Abstract

Background: Guidance on clozapine dosing in treatment refractory schizophrenia is based largely on data from young adult male Caucasian patients.

Aim: To audit the plasma clozapine and *N*-desmethylclozapine (norclozapine) concentrations attained in male and female patients of different ethnicity and smoking habit.

Method: The effect of dose, sex, ethnicity, age, body weight, and smoking habit on plasma clozapine and norclozapine concentrations were studied using data from a TDM service, 1993–2017.

Results: There were 371,610 samples (48,098 patients, 32,855 male). Ethnicity was recorded for 763 Afro-Caribbean, 536 Asian, and 7,940 Caucasian patients. Males were prescribed significantly higher median doses than females, but attained significantly lower median plasma clozapine and norclozapine concentrations. Asian and Afro-Caribbean males were prescribed significantly lower and higher median doses, respectively, than Caucasian males, but attained significantly higher and lower median plasma clozapine and norclozapine concentrations, respectively. Data from 78,431 samples (23,516 patients) were analysed using a linear mixed model. The predicted dose to attain a pre-dose plasma clozapine concentration of 0.35 mg/L in a non-smoking male Caucasian aged 40 yr, weight 70 kg, and plasma clozapine:norclozapine ratio 1.32 was 344 (95% CI 227, 526) mg/d. The predicted dose was 33% higher and 20% lower in otherwise analogous Afro-Caribbean and Asian patients, respectively. In all cases the predicted dose was increased by 36% in smokers, and decreased by 22% in females.

Conclusions

Research is needed to further investigate the complex relationships between dose, sex, ethnicity, plasma clozapine and norclozapine concentrations, and clinical outcome such as weight gain.

Keywords: Clozapine dose prediction; Therapeutic drug monitoring; Treatment refractory schizophrenia; Cytochrome CYP1A2; Harm reduction

Introduction

Clozapine has proven efficacy in schizophrenia that has not responded to other antipsychotics (treatment refractory schizophrenia, TRS). Clozapine is also indicated in patients with schizophrenia who show adverse neurological reactions to other antipsychotics, including second-generation antipsychotics.[1]

In the UK and in many other parts of the world, clozapine is licenced to treat TRS in patients aged 18 years and older at doses up to 900 mg/d and, at doses 5- to 10-fold lower, Parkinson disease psychosis.[2] Off-label uses of clozapine include treatment of schizophrenia in patients younger than 18 years, bipolar disorder, depressive disorders, borderline personality disorder, substance misuse disorders, suicidality, aggression, tardive dyskinesia, and tardive dystonia.[3]

Clozapine dose assessment is not straightforward. There is wide between- and within-subject variability in the plasma concentrations of clozapine and of its principal plasma metabolite *N*-desmethylclozapine (norclozapine) attained after a given dose. This is partly explained by factors that either activate, or inhibit the principal clozapine metabolising enzyme, cytochrome (CYP) 1A2. These include smoking (polycyclic aromatic hydrocarbons induce CYP1A2), female sex (oestrogen inhibits CYP1A2), co-prescription of drugs that either inhibit (e.g. fluvoxamine), or enhance (carbamazepine, omeprazole, phenobarbital, phenytoin) clozapine clearance, and. South Asian ancestry, which is associated with lower CYP1A2 activity compared to Caucasians.[4,5] Patients of Afro-Caribbean ethnicity have not been studied in this regard, although it has been suggested that clearance of olanzapine, also a CYP1A2 substrate, is increased in Afro-Caribbean as compared to Caucasian patients.[5] The plasma clozapine:norclozapine (C:NC) ratio has been used as an index of clozapine metabolic capacity.[6]

Stopping smoking is associated with on average a 30% decrease in clozapine dose requirement.[7] On the other hand, during infection down-regulation (phenoconversion) of CYP1A2 and also of CYP3A4 and CYP2C9 (other enzymes implicated in clozapine metabolism) via release of proinflammatory cytokines may occur with the attendant risk of clozapine intoxication.[8] A potential complication is that the contribution of passive smoking to the observed effects is always unknown. Differences in clozapine formulation is a further potential complication. From 2004 three different clozapine preparations were available for prescription in the UK, but bioequivalence data are limited.[9] Finally, in practice patients prescribed clozapine as either crushed tablets suspended in an appropriate medium, or proprietary suspension may attain lower plasma clozapine and norclozapine concentrations for a given prescribed dose than those given tablets.[10]

Clozapine therapeutic drug monitoring (TDM), i.e. the measurement of the plasma concentrations of clozapine and norclozapine in an appropriate sample, can help monitor adherence, guide dosage, and minimise the incidence of dose-related adverse drug reactions

(ADRs). Many people with TRS respond at pre-dose ('trough') plasma clozapine concentrations between 0.35–0.60 mg/L, but there is considerable variation in both response and ADRs. It is accepted that 0.35 mg/L is the threshold to ensure a fair trial of the drug.[11,12] However, some patients may show a good response at pre-dose concentrations of 0.25 mg/L when *inter alia* a lower incidence of ADRs might be expected.[13] On the other hand, there is thought to be an increased risk of confusion, constipation, delirium, excess sedation, grand mal seizure, myoclonus, and orthostasis if the pre-dose plasma clozapine is >1 mg/L.[12]

The aim of this paper was to explore the influence of dose, mode of administration, sex, ethnicity, age, body weight, smoking status, and the plasma C:NC ratio on plasma clozapine and norclozapine concentrations in the UK and Ireland in clinical practice.

Patients and Methods

Patients

We studied data arising from the analysis of plasma, serum, or blood samples (use of EDTA anticoagulant requested) submitted for clozapine TDM from patients from Great Britain and Ireland, November 1993–December 2017. Patient samples that had been referred during investigation of (1) death during clozapine treatment, (2) suspected self-poisoning or accidental poisoning requiring medical admission, for example, via a hospital Emergency Department, and (3) suspected clozapine exposure in neonates were excluded. Bioequivalence of clozapine brands was assumed.

A pre-dose blood sample was requested, taken either in the morning before a morning dose, or in the morning after the evening dose if dosage was once daily. Information requested when submitting samples was time and date of sample, time and date of last clozapine dose, clozapine dose prescribed, mode of administration (tablets or suspension), duration of clozapine treatment, age, sex, body weight, smoking habit, clozapine service registration number, and other information that could aid interpretation of the result such as co-prescribed drugs. A customized database was used to facilitate data analysis.

Clozapine Assay

Samples were analysed promptly on receipt in the laboratory. Whole blood was separated by centrifugation prior to the analysis. Total plasma or serum clozapine and norclozapine were measured by high performance liquid chromatography with ultraviolet absorption detection (HPLC–UV) (240 nm),[14] or from April 2016 by liquid chromatography-tandem mass spectrometry (LC–MS/MS) or flow-injection analysis-MS/MS[15,16] after either liquid-liquid, or extraction plate extraction (pH 10.6). If interference in the HPLC-UV assay was thought a possibility, samples were reanalysed by LC-MS/MS from 2012. Assay implementation and validation conformed throughout

to the standards set by the US Food and Drug Administration[17] and accuracy and precision monitoring was as documented by the Clinical and Laboratory Standards Institute.[18]

Assay calibration was by duplicate analysis of solutions containing clozapine and norclozapine (0.05, 0.10, 0.20, 0.50, 1.00, and 2.00 mg/L free base) in analyte-free calf or pooled human serum and plotting the ratio of peak height of analyte to that of an internal standard against analyte concentration. Internal quality control (IQC) samples (0.15, 0.40, and 1.20 mg/L both analytes) prepared in analyte-free pooled human serum were analyzed with each sample batch and between every 10 sample extracts. Samples with plasma clozapine markedly (approximately 15 % or more) above the calibration range were diluted in pooled analyte-free human serum and reanalyzed provided that sufficient sample was available. The limit of detection (limit of accurate measurement) was 0.01 mg/L for either analyte (100 µL sample). Intra-assay precision at analyte concentrations 0.15, 0.40, and 1.20 mg/L (N = 6 at each concentration) was between 3.8 and 6.6%. Corresponding inter-assay precision (N = 63 over 3 months) was between 5.6 and 15.2%. Patient results were reported to the nearest 0.01 mg/L. External quality control (EQC) materials containing clozapine and norclozapine at known concentrations were from UTAK (Valencia, CA) or Chromsystems (Munich, Germany). Proficiency testing (PT, also known as External Quality Assessment, EQA) was via Heathcontrol, now LGC Standards (www.lgcstandards.com/GB/en/PS04-Clozapine-Norclozapine/p/PT-TM-PS04), from 1993. One sample of lyophilised plasma or serum containing clozapine and norclozapine at concentrations known only to the scheme organiser were circulated per month. The analytical results were reported to the scheme organiser without prior knowledge of the weighed-in values.

Statistical analysis

Chi-square and Mann-Whitney U-tests for nonparametric data were used to assess group differences.[19] Linear mixed models, which accounted for repeated sampling in the same patient, were used to investigate the contribution of selected variables to plasma clozapine and to plasma norclozapine concentrations.[20,21] Akaike information criterion and explained variance were also used to evaluate candidate models.

The variables studied were (a) clozapine: $\log_{10}$ dose (mg/d), $\log_{10}$ plasma C:NC ratio, sex, age (yr), smoking habit, body weight (kg), formulation (tablet, suspension, formulation not recorded), ethnicity (Afro-Caribbean, Asian, Caucasian, mixed ethnicity, ethnicity not recorded), and (b) norclozapine: as for clozapine except that $\log_{10}$ plasma clozapine was used in place of $\log_{10}$ plasma C:NC ratio. Marginal R^2 values were calculated for each model.[20]

Samples for which co-prescription of fluvoxamine, phenytoin, carbamazepine, or phenobarbital was recorded were not included in the linear mixed model analysis. Samples with one or more missing values were likewise not included. Patient unique identifiers were specified as a grouping variable to control for their random effects. The coding used was as follows: sex (male

1, female 0), ethnicity (true 1, false 0), smoking status (smoker 1, non-smoker 0), and formulation (true 1, false 0).

The doses predicted to give a pre-dose plasma clozapine concentration of 0.35 mg/L were generated from the final model equation by holding each parameter constant and varying dosage. Upper and lower confidence bounds for parameter estimates were used to construct confidence intervals surrounding plasma clozapine concentration estimates for the given doses.

Results

There were 371,610 samples from 48,098 distinct patients [32,855 male, median age at first sample 37 (range 9–107) yr; 15,243 female, median age at first sample 41 (range 11–100)] yr. Ethnicity was recorded for 9,412 patients (763 Afro-Caribbean, 536 Asian, 7,940 Caucasian, 173 mixed-race). The results are summarised in Tables 1 and 2. The age distributions at the time of sampling for males and for females are given in Supplementary Table 1. No clozapine was detected (plasma clozapine <0.01 mg/L) in 3,933 samples (1.1% of all samples from males and 1.0% of all samples from females; Supplementary Table 2).

Although the median dose was significantly higher in the males as compared to the females in all of the groups studied, the median plasma clozapine and norclozapine concentrations were significantly higher in the females except that the median norclozapine concentration was not significantly different in the Afro-Caribbean patients (Supplementary Table 3). Afro-Caribbean men were prescribed significantly higher median doses than Caucasian men, but attained significantly lower median plasma clozapine and norclozapine concentrations. The converse applied to Asian men. Asian women were prescribed a significantly lower median dose than Caucasian women, but attained significantly higher plasma clozapine and norclozapine concentrations. The opposite pattern was observed in Afro-Caribbean women.

At the time of sampling the median weight of the males was significantly higher and the median age significantly lower than that of the females in all groups except that in the mixed-race group the median ages were not significantly different (Supplementary Table 3). As regards the Afro-Caribbean, Asian and Caucasian groups, the proportions of samples with body weight, dose, and smoking habit recorded were not significantly different between the males and females, except that for the Afro-Caribbean females the proportion of samples with body weight recorded was significantly higher (Supplementary Table 3). The proportions of patients recorded as given either tablets, or crushed tablets/suspension was similar between the ethnic groups (Supplementary Table 4).

Trends over Time

Although there were relatively few samples submitted for analysis in 1993 (52), samples/year increased from 118 (1994) to 32,354 (2017). Except for 1994–6 when there were relatively few

samples (1995, 452 samples; 1996, 926 samples), the median annual dose in males remained constant at 450 mg/d 1997–2006, then dropped by 25 mg/d 2007–8 and thereafter remained constant at 400 mg/d. The median annual dose in females was always 50 mg/d lower than males, except that there was a further drop to 325 mg/d in 2015–7 (Figure 1). In contrast, the annual median plasma clozapine concentration was always lower in males (average difference 0.06 mg/L) and the median norclozapine concentration was almost always lower (average 0.02 mg/L). The average median C:NC ratio was 1.48 in males and 1.59 in females. The median ratio fell (both sexes) from 1993–1999 (1.05 males, 1.18 females) and then rose steadily to 2017 (1.86 males, 1.94 females, Supplementary Table 5).

The falls in median annual dose corresponded approximately to a reduction in the proportion of samples received for analysis from smokers of both sexes that began in 2005 and continued thereafter. In contrast the median annual clozapine and norclozapine concentrations increased steadily 1994–2009 and steadily fell thereafter in line with the trend to fewer samples from smokers (2017 values: males median clozapine and norclozapine 0.39 and 0.20 mg/L, respectively, 55 % smokers; females clozapine and norclozapine 0.43 and 0.22 mg/L, respectively, 43 % smokers).

The median age at the time of sampling increased year on year from 30–40 yr (male) and from 30.5–45 yr (female) over the period 1993–2017 (males: $y = 0.35x – 66.23$; $R^2 = 0.94$; females: $y = 0.45x – 858.38$, $R^2 = 0.93$). The median weight at the time of sampling also increased linearly from 70–95 kg (male 1995–2017) and from 71–84 kg (female 1996–2017) (males: $y = 0.96x – 1845.4$; $R^2 = 0.88$; females: $y = 0.67x – 1274$, $R^2 = 0.81$) (Figure 2).

Proficiency Testing Data

PT data were available (one circulation per month) for 2012–2017, inclusive. Six circulations were identified correctly as ‘negative’ (both analytes <0.01 mg/L). Accuracy (SD) compared to the weighed-in (target) value (mg/L) for clozapine (range 0.01–2.14) and for norclozapine (range 0.01–2.19) averaged 98 (8) and 105 (12) %, respectively (N = 66 in each case).

Differences Between the Ethnic Groups

Data from 78,431 samples (23,516 patients) were analysed using a linear mixed model. We were unable to estimate the coefficients for mixed ethnicity and oral tablets as independent variables, hence these variables were not incorporated into the final model.

The equations generated from the final model were as follows. Log_{10} transformation was used to normalise the specified variables.

*Clozapine: Log_{10} plasma clozapine concentration = (intercept * -2.524) + (log_{10} dose * 0.7252) + (sex * -0.07650) + (age * 0.001049) + (body weight * 0.001824) + (smoking status * -0.09546) + (Caucasian * 0.03422) + (Afro-Caribbean * -0.05644) + (Asian * 0.1064) + (unknown ethnicity *

*0.03741) + (log_{10} plasma C:NC ratio * 0.8553) + (crushed tablets/suspension * -0.05578) + (unknown formulation * -0.003147).*

*Norclozapine: Log_{10} plasma norclozapine concentration = (intercept * -0.8585) + (log_{10} dose * 0.2416) + (sex * -0.0005731) + (age * -0.001022) + (body weight * -0.0002589) + (smoking status * -0.01026) + (Caucasian * 0.03688) + (Afro-Caribbean * -0.04186) + (Asian * 0.01852) + (unknown ethnicity * 0.01693) + (log_{10} plasma clozapine concentration * 0.6918) + (crushed tablets/suspension * -0.007826) + (unknown formulation * -0.005887).*

As regards the random effects associated with repeated sampling, individual intercepts had a variance of 0.033 and a standard deviation (SD) of 0.18 with respect to log_{10} plasma clozapine concentration. All variables entered had a significant effect on plasma clozapine concentration ($p < 0.05$) except unknown formulation ($p > 0.05$). Individual intercepts varied with a variance of 0.001 and an SD of 0.01 with respect to log_{10} plasma norclozapine concentration. All variables entered had a significant effect on plasma norclozapine concentration ($p < 0.001$) except age, Asian ethnicity, unknown ethnicity, and crushed tablets formulation ($p > 0.05$).

The first linear mixed model explained 40% of the variance in the plasma clozapine concentration. The second linear mixed model explained 73% of the variance in the plasma norclozapine concentration. Further analyses were carried out using only samples pertaining to ages 18-65 yr, but the results obtained were almost identical to those given by the data as a whole.

The predicted dose to attain a pre-dose plasma clozapine concentration of 0.35 mg/L in a non-smoking male Caucasian aged 40 yr, weight 70 kg, and plasma C:NC ratio 1.32 was 344 (95% CI 227, 526) mg/d. The predicted dose was 33% higher and 20% lower in otherwise analogous Afro-Caribbean and Asian males, respectively. In all cases the predicted dose was 36% higher in smokers and 22% lower in females (Table 3). Finally, the predicted dose increased or decreased by 1.7% for every 5 yr below or above 40 yr, by 5.8% for every 10 kg weight above or below 70 kg, and by 8.6% for every 0.1 change in the C:NC ratio below or above 1.32.

Discussion

The data presented here summarise results from a clozapine TDM service. Selective liquid chromatographic methodology was used throughout together with EQC and PT to FDA/CLIA standards as these became available. Samples were sent for analysis for a variety of reasons (adherence check/incomplete response, suspected ADR, baseline measurement, etc). Given the doses used once treatment was established, it is clear that the vast majority of the patients studied were receiving treatment for TRS. Dose adjustment will in all cases have been under the control of a consultant psychiatrist and based not only on the TDM results, but also on patient response including the incidence and severity of ADRs.

In collating the data, no attempt was made to control for potential issues such as adherence, sample timing in relation to the last dose, use of crushed tablets/suspension rather than tablets,[10] unrecorded changes in smoking habit/covert or passive smoking, concomitant therapy with omeprazole,[22] and the possible presence of infection/inflammation,[8] clozapine-induced gastrointestinal hypomotility (CIGH),[23] or obesity.[24] However, given the large number of patients/samples studied, it is not unreasonable to assume that the effect of these potential variables will have been distributed at random throughout the data. In addition, the linear mixed model approach accounted for repeated sampling of individual patients.

The data are derived almost entirely from the analysis of EDTA plasma performed within 1–3 days of sample receipt in the laboratory. Although clozapine and norclozapine concentrations are some 5% lower in serum obtained from blood collected into Vacuette serum separator collection tubes as compared to EDTA plasma,[25] this difference is within the accuracy limits of the assay. In contrast, whole blood clozapine and norclozapine concentrations in patient samples are some 15% lower and 30% higher, respectively, than plasma concentrations.[26] Finally, norclozapine has limited stability especially in serum hence prompt analysis is important to ensure the reliability of results.[27]

In one audit of predominantly Caucasian patients that incorporated data used in this paper up to December 1999 (3,689 samples) multiple regression analysis of the effect of $\log_{10}$ dose, age, sex, body weight, smoking habit, and the plasma C:NC ratio (used simply as an index of a patient's ability to metabolize clozapine), explained some 48 % of the observed variation in plasma clozapine.[6] In a similar study, Perry et al.[28] included dose, smoking habit, sex, and a 'dose–gender interaction variable' and explained 47% of the observed variance in plasma clozapine in a group of 44 male and 19 female patients. Paz et al.[29] compared these two methods of clozapine dose prediction and also that of Oyewumi et al.[30] and reported no great difference between them.

The data presented in Table 3 add to the work of Rostami et al.[6] by giving separate dosage guidance for Afro-Caribbean, Asian, and Caucasian patients. In this context we have taken 'Asian' to be people of South Asian ethnicity. Unfortunately, there were not sufficient data to attempt similar recommendations for patients of mixed ethnicity and moreover here was no information on what was meant by 'mixed' ethnicity. The major differences in the results from the linear mixed model presented here and the multiple regression analysis reported by Rostami et al. are (i) that the predicted increase in the clozapine dose requirement in smokers was 36% as opposed to 48% and (ii) the effect of age was an increase or decrease of 1.7% as opposed to 4% for every 5 years below or above 40 yr. The effect of smoking reported here is much more in line with other reports.[7]

Guidance based on a pre-dose target plasma concentration of 0.35 mg/L to ensure a fair trial of the drug in TRS was provided at the commencement of the service.[11] However, neither the lower, nor the upper limits are fixed as regards the optimal response in individual patients.[31] The

target range of 0.35–0.60 mg/L takes no account of possible sex or ethnic differences in response. Although we have no information on the use of either oral contraceptives, or hormone replacement therapy in the patients studied (67 % of samples from patients aged 18-47 yr, with 30 % from patents aged 48-67 yr; Supplementary Table 1) the data presented here do emphasise that women in all ethnic groups attain higher median plasma clozapine and norclozapine concentrations than men in clinical practice despite lower median clozapine dosage. This difference is especially noticeable at higher plasma clozapine concentrations (Table 2). Whilst influenced to an extent by differences in smoking habit, and by body weight and age, these differences likely reflect sex differences in body composition, notably in the proportion and distribution of adipose tissue.[32] Reduced hepatic metabolism due to oral contraceptive and/or oestrogen-mediated inhibition of CYP1A2, reduced hepatic blood flow and liver size, and possibly differential expression of other metabolising enzymes may also contribute to the observed differences.[33]

In a large survey (104,127 samples, 26,796 patients) although the median plasma C:NC ratio was 1.25 at plasma clozapine concentrations <0.35 mg/L, the median ratio was 2.08 at plasma clozapine concentrations >1.0 mg/L suggesting saturation of clozapine *N*-demethylation at higher plasma clozapine concentrations.[34] Whilst it has been reported that the plasma C:NC ratio is not a measure of CYP1A2 activity from the study of 2,317 patients,[35] in the present analysis (78,431 samples, 23,516 patients) use of $\log_{10}$ C:NC ratio as a variable in the linear mixed model for plasma clozapine simply as an index of clozapine metabolic capacity did make a significant contribution. However, it should be noted that the C:NC ratio in individual samples may be affected by such variables as adherence and time since last dose, hence the measurement of this parameter for individual patients is not always straightforward.

The move to non-smoking hospitals and care homes in the UK is clearly a likely factor in the reduction in the proportion of samples from smokers sent for analysis apparent from 2004 (Figure 1). Treatment with clozapine is itself associated with a reduction in cigarette smoking.[37] This apparent reduction in smoking behaviour is the likely reason for the reduction in the median clozapine dose for men and for women apparent since 2006. The annual variation in median plasma clozapine and norclozapine 1994–2017 (Figure 1) is less easy to explain, although it is clear that in every year median plasma clozapine and norclozapine were higher in women despite lower median dosage.

Possible explanations for these annual variations in plasma clozapine and norclozapine are analytical, formulation-related, and patient/treatment-related. It is unlikely that the observed variations are analysis-related because, although the analytical methods used did change in late 2016/2017, calibration standards, IQC, EQC, and PT performance remained constant throughout

and the MS/MS methods were cross-validated against the original HPLC method. As to formulation, there is no evidence from other sources that there have been changes in the formulation of the clozapine tablets available in the UK, although equally there is no evidence that there have not been changes.

As to patient factors, the increase in annual median clozapine and norclozapine up to 2009 could possibly be related to improved adherence, but thereafter the reduction in the proportion of samples from smokers and the reduction in the median dose apparent since 2006 should have acted to keep median plasma clozapine and norclozapine constant. Increasing age at constant dose could have been associated with increasing plasma clozapine and norclozapine, but on the other hand increasing body weight could have been associated with some decrease in these parameters. Another possible explanation for the reduction in these parameters that occurred since 2009 are an effect on clozapine bioavailability that affected man and women equally such as might be expected with an increased incidence of potentially harmful CIGH with length of time on the drug.[23]

It remains unclear whether females require higher pre-dose plasma clozapine and norclozapine concentrations for optimal benefit from clozapine than their male counterparts, or whether they are, on average, receiving more drug than they need. Martini et al.[38] observed a significantly higher prevalence of tachycardia and hypertriglyceridemia, but lower HDL-cholesterol in men (N = 93), whereas females (N = 54) had a higher prevalence of orthostatic hypotension, constipation, and abdominal obesity. In a further survey, mean BMI and blood glucose concentrations were higher in females than males.[39] This may relate to higher tissue exposure over time. It is clear that plasma clozapine concentrations well above the suggested (sex-independent) target ranges are more frequent in females (Table 2) with the attendant metabolic risks such as diabetes.[40]

Ethnic group differences may be accounted for by differences in body composition, variation in CYP1A2 genetics or epigenetics (heritable changes in gene function that are not explained by DNA sequence) including DNA methylation and histone modifications, or other environmental factors.[41] It is of note that intermediate results were found in patients of mixed ethnicity (Table 1).

Whether Asian and Afro-Caribbean patients of either sex require lower or higher average doses, respectively, than their Caucasian counterparts remains uncertain, other factors such as smoking habit notwithstanding. A factor here may be that response may not be linearly related to plasma concentration hence what appear to be marked differences in plasma concentration may be relatively minor as far as the body is concerned. Further factors may de differences in plasma protein binding – total, i.e. protein-bound and unbound (‘free’) drug, is normally measured –

between the sexes/ethnic groups and there may be differences in receptor binding of clozapine and/or active moieties derived from clozapine between the sexes/ethnic groups.

Implications

Research is needed to further investigate the complex relationships between dose, sex, ethnicity, plasma clozapine and norclozapine concentrations, and clinical outcome. At present it remains unclear whether females and patients of south Asian ethnicity are prescribed higher doses than they need, or whether excessive reliance on plasma and norclozapine concentrations is misleading in this context Psychiatrists should nevertheless consider sex and ethnic differences when prescribing clozapine, and should make use of TDM to guard against clozapine accumulation if people stop smoking, for example, which may be more of a risk in females and those of south Asian ethnicity.

At first sight the data might suggest that weight gain is related more to dose than to plasma clozapine and norclozapine concentrations. Men were consistently prescribed higher median doses and gained more weight than women despite consistently lower median plasma clozapine and norclozapine concentrations. In contrast, women gained less weight and were prescribed lower median doses yet attained higher median clozapine and norclozapine concentrations. These differences did not seem to be related to the median age of the cohorts studied (Figure 2). However, the proportion of female smokers was some 10% lower than in the males suggesting a partial explanation for the lower median clozapine doses prescribed and the higher median plasma clozapine and norclozapine concentrations in the females.

Acknowledgements

Novartis for help in establishing the clozapine TDM service, Mr Harley Quilliam for setting up the database used, Dr Anwar Alhaq (King's College Hospital) for facilitating database and statistician support, the healthcare professionals who requested clozapine TDM and filled in the associated forms, and Prof Gerome Breen (King's College London) for assistance.

Declaration of Interest

None

Funding Statement

This research received no specific grant from any funding agency, commercial, or not-for-profit sectors.

Ethics/Consent Statement

Anonymised audit of data collected during routine service operation (Guy's Research Ethics Committee reference 05/Q0704/158).

Author Contribution Statement

RJF was responsible for the operation of the clozapine assay service 1993-2017, planned the research and drafted the paper, SH was responsible for the statistical analysis, SO managed the audit database and extracted the data, and SR provided statistical and clinical advice and helped draft the paper.

References

1. NICE (National Institute for Health and Care Excellence). Psychosis and schizophrenia in adults. Quality standard [QS80], 12 February 2015 (available at: www.nice.org.uk/guidance/qs80, accessed 10 October 2022).
2. Lutz UC, Sirfy A, Wiatr G, et al. Clozapine serum concentrations in dopamimetic psychosis in Parkinson's disease and related disorders. Eur J Clin Pharmacol 2014; 70: 1471–1476.
3. Newman WJ, Newman BM. Rediscovering clozapine: clinically relevant off-label uses. Curr Psychiatr 2016; 15: 51-62.
4. Ruan CJ, Zang YN, Wang CY, et al. Clozapine metabolism in East Asians and Caucasians: a pilot exploration of the prevalence of poor metabolizers and a systematic review. J Clin Psychopharmacol 2019; 39: 135–144.
5. de Leon J, Schoretsanitis G, Smith RL, et al. An international adult guideline for making clozapine titration safer by using six ancestry-based personalized dosing titrations, CRP, and clozapine levels. Pharmacopsychiatry 2022; 55: 73-86.
6. Rostami-Hodjegan A, Amin AM, Spencer EP, et al. Influence of dose, cigarette smoking, age, sex, and metabolic activity on plasma clozapine concentrations: a predictive model and nomograms to aid clozapine dose adjustment and to assess compliance in individual patients. J Clin Psychopharmacol 2004; 24: 70-78.
7. Wagner E, McMahon L, Falkai P, et al. Impact of smoking behavior on clozapine blood levels - a systematic review and meta-analysis. Acta Psychiatr Scand 2020; 142: 456-466.
8. Thorn CF, Müller DJ, Altman RB, et al. PharmGKB summary: clozapine pathway, pharmacokinetics. Pharmacogenet Genomics 2018; 28: 214-222.
9. Couchman L, Morgan PE, Spencer EP, et al. Plasma clozapine and norclozapine in patients prescribed different brands of clozapine (Clozaril, Denzapine, and Zaponex). Ther Drug Monit 2010; 32: 624-627.
10. Flanagan RJ, Hunter S, Obee S, et al. Plasma clozapine and norclozapine after use of either crushed tablets or suspension compared with tablets. J Clin Psychopharmacol 2022; 42: 400-404.
11. Eilers R. Therapeutic drug monitoring for the treatment of psychiatric disorders. Clinical use and cost effectiveness. Clin Pharmacokinet 1995; 29: 442-450.
12. Hiemke C, Bergemann N, Clement HW, et al. Consensus guidelines for therapeutic drug monitoring in neuropsychopharmacology: Update 2017. Pharmacopsychiatry 2018; 51: 9-62.

13. Ellison JC, Dufresne RL. A review of the clinical utility of serum clozapine and norclozapine levels. Ment Health Clin [Internet] 2015; 5: 68-73.
14. McCarthy PT, Hughes S, Paton C. Measurement of clozapine and norclozapine in plasma/serum by high performance liquid chromatography with ultraviolet detection. Biomed Chromatogr 1995; 9: 36–41.
15. Fisher DS, Partridge SJ, Handley SA, et al. LC-MS/MS of some atypical antipsychotics in human plasma, serum, oral fluid and haemolysed whole blood. Forensic Sci Int 2013; 229: 145-150.
16. Couchman L, Subramaniam K, Fisher DS, et al. Automated analysis of clozapine and norclozapine in human plasma using novel extraction plate technology and flow-injection tandem mass spectrometry. Ther Drug Monit 2016; 38: 42-49.
17. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, Center for Veterinary Medicine. Guidance for Industry. Bioanalytical Method Validation. May 2018. Available at: www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070107.pdf . Accessed: 1 July 2022.
18. Clinical and Laboratory Standards Institute. Evaluation of precision of quantitative measurement procedures: approved guideline. 3rd ed. Document EP05-A3, 2014. Available at: https://clsi.org/media/3396/ep05a3e_sample.pdf, accessed 19 May 2022.
19. Virtanen P, Gommers R, Oliphant TE, et al. SciPy 1.0: Fundamental algorithms for scientific computing in Python. Nature Meth 2020; 17, 261-272.
20. Edwards LJ, Muller KE, Wolfinger RD, et al. An R2 statistic for fixed effects in the linear mixed model. Statist Med 2008; 27: 6137-3157.
21. Bates D, Mächler M, Bolker B, et al. Fitting linear mixed-effects models using lme4. J Statist Software 2015; 67: 1–48.
22. Mookhoek EJ, Loonen AJ. Retrospective evaluation of the effect of omeprazole on clozapine metabolism. Pharm World Sci 2004; 26: 180–182.
23. Handley SA, Every-Palmer S, Ismail A, et al. Clozapine-induced gastrointestinal hypomotility: presenting features and outcomes, UK pharmacovigilance reports, 1992-2017. Br J Psychiatr 2022; Feb 15: 1-9.
24. Kuzin M, Haen E, Hiemke C, et al. Body mass index as a determinant of clozapine plasma concentrations: a pharmacokinetic-based hypothesis. J Psychopharmacol 2021; 35: 273-278.
25. Handley SA, Silk SW, Handley DS, et al. Clozapine and norclozapine concentrations in paired human plasma and serum samples. Ther Drug Monit 2018; 40: 148-150.
26. Flanagan RJ, Yusufi B, Barnes TR. Comparability of whole-blood and plasma clozapine and norclozapine concentrations. Br J Clin Pharmacol 2003; 56: 135-138.

27. Fisher DS, Partridge SJ, Handley SA, et al. Stability of some atypical antipsychotics in human plasma, haemolysed whole blood, oral fluid, human serum and calf serum. Forensic Sci Int 2013a; 229: 151-156.
28. Perry PJ, Bever KA, Arndt S, et al. Relationship between patient variables and plasma clozapine concentrations: a dosing nomogram. Biol Psychiatr 1998; 44: 733–738.
29. Paz E, Bouzas L, Hermida J, et al. Evaluation of three dosing models for the prediction of steady-state trough clozapine concentrations. Clin Biochem 2008; 41: 603-606.
30. Oyewumi LK, Freeman DJ, Vollick D. Can low-dose clozapine pharmacokinetics predict steady-state plasma concentrations. Ther Drug Monit 1995; 17: 137–141.
31. Flanagan RJ. Clozapine therapeutic drug monitoring. Why is it important to monitor clozapine doses effectively? Br J Clin Pharm 2011; 3: 18-20.
32. Karastergiou K, Smith SR, Greenberg AS, et al. Sex differences in human adipose tissues - the biology of pear shape. Biol Sex Differ 2012; 3: 13.
33. Bies RR, Bigos KL, Pollock BG. Gender differences in the pharmacokinetics and pharmacodynamics of antidepressants. J Gend Specif Med 2003; 6: 12-20.
34. Couchman L, Morgan PE, Spencer EP, et al. Plasma clozapine, norclozapine, and the clozapine:norclozapine ratio in relation to prescribed dose and other factors: data from a therapeutic drug monitoring service, 1993-2007. Ther Drug Monit 2010; 32: 438-447.
35. Schoretsanitis G, Kane JM, Ruan CJ, et al. A comprehensive review of the clinical utility of and a combined analysis of the clozapine/norclozapine ratio in therapeutic drug monitoring for adult patients. Expert Rev Clin Pharmacol 2019; 12: 603-621.
36. Bush T, Lovejoy JC, Deprey M, et al. The effect of tobacco cessation on weight gain, obesity, and diabetes risk. Obesity (Silver Spring) 2016; 24: 1834-1841.
37. Abela AR, Li Z, Lê AD, et al. Clozapine reduces nicotine self-administration, blunts reinstatement of nicotine-seeking but increases responding for food. Addict Biol 2019; 24: 565-576.
38. Martini F, Spangaro M, Buonocore M, et al. Clozapine tolerability in treatment resistant schizophrenia: exploring the role of sex. Psychiatr Res 2021; 297: 113698.
39. Anderson SG, Livingston M, Couchman L, et al. Sex differences in plasma clozapine and norclozapine concentrations in clinical practice and in relation to body mass index and plasma glucose concentrations: a retrospective survey. Ann Gen Psychiatr 2015; 14: 39.
40. Fève B, Scheen AJ. When therapeutic drugs lead to diabetes. Diabetologia 2022; 65: 751-762.
41. Gunes A, Dahl ML. Variation in CYP1A2 activity and its clinical implications: influence of environmental factors and genetic polymorphisms. Pharmacogenomics 2008; 9: 625-637.