Zaponex Fact Sheet
Clozapine metabolism and plasma level monitoring

Important Information

The information provided in this fact sheet is intended for healthcare professionals and should not be used as a patient information leaflet.

The information in this document is not intended as a definitive treatment strategy, but as a suggested approach for clinicians. It is based on information from scientific literature and previous successful experience. Each case should, of course, be considered individually.

This fact sheet covers the following subjects (click to jump to the corresponding section):

Pharmacokinetic properties of clozapine

Summary of Product Characteristics statements

The Zaponex® Summary of Product Characteristics (section 5.2) states that:
"The absorption of orally administered clozapine is 90 to 95%; neither the rate nor the extent of absorption is influenced by food. Clozapine is subject to moderate first-pass metabolism, resulting in an absolute bioavailability of 50 to 60%."[1]

"In steady-state conditions, when given twice daily, peak blood levels occur on an average at 2.1 hours (range: 0.4 to 4.2 hours), and the volume of distribution is 1.6 l/kg. Clozapine is approximately 95% bound to plasma proteins."[1]

"Clozapine is almost completely metabolised before excretion by CYP1A2 and CYP3A4, and to some extent by CYP2C19 and CYP2D6. Of the main metabolites only the demethyl metabolite" (norclozapine) "was found to be active."[1]

"Its elimination is biphasic, with a mean terminal half-life of 12 hours (range: 6 to 26 hours). After single doses of 75 mg the mean terminal half-life was 7.9 hours; it increased to 14.2 hours when steady-state conditions were reached by administering daily doses of 75 mg for at least 7 days. Only trace amounts of unchanged drug are detected in the urine and faeces, approximately 50% of the administered dose being excreted as metabolites in the urine and 30% in the faeces."[1]

"Dosage increases from 37.5 mg to 75 mg and 150 mg given twice daily were found to result during steady state in linearly dose-proportional increases in the area under the plasma concentration/time curve (AUC), and in the peak and minimum plasma concentrations."[1]

Cytochrome P450 metabolism

Clozapine is mainly metabolised in the liver. The initial step involves phase I oxidative reactions, catalysed by cytochrome P450 isoenzymes, creating clozapine's main metabolites N-desmethylclozapine (norclozapine) and clozapine N-oxide. A reverse metabolism, back into the parent compound clozapine, has been shown for the clozapine-N-oxide metabolite.[2,3] Bioactivation to reactive nitrenium or iminium ions may also occur.[4,5] Oxidative reactions are followed by phase II glucuronide conjugation, mediated by UDP-glucuronosyltransferases.[6,7]

The main cytochrome P450 isoenzyme involved in clozapine metabolism is CYP1A2, but CYP2C19, CYP3A4 and CYP2D6 also play a role.[6,8] CYP1A2 is important in the metabolism of clozapine to N-desmethylclozapine, while CYP3A4's dominant role is oxidation to clozapine N-oxide.[8,9] CYP3A4 is probably also the main enzyme leading to reactive nitrenium metabolites of clozapine in the liver.[5] CYP2D6 appears the most active enzyme involved in bioactivation of clozapine to the reactive iminium ion. CYP2D6 expression accounts for only a small percentage of the total CYP content of the liver in most individuals[5,10] and therefore, this enzyme is expected to have little clinical relevance in clozapine pharmacokinetics and bioactivation to reactive metabolites in the majority of patients.[5] In situations where CYP1A2 is functionally impaired, other CYPs like CYP3A4 get a more significant role.[9]

The various metabolites are thought to have a different capacity to induce side effects.[5] For instance, the reactive nitrenium ion is probably directly involved in neutrophil[11] and liver toxicity.[5] Bioactivation of clozapine to a reactive iminium ion might also contribute to hepatotoxicity.[5] Clozapine N-oxide exerted no neutrophil toxicity in an *in vitro* study.[11] In addition, N-desmethylclozapine is suspected to be involved in certain side effects,[12] such as weight gain.[13] Certain UDP-glucuronosyltransferases and glutathione S-transferases may protect against toxicity from (reactive) clozapine metabolites.[7,14] Individual variation in metabolic pathways may therefore correlate with a different adverse event profile, although there is very little evidence to corroborate this theory.

Cellular uptake transporters

Clozapine uptake into the brain is thought to be mediated by a carrier. However, it was found to be independent from currently known drug transporters and the transporter responsible is yet to be identified. Transporter-mediated uptake in the brain may partly explain clozapine's high unbound accumulation in the brain and its drug-drug interaction profile.[15]

Microkinetics

Latest insights indicate that the cell membrane plays a role in 'microkinetics' of drugs. At high free drug concentrations, clozapine, as a highly lipophilic molecule, is expected to accumulate within the hydrocarbon core of the cell membrane. At lower levels, constant exchange of drug molecules between the membrane and the surrounding aqueous medium may allow the membrane to act as a repository and, in this manner, prolong the exposure of the target receptor to sufficiently high concentrations of the drug. The cell membrane also affects the conformation of the drug. Furthermore, the membrane may facilitate the approach (and rebinding) of the drug to the target receptor and may even participate in the binding process.[16]

Interpersonal variation in the here described pharmacokinetics make it hard to predict an individual therapeutic dose and plasma level.

Relation clozapine dose and plasma levels

Many studies have identified a linear relationship between dosage and clozapine plasma level within individual patients.[17–20] On the other hand, it also appears that there is notable intra-individual variability in plasma clozapine and norclozapine levels.[21]
Inter-individual differences are even bigger; clozapine plasma levels between patients can vary as much as 45-fold for the same dose.[22,23] Several factors are known to affect clozapine's metabolic rate, including ethnicity, gender, age, weight, genetic polymorphisms, smoking, caffeine intake, diet, infection/inflammation and co-medication, which are further elucidated below.

Factors affecting clozapine plasma levels

Gender

Women tend to have higher clozapine plasma levels than men when administered the same clozapine dose.[17,20,24–27] This can be explained predominantly by a slower clearance rate of clozapine from the female body,[27] Pharmacokinetic gender differences include lower CYP1A2 activity; slower gastric emptying; lower glomerular filtration rate, renal tubular secretion and reabsorption; more adipose tissue;[28] and lower liver blood flow in women[29,30] Other potential factors include CYP inhibiting effects of female natural and synthetic hormones (more on that further on in this chapter).
Although this information has been known for years, women usually still get prescribed the same doses as men.[31] We advise to aim for a lower target dose in females, compared to males. See also "Fact sheet Dosing".

Ethnicity

Studies among several Asian ethnicities, including Chinese,[32] Korean[33] and Taiwanese,[23] reported that Asian patients tend to have higher clozapine plasma levels compared to Caucasian patients. One study reported that Asian patients required less than half the dose taken by Caucasians to reach similar plasma levels.[34] One explanation may be that certain CYP2C19 polymorphisms, leading to substantial higher clozapine levels,[35] are more common among Asians.[6,36]

Weight

There is some inconsistency between studies on the correlation between weight and clozapine plasma levels, with some reporting a correlation, and others reporting no relationship. However, the studies reporting no relationship were smaller (< 162 patients).[25,37,38] A large study including 3789 patients conducted in the UK and Ireland showed that plasma levels were generally higher in patients with high body weight.[20] A small study (n=46) measuring the clozapine plasma level kinetics 10-14 hours after dosing indicated that patients with a higher BMI may indeed have slower clozapine elimination.[3]
Another study (n=47; 424 plasma concentrations) showed that weight increases during clozapine treatment, which probably reflect increases in fat tissue, were associated with increases in total plasma concentrations, especially in females.[39] These studies support the idea that clozapine may deposit in fat tissue and that this increases clozapine half-life.[39,40]

Age

Several studies have indicated that plasma levels increase with age.[25,27,41] More specifically, Ismael *et al*. reported that clozapine clearance decreased exponentially with increasing age, starting from an age of 39 years old.[27] The clozapine dose in ageing patients on long-term treatment should therefore be periodically reconsidered.[27,41] For further advice on clozapine treatment in elderly patients, see our fact sheet "Elderly patients".

Genetic polymorphisms

The available information on the effect of various polymorphisms on clozapine plasma levels and the clinical usefulness of geno- or phenotyping is inconsistent.
CYP1A2 variants *1C and *1D were associated with higher serum clozapine concentrations in a small study including 17 patients.[42] In smokers carrying a CYP1A2*1F variant, low plasma levels[43] and nonresponse to clozapine were associated with ultra-rapid CYP1A2 activity.[44] On the contrary, a recent systematic review and meta-analysis suggests that CYP1A2 genetic polymorphisms have no significant impact on the pharmacokinetics of CYP1A2-metabolised antipsychotic drugs and that CYP1A2 genotyping may have no clinical implications for personalised dosing of these drugs.[45]
CYP2C19 poor metabolisers are reported to have 2.3 fold higher plasma concentrations.[35] Plasma levels do not seem to be related to the CYP2D6 genotype.[42] Polymorphisms in the ABCB1 gene encoding P-glycoprotein, a transmembrane transporter affecting drug absorption and distribution, were associated with lower plasma levels[46] and non-response to treatment.[47]
Results of a pre-clinical study indicated that prediction of drug-drug interactions in individual patients using *in vivo* phenotyping is unlikely to be informative.[9]

Caffeine intake

Both caffeine and clozapine are metabolised by, and compete for, the CYP1A2 enzyme. As a result of this competitive interaction, caffeine intake increases the plasma concentration of clozapine,[38,48] as there is less CYP1A2 available to metabolise clozapine. Consequently, the interruption of habitual caffeine intake may increase clozapine metabolism.
The degree by which caffeine affects clozapine pharmacokinetics varies between individuals, as demonstrated in a study of healthy volunteers. In this study, caffeine intake in the range of 400–1000 mg/day was found to inhibit clozapine metabolism to an extent that might be clinically significant in certain individuals.[49] Mean plasma concentration of clozapine was decreased by 37% following a 5-day caffeine-free period in another study.[50]

Contradictory, there is also literature that reports on a relation of high intake of caffeinated beverages and poor clozapine response,[51] or lower clozapine levels.[52] It is not completely sure if there is a causative relation in these studies; it may very well be that the lower levels and response are actually caused by cigarette smoking,[51] which often coincides with high caffeine intake.[53] Moreover, it is theorised that other constituents in caffeinated beverages may also induce CYP1A2 enzyme activity.[52]

At treatment initiation, it is recommended that patients are questioned regarding their caffeine intake and informed about the possible effects of changing their regular caffeine-drinking habits. When considering caffeine intake, it must be remembered that, apart from coffee and tea, carbonated drinks such as cola and energy drinks (e.g. Red Bull™) also contain caffeine. There is an estimated 75–165 mg of caffeine in an 8 ounce (237 mL) cup of coffee (with filter coffee containing the most caffeine[54]) or can of energy drink. Tea and cola contain less caffeine, up to 47 mg per cup/glass.[55] Dose adjustments may be required during therapy as a result of substantial changes in caffeine intake.[1]

Smoking

Polycyclic aromatic hydrocarbons in cigarette smoke are associated with the induction of some hepatic enzymes, particularly CYP1A2,[56] increasing the rate of metabolism of clozapine through this enzyme.[57] This leads to around 50% lower dose-adjusted serum concentrations in smokers compared to non-smokers[58–60] and is consistent with reports on heavy smokers requiring higher doses of antipsychotic medication.[59] This also means that patients who smoke and who are not responding to clozapine treatment should have their plasma levels checked to ensure that their dose is adequate for obtaining full therapeutic effect.

Smoking cannabis has the same effect as smoking tobacco[61,62] and also second-hand smoke appears to induce CYP1A2 metabolism.[63]

Accordingly, the impact of smoking cessation or severe reduction on plasma levels of clozapine needs to be considered.[1] There are several reports describing increased plasma levels of clozapine following smoking cessation and a subsequent increase in dose-related side effects, some of which were serious.[61,64,65] Patients should be reminded to inform their treatment team if they intend to give up smoking. Therapeutic drug monitoring of clozapine plasma levels and dose-reductions may be necessary to minimise side effects.[66]

In general, stepwise dose reductions are recommended immediately after smoking cessation,[67,68] especially in patients with high clozapine levels prior to cessation,[64] since acting upon clozapine assay test results would delay dose reductions with at least a couple of days. Cormac *et al*.[64] retrospectively evaluated the effect smoking cessation had on both plasma levels and dose adjustments after the introduction of a smoking ban in a UK hospital. During the study period, there was a significant reduction in mean clozapine dosage with 60% of patients having a dose reduction up to 25%; 11% of patients had a dose reduction of over 50%. As a rule of thumb, Faber *et al*. propose a stepwise daily dose reduction of approximately 10% until the fourth day after smoking cessation.[68]

Please note that e-cigarettes and nicotine patches do not have the enhancing effect on clozapine metabolism.[62] Nicotine itself is metabolised by the CYP2A6 and CYP2B6 enzymes,[69] which are not involved in clozapine metabolism. Thus, a switch from smoking to these quit-smoking aids can also lead to clozapine toxicity.

Infection and inflammation

Inflammation, *e.g.* as a result of bacterial or viral infections, has been reported to increase plasma clozapine levels.[70–74] A systemic review and analysis of published case reports learned that the mean clozapine level during infection increased with a mean of 234% (±SD 165%).[75] Severe side effects were rare, despite of the substantial increases in clozapine levels.[75]
Based on published case reports and their own clinical experience, Ruan *et al.* recommend that for clozapine patients with severe infection or inflammation (with CRP being an established marker), clinicians should consider cutting the clozapine dose in half until the results of a clozapine plasma assay have come back to guide further dosing adjustments.[76,77]

The increase in clozapine levels is thought to be mediated by suppression of CYP enzymes (CYP1A2, CYP3A4, and CYP2C19)[77,78] by inflammatory cytokines such as IL-6, TNF-α and interferon.[74,75,78,79] It is possible that alterations to drug transporters and other metabolic pathways in the setting of inflammation also contribute to increased levels of clozapine.[80]
It has been hypothesised that infection and inflammation-associated increase of the acute phase protein α1-acid glycoprotein may counteract this effect by increasing the plasma protein binding

capacity for both clozapine and norclozapine, thereby reducing the amount of free 'active' clozapine.[71,75,81,82] The net clinical impact may therefore not be as high as would be expected based on clozapine levels alone.[75] The rate and level of increase of α1-acid glycoprotein is thought to be individually and situationally variable.[72,81,83]

Note that non-infectious inflammation, caused for instance by surgery,[84] injury, auto-immune disease, or chronic obstructive pulmonary disease exacerbation,[84] may also lead to increased clozapine levels.

An important factor that can aggravate the risk of increased plasma clozapine levels during infection is treatment with certain antibiotics. Fluoroquinolones (*e.g.* ciprofloxacin) are known to inhibit both CYP1A2 and CYP3A4 and macrolides suppress the action of CYP3A4. Ciprofloxacin is most notorious and has been described to increase clozapine levels to varying, though substantial, extents; underlying infections may have confounded these increases.[75,85–87]

Hospitalisation and serious illness carry an added risk of clozapine toxicity since patients may temporarily reduce or stop smoking. Thus, extra attention needs to be provided during infection or hospitalisation to prevent possible clozapine toxicity.[75]

Pfuhlmann *et al.* recommended measuring CRP if an unexpectedly high serum clozapine concentration is found.[70] The other way around is also advisable: if CRP is found to be elevated, a clozapine plasma assay may be performed to detect a possibly raised clozapine level.

Hormones

There have been several case reports showing that oestrogens, progestogens, and their synthetic analogues in contraceptives can increase clozapine plasma levels.[86,88,89] Oestrogens are probably inhibitors of CYP2C19 and CYP1A2, and, to a lesser extent, of CYP3A4. Progestogens also appear to variably inhibit CYP3A4.

These interactions should be taken into account in any patient who starts or stops contraceptives. Contraceptives with intermittent dosing (3 weeks therapy, 1 stop week) are probably best avoided. Note that hormone replacement therapy usually also consists of oestrogens and progestogens, (although hormone levels are much lower than in contraceptives).

Pregnancy

Consequently, it can be expected that pregnancy will also affect clozapine plasma levels, as natural oestrogens and progesterone are raised during pregnancy. Indeed, CYP1A2 and CYP2C19 activity is decreased during pregnancy.[90] However, volume of distribution will also be altered due to the pregnancy,[90] possibly leading to lower levels. It is unknown how these different factors will affect the clozapine plasma levels throughout the full-time course of the pregnancy,[90] and there may be large differences between patients. Therefore, we strongly advise to routinely monitor plasma levels during pregnancy in patients in whom clozapine is continued.[91] Dose adjustments may be needed in order to treat the patient on the lowest effective clozapine level.[91]

Grapefruit juice does not affect clozapine plasma levels

Although grapefruit juice is a known inhibitor of CYP3A4 and it is known to affect drug metabolism, there is no evidence that it substantially affects clozapine plasma levels, clinical efficacy, or tolerability.[92]

Clozapine and drug interactions

Concomitant use of clozapine with pharmaceutical drugs affecting CYP enzymes, may also have an effect on clozapine plasma levels. An overview of the most common interactions is shown in Table 1. Caution should be observed if clozapine is used concomitantly with these substances and dosage adjustments of clozapine and/or other drugs may be necessary.[1] It should also be remembered that upon discontinuation of these drugs, dose adjustments may be needed again.

In general, any inhibitor of clozapine's major CYP1A2 pathway is likely to have an effect on clozapine plasma levels. The effect of inhibition on CYP2C19, CYP2D6 and CYP3A4 may vary between patients.[93] Interaction at these minor pathways can be expected in patients with reduced CYP1A2 activity. Competition for clozapine's minor metabolic pathways will usually only have significant effects when multiple competitors and/or inhibitors are present.

Accordingly, please note that polypharmacy can lead to unpredictable interactions: Interactions that are insignificant when used alone with clozapine, suddenly have substantial effects when other pathways of clozapine metabolism are attenuated by inhibition or competition by multiple other medications.[94] Routine therapeutic drug monitoring is recommended.

Importantly, when introducing a strong CYP1A2 inhibitor such as fluvoxamine or ciprofloxacin, or discontinuing a major CYP1A2 inducer as with smoking cessation, immediate gradual dose reductions are advised. Dose reductions that act upon plasma assay results may come too late, as toxic levels may arise within days after the change.[67,68] As a rule of thumb, the following dose reductions have been proposed for the aforementioned situations in the scientific literature:

- A stepwise daily dose reduction of approximately 10% until the fourth day after smoking cessation has been proposed by Faber *et al*.[68] See also "Smoking" on page 3 of this fact sheet.
- When adding a strong CYP1A2 inhibitor, a clozapine dose reduction of at least two-thirds has been suggested by Meyer *et al.*[95]
- A plasma clozapine level needs to be obtained at baseline, prior to the medication/smoking change, and rechecked after the new steady state has been reached, that is 5-7 days after the last (dose) change. Clozapine dose must be adjusted accordingly.[95]

Table 1. ***Patient characteristics, lifestyle and substances affecting clozapine plasma levels***	
Factors that are associated with **increased** plasma concentrations of clozapine	Factors that are associated with **decreased** plasma concentrations of clozapine
Female gender Asian origin Old age High body weight	Male gender Young age Low body weight
CYP1A2 variants *1C and *1D CYP2C19 poor metabolisers	CYP1A2 variant *1F in smokers Polymorphisms in ABCB1
Infection/Inflammation	
Drugs that inhibit the CYP enzyme system, and may result in **increased** clozapine plasma levels	Drugs that induce the CYP enzyme system, and may result in **decreased** clozapine plasma levels
Caffeine	Smoking
Cimetidine,[1,96] ciprofloxacin,[87,95,97] erythromycin,[1,98] minocycline,[99] certain antidepressants [e.g. SSRIs; fluoxetine,[100] fluvoxamine,[100] paroxetine,[100] sertraline,[101] citalopram[102], pregabalin[103–105] (type of interaction unclear), amiodarone,[106] isoniazid,[107] oestrogen, ethinyloestradiol, and progesterone (e.g. in contraceptives)[86,88,89]	Carbamazepine,[1,108,109] phenytoin,[1] rifampicin,[1,110–113] phenobarbital,[100] aspirin (possibly mediated by valproic acid),[114] omeprazole in non-smokers,[1,115] St. John's wort[116]
Valproic acid Reports on the effect of valproic acid on clozapine plasma levels are unequivocal. In some reports the clozapine levels were increased,[117] whereas in others decreased levels have been reported after starting sodium valproic acid.[118–120] The inconsistent results found in the literature may be partially explained by the observation that valproic acid increases clozapine plasma levels in non-smokers, but decreases clozapine levels in smokers.[100,121] The mechanism of this interaction is not clear, but the net effect of valproic acid may be a sum of both inducing and inhibiting actions on clozapine metabolism, influenced by smoking status, valproic acid levels, and valproic acid treatment duration.[122]	

More information about interactions can be found in the Zaponex® Summary of Product Characteristics, section 4.5.[1] For additional questions about possible drug interactions, please contact the Zaponex Medical Information Service / ZTAS help desk.

Measuring clozapine plasma levels

As said, plasma levels differ greatly between individual patients. Unfortunately, clozapine has a relatively narrow gap between the therapeutic range and excessive levels associated with increased side effect risk and toxicity. Therefore, the monitoring of clozapine levels in blood plasma can be a useful tool in the adjustment and the maintenance of the right dose.[67]

We suggest considering plasma level determination in the following situations:

- Halfway during clozapine titration; for instance at a daily dose of 150 mg to identify fast or slow metabolisers[6,123]
- At steady state (approximately 5 days) after reaching the target dose
- After dose changes (at steady state, 5 days after the last change)
- After 6 months and then at least yearly to prevent unnoticed decreases or increases in plasma levels
- When patients change lifestyle habits or pharmaceutical drug use that can affect the function of cytochrome P450 enzymes, involved in clozapine metabolism (see table 1). Preferably, a baseline

clozapine level is determined before such changes occur, to facilitate adequate dose adjustments if necessary
- In case of infection or inflammation (or raised CRP as a marker of this)
- Patients with polypharmacy, especially in case of multiple drugs known to modulate or compete for CYP1A2, CYP2C19, CYP2D6 and/or CYP3A4
- Elderly patients, as they are at increased risk of having high plasma levels[25,27,41]
- Patients being prescribed higher doses (> 600 mg/day) to help in assessing the risk of toxicity[124]
- If toxicity is suspected[67]
- If a drug interaction is suspected[67]
- In case of sub-optimal response to treatment[124]
- To confirm compliance,[67] although it is often difficult to draw definitive conclusions based on clozapine assays
- In case of overdose, it is advised to take a sample as soon as possible after discovering the overdose[67]

Practical information

ZTAS facilitates a service for measuring clozapine plasma levels (clozapine plasma assay). Healthcare Professionals wishing to measure clozapine plasma levels can request this service (at a small additional charge) from the ZTAS Central Laboratory, provided by Magna Laboratories.

For a clozapine plasma assay, a minimum of 2 mL of venous blood should be drawn into an EDTA tube (provided via Magna Laboratories) and be sent, together with a request form, to Magna Laboratories for analysis. Please note that a separate sample should be provided for clozapine plasma assays. Capillary samples are not suitable for the purpose of clozapine assays.

The ZTAS website (www.ztas.co.uk) contains a dedicated online order form that can be used for the (re-)ordering of laboratory support materials.

Conversion of plasma concentrations: mass or mole per volume

0.1 mg/L equals 100 µg/L equals 100 ng/mL. Sometimes plasma levels are expressed in mole; in this case a factor of 3.06 may be used for conversion;[125] *e.g.* nmol/L = µg/L x 3.06 and nmol/L x 0.3268 = µg/L; µmol/L = mg/L x 3.06 and µmol/L x 0.3268 = mg/L.

When to measure: steady state after dose adjustments

After titration and dose adjustments, clozapine plasma levels should be measured at steady-state. "The Maudsley prescribing guidelines" states that this usually takes 4-5 drug half-lives and, as clozapine has a drug half-life of 12-14 hours, in most patients the estimated steady state could already be achieved after 2-3 days.[67] However, since some patients have slower elimination with longer half-lives (up to 26 hours),[1] a steady state should have been reached for virtually all patients after 5 days.

When to measure: steady state after change in factors associated with clozapine metabolism (CYP450)

It is strongly advised to measure the clozapine plasma levels before introducing factors that affect clozapine metabolism. This will allow future dose adjustments targeting the same individual therapeutic range.

In general, CYP450 enzyme inhibition is mediated by a direct interaction with the metabolising enzyme, either by competitive, non-competitive or uncompetitive actions, which can be reversible or

irreversible.[126,127] Inhibition usually starts immediately, but the effect can increase in time along with blood plasma levels of the inhibiting drug. With a reversible inhibitor, the enzyme can resume its activity when the inhibitor is stopped, depending on the drug half-life. However, with an irreversible inhibitor, the enzyme is permanently dysfunctional.[127] After stopping an irreversible inhibitor, it may therefore take several weeks before the enzyme returns to its full level of activity, since new enzyme needs to be synthesised.

CYP450 enzyme induction generally involves gene regulation and increased gene expression, followed by increased synthesis of the enzyme. Therefore, it usually takes a longer time before the full effect is apparent; this largely depends on the specific drug or substance involved.[126,127]

Since the nature of interactions is not always known in detail, and/or the data are not easily to retrieve, it is usually clinically impractical to adjust the timing of the sampling to the individual interaction. In most situations it is appropriate to measure plasma levels 2 weeks after a mild inducer or inhibitor is started, as it will cover the effect of most interactions.[123] Dose adjustments can follow accordingly.

Importantly, in case of factors strongly affecting clozapine levels such as smoking cessation or addition of fluvoxamine or ciprofloxacin, dose reductions acting upon plasma assay results may come too late: Toxic levels may arise within days and immediate gradual dose reductions are advised (see also "Smoking" on page 3 of this fact sheet).[67,68] A plasma assay may be taken a week after smoking cessation[67] or the addition of fluvoxamine to ensure dose adjustments have been appropriate. It is recommended to repeat the assay 2 weeks after cessation, since in most patients the final new steady state for clozapine will be reached by the end of the second week.[64,68,128,129] Note that in some patients, clozapine plasma levels can remain unstable for a number of months,[64,128] so a repeat assay is recommended if there is any suspicion of toxicity or reduced clinical benefit.

When to measure: sampling time

Maintaining a fixed sampling regime is essential for achieving reproducible and reliable plasma sample data.[3,67] The time of sampling and the time of last dose, therefore, should always be recorded.[3,67] A sampling time of 12 hours post-dose (and before taking the next dose) is generally recommended.[67,91] Importantly, if a sample is not taken within 1-2 hours of the required time, it has the potential to mislead rather than inform.[67]

In a study by Jakobsen *et al*. in 46 patients, clozapine and norclozapine plasma levels were measured 10, 11, 12, 13 and 14 hours after the evening clozapine dose, in order to assess the impact of sampling time on clozapine assay results. The clozapine concentration seemed to generally decrease in time, but median and mean percentage differences in clozapine and norclozapine concentrations within the 4-h time span were minor. However, some individuals experienced substantial variations in clozapine and norclozapine levels between 10 and 14 hours post-dose. Furthermore, in nearly one quarter of the subjects the clozapine concentration unexpectedly increased during this 4-hour period. Erratic absorption, gastro-intestinal side effects, food-intake, or reverse transformation of clozapine-N-oxide and some less important protein-reactive metabolites into the parent compound clozapine are mechanisms proposed by the authors as explanation for this observation. These data stress the need to adhere to a fixed sampling time.[3]

For patients with a twice daily dosing schedule, 12 hours post-dose usually is equal to trough phase. For patients taking their dose once a day, it is also recommended to adhere to the 12-hour post-dose sampling time, in order to compare the results with general therapeutic ranges.[91] But note that

around 23% higher results can be expected 12-hour post-dose in case of once daily dosing and correcting for this may be appropriate.[130]

If toxicity is suspected, a sample should be taken immediately.[67]

Interpreting clozapine plasma levels

The basic rule for sample level interpretation is to act upon assay results in conjunction with reliable clinical observation (*'treat the patient, not the level'*).[67] For example, if a patient is responding adequately to a drug but has a plasma level below the accepted target range, then the dose should not normally be increased. If a patient has intolerable adverse effects but a plasma level within the target range, then a dose decrease may be appropriate.[67] (see also below in section: "*Actions to take after a plasma assay result outside the therapeutic target range"*)

When a plasma level result is substantially different from previous results, a repeat sample is usually advised. Check the dose, the time of dosing, recent compliance and ensure, in particular, the correct timing of the sample. Many anomalous results are the consequence of changes in sample timing.[67] In addition, be aware of intra-individual variability; do not assume that plasma levels are static. If plasma levels are used to guide dosing of clozapine, serial measurements rather than a single level might be necessary to make an informed clinical decision.[21]

Based on an assay result, it is impossible to draw definitive conclusions on compliance. Bear in mind that a plasma level of zero indicates only that the drug has not been taken in the past several days. Plasma levels above zero may indicate erratic compliance, full compliance or even long-standing non-compliance disguised by recent taking of prescribed doses.[67]

Therapeutic target range in schizophrenia spectrum disorders

The ZTAS Central Laboratory (Magna Labs) quotes a clozapine concentration reference interval of 0.35-0.6 mg/L on their reports, which is a generally accepted target range for schizophrenia.[91] In those not responding to clozapine, dose may be adjusted to acquire plasma levels in the therapeutic target range. Those not tolerating clozapine may benefit from a reduction to a dose corresponding to plasma levels in this range.[67]

As previously mentioned, many patients achieve optimal therapeutic benefits at levels above or below therapeutic target range. A study in 26,796 clozapine treated patients indicated that plasma clozapine was either below 0.35 mg/L or greater than 0.6 mg/L in 42.5% and 28.4% of samples, respectively.[62] A study by Van der Zwaag *et al.* comparing treatment groups with different ranges of plasma levels reported no clinical advantage for 0.35-0.45 mg/L over 0.2-0.3 mg/L.[130]

Limited data suggest that a level of at least 0.2 mg/L is required to prevent relapse.[131] An upper limit to the clozapine target range is ill-defined. There is some evidence that plasma levels above 0.6-0.838 mg/L do not improve clinical response,[19] although some cases suggest that some patients benefit from clozapine levels above the therapeutic target range.[132] In any case, plasma levels are best kept well below 1.0 mg/L.[67]

If clozapine is administered once daily in the evening, the target level 12 hours post-dose may be increased by 23% due to a later trough phase.[130]

NB: A clozapine plasma assay provides the total clozapine plasma level, which is the free (unbound) clozapine portion and the (protein-)bound portion together. Clozapine is highly protein bound; it is for approximately 95% bound to plasma proteins.[1] The unbound clozapine concentration in the brain would be the true exposure responsible for specific target receptor occupancy.[133] Transporter-mediated clozapine uptake in the brain is a limiting factor thereby.[15] These are of course variable factors, complicating the interpretation of clozapine plasma level results.

Therapeutic target range in Parkinson's disease psychosis and related disorders

No official therapeutic target range has been established for treatment of psychosis during the course of Parkinson's disease (PD) and related disorders. A retrospective chart review of 35 patients (mean age 72 years) suffering from dopamimetic psychosis in PD and related disorders treated with clozapine found a mean clozapine serum concentration of 0.078 mg/L (77.9 ng/mL; standard deviation (SD) 63.4 ng/mL). Clozapine dose was significantly correlated with clozapine serum concentration. Based on this study the authors suggest an orienting indication-specific therapeutic reference range of 0.015-0.141 mg/L (15–141 ng/mL) (equivalent to mean±1SD of clozapine levels obtained in this study) among PD patients with dopamimetic psychosis.[134]

Side effects at high plasma concentrations

It is important to note that toxicity is more likely in novice users who have not yet developed tolerance to clozapine or in non-adherent patients inconsistently exposed to their prescribed dose.[124]

Typical side effects associated with high plasma levels or clozapine toxicity are orthostatic hypotension, dizziness, hypersalivation, sedation, tachycardia, nocturnal enuresis, irritability, and confusion / mental status change.[1,67,84,125]

High plasma levels also seem to predict EEG changes and seizures.[135–137] The threshold is unclear and estimations varied greatly in different studies (from as low as 0.24 mg/L to 1.3 mg/L.[19] Stark *et al*. conclude in their review that the risk of seizures significantly increases with clozapine plasma levels greater than 0.6 mg/L.[124]

The risk of constipation also seems to be correlated to clozapine plasma levels[138] and clozapine doses were found to be higher among patients who died from gastrointestinal hypomotility.[139]

In addition, there is a relation between elevated liver function tests and clozapine dose[140] or plasma levels,[141] although this typically is a problem occurring in the first months of clozapine therapy.[141,142]

The risk of developing clozapine-induced neutropenia/agranulocytosis is not correlated to dose or plasma levels.[143–146] Many other serious side effects, such as diabetic ketoacidosis, cardiomyopathy, QTc interval prolongation, fulminant hepatic failure and venous thromboembolism, also seem not clearly associated with high plasma levels.[19]

Clozapine/norclozapine ratio

"Clozapine is almost completely metabolised before excretion. Of the main metabolites only the desmethyl metabolite (norclozapine) was found to be active."[1]
Clozapine and norclozapine plasma levels are both reported by clinical labs. Whereas clozapine levels are correlated to antipsychotic effect, norclozapine levels are not.[147,148] However, norclozapine does seem to enhance cognition.[149–153]
The clozapine/ norclozapine ratio can provide some insight on the metabolism of clozapine: clozapine metabolism may become saturated at higher doses or when enzymatic clearance is diminished and

therefore the ratio of clozapine to norclozapine rises with increasing plasma levels, suggesting saturation.[67] In a review study, plasma levels in 3782 patients with a median dose of 300 mg/day showed an average ratio of 1.3.[20] Another large study reported that median plasma clozapine/norclozapine ratio was 1.25 at plasma clozapine concentrations below 0.35 mg/L, and the median ratio was 2.08 at plasma clozapine concentrations above 1.0 mg/L.[62] In addition, norclozapine may give some information concerning compliance, as the half-life of norclozapine is around 1.8 times longer than clozapine's.[154]

If the clozapine plasma level is within normal limits and the patient is responding well to clozapine without side effects, there is absolutely no need to change the dose in order to reach a certain clozapine/norclozapine ratio. The ratio could be used to alert clinicians for possible non-compliance issues, saturation of clozapine metabolism or erroneous timing of sampling, especially in case of very high or very low clozapine plasma levels.

A clozapine/norclozapine ratio greater than 2 may suggest either a non-trough sample or saturation of clozapine N-demethylation.[62] There is some risk involved with saturated clozapine metabolism, as relatively small dose increases can result in disproportionate increases in clozapine levels in some patients.[62]

A ratio below 0.5 suggests either poor adherence within the last 24 hours or so, or that alterations in dose schedule would probably be beneficial.[62] A relatively high ratio in combination with a normal clozapine level (*e.g*. low nor-clozapine level) could indicate non-compliance disguised by recent taking of prescribed doses, but other explanations are possible. For interpretation it is vital that other aspects indicating non-compliance are also taken into account.

According to a small study recently performed by Molins and colleagues, lower clozapine/norclozapine ratios are associated with better executive functioning. The authors suggest that diminishing the clozapine/norclozapine ratio could improve cognitive functioning in clinically stable patients with cognitive complaints.[153] However, this could represent a problem in those patients with metabolic syndrome, since it has been demonstrated that higher clozapine/norclozapine ratios obtained by adding the potent CYP1A2 inhibitor fluvoxamine reduce metabolic adverse effects of clozapine.[129]

Actions to take when a clozapine assay result is outside the average therapeutic range

If clozapine plasma levels are below the therapeutic range, and non-compliance can be excluded, dose increases should only be considered in case of incomplete clinical response.

Levels above 1.0 mg/L are possibly toxic and are probably not contributing to drug effectivity. We would recommend dose reductions. In case of plasma levels above 0.6 mg/L, but below 1 mg/L, dose reductions would be a clinical decision, based on the patient's clinical response and side effect profile. An aid to interpret and act on plasma level results is added as appendix to this document.

In general, dose reductions should be done gradually. Very high plasma levels may result in heavy saturation of the clozapine metabolism and a longer elimination time.[155] Therefore, in patients with extremely high plasma levels it may be appropriate to omit a dose and/or to immediately continue on a lower dose, to allow the excess clozapine to clear. This off course would be a clinical decision.

In case dose reductions are not desirable and the patient continues treatment on high plasma levels, it may be considered to prescribe a prophylactic anticonvulsant,[67] especially in case of other risk factors for seizures, such as a history of seizures, concurrent use of epileptogenic medication, stuttering, myoclonic jerks, or EEG abnormalities. However, the recommendations regarding prophylactic use of antiepileptic drugs are not univocal.[156] Some discourage the prophylactic use for reasons including the risk of drug-drug interactions and the increased risk of other side effects.[156] Others advise to use antiepileptic drugs remedially after the occurrence of one seizure[157] or after two seizures.[158,159] See for further information on specific anticonvulsives the fact sheet "Seizures".

Advice for daily practice:

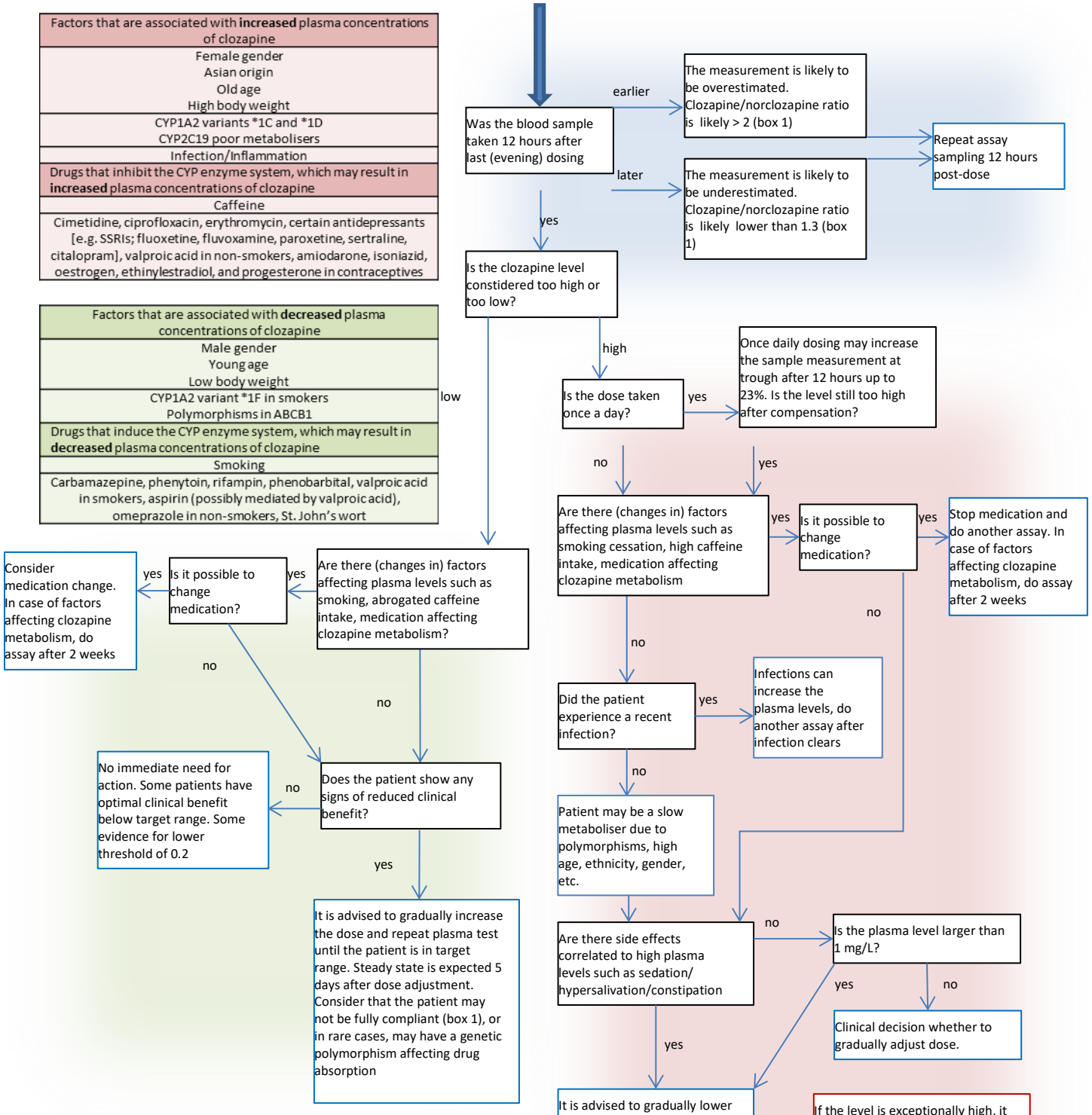
- The main cytochrome P450 isoenzyme involved in clozapine metabolism is CYP1A2, but CYP2C19, CYP2D6 and CYP3A4 also play a role
- Several factors can affect clozapine plasma levels, such as gender, ethnicity, weight, age, genetic polymorphisms, caffeine intake, smoking, infection and inflammation, hormones and several pharmaceutical drugs
- The monitoring of clozapine levels in blood plasma can be a useful tool in the adjustment and the maintenance of the right dose
- A separate sample of venous blood is needed for a clozapine plasma assay
- A blood sample should be taken 12 hours after the last dose, but before the next dose
- After dose adjustments, a new steady state is reached after 5 days
- After a change of factors affecting clozapine metabolism, reaching a new steady state may take up to approximately 14 days
- The optimal clozapine plasma level range for most patients with schizophrenia spectrum disorders is 0.35-0.6 mg/L. A plasma clozapine level of at least 0.2 mg/L is probably required to prevent relapse
- An orienting therapeutic range of 0.015- 0.141 mg/L (15–141 ng/mL) has been suggested in medical-scientific literature for Parkinson's disease psychosis and related disorders
- The clozapine/norclozapine ratio could in some cases provide information on clozapine metabolism and patient compliance
- High clozapine plasma levels or clozapine toxicity are typically associated with orthostatic hypotension, dizziness, hypersalivation, sedation, tachycardia, nocturnal enuresis, irritability, and confusion / mental status change. The risk of seizures, constipation and elevated LFTs is probably increased
- It is recommended to keep the clozapine plasma levels well below 1.0 mg/L
- Side effect profile and clinical response should be leading in the decision to adjust the dose

Zaponex Fact Sheet
Clozapine metabolism and plasma level monitoring

Troubleshooting clozapine assay results – flowchart

Factors that are associated with **increased** plasma concentrations of clozapine
Female gender Asian origin Old age High body weight
CYP1A2 variants *1C and *1D CYP2C19 poor metabolisers
Infection/Inflammation
Drugs that inhibit the CYP enzyme system, which may result in **increased** plasma concentrations of clozapine
Caffeine
Cimetidine, ciprofloxacin, erythromycin, certain antidepressants [e.g. SSRIs; fluoxetine, fluvoxamine, paroxetine, sertraline, citalopram], valproic acid in non-smokers, amiodarone, isoniazid, oestrogen, ethinylestradiol, and progesterone in contraceptives

Factors that are associated with **decreased** plasma concentrations of clozapine
Male gender Young age Low body weight
CYP1A2 variant *1F in smokers Polymorphisms in ABCB1
Drugs that induce the CYP enzyme system, which may result in **decreased** plasma concentrations of clozapine
Smoking
Carbamazepine, phenytoin, rifampin, phenobarbital, valproic acid in smokers, aspirin (possibly mediated by valproic acid), omeprazole in non-smokers, St. John's wort



Box 1: interpreting clozapine/norclozapine ratio

Clozapine and norclozapine plasma levels are both reported by clinical labs. Whereas clozapine levels are correlated to therapeutic benefit, norclozapine is not. Norclozapine may give some information concerning compliance, as the half-life of norclozapine is around 1.8 times longer than clozapine's. Clozapine metabolism may become saturated at higher doses or when enzymatic clearance is diminished and therefore the ratio of clozapine to norclozapine rises with increasing plasma levels, suggesting saturation. A ratio is usually between 1 and 2, and the average ratio is 1.3. A ratio greater than 2 may suggest either a non-trough sample, or that clozapine N-demethylation has become saturated. A ratio below 0.5 suggests either poor adherence within the last 24 hours or so, or that alterations in dose schedule would probably be beneficial. A relatively high ratio in combination with a normal clozapine level (*e.g.* low nor-clozapine level) could indicate non-compliance disguised by recent taking of prescribed doses, but other explanations are possible. For interpretation it is vital that also other aspects indicating non-compliance are taken into account.

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