ORIGINAL ARTICLE

Incidence and risk for neutropenia/agranulocytosis among clozapine users: A retrospective cohort study

CHAVEEWAN RATANAJAMIT1, CHUTIMA MUSAKOPAS1, SORAYUT VASIKNANONTE2 & WANTANA REANMONGKOL1

1Department of Clinical Pharmacy, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Thailand, and 2Department of Psychiatry, Faculty of Medicine, Prince of Songkla University, Hat Yai, Thailand

Abstract

Objective. To estimate the incidence and the risk of neutropenia or agranulocytosis (the outcome) associated with clozapine use (the exposure), and to identify risk factors. Methods. All data were derived from the computerized hospital database. Adult psychiatric patients were identified, and 95 incident clozapine users and 884 non-clozapine users were included. Cox proportional hazards regression was used to estimate the hazards ratio (HR) of developing the outcome after clozapine use adjusted for confounders. The interaction between clozapine and valproic acid was assessed a posteriori. Results. Throughout the 24-month follow-up, the incidence of neutropenia was 6.3% in the clozapine group and 5.8% in the non-clozapine group. One agranulocytosis was found in the non-clozapine group. The HR (95% CI) for neutropenia were: clozapine 1.33 (0.54–3.25) and age > 45 years 2.99 (1.63–5.48). Lithium, as an independent protective factor, reduced the risk for neutropenia by 85% compared with patients who did not receive lithium, HR 0.15 (95% CI 0.02–1.09). Valproic acid might potentiate the clozapine-associated neutropenia (HR 5.10, 95% CI 0.70–37.12). Conclusion. Clozapine might slightly increase the risk of neutropenia in psychiatric patients. Concerning clozapine-associated neutropenia, older patients are at increased risk and use of valproic acid concurrently with clozapine should be avoided.

Key Words: Clozapine, neutropenia, agranulocytosis, cohort, valproic acid, psychosis

Introduction

Neutropenia and agranulocytosis are the most important drug-related dyscrasias [1]. Due to a higher incidence of neutropenia/agranulocytosis associated clozapine, this drug is restricted to refractory schizophrenia and recurrent suicidal behavior [1]. The cumulative incidence of agranulocytosis among clozapine users was 0.8% (1-year follow-up) to 0.9% (3-year follow-up) [1–3], and the cumulative incidences of neutropenia were 1.7% (over 3 years), 2.9% (over 4.5 years) [4], and 19.8% (over 8 years) [5]. The US FDA suggests any patient prescribed with clozapine should have weekly white blood cell (WBC) monitoring during the first 18 weeks, and then monthly monitoring for the whole period of clozapine therapy. In addition to clozapine, many antipsychotics (chlorpromazine, haloperidol, thiouridazine, quetiapine, risperidone and ziprasidone), hypnosedatives (barbiturates and benzodiazepines), and antiepileptics (carbamazepine and valproate) are associated with higher risks of agranulocytosis and neutropenia [1,6,7]. Drug therapy in psychiatric patients might therefore increase the risk of agranulocytosis or neutropenia.

This study aimed (1) to examine the incidence and the cumulative hazard of agranulocytosis/neutropenia among Thai incident users of clozapine in the first 24 months of use, (2) to examine the risk for developing neutropenia/agranulocytosis in psychiatric patients treated with clozapine compared with those who were not, and (3) to identify the risk factors of neutropenia/agranulocytosis in psychiatric patients. In addition, post hoc analysis was done to assess whether valproic acid was an effect modifier of clozapine-associated neutropenia or agranulocytosis. This is probably the first controlled study that
examined the risk of neutropenia/agranulocytosis associated with clozapine in psychiatric patients who were not exposed to chemotherapy.

**Methods**

**Design**

The association between the use of clozapine and the development of agranulocytosis or neutropenia was studied in a cohort of in- and out-psychiatric patients who were treated at Songklanagarind Hospital, a medical teaching hospital in southern Thailand. The reference cohort consisted of psychiatric patients who did not receive clozapine. The study was approved by the Ethics Committee of the Faculty of Medicine, Prince of Songkla University (SUB.EC 50/400-028).

**Study population and setting**

The hospital computerized database of Songklanagarind Hospital was initiated before 2000. It includes a population of approximately 1,500,000 patients. The study included 95 incident clozapine users and 884 non-clozapine users, identified using the hospital computerized database. The eligible subjects were adult patients diagnosed with psychosis or mood disorders and treated between 1 January 2002 and 31 December 2007. All diagnoses were recorded according to the International Classification of Diseases 10th revision (ICD-10) [8]. Patients were excluded if they had any of the following criteria: (1) history of diseases of the blood or blood-forming organs (ICD-10 code D50–D89) including agranulocytosis or neutropenia (ICD-10 code D70) diagnosed prior to the study entry; (2) history of malignant neoplasms (ICD-10 code C00-C97) treated with chemotherapy within 3 months prior to the study; (3) human immunodeficiency virus (HIV) disease (ICD-10 code B20–B24); (4) history of systemic lupus erythematosus (ICD-10 code M32); (5) history of hyperthyroidism (ICD-10 code E05); or (6) no available medical information, medication record, or hematology record.

**Sample size**

The study required 94 clozapine incident users for incidence estimation. It was calculated under the assumed incidence of 1.4% with a 95% confidence interval (CI) ± 0.7%. The sample size to detect the relative risk of 2.0, with the exposed to non-exposed ratio of 1:10, 80% power and significance level of 0.05 required 164 clozapine-exposed subjects and 1,640 clozapine-nonexposed subjects. This study planned to include not less than 164 incident clozapine users.

**Data collection**

From the hospital computerized database, demographic information, such as date of birth, gender, weight, diagnoses, etc., was collected. Exposure data, outcome data, as well as potential confounding variables, were likewise extracted from the same database.

**Exposure data.** The dose and duration of clozapine, Anatomical and Therapeutic Classification (ATC) code N05AH02, prescribed were recorded in the computerized database by the doctors. The prescription had to be redeemed at the pharmacy department within the next 3 days, otherwise it would be automatically deleted from the database. As drug-induced neutropenia usually occurs after 1–2 weeks of use, and agranulocytosis after 3–4 weeks [1], only the incident users of clozapine who were prescribed at least 3 weeks were included in the clozapine-exposed group, otherwise they were included in the other group.

**Outcome data.** The outcome data including diagnosis of agranulocytosis or neutropenia (ICD-10 code D70) were recorded in the computerized database by the doctors. WBC count and polymorphonuclear (PMN) cell count were recorded by the hematology technicians. Agranulocytosis was defined as a neutrophil count (ANC) \( \leq \) 500/mm\(^3\) and neutropenia was defined as ANC = 500–2,000/mm\(^3\). To ascertain the occurrence of agranulocytosis or neutropenia, both the diagnosis and the WBC count at the time of diagnosis were considered. In the clozapine group, at least 21 days of clozapine exposure was required for the induction time of agranulocytosis/neutropenia associated with clozapine. The time that each patient was included in the study and the time of diagnosis of the outcome were also extracted from the database, so that time until neutropenia or agranulocytosis could be calculated. To study the risk for neutropenia/agranulocytosis associated with clozapine, only the incident outcome of neutropenia or agranulocytosis was included.

**Potential confounders.** Data on potential confounding factors, including the patient’s age, gender, other antipsychotics (first generation and atypical antipsychotics) known to be associated with neutropenia/agranulocytosis, and other concomitantly prescribed
Multivariate analysis was performed by use of backward Cox proportional hazards regression modeling to estimate the adjusted hazard ratio (HR) of developing neutropenia/agranulocytosis after clozapine use, adjusted for other confounding factors such as age (≤45 and >45 years), other antipsychotics, and other concomitant drugs (received or not received), and including an interaction term to evaluate the possibility of effect modification. The covariates were deleted from the model one at a time and the new model was tested for significance against the previous one by likelihood ratio test. Since the study was directed towards identifying the effects of clozapine, only variables that caused substantial confounding were included in the final model. All analyses were performed on the commercial statistical software (STATA program, version 8.0, Stata Corporation, Texas, USA).

Results

The characteristics of the study cohorts at baseline are presented in Table I. Although the sample size was large enough for incidence estimation, it was too small for risk estimation. Clozapine was prescribed more than 3 weeks to all clozapine users, with the mean [range] of clozapine maintenance dose of 187.2 (25–500) mg/day. Among the non-clozapine group, in addition to schizophrenia that was treated with first generation antipsychotics, other diagnoses were major depression (most of them were treated medications, such as lithium, spironolactone, carbamazepine, valproic acid, phenytoin, and ticlopidine [9-12], were extracted.

Table I. Characteristics and outcomes of study cohorts.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Incident clozapine users (n = 95)</th>
<th>Non-clozapine users (n = 884)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>40 (42.1)</td>
<td>555 (62.8)</td>
<td>0.0005a</td>
</tr>
<tr>
<td>Male</td>
<td>55 (57.9)</td>
<td>329 (37.2)</td>
<td></td>
</tr>
<tr>
<td>Age (years), (mean ± SD)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤45 years, n (%)</td>
<td>34.0 ± 10.9</td>
<td>44.4 ± 15.6</td>
<td>0.0005</td>
</tr>
<tr>
<td>&gt;45 years, n (%)</td>
<td>81 (85.3)</td>
<td>456 (51.6)</td>
<td>0.0005a</td>
</tr>
<tr>
<td>Weight (kg), (mean ± SD)</td>
<td>61.9 ± 12.4</td>
<td>58.9 ± 10.9</td>
<td>0.1035b</td>
</tr>
<tr>
<td>Diagnosis, n (%)</td>
<td></td>
<td></td>
<td>0.0005a</td>
</tr>
<tr>
<td>Schizophrenia (F20)</td>
<td>59 (62.1)</td>
<td>143 (16.2)</td>
<td></td>
</tr>
<tr>
<td>Schizo-affective disorder (F25)</td>
<td>17 (17.9)</td>
<td>25 (2.8)</td>
<td></td>
</tr>
<tr>
<td>Bipolar disorders (F31)</td>
<td>8 (8.4)</td>
<td>139 (15.7)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>13 (13.7)</td>
<td>588 (65.6)</td>
<td></td>
</tr>
<tr>
<td>Baseline WBC/mm³, (median) [range]</td>
<td>7,600 [3,700, 14,200]</td>
<td>7,400 [2,800, 19,800]</td>
<td>0.9915e</td>
</tr>
<tr>
<td>Concomitant drugs, n (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithium</td>
<td>6 (6.3)</td>
<td>109 (12.3)</td>
<td>0.0840a</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>21 (22.1)</td>
<td>74 (8.4)</td>
<td>0.0005a</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>6 (6.3)</td>
<td>40 (4.5)</td>
<td>0.4330a</td>
</tr>
<tr>
<td>Ticlopidine</td>
<td>0 (0.0)</td>
<td>6 (0.7)</td>
<td>0.4210b</td>
</tr>
<tr>
<td>Phenytoin</td>
<td>0 (0.0)</td>
<td>8 (0.9)</td>
<td>0.3520a</td>
</tr>
<tr>
<td>Outcome developed, n (%)</td>
<td></td>
<td></td>
<td>0.897a</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>6 (6.3)</td>
<td>51 (5.8)</td>
<td></td>
</tr>
<tr>
<td>Agranulocytosis</td>
<td>0 (0.0)</td>
<td>1 (0.1)</td>
<td></td>
</tr>
</tbody>
</table>

*aChi-square test; bStudent’s t-test; cMann–Whitney U-test; dFisher’s exact test.
these corresponding times were 2 and 711 days. Among psychiatric patients, neutropenia seemed to be higher in females than in males (67.2 vs. 32.8%, \( P = 0.2980 \)).

From crude analysis, the HR for developing neutropenia associated with clozapine was 1.05. After adjustment for other potential confounders it increased approximately 30%, to 1.33, but with low precision, compared with psychiatric patients who did not receive clozapine (Table II). This study had only 12% power to detect a HR of 1.33. Age \( \geq 45 \) years significantly increased the risk of neutropenia 3-fold (HR 2.99, 95% CI 1.63 – 5.49, \( P = 0.0005 \)), while lithium seemed to reduce the risk (HR 0.15, 95% CI 0.02 – 1.10, \( P = 0.0620 \)). There was no evidence that other antipsychotics increased the risk of neutropenia (HR 1.27, 95% CI 0.67 – 2.42, \( P = 0.456 \)). Other variables, such as gender, benzodiazepines, antidepressants, carbamazepine, spironolactone, ticlopidine, and phenytoin were not significant confounders and were excluded from the model.

Although assessment of interaction (effect modification) was not designed ad hoc, there was likely to be potential interaction (synergism) between valproic acid and clozapine in the development of neutropenia since the effect of combination therapy was more than a simple addition of the effects of individual drugs (HR 5.10, 95% CI 0.70 – 37.12, interaction \( P = 0.1070 \)).

Discussion

This hospital-based retrospective cohort study found 6.3% incidence of neutropenia among clozapine users throughout the 24-month follow-up and no cases were reported after 1 year. Clozapine might only slightly increase the risk of neutropenia compared with psychiatric patients who did not receive clozapine. The risk of neutropenia increased in patients age \( \geq 45 \) years, but it seemed to decrease by lithium use. Valproic acid might potentiate the effect of clozapine on the development of neutropenia.

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>Crude HR (95% CI)</th>
<th>( P ) value</th>
<th>Adjusted* HR (95% CI)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>clozapine use</td>
<td>1.05 (0.45–2.44)</td>
<td>0.9095</td>
<td>1.33 (0.54–3.25)</td>
<td>0.5360</td>
</tr>
<tr>
<td>Age &gt; 45 years</td>
<td>3.08 (1.73–5.48)</td>
<td>0.0005</td>
<td>2.99 (1.63–5.48)</td>
<td>0.0005</td>
</tr>
<tr>
<td>Concomitant drug use</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lithium</td>
<td>0.11 (0.02–0.82)</td>
<td>0.0315</td>
<td>0.15 (0.02–1.09)</td>
<td>0.0625</td>
</tr>
<tr>
<td>Valproic acid</td>
<td>1.75 (0.83–3.68)</td>
<td>0.1445</td>
<td>1.62 (0.58–4.53)</td>
<td>0.3580</td>
</tr>
</tbody>
</table>

*HR adjusted for age group (\( \leq 45 \) versus > 45 years), valproic acid, lithium use, and interaction term (clozapine_valproic acid).
The incidence of clozapine-associated neutropenia in the first year of use found in this study is similar to a 7.4% incidence in 6,782 Koreans, using the same definition of neutropenia [5]. The cumulative incidences of reported clozapine-induced neutropenia ranged between 0.9% [17] and 19.8% (8-year cumulative incidence) [5]. The differences in these figures might be partly due to the differences in the definition of neutropenia, ethnicity, duration of the study, or the hematological monitoring practices, which varied among settings [12]. In transient neutropenia WBC can return to normal within an average of 1.4 weeks without dose adjustment [18], and cases would be missed if the hematological monitoring was not frequently performed. Among a subgroup of 5,199 patients (41% of 12,760 clozapine users actively monitored in the UK and Ireland) the hematological safety of clozapine in patients monitored at 2-weekly intervals and those monitored at 4-weekly intervals after at least 1 year of treatment were compared. The incidences of neutropenia were 0.25% versus 4.7% in 4- and 2-weekly monitoring intervals, respectively [12]. As the incidence of neutropenia related to clozapine was highest during the first 6 months of use, it was suggested that WBC monitoring of clozapine was not cost-effective after 6 months [19], but this study shows a steadily increasing cumulative incidence over at least the first 12-month follow-up, that might confirm the recommendation of a regular monthly WBC monitoring after the first 18 weeks of clozapine use.

The cumulative incidence of neutropenia in the non-clozapine group was very similar to that found in the clozapine group. This might indicate the risk of neutropenia related to psychoactive drugs, such as valproic acid, carbamazepine or others [1]. In addition, it might be attributable to selection bias as this study included only psychiatric patients whose hematological data were available. Such a selection bias might overestimate the risk of neutropenia among non-clozapine users because WBC surveillance in this group was less intensive than in the other group as regular monitoring was not recommended and patients presenting with signs of neutropenia or infection (such as fever, chill, or sore throat) were more likely to have WBC data.

The incidence of agranulocytosis associated with clozapine was not found in this study, like previous studies among Thais [20,21]. Apart from random variability, the study was too small to detect agranulocytosis as the reported incidence has been between 0.3 and 1.0% [1,9,9].

The estimate of clozapine on the risk of neutropenia in psychiatric patients lacked precision as the observed HR (1.33) was smaller than the assumed one (2.0) and would have required a larger sample size to achieve the desired precision of the estimate. The estimated risk was expected to be confounded by indication as clozapine prescribing guideline requires WBC to be > 3500/mm³ and thus could lead to underestimation of the HR. Moreover, as discussed earlier, the risk of neutropenia for the non-clozapine group was likely to be overestimated due to selection bias, and this could also bias the HR towards the null. Rigorously designed studies assessing the risk of neutropenia related to clozapine were not identified, the consistency of our findings with others was therefore not examined.

We found borderline evidence of the protective effect of lithium against neutropenia. Although the reported effects of lithium on neutropenia in patients treated with clozapine have been contradictory [22–28], most studies have documented the benefit of lithium on clozapine-associated neutropenia, either on prevention [27,29] or treatment [24–26,30]. Epidemiological studies suggest that there may be different risk factors for clozapine-induced neutropenia and agranulocytosis [1]. For example, the risk of agranulocytosis increases with age [4,9,12], but the risk of neutropenia does not increase and may even decrease with increasing age [1,4]. However, this study found that age over 45 years significantly increased the risk of neutropenia 3-fold. Several studies have demonstrated an increased risk of neutropenia or agranulocytosis in females [2,9,11]; this was not confirmed in our study.

The interaction between clozapine and valproic acid on the development of neutropenia was assessed a posteriori. However, the HR of 5.1 was relatively large reflecting a potential effect modification and not chance variation alone. Evidence suggests that a combination of clozapine and valproic acid was associated with a greater risk for neutropenia [31–34]. Although the data on clozapine–valproic acid effect modification remain controversial [35–37], coadministration of valproic acid might slightly increase serum concentration of toxic metabolite norclozapine that increased the propensity for neutropenia development [35]. The effect modification should be further assessed as an a priori hypothesis.

This study found one clozapine user died of cardiovascular complication (myocarditis and cardiomyopathy, data not shown), a rare but serious adverse effect that has previously been reported [38–40].

Besides a too small sample size, this study was limited by the paucity of data on potential confounding variables and the lack of linkage of the data between hospitals or other health care settings. Some other drugs recognized to be associated with an increased risk of neutropenia or agranulocytosis were not measured in this study, and thus adjustment for confounding was impossible. In addition, only drugs...
available in the hospital computerized database were collected, those dispensed from other sources were not accessed. However, the probability of exposure to any drug classes were less likely in this population and should be similar in both groups of psychiatric patients and thus might not substantially bias the results. In addition, because of the reliance on prescription information, we do not know whether the patients actually took the drugs prescribed. Any non-compliance is probably non-differential between the two groups and this could lead to an underestimation of the HR. Moreover, the hospital computerized database does not cover patients seen by private clinic or other hospitals, therefore, some cases might be missed.

The strength of this study was the cohort design in which exposure data and outcome (WBC) data were recorded independently, thereby reducing information bias. This study demonstrated that the risks of neutropenia/agranulocytosis in psychiatric patients not treated with clozapine might be similar to those receiving clozapine, and safety concern should be paid to this group of patients.

In conclusion, the incidence of neutropenia associated with clozapine is similar to that reported previously. The risk of neutropenia due to clozapine among psychiatric patients might increase 30%, but not precise enough to reach statistical significance. Older age is a significant risk factor, while lithium use is likely to be a protective factor against neutropenia. Use of valproic acid concomitantly might potentiate the risk of neutropenia due to clozapine. Confounding by indication could not be ruled out from this retrospective study as clozapine is contraindicated in high risk patients, and thus might underestimate the risk. All patients receiving clozapine should be regularly monitored for WBC, especially those with old age and concomitantly prescribed with valproic acid. Due to insufficient power, the risk of neutropenia associated with clozapine as well as the clozapine–valproic acid interaction should be demonstrated in a larger study.

Key points

- The first year incidence of clozapine-associated neutropenia is 6.3% and similar to those previously reported
- The risk of neutropenia due to clozapine among psychiatric patients might increase 30%
- Older age is a significant risk factor for neutropenia, but lithium use is likely to be a protective factor against neutropenia
- Concomitant valproic acid might potentiate the effect of clozapine-associated neutropenia, and this should be further assessed as an a priori hypothesis
- Selection bias and confounding by indication could not be ruled out, and therefore might underestimate the risk of neutropenia associated with clozapine

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References

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