

Clozapine- and non-clozapine-associated neutropenia in patients with schizophrenia: a retrospective cohort study

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Abstract

Introduction: The antipsychotic drug clozapine remains underutilized partly because of the risk of life-threatening adverse effects, such as neutropenia. Therefore, an extensive hematological monitoring program was set up to detect neutropenia.

Methods: In this retrospective cohort study, we used registry-based data from the Capital Region of Denmark to investigate incidence rates of neutropenia among patients with a diagnosis of schizophrenia or other psychotic disorders and treated with clozapine for the first time. In a within-subject design, we compared rates of neutropenia in time periods where patients were exposed to clozapine *versus* time periods, where they were not exposed to clozapine. We also investigated whether the lengths of clozapine-associated neutropenia (CAN) were related to discontinuation of clozapine treatment.

Results: Data from 520 clozapine users were included. The incidence rate of CAN was 3.2 cases per 100 person-years (95% confidence interval [CI]: 2.1–4.8) throughout the entire study. There was no significant difference in incidence rates of neutropenia during clozapine exposure and non-clozapine exposure, with an incidence rate ratio of 0.7 (95% CI: 0.4–1.3). One episode of severe neutropenia was detected. Episodes of CAN with only one sub-threshold neutrophil count were not associated with higher clozapine discontinuation (26%) than CAN episodes of more than one sub-threshold neutrophil count (28%).

Conclusion: In the present study, we could not confirm that clozapine treatment was associated with neutropenia.

Keywords: antipsychotics, clozapine, neutropenia, schizophrenia

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Introduction

A recent survey among psychiatrists reported that 30% of all patients with schizophrenia were classified as treatment-resistant.¹ Clozapine is a second-generation antipsychotic drug with superior efficacy against treatment-resistant schizophrenia.^{2,3} However, clozapine is associated with potentially life-threatening adverse effects, such as neutropenia, where neutropenia is defined as a neutrophil count below $1.5 \times 10^9/L$.⁴ Mild neutropenia (1.0 to less than $1.5 \times 10^9/L$) is generally perceived as harmless. Moderate neutropenia (0.5 to less than

$1.0 \times 10^9/L$) increases risk of infection, especially in immunocompromised patients, and severe neutropenia (less than $0.5 \times 10^9/L$) can result in life-threatening opportunistic infections even in immunocompetent patients.⁵ Increased duration of neutropenia is linked to an increased risk of infections.⁶ Clozapine-associated neutropenia (CAN) occurs most frequently in the first month after clozapine initiation.⁷ The pathophysiological underpinnings of CAN are not elucidated but suspected to be either an autoimmune reaction directed against neutrophil surface antigens or direct

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toxicity of clozapine metabolites on bone marrow and neutrophils.⁸ Previous studies have mainly reported cumulative incidences of CAN ranging from 2.9% to 8.5% of the patients included, with observation periods lasting from 12 to 132 months.^{9–13} Difference in sample sizes and observation time periods as well as inconsistent case definitions limit comparability of those studies. These issues are largely solved by calculating incidence rates instead as well as using homonymous case definitions. The incidence rate of CAN, defined as the number of new cases in a population over a defined period of time, is reported to be 0.4–0.8 cases per 100 patient-years.^{14,15} In comparison, the prevalence of neutropenia in the general population of Denmark and the United States has been estimated to be about 1%.^{16,17}

Among the hematological adverse reactions leading to clozapine discontinuation, CAN is the most important contributor, with studies reporting that 4.5–14.2% of all clozapine discontinuations are due to CAN.^{18–22} The clinician-based decision to discontinue clozapine treatment due to CAN is a consequence of current European guidelines that mandate discontinuation of clozapine treatment in all patients with CAN in order to prevent fatal outcomes.²³ It has been reported that mild or moderate CAN should not generally warrant clozapine discontinuation, but could be managed with other strategies, for example, bone-marrow-stimulating drugs such as granulocyte colony-stimulating factor, lithium or filgrastim.²⁴ However, it remains unknown how often clozapine discontinuation is due to mild/moderate CAN or due to severe CAN. It is also unclear whether the duration of a given CAN episode is associated with clozapine discontinuations. Shorter CAN episodes could be more likely to reflect non-clozapine-related variations in neutrophil count of lesser clinical significance.

Aims of the study

The primary aim of this study was to investigate in a within-subject longitudinal study design whether clozapine exposure is associated with higher incidence of neutropenia as compared with time periods without clozapine exposure. The secondary aim was to investigate whether length of blood-verified neutropenia was associated with an increased incidence of clozapine discontinuation.

Methods

Study design

We conducted a retrospective cohort study on psychiatric inpatients or outpatients from psychiatric hospital clinics in the Capital Region of Denmark from 1 January 2012 to 1 March 2017. To ensure that patients were clozapine treatment-naïve for a minimum of 1 year prior to inclusion, the study period ranged from 1 January 2013 to 1 March 2017. Patients were included in our cohort on the date of their first available neutrophil count. Incidence rates of neutropenia between clozapine and non-clozapine exposure time periods were compared in a within-subject comparison.

We included patients with F20-spectrum diagnosis of psychotic disorders according to the *International Classification of Diseases, 10th revision (ICD-10)*,²⁵ who were clozapine treatment-naïve for a minimum of 1 year prior to inclusion and had blood tests taken according to the mandatory hematological monitoring program for clozapine-treated patients.²³ In Denmark, white blood cell counts are performed on all clozapine users every week during the first 18 weeks of treatment and thereafter every month. When clozapine is discontinued, white blood cell counts continue on a weekly basis for 4 weeks. White blood cell counts taken more than 4 weeks after clozapine discontinuation are not part of the routine monitoring for hematological adverse effects of clozapine. Indication for the white blood cell counts was not provided in the data registries. The exclusion criteria were diagnoses of cancer in lymphatic or hematopoietic tissue, bone marrow metastasis, liver fibrosis, liver cirrhosis, malnutrition, and anemia caused by vitamin B12 deficiency or other vitamin B deficiencies known to reduce white blood cell count up to 6 years prior to study entry (see Supplementary Table 1S).

Data on clozapine utilization and demographics within 1 year from the first possible date of inclusion and throughout the study period were obtained from the Electronic Patient Medication module, which is the database for inpatient and outpatient drug use in the Capital Region of Denmark until May 2017.²⁶ Data on ethnicity were not available. Data on hematologic surveillance, that is, neutrophil counts, were retrieved from the Clinical Laboratory Information System, systemet for sygehus-laboratorier og klinisk biokemiske afdelinger (LABKA).²⁷ We used the mean value of available hematologic blood tests

taken 60 days prior to clozapine initiation as the hematological baseline for each patient. Data sources were linked using the unique and permanent Danish identification number.²⁸

Definition of CAN

It is well known that prolonged neutropenia increases the risk of infections.⁶ We therefore hypothesized that longer lasting episodes of CAN result in more clinical awareness and therefore have a different pattern of development. We have therefore used two definitions of a neutropenia episode:

Definition A: Two or more consecutive neutrophil counts of less than $1.5 \times 10^9/L$ not separated by more than 7 days.

Definition B: A single neutrophil count of less than $1.5 \times 10^9/L$.

CAN was defined as neutropenia occurring in a patient with an active clozapine prescription or neutropenia occurring less than 7 days after discontinuation of clozapine. Two factors were included in this definition: elimination half-life and a cytotoxic or autoimmune response.²⁹ First, clozapine has an elimination half-life of 9–17 h.³⁰ We expected that most patients would have metabolized clozapine 3–4 days following treatment discontinuation and even patients with slower metabolism would have metabolized clozapine no later than 7 days after treatment discontinuation. Second, autoimmune pathogenesis is not necessarily connected to clozapine plasma levels but can be related to rare HLA-DQB1 and HLA-B alleles.³¹ Non-CAN was defined as neutropenia occurring before commencement of clozapine treatment or neutropenia occurring after the end of the seventh day after discontinuation of clozapine.

Neutropenia episodes were stratified into mild neutropenia (neutrophil count of 1.0 to less than $1.5 \times 10^9/L$), moderate neutropenia (neutrophil count of 0.5 to less than $1.0 \times 10^9/L$), and severe neutropenia (neutrophil count of less than $0.5 \times 10^9/L$). A neutropenia episode with changing level of severity was classified by the most severe degree of neutropenia that occurred in this neutropenia episode.

We compared the number of clozapine discontinuations associated with episodes of neutropenia with different time lengths. Clozapine

discontinuation was defined as the end of a clozapine prescription. Treatment discontinuation was defined as neutropenia-dependent when the clozapine discontinuation occurred from 1 day prior to neutropenia to 14 days after the start of neutropenia.

Statistical analysis

Categorical variables were reported as frequencies and percentages. Normal distributed continuous variables were reported as mean and standard deviation and non-normal distributed variables as medians and interquartile ranges. Categorical variables and continuous variables were compared with chi-square test and Kruskal–Wallis test, respectively. Mixed-effects logistic regression was used to analyze potential individual predisposing risk factors for CAN. Due to our small sample size, it was not possible to perform an adjusted regression analysis. Time to first CAN (mild, moderate, or severe) was visualized using a Kaplan–Meier estimation.

Possible confounders, including age, gender, baseline hematology, seasonal variation, concomitant medication with lithium or valproate, incidence of previous episodes of neutropenia, and dose of clozapine at neutropenia event, were included in the risk factor analysis.

Statistical analyses were conducted using R version 3.6.1.³²

Ethics

According to the Danish ‘Act on Research Ethics Review of Health Research Projects’ Section 14 (2), retrospective register-based studies do not require ethical approval or patient consent (assuming anonymous handling of patient data) in Denmark.³³ The study was approved by the Danish Data Protection Agency (BFH-2016–058, I-Suite nr.: 04906) and the Danish Patient Safety Authority (3-3013-1884/1/).

Compliance with ethics guidelines. This article is based on previously collected human health data. No human participants were recruited for this study.

Data availability. The dataset used in this study cannot be made available in accordance with Danish Law.

Results

Baseline comparison of clozapine users with and without neutropenia

The main characteristics of the 520 clozapine users included in our study are shown in Table 1. Clozapine users were grouped according to if they developed CAN or not. The clozapine users that developed CAN were further categorized into three subgroups: clozapine users that only developed CAN definition A episodes, clozapine users that only developed CAN definition B episodes, and finally clozapine users that developed both CAN definition A episodes and CAN definition B episodes. Clozapine users that developed CAN had significantly lower baseline levels of leukocytes, neutrophils, thrombocytes, and monocytes compared with clozapine users that did not develop neutropenia. No differences with respect to gender, age at onset of clozapine treatment, prevalence of other diagnoses, number of treatment discontinuations, or length of clozapine treatment were detected. Two clozapine users with CAN received concomitant treatment with valproic acid and three clozapine users with CAN received concomitant treatment with lithium during the study period. Additional demographic variables are reported in Supplementary Tables 2S and 3S.

Incidence of CAN in patients on versus off clozapine

Entire study period. The total length of the observation period was 1787 person-years with 773 person-years of clozapine exposure. Table 2 compares the number of CAN definition A and definition B episodes with or without clozapine exposure.

Fifteen clozapine users had in total 25 episodes of CAN (definition A) with an incidence rate of 3.2 per 100 person-years [95% confidence interval, CI: 2.1–4.8]. Sixteen CAN definition A episodes were characterized as mild, eight as moderate, and one as severe. Twenty-four clozapine users had 38 CAN definition B episodes with an incidence rate of 4.9 per 100 person-years [95% CI: 3.5–6.7]. Of these episodes, 36 were characterized as mild, 2 as moderate, and none as severe. Seven clozapine users developed both CAN definition A episodes and CAN definition B episodes. In time periods with no clozapine exposure, 19 clozapine users developed 24 CAN definition A episodes with an incidence rate of 2.4 per 100 person-years [95% CI: 1.5–3.5]. Thirty-eight clozapine users had 64 CAN definition B episodes with

an incidence rate of 6.3 per 100 person-years [95% CI: 4.9–8.1]. There was no statistically significant difference between incidence rates of CAN definition A episodes, CAN definition B episodes, or a combination among clozapine users when exposed to clozapine compared with when not exposed to clozapine.

Figure 1 shows the number of CAN definition A episodes and CAN definition B episodes, and the number of these episodes that were associated with treatment discontinuation. The larger number of sub-threshold neutrophil counts per episode in CAN definition A episodes was not more strongly associated with clozapine treatment discontinuation. Twenty-eight percent of CAN definition A episodes were associated with clozapine discontinuation, while 26% of the CAN definition B episodes were associated with clozapine discontinuation.

Forty-seven percent of the CAN definition A or CAN definition B episodes that were associated with clozapine discontinuation were the first ever neutropenia episode in the respective clozapine user. Sixty-three percent of these episodes were characterized as mild neutropenia. Fifteen clozapine users had more than one episode of CAN definition A or CAN definition B through the study period. Forty percent of these clozapine users discontinued clozapine treatment in relation to a later episode of CAN definition A or CAN definition B, whereas the remaining 60% continued clozapine treatment despite developing as many as nine new episodes of CAN definition A or CAN definition B.

The CAN definition A episodes that were not associated with clozapine discontinuation were followed by another episode of CAN definition A or CAN definition B episode in 78% of cases, 11% continued treatment after the CAN episode and discontinued treatment later in the study period, and 11% continued treatment throughout the study period. CAN definition B episodes that were not associated with clozapine discontinuation were followed by another episode of CAN definition A or CAN definition B in 53% of cases, 10% continued treatment after the CAN episode and discontinued treatment later in the study period, and 37% continued treatment throughout the study period. Fifty-six percent of all CAN definition B episodes were followed by a normalized neutrophil count within 7 days. Forty-four percent of all CAN definition B episodes were

Table 1. Demographics, baseline hematology, and clozapine treatment..

	No CAN	≥1 episodes of CAN			p value
		Definition A only (≥2 sub-threshold neutrophil counts, taken <7 days apart)	Definition B only (1 sub-threshold neutrophil count)	Both definition A and definition B	
	n = 488	n = 8	n = 17	n = 7	
Female, No (%)	212 (43.4%)	4 (50.0%)	7 (41.2%)	3 (42.9%)	0.538
Age (years)	37.4 [26.5–50.1]	30.8 [29.2–35.8]	28.6 [21.1–44.4]	33.4 [29.7–35.2]	0.179
Schizophrenia	429 (87.9%)	6 (75.0%)	15 (88.2%)	7 (100%)	0.529
Clozapine discontinuation (all causes)	203 (41.6%)	7 (87.5%)	7 (41.2%)	4 (57.1%)	0.060
Length of clozapine treatment (days)	504 [145–951]	100 [39–357]	484 [96–872]	309 [171–883]	0.309
Baseline leukocyte count (×10 ⁹ /L)	7.72 [6.22–9.25]	4.56 [4.06–4.93]	5.52 [4.47–7.72]	4.58 [3.87–5.63]	<0.001
Baseline neutrophil count (×10 ⁹ /L)	4.49 [3.53–5.88]	1.90 [1.55–2.07]	2.88 [2.06–4.00]	1.95 [1.82–2.09]	<0.001
Baseline eosinophil count (×10 ⁹ /L)	0.18 [0.10–0.27]	0.11 [0.09–0.17]	0.16 [0.09–0.23]	0.11 [0.06–0.23]	0.443
Baseline thrombocyte count (×10 ⁹ /L)	251 [209–293]	225 [211–237]	207 [175–226]	197 [185–204]	0.005
Baseline lymphocyte count (×10 ⁹ /L)	2.09 [1.63–2.56]	1.94 [1.77–2.27]	2.08 [1.78–2.72]	1.79 [1.73–2.11]	0.823
Baseline monocyte count (×10 ⁹ /L)	0.55 [0.43–0.69]	0.41 [0.36–0.43]	0.45 [0.36–0.53]	0.44 [0.38–0.56]	0.014

CAN, clozapine-associated neutropenia; IQR, interquartile range. Categorical variables are reported as number (%), and constant variables were evaluated graphically and reported as median and IQR; p value refers to the comparison of all four groups. Significant p values equal significant difference between one or more groups compared with the remaining groups.

followed by one or more sub-threshold neutrophil counts within 7 days.

Initial 18 weeks of clozapine treatment. The incidence rate (IR) of CAN definition A in the first 18 weeks of clozapine treatment was higher than in the entire study period: IR of 7.5 per 100 person-years [95% CI: 3.7–14.5]. The cumulative incidence of CAN definition A in the first 18 weeks of clozapine treatment was 2.1%.

Time to first CAN episode

Approximately two-thirds of the clozapine users' first CAN definition A episodes occurred during the first 18 weeks of clozapine treatment. A Kaplan–Meier visualization of time to first CAN

definition A episode is provided in Supplementary Figure 1S.

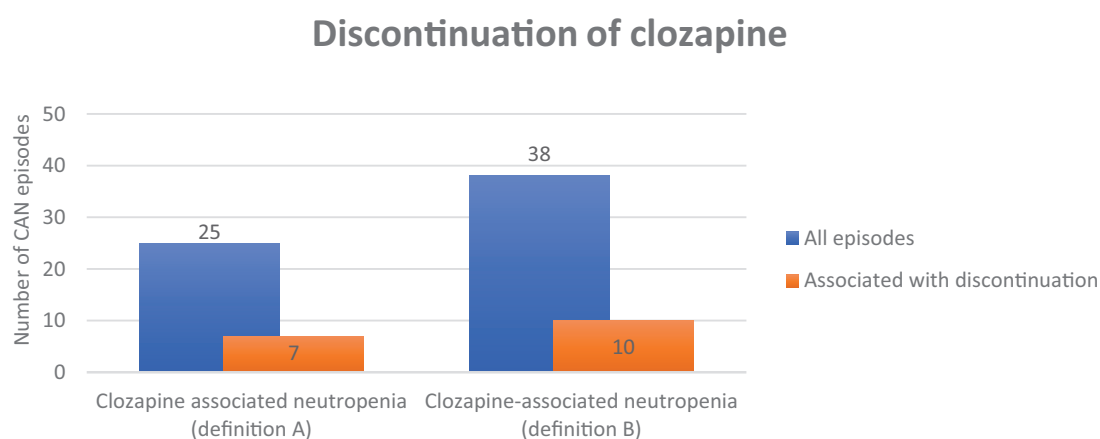
Analysis of predictive risk factors for CAN

Table 3 provides the results of the analysis of potential predictive risk factors for CAN definition A (all types of severity). We identified low baseline neutrophil count and previous history of neutropenia as risk factors for a new episode of CAN. The number of clozapine users that received concomitant treatment with either valproic acid or lithium was too small for meaningful risk factor analysis and is therefore not reported. The limited sample size resulted in wide CIs which hamper firm interpretation of data.

Table 2. Comparison of incidence rates of definition A and definition B neutropenia among clozapine users in periods of clozapine exposure and periods of no exposure.

	Clozapine exposure			No clozapine exposure			Incidence rate ratio [95% CI]
	Clozapine users (n)	Episodes (n)	Incidence rate (per 100 person-years) [95% CI]	Clozapine users (n)	Episodes (n)	Incidence rate (per 100 person-years) [95% CI]	
Definition A	15	25	3.2 [2.1–4.8]	19	24	2.4 [1.5–3.5]	0.7 [0.4–1.3]
Definition B	24	38	4.9 [3.5–6.7]	38	64	6.3 [4.9–8.1]	1.3 [0.9–1.9]
Definition A and definition B	32	63	8.1 [6.3–10.4]	41	88	8.7 [7.0–10.7]	1.1 [0.8–1.5]

CI, confidence interval.

**Figure 1.** Comparison of the number of CAN definition A episodes and CAN definition B episodes as well as the number of episodes associated with clozapine discontinuation.

Incidence of clozapine-associated eosinopenia

Although female clozapine users had significantly lower median baseline eosinophil counts than male clozapine users (0.15 versus $0.20 \times 10^9/L$, $p = 0.031$), none of the clozapine users experienced eosinopenia (eosinophil count $< 0.04 \times 10^9/L$) throughout the observation period.

Discussion

In the present study, we did not find that clozapine exposure was associated with higher incidence of neutropenia nor that the length of a neutropenia episode was associated with clozapine discontinuations.

To the best of our knowledge, a generally accepted definition of a CAN episode does not exist. Often, episodes are just defined as the occurrence of neutrophil counts below $1.5 \times 10^9/L$. Previous

retrospective studies on CAN solely defined an episode of neutropenia based on occurrence of sub-threshold neutrophil counts without taking episode duration into account.^{9–15} When the definition of an episode is based only on the threshold neutrophil count value, low specificity will occur. Many clozapine users with single sub-threshold neutrophil counts are most likely not experiencing a clinically significant episode of neutropenia.³⁴ In order to use a case definition with higher specificity (definition A), we defined a neutropenia episode as at least two consecutive sub-threshold neutrophil counts not separated by more than 7 days. This might have reduced sensitivity by misclassifying clinically significant episodes consisting of single neutrophil counts, although we found this unlikely. We assume that many clinicians would perform at least one more neutrophil count of patients with a single sub-threshold neutrophil count.

Table 3. Predictive risk factor analysis by mixed-effects logistic regression analysis.

Predictive risk factors for clozapine-associated neutropenia	OR	95% CI	p value
Baseline neutrophil count $<2.0 \times 10^9/L$	24.4	[6.4–215.0]	<0.001
Neutropenia (any type) in previous 90 days	11.4	[3.9–34.9]	<0.001
Age at clozapine start (change per 10 years)	0.7	[0.4–1.1]	0.196
Female sex	1.5	[0.5–5.7]	0.493
Winter (15 December–15 March) <i>versus</i> other seasons	0.6	[0.3–6.7]	0.744

CI, confidence interval; OR, odds ratio.

To the best of our knowledge, the literature did not provide a definition of when an episode of neutropenia is related to clozapine treatment or not. In the present study, we have defined CAN as neutropenia occurring while the patient is treated with clozapine or up to 7 days following clozapine discontinuation. It is possible that some of the CAN episodes observed in our total cohort were in fact not associated with clozapine treatment but caused by other factors. To address this, we excluded clozapine users that had diagnoses of hematological cancers, bone marrow metastases, nutritional deficiencies, and liver diseases, all able to reduce the white blood cell count. None of the episodes occurred while patients were hospitalized for a somatic condition and none of the patients with neutropenia episodes were diagnosed with a concomitant upper respiratory infection or influenza. It is still possible that clozapine users had contracted a virus responsible for their lowered neutrophil count. However, this kind of information was not available in the patients' electronic health records. We found no seasonal variation in the occurrence of CAN which either indicates that seasonal virus infections had no impact on the results or that the sample size was too small to detect a possible difference.

Because our data sources did not provide data on patients' ethnicity, we could not estimate rates of benign ethnic neutropenia in our cohort. Benign ethnic neutropenia is prevalent among people of African and Middle Eastern ethnicity and is not a risk factor for infectious diseases.³⁵ Immigrants in Denmark are known to have higher incidence of schizophrenia than native Danes.³⁶ As of 2017, people of non-Western ethnicity account for 12% of the population of the Capital Region of Denmark with the majority being of Middle Eastern ethnicity.³⁷ It is therefore plausible that a

corresponding part of the included clozapine users in our study is of an ethnicity that predisposes to benign ethnic neutropenia. This might have led to an overestimation of the incidence of CAN and non-CAN as benign ethnic neutropenia episodes might have been misclassified as CAN or non-CAN. We did not include concomitant treatment with other antipsychotics in our analysis, since a recent meta-analysis found that the risk of neutropenia for patients treated with additional antipsychotics was comparable with the risk of neutropenia for patients treated only with clozapine.³⁸ However, valproic acid has earlier been reported to decrease white blood cell count,³⁹ but in the present study, we did not find valproic acid to be associated with CAN. Lithium which can be used to increase neutrophil count in patients with CAN⁴⁰ was neither associated with CAN.

CAN definition A episodes were equally associated with clozapine discontinuation as CAN definition B episodes. This finding is notable since it reflects a difference between the European clozapine guideline, which mandates clozapine discontinuation in patients with all severity levels of neutropenia, and current clinical practice as shown through our data.²³ However, our study design and the low number of CAN events do not allow evaluation of all aspects of the clinical decision making and can therefore not explain this difference. We believe that some psychiatrists are likely to accept the risk of continuing clozapine treatment in patients with mild and moderate neutropenia because the benefits of clozapine may outweigh the potential risk of CAN. Other psychiatrists might follow the guidelines more rigorously and, for example, discontinue clozapine in patients with a single count of mild neutropenia. Our study does not allow us to conclude on

whether it would be advisable to continue or discontinue clozapine treatment based on only one measurement, that is, CAN definition B episode. However, mild neutropenia is in general not considered dangerous,⁵ and clinicians should note that the US Food and Drug Administration (FDA) recently changed its guidelines on clozapine treatment and now allows continuation of clozapine use in patients with mild neutropenia.⁴¹ Our study showed that 63% of CAN episodes were of mild severity. Only one episode of severe CAN was detected. Duration of most CAN episodes was short as 60% of CAN episodes consisted of merely one sub-threshold neutrophil count. A substantial part of clozapine discontinuation occurred following CAN episodes of mild severity. In order to counteract the current underutilization of clozapine, we believe it would be relevant for the European Medicines Agency (EMA) to reconsider their recommendations regarding discontinuation of clozapine treatment following mild neutropenia. Moreover, based on our present data which show that 56% of CAN definition B episodes are followed by normal neutrophil counts, we believe that one blood sample showing sub-threshold neutrophil counts should be followed up by a second neutrophil count which is in accordance with a number of hematological guidelines, for example, the Mayo Clinic.⁴² Risk factor analysis showed that patients with low baseline neutrophil count or previous neutropenia episodes were at increased risk for new episodes of CAN. Clinicians need to evaluate if the benefits of clozapine outweigh the risks of neutropenia in such patients before prescribing clozapine. Our study could not confirm findings from previous studies that age and female gender increase the risk for new CAN episodes.⁴³

The extensive hematologic monitoring of patients treated with clozapine may well contribute to its underutilization too.⁴⁴ Kaplan–Meier estimation showed that two-thirds of all episodes in our study occurred within the first 18 weeks of clozapine treatment (Supplementary Figure 1S), which is in accordance with the literature.⁷ Whether the frequency of hematologic monitoring is appropriate cannot be concluded based on our study.

In discordance with our earlier report on gender-specific eosinopenia in clozapine users, we found no patients with clozapine-associated eosinopenia defined as at least 2 consecutive eosinophil counts of less than $0.04 \times 10^9/L$, occurring in a patient that had an active prescription of clozapine or had

discontinued clozapine less than 7 days before the start of the eosinopenia episode.⁴⁵

Strengths and limitations

Among the strengths of our study are that the neutrophil counts of clozapine users were collected *via* a mandatory surveillance program and hence were less likely to be confounded by other indications. We included all available clozapine users and therefore minimized selection bias. Patients included had not been treated with clozapine for at least 1 year, and therefore, results are more likely to reflect the effect of exposure to clozapine. Our definition of a CAN episode consists of both the neutrophil count and a time component, resulting in higher specificity. In our analysis, we included several potential confounders and effect modifiers such as season of year, hospitalizations, and viral infections. Concomitant treatment with valproic acid or lithium was not controlled for but would ideally be in a larger sample.

The study limitations include the relatively small sample size. Due to the within-subject comparison design, there is a potential risk that observation of several episodes of neutropenia occurring in the same clozapine-treated patient is not independent. In addition, causal relation between clozapine and neutropenia episodes could not be evaluated due to our retrospective observational study design. We think that our case definition still lacks specificity and leads to overestimation of the number of CAN episodes because the incidence rate of CAN in our study is four to eight times higher than what has been reported in previous studies. Some of the CAN episodes consisting of two or more consecutive sub-threshold neutrophil counts might in fact be clinically non-significant, with diurnal variations (pseudo-neutropenia) or preexisting benign ethnic neutropenia being possible explanations.^{34,46} Since data on ethnicity were not available, we could not investigate the possible occurrence of preexisting benign ethnic neutropenia. We were not able to conclude whether an episode of CAN caused discontinuation of clozapine treatment. Despite this, we were able to adjust for some confounders albeit not all, for example, other antipsychotic medication or the ethnicity of clozapine users. Forty percent of patients on clozapine did not have all 18 neutrophil counts taken during the first 18 weeks of treatment, resulting in missing data. The blood tests taken during clozapine treatment and the first 4 weeks past discontinuation of clozapine

treatment are part of the hematological monitoring for clozapine adverse effects in Denmark. Patients that did not have an active clozapine prescription yet or had discontinued clozapine more than 4 weeks before the blood test was taken had blood tests taken for other reasons than screening for clozapine-associated hematological adverse effects. These blood tests therefore have been confounded to a greater extent than those taken when the patients were taking clozapine or had just discontinued it. It cannot be estimated if this confounding has increased or decreased incidence of neutropenia in our study.

Conclusion

In the present study, we could not confirm that clozapine exposure was associated with neutropenia. Instead, the episodes of neutropenia were associated with other risk factors: low baseline neutrophil count and previous episodes of neutropenia. Duration of CAN episodes was not associated with clozapine discontinuations, and two-thirds of the first CAN episodes occurred during the first 18 weeks of treatment, in accordance with previous reports. To increase comparability of future studies on CAN, we propose that a common case definition consisting of both the neutrophil count and a time interval is adopted. Studies with larger sample sizes are needed to further investigate the association between clozapine exposure and neutropenia.

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Author contributions

Claas-Frederik Johannsen: Conceptualization; Formal analysis; Investigation; Methodology; Project administration; Software; Visualization; Writing – original draft; Writing – review & editing.

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Supplemental material

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