6.

40. SPINA E, AVENOSO A, FACCIOLA G, et al. Relationship between plasma concentrations of clozapine and norclozapine and therapeutic response in patients with schizophrenia resistant to conventional neuroleptics. Psychopharmacology (Berl). 2000 Jan;148:83-9.

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41. TANG YL, MAO P, LI FM, et al. Gender, age, smoking behaviour and plasma clozapine concentrations in 193 Chinese inpatients with schizophrenia. British journal of clinical pharmacology. 2007 Jul;64:49-56.

42. YUE Y, XU YF, SONG LS, YI ZH, LU WH. Association of smoking with the therapeutic effects of the drug, side effects and plasma level of drug in patients with schizophrenia. [Chinese]. Chinese Journal of Clinical Rehabilitation. 2005 28-May;9:44-5.

43. PALEGO L, BIONDI L, GIANNACCINI G, et al. Clozapine, norclozapine plasma levels, their sum and ratio in 50 psychotic patients: influence of patient-related variables. Progress in neuro-psychopharmacology & biological psychiatry. 2002 Apr;26:473-80.

44. FABER MS, FUHR U. Time response of cytochrome P450 1A2 activity on cessation of heavy smoking. Clinical pharmacology and therapeutics. 2004 Aug;76:178-84.

45. WILLIAMS JM, GANDHI KK. Use of caffeine and nicotine in people with schizophrenia. Current drug abuse reviews. 2008 Jun;1:155-61.

46. TSUDA Y, SARUWATARI J, YASUI-FURUKORI N. Meta-analysis: the effects of smoking on the disposition of two commonly used antipsychotic agents, olanzapine and clozapine. BMJ open. 2014 Mar 4;4:e004216.

Tables and Figures:

Table 1: Description of included studies

Values of age, clozapine dose and clozapine blood levels were rounded to whole numbers.

In the publication from Rostami et al., TDM sample cases were reported instead of number of patients.

Legend:

*mean cigarettes per day (and SD) in smoking group

**except organic mental disorders

*** during the treatment period (46.2±25.4 months), the medication dose was adjusted by physicians based on therapeutic response and adverse effects (…) When the clozapine dose was

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changed, the steady-state serum concentration was measured after at least 7 days of constant dosing“ (Lee, 2009).

#: From n=14, those excluded where dose pre-post not stable or who were caught smoking after the ban (n=10), age mean =39.75 yrs, SD=9.11, 3 men, 1 woman.

##: multiple samples/patient

###: SD calculated from 95%CIs: smokers 38.8 years 95%CI (19.0-72.0), non-smokers 44.32 years 95%CI (19-88).

levels in week 8 were taken for analyses.

Abbrev.: BD=bipolar disorder, cigs=cigarettes, d=day, DSM-IV=Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, DSM-III= Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition, DSM-III-R= Diagnostic and Statistical Manual of Mental Disorders, 3rd Edition, Text Revision, f=female, ICD-10=International Classification of Diseases, 10th Revision, m=male, m:f=male to female ratio, mg/d=milligram per day, n=no, ns=not specified, SD=standard deviation, Sz=schizophrenia, TDM=therapeutic drug monitoring, TR-Sz=treatment-resistant schizophrenia, y=yes, yrs=years.

Figure 1: PRISMA-Flowchart (study selection process)

Systematic literature search according to Moher D et al., 2009.

Figure 2: Clozapine blood levels (ng/ml) in between-subject comparisons of smokers vs. non-smokers

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Author (year)	*Country*	*CYP-450 comedication excluded in the analysed cohort (y/n)*	*Proportions smoker vs. non-smoker balanced (y/n), ratio*	*Setting*	*Type of study*	*Diagnosis*	*Diagnostic tool*	*Smoking quantity (mean, SD) in smoking group**	*Clozapine dose (mean, SD) in smoking group (mg/d)*	*Clozapine dose (mean, SD) in non-smoking group (mg/d)*	*Clozapine level (mean, SD) in smoking group (ng/ml)*	*Clozapine level (mean, SD) in non-smoking group (ng/ml)*	*Age (mean, SD) in smoking group (yrs)*	*Age (mean, SD) in non-smoking group (yrs)*	*M:f ratio in smoking group*	*M:f ratio in non-smoking group*
Augustin (2019)	Germany	y	y, 43:28	Inpatient	retrospective	Sz	Chart, TDM database	ns	339, 150	286, 123	315, 192	454, 183	40, 13	46, 14	27:16	11:17
Dettling (2000)	Germany	y	n, 25:9	Inpatient + outpatient	prospective	Sz	DSM-III-R	≥10cigs/d	ns	ns	ns	ns	ns	ns	ns	ns
González-Esquivel (2011)	Mexico	n	n, 16:53	Inpatient + outpatient	prospective	Sz	DSM-IV	ns	265, 123	236, 134	329, 274	394, 274	ns	ns	ns	ns
Haring (1990)	Austria	y	y, 81:67	Inpatient	retrospective	Sz + BD	DSM-III	>5cigs/d	262, 132	304, 150	141, 114	183, 16	30, 7	32, 11	ns	ns
Haslemo (2006)	Norway	y	n, 28:5	psychiatric nursing homes	prospective	Sz	ns	7->20 cigs/d	495, 1012	415, 228	548, 1586	825, 291	ns	46, 9	18:10	3:2
Kuzin (2019)	Germany	y	n, 326:250	Inpatient + outpatient	retrospective	mental disorders **	Chart, TDM database	ns	363, 181	291, 148	294, 209	392, 218	39, 244 ###	44, 278 ###	240: 86	145: 105
*Lee (2009)****	Korea	y	n, 17:53	clinic	retrospective	Sz	DSM-IV	>10cigs/d	384, 132	314, 121	536, 412	632, 372	37, 8	31, 8	ns	ns
Lin (2006)	Taiwan	n	n, 34:68	clinic	prospective	Sz	ns	ns	295, 95	277, 145	387, 235	549, 329	ns	ns	ns	ns
Lu	Taiwan	y	y, 10:8	Inpatient	prospective	TR-Sz	DSM-IV	>10cigs/d	100,	100,	154,	212,	ns	ns	ns	ns

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(2000)					open-label				0	0	99	96				
Mayerova (2018)	Czech Republic	n	y, 46:52	Inpatient	prospective	TR-Sz	ICD-10	24.20cigs /d, SD=11.21	363, 122	327, 121	338, 178	607, 388	36, 12	35, 12	38:8	30:22
Meyer (2001)	USA	y	y, 11:11	Inpatient	prospective within-subject	Sz	ns	ns	568, 123	568, 123	550, 160	993, 713	42, 5	42, 5	8:3	8:3
Murayama (2011)#	USA	n	y, 4:4	Inpatient	prospective within-subject	Sz, 1 BD	ns	5.25cigs/d, SD=2.06	681, 312	681, 312	570, 188	749, 157	40, 9	40, 9	3:1	3:1
Olmos (2019)	Uruguay	n	y, 46:52	ns	prospective	Sz	DSM-IV	ns	375, 104	373, 106	382, 269	462, 212	39, 8	38, 10	37:9	39:13
Palego (2002)	Italy	y	y, 22:27	Inpatient + outpatient	prospective	Psychiatric disorders	DSM-IV	>5cigs/d	ns	ns	ns	ns	ns	ns	ns	ns
Rostami (2004)	UK, Ireland	Probably not	n, 4139:1360	Inpatient + outpatient	retrospective	ns	ns	ns	491, 9662 (m), 458, 5878 (f)	428, 5701 (m), 398, 4182 (f)	ns	ns	35, 494 (m), 38, 368 (f)	35, 285 (m), 38, 247 (f)	3021: 1118	852: 508
Ruan Beijing 1 (2019) ##	China	y	n, 26:100	Inpatient	retrospective	Sz	ns	ns	271, ns (m), 301, ns (f)	230, ns (m), 202, ns (f)	ns	ns	47, ns (m), 55, ns (f)	37, ns (m), 45, ns (f)	22:5	35:64
Ruan Beijing 2 (2019)	China	y	n, 51:140	Inpatient	retrospective	Sz	ns	ns	341, ns (m), 313, ns (f)	262, ns (m), 297, ns (f)	ns	ns	41, ns (m), 39, ns (f)	43, ns (m), 47, ns (f)	49:2	65:75
Ruan Taipei (2019)	China	y	n, 25:60	Outpatient	retrospective	Sz	ns	ns	286, ns (m), 325, ns (f)	300, ns (m), 254, ns (f)	ns	ns	36, ns (m), 36, ns	36, ns (m), 39, ns	22:3	29:31

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													(f)	(f)		
Ruan Seoul (2019)	China	y	n, 16:51	Outpatient	retrospective	Sz	ns	ns	378, ns (m), 450, ns (f)	347, ns (m), 282, ns (f)	ns	ns	37, ns (m), 38, ns (f)	31, ns (m), 32, ns (f)	15:1	26:25
Ruan Vellore (2019)	China	y	n, 19:82	Inpatient + outpatient	retrospective	Sz	ns	ns	401, ns (m), no f	329, ns (m), 323, ns (f)	ns	ns	39, ns (m), no f	34, ns (m), 36, ns (f)	19:0	54:28
Salazar-Pereyra (2011)	Mexico	y	n, 7:18	ns	prospective	Sz	DSM-IV	>5cigs/d	314, 175	226, 123	268, 188	384, 261	ns	ns	ns	ns
Scherf-Clavel (2018)	Germany	Probably not	n, 34:72	Inpatient + outpatient	prospective	any psychiatric disorder	ns	ns	319, 187	252, 117	296, 171	397, 214	39, 11	45, 14	34:11	32:29
Seppala (1999)	Finland	y	n, 34:10	Inpatient	prospective	Sz	clinical diagnosis	18cigs/d, ns	184, 97	298, 127	184, 97	298, 127	39, 12	37, 7	26:8	5:5
Spina (2000a)	Italy	y	y, 18:27	Hospital + community	prospective	TR-Sz	DSM-IV	>10cigs/d	ns	ns	ns	ns	ns	ns	ns	ns
Tang (2006)	China	y	y, 50:66	Inpatient	Retrospective TDM analysis	Sz	DSM-IV	ns	339, 135	264, 113	432, 355	351, 206	41, 9	43, 13	55:0	66:0
Van der Weide (2003)	Netherlands	n	y, 45:35	ns	prospective	Sz	ns	≥15cigs/d	382, 147	197, 138	ns	ns	ns	ns	ns	ns
Yue (2005)####	China	n	y,16:21	Inpatient	prospective	Sz	ICD-10	ns	239, 88	250, 84	273, 121	501, 198	ns	ns	ns	ns

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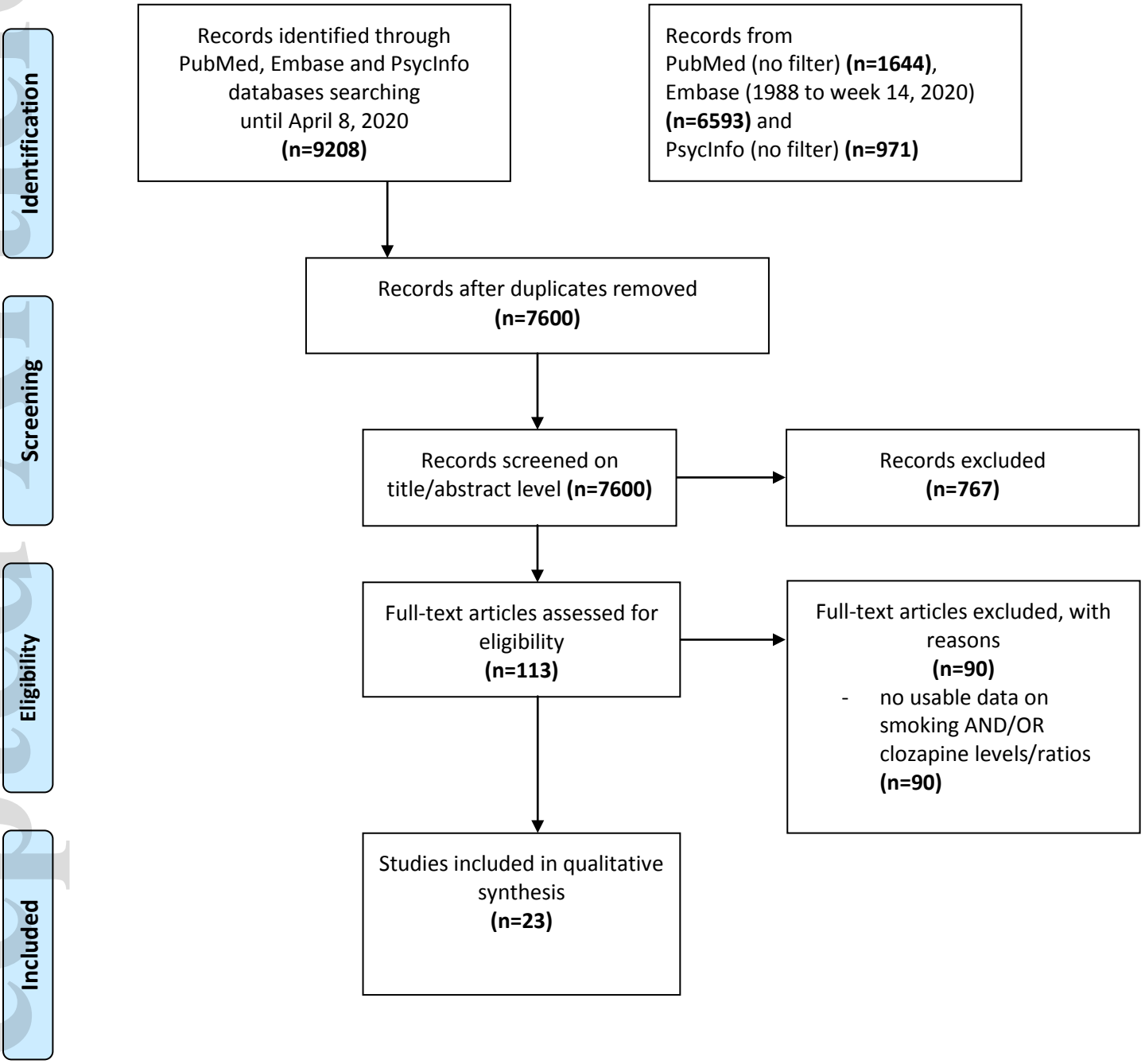

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). *P*referred *R*eporting *I*tems for *S*ystematic Reviews and *M*eta-*A*nalyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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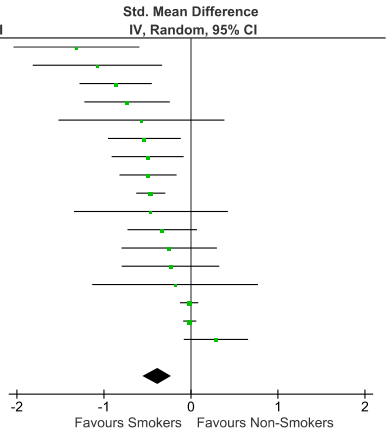
Study or Subgroup	Smoker Mean	SD	Total	Non-Smoker Mean	SD	Total	Weight	Std. Mean Difference IV, Random, 95% CI
Yue 2005	273.06	120.52	16	500.74	198.21	21	3.6%	-1.32 [-2.04, -0.59]
Seppala 1999	184	97	34	298	127	10	3.4%	-1.07 [-1.82, -0.33]
Mayerova 2018	338.2609	177.7676	46	606.5385	387.8594	52	6.4%	-0.86 [-1.28, -0.45]
Augustin 2019	314.56	191.947	43	454.43	183.07	28	5.5%	-0.73 [-1.23, -0.24]
Lu 2000	153.5	99.4	8	211.6	95.5	10	2.4%	-0.57 [-1.52, 0.38]
Lin 2006	387.2	234.9	34	549.3	328.6	68	6.4%	-0.53 [-0.95, -0.12]
Scherf-Clavel 2018	296.441	171.3895	34	397.403	213.8301	72	6.4%	-0.50 [-0.91, -0.08]
Haring 1990	141	114.2	81	183.2	15.5	67	7.5%	-0.49 [-0.82, -0.16]
Kuzin 2019	293.98	208.83	326	392.33	218.42	250	9.6%	-0.46 [-0.63, -0.29]
Salazar-Pereyra 2011	268	188	7	384	261	18	2.7%	-0.46 [-1.34, 0.42]
Olmos 2019	382	269	46	462	212	52	6.6%	-0.33 [-0.73, 0.07]
Lee 2009	535.5	411.5	17	631.8	371.8	53	5.0%	-0.25 [-0.80, 0.30]
González-Esquivel 2011	329	274	16	394	274	53	4.8%	-0.23 [-0.80, 0.33]
Haslemo 2006	548	1,586.371	28	825.2	290.917007	5	2.4%	-0.18 [-1.13, 0.77]
Rostami 2004 (female)	420	9,404.01759	1118	610	8,892.28277	508	10.1%	-0.02 [-0.13, 0.08]
Rostami 2004 (male)	370	9,232.17127	3021	500	9,235.59438	852	10.3%	-0.01 [-0.09, 0.06]
Tang 2006	432.1	354.6	50	351	206.1	66	7.0%	0.29 [-0.08, 0.66]
Total (95% CI)			**4925**			**2185**	**100.0%**	**-0.39 [-0.55, -0.22]**

Heterogeneity: Tau² = 0.07; Chi² = 81.42, df = 16 (P < 0.00001); I² = 80%
Test for overall effect: Z = 4.51 (P < 0.00001)



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