Supplementary Files.

Supplementary Information: Clozapine concentration measurement

Clozapine and N-desmethylclozapine concentrations were measured in plasma or serum by Liquid Chromatography tandem Mass Spectrometry (LCMSMS) using multiple reaction monitoring in positive ion mode for the mass to charge ratio transitions of 327 > 270 and 313 > 275 respectively, and using isotopically labelled internal standards with 8 deuterium atoms for each compound. The analytical instrumentation consisted of a Waters Acquity Ultra High Pressure Liquid Chromatography (UPLC) system coupled with a Waters Premier XE triple quadrupole mass spectrometer (Waters, Ireland). A Waters Acquity UPLC BEH C18 1.7um, 2.1 x 100mm chromatography column was used for the elution of the compounds with a gradient between Mobile Phase A and B, which consisted of 2mM Ammonium Acetate/0.1% formic acid in water and methanol respectively, at a flow rate of 0.4mL/min and column temperature of 55 degrees C. The mass spectrometry conditions included capillary voltage 1.00kV, cone voltage 30V, source desolvation temperature 400°C, with nitrogen desolvation gas 400L/hr. Collision energies for clozapine (and clozapine-D8) and N-desmethylclozapine (and N-desmethylclozapine-D8) were 25eV and 45eV respectively. 5μL of patient plasma or serum was added to 1000μL of methanol containing both internal standards, vortexed after protein precipitation, followed by centrifugation at 14,000 rpm for 5 minutes, and 6uL of supernatant were injected onto the LCMSMS system.

The method was linear between lower limit of quantitation (20μg/L) and upper limit of quantitation (2300μg/L) for both compounds. Intra-day imprecision (5 replicates at low, medium and high control concentrations), inter-day imprecision (5 replicates for the control concentrations, each over 5 days) and instrument imprecision were less than 4% for both compounds. Accuracy was maintained through within-laboratory and external proficiency quality assurance programs (RCPA Australia). Specificity testing, including an extensive suite of psychotropic drugs, had not identified

25 interfering drug species. The use of isotopically labelled internal standards fully compensated for
26 matrix effects on the electrospray ionisation in the source of the mass spectrometer.

Supplementary Table 1. Population-derived estimates for pharmacokinetic parameters from published models for clozapine that were subjected to external validation.

Source & Population	N (M/F)	Samples	Covariates tested	CL/F (M/F)	CL/F Non-Smoker (M/F)	CL/F Smoker (M/F)	CL IIV %	V/F (M/F)	V/F IIV %	Ka (M/F)	Ka IIV %	tlag	Q/F	F	Residual Error %
Li et al (2012)[43] China	162 (74/88)	809	Sex, Age, weight, smoking		26.46/21.9	38.37/31.76	42.9	526	65.7	1.3	Fixed				26.6
Shang et al (2012)[16] China	198 (125/73)	1391	Sex Age Smoking Weight		31.1/21.9	37/-	45.1	V_1: 314 V_2: 272	V_1: 32.7 V_2: 90.3	1.3	145.6	0.245	83.3		28.4
Ismail et al, 2012[40] Canada	391 (278/113)	1142	Sex Age Height Weight Formulation	36.7/27			44.5	950 Fixed	93.2	0.8	Fixed				545.9 nMol/L*
Olmos et al, 2019[44] Uruguay	98 (76/22)	171	Smoking, age, caffeine		28.1	36.5	43.3	750	Fixed	1.24	Fixed				9.54
Jerling et al (1997)[41] Sweden	241 (159/82)	391	Sex Age	47.9/39.9			Not stated	719/564	Not stated	1.37/1.24	Not stated				Not stated
Ng et al (2009)[29] Canada	197 (138/59)	519	Sex, age, weight, smoking, formulation		52.46/18	28.46/24	60.8	7 L/kg[§] Fixed	131.5	0.14 Tablet	Not stated			0.817	11.5

* Mean measured [clozapine] = 1603.2 nMol/L, SD = 997.8 nMol/L.

§ Mean weight = 80.8 kg, SD = 18.5 kg

Supplementary Figure 1. Structural model for population pharmacokinetics. K_{ABS}: absorption rate constant; t_{LAG}: absorption time lag; CL: clearance of clozapine; V: volume of distribution for clozapine. Clozapine transformation to N-desmethylclozapine is shown, but models incorporating the step did not improve residual error, so it was not included in the final model.

Supplementary Figure 2. Comparisons between concentrations observed at later intervals and concentrations predicted from modelling data collected in the first 6 study weeks, employing predicted pharmacokinetic values for the individual. Left panels show observed and individual predicted concentrations. Dashed lines represent lines of equality. Right panels show percentage error for each data point.

Supplementary Figure 3. Visual predictive check plots for relationships between observed plasma clozapine concentrations and concentrations predicted by published models over 24 hours from the time of last dose. Solid blue line represents the median observed concentrations (50^{th} percentile) and upper and lower broken blue lines represent 5^{th} and 95^{th} percentiles of observed values. Solid red lines represent median estimates from each model, based on dose, sample time and covariate data of the study set patients. Red shaded area represents the 95% confidence intervals for the median estimates at each time point. Upper and lower broken lines red lines represent 5^{th} and 95^{th} percentile estimates for the prediction- and variance-corrected distributions of predicted concentrations and grey shaded areas represent the 95% confidence intervals for 5^{th} and 95^{th} percentile estimates. Fifteen observations (of 1048) lying between 24 and 40 hours were included in the analysis, but are omitted from figures for clarity. Lower confidence intervals that fell below 0.1 $\mu g.L^{-1}$ in some models are not depicted.

Supplementary Figure 4. Observed clozapine concentrations in the study data set and population concentrations predicted from each of the tested population pharmacokinetic models.