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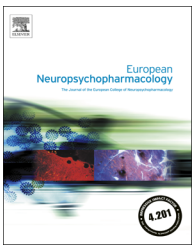

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Fluorescence of neutrophil granulocytes as a biomarker for clozapine use

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Abstract
Non-adherence to medication is a major issue in the treatment of schizophrenia in general and in particular for those treated with clozapine. A reliable tool to quantify patients long-term adherence to clozapine is currently unavailable. Enhanced FL3 neutrophil granulocyte fluorescence was serendipitously observed in a small population of schizophrenic patients treated with clozapine. The present study was aimed at assessing the association between clozapine use and FL3-fluorescence.
A cross-sectional study was performed using data from the Utrecht Patient Oriented Database (UPOD). A total of 38,390 inpatients were included, of which 124 (0.33%) used clozapine.
FL3-fluorescence was significantly higher ($U=240{,}179$, $P<0.001$) in clozapine users (mean (SD) $=90.5$ (11.8)) than in non-users (mean (SD)$=69.8$ (3.3)). Observed FL3-fluorescence was found to increase with increasing clozapine dose. The area under the receiver operating characteristic curve was 0.95.
Our results confirm the association between use of clozapine and elevated FL3-fluorescence. Further research is needed to unravel the underlying mechanism and to investigate the true potential of FL3-fluorescence as a clozapine-adherence biomarker in clinical practice.

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1. Introduction

Schizophrenia is a disabling chronic form of mental illness affecting about 1% of the world population (Bromet and Fennig, 1999). Use of antipsychotics is an essential part in the treatment of schizophrenia.

Among all antipsychotics, the atypical antipsychotic agent clozapine is the only drug with demonstrated efficacy in patients with refractory schizophrenia and the only agent with demonstrated antisuicidal properties(Chakos et al., 2001; Kane et al., 1988; Meltzer et al., 2003; Pickar et al., 1992; Reid et al., 1998). Given these features, clozapine is considered to be of great importance in the treatment of schizophrenia. However, its use is hampered by its adverse effect profile, including the risk for potentially fatal agranulocytosis (0.8% within 4–5 years, with a peak incidence in the first 6–18 weeks of treatment) (Atkin et al., 1996; Dibonaventura et al., 2012; Reid et al., 1998), necessitating weekly blood monitoring in the first 18 weeks of treatment followed by blood monitoring once every 4 weeks according to European guidelines and regulatory authorities (Schulte, 2006).

Apart from the adverse effects of antipsychotic therapy, non-adherence is a major issue in the treatment of schizophrenia. An estimated 25% of the patients is non-adherent within the first 7–10 days of treatment (Keith and Kane, 2003) and studies estimate the mean overall prevalence of non-adherence between 40% and 50% (Lacro et al., 2002). Results of one study reveal that half of the ambulant schizophrenic patients take less than 70% of their medication (Cramer and Rosenheck, 1998). A recent study showed that low treatment adherence is associated with a two-fold increased risk for relapse in psychotic disorders within six months after discharge (Laan et al., 2010).

Physicians apply different strategies to improve treatment adherence including therapeutic drug monitoring (TDM) (Mennickent et al., 2010). TDM consists of determining the ratio of clozapine and N-desmethylclozapine levels, however these measurements have limited value for detection of poor adherence. Due to the elimination half-life of clozapine of about 14 h (Flanagan et al., 2005) and its main metabolite N-desmethylclozapine (half-life of 18 h (Flanagan et al., 2005)), serum level monitoring only provides information on clozapine intake for a few days prior to blood sampling at most. Moreover, merely a suspicion for poor-adherence in the last few days can be raised if a low clozapine:norclozapine ratio is found, because this low ratio could also be explained by rapid metabolising individuals or drug-drug interactions (Couchman et al., 2010). Therefore, a biomarker providing long-term information on adherence would be of great value, such as glycosylated haemoglobin (HbA_{1c}), a long-term biomarker of glucose control in diabetes. In this regard a serendipitous observation by Hoffmann and Lillhom (2000) could be of interest. They described elevated FL3-fluorescence of neutrophil granulocytes in a small Dutch population of schizophrenic patients treated with clozapine. The objective of the current study is to confirm this observation and to further assess the association between clozapine use and FL3-fluorescence of neutrophil granulocytes.

2. Experimental procedures

2.1. Setting

For this study data from the University Medical Center Utrecht (UMC Utrecht) were used. The UMC Utrecht is a 1042-bed academic teaching hospital in the centre of The Netherlands, with annually approximately 28,000 clinical and 15,000 day-care hospitalisations and 334,000 outpatient visits. Data were extracted from the Utrecht Patient Oriented Database (UPOD). UPOD is an infrastructure of relational databases comprising data on patient characteristics, hospital discharge diagnoses, medical procedures, medication orders and laboratory tests for all patients treated at the UMC Utrecht since 2004 (ten Berg et al., 2007). UPOD data acquisition and data management are in line with current Dutch regulations concerning privacy and ethics. The data used for this cross-sectional study were collected for patient care purposes and were used retrospectively. The structure and content of UPOD have been described in more detail elsewhere (ten Berg et al., 2007).

2.2. UPOD haematology database: FL3-fluorescence of neutrophil granulocytes

Next to clinical laboratory data from the laboratory information system, UPOD contains specific haematology data of automated blood cell analyses performed by the Abbott Cell-Dyn Sapphire automated haematology analyser (Abbott Diagnostics, Santa Clara, CA, USA) used at the UMC Utrecht (Groeneveld et al., 2012; Kang et al., 2008; Muller et al., 2006). A feature of this haematology analyser is that all parameters of the complete blood count (CBC) are measured irrespective of the requested parameter. The analyser is equipped with an integrated 488 nm blue diode laser and uses spectrophotometry, electrical impedance and laser light scattering (multi angle polarised scatter separation, [MAPPS]), to measure the CBC and to classify the white blood cell counts (WBCs). For the WBC differential count the following 5 optical scatter signals are measured for each individual cell: cell size (0° scatter, axial light loss [ALL]), cell complexity and granularity (7° scatter, intermediate angle scatter [IAS]), nuclear lobularity (90° scatter, polarised side scatter [PSS]), depolarisation (90° depolarised side scatter [DSS]) and red fluorescence (90° (FL3), 630 ± 30 nm). Per measurement these parameters are stored within UPOD. The FL3-fluorescence parameter measures Nucleated Red Blood Cells and WBC viability by propidium iodide (PI) staining. PI stains nucleic acids and is part of the reagents used by the analyser. PI is capable of crossing the cell-membrane of non-viable WBCs and stains nucleic acids (RNA and DNA). The WBC viability is reported by the analyser as the WVF (the fraction of viable white blood cells within the total population of white blood cells). Older blood samples have a lower WVF. A WVF of 0.95 was considered as the optimal cut-off value to differentiate between fresh and old samples (Huisman et al., 2004). FL3-fluorescence measurement of neutrophil granulocytes is performed by the analyser as part of the routine CBC measurement. Analysis of the WBC scatterplots shows that the FL3-fluorescence is only observed in neutrophil granulocytes and not in eosinophilic granulocytes, basophilic granulocytes, lymfocytes and monocytes. Using a PI free WBC reagent we proved that neutrophil FL3-fluoresence was independent of PI. The reliability and validity of all laboratory results was monitored with built-in quality flags, daily quality control samples and external quality assessment schemes.

2.3. Study population

All inpatients of 18 years or older for whom at least one FL3-fluorescence measurement with a WVF $>$ 0.95, and a history of in-hospital prescribed drugs was available between 2007 and 2010,