Therapeutic drug monitoring (TDM) of clozapine and norclozapine using a multiplex UHPLC-MS/MS method

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Objective

TDM is of great importance for drugs with a high interindividual variability in serum concentration, a narrow therapeutic range or serious toxic side effects. Clozapine, an atypical antipsychotic, meets these requirements. According to the AGNP-TDM consensus guidelines, TDM of clozapine and its metabolite norclozapine is strongly recommended.1 330 serum samples, sent to the toxicological laboratory of Ziekenhuis Netwerk Antwerpen for monitoring of clozapine, were tested with a new ultra-high performance liquid chromatography-tandem mass spectrometric method (UHPLC-MS/MS) for analysis of 16 antipsychotics and 8 of their metabolites.2 The aim of this research was to evaluate this method for TDM of clozapine and norclozapine, and determine other antipsychotics present in these serum samples.

Method

Serum clozapine and norclozapine concentrations

323 of the 330 serum samples contained clozapine and 324 of the 330 contained norclozapine. For clozapine, only 22.3 % of the serum concentrations were within the therapeutic range. 21.8 % of the serum concentrations were between 250-350 ng/ml, which can still be adequate when symptoms are controlled. Overall, 67.9 % of the serum results were < 350 ng/ml. In comparison, Couchman et al. found 42.5 % of serum samples < 350 ng/ml. Since the administered dose was lacking, it is difficult to address these subtherapeutic concentrations to non-compliance, to underdosing or to start-up of the therapy.

68.4 % of the norclozapine concentrations were found within the proposed therapeutic range. Despite its doubtful activity, monitoring of this metabolite has some advantages, because norclozapine has a longer t1/2 and less day-to-day variability.4

Patient samples

330 serum samples from 171 patients (62.6 % male; 37.4 % female), aged 19 to 74 years, were collected between November 2012 and September 2013. Serum samples were taken just prior to the morning dose of the antipsychotic (trough concentration).

Sample preparation: liquid-liquid extraction

200 µl serum
20 µl IS mix
50 µl buffer pH 9.5
1 ml MTBE

Centrifugation
Transfer solvent layer
Evaporate
Reconstitution in 0.3 µl injection on UHPLC-MS/MS

UHPLC-MS/MS assay

The assay, used for monitoring of clozapine, was a UHPLC-MS/MS method which was able to quantify 16 antipsychotics and 8 of their metabolites: amisulpride, aripiprazole, dehydroaripiprazole, asenapine, bromeridol, clozapine, norclozapine, haloperidol, reduced haloperidol, loperidone, hydroxy-loperidone, lurasidone, levosulpiride, olanzapine, norclozapine, paliperidone, pipamperone, quetiapine, 7- hydroxy-N-desalkyl-quetiapine, 7-hydroxy-quetiapine, risperidone, sertindole and zuclopenthixol. Except for levosulpiride and bromeridol, labeled IS were used for each individual compound. The method was validated according to EMA and FDA guidelines.2

For the determination of clozapine and norclozapine, a UHPLC-MS/MS method was used which can be applied to 16 antipsychotics and 8 of their metabolites. The assay was validated according to EMA and FDA guidelines.2

AGNP Consensus Guidelines for TDM

For clozapine, the therapeutic range defined by the AGNP Consensus Guidelines is 350 to 600 ng/ml. The most frequently prescribed and most effective dose of 350-550 mg clozapine per day, is associated with mean serum concentrations of 350 ng/ml.1 According to literature, concentrations of the order of 250 ng/ml can be adequate once symptoms are controlled. Concentration above 600-1000 ng/ml are associated with serious side effects.1

For norclozapine, the therapeutic range defined by AGNP is 100-600 ng/ml.1

Results

Clozapine:norclozapine ratio

The clozapine:norclozapine ratio can be a valuable parameter for TDM. For most serum samples, the calculated ratio was between 0.5-3, which was in correlation with the findings of Couchman et al. The mean ratio was 1.7 (range 0.1-5.9). 24 samples had a ratio > 3, only 2 samples had a ratio < 0.5. The influence of co-medication on the metabolism can be evaluated by the clozapine:norclozapine ratio.

Other antipsychotics

218 of the 330 serum samples (66.1 %) contained other antipsychotics than clozapine and its metabolite. As can be seen in figure 4, 10 antipsychotics were found and about half of the concentrations (mean 52.5 %) were outside the therapeutic range as defined by the AGNP Guidelines.

Conclusion

57 % of the serum concentrations of all antipsychotics found in 330 serum samples (sent for TDM of clozapine) were outside the proposed therapeutic range. However, additional information, like dose and clinical diagnosis, was lacking in this retrospective study and makes interpretation more difficult. Clinicians should be aware of these suboptimal concentrations and correlate the result with the clinical effect.