Sodium valproate and clozapine induced neutropenia: a case control study using register data.

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Abstract:

Background:

The use of clozapine is limited due to the occurrence of neutropenia, and the rare but life threatening adverse event of agranulocytosis. There is little epidemiological research into clinical factors that may impact on this risk. We conducted a case control study examining the clinical risk factors for neutropenia patients treated with clozapine.

Method:

A case-control study was conducted within a database of anonymised electronic clinical records. All patients who discontinued clozapine due to a neutropenic event were included as cases. Matched controls were selected from patients with a documented clozapine exposure at the time of the clozapine neutropenic event of the case patient, matched by duration of clozapine treatment.

Results:

136 cases and 136 controls were included. In multivariable analysis, the concurrent use of sodium valproate was associated with neutropenia (Odds Raito (OR) 2.28, 95%CI:1.27-4.11, p=0.006). There was a dose-response effect, with greater associations for higher doses. Patients who discontinued clozapine due to neutropenia were more likely to be of black ethnicity (OR 2.99, p<0.001), were younger (t=5.86, df=267, p<0.001), and received lower doses of clozapine (t=-2.587, p=0.01) than those who did not develop neutropenia.

Conclusion:

We identified an association between the concurrent use of sodium valproate and an increased risk of clozapine associated neutropenia. These results, taken in combination with the results from previous case series, suggest that the risk of clozapine associated neutropenia could be reduced by avoiding concurrent valproate treatment.

Keywords:

Treatment resistant; schizophrenia; clozapine; valproate; neutropenia; agranulocytosis

1. Introduction

Treatment resistant schizophrenia (TRS) affects approximately 1/3 of patients with schizophrenia, with 70% of TR cases not responding to antipsychotics from illness onset (Lally et al. 2016a). Clozapine is the only evidence based medication in treatment resistant schizophrenia (TRS) (Essali et al. 2009) with 60-70% of those treated with clozapine showing a response (Chakos et al. 2001;Meltzer 1992;Lieberman et al. 2005). Clozapine is not only an effective treatment in TRS (Siskind et al. 2016) but is associated with long term reductions in mortality (Hayes et al. 2015;Tiihonen et al. 2009).

However, despite this, clozapine is underutilised (Stroup et al. 2014;Manuel et al. 2012;Nielsen et al. 2016), with delays of up to four years in its use in clinical practice (Howes et al. 2012;Taylor et al. 2003;Nielsen et al. 2016). Despite its beneficial effects, approximately 25% of clozapine users discontinue treatment due to adverse effects (Legge et al. 2016). Although the more common clozapine adverse events can generally be symptomatically managed, one of the primary reasons for clozapine underprescribing and delays in its use, is patient and clinician fears about the emergence of life threatening adverse events, and in particular agranulocytosis (Nielsen et al. 2010).

Clozapine induced agranulocytosis (CIA) occurs with a period prevalence of 0.8% in the first year of clozapine treatment (Alvir et al. 1993), with peak incidence at 6-18 weeks after commencing clozapine (Atkin et al. 1996). The one year prevalence of clozapine-induced neutropenia is 2.7% in the first year, with the peak incidence occurring at 6-18 weeks (Munro et al. 1999). In the UK, stringent and mandatory full blood count (FBC) monitoring is a requirement for continuing clozapine therapy so that the emergence of neutropenia can be detected early and clozapine treatment can be promptly discontinued if indicated (Atkin et al. 1996;Patel et al. 2005).

The occurrence of clozapine associated neutropenia and agranulocytosis necessitates discontinuation of clozapine. However, if the risk neutropenia could be reduced, this would have the additional effect of denying fewer patients the potential long term benefits of clozapine treatment. Recent evidence suggests that psychotropic medications and other drugs when, administered with clozapine may contribute to the development of neutropenia. Olanzapine, chlorpromazine, benzodiazepines, and anticonvulsants have been shown to be independently associated with increased incidence of neutropenia (Meyer et al. 2015;Garbe 2007;Andres and Maloisel 2008;Andres et al. 2008).

There is little research into clinical factors that may impact on the risk of clozapine associated neutropenia. The aim of the study was to identify concurrent medication use that increases

the risk of clozapine associated neutropenia. We analysed factors related to clinical management (i.e. concomitant medication) and demographic characteristics (gender, age, ethnicity) which may predispose to the development of neutropenia in individuals treated with clozapine.

2. Methods

2.1 Data Source and Ethical approval

A case control study was performed using de-identified clinical records at South London and Maudsley NHS Foundation Trust (SLaM). This anonymised data was accessed using the Clinical Record Interactive Search (CRIS) facility. This was developed by the National Institute for Health Research, Biomedical Research Centre at SLaM and full details of this data resource have been provided elsewhere (Stewart et al. 2009). Governance for all CRIS projects is provided by a patient-led oversight committee reporting to the SLaM Caldicott Guardian. CRIS received ethical approval as an anonymised data resource for secondary analysis by Oxfordshire Research Ethics Committee C in 2008. The CRIS oversight committee application number corresponding to this particular study is 15-027 and was approved on 20th March 2015.

2.2 Documentation of cases and controls

Two thousand four hundred and seventy-three patients treated in SLaM with a clozapine prescription start date between January 2007 and May 2015 (inclusive) were screened for inclusion in the study using CRIS.

An exhaustive automated free text search of clinical records using the search terms “neutropenia”, “neutropenia+red”, “neutropenia+amber” and “agranulocytosis” in CRIS was completed. The selection of these terms was based on commonly used terminology referring to a neutropenic event by SLaM clinicians and was therefore best suited in identifying a neutropenic event. In the UK, clozapine monitoring uses a ‘traffic light system’. When neutrophil counts are greater than 2.0 x 10^9 /L (and white cell count (WCC) >3.5 x 10^9 /L), a “green” result is recorded and clozapine is prescribed. If a neutrophil count 1.5–2.0 x 10^9 /L is recorded, denoted “amber”, clozapine can be prescribed but with twice weekly FBC monitoring. A “red” result refers to a neutropenia with a neutrophil cell count below 1.5 x 10^9 /L and/or a leucopenia with a WCC below 3.0 x 10^9 /L. Similarly, a neutrophil count below 0.5 x 10^9 /L is also referred to as a “red result” or “agranulocytosis”. Under the UK monitoring rules, clozapine must be stopped in the event of a red result.

This search identified 544 cases with suspected neutropenia.

2.3 Ascertainment of clozapine associated neutropenia cases

For inclusion as a case, patients were required to have at least one discontinuation of clozapine due to a neutropenic event i.e. a reduction in neutrophil and/or white cell count below 1.5 x 10^9 /L, and 3.0 x 10^9 /L respectively.

All 544 patients identified as potential cases had their clinical records manually reviewed. 136 patients had a confirmed clozapine related neutropenic event (i.e. all neutropenic events occurring during clozapine rechallenges were excluded) necessitating cessation of clozapine treatment between January 2007 and May 2015 (inclusive). For patients with more than one discontinuation, only the first event was included, thus clozapine rechallenges were excluded.

2.4 Matching to controls

For each case, an index date was defined as the date on which the neutropenic event occurred. Each case was matched 1:1 to a control drawn from the 1929 patients with no mention of neutropenia in their records using risk set sampling (Goldstein and Langholz 1996), matched by duration of clozapine therapy at the index date (< 6 months; 6-12 months; or >12 months of clozapine treatment). This sampling strategy was designed to randomly match each case with a control with a similar duration of exposure to clozapine as the case. We selected controls from the cohort of 1929 patients that had a continuous repeat clozapine prescription ± 3 months from the index date when matching to cases. This was done to ensure the ongoing use of clozapine in controls at the time of the index neutropenic event.

2.5 Data collection

Data collected for cases and controls included gender, age at time of neutropenic event and ethnicity as documented in clinical records [categorised as: White (British, Irish, White and Asian and any other White background), Black (African, Caribbean, White and Black African, White and Black Caribbean and any other Black background) and other (Bangladeshi, Chinese, Indian, Pakistani, any other Asian background, any other ethnic group and any other mixed background)]. We also documented clozapine start date, daily dose of clozapine administered at the index date of neutropenic event, and concurrent medication used at the index date. The 136 cases were categorised by duration of clozapine treatment at the time of the neutropenic event (< 6 months; 6 to 12 months; and > 12 months).

Concurrent medication use represents a time-varying element of treatment. We collected in both cases and controls concurrent medication use on the index date. These included

concomitant psychotropic medications (e.g. first generation antipsychotics, second-generation antipsychotics, mood stabilisers, antidepressants and anxiolytics).

In addition, to ensure reliability, a second author (JL) reviewed the extracted data. Any disagreement was discussed, decisions documented and, if necessary, we planned that a third author (JM) would help clarify issues and these final decisions would be documented. However, this did not happen

2.6 Statistical analysis

Analyses were conducted using STATA (StataCorp, Stata Statistical Software, Version 12). Crude ORs for neutropenia associated with concurrent medication exposure were estimated using conditional logistic regression. Multivariable analysis was conducted for each variable adjusted for age, ethnicity and concurrent sodium valproate use.

3. Results

136 cases met the case definition for clozapine associated neutropenia and were matched to 136 controls. A similar number of males were found in cases (n=89) and controls (n=91). Cases were significantly younger (mean age 35.7±12.5years, range 21-77 years) than controls (mean age 44.2 ± 11.4years, range 17-74 years; t=5.86, df=267, p<0.001).

Thirty nine percent (n=53) developed neutropenia within the first 6 months of clozapine treatment, 17.6% (n=24) between 6 – 12 months of treatment, and 43.4% (n=59) after one year of treatment. None of the cases died due to the neutropenic event.

In univariate analysis, the risk of clozapine associated neutropenia was higher in younger patients and in those of black ethnicity. Multivariable analysis found a strong association for younger age only (17-29), with odds for those aged 17-29 years 6 times greater than those aged 60 or older (Odds Ratio (OR) 6.3, p=0.001); for those of black ethnicity the odds of developing neutropenia was 3 times greater than those of white or Asian ethnicity (OR 2.99, p<0.001), (table 1).

Insert table 1 here

3.1 Concurrent medication use and risk of neutropenia (table 2)

Twenty seven percent of cases (n=36) and 23% of controls (n=30) were treated with at least one other antipsychotic concurrent to clozapine.

In multivariable analysis, when adjusting for age and ethnicity, anticonvulsants were associated with an increased risk of neutropenia (OR 2.01, p=0.01).

Insert table 2 here

3.2 Sodium valproate use and risk of neutropenia (table 3)

Sodium valproate was associated with significantly increased odds of neutropenia with an adjusted odds ratio of 2.28 (95% CI 1.27-4.11, p=0.006). There was a dose dependent relationship between sodium valproate use and odds of neutropenia. The adjusted ORs for neutropenia were 5.59 (95% CI 1.64-19.07) and 13.2 (95% CI 1.96-88.90, p = 0.008) for those treated concurrently with 1000-1999mg and ≥2000mg daily compared to those treated with less than 1000mg daily.

Insert table 3 here

3.3 Other medications and neutropenia risk (table 2)

The use of antidepressants appeared protective against the development of neutropenia (OR 0.44, 95% CI 0.22–0.91, p= 0.03). More specifically, this effect was driven by the use of SSRIs (OR 0.33, 95%CI: 0.15-0.75, p=0.008). In univariate analysis, statins were associated with decreased odds of neutropenia, with an unadjusted OR of 0.51 (95% CI 0.23 – 1.11, p= 0.09), but controlling for age, ethnicity and sodium valproate use eliminated this association.

Other concurrent medication use, including antipsychotic medication, anticholinergics, analgesics, diabetic medication and beta blockers, was not significantly associated with the odds of neutropenia. In univariate analyses lithium carbonate (OR 1.98, 95% CI 0.81-4.85, p=0.13), lamotrigine (OR 0.79, 95% CI 0.21-3.02, p=0.74) and clonazepam (OR 0.43, 95%CI 0.13-1.42, p=0.17) were not associated with neutropenia.

3.4 Clozapine dose and neutropenia

Patients who developed neutropenia received lower doses of clozapine than those who did not (mean clozapine dose in cases: 339.2±146.5 mg vs mean clozapine dose in controls: 347.9±171.6mg (t=-2.587, df=256, p=0.01).

4. Discussion

This study found that sodium valproate is a significant risk factor for the development of clozapine associated neutropenia. This supports the findings of previous case series and reports suggesting an association between neutropenia and concordant clozapine and valproate use (Imbarlina et al. 2004;Madeb et al. 2002;Meyer et al. 2015;Pantelis and Adesanya 2001).

The first cases of sodium valproate associated neutropenia were reported in paediatric populations in the 1970s (Jaeken et al. 1979). The incidence of leucopenia associated with sodium valproate is estimated to be 0.4% (Tohen et al. 1995), though an association between sodium valproate use and a dose dependent risk of thrombocytopaenia is more widely known (Blackburn et al. 1998;Vasudev et al. 2010). In a recent case series of clozapine rechallenges (n=19 patients), rechallenge was successful (i.e. with no reoccurrence of neutropenia) in 15 cases (79 %). There is also evidence that valproate may be associated with failure of clozapine rechallenge after neutropenia. In a separate study from our group, Meyer et al reviewed the outcomes of patients rechallenged on clozapine following neutropenia. Of the 4 patients who had a further neutropenia following rechallenge, three were prescribed sodium valproate, compared to only three patients treated with valproate in the fifteen who were successfully rechallenged (Meyer et al. 2015).

4.1 Sodium valproate dose effect

We found a dose dependent effect of sodium valproate and risk of neutropenia. Sodium valproate has been previously shown to be associated with a dose dependent thrombocytopaenia (Nasreddine and Beydoun 2008). Valproate associated thrombocytopaenia occurs at an incidence of 5%, and there is an increased risk with serum valproate levels above 80 g/L, especially in females (Vasudev et al. 2010).

Similar to its associations with neutropenia, the mechanisms of valproate dose effect on other haematological adverse events is unclear. Putative mechanisms in thrombocytopenia include a dose-dependent suppression of bone marrow production of platelets or peripheral platelet destruction due to the development of a platelet antibody brought about by sodium valproate or one of its metabolites (Verrotti et al. 2014;May and Sunder 1993). This may be due to a direct effect of sodium valproate on platelets because of its branched chain carboxylic acid structure that is similar to fatty acid constituents of cell membranes (Barr et al. 1982) or an indirect effect of sodium valproate or one of its metabolites through the production of an IgM/IgG platelet antibody response (Acharya and Bussel 2000).

4.2 SSRIs

A survey of a drug surveillance program in 122,000 psychiatric inpatients did not find SSRI use to be associated with neutropenia (Stubner et al. 2004). In our study, we identified a reduced risk of neutropenia in those treated with SSRIs. This was an unexpected finding. We might speculate that antidepressants with putative immunostimulatory and anti-prostaglandin effects (Lieb 2004) may have a role to play in promoting proliferation of neutrophils. Granulopoiesis is associated with pro-inflammatory cytokines such as Interleukin (IL)-6 and GM-CSF (Kenis and Maes 2002). SSRIs have been shown in some studies to increase IL-6

(Munzer et al. 2013) which may promote granulocyte production, though this evidence for a pro-inflammatory effect is inconsistent (Kenis and Maes 2002). However, this finding remains speculative, and we cannot rule out the possibility of a false positive finding due to effects of multiple testing.

4.3 Lithium carbonate

Previous studies have found a protective effect for lithium (Kanaan and Kerwin 2006). The lack of such an effect in this study may be evidence of confounding by indication, in that those patients deemed to be at higher risk of clozapine associated neutropenia, particularly those with low baseline neutrophil counts, were prescribed lithium carbonate as prophylaxis against neutropenia.

4.4 Clozapine dose and neutropenia

We observed an inverse relationship between mean clozapine dose and the development of neutropenia. One previous study reported a similar relationship, but it was unclear if this analysis was adjusted for the duration of clozapine treatment at the time of the index neutropenia (Munro et al. 1999). We adjusted for the effect of clozapine dose titration over the first two weeks of clozapine treatment, when investigating the relationship between clozapine dose and neutropenia. This is potentially an important difference from the earlier study of Munro et al, in which their findings may have been reflective of the early time to onset of the index neutropenia or agranulocytosis during the first 6-18 weeks of clozapine treatment, without accounting for the clozapine titration period.

4.5 Duration of clozapine treatment

Since we matched on length of clozapine treatment, we could not compare the length of clozapine treatment between cases and controls. However, it is striking that 43.4% of discontinuations due to clozapine-induced neutropenia occurred in patients who were taking clozapine for more than one year. This finding is potentially important, given that only 21 cases of late onset neutropenia (occurring greater than one year after clozapine commencement) have been reported in the literature (Cohen and Monden 2013;Singh et al. 2016;Ratanajamit et al. 2010). Previous studies have suggested that neutropenia after the first 12 months is a rare occurrence(Cohen and Monden 2013;Kumar 2013;Schulte 2006), but it is not clear whether attrition was accounted for in the primary studies, given the high rates of clozapine discontinuation(Legge et al. 2016). In a retrospective study of clozapine treated patients, 28% (n=195) developed neutropenia and 26% (n=14) developed agranulocytosis after one year of treatment, with 102 cases of late onset neutropenia occurring during 13,594 years of cumulative, giving a crude incidence of late onset neutropenia of 7.5 per 1,000 patient

years(Kang et al. 2006). This is a higher incidence rate than that previously identified in the UK (1.9-2.6 patients per 1,000 patient years) and the USA (0.28-0.45) (Kumar 2013), and provides support for late onset neutropenia being more common than generally thought.

4.6 Clinical implications

After a patient has a clozapine associated neutropenia, clozapine must be stopped and the patient should not be re-exposed to clozapine. Despite this, some patients who have had a neutropenia (which would usually preclude its use) may be re-exposed to clozapine as part of a planned clozapine rechallenge. Rechallenge should only be contemplated either if it is thought that clozapine was not the cause of the dyscrasia, i.e. there are thought to be alternative explanations apart from clozapine for the first neutropenia, such as concurrent medication or infection, or if other treatment options are severely limited or non-existent. Our study findings provide robust evidence for an association between valproate use and clozapine associated neutropenia. If patients are concurrently treated with valproate at the time of a clozapine associated neutropenia, then consideration for the effect of valproate as an alternative explanation for the neutropenia should be made. If it is thought to be a contributing factor, then consideration for a clozapine rechallenge may be made (after a careful risk/benefit analysis and consultation with a haematologist), with valproate discontinued before any clozapine rechallenge.

Sodium valproate is frequently used in clinical practice with clozapine. Thirty six percent of the cases and 21% of controls in our study were concurrently treated with valproate. Valproate is used as a clozapine augmentation agent (though with limited evidence to support its efficacy) (Basan et al. 2004), for mood stabilisation (Cipriani et al. 2013), for seizure prophylaxis, and in the management of myoclonic jerks.

Our study follows on from a previous case-control study that identified an association between sodium valproate use and an increased risk of myocarditis in people commencing clozapine (Ronaldson et al. 2012). The findings from both studies indicate that for patients taking sodium valproate, consideration should be given to its discontinuation before initiation of clozapine.
Alternatives to valproate treatment exist for all of the above clinical indications (Lally et al. 2016b;Sommer et al. 2012). However, similar to valproate, none of the alternative medication augmentation strategies have a strong evidence base. Clozapine at higher doses and plasma concentrations has an increased risk for generalised seizures, for which lamotrigine is a recommended first line treatment (as is valproate) (NICE 2016) and may be used as an alternative to valproate for this indication.

4.7 Putative mechanism of clozapine and valproate associated neutropenia

The exact mechanism of clozapine-induced neutropenia is yet to be elucidated. As with most drug induced haematological events, it is thought to have a significant idiosyncratic component. We propose a putative mechanism for the increased risk of neutropenia seen with clozapine and valproate co-use. It has previously been postulated that the initial step in events leading to clozapine-associated neutropenia, may be the activation of clozapine and norclozapine to electrophilic nitrenium ions (Pirmohamed and Park 1997;Pereira and Dean 2006). These nitrenium ions may either bind to neutrophils to cause cell death, or cause oxidative stress-induced neutrophil apoptosis (Fehsel et al. 2005;Husain et al. 2006). A similar process involving the oxidative metabolism of valproate to free radical intermediates has been postulated as an aetiological factor in valproate related haematolgocial adverse events, including neutropenia (Fehsel et al. 2005), and sodium valproate has been shown to similarly promote oxidative stress (Zhang et al. 2011). Further, sodium valproate has been shown to decrease the synthesis of glutathione peroxidase, thus inhibiting the production of the antioxidant glutathione (Pippenger et al. 1992). The rapid reduction of nitrenium ions may be limited by valproates inhibition of glutathione production, which may contribute to or trigger the generation of excess free ions, leading to neutropenia (Linday et al. 1995). This suggests that the combined effect on cell activation by clozapine and sodium valproate, combined with reduced glutathione production induced by valproate, may lead to increased reactive metabolites, leading to neutrophil toxicity and apoptosis.

Another potential mechanism for the increased risk for neutropenia relates to shared actions on cytokines. Both clozapine (Sperner-Unterweger et al. 1993) and sodium valproate (Linday et al. 1995) have been shown to inhibit the production of granulocyte-macrophage colony stimulating factor (GM-CSF) in vitro, thus reducing granulopoiesis and thus limiting the compensatory mechanisms against the increased rate of neutrophil destruction.

It is possible that both mechanisms may be relevant; namely the increased risk of neutropenia seen with clozapine and valproate use may be due to an additive or synergistic effect of sodium valproate which increases the degree of neutrophilic oxidative stress and apoptosis, along with a reduced ability of bone marrow to produce granulocytes due to inhibition of GM-CSF.

5. Limitations

Limited data on plasma clozapine concentrations prevented us from further exploring the inverse association between clozapine dose and risk of developing neutropenia, though

previous research has found no difference in plasma clozapine and norclozapine concentrations in patients who either do or do not develop neutropenia (Hasegawa et al. 1994). Further, a lack of data on smoking habits of patients limited us in evaluating the effects of smoking on the relationship between sodium valproate and clozapine plasma concentrations (Diaz et al. 2014). Similarly, while an association between higher doses of valproate and neutropenia was identified, lack of data on serum valproate levels precluded an investigation of how serum levels influenced this risk.

Lack of availability of all haematological cell lines further restricted us from measuring the duration and severity of the neutropenic episode and from investigating haematological markers predictive of neutropenia.

6. Conclusion

Our findings indicate a strong association between clozapine and sodium valproate use and neutropenia risk. These findings have clinical implications, both when investigating possible causes of severe neutropenia on clozapine, and on the choice of anticonvulsant used for seizure prophylaxis.

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Contributors

We affirm that the manuscript is an honest, accurate, and transparent account of the study being reported and that no important aspects of the study have been omitted. SM, JL and JHM made substantial contributions to the conception and design of the work. SM, JL, MP, HS were involved in the acquisition of the data and SM, JL and OA in the analysis of the data. SM, JL, AK, FG, RJF, and JHM also contributed towards data interpretation for this study. SM and JL created the first draft of the work and SM, JL, AK, FG, RJF, and JHM were involved in its revision.

All authors gave final approval of the version to be submitted and published and are in agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The corresponding author had full access to all the data and had the final decision to submit for publication.

Conflicts of interest

Only 1 authors (FG) has a potential conflict of interest, although not in relation to this work. FG has received support or honoraria for CME, advisory work and lectures from Bristol-Myers Squibb, Janssen, Lundbeck, Otsuka, Roche, and Sunovion, has research funded by an NHS Innovations/Janssen-Cilag award and has a family member with professional links to Lilly and GSK, including shares.

All other authors (SM, JL, OA, MP, AK, HS, RJF, and JHM) declare no conflict of interest. The other authors have no financial relationships with any organisations that might have an interest in the submitted work in the previous 3 years.

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