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ORIGINAL ARTICLE

Clozapine and COVID-19 Vaccination: Effects on blood levels and leukocytes. An observational cohort study

Selene R. T. Veerman[1,2] | **Timo Moscou**[3] | **Jan P. A. M. Bogers**[2,4] | **Dan Cohen**[2,5] | **Peter F. J. Schulte**[1,2]

[1]Department of Community Mental Health, Mental Health Service Noord-Holland Noord, Alkmaar, The Netherlands

[2]Dutch Clozapine Collaboration Group, Oegstgeest, The Netherlands

[3]High Intensive Care Clinic, Mental Health Service Noord-Holland Noord, Heiloo, The Netherlands

[4]High Care Psychiatric Clinics, Mental Health Service Rivierduinen, Leiden, The Netherlands

[5]Department of Community Mental Health, Mental Health Service Noord-Holland Noord, Heerhugowaard, The Netherlands

Correspondence
Department of Community Mental Health, Mental Health Service Noord-Holland Noord, Alkmaar 1816 BT, The Netherlands.
Email: s.veerman@ggz-nhn.nl

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Abstract

Objective: To investigate the safety of COVID-19 vaccination in patients on clozapine as regards plasma clozapine concentration and haematological parameters.

Methods: We conducted a multicentre observational cohort study from 22 February 2021 to 2 September 2021. Primary outcomes were clinically relevant increase in clozapine blood levels (>100 μg/L increase compared to baseline) and clozapine alert levels (>1000 μg/L). Secondary outcomes were granulocytopenia, leukocytopenia and lymphocytopenia. Outcomes were measured approximately 5 days after the first and (where applicable) second dose of COVID-19 vaccine.

Results: This study included 139 patients. Compared to baseline, clozapine blood levels increased significantly (ES = 0.28, $p = 0.003$) after the second vaccination. Clinically relevant increases in clozapine blood levels occurred in 20/92 patients (22%) and in 16/56 patients (29%) during the first and second phases, respectively. Clozapine alert levels developed in one patient (1%) following the first dose and in three patients (5%) after the second dose. In both phases, changes in white blood cells (WBC) were limited to mild granulocytopenia (3% and 5%), moderate granulocytopenia (1% and 0%) and leukocytopenia (2% and 3%) without cause for extra monitoring according to the guideline.

Conclusion: In general, as regards WBC counts COVID-19 vaccination seems to be safe in patients with SMI. Changes in WBC had no clinical implications. Psychoeducation on the symptoms of clozapine intoxication is recommended, especially in patients with clozapine blood levels approaching the upper limit of the therapeutic range. Increase in the C-reactive protein (CRP) level can signal inflammation rapidly and help to prevent clozapine intoxication following vaccination.

KEYWORDS

clozapine, COVID-19 vaccine, plasma concentration, safety

1 | INTRODUCTION

The outbreak of the pandemic of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection has several consequences for mental health care. Firstly, the infection rate with SARS-CoV-2 is higher in patients with severe mental illness (SMI) compared to people without a mental health disorder.[1,2] Secondly, patients with a mental health disorder are more frequently admitted to hospital for this infection. Thirdly, patients with a mental health disorder have a higher Coronavirus Disease 2019 (COVID-19) mortality rate compared to patients without a mental health disorder.[3] Mortality related to COVID-19 is particularly increased in patients with a schizophrenia spectrum disorder (SSD) or a bipolar disorder.[4,5]

At the same time, a growing body of evidence indicates that psychotropics have protective effects against infection with SARS coronaviruses.[6–8] A recent cohort study ($n = 7348$) of adults with SMI diagnosed with COVID-19 in New York City indicated that the higher mortality rates in this patient population were not associated with antecedent antipsychotic use.[9] The evidence for potential anti-SARS-CoV-2 effects of clozapine is unsatisfactory due to contradictory results. No negative effects of clozapine were shown in three studies with small numbers of clozapine users.[10–12] In a German retrospective inpatient study of the possible effects of psychiatric and somatic medication on the duration and severity of COVID-19 ($n = 96$), four patients who used clozapine (4.2%) all survived.[10] In an Indian study of 594 ambulatory SSD patients of whom 356 received clozapine, no difference in incidence of COVID-19 was found between patients receiving clozapine ($n = 23$) and those receiving other antipsychotic medication ($n = 9$).[11] Unfortunately, a control group was lacking. Clozapine showed protective effects for infection and the course of disease in a small case-control study ($n = 142$) in Argentina with 60% less SARS-CoV-2 infection in clozapine users ($n = 13$) compared with non-clozapine users ($n = 102$) and with the disease being only half as symptomatic in patients using clozapine as in patients on other medication (69.2% vs. 30.6%).[12] A cohort study in Stockholm showed no differences in inpatient care, ICU admission or death due to COVID-19 after adjusting for age, sex and residence in a retirement home in patients on clozapine treatment ($n = 966$) and those on other antipsychotic medication ($n = 7276$).[13] Due to the small number of cases ($n = 147$), the statistical power was low. However, in a large UK study ($n = 6309$), after adjusting for gender, age, ethnicity, body mass index (BMI), smoking status and use of mental health care, clozapine use ($n = 1282$) was associated with a higher risk of SARS-CoV-2 infection (adjusted hazard ratio [AHR] = 1.76) compared with patients on other antipsychotic medication.[14]

Significant outcomes

- Clinicians should inform clozapine users that they seem to be at high risk for COVID-19 and vaccination is safe.
- Routine extra monitoring of haematological parameters is not warranted following COVID-19 vaccination in clozapine users.
- Psycho-education on symptoms of clozapine intoxication is recommended, because clozapine blood levels can rise due to the inflammatory response after COVID-19 vaccination. This can be monitored quickly with a CRP test.

Limitations

- The study cohort was small and there was no control group, so that the statistical power to detect clinically relevant changes in clozapine blood levels and haematological parameters was low.
- Rare complications of vaccination were not observed, which is consistent with both the relatively small sample size and the rarity of the complications.
- The three Dutch mental health centres used different methods to monitor clozapine blood levels and at one centre, different methods were used in the same patient at different measurement points.

Clozapine has effects on immune response. There is limited evidence of possible anti-inflammatory effects of clozapine in critically ill COVID-19 patients. Although clozapine is a strong histamine-1 (H1) antagonist and reduces interleukin-6 (IL6), it has not been shown to be effective in preventing cytokine storm syndrome.[6] Moreover, clozapine may be associated with secondary antibody deficiency (SAD), explaining the more frequent complications of a SARS-CoV-2 infection with pneumonia.[15] Increased clozapine blood levels (with a high metabolic ratio of clozapine/norclozapine) are associated with decreased neutrophils in long-term recipients of clozapine with refractory schizophrenia.[16] Both genetic factors (with increased neutropenia in patients with schizophrenia of African ethnicity) and combination therapy with fluvoxamine, reducing the formation of norclozapine by CYP1A2 inhibition, are associated with high clozapine to norclozapine ratios.[17]

While the possible protective or unprotective properties of clozapine remain unclear, clozapine use and

SARS-CoV-2 infection can lead to complications, since clozapine blood levels rise due to inflammatory responses with cytokines such as IL-6 and IL-1, which inhibit the cytochrome P450 enzyme CYP1A2.[18] In our systematic review, we found that a dangerous increase in clozapine blood levels is a common complication in critically ill COVID-19 patients.[19] We also found that lymphocytopenia and granulocytopenia are generally mild and transient and a result of COVID-19 rather than of clozapine treatment. Other complications of COVID-19 associated with clozapine are delirium and COVID-19 pneumonia. A more recent review of literature on clozapine and COVID-19 also found an increased need for treatment in a critical care unit, and discontinuation of clozapine because of COVID-19-induced lymphopenia.[20] Due to these extra risks, as well as a high risk of COVID-19 in clozapine users, vaccination against SARS-CoV-2 is even more imperative for this patient group than for people with SMI in general.[21,22]

Since we know that in the general population, inflammation and fever may occur after administration of a COVID-19 vaccine, with a higher risk of fever after the second dose due to a stronger immune response, complications such as high clozapine blood levels or WBC count abnormalities can be expected in clozapine users.[23] At present, there is not much evidence as to whether COVID-19 vaccination is more dangerous for clozapine users than for the general population. Based on one study among 14 SSD patients with measurements before and after 2, 4, 7 and 14 days of vaccination, we may conclude that common influenza vaccination does not seem to affect plasma concentrations of clozapine and is not associated with C-reactive protein (CRP) elevations.[24] One clozapine case report described a fever of 40.3°C and a generalized tonic–clonic seizure 72 h after the first dose of a BioNTech/Pfizer vaccine.[25] Unfortunately, clozapine blood levels were not determined after vaccination. Another case report refers to elevated clozapine levels with toxic effects and mild, transient lymphocytopenia 4 days after the first dose of a BioNTech/Pfizer vaccine.[26] A direct causal relationship between COVID-19 vaccination and these adverse events could not be established.

As regards haematological effects, there is some evidence that influenza vaccination suppresses white blood cell (WBC) proliferation. One case report and two small prospective studies among 25 healthy volunteers and 70 people aged over 65 respectively showed transient lowering of lymphocyte and WBC counts, mostly not clinically significant, up to one month after vaccination.[27,28] Hazardous complications of immunization with influenza vaccines are rare. We found one report of agranulocytosis in an elderly woman known to have acquired haemophilia A and another of sudden very late-onset clozapine-induced agranulocytosis following vaccination after periods of 3 and 7 weeks, respectively.[29,30] We also found several case reports of aplastic anaemia onset or relapsing aplastic anemia.[31–34]

Data on adverse events following COVID-19 vaccination are limited. In the United Kingdom, 141,866 spontaneous reports were received between 09/12/20 and 08/12/21 for the Pfizer/BioNTech vaccine, with one fatality due to aplastic anaemia, four reports of leukopenia with one fatality, and four reports of lymphopenia.[35] Leukopenia was found in 0.01% in phase IV clinical trials of the Pfizer/BioNTech vaccine (16/179,630 reports on side effects), the Moderna vaccine (16/183,219 reports) and the Johnson & Johnson vaccine (4/41,403).[36–38] Fatality rates were 0% for both Moderna and Johnson & Johnson vaccines and 6.25% for people who developed leukopenia after administration of the Pfizer/BioNTech vaccine. These findings suggest that leukopenia as a side effect of COVID-19 vaccines is extremely rare and exceptionally rarely causes death. Moreover, there is no consistent evidence that an additional risk for haematological changes exists in clozapine users post-COVID-19-vaccination.

1.1 | Aims of the study

Thus far, the safety of COVID-19 mRNA vaccines (Moderna and BioNTech/Pfizer) and viral vector vaccines (AstraZeneca and Johnson & Johnson) has not been investigated in clozapine users. Hence, our primary research question was whether a clinically relevant increase in clozapine levels (>100 μg/L), or even a clozapine alert level (>1000 μg/L),[39] would occur approximately 5 days after COVID-19 vaccination. We hypothesized that a clinically relevant rise in clozapine blood levels would occur more often after administration of the second vaccine due to a stronger inflammatory response. We did not anticipate a significantly higher risk of clozapine levels above the upper limit of the therapeutic range after the first or second COVID-19 vaccination.

Our secondary research question was whether granulocytopenia (mild, moderate or severe granulocytopenia or agranulocytosis), leukocytopenia or lymphocytopenia would occur approximately 5 days after COVID-19 vaccination in patients on clozapine. Considering the small chance of a decline in WBC count, we hypothesized that following COVID-19 vaccination no dangerous decrease in neutrophils, total leukocytes or lymphocytes would occur in patients on clozapine.

2 | MATERIAL AND METHODS

2.1 | Study design

This prospective, observational cohort study (researchregistry6726) was conducted from 22 February 2021 to 2 September 2021. The trial consisted of only one phase for subjects receiving the single Johnson & Johnson vaccine and of two phases for subjects receiving Moderna, BioNTech/Pfizer or AstraZeneca vaccines. In each phase, clinical assessments were performed after vaccination. Clozapine dosage and use of concomitant medications were at the discretion of the treating psychiatrist. Throughout the study we documented changes in clozapine dose and other factors which might affect clozapine blood levels, such as inflammation (sometimes because of infection), fluvoxamine, carbamazepine or omeprazole addition, caffeine use and smoking habits. Inflammatory cytokines, fluvoxamine and caffeine cause elevated clozapine levels because of inhibition of the clozapine metabolizing enzyme CYP1A2 or competition for the availability of this enzyme (caffeine), and carbamazepine, omeprazole and polycyclic aromatic hydrocarbons in cigarette smoke cause lowered clozapine levels because they stimulate CYP1A2.[18,40]

2.2 | Study population, inclusion and exclusion criteria

Outpatients and long-stay inpatients of the Noord-Holland Noord, Rivierduinen and Reinier van Arkel Dutch Mental Health Centres who used clozapine and received COVID-19 vaccinations were recruited by their treating psychiatrist, clinical nurse specialist or case manager. There were no exclusion criteria. The protocol was approved by the institutional review boards of the participating mental health centres. Written consent was obtained from the participating subjects or their legal representatives if the decision-making capacity of the patient was impaired, as assessed by the treating physician. Ethical approval was not necessary, because this study did not examine an intervention, but entailed additional monitoring of the safety of COVID-19 vaccination in clozapine users in a context of good clinical care.

2.3 | Clinical assessments

Clozapine levels, WBC and differential were determined 5 days after the first and—where applicable—the second COVID-19 vaccination, unless this time frame was impossible due to practical issues. Primary outcomes were clinically relevant increase in clozapine blood level (>100 μg/L increase compared to baseline, which was the last clozapine blood level determined before vaccination) or clozapine alert level (>1000 μg/L) after a vaccination. Secondary outcomes were mild granulocytopenia ($1.5–<2.0 \times 10^9$/L), moderate granulocytopenia ($1.0–<1.5 \times 10^9$/L), severe granulocytopenia ($0.5–<1.0 \times 10^9$/L) or agranulocytosis ($<0.5 \times 10^9$/L), leukocytopenia ($<3.5 \times 10^9$/L) or lymphocytopenia ($<1.5 \times 10^9$/L) following vaccination.

2.4 | Statistical analysis

To determine the effects of COVID-19 vaccination on the hypothesized clozapine blood levels and leukocyte differentiation, the two phases (after the first and second vaccine) of the trial were compared using a Wilcoxon signed-rank test in SPSS Statistics version 27.0 (SPSS Inc., 2020).[41,42] The effect sizes (ES) of significant differences were calculated as Z/sqrt N.[43] A McNemar's test was used to test if a significant change in proportions occurred following the first vaccination compared with the second vaccination, after dichotomizing the outcomes (clozapine alert level, severe granulocytopenia, leukocytopenia or lymphocytopenia).[44] We conducted analyses separately for both outcomes, and phases using a per-protocol analytic approach. Protocol completion for both primary outcomes (clozapine blood levels) and secondary outcomes (WBC count and differential) was defined as having received a COVID-19 vaccine and having had blood samples taken between 3 and 9 days following vaccination. For the primary outcome, an extra condition was no change in clozapine, fluvoxamine, carbamazepine or omeprazole dose. Additional descriptive analyses were conducted for both primary and secondary outcomes.

2.5 | Reliability of analyses

Possible confounders were analysed. Changes in clozapine dose, the occurrence of fever or inflammation not caused by the vaccination, changes in caffeine or tobacco consumption, and comedication that might affect CYP1A2 activity in the period between baseline and second COVID-19 vaccination were monitored. The sampling time for blood tests to check clozapine plasma levels was standardized at 12 h (± 1 h) after the last clozapine dose.

Another step was taken to address sources of confounding: the methods for monitoring clozapine blood levels (high performance liquid chromatography [HPLC] or liquid chromatography-mass spectrometry [LC-MS]) were determined at baseline, and following

the first and second vaccination. The LC-MS procedure is more sensitive and specific, and gives more accurate results with interassay coefficients of variation (CV) of 3.04%–4.94% for clozapine compared with HPLC, with interassay CVs ranging from 0.99% to 10.14% for clozapine.[45,46] After internal analysis of both HPLC and LC-MS, we found LC-MS to be more accurate for purification and quantification with an average higher outcome of 22% (Unpublished data analytical method validation Laboratory of Clinical Pharmacy Northwest Hospital; [LC-MSMS; Recipe TCA calibrators, internal standard and controls; Waters Acquity UPLC® BEH C18, 1.7 μm, 2.1 × 100 mm; mobile phase A: ammoniumacetate, methanol, aquadest Milli-Q; mobile phase B: ammoniumacetate, methanol]). Therefore, we corrected all results obtained with HPLC with a factor of 1.22.

3 | RESULTS

3.1 | Baseline characteristics

Figure 1 presents enrolment flow data. A total of 139 patients were included, of whom 2 refused COVID-19 vaccination; 4 eligible patients refused participation after enrolment. Clozapine dosage remained unaltered in 108 subjects. Infection occurred in one subject who was admitted to the hospital on the day after his second vaccination because of a severe gallbladder inflammation. Table 1 presents demographic and clinical characteristics of the study population.

3.2 | Clozapine blood levels

An analysis of effects using a per-protocol approach is presented in Table 2. Wilcoxon signed-rank tests showed no significant differences (ES = 0.11, $p = 0.123$) between clozapine blood levels at baseline and 3 and 9 days after the first vaccine. After the same interval between the second vaccination and blood monitoring, a small significant increase (ES = 0.28, $p = 0.003$) was found for clozapine blood levels compared with baseline.

Clinically relevant increases in clozapine blood levels were found in 20 patients (22%) after the first vaccine and in 16 patients (29%) following the second vaccine against SARS-CoV-2. In the first phase, a maximum increase of 548 μg/L (159% increase compared to baseline) in the clozapine blood level was found following a Johnson & Johnson vaccine. In the second phase, a maximum increase of 420 μg/L (76% increase compared to baseline) in the clozapine blood level was found after administration of a Moderna vaccine.

Analysis of decrease in clozapine blood levels showed a significant reduction of >100 μg/L in 12% (range 112–362 μg/L) and 9% of participants (range 113–226 μg/L) in the first and second phases, respectively.

A post-vaccination clozapine alert level occurred in one patient (1%) in the first phase (blood levels and symptoms are shown in Table 3). In this patient, a significant increase in clozapine blood level of 430 μg/L (64% increase compared to baseline) was found which could not be explained by different laboratory techniques. In the second phase, clozapine alert levels developed in three patients (5%) without any symptoms of intoxication. Two of these patients showed a significant increase in clozapine blood level compared with baseline of 342 μg/L (51%) and 394 μg/L (56%), respectively (see Table 3). The clozapine blood levels at baseline and T1 of these three patients have already been corrected by a factor of 1.22, in view of the change in method from HPLC to LC-MS in the second phase.

3.3 | Haematological parameters

The Wilcoxon signed-rank test determined that there were no significant differences (ES = −0.10, $p = 0.142$) between the neutrophil count at baseline and after the first vaccine (Table 4). However, after the second vaccine, compared with baseline, a small but significant decrease (ES = −0.26, $p = 0.002$) was found in neutrophils. Additionally, the Wilcoxon signed-rank test determined that compared with baseline, the WBC count showed a significant decrease after both the first (ES = −0.16, $p = 0.018$) and second vaccines (ES = −0.25, $p = 0.002$). There were no significant changes in lymphocytes compared with baseline following the first (ES = −0.13, $p = 0.064$) or second vaccine (ES = −0.02, $p = 0.982$).

No severe granulocytopenia or agranulocytosis occurred following COVID-19 vaccination in either phase (Table 4). However, mild granulocytopenia occurred in three patients (3%) after the first vaccine and in four patients (5%) after the second vaccine. Moderate granulocytopenia was found in one patient following first vaccination (1%). One patient was already known to have benign ethnic leukocytopenia (WBC range = 2.4–4.7 × 10^9/L in the last 3 years, WBC = 3.3 × 10^9/L at baseline). In another patient, leukocytopenia was found after the first vaccine (3.3 × 10^9/L). Another patient showed leukocytopenia after the second vaccine (WBC = 3.2 × 10^9/L). We found no significant change in the proportion of patients with lymphocytopenia from baseline (22 patients, 17%) to first vaccination (19 patients, 17%) or second vaccination (13 patients, 17%). In short, we found no significant difference in categorical

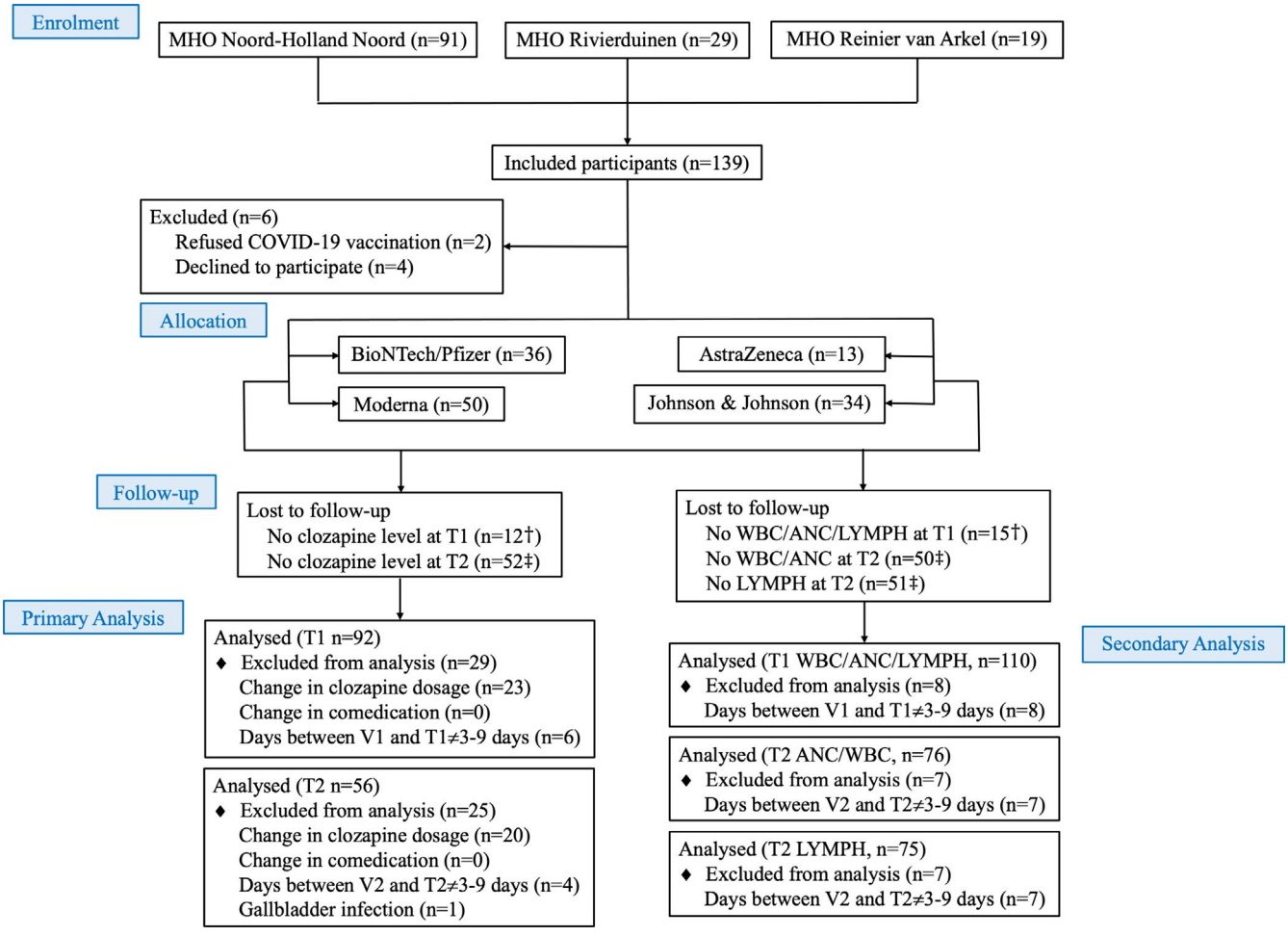

FIGURE 1 Flow of participants in a multicentre observational cohort study on the safety of COVID-19 vaccination in clozapine users. ANC, absolute neutrophil count; LYMPH, lymphocytes; *n*, number; T1, first phase; T2, second phase; V1, first vaccination; V2, second vaccination; WBC, white blood cell count. †No V1 due to earlier COVID-19 infection ($n = 4$); ‡No V2 due to Johnson & Johnson ($n = 34$)

distribution in severe granulocytopenia, leukocytopenia and lymphocytopenia between the first and second phase.

4 | DISCUSSION

As no evidence exists about the risks involved with COVID-19 vaccination in clozapine users, we decided to conduct a prospective observational cohort study in this population in three Dutch mental health centres. Changes in clozapine median blood levels following the first and second vaccination compared with baseline, only showed a significant increase in the second phase (ES = 0.28, $p = 0.003$). In our study group, we measured a clozapine blood level increase of >100 μg/L in 22% and 29% of participants in the first and second phase, respectively. Given that a clinically relevant decrease occurred in 12% and 9% of participants in the first and second phases, it seems that elevation of clozapine blood levels following vaccination occurs more often than reduction. Clozapine alert levels were found in four patients (three patients at T2), but with only minor symptoms or none at all. The results during the second phase were more prominent compared with the first phase as regards clozapine blood level increase, and more patients reached alert levels for clozapine, confirming our hypothesis of a stronger inflammatory response following the second vaccination due to alertness of the immune system.

With regard to white blood cell counts, we found a transient, mostly not clinically significant decline in lymphocytes and neutrophils, which is in agreement with earlier findings following immunization with influenza vaccines and Pfizer/BioNTech, Moderna and Johnson & Johnson vaccines.[24,35–38] The only clinically relevant changes in WBC count were mild granulocytopenia (3% of participants at T1 and 5% at T2) and moderate granulocytopenia (1% of participants at T1) and leukocytopenia (2% of participants at T1 and 3% at T2), which did not necessitate extra monitoring according to the guideline.

To our knowledge, this is the first study evaluating the safety of vaccination against the SARS-CoV-2 in clozapine users. Our main limitation was the sample size, which possibly excluded finding more rare complications of COVID-19 vaccination. Sample sizes were also too small to analyse the effects of mRNA and viral vector vaccines in clozapine users, separately. We chose to combine the results of the Johnson & Johnson vaccine with the first phase of Moderna, BioNTech/Pfizer and AstraZeneca under the assumption of an equal inflammatory response following vaccination with Johnson & Johnson. However, at present there is still no robust evidence suggesting an equally strong immune response in general following a single dose of Johnson & Johnson vaccine compared to the first vaccination with the other COVID-19 vaccines. When the Johnson & Johnson results were excluded from the results of the first phase of Moderna, BioNTech/Pfizer and AstraZeneca vaccination, there was still no clinically relevant increase in clozapine blood levels or change in WBC count following vaccination ($p = 0.150$).

Another limitation was the failure to monitor the level of CRP, which increases in the event of inflammation.[24] CRP elevation would make it very unlikely that increased clozapine blood levels or even clozapine alert levels were caused by laboratory error or random variation. The different methods used to monitor clozapine blood levels were also important. In three of four cases of clozapine alert level (at T2), the method used to measure the clozapine blood level had changed. We corrected based on an internal analysis, comparing results obtained by HPLC and LC-MS of the same blood samples. However, a confounding factor for this correction is the difference in interassay CVs for the HPLC and procedures LC-MS. CVs are determined by both measurement errors and intra-individual variety. Intra-individual fluctuations of clozapine plasma concentrations are a normal phenomenon. Therefore, increase in clozapine levels can be explained by several other factors besides COVID-19 vaccination. In the absence of change in clozapine dosage or in substances which inhibit or induce clozapine over CYP1A2 (and to lesser extent CYP2C19), passive smoking, minor infections, and long-term action of clozapine with inhibition over CYP2D6 can cause intra-individual variability of clozapine blood levels as well.[47] Moreover, mean CVs seem to

TABLE 1 Patient demographics and baseline characteristics

Characteristic	*n* (%)	Mean (SD)
Sex		
Male	85 (63.9)	
Female	48 (36.1)	
Age		52.15 (12.91)
Living conditions		
Inpatient	85 (63.9)	
Outpatient	48 (36.1)	
Diagnosis		
Schizophrenia	102 (76.7)	
Schizoaffective disorder	12 (9.0)	
Bipolar disorder	7 (5.3)	
Other	12 (9.0)	
Clozapine blood level at baseline, μg/L		451.91 (225.78)
Method for monitoring clozapine blood levels		
HPLC	82 (61.7)	
LC-MS	51 (38.3)	
COVID-19 vaccine		
Moderna	50 (37.6)	
BioNTech/Pfizer	36 (27.1)	
AstraZeneca	13 (9.8)	
Johnson & Johnson	34 (25.6)	

Abbreviations: HPLC, high performance liquid chromatography; LC-MS, liquid chromatography–mass spectrometry; *n*, number; SD, standard deviation.

TABLE 2 Primary outcomes of clinically relevant clozapine increase compared to baseline and clozapine alert levels following the first and second vaccination against SARS-CoV-2

	Clozapine blood level (μg/L)	Clinically relevant increase in clozapine blood levels (>100 μg/L)	Increase in clozapine blood levels compared to T0 (μg/L)		Clozapine alert level (>1000 μg/L)
T	Mdn	*n/N* (%)	ES	*p*	*n/N* (%)
T0	460	n.a.	n.a.	n.a.	1/133 (0.8)
T1	454	20/92 (21.7)	0.11	0.123	1/133 (0.8)
T2	503	16/56 (28.6)	**0.28**	**0.003**	3/56 (5.4)

Note: Bold values statistically significant at $p < 0.05$.

Abbreviations: ES, effect size; Mdn, median; n.a., not applicable; *n/N*, number of affected patients out of the total number of patients; *p*, *p*-value; T, time of measurement; T0, baseline; T1, first phase; T2, second phase.

TABLE 3 Characteristics of patients who developed clozapine alert levels following COVID-19 vaccination

	Clozapine blood levels (μg/L)			Clozapine blood level increase (μg/L)			
Subject number	**T0**	**T1**	**T2**	**T0-T1 (%)**	**T0-T2 (%)**	**Vaccine**	**Symptoms**
111	670	**1100**	—	**430 (64)**	—	Johnson & Johnson	Fever, dizziness
20	920	804	**1025**	−116 (−13)	**105 (11)**	Pfizer	No symptoms
22	667	671	**1009**	4 (0)	**342 (51)**	Pfizer	No symptoms
9	698	756	**1092**	58 (8)	**394 (56)**	Moderna	No symptoms

Note: Bold values clozapine alert level or increase to clozapine alert level.

Abbreviations: T0, baseline; T1, first phase; T2, second phase.

be dose-dependent with higher CVs in lower dosages.[48] Unfortunately, data on confounding factors such as use of caffeine and cigarette smoking were incomplete, because many patients were not able to recollect precisely whether a change in caffeine use or cigarette smoking had occurred. In short, the observed changes in clozapine blood levels may be partly due to intra-individual variety, caused by factors, which the prescribing specialist could not totally be aware of. And the lower the dose, the higher the intra-individual variety, the greater the dispersion around the mean clozapine blood level.

Interpretation of haematological parameters was hampered by normal fluctuation of WBC counts. In order to avoid extra strain on patients and patient laboratory service centres, no baseline measurements of clozapine blood level, WBC and differential were taken on the day of administration of both COVID-19 vaccinations. Considering the negative association of clozapine/norclozapine ratio with neutrophil concentrations, observed decline of granulocytes may also be due to alterations in metabolization of clozapine.[16] Unfortunately, norclozapine levels were not analysed in measurements conducted by HPLC. Therefore, we were not able to correct for changes in clozapine/norclozapine ratio. When results on WBC counts in patients with alterations of clozapine dosage were excluded from the analysis ($n = 92$ at T1 and $n = 56$ at T2), we still found no clinically significant decline in neutrophil concentrations following the first vaccination ($p = 0.119$) and the decrease after the second vaccination remained significant ($p = 0.016$).

Finally, the lack of a control group is a limitation which we tried to tackle by using median clozapine blood levels and median concentrations of leukocytes, neutrophils and lymphocytes. In our setting, the interval between baseline and the prior available blood samples varied widely. We were unable to analyse the variability of clozapine levels, WBC and differential because we could not correct for confounding factors at the time of monitoring before baseline, such as infection, compliance with any alterations of clozapine dosage and comedication, or variation in use of caffeine and cigarette smoking.

In conclusion, in this cohort study, we found a clinically relevant rise (>100 μg/L) of clozapine blood levels with 22% resp. 29% of participants after the first and second vaccination. Clozapine alert levels cannot be ruled out in all patients, especially those with pre-existing upper limit high plasma clozapine concentrations. Therefore, although alert levels following vaccination are rare, repetition of psycho-education on the symptoms of clozapine intoxication is both a safe and recommended precautionary measure, especially in patients with clozapine blood levels near the upper limit of the therapeutic range. We also recommend that in the event of flu-like symptoms after vaccination clinicians should measure CRP as well as WBC and differential, because CRP can be determined the same day, whereas clozapine blood levels are usually determined only after a few days. In this way, inflammation can be detected in time to prevent clozapine intoxication.

Concerning WBC counts, we found no reason for alarm as regards the safety of COVID-19 vaccination in clozapine users. Our data did not warrant routine extra monitoring of haematological parameters following COVID-19 vaccination in clozapine users. Indeed, no severe granulocytopenia or agranulocytosis occurred. When after immunization a mild or moderate granulocytopenia is found—in contradiction with the European Medicines Evaluation Agency (EMEA) text—ongoing clozapine treatment with extra monitoring of WBC and granulocytes should be considered in long-term recipients of clozapine.[19] A transient, not clinically significant, decline in lymphocytes and neutrophils is, after all, most likely caused by the vaccine.

Finally, additional epidemiological studies are necessary to investigate the effects of vaccination against SARS-CoV-2 on clozapine blood levels and WBC and differential in patients on clozapine. We recommend that future studies monitor CRP at the same time as clozapine blood

TABLE 4 Secondary outcomes of granulocytopenia, leukocytopenia and lymphocytopenia compared to baseline following the first and second vaccinations against SARS-CoV-2

	Mild granulocytopenia ($1.5–2.0 \times 10^9$/L)	Moderate granulocytopenia ($1.0–<1.5 \times 10^9$/L)	Severe granulocytopenia ($0.5–<1.0 \times 10^9$/L)	Agranulocytosis ($<0.5 \times 10^9$/L)	Leukocytopenia ($<3.5 \times 10^9$/L)	Lymphocytopenia ($<1.5 \times 10^9$/L)
T	*n/N* (%)	*n/N* (%)	*n/N* (%)	*n/N* (%)	*n/N* (%)	*n/N* (%)
T0	3/130 (2.3)	0/130 (0)	0/130 (0)	0/130 (0)	1/131 (0.8)	22/130 (16.9)
T1	3/110 (2.7)	1/110 (0.8)	0/110 (0)	0/110 (0)	2/110 (1.8)	19/110 (17.3)
T2	4/76 (5.3)	0/76 (0)	0/76 (0)	0/76 (0)	2/76 (2.6)	13/75 (17.3)

	Neutrophils compared to T0			Leukocytes compared to T0			Lymphocytes compared to T0		
	Mdn ($\times 10^9$/L)	ES	*p*	Mdn ($\times 10^9$/L)	ES	*p*	Mdn ($\times 10^9$/L)	ES	*p*
T0	4.1			7.2			2.1		
T1	4.2	−0.10	0.142	**7.1**	**−0.16**	**0.018**	2.0	−0.13	0.064
T2	**3.5**	**−0.26**	**0.002**	**6.5**	**−0.25**	**0.002**	2.0	−0.02	0.982

Note: Bold values statistically significant difference at $p < 0.05$ compared to baseline.

Abbreviations: ES, effect size; Mdn, median; *p*, *p*-value; T, time of measurement; T0, baseline; T1, first phase; T2, second phase.

levels, using LC-MS. Clozapine blood levels, WBC and differential before administration of a vaccine could be used to control for natural variability. We also recommend further investigation into genetic determinants of clozapine pharmacokinetics and comedication with fluvoxamine, causing increased levels of clozapine proportional to norclozapine, which are negatively associated to neutrophil concentrations.

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CONFLICT OF INTEREST

The authors have declared that there are no conflicts of interest in relation to the subject of this study.

PEER REVIEW

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DATA AVAILABILITY STATEMENT

Derived data supporting the findings of this study are available from the corresponding author on request.

ORCID

Selene R. T. Veerman https://orcid.org/0000-0002-7604-6529
Jan P. A. M. Bogers https://orcid.org/0000-0001-7273-0027
Dan Cohen https://orcid.org/0000-0001-8804-4806
Peter F. J. Schulte https://orcid.org/0000-0003-4931-3393

REFERENCES

1. Wang Q, Xu R, Volkow ND. Increased risk of COVID-19 infection and mortality in people with mental disorders: analysis from electronic health records in the United States. World Psychiatry. 2021;20:124-130.
2. Jeon HL, Kwon JS, Park SH, Shin JY. Association of mental disorders with SARS-CoV-2 infection and severe health outcomes: nationwide cohort study. Br J Psychiatry. 2021;7:1-8.
3. Vai B, Mazza MG, Delli Colli C, et al. Mental disorders and risk of COVID-19-related mortality, hospitalisation, and intensive care unit admission: a systematic review and meta-analysis. Lancet Psychiatry. 2021;8:797-812.
4. Toubasi AA, AbuAnzeh RB, Tawileh HBA, Aldebei RH, Alryalat SAS. A meta-analysis: the mortality and severity of COVID-19 among patients with mental disorders. Psychiatry Res. 2021;299:113856.
5. Fond G, Nemani K, Etchecopar-Etchart D, et al. Association between mental health disorders and mortality among patients with COVID-19 in 7 countries: a systematic review and meta-analysis. JAMA Psychiatry. 2021;78(11):1208.
6. Khosravi M. Candidate psychotropics against SARS -CoV-2: a narrative review. Pharmacopsychiatry. 2022;55:16-23. doi: 10.1055/a-1551-3756
7. Canal-Rivero M, Catalán-Barragán R, Rubio-García A, Garrido-Torres N, Crespo-Facorro B, Ruiz-Veguilla M. Lower risk of SARS-CoV2 infection in individuals with severe mental disorders on antipsychotic treatment: a retrospective epidemiological study in a representative Spanish population. Schizophr Res. 2021;229:53-54.

8. Dratcu L, Boland X. Can antipsychotic use protect from COVID-19? Schizophr Res. 2021;236:1-2.
9. Nemani K, Conderino S, Marx J, Thorpe LE, Goff DC. Association between antipsychotic use and COVID-19 mortality among people with serious mental illness. JAMA Psychiatry. 2021;78:1391-1393.
10. Bonnet U, Claus B, Schaefer M, et al. Impact of psychiatric and related somatic medication on the duration and severity of COVID-19: a retrospective explorative multi-center study. Pharmacopsychiatry. 2022;55:30-39.
11. Grover S, Kathiravan S, Suman A, et al. Incidence and outcome of COVID-19 in patients with schizophrenia: a study from India. Schizophr Res. 2021;236:38-40.
12. Prokopez CR, Vallejos M, Lopredo LS, et al. An analysis of the possible protective effect of antipsychotics for SARS-CoV-2 in patients under treatment for severe mental illnesses. Schizophr Res. 2021;64(21):99-100.
13. Ohlis A, Sörberg Wallin A, Sarafis A, et al. Clozapine treatment and risk of severe COVID-19 infection. Acta Psychiatr Scand. 2022;145:79-85.
14. Govind R, Fonseca de Freitas D, Pritchard M, Hayes R, MacCabe J. Clozapine treatment and risk of COVID-19 infection: retrospective cohort study. Br J Psychiatry. 2021;219:368-374.
15. Ponsford MJ, Pecoraro A, Jolles S. Clozapine-associated secondary antibody deficiency. Curr Opin Allergy Clin Immunol. 2019;19:553-562.
16. Willcocks IR, Legge SE, Nalmpanti M, et al. Clozapine metabolism is associated with absolute neutrophil count in individuals with treatment-resistant schizophrenia. Front Pharmacol. 2021;12:658734.
17. Shymko G, Clark V, Shymko L, Waters F. Navigating the coprescription of clozapine and fluvoxamine. Schizophr Res. 2018;202:391-393.
18. Clark SR, Warren NS, Kim G, et al. Elevated clozapine levels associated with infection: a systematic review. Schizophr Res. 2018;192:50-56.
19. Veerman SRT, Bogers JPAM, Cohen D, Schulte PFJ. COVID-19: risks, complications, and monitoring in patients on clozapine. Pharmacopsychiatry. 2022;55(01):48-56. doi: 10.1055/a-1562-2521
20. Dragoi AM, Radulescu I, Năsui BA, Pop AL, Varlas VN, Trifu S. Clozapine: an updated overview of pharmacogenetic biomarkers, risks, and safety-particularities in the context of COVID-19. Brain Sci. 2020;1:840.
21. Mazereel V, Van Assche K, Detraux J, De Hert M. COVID-19 vaccination for people with severe mental illness: why, what, and how? Lancet Psychiatry. 2021;8(5):444-450.
22. Silva E, Gee S, Smith S, Gaughran F. Clozapine and COVID-19. Bjpsych Bull. 2020;44:179-180.
23. Teijaro JR, Farber DL. COVID-19 vaccines: modes of immune activation and future challenges. Nat Rev Immunol. 2021;21:195-197.
24. Raaska K, Raitasuo V, Neuvonen PJ. Effect of influenza vaccination on serum clozapine and its main metabolite concentrations in patients with schizophrenia. Eur J Clin Pharmacol. 2001;57:705-708.
25. Makhlouf AT, Van Alphen MU, Manzano GS, Freudenreich O. A seizure after COVID-19 vaccination in a patient on clozapine. J Clin Psychopharmacol. 2021;41(6):689-690.
26. Thompson D, Delorme CM, White RF, Honer WG. Elevated clozapine levels and toxic effects after SARS-CoV-2 vaccination. J Psychiatry Neurosci. 2021;46:E210-E211.
27. Faguet GB. The effect of killed influenza virus vaccine on the kinetics of normal human lymphocytes. J Infect Dis. 1981;143:252-258.
28. Cummins D, Wilson ME, Foulger KJ, Dawson D, Hogarth AM. Haematological changes associated with influenza vaccination in people aged over 65: case report and prospective study. Clin Lab Haematol. 1998;20:285-287.
29. Griffin M, Makris M. Vaccination induced neutropenia. Int J Lab Hematol. 2013;35:e33.
30. Jean S, Romain L, Florvil S, Heyliger FG, Mandal A. A case of sudden very late onset of severe clozapine-induced agranulocytosis following an influenza vaccine. Cureus. 2019;11:e4682.
31. Curzon PG, Muers MF, Rajah SM. Aplastic anaemia associated with influenza A infection. Scand J Haematol. 1983;30:232-234.
32. Hendry CL, Sivakumaran M, Marsh JC, Gordon-Smith EC. Relapse of severe aplastic anaemia after influenza immunization. Br J Haematol. 2002;119:283-284.
33. Shah C, Lemke S, Singh V. Case reports of aplastic anemia after vaccine administration. Am J Hematol. 2004;77:204.
34. Ritz C, Meng W, Stanley NL. Postvaccination graft dysfunction/aplastic anemia relapse with massive clonal expansion of autologous CD8+ lymphocytes. Blood Adv. 2020;4:1378-1382.
35. COVID-19 mRNA Pfizer- BioNTech vaccine analysis print. Accessed December 17, 2021. https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/1041483/Pfizer-_BioNTech_vaccine_analysis_print.pdf
36. Pfizer BioNTech Covid vaccine and leukopenia – a phase IV clinical study of CDC and FDA data. Accessed December 22, 2021. https://www.ehealthme.com/vs/pfizer-biontech-covid-vaccine/leukopenia/
37. Moderna COVID vaccine and leukopenia – a phase IV clinical study of CDC and FDA data. Accessed December 22, 2021. https://www.ehealthme.com/vs/moderna-covid-vaccine/leukopenia/
38. Johnson and Johnson Covid vaccine and leukopenia - a phase IV clinical study of CDC and FDA data. Accessed December 22, 2021. https://www.ehealthme.com/vs/johnson-and-johnson-covid-vaccine/leukopenia/
39. Schoretsanitis G, Kane JM, Correll CU, et al. Blood levels to optimize antipsychotic treatment in clinical practice: a joint consensus statement of the American Society of Clinical Psychopharmacology and the Therapeutic Drug Monitoring Task Force of the Arbeitsgemeinschaft für Neuropsychopharmakologie und Pharmakopsychiatrie. J Clin Psychiatry. 2020;81:19cs13169.
40. Murray M, Zhang WV, Edwards RJ. Variation in the response of clozapine biotransformation pathways in human hepatic microsomes to CYP1A2- and CYP3A4-selective inhibitors. Basic Clin Pharmacol Toxicol. 2018;122:388-395.
41. Miller E. The signed-rank (wilcoxon) test. Lancet. 1969;293(7590):371.
42. IBM Corp. IBM SPSS Statistics for Windows, Version 27.0. IBM Corp. Released 2020.
43. Tomczak M, Tomczak E. The need to report effect size estimates revisited. An overview of some recommended measures of effect size. Trends Sport Sci. 2014;21:19-25.

44. Siegel S, Castellan NJ. Nonparametric Statistics for Behavioral Sciences. McGraw-Hill Book Company Inc; 1988.
45. Ming DS, Heathcote J. Therapeutic drug monitoring of clozapine and norclozapine in human serum using ultra-performance liquid chromatography- tandem mass spectrometry. J Anal Toxicol. 2009;33:198-203.
46. Akamine Y, Sugawara-Kikuchi Y, Uno T, Shimizu T, Miura M. Quantification of the steady-state plasma concentrations of clozapine and N-desmethylclozapine in Japanese patients with schizophrenia using a novel HPLC method and the effects of CYPs and ABC transporters polymorphisms. Ann Clin Biochem. 2017;54:677-685.
47. Turrion MC, Perez J, Bernardo M, Fernandez-Egea E. Intra-individual variation of clozapine and norclozapine plasma levels in clinical practice. Rev Psiquiatr Salud Ment. 2020;13:31-35.
48. Diaz FJ, de Leon J, Josiassen RC, Cooper TB, Simpson GM. Plasma clozapine concentration coefficients of variation in a long-term study. Schizophr Res. 2005;72:131-135.

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