Graylands Hospital Drug Bulletin

Interpretation of Clozapine Levels

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Introduction

The use of blood or plasma levels of medications in psychiatry has been a common practice for many years.[1] The fundamental assumptions behind the use of drug levels in psychiatry is that the effects of the drug are concentration dependent and that the plasma level correlates with levels of the drug at the site of action, the Central Nervous System (CNS).

In a 2014 survey, 83% of clinicians reported they routinely used therapeutic drug monitoring (TDM) for clozapine.[2] This drug bulletin seeks to explain the interpretation of clozapine TDM and also to consider some theoretical advantages in using TDM for clozapine.

Clozapine Pharmacokinetics

The absorption of clozapine is unaffected by food and is essentially complete but the percentage of clozapine reaching the systemic circulation is estimated at between 27% and 47% reflecting a large and variable first pass effect.[3]

Volume of distribution is large and protein binding is extensive. Clozapine is a low extraction ratio drug[4] and displacement from protein binding should not affect unbound levels of clozapine although there has been suggestion of triglyceride levels affecting changes in unbound clozapine.[4]

Clozapine is largely metabolised by the cytochrome P450 system (CYP) by N-demethylation and N-oxidation.[5] The main enzymes involved are CYP_{1A2} and CYP_{3A4}.[6, 7] Most of the variation in activity of the metabolism of clozapine is thought to be due to variations in CYP_{1A2} due to factors such as genotype, age, sex, ethnicity and smoking status.[6, 8] However, some research has indicated that clozapine levels are more strongly correlated with CYP3A4 expression.[9] The main metabolite of CYP_{1A2} is n-desmethylclozapine, also known as norclozapine. (NOR) but both enzymes may play a part in the formation of NOR depending on the clozapine concentration.[9] A role for p-glycoprotein

Box 1. Response to High clozapine plasma concentration result

1) Check your patient
   a) Ascertain the presence and evaluate specific dose related side effects e.g. sedation, hypersalivation, tachycardia, cognitive state, seizures
2) Ensure it was a trough level
   a) Check time between last dose and sample
   b) Check if once a day or twice a day dosing
   c) Check if morning dose was withheld before the blood was taken
   d) Look at ratio of Clozapine to Norclozapine
3) Consider efficacy
   a) Has patient improved on this dose
   b) Evaluate is the patient's current mental state
4) Consider Reasons for High Level
   a) Drug Interactions
   b) Serious illness
5) Consider the trends of previous results for this patient
6) Make appropriate dosage adjustment

has also been proposed and ABCB1 polymorphisms have been associated with regulation of clozapine availability.[7, 8]

Ideally, for clozapine monitoring you should first determine your patient's phenotype of CYP_{1A2} and CYP_{3A4}.[1] Caffeine can be used as a probe to determine the activity of CYP_{1A2} and

CYP_{3A4} can be determined using midazolam as a probe.[1] In clinical practice, this is never done but it remains important to remember that clozapine is primarily metabolised by CYP_{1A2} in most patients but some patients may have the $CYP_{3A3/4}$ enzyme as the major metabolic pathway. [6]

The reported elimination half-life of clozapine has varied significantly over the years. It appears that the elimination half-life varies with the time of the last data point.[10] MIMS describes elimination as "biphasic with a mean terminal half-life of approximately fourteen hours (range 7.9-29.1 hours)"[11] however, based on the information presented by Fang et al[10], it is suggested that 10 hours be used for calculation as this is their average elimination half-life at 12 hours post dose.[10]

Clozapine nomograms have been developed[12] for estimation of clozapine dose but these are not in common use in Western Australia as it is far simpler to titrate the dose to clinical response, supported by plasma levels..

Guidelines for Clozapine Levels

The use of any TDM results in clinical practice carries the assumption that the underlying guidelines have been followed. Results from samples that do not meet these criteria are essentially useless.[13]

For clozapine, the requirements are

- the sample analysed is plasma,
- it is a trough level
- clozapine has reached steady state levels.

Time of the last dose has a significant influence on serum clozapine levels and clozapine to NOR ratios. NOR levels are less influenced by the time because it has a longer plasma half-life and shows less day-to-day variation when compared with clozapine.[13]

Clozapine concentrations are measured in plasma rather than blood. The two samples are not interchangeable as concentrations differ in whole blood and serum.[14, 15] Clozapine levels in red blood cells are lower than in plasma while NOR levels are higher in red blood cells.[15]

Therapeutic Range Evidence

There is broad consensus for a therapeutic threshold concentration of 350 to 370 mcg/L.[8, 13, 14, 16, 17] Data also suggests that the minimum level to prevent relapse could be as low as 200mcg/L.[18] Substantial variation in clozapine plasma level may also predict relapse.[18]

The upper limit for clozapine efficacy remains undefined, therefore most literature dealing with an upper limit of clozapine levels are concerned with toxicity rather than efficacy.

It is clear that many side effects of clozapine are related to both dose and rate of titration and most sources suggest levels greater than 600mcg/L are more likely to cause adverse effects.[19] The risk of developing seizures appears to increase with higher concentrations (>1000 mcg/L).[14] Plasma concentration related side effects include seizures, sedation, constipation, enuresis, hypersalivation, tachycardia and hypotension.[5, 14]

The PathWest reference range is given as 350mcg/L to 1000mcg/L. The lower, therapeutic limit is well supported in the literature.. The 1000mcg/L upper limit used by PathWest is taken from the Laboratory Alert Level recommendation of the AGNP Consensus Committeel.[20] It was thought that their proposal for the upper limit of the therapeutic range (600 mcg/L) was restrictive for a last resort

drug, where the relationship between adverse effects and plasma concentration is still indistinct. The higher 1000mcg/L limit, which was proposed in earlier literature[17], was thus retained.

The serious toxic effects of agranulocytosis and myocarditis appear to be unrelated to plasma concentrations of clozapine.[14, 16] It is believed that these effects as well as pancreatitis, serositis, eosinophilia are associated with increased pro-inflammatory cytokines.[21]

Causes of Variation in Clozapine Levels

There is wide variation in the relationship between clozapine dose and plasma levels from individual to individual.[5, 8, 18, 22] Published literature does support some generalisations;

- Females tend to have higher concentrations of clozapine compared to males.[18, 22]
- Smokers tend to have lower concentrations of clozapine.[18, 22] In older smokers, both men and women tend to have lower doses of clozapine prescribed which accords with the reported decline in activity of the CYP_{1A2} enzyme with age.[12, 23]

Apart from drug interactions, other factors that have been proposed to affect clozapine levels include [5]

- Inflammatory reactions causing slower metabolism
- Plasma clozapine to NOR ratio increases at higher clozapine plasma concentrations in some patients (usually at > 600mcg/L)
- Caffeine can inhibit CYP1A2 but the caffeine dose required to make this significant is probably very high
- Serious respiratory infections can be associated with increases in plasma clozapine. It is also possible that respiratory infections in smokers can lead to reduced smoking or reduced absorption of polyaromatic hydrocarbons so that enzyme induction is lost.
- In some patients, clozapine can show dose dependent saturation of metabolism at or slightly above the plasma clozapine concentration associated with efficacy in many patients[5]

After changing the clozapine dose, changes in plasma levels may be delayed. Delayed absorption of clozapine may be due to gastrointestinal hypomotility, a common adverse effect of the drug. Absorption time may be extended while constipation resolves and this may be an explanation for the observation that in some patients, after reducing the dose, clozapine plasma levels appear to continue to increase for some days. This is more often seen when the plasma level has been over 1000mcg/L: [5].

Use of Norclozapine Levels

Norclozapine, the main metabolite of clozapine, is regarded as an active metabolite.[14] NOR is believed to be associated more with the adverse effects of neutropenia and myocarditis while clozapine itself tends to cause more sedation.[1]

Estimates of the usual ratio of clozapine to NOR vary. The theory has been proposed that a clozapine to NOR ratio of at least two will offer increased clinical benefit and minimise side effects.[24] It is also believed that a ratio of clozapine to NOR of greater than 2 indicates saturation of the CYP_{1A2} enzyme.[1] In regular, stable dosing, the ratio should remain roughly the same for a patient although it does increase to some extent with increasing plasma levels.[5, 15]

Plasma NOR is less affected than clozapine during the absorption phase and NOR has a longer half-life resulting in less day to day variation.[5]

The clozapine to NOR ratio can have practical uses. Changes in the ratio are an indication that something significant has changed in the patient's therapy. A sudden drop in ratio could indicate either non-adherence or the addition of an enzyme inducer e.g. starting smoking. A sharp rise in ratio can indicate a peak level, rather than a trough level or the addition of an enzyme inhibitor e.g. fluvoxamine.

A high ratio of around 3 can also suggest that absorption of clozapine from the last dose may not have been completed at the time the sample was obtained, or that clozapine metabolism is saturated. Some use of professional expertise must be made in interpretation of results as saturation of metabolism only becomes evident at higher plasma clozapine concentrations[5]

How to Respond to Aberrant Clozapine Levels

Although clozapine plasma concentration monitoring is ordered more often than any other antipsychotic, clinicians sometimes have difficulty interpreting results. Box 1 provides steps that could be followed when interpreting plasma levels for drugs like antipsychotics.

It is most important to remember that clozapine plasma levels are only an aid to treatment and clinical response and signs and symptoms should be the primary means for evaluating treatment.

If a high clozapine plasma level is returned, the first action to take is to check on the status of the patient as urgent treatment could be required. Only dose-related side effects should be considered at this stage. Looking for signs of side effects that are believed to be immunogenic in origin (e.g. myocarditis, neutropenia) is not helpful at this stage. If there are signs of clozapine toxicity, the patient should be prescribed symptomatic treatment and further investigation should be made for more serious sequelae e.g. ECG for cardiac arrhythmias.

If the patient is physically well and there are no obvious signs of toxicity, then it is

Box 2 The PathWest Assay

The Clinical Pharmacology and Toxicology Laboratory at PathWest uses liquid chromatography tandem mass spectrometry (LC-MS/MS) to perform Therapeutic Drug Monitoring (TDM) of clozapine. The concentrations of clozapine and norclozapine in serum or plasma are reported

The range of the assay covers the expected therapeutic range for clozapine, and the laboratory can report values from approximately 20 mcg/L up to 1800 mcg/L, with a similar range for norclozapine. Analytical imprecision of the assay has been determined to be 3.3% and 4.9% at the lower reporting limit for clozapine and norclozapine respectively. LC-MS/MS analytical technology offers excellent specificity, and the laboratory reports no known interferences for the assay from either endogenous or other compounds such as co-administered medication.

The estimation of Measurement Uncertainty (MU) is a parameter of assay performance in terms of overall precision and accuracy. The MU for the clozapine assay is estimated to be in the order of +/-20%. This is an estimate of the worst case variation. In reality the method performs much better than +/- 20% as demonstrated by validation procedures.

The MU is much smaller than the reference interval but around the "borders" of the therapeutic range, accounting for the MU may allow a result to be viewed as within or outside the range. The clinician must monitor levels rather than decide actions based on a spot test and this result should be interpreted in the light of the trends of this patient's previous results. The clinical response to a high level would range from repeating the test to reducing the dose. The main criteria for analysis must be the clinical response and side effects the patient experiences.

important to ensure the result that has been reported is a trough level. Any significant changes in ratio of clozapine to NOR for that result can give a clue as to whether the reported result is a peak or a trough. A peak clozapine level will see the ratio skewed towards clozapine. The clozapine ratio should be compared to previous ratios for that patient. Changes can also indicate inhibition or induction of metabolising enzymes e.g. from other medications or changes in smoking habits. If the time between the last dose and sample is known and it is significantly different to 12 hours, an approximate 12 hour level can be calculated using the first order decay equation

$$C = C_o e^{-kt}$$

where the elimination rate constant, k is estimated by

$$k = \frac{0.693}{T½}$$

and the half-life is assumed to be 10 hours.[3, 10, 25]

Consideration of efficacy at this plasma level follows and then other reasons as to why the level may be high should be sought. Some guidelines like The Maudsley Prescribing Guidelines in Psychiatry suggest the use of prophylactic anticonvulsants should be considered.[18]

Low clozapine levels could be a result of low dose, non-adherence or rapid metabolism. There is little point in requesting a clozapine level early in titration unless there are clear signs of dose-related side effects. If the clozapine dose is more than 100mg per day and no clozapine was detected, it is likely that clozapine has not been taken for several days.[5]

It is possible that the result indicates clozapine is in the therapeutic range but clozapine has only been taken just before the blood sample. The clozapine to NOR ratio will allow some interpretation of this type of result. An unexplained change in the patient's ratio to higher clozapine would indicate the result is more likely a peak level.

Table 1 Theoretical Receptor Affinity of Norclozapine

Receptor	Affinity compared to clozapine	Possible Effect of Increasing Norclozapine
Dopamine D2 and D3	Partial Agonist	Less EPSE and better effect on cognition and negative symptoms
Serotonin 5-HT_{2C}	Higher	Increased seizure risk, weight gain, less neutropenia
adrenergic α_1	Lower	Less Postural Hypotension
Histamine H_1	Lower antagonist effect	Less Sedation, less weight gain
Muscarinic M_1	Partial agonist	Increased salivation, improved cognition
δ opioid receptors	Stronger agonism	Benefit mood, cognition and emotional functions

Some variability in results can be attributed to the assay itself, however, in most cases, variability is mainly due to changes in pharmacokinetic parameters as described in Box 2. It remains important to view each result in the context of the trends of previous results for that patient.

When to Add Fluvoxamine

Using fluvoxamine as augmentation to ongoing clozapine treatment may be useful in certain clinical situations e.g. patients who

- do not respond adequately and have difficulty achieving therapeutic levels of clozapine,
- have prominent negative or depressive symptoms

Fluvoxamine potently inhibits CYP_{1A2} in a dose dependent manner and significantly and irreversibly inhibits CYP_{3A4} by forming a metabolic intermediate complex.[9] The addition of 50 mg can increase clozapine plasma levels by up to120 % [6] but the magnitude of the effects is variable and unpredictable.[26] It is thought that patients with fast CYP_{1A2} enzymes will experience a more dramatic increase in clozapine levels from the addition of fluvoxamine.[27]

The addition of an enzyme inhibitor like fluvoxamine will also alter the clozapine to NOR ratio. Reducing the proportion of NOR may reduce some adverse effects, as NOR has higher affinity to serotonin 5-HT_{2C} receptors which are believed to contribute to seizure risk, weight gain and possible risk of the less severe forms of neutropenia than agranulocytosis.[6] NOR is considered a less potent antagonist at adrenergic α_1 and histamine H_1 receptors than clozapine and is less likely to cause adverse effects such as orthostatic hypotension and sedation.[6, 24, 28]

Agranulocytosis is generally regarded to be a result of an immunological reaction.[19] Bone marrow toxicity for both clozapine and its metabolites have been measured and NOR has been found to be up to 10 times more potent than clozapine.[19]

Table 1 summarises different receptor affinities between NOR and clozapine and gives a theoretical outcome if the ratio is altered.

It is also possible that the clozapine to NOR ratio may differ between a patient's plasma and central nervous system as clozapine and NOR have differences in affinity to p-glycoprotein and so could penetrate the blood brain barrier to different extents.[29]

The use of fluvoxamine may also reduce the number of tablets a patient is prescribed. Apart from potentially reducing the number of tablets that could be available for misuse by a third party, less tablets to take could also improve adherence. However, addition of fluvoxamine adds another medication the regimen, increasing its complexity with the possibility of worsened adherence.

Another risk of using fluvoxamine in this way is increasing the risk of side effects as the plasma level increases. Since the effect of fluvoxamine of clozapine is difficult to predict, extremely high plasma levels can be produced with the consequent adverse effects. For this reason, it is recommended that if fluvoxamine is to be added to clozapine it is done cautiously.

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