NIKO SEPPÄLÄ

Clozapine

Clinical studies on adverse effects and interactions

ACADEMIC DISSERTATION
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UNIVERSITY OF TAMPERE

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University of Tampere, School of Medicine
Satakunta Hospital District, Department of Psychiatry
Pirkanmaa Hospital District, Department of Psychiatry
Tampere City, Mental Health Center
Etelä-Pohjanmaa Hospital District, Department of Psychiatry
Finland

Supervised by
Professor Esa Leinonen
University of Tampere
Finland
Professor Olli Kampman
University of Tampere
Finland

Reviewed by
Docent Björn Appelberg
University of Helsinki
Finland
Docent Grigori Joffe
University of Helsinki
Finland

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To my Family and Friends

Contents

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II. Seppälä NH, Leinonen EV, Lehtonen ML, Kivistö KT. Clozapine serum concentrations are lower in smoking than in non-smoking schizophrenic patients. Pharmacol Toxicol 1999 Nov;85(5): 244-6

III. Seppälä NH, Leinonen E, Solismaa A, Nuolivirta T, Viikki M, Kampman O. Factors associated with subjective side effects during clozapine treatment. Nord J Psychiatry (in press)

IV. Seppälä N, Leinonen E, Viikki M Kampman O. Smoking and weight among patients using clozapine. Nord J Psychiatry 2014; May7 (Epub ahead on print)

ABBREVIATIONS

5HT	5-Hydroxytryptamine (serotonin)
ANNSERS	Antipsychotic Non-Neuroleptic Side Effects Rating Scale
BMI	Body Mass Index
CLOZ	Clozapine
CGI	Clinical Global Impression Scale
CPZ	Chlorpromazine
CVD	Cardiovascular disease
CYP	Cytochrome P450 enzyme
DMCLOZ	Desmethylclozapine (or norclozapine)
ECT	Electroconvulsive Therapy
E-EPA	Ethyl Eicosapentaenonic Acid (fish oil)
EPS	Extrapyramidal side effects
FGA	First generation antipsychotic
FMO	Flavin containing mono-oxygenase enzyme
GABA	Gamma Amino Butyric Acid
GLM	General linear model
LUNSERS	Liverpool University Neuroleptic Side Effect Rating Scale
MANCOVA	Multivariate covariance analysis
nAChR	Nicotinic Acetylcholinesterase receptor
NMS	Neuroleptic Malignant Syndrome
PANSS	Positive and Negative Syndrome Scale for Schizophrenia
rTMS	Repetitive Transcranial Magnetic Stimulation
SANS	Scale for the assessment of negative symptoms
SAPS	Scale for the assessment of positive symptoms
SGA	Second generation antipsychotic, atypical antipsychotic
SSRI	Selective Serotonin Reuptake Inhibitor
SPSS	Statistical Package for the Social Sciences
UGT	UDP-glucurionocyltranferase
UKU	UKU (“Udvalg for Kliniske Undersøgelser”) -scale.

ABSTRACT

Clozapine is the most effective antipsychotic agent in treatment-resistant schizophrenia. It moreover seems to have specific antiaggressive and antisuicidal properties. Numerous serious adverse effects limit the use of clozapine, the most salient of these being agranulocytosis. Myocarditis, aspiration pneumonia, ileus and weight gain are potentially be fatal and may actually cause more fatalities than agranulocytosis. Granulosytopenia and agranulocytosis and myocarditis require immediate withdrawal of clozapine therapy, when the risk of relapse and exacerbation of psychotic symptoms is high. There are also other unpleasant adverse effects associated with clozapine, such as sedation, hypersalivation and constipation.

Clozapine also interacts with numerous drugs. It is metabolized mainly by CYP1A2 cytochrome enzyme and many other drugs and living habits, like smoking, may affect the metabolism of clozapine.

In the first of the present series of studies (Study I) abrupt clozapine withdrawal was explored retrospectively when the medication had to be discontinued for reasons not related to the patient. The study population consisted of 28 patients with schizophrenia treated in Pitkäniemi Hospital, Tampere, Finland between February and July 1975, when clozapine was banned and suddenly withdrawn from the market due to eight fatal cases of agranulocytosis. After this withdrawal, psychiatric symptoms increased rapidly in nearly half of the patients. The probability of deterioration was decreased by anticholinergic medication or other antipsychotic medication after discontinuing clozapine.

In the second study (Study II) the effects of smoking on serum clozapine concentration were detected in 44 hospital treated patients. The smokers had significantly (38%) lower serum concentrations than non-smokers. The smokers had higher

body weight related clozapine doses although their clozapine serum concentration was lower.

Studies three (III) and four (IV) consisted of 237 patients on long-term clozapine treatment. In the third study the subjective side effects of clozapine were studied using the Liverpool University Neuroleptic Side Effect Rating Scale (LUNSERS). I

In the multiple regression analysis a direct correlation between serum clozapine concentration and reported depression-anxiety–like side effects was found. In multiple covariate analysis (MANCOVA) there was a relationship between younger age and reported sedation as well as increased dreaming. Patients on antipsychotic polypharmacy reported more sympatichotonia-tension–like symptoms than patients on clozapine monotherapy.

In the fourth study the relationship between smoking and body mass index (BMI) in 237 patients receiving clozapine treatment was explored. There was no statistically significant difference in BMI between smokers and non-smokers contrary to earlier samples representing normal population. There was a trend for the clozapine treated patients who smoked most also to be the most overweight.

In the present series of studies, abrupt withdrawal of clozapine was related to sudden psychotic relapses. Their probability could be diminished by concomitant anticholinergic medications. Smoking markedly lowered clozapine serum concentration and resulted in higher doses in clinical practice. Clozapine serum concentration, age and antipsychotic combination treatments were related to the reported side effects of clozapine. In clinical settings patients with more symptomatology are to be prescribed higher doses and neuroleptic combination treatments. There was no difference in BMI between smokers and non-smokers in clozapine treated patients, but there was a subgroup of heavy smokers whose weight was extremely high. Smoking and overweight are both important risk factors for cardiovascular disease (CVD) and special attention should be paid to this patient group.

TIIVISTELMÄ

Klotsapiini on tehokkain psykoosilääke muille psykoosilääkkeille hoitoresistentissä skitsofreniassa. Klotsapiinilla on aggressiivisutta sekä itsetuhoisuutta vähentävä vaikutus. Sen käyttöä rajoittavat kuitenkin lukuisat sivuvaikutukset, joista tunnetuin on agranulosytoosi. Myös klotsapiinin aiheuttamien myokardiiti, aspiraatiopneumonia, ileus sekä painon nousu voivat olla hengenvaarallisia ja ne voivat aiheuttaa enemmän kuolemantapauksia, kuin agranulosytoosi. Klotsapiinin käyttöön liittyvä granulosytopenia ja agranulosytoosi sekä sydäntulehdus voivat edellyttää klotsapiinihoidon välitöntä keskeyttämistä, jolloin psykoosin uusiutumis- ja pahenemisriski ovat suuria. Klotsapiinin käyttöön liittyy myös monia muita epämiellyttäviä hoitoa haittaavia sivuvaikutuksia, joita ovat esimerkiksi sedaatio, syljenerityksen lisääntyminen ja kouristuskynnyksen aleneminen.

Klotsapiinilla on paljon yhteisvaikutuksia esimerkiksi muiden lääkkeiden kanssa. Se metaboloituu pääosin maksan sytokromijärjestelmän ja erityisesti CYP1A2:n kautta, joiden kautta monet lääkeaineet ja esimerkiksi tupakointi voivat vaikuttaa klotsapiinin metaboliaan.

Ensimmäisessä osatyössä tutkittiin retrospektiivisesti äkillisen klotsapiinihoidon lopettamisen vaikutusta psykoosioireisiin kun lääkitys jouduttiin lopettamaan ilman potilaasta johtuvaa syytä. Aineiston muodostivat 28 Pitkäniemen sairaalassa hoidettua skitsofreniapotilasta, joiden klotsapiinilääkitys lopetettiin heinäkuussa 1975, kun klotsapiinin käyttö kiellettiin Lääkintöhallituksen määräyksellä kahdeksen klotsapiinihoitoa saaneen potilaan menehdyttyä agranulosytoosiin. Lopettamisen seurauksena psykiatriset oireet lisääntyivät nopeasti noin puolella potilaista. Oireiden lisääntymisen todennäköisyyttä vähensi antikolinergisen lääkityksen tai muun psykoosilääkkeen käyttö.

Toisessa osatyössä tutkittiin tupakoinnin vaikutusta seerumin klotsapiinipitoisuuteen 44 sairaalahoidossa olevalla potilaalla. Tupakoivien potilaiden seerumin klotsapiinipitoisuudet olivat merkitsevästi matalampia (38 %) kuin tupakoimattomien potilaiden. Tupakoivilla potilailla oli tilastollisesti merkitsevästi suuremmat painoon suhteutetut klotsapiiniannokset, vaikka heidän seerumin klotsapiinipitoisuutensa oli pienempi.

Kolmannessa ja neljännessä osatyössä käytettiin samaa kolmen sairaanhoitopiirin alueelta kerättyä 237:n klotsapiinipotilaan aineistoa. Kolmannessa osatyössä tutkittiin klotsapiinin subjektiivisia sivuvaikutuksia LUNSERS-itsearviointilomaketta käyttäen. Monimuuttuja-analyysissä todettiin yhteys seerumin klotsapiinipitoisuuden ja ilmoitettujen masennus-ahdistussivuvaikutuksen, nuoremman iän ja lääkityksestä koetun sedaation sekä lisääntyneen unien näkemisen välillä. Lisäksi psykoosilääkkeiden yhdistelmiä käyttävät potilaat raportoivat enemmän sympatikotonia-tensio -tyyppisiä oireita.

Neljännessä osatyössä tutkittiin tupakoinnin ja painoindeksin välistä suhdetta klotsapiinihoitoa saavilla potilailla. Tupakoivien ja tupakoimattomien potilaiden painoindekseissä ei todettu eroa toisin kuin aiemmissa, normaaliväestöä koskevissa tutkimuksissa. Kuitenkin runsaimmin tupakoivat klotsapiinihoitoa saavat potilaat olivat kaikkein eniten ylipainoisia.

Väitöskirjatutkimuksissa todettiin klotsapiinin äkillisen lopettamisen olevan yhteydessä sekavuuteen ja nopeisiin psykoosirelapseihin, joiden todennäköisyyttä pienensi samanaikainen antikolinergin ja antipsykootin käyttö. Tupakointi alensi seerumin klotsapiinipitoisuutta ja oli yhteydessä korkeampiin klotsapiiniannoksiin. Klotsapiinipitoisuudella, psykoosilääkkeiden yhdistelmähoidoilla ja iällä todettiin yhteys tiettyihin subjektiivisiin haittavaikutuksiin kuten masennus-ahdistuneisuus – sekä sympatikotonia-tensio tyyppisiksi määriteltyihin sivuvaikutuksiin sekä lisääntyneeseen unien näkemiseen. On mahdollista, että kliinisessä aineistossa vahvemmin oireilevat potilaat saattoivat valikoitua käyttämään suurempia lääkeannoksia sekä eri lääkkeiden yhdistelmiä. Tupakoivien ja tupakoimattomien painoindekseissä ei todettu eroa, mutta paljon tupakoivien potilaiden joukosta joutui alaryhmä, jossa painon nousu oli poikkeuksellisen suurta. Tupakointi, kuten ylipainokin ovat merkittävimpiä sydän- ja verisuonisairauksien riskitekijöitä, näin ollen tähän potilasryhmään tulisikin kiinnittää erityistä huomiota.

1. INTRODUCTION

Clozapine, nearly 40 years after its introduction, remains the drug of choice in treatment-resistant schizophrenia despite a wide range of adverse effects. Many of these, like agranulocytosis, thromboembolism, myocarditis and cardiomyopathy are serious or potentially life-threatening. However, clozapine is known to be more effective than any other first or second generation antipsychotic (Essali et al. 2009; Tuunainen and Wahlbeck 2010). Despite these risks it may also be associated with the lowest mortality of all antipsychotics (Tiihonen et al. 2009) .

Agranulocytosis, myocarditis or cardiomyopathy may necessitate immediate withdrawal of clozapine medication, which may be problematic because of confusion and rapid psychotic relapse i.e. relapse during the first month. Indeed, this is more common in clozapine treated patients than with other antipsychotics and such abrupt clozapine withdrawal is clinically a more important topic than the abrupt withdrawal of most other antipsychotics. Because of its potentially dangerous adverse effects clozapine is nowadays reserved as a third-line antipsychotic. Clozapine has also a wide range of other common neurological, metabolic, gastrointestinal and cardiovascular adverse effects, which may cause significant distress thus affecting medication adherence and treatment outcome. So far there is limited research on the subjective distress caused by these adverse effects.

Patients with schizophrenia are more often smokers, begin smoking at a younger age, prefer higher tar cigarettes and have reduced smoking cessation rates compared to people in general (Kelly and McCreadie 1999). From a pharmacokinetic point of view tobacco hydrocarbon compounds are significant CYP1A2 inducers, which means that the effect of smoking should be taken into account when initiating clozapine treatment or any other medication affected by CYP1A2. In addition to

changes in serum drug concentratrations, smoking may also have clinical effects when a patient either discontinues or initiates smoking during clozapine treatment.

Poor treatment adherence in patients with schizophrenia increases the risk of psychotic relapse, which may further lead to impaired social and cognitive functioning, psychiatric hospitalizations and increased treatment costs. Unpleasant subjective side effects are a common reason for discontinuing medication.

Patients with schizophrenia suffer from significantly excessive mortality compared to general population, which means about 12-15 years shorter life expectancy. A significant part of this excessive mortality is explained by cardiovascular disease. Weight gain is a common adverse effect of clozapine and overweight is also the most important risk factor for metabolic syndrome. Thus the risks of smoking and overweight for cardiovascular disease are presumably additive.

2. REVIEW OF THE LITERATURE

Schizophrenia is the most severe psychiatric disorder with multiple aetiologies. Onset is usually early in adult life, causing impairments in social, occupational, cognitive and global functioning. Schizophrenia is a major public health problem affecting slightly less than 1% of people worldwide (Perala et al. 2007). Overall patients with schizophrenia have two to three fold higher mortality rates than general population (Talaslahti et al. 2012; Crump et al. 2013).

Schizophrenia is a major psychiatric disorder, or cluster of disorders, characterized by psychotic symptoms that alter a person's perception, thoughts, affect and behaviour. Each person with this disorder will have a unique combination of symptoms and experiences. Typically there is a prodromal period often characterized by some deterioration in personal functioning. This includes problems in attention, unusual behaviour and ideas, disturbed communication and affect, and social withdrawal, apathy and reduced interest in daily activities. These are commonly called negative symptoms. The prodromal period is usually followed by an acute episode with hallucinations, delusions, and behavioural disturbances. These are called positive symptoms, and are often accompanied by agitation and distress. Following resolution of the acute episode, usually after pharmacological, psychological and other interventions, symptoms diminish and often disappear, although sometimes a number of negative symptoms may remain as chronic. This phase, which can last for years, may be interrupted by recurrent acute episodes, which usually require additional interventions (National Collaborating Centre for Mental Health (Great Britain) and National Institute for Health and Clinical Excellence (Great Britain) 2009) However, the interindividual course of schizophrenia varies considerably. Some patients have positive symptoms only briefly while others experience them chronically. A

few patients have no prodromal period and the disorder begins suddenly with an acute episode (National Collaborating Centre for Mental Health (Great Britain) and National Institute for Health and Clinical Excellence (Great Britain) 2009)

2.1 Drug treatment of schizophrenia

Before the introduction of conventional antipsychotic medications (neuroleptics) in the early 1950s, the majority of patients with schizophrenia were cared for in psychiatric hospitals. The introduction of first generation antipsychotic (FGAs) also called conventional antipsychotic, first of all chlorpromazine, for the first time offered an effective option to control psychotic symptoms. Some of the patients who had previously been treated in psychiatric hospitals for years could now be discharged to live in the community (Tuteur et al. 1959). FGAs, dopamine-2 receptor antagonists are still effective therapeutic pharmacological agents and also currently available to treat psychotic symptoms (Tandon et al. 2008)

Although FGAs provided efficacious treatment for psychotic symptoms, their use often led to extrapyramidal symptoms (EPS). These are various neurological movement disorders characterized by repetitive involuntary muscle movements, restlessness or an inability to initiate movements. FGAs may also cause prolactin elevation even in therapeutic doses. Dry mouth and sedation are also side effects of FGAs. On rare occasions serious side effects such as neuroleptic malignant syndrome (NMS) may occur.

Second generation antipsychotics (SGAs) have mainly replaced conventional antipsychotics since the 1990s. Generally they are considered at least as effective as FGAs. SGAs have a much lower risk for tardive dyskinesia but weight gain is more common with some SGAs such as olanzapine and clozapine. Most SGAs have little effect on cardiac functioning and are not associated with sudden death (Luft and Taylor 2006).

2.2 Non-response to antipsychotics in schizophrenia

Since the introduction of the first antipsychotic drug, chlorpromazine, it has become evident that a large number of patients with schizophrenia are treatment resistant to conventional neuroleptics. Between 20% and 60% of patients with schizophrenia do not respond sufficiently to ordinary treatment (Melzer and Kostacoglu 2001; Miller et al. 2006) .

The definition of treatment resistant schizophrenia is problematic, as schizophrenia is by definition a chronic disease and long-term studies have shown wide variation, 2.6-40 % of the patients achieve recovery or remission depending on the recovery or remission criteria (Lauronen et al. 2005). Chronicity is also often used interchangeably with a synonym for refractoriness and many clinicians believe that refractoriness and total deterioration are the inevitable outcome of schizophrenia (Elkis 2010).

Poor treatment adherence, also typical for patients with schizophrenia, is a critical factor when considering possible treatment resistance (Citrome 2013). Poor treatment adherence should be distinguished from treatment resistance, although the consequences may be similar. Poor adherence is usually associated with more adverse effects, poor insight and a weak therapeutic alliance or treatment continuity (Kampman and Lehtinen 1999). Comorbid physical and psychiatric disorders and inadequate social support are factors that may also lead to inadequate treatment and unfavorable outcome (Goff et al. 2010).

It is also necessary to distinguish between response to treatment and remission. Response to treatment in psychosis has commonly been defined as a 20% reduction in the severity of symptoms measured with the Positive and Negative Symptom Scale for Schizophrenia (PANSS) (Kay et al. 1987). This goal is far from a symptom-free or nearly symptom-free state, whereas remission refers to a total, or almost total symptom-free period and "recovery" refers to the absence of the disease for a long period (Leucht et al. 2009). The distinction between incomplete recovery and treatment refractoriness is likewise not unambiguous (Kane et al. 2001).

The Schizophrenia Working Group has proposed widely accepted remission criteria for schizophrenia. The working group proposed as a definition of symptomatic remission of schizophrenia a maintenance of at most mild or less simultaneous ratings concerning psychotism (reality distortion), disorganization and negative symptom (psychomotor poverty) items in the most widely used schizophrenia scales

like PANSS or Scales for the Assessment of Positive Symptoms (SAPS) and negative symptoms (SANS) over a six-month period (Andreasen et al. 2005).

Although there are no generally accepted criteria of treatment resistance in schizophrenia, the general consensus is that adequate drug treatment requires a duration of four to ten weeks, a dosage equivalent to 1,000 mg/d of chlorpromazine and trials of two to three different classes of antipsychotic drugs (Kane et al. 2001).

One or two thirds of those patients who do not respond adequately to treatment with FGAs or other SGAs respond adequately to treatment with clozapine (see below). Thus clozapine is widely considered the treatment of choice in treatment-resistant schizophrenia (Essali et al. 2009). In addition, clozapine may alleviate cognitive deficits (Meltzer and McGurk 1999; Kane et al. 2001; Peuskens et al. 2005), reduce suicidality (Hennen and Baldessarini 2005) and may be associated with lower total mortality than other antipsychotic agents (Tiihonen et al. 2009).

2.2.1 Clozapine in treatment resistant schizophrenia

The limited use of clozapine for treatment resistant patients has also been criticized because it is superior to both FGAs and SGAs (Essali et al. 2009; Leucht et al. 2009; Asenjo Lobos et al. 2010; Tuunainen and Wahlbeck 2010). There are also reports on its beneficial effects on anxiety, mood and negative symptoms (Breier et al. 1999), as well as on hostile behavior compared to other antipsychotic agents (Citrome et al. 2001). Moreover, it has been reported that patients on clozapine have a better treatment adherence than with other SGAs and it has also antisuicidal properties (Meltzer et al. 2003; Cooper et al. 2007; Ascher-Svanum et al. 2008; Tiihonen et al. 2009).

Although clozapine is considered the standard pharmacotherapy as a last resort in the management of treatment-resistant schizophrenia, 40% to 70% of these patients also fail to respond to clozapine treatment (Buckley et al. 2001; Lieberman et al. 2005). Clozapine-resistant schizophrenia characteristics include persistent active psychotic features despite daily doses of 300 to 900 mg/d for eight weeks to six months, with plasma drug levels of 1.0 nmol/l of the parent drug or higher (Umbricht et al. 2002).

The evidence supporting the efficacy of clozapine augmentation with other psychotropic drugs is weak (Sommer et al. 2012; Taylor et al. 2012). Probably the best evidence is that for lamotrigine and sulpiride**,** and there is also some support

for citalopram and a glutamate agonist CX516 (Tiihonen et al. 2009; Wang et al. 2010; Sommer et al. 2012). Combinations with amisulpiride, risperidone, haloperidol, ziprasidone, topiramate, aripiprazole, memantine and mirtazapine may be also useful (Zoccali et al. 2004; Kontaxakis et al. 2006; Assion et al. 2008; Englisch and Zink 2008; de Lucena et al. 2009; Tiihonen et al. 2009; Zink et al. 2009; Kim et al. 2010; Wang et al. 2010; Barbui et al. 2011; Sommer et al. 2012; Hahn et al. 2013).

2.3 Second generation antipsychotics

Unlike clozapine (see below), which was synthetized as early as in 1958, most SGAs emerged in the 1980s and 1990s. They generally have a lower risk of motor side effects than FGAs, but some of them are associated with significant weight gain, lipid and prolactin elevation and the development of type 2 diabetes (Melkersson and Dahl 2004; Miyamoto et al. 2005).

In earlier meta-analysis FGAs were compared with SGAs (Leucht et al. 2009). It was found that some SGAs, notably clozapine, but also olanzapine, amisulpride and risperidone may be more efficacious than FGAs in reducing the overall symptoms. Some SGAs, such as aripiprazol and quetiapine, may also be also more efficacious than FGAs in treating depression (Komossa et al. 2010).

Another more recent meta-analysis (Leucht et al. 2012) compared placebo controlled studies with SGAs and two standard FGAs namely typical high potency FGA haloperidol and low potency FGA chlorpromazine. In this meta-analysis the superior efficacy of clozapine was established and to a lesser extent that of amisulpride, olanzepine and risperidone, while other atypicals did not differ from haloperidol or chlorpromazine. It also seemed that SGAs might be generally more easily tolerated and cause less EPS but more weight gain than FGAs.

2.4 Clozapine

Clozapine is a prototype of SGAs (Kane et al. 1988). It is known to be more efficacious than FGAs and other SGAs (Essali et al. 2009; Tuunainen and Wahlbeck 2010).

Unfortunately, due to its many adverse effects – some of them life-threatening - it is usually considered a third-line antipsychotic (Wang et al. 2004).

Clozapine is more effective than other antipsychotics for treatment-resistant positive symptoms and suicidality (Meltzer et al. 2003; Essali et al. 2009). Olanzapine may also be somewhat more efficacious for positive symptoms (hallucinations, delusions and agitation) than the other available antipsychotics (Lieberman et al. 2005; Kahn et al. 2008; Leucht et al. 2009). Unfortunately the efficacy of antipsychotics, including clozapine, on cognitive and negative symptoms is limited.

2.4.1 History of Clozapine

Since the introduction of chlorpromazine several other dopamine D2-antagonists, like haloperidol, trifluperazine, thioridazine and fluphenazine have become available. They have comparable efficacy, but unfortunately they all have serious neurological side effects. Thus there was a need for safer neuroleptics with fewer side effects. In 1958 a group of tricyclic compounds was synthetized based on the antidepressant imipramine by a small Swiss pharmaceuticals company Wander in order to develop new antidepressants, but one of these compounds, clozapine, was surprisingly found later to have antipsychotic properties (Lopez-Munoz et al. 2005; Crilly 2007; Ramachandraiah et al. 2009).

Unlike the earlier neuroleptics, clozapine did not cause catalepsy in animal studies and this raised doubts about its antipsychotic properties. The first study conducted on humans by Gross and Lagner 1962 found that clozapine was ineffective in psychosis but the second trial by these authors, published in 1966, was successful (Gross and Lagner 1966). In addition, a German researcher, Hans Hippius, likewise reported in 1966 that clozapine was an effective antipsychotic without disabling neurological side effects. The psychiatric community was skeptical: Because clozapine does not cause EPS it could not be an effective antipsychotic. Despite this suspicion clozapine treatment was initiated for many patients (Crilly 2007; Ramachandraiah et al. 2009).

Finally, after several positive trials (Stille and Hippius 1971), clozapine was launched in the USA, Switzerland and Austria in 1972, in West Germany in 1974 and in Finland in 1975. At the same time the first open-label study started up in the USA (Honigfeld et al. 1984).

The introduction to the Finnish market began in February 1975. The usage of clozapine expanded quickly and by the middle of 1975 some 3,000 patients were on clozapine treatment. By July 1975 it was reported that clozapine was implicated in 17 cases of neutropenia or agranulocytosis in Finland – eight of them fatal (Idanpaan-Heikkila et al. 1977). Clozapine was immediately withdrawn from the market in Finland and western countries.

The sudden discontinuation of clozapine caused severe psychotic exacerbations in all psychiatric hospitals. It was reported that 39% of the patients taking clozapine as outpatients were rehospitalized after cessation due to exacerbation of their psychotic symptoms (Kuha 1977). Therefore, the re-introduction of clozapine was later permitted for many patients who did not respond to any other antipsychotic drug. In the mid-1980s clozapine was finally suggested to be more effective than chlorpromazine in a controlled double blind study (Honigfeld et al. 1984) and later on a methodologically more restrictive study demonstrated the superiority of clozapine over chlorpromazine in a group of 286 treatment-resistant patients with schizophrenia (Kane et al. 1988). Consequently clozapine was launched in the UK and USA. Since the introduction of chlorpromazine this has been the greatest advance in the pharmacotherapy of schizophrenia. Now the superiority of clozapine is commonly accepted over both conventional and other second generation antipsychotics (Joffe 1999; Meltzer et al. 2003; Essali et al. 2009; Leucht et al. 2009; Asenjo Lobos et al. 2010; Tuunainen and Wahlbeck 2010).

2.4.2 Pharmacology of clozapine

Clozapine is a derivative of dibenzepine (8-chloro-11-(4-methyl-1-piperazinyl)-5H-dibenzo(b,e)(1,4)diazepine) with antipsychotic properties. It is structurally related to some SGAs like olanzapine and quetiapine and it has a broad receptor affinity (Markowitz et al. 1999).

It has a low antagonistic affinity for dopamine D2 receptors and high affinity for D1 and D3-5 receptors. It has also high affinity for serotonergic 5HT2A, 5HT2C, 5HT1A, histaminegic H1, adrenergic alpha1 and alpha2 receptors and muscarinic M1 receptors (Richelson and Souder 2000; Farah 2005).

2.4.3 Pharmacokinetics of clozapine

In therapeutically relevant concentrations clozapine has a linear, or first order kinetics, although interindividual variation in concentrations is very high. (Haring et al. 1989; Haring et al. 1990; Potkin et al. 1994; Guitton et al. 1998; Perry et al. 1998). This means that its rates of absorption and elimination are proportional to drug concentrations. The peak concentration is reached in one to three hours and mean elimination half-life (T1/2) is 10-17 hours (range 5-60 hours), mean volume of distribution (Vd) is 2 to 5 l/kg (range 1-10 l/kg) and mean plasma clearance is 13 to 57 l/h ranging 11-435 l/h (Byerly and DeVane 1996).

There is a wide variation in the bioavailability of clozapine. After oral dosage 27 to 47% of the clozapine dose reaches the systemic circulation (Cheng et al. 1988; Choc et al. 1990) but the steady state plasma concentration may show up to 45-fold interindividual variation with the same dose of clozapine (Potkin et al. 1994).

Clozapine metabolism to its active N-Desmethyl (=norclozapine) is catalyzed by Cytochrome P450 (CYP) -enzymes CYP1A2, CYP2D6, CYP2C19 and CYP3A4 *in vitro*, while CYP3A4, and flavin containing mono-oxygenases (FMOs) mediate its N-oxidation (Fang et al. 1998; Olesen and Linnet 2001). Uridine 5'-diphospho-glucuronosyltransferase 1A3/4 catalyzes the glucuronidation of clozapine (Mori et al. 2005). *In vivo* studies suggest that medications and substances which induce CYP1A2 accelerate the metabolism of clozapine (Raaska and Neuvonen 2000; Kroon 2007) rather than e.g. CYP3A4 (Raaska and Neuvonen 1998).

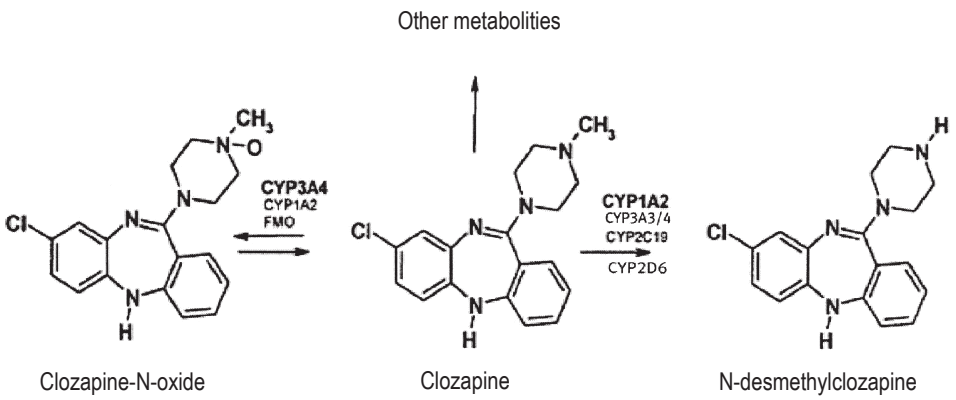

Figure 1. Clozapine metabolism. Reprinted by permission (Raaska 2003).

2.4.4 Clozapine side effects

The side effect profile of clozapine differs from that of conventional FGAs and most of the SGAs, but the SGAs are a more heterogeneous group of drugs. The most salient life-threatening side effect is potentially fatal agranulocytosis in about 1% and the risk is highest in patients taking concomitant agranulocytosis-risk drugs. Other side effects like myocarditis, aspiration pneumonia, ileus and weight gain may actually cause more deaths than agranulocytosis (Idanpaan-Heikkila et al. 1977; Alvir et al. 1993; Merrill et al. 2006; Lahdelma 2009; Taylor et al. 2009; Nielsen et al. 2013). Other serious and life-threatening side effects include cardiomyopathy, pericarditis, alveolitis, pancreatitis, hepatitis, nephritis, colitis, drug-induced lupus erythematosus, status epilepticus, diabetic ketoacidosis and hyperosmolar coma and NMS (Miller et al. 1991; Au et al. 2004; Pieroni et al. 2004; Reinders et al. 2004; Bayard et al. 2005; Rami et al. 2006; Pelizza and Melegari 2007; Rathore et al. 2007; Gandelman-Marton et al. 2008; Chang et al. 2009; Cohen and Correll 2009; Arias et al. 2011). Unlike all FGAs and some SGAs, clozapine has a low propensity to cause extrapyramidal side effects and does not raise serum prolactin or corticotrophin levels (Wagstaff and Bryson 1995).

Most adverse effects of clozapine are related to its pharmacological properties. Sexual dysfunction and orthostatic hypotension are linked to adrenergic alpha-blockage. Sedation is mostly due to H1-blockage and muscarinic M1-antagonism causes anticholinergic effects, such as constipation, tachycardia, blurred vision and urinary retention (Markowitz et al. 1999).

Moreover, the the metabolic abnormalities caused by clozapine seem to be linked affinities of several receptors. Clozapine has a high affinity for the 5HT2C and histamine H1 receptors, which are linked to weight gain. The central 5HT2C and peripheral M3 muscarinic receptor effects may also contribute to obesity-independent risk of diabetes. There may also be other additive or synergistic effects, such as dopamine D2 receptor antagonism (although not essential with clozapine), which may reinforce the 5HT2C-mediated effect on food intake. In addition, side effects such as dizziness, transient eosinophilia, hypersalivation, hyperthermia, nausea and seizures are also common (Wagstaff and Bryson 1995; Lahdelma 2009).

Although the interindividual variation in serum concentrations may vary as much as 45-fold (Potkin et al. 1994). Side effects generally correlate only weakly with clozapine serum concentrations, although some side effects like seizures can be controlled by reducing the dosage (Pacia and Devinsky 1994; Yusufi et al. 2007).

2.5 Abrupt clozapine withdrawal

Due to treatment-emergent adverse effects and poor adherence, a high percentage of patients discontinue clozapine (Tollefson et al. 1999). Abrupt withdrawal of clozapine can result in confusion and psychotic symptoms and/or dystonic symptoms much faster than discontinuation of an FGA or any other SGA (Gerlach et al. 1974; Kuha 1977; Borison et al. 1988; Tollefson et al. 1999). There are also some reports according to which sudden clozapine discontinuation has caused catatonia (Thanasan and Jambunathan 2010; Wadekar and Syed 2010). Some patients may not have any response to other antipsychotics for several weeks after clozapine withdrawal. Thus gradual tapering-off is strongly recommended if possible (Szafranski and Gmurkowski 1999; Miodownik et al. 2006).

Because clozapine has effects on various central receptor systems, several mechanisms, such as D2 receptor supersensivity, cholinergic rebound and serotonergic hyperactivity may be underlie withdrawal symptoms (Verghese et al. 1996; Meltzer 1997).

The dopaminergic hypersensititity hypothesis is based on animal and human positron emission tomography findings that long term exposure to antipsychotic drug causes increased dopamine D2 receptor binding. In clozapine, however, a short half life and rapid elimination from brain tissue or loose binding and rapid detatchment from from D2 receptors could cause dopaminergic rebound after rapid clozapine withdrawal (Baldessarini et al. 1995; Seeman and Tallerico 1999; Moncrieff 2006).

The cholinergic rebound theory is based on the strong antagonism of clozapine to muscarine receptors and the consequent cholinergic rebound when clozapine is replaced with the non-anicholinergic or with less potent antipsychotic (Buckley 2007). This theory is supported by clinical findings that acute exacerbation of psychiatric symptoms during clozapine withdrawal can be reversed with anticholinergic agents (Shiovitz et al. 1996).

There is also some evidence of serotonergic rebound or hypersensitivity because clozapine is a 5HT receptor 2A antagonist and the rapid discontination of clozapine may lead to serotonergic rebound. This theory is supported by data that cyproheptadine, an antihistamine with anticholinergic properties and 5HT2A antagonism, can rapidly reverse the withdrawal symptoms of clozapine. (Meltzer et al. 1996; Stevenson et al. 2013).

2.6 Schizophrenia and smoking

Smoking is much more common among patients with schizophrenia worldwide and the prevalence of smoking is 2-3 times higher among patients with schizophrenia than in general population (de Leon and Diaz 2005). Smoking is also a leading cause of preventable mortality in developed countries (Bobes et al. 2010). Patients with schizophrenia may spend up to 30% of their monthly income on tobacco products (Steinberg et al. 2004).

Patients with schizophrenia live 12-15 years shorter lives than people in general. The leading causes of the markedly premature mortality is cardiovascular disease, diabetes, pulmonary diseases, suicide and accidents (Talaslahti et al. 2012; Crump et al. 2013). Smoking and smoking-related diseases like cardiovascular disease, COPD and pulmonary cancer are considered to explain significant number of excess mortality related to schizophrenia and life expectancy of schizophrenia, and smoking implies a two-fold higher risk for coronary heart disease in patients with schizophrenia (Bobes et al. 2010; Kelly et al. 2011; Crump et al. 2013). However, patients with schizophrenia are less motivated to stop smoking and have less appreciation of smoking related health-risks than general population. They also report greater social facilitation and greater stimulation or state enhancement from smoking than do normal population (Kelly et al. 2012).

It has been pointed out that patients with schizophrenia have too much leisure time and too few activities other than smoking (Roick et al. 2007). Patients with schizophrenia are three times more likely to initiate smoking and five times less likely to stop smoking than people in general (Diaz et al. 2006). Patients who smoke are often heavily nicotine dependent, as indicated by studies reporting greater carbon monoxide boost, deeper puffs, an increased number of puffs per cigarette and increased saliva excretion in smokers with schizophrenia than in smokers without schizophrenia (Hitsman et al. 2005; Strand and Nyback 2005; Tidey et al. 2005; Williams et al. 2005).

The psychopharmacological and pharmacokinetic self-medication hypothesis proposes that patients with schizophrenia medicate their positive and negative symptoms by smoking. They may also attempt to relieve antipsychotic associated side effects like neuroleptic induced parkinsonism (Winterer 2010). The addiction vulnerability hypothesis proposes that there are common genetic factors and abnormalities in brain reward pathways in schizophrenia that predispose patients to tobacco consumption (Wing et al. 2012). In a very recent genetic study there

was tentative evidence that at least one specific mechanism could be disturbance in Neuregulin/ErbB signalling of tyrosine kinase providing a potiential link between the high co-morbidity of schizophrenia and nicotine dependence (Loukola et al. 2013).

Pharmacokinetic explanations are based on the property of antipsychotic drugs to block dopaminergic D2-receptors. Smoking may restore the blocked dopamine effects through the central action of nicotine on dopaminergic neurons. It has also been proposed that nicotine could alleviate the common side effects of antipsychotics including extrapyramidal symptoms and pharmacogenic depression (Anfang and Pope 1997; Dalack et al. 1998). Some carbohydrate burning products of tobacco induce CYP1A2 and UGT enzymes. CYP1A2 and UGT enzymes are involved in the metabolism of many antipsychotics like olanzapine and clozapine (Zevin and Benowitz 1999). Thus smoking may be a method of self-regulating antipsychotic levels and consequently antipsychotic side effects.

There is also evidence that smoking and nicotine may relieve negative symptoms possibly through normalizing auditory sensory gating (P50) deficits (Adler et al. 1992; Adler et al. 1993; Griffith et al. 1998), some additional sensory deficits like prepulse inhibition abnormalities and eye-tracking deficits (Kumari et al. 2001).

Recent studies suggest a common neurobiological background for smoking and schizophrenia. It has been proposed that genetic and environmental factors lead to deficient Nicotinic Acetylcholinesterase receptor (nAChR) lead signaling with the concurrent Gamma Amino Butyric Acid (GABA) and glutamatergic imbalances, and that this imbalance leads to multiple cognitive deficits which may further be alleviated, at least temporarily, by smoking. It has also been proposed that mesolimbic dopaminergic mechanisms could lead to increased tobacco craving and sensitivity to tobacco reinforcing effects by increasing dopamine in the Nucleus Accumbens (Wing et al. 2012).

There is some evidence that patients taking clozapine smoke less than patients taking conventional neuroleptics and that the initiation of clozapine may reduce smoking in patients with schizophrenia (George et al. 1995; McEvoy et al. 1999; Procyshyn et al. 2001).

2.7 Clozapine and weight gain

Clozapine and olanzapine are associated with weight gain and metabolic abnormalities more frequently than other antipsychotics (Rummel-Kluge et al. 2010).

The relationship between clozapine dose or serum concentration and metabolic adverse effects is controversial (Simon et al. 2009). Little is known about changes in food intake and appetite behaviour in these patients. Clozapine and olanzapine have been associated with increased self-reported binge-eating and food craving in hospitalized patients (Kluge et al. 2007). It has been found that one-third of patients taking clozapine, gain 20% of their baseline weight in two years (Covell et al. 2004).

In animal studies, chronic doses of clozapine had no effect on food intake in female rats (Cooper et al. 2008). Small doses of clozapine actually caused weight loss, but increased visceral adiposity.

The exact mechanism of clozapine induced weight gain is unknown. Histamine H1 receptor affinity, 5HT2C receptor antagonism and dopamine D2 antagonism have been implicated (Reynolds and Kirk 2010). Genetic factors may also be important in clozapine induced weight gain. It has been proposed that a certain adipocyte derived hormone, leptin genotype, may be involved in clozapine induced weight gain (Zhang et al. 2007)

AIMS OF THE STUDY

The overall aim of the present study was to investigate some common and clinically important problems associated with clozapine treatment. The aims were:

1. To investigate the effect of abrupt clozapine discontinuation on the manifestation of psychiatric symptoms and the effect of co-medications on the clozapine withdrawal symptoms (Study I). A more specific question was: *Does concomitant anticholinergic medication prevent acute deterioration after abrupt withdrawal of clozapine in patients with schizophrenia?*

2. To investigate the effect of smoking on clozapine serum levels (Study II). A more specific question was: *Are there differences in weight adjusted clozapine serum levels between smokers and non-smokers?*

3. To investigate the association of clozapine serum concentrations and reported side effects (Study III). A more specific question was: *What single subjective side effects or side effect clusters are dependent on drug concentrations?*

4. To investigate the relationship between smoking and weight gain in patients treated with clozapine (Study IV). A more specific question was: *Does smoking affect clozapine induced weight gain?*

3. MATERIALS AND METHODS

The studies of which this dissertation is composed are referred to in the text by the Roman numerals I to IV and they are based on three different patient samples. Original publications I and II are separate studies and the third and fourth papers share the same patient population. They are produced here with the permission of the copyright holders

3.1 Study I

The Study I population comprises a series of cases where clozapine was suddenly withdrawn. The outcome of patients receiving or not receiving concomitant anticholinergic and antipsychotic medications at time of withdrawal was studied.

3.1.1 Patients

Clozapine first came on the market in Finland between February and July 1975 (Idanpaan-Heikkila et al. 1975). All patients receiving clozapine for at least two weeks during this period and suffering from schizophrenia group disorders treated in Pitkäniemi Hospital, Tampere, Finland were assessed retrospectively.

Altogether 36 patients fulfilled these inclusion criteria but eight of them had discontinued clozapine medication before clozapine was officially banned. Thus 28 patients who had to discontinue clozapine treatment abruptly after the clozapine

was officially forbidden on 28 July 1975 were included in the analyses. Patient characteristics are shown in Table 1.

Table 1. Basic characteristics of the patients (n=28) in Study I

Men	14
Women	14
Age(yrs)[1]	28.7 (18-60)
Duration of illness[2]	5.82 (4.26)
Number of hospitalizations [1]	5.04 (0-14)
Total hospitalization time in months[1]	27.7 (0-90)

[1]Mean (range)
[2]Mean (SD)

At the time of clozapine discontinuation 12 patients (42.9%) were on clozapine monotherapy, 10 patients (35.7%) were taking clozapine with one FGA (mean FGA dose 235 mg/d, range 25-538 mg/d in chlorpromazine (CPZ) equivalents (Aronson 2009) and 6 patients (21.4 %) were taking clozapine with two FGAs (cumulative dose 328 mg/d, range 66-433 mg/d) respectively. Total antipsychotic dosages in the different phases of the study are shown in Table 2.

Table 2. Total antipsychotic doses in CPZ equivalents (mg/d) in the various study phases and clozapine doses at the time of discontinuation.

	Total	*Clozapine*
Before initiation of clozapine	605 (200-1000)	
End of therapy	811 (200-1300)	329 (100-600)
One month after	478 mg (150-850)	
End of the year	491 mg (332-1033)	

CPZ equivalent of clozapine is taken to be 2:1 corresponding to 100 mg of clozapine as equal to 200 mg of CPZ. The clozapine dosage is represented as it is – not in CPZ equivalents.

3.1.2 Methods

The psychiatric state of the patients was assessed retrospectively from the case notes by two experienced psychiatrist researchers (Esa Leinonen, Niko Seppälä) using the Clinical Global Impression Severity of Illness Scale (CGI/SI) (Guy 1976). The evaluations were made at four time points: at the beginning of the clozapine trial (<1 week before), before discontinuation of the drug (<1 week; end of July 1975), one month after discontinuation (±2 weeks; end of August 1975) and at the end of 1975 (±1 month) by both researchers at every time point. In cases of disagreement between the results of the evaluations a more thorough evaluation was made together to reach a consensus assessment. The type of care was divided into three categories 1) closed ward 2) open ward 3) outpatient care. The doses of antipsychotic drug treatments were assessed simultaneously with the CGI. The signs of rapid relapse shortly after clozapine discontinuation were assessed by modified Linszens criteria (see below). Adverse effects, such as EPS were categorized as present or absent.

The case notes included no detailed descriptions of symptoms observed using rating scales. However, the patients were generally described as "anxious" and "having sleeping problems" or as "complaining of stomach pain" and more frequently "more psychotic", "more restless" or simply as "worse".

The modified narrow criteria of Linszen were used as relapse criteria to define rapid relapse in the patient records (Linszen et al. 1994). These include: 1) hospital readmission due to psychotic symptoms 2) an explicit statement of relapse or exacerbation in the patients record or 3) a significant increase in antipsychotic medication dose during the first two weeks after discontinuation of clozapine. In this study only criteria 1 and 2 were used because criterion 3 was indistinguishable from medication changes due to clozapine withdrawal.

After discontinuation of clozapine conventional neuroleptic treatment was immediately initiated for all hospitalized patients. For outpatients this was done as soon as possible, which, according to the records, was usually within a few days.

3.1.2.1 The Clinical Global Impression Scale (CGI)

The CGI rating scale was used retrospectively using patient records to evaluate the patients' psychiatric state. The CGI is commonly used in the assessment of symptom severity and efficacy of treatments (Guy 1976). It is a two-dimensional scale divided into severity of illness (CGI-SI) and global improvement (CGI-GI) scales.

In the present study the CGI-SI scale was used. This is a 7-point scale that requires the evaluator to rate the severity of the patient's illness at the time of assessment relative to the evaluator's past experience of patients with the same diagnosis. The patient is assessed according to the severity of mental illness at the time of rating as: 1=normal and not at all ill, 2=borderline mentally ill, 3=mildly ill, 4=moderately ill, 5=markedly ill, 6=severely ill, or 7=among the most extremely ill patients.

3.2 Study II

To detect the effect of smoking on clozapine serum levels, patients with schizophrenia and on clozapine treatment and hospitalized in Pitkäniemi Hospital, Tampere were included in the study. However, patients taking concomitant medications possibly affecting serum clozapine concentrations, such as inducers or inhibitors of CYP enzymes (e.g. omeprazole, SSRI, carbamazepine) were excluded. The patients' weight was measured and their smoking status and number of cigarettes smoked daily was assessed by the nursing staff.

Of the patients screened, 44 (31 men and 13 women) agreed to participate in the study. Of these 34 (26 men and 8 women) were smokers and ten (5 men and 5 women) were non-smokers. The cigarette consumption varied between 10 and 40 cigarettes per day. Mean consumption was 18.0 cigarettes per day. The patient characteristics are presented in Table 3. There were no differences in age, weight, duration of hospitalization or clozapine therapy between non-smokers and smokers.

Table 3. Basic characteristics of the patients (n=44) in Study II

	Men	Women	All
Patients	31	13	44
Age, years*	38.4 ±11.0	38.2 ±12.5	38.3 ±10.9
Weight, kg*	76.4 ±12.5	74.2 ±11.2	75.8 ±12.1
Clozapine dose, mg*	515 ±148	535 ±189	520 ±159
Clozapine dose, mg/kg*	6.86 ±2.1	7.3 ±2.8	7.0 ±2.3
Clozapine monotherapy, n (%)	18 (58%)	6 (46%)	24 (54%)
Total antipsychotic dose mg*#	1194 ±463	1215 ±433	1200 ±449
Number of smokers (%)	26 (83%)	8 (61%)	34 (77.2%)

*Mean±SD
CPZ equivalents

3.3 Studies III and IV

Reported side effects during clozapine treatment in schizophrenia patients were explored in association with clozapine doses and serum concentrations (Study III) and smoking (IV) in the same sample.

3.3.1 Patients

The patient sample was collected in three hospital districts in Western Finland (Satakunta, Pirkanmaa and Seinäjoki). The recruited patients were treated at hospitals, psychiatric secondary outpatient clinics and supportive housing units.

Inclusion criteria for the studies were: 1) Age ≧18 years; 2) An ICD-10 diagnosis of schizophrenia, schizo-affective or delusional disorder; 3) Ongoing clozapine treatment; 4) ability to understand Finnish.

All participants gave informed consent on entry to the study. The study protocol was approved by Satakunta Hospital District Ethics Committee and confirmed by Pirkanmaa and Etelä-Pohjanmaa Hospital District Ethics Committees and the relevant institutional authorities. Selection of the patients is given in Figure 2 (page 34).

All patients completed the Liverpool University Neuroleptic Side Effect Rating Scale (LUNSERS) (Day et al. 1995) and an additional questionnaire including patients' estimates of their current height, weight and possible weight changes during clozapine treatment, and data on smoking status.

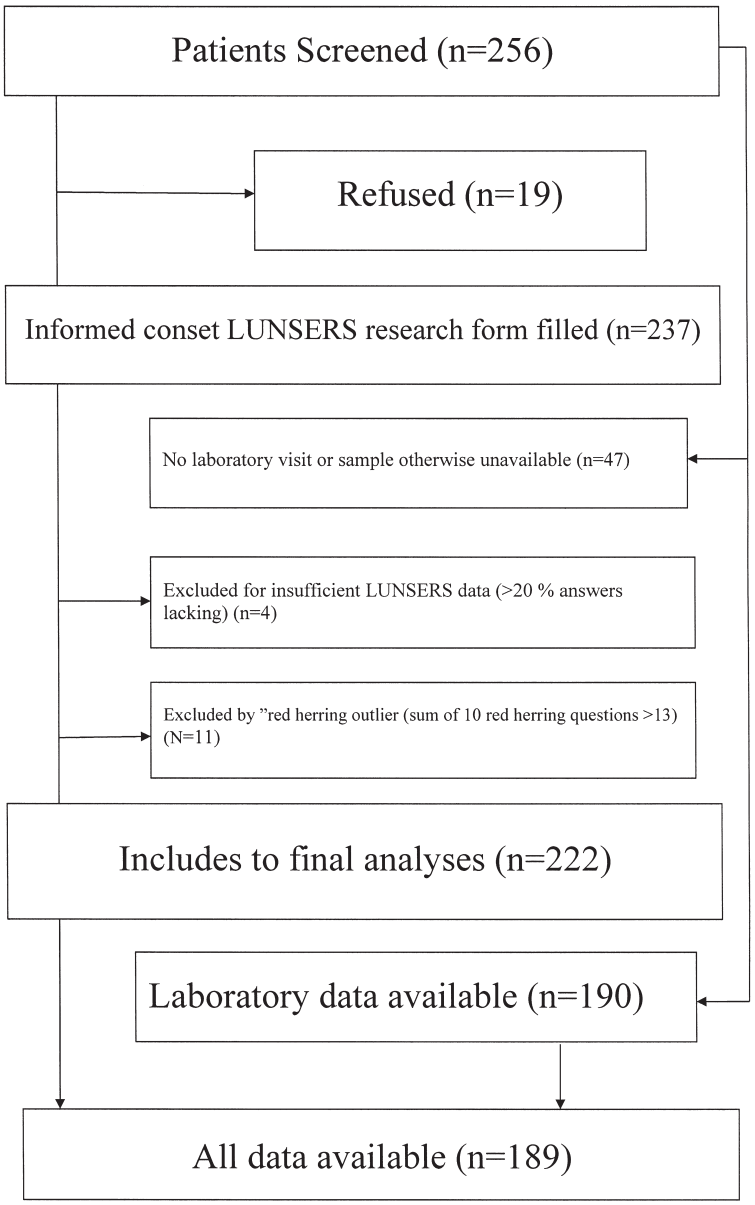

Figure 2. Patient screening in Studies III and IV.Methods

3.3.1.1 LUNSERS

LUNSERS is a 51-item self-report questionnaire with each item describing the intensity of a certain side effect of medications (0=not at all, 4= very much). Forty-one of the items deal with known antipsychotic side effects and ten of the items function as 'red herring' questions for testing possible over-reactivity in responding to the questionnaire. The scale has been considered an efficient, reliable and valid method for assessing antipsychotic side effects (Lambert et al. 2003). The original UKU based subscales were not used.

Serum clozapine concentrations were also measured. Information about medication and history of illness was also collected from patient records.

A total of 256 patients were screened and 237 patients gave informed consent. After several exclusions for various reasons 222 patients were included in the analyses (Figure 2). All patients were of native Finnish origin. The characteristics of the patients are shown in Table 4.

Table 4. Basic characteristics of the patients (n=237) in Studies III-IV

	Men	Women	All	
Number of patients	136	101	237	NS
Age, years*	41.8 ±10.7	43.5 ±11.38	42.7 ±11.1	NS
BMI (kg/m2)*	29,0 ±5.6	31.1 ±6.7	29.9 ±6.2	0.01
Clozapine dose, mg*	410 ±153	394 ±151	403 ±152	NS
Clozapine dose, mg/kg*	4.69 ±2.18	5.00 ±2.25	4.82 ±2.21	NS
CLOZ+DMCLOZ concentration (µmol/l)	2.21 ±1.12	2.62 ±1.42	2.62 ±1.42	0.03
On clozapine monotherapy, %	66.7%	64.4%	65.6%	NS
Total antipsychotic dose, CPZ equivalent, mg*	921 ±380	900 ±412	911 ±304	NS
Proportion of smokers, %	51.1%	44.5 %	48.2 %	NS
Time from first hospitalization due to psychosis, years*	18.4	16.5	17.3 ±10.0	NS

*Mean ±SD

Mean clozapine dose was 403 mg/d and there were no differences in doses between genders. When the total dose of antipsychotics was converted to CPZ equivalents (Aronson 2009) it was 911 mg/day (SD±304 mg). Of the 237 patients included in the study, 190 patients (112 men and 78 women) gave a sample of serum clozapine + desmethylclozapine concentration measurement for analysis in a commercial laboratory (Laakkonen and Heiskanen 1991).

Most patients (94%, n=223) had a diagnosis of schizophrenia and the rest a diagnosis of schizo-affective psychosis or delusional disorder. Most patients (65.4%, n=155) were on clozapine monotherapy, and the rest on antipsychotic combination treatment. Clozapine was combined with SGA in 53 (22.5%) patients, while 23 (9.7%) had FGA combined and four (1.7%) received a combination treatment of all three (CLOZ+FGA+SGA). Information on the duration of clozapine therapy was available for 92% of the patients. Most (n=137, 57.8%) had been taking clozapine for at least five years, 77 (32.5%) for 1-5 years and 4 (1.7 %) for less than a year. The shortest period of use was three months.

3.3.2 Patients' weight and smoking status

Of all patients in the study (n=237) only two did not answer the question on their smoking habits. Nearly half (n=114, 48.1%) were non-smokers and the same number were daily smokers. Seven patients (3.7%) reported being occasional or non-daily smokers. The estimated BMI of regular smokers was 29.7 kg/m^2 (SD±6.1) and of non-smokers or occasional smokers 30.1 kg/m^2 (SD±6.3) (p=0.673 between groups, t-test).

3.4 Statistical Methods

In Study I, Pearson's chi-square test was used in outcome comparisons between groups with and without concomitant anticholinergic medications. This test was also used in outcome comparisons between groups with and without typical antipsychotic medications. Analysis of variance (ANOVA) was used to compare ratings of CGI scale between different time points in Study I.

Two-tailed t-test was used to compare clozapine serum levels between smokers and non-smokers in Study II. For analyses, the obtained serum clozapine and desmethylclozapine concentrations were divided by the daily dose (mg) per body weight (kg) and to reach normal distribution, the appropriate logarithmic transformation to reach the normal distribution was used.

For predicting current BMI in Study III, a general linear univariate model (GLM) was used with age, number of cigarettes smoked daily and sedation factor as covariates and gender as a factor. Chi-square statistics was used in calculations between different subgroups of smoking and weight gain. The association between the number of cigarettes smoked daily and weight change was calculated with ANOVA. T-test was used to calculate mean differences of BMI between different subgroups (smoking status, feeling of hunger). Pearson's correlation coefficients were calculated between continuous variables, such as BMI, clozapine concentration, total antipsychotic dose, number of cigarettes smoked daily and sedation factor.

In Study IV, principal component factor analysis with Varimax rotated solution was performed with LUNSERS items. In the factor analysis a factor loading of >0.45 was taken into account in single items. Resulting factors and remaining single items were used as dependent variables in the multivariate analysis of covariance (MANCOVA) in Study IV. Weakly reacting questions (mean <0.7) were not taken into account in the factor analysis. The items not loading on any of the clinically coherent factors were the items headache (5), dry mouth (6), increased sex drive (17), losing weight (22) and involuntary movements (48).

Patients with a sum of more than 12 points in LUNSERS "red herring" answers (n=11) were considered outliers, and were excluded from final analyses. Patients with at least 20% of LUNSERS items unanswered (n=4) were excluded from the final analyses.

For the factor analysis, a missing value replacement was performed according to the following procedure. In one or two item factors (5,7,8) and in the individual items median replacement of series was used. In all other clinically coherent factors (1,2,3,4,6 and 8) the missing values were replaced with the values closest to the mean in each factor. In gender specific items "tenderness of the breast" (7), "period pains" (13) and "menstrual irregularities" (50), the missing values in males were replaced with a value of 0 (no symptom). Unanswered 'red herring' items were replaced with a value of 0. The total proportion of replaced values in the total sample was < 5% of all item ratings.

A multivariate analysis of covariance (MANCOVA model) was used for predicting differences in different LUNSERS factors (Study IV). Age and the sum of clozapine and desmethylclozapine serum concentrations were used as covariates, and gender and the use of combined antipsychotic medications as factors in the model used.

All calculations were made with SPSS statistical software (versions 9.0, 17.0 and 19.0, SPSS Inc.). The limit of statistical significance was set at <0.05.

4. RESULTS

4.1 Abrupt withdrawal of clozapine worsens patients clinical state (I)

After withdrawal of clozapine, 13 (46.4 %) out of 28 patients were considered to have deteriorated during the first two weeks according to Linszens criteria and according to the information in the case notes. Of all these patients, five (17.9%) improved during the first two weeks but eight (28.6%) did not. Three patients (10.7%) developed significant EPS. Four patients (14.3%) improved after clozapine discontinuation. Of the eleven patients who could be discharged during clozapine treatment, five were rehospitalized during the first month and three more by the end of 1975.

4.1.1 Use of concomitant anticholinergic medications and deterioration of clinical state.

Of the 28 study patients 14 were taking another anticholinergic medication, like low potency neuroleptic, anticholinergic antidepressant or an antiparkinsonian drug in addition to clozapine. Those patients were compared to the other 14 patients not taking such medications before clozapine withdrawal. It was found that only three (21.4%) of the patients taking anticholinergic medication deteriorated against ten out of the 14 patients not taking such medication. One month after the discontinuation of clozapine the corresponding numbers were 1/14 (7.1 %) and 8/14 (57.1 %) (p=0.005, chi-square test). A similar trend persisted to the end of the same calendar year i.e. at five months (2/14 vs. 6/14 p=0.094).

4.1.2 Use of concomitant neuroleptics and deterioration of clinical state.

Of those 12 patients on clozapine monotherapy, nine (75 %) deteriorated rapidly during the first week after clozapine discontinuation when compared to those four (25%) of the 16 patients on antipsychotic polypharmacy (p=0.009, chi-square test). The concomitant neuroleptics were levomepromazine and haloperidol each in six patients, chlorpromazine in four, chlorprothixene in three, fluphenazine in two and pipotiazine palmitate in one patient. Clozapine monotherapy also predicted poorer outcome at the one-month and five-month follow-up points (by the end of 1975) than a combination of clozapine + typical neuroleptics. Of the 12 patients on clozapine monotherapy at the time of withdrawal, eight (66.7%) had deteriorated at one-month follow-up, while only one (7.1%) patient also receiving other neuroleptics at clozapine withdrawal had deteriorated (p=0.001, chi-square test). This result persisted for up to five months (6/12 vs. 2/16, p=0.03). Smoking lowers serum clozapine concentrations (II).

Non-smokers had lower mean clozapine doses than smokers. The non-smokers' mean clozapine doses were only 72% of the smokers'. After adjusting for body weight, the male non-smokers still had 61% higher clozapine concentrations (Table 5). However, a significant difference was only found between smoking and non-smoking females, but there was a non-significant trend to lower clozapine concentrations in smokers. The desmethylclozapine concentrations did not differ between genders among non-smokers or smokers although a similar trend of lower concentrations was seen in both men and women. No relation was found between drug concentrations and number of cigarettes smoked daily.

Table 5. Clozapine doses and concentrations in non-smoking and smoking patients (Study II)

	Non-smokers (n=10)	Smokers (n=34)	P-value
Clozapine dose (mg/d)	410 ±131	553 ±154	0.010
Clozapine dose per body weight (mg/kg/d)	5.4 ±1.4	7.5 ±2.4	0.012
S-Clozapine (nmol/l per mg/kg)	298 ±127	184 ±97	0.021
S-Desmethylclozapine (nmol/l per mg/kg)	260 ±150	165 ±89	0.016

4.2 Use of concomitant antipsychotics and high clozapine concentrations increase subjective side effects (III).

To explore the clinical validity of side effects according to the LUNSERS questionnaire in the patient sample a factor analysis was performed. This analysis revealed 13 factors, of which the researchers considered eight to be a clinically coherent combination. These were named sympatichotony/tension, depression/anxiety, sedation, orthostatism, menstrual problems, dermatological symptoms, sexual dysfunction and urinary problems. All thirteen factors explained 66.8% of the variance and the eight clinically coherent factors 45.3% of the variance of all included items. The distribution of factors and their loadings are presented in Table 6.

Table 6. Clinically coherent factors and loadings of single items in the analysis of the LUNSERS questionnaire.

Factor	*Explanatory proportion*	*Items in factor (loadings >0.45)*	*Item loading in factor*
Sympatichotony/ tension	7.4 %	14 Tension	0.470
		19 Muscle stiffness	0.663
		20 Palpitations	0.586
		29 Slowing of movements	0.468
		36 Diarrhoea	0.494
		43 Shakiness	0.705
		44 Pins and needles	0.507
Depression/anxiety	7.4 %	9 Difficulty in concentrating	0.610
		14 Tension	0.589
		21 Difficulty remembering things	
		296 Depression	0.507
		40 Restlessness	0.742
		41 Difficulty getting to sleep	0.530
			0.579
Sedation	5.6 %	2 Difficulty staying awake during the day	
		18 Tiredness	0.763
		31 Sleeping too much	0.635
			0.678
Orthostatism	5.4 %	15 Dizziness	0.570
		16 Feeling sick	0.762
		38 Blurred vision	0.543
Menstrual problems	5.5 %	13 Period pains	0.831
		50 Periods less frequent	0.832
Dermatological symptoms	4.9 %	1 Rash	0.830
		47 New or unusual skin marks	0.745
		49 Itchy skin	0.495
Sexual dysfunction	4.7 %	24 Difficulty achieving climax	0.792
		46 Reduced sex drive	0.530
Urinary problems	4.5 %	32 Difficulty passing water	0.761
		51 Passing a lot of water	0.645

The eight clinically coherent factors and single items were used as dependent variables in the MANCOVA analysis and patient and medication related variables as independent variables. All variables loading significantly on the eight clinically coherent factors in MANCOVA model are shown in Table 7.

Table 7. Variables associated with LUNSERS clinical factors and single items.

Dependent variable	*Independent variable*	*eta-square*	*Statistical Power*	*p*
Depression-anxiety factor	Clozapine serum concentration	6.6 %	0.93	0.001
Sedation factor	Age	5.8 %	0.89	0.002
Increased dreaming -item	Age	3.4 %	0.68	0.016
Period problems factor	Gender	21.2 %	1	<0.001
Sympatichotony/tension factor	Clozapine monotherapy-antipsychotic combinations	5.2 %	0.85	0.003
Lack of emotions -item	Clozapine monotherapy-antipsychotic combinations	4.7 %	0.81	0.005

In confirmatory bivariate analyses, depression/anxiety factor score was higher in patients with clozapine + desmethylclozapine serum concentrations of at least 2.2 µmol/l (n=92) compared with patients having a lower serum concentration (n=95) (9.7 ±4.8 vs. 7.5 ±5.1 points, p=0.004). The patients on clozapine monotherapy (n=152), had lower sympatichotonia factor scores than the patients on antipsychotic combination therapy (n=81) (6.2 ±4.5 vs. 8.0 ±5.2 points, p=0.007). Sedation factor score was associated with younger age: patients aged 20-43 years (n=107) had higher sedation scores than patients aged 44-65 years (n=104) (5.4 ±2.8 vs. 4.3 ±2.7 points, p=0.002, t-test). The increased dreaming item was also associated with younger age (p=0.006), but the lack of emotions item was no longer associated with clozapine monotherapy/antipsychotic combination in the bivariate analysis (p=0.16, Mann-Whitney U-test).

A correlation was found between LUNSERS total score and combined clozapine and desmethylclozapine serum concentration (r=0.19, p=0.03).

4.3 Heavy smoking is associated with high body mass index in clozapine treated patients (IV).

Reported weight gain correlated moderately with estimated BMI (r=0.57, p=0.01). Number of cigarettes smoked daily was not associated with reported weight change (p=0.17, ANOVA).

BMI correlated weakly with sedation, number of cigarettes smoked daily, clozapine serum level and total neuroleptic dose (r=0.11, r=0.16, r=0.18, r=-0.05, r=-0.15 respectively). The correlation between reported weight gain and sedation was also weak (r=0.11).

In the analysis of covariance (ANCOVA) with BMI as a dependent variable, the best fitting model comprised age, sex, intensity of sedation, and reported amount of daily smoking as explanatory variables (ηp^2=0.116, p=0.029, power=0.750). In the model, the explanatory proportions of age, sex (ηp^2=0.042, p=0.056; ηp^2=0.037, p=0.074) and the number of cigarettes smoked daily (ηp^2=0.039, p=0.066) were close to significant. The explanatory proportion of the sedation factor of the model was not significant (ηp^2=0.014, p=0.279). Adding the variables "continuous feeling of hunger" or serum clozapine concentration into the model as independent variables resulted in non-significant models.

The distribution of reported weight change was similar in regular smokers and other patients. (Table 8)

Table 8. Distributions of reported weight changes after clozapine initiation in non-smokers and smokers in Study IV

Change in weight	*Non-smoker or occasional smoker*	*Regular smoker*
No change	30.1 % (35)	24.3 % (27)
Weight loss	17.2 % (20)	15.3 % (17)
Some weight gain	22.4 % (26)	29.7 % (33)
Marked weight gain	30.1 % (35)	30.6 % (34)

Chi-square (p=0.570 between groups)

Regular smokers had higher clozapine and total antipsychotic doses than other patients, but there was no difference in combined clozapine+desmethylclozapine concentrations (Table 9).

Table 9. Mean clozapine dose (mg/d) and total antipsychotic dose (measured in chlorpromazine equivalents mg/d) and clozapine+desmethylclozapine concentrations among non-smoking and smoking patients.

	Non-smoker or occasional smokers (n=116)	***Regular smokers (n=111)***	***p-value***
Clozapine dose (mg/d)	361.1 ±131.8	452.6 ±157.8	<0.001
Total antipsychotic dose (CPZ equivalents)*	808.6 ±327.9	1029.0 ±430.3	p<0.001
CLOZ + NCLOZ concentration (nmol/l)	2.33 ±1.23	2.42 ±1.31	0.64

*Aronson (2009)

Men had higher weight and dose adjusted S-clozapine concentrations than women (4.32±2.96 vs. 3.73±2.52 mg/kg per ng/ml, p 0.047 Mann-Whitney U-test), but not higher S-desmethylclozapine concentrations (6.32±3.32 vs. 6.27±4.21 mg/kg per ng/ml, p=0.387 Mann-Whitney-U-test).

5. DISCUSSION

This dissertation was concerned with the adverse effects associated with clozapine treatment (I, III, IV) and problems concerning smoking during clozapine treatment (Studies II and IV).

5.1 Adverse effects in clozapine treatment

Clozapine, although a most effective antipsychotic, has many problematic adverse effects which limit its use. Some adverse effects, like agranulocytosis or cardiac effects may be life-threatening and necessitate immediate clozapine withdrawal.

Although not immediately life-threatening, there are many other unpleasant side effects with clozapine, such as sedation, hypersalivation and constipation which may lead to non-adherence. Weight gain leading to significant obesity is also a common adverse effect of clozapine. Obesity is a significant risk factor for diabetes and thus also a risk factor for cardiovascular disease.

5.1.1 Clozapine serum concentrations and side effects (III)

In Study III the main purpose was to evaluate the relationship between clozapine serum concentrations and subjective side effects. Almost all patients (94%) had a diagnosis of schizophrenia or schizo-affective disorder and their illness was of long duration. Of these patients, two thirds were on clozapine monotherapy, which is in

line with an earlier Nordic sample of clozapine prescribing practice, but the practices worldwide in this sense are somewhat variable (Peacock and Gerlach 1994; Chong et al. 2000).

Only two out of 190 patients (1.5%) had clozapine serum level below the recommended treatment range (0.3 nmol/l). There may be a selection bias towards the most adherent patients in the present study sample because the consciously unadherent may not visit the laboratory or decline to participate in the study. Most of the patients were also on controlled medication under the surveillance of nursing staff in hospital, sheltered accommodation or outpatient care using at least a pill dispenser. Regular blood monitoring and more regular contacts with medical personnel could also have improved the adherence.

In Study III clinically coherent factors were calculated from single LUNSERS items which were then analysed with MANCOVA. These sum variables were considered to reflect the subjective distress of various side effects more reliably than single semi-quantitative LUNSERS items. Confirmatory bivariate analyses were used to ascertain the direction of associations. The LUNSERS scale was originally developed in comparison with the UKU ("Udvalg for Kliniske Undersøgelser") –a rater based neuroleptic medication side-effect scale (Lingjaerde et al. 1987; Day et al. 1995) and the subscales of UKU were developed to cover the most common side effects of FGAs. Because the validity of the LUNSERS subscales has not been tested specifically with SGAs these factors were used as target variables and the original UKU-based subscales were not used.

Higher clozapine+desmethylclozapine concentrations were related to the factor called "depression and anxiety" in the present patient sample. This factor consisted of single items concerning concentration, memory problems, tension, restlessness and sleeping. Clozapine is perhaps prescribed in higher doses to patients with anxiety or depression-like symptoms. This hypothesis is supported by earlier reports, where clozapine reduced symptoms of depression and suicidality (Meltzer et al. 2003; Chang et al. 2006; Hodge and Jespersen 2008; Tiihonen et al. 2009). The other possible explanation may also include, that the higher clozapine levels impairing cognitive function such as ability to concentrate and mamory functions such as ability ti concentrate and it may cause anxiety. There is some evidence that cognitive impairment may be associated with high total clozapine levels especially if serum CLOZ/DMCLOZ ratio is high (Rajji et al. 2010).

It was also found that sedation and increased dreaming were negatively associated with age (III). Younger patients may be more susceptible to the sedative side

effect of clozapine but young people in normal population also sleep more. Young adults may also consider sedation more troublesome than older ones, because they usually have more daily activities. It is also known that sedation is more common at the beginning of treatment and usually decreases with time (Taylor et al. 2012) Elderly patients with sedation may have discontinued clozapine more often. However, all patients in the present study were on stable and long-lasting clozapine therapy.

Sympatichotonia-tension type side effects were related to antipsychotic polypharmacy. This factor consisted of seven items, namely tension, muscle stiffness, palpitations, slowing of movements, diarrhea, shakiness and pins and needles. It is likely that some of these items could be associated with extrapyramidal adverse effects and others with the anticholinergic effects of antipsychotics. From single LUNSERS items a higher score on the "lack of emotions item" was also associated with antipsychotic combination treatment and this could be a consequence of decreased striatal presynaptic dopaminergic function caused by antipsychotics other than clozapine (Bragulat et al. 2007).

As anticipated, the total antipsychotic dose measured in chlorpromazine equivalents was higher in patients on antipsychotic combination treatments than in patients on clozapine monotherapy, which may also contribute to affect flattening. Antipsychotic combination treatment has also been proposed to be associated with more extrapyramidal symptoms than monotherapy (Xiang et al. 2007).

Period problems were reported by some of the patients. However, clozapine has only a minor affinity with tuberoinfundibular dopaminergic pathway (Turrone et al. 2002), and thereby clozapine associated amenorrhoea has not been reported in the literature so far and menstrual irregularities were less common in clozapine treated patients than in patients taking risperidone or haloperidol (Chitaia et al. 2009). Thus these problems may rather be associated with concomitant medications than with clozapine.

5.1.2 The relationship between clozapine, smoking and weight gain (IV)

The relationship between smoking, weight and weight change were the main focuses in Study IV. Number of cigarettes smoked daily, age and gender explained BMI in the GLM model. The well-known factors age and gender had an effect to BMI almost equal to that of the extent of smoking. Some population based studies have suggested that light smokers have lower body weight than heavy smokers (Bamia et

al. 2004; John et al. 2005; Chiolero et al. 2007). A study by Wehring et al. (2012) compared 319 heavy (>20 cigarettes/day) and 426 non-heavy smokers suffering from schizophrenia (Wehring et al. 2012) and found no differences in weight or BMI in heavy or non-heavy smokers but their patients had heterogeneous medication and dichotomous evaluation of extent of smoking.

Some studies have also suggested that smoking affects body fat distribution and may cause central obesity and insulin resistance (Eliasson 2003; Houston et al. 2006). In people with lower socioeconomic status smoking and obesity have been found to be clustered (Wild and Byrne 2006). The patients in the present study were overweight or obese and their BMI estimate was higher (mean 29.9) than in general Finnish population (mean 27.6) (Salopuro et al. 2011). This finding was expected because clozapine is known to be one of the most weight increasing antipsychotics and may be associated with binge eating and craving for food (Theisen et al. 2001; Kluge et al. 2007). In a Greek study, a difference in BMI between 105 patients with schizophrenia and their matched normal controls was reported, but no association between cigarette consumption and weight was found (Fontoulakis et al. 2010). In general population smokers weigh approximately 4-5 kg less, or their BMI is 1 kg/m^2 lower than that of non-smokers (Williamson et al. 1991; Kruger et al. 2009). In the present patient sample no such difference was found. This finding can be explained either by the factors associated with medication and the mental disorder or with overrepresentation of heavy smokers in the sample. However, the weight gaining effect of clozapine may be so powerful that the possible weight control effect of smoking becomes relatively neglible.

In an earlier study weight gain was compared in patients taking olanzapine or risperidone. Smoking did not affect weight gain in patients taking olanzapine, but smokers taking risperidone gained less weight than non-smokers taking risperidone (Lasser et al. 2004). As clozapine and olanzapine are similar in molecular structure, and are the most weight gaining antipsychotics, this finding may also apply to clozapine. Clozapine increases leptin concentration (Kivircik et al. 2003; Zhang et al. 2007), which has been reported to be associated with both obesity and nicotine dependency (von der Goltz et al. 2010).

The findings on the relationship of clozapine dose and weight gain are somewhat contradictory. In a double blind study with 50 patients weight gain during clozapine treatment was found to be dose dependent (de Leon et al. 2007). In a study with 68 patients patients on clozapine monotherapy and patients on clozapine-fluvoxamine combination plasma levels of clozapine were compared (Lu et al. 2004). The patients

on clozapine monotherapy received higher doses of clozapine and the weight gain was greater in the clozapine monotherapy group. Responders to smaller doses of clozapine gained more weight than patients requiring higher doses (Jalenques et al. 1996). On the other hand, three other studies did not find a relationship between clozapine dose and weight gain (Hummer et al. 1995; Frankenburg et al. 1998; Henderson et al. 2005; Bai et al. 2011). In the present sample there was an association between weight gain and sedation. In the present study, however, neither the dose nor the concentration of clozapine was a main contributor to sedation or overweight. It has been proposed that the sedative and weight gaining effects of clozapine may have a common biochemical origin in the histaminergic system of the brain (Humbert-Claude et al. 2011).

Both obesity and smoking are major cardiovascular risk factors and they act synergistically (Benowitz 2003). Both obesity and smoking cause an inflammatory state – followed by a rise in blood pressure and serum lipid levels and increased risk for adult-type diabetes. In smokers the ratio of the visceral/subcutaneous fat is more unfavourable than in non-smokers, which further increases the risk for cardiovascular disease (Han et al. 2006). In the present study these risk factors seemed to accumulate in heavy smokers.

5.1.3 Effect of abrupt clozapine withdrawal (I)

Rapid deterioration after withdrawal of clozapine was more common among patients not taking any concomitant anticholinergic medications and this deterioration occurred in almost half of the patients. There was also less deterioration in patients taking other antipsychotics in combination with clozapine than in patients taking clozapine alone at the time of discontinuation. However, this difference seemed to be less prominent than the difference found between patients taking anticholinergic vs. not taking anticholinergic medications. However, these frequencies did not differ statistically at any evaluation point.

After discontinuation of clozapine, antipsychotics (FGAs) were initiated, if possible immediately or at least the next day for hospitalized patients, and during the very first days for outpatients. In many cases, however, neuroleptic medication could not prevent acute deterioration.

The reported incidence of rapid deterioration after clozapine withdrawal has varied widely between different studies. A low incidence of 7.5% was found in pa-

tients whose clozapine medication was tapered off and immediately replaced with olanzapine, whereas 24.4% of patients deteriorated when clozapine was replaced by a placebo (Tollefson et al. 1999). A high deterioration incidence of 88.9% was found in patients responsive to FGAs and whose clozapine dose was partially withdrawn without cross-tapering to another antipsychotic drug (Meltzer et al. 1996). In other studies, as in the present one, intermediate deterioration rates have been reported (Borison et al. 1988; Shiovitz et al. 1996). There are several treatment options for treating clozapine withdrawal symptoms, such as with anticholinergics, cyproheptadine and risperidone (de Leon et al. 1994; Baldessarini et al. 1995; Meltzer et al. 1996; Stanilla et al. 1997). The best evidence with this indication has been found with olanzapine (Tollefson et al. 1999).

After discontinuation of clozapine, four patients out of 28 in the present study experienced an improvement. It is obvious that not all patients benefited from clozapine treatment more than from other antipsychotics. Some patients may have experienced more side effects with clozapine than with other antipsychotics.

In the present study, the patients who deteriorated when clozapine was discontinued recovered slightly during the next half year while taking typical antipsychotics. Some studies have reported that after clozapine withdrawal and relapse, some patients responded even less successfully to FGAs than before the use of clozapine (Meltzer et al. 1996). This study, although limited by the retrospective design, does not necessarily support this finding because most of the patients improved somewhat during follow-up. However, the patients treated in 1975 were not selected on the basis of treatment refractoriness and therefore they likely represent a different population from subsequent samples, in which response to antipsychotic agents seems to be less satisfactory after the clozapine trial. In the study by Melzer and co-workers the patients were selected specifically according to their antipsychotic non-response and intolerance using the systematic criteria introduced by Kane (Kane et al. 1988; Meltzer et al. 1996). At the beginning of 1975 the risk of agranulocytosis was unknown and clozapine was marketed as a first line antipsychotic as well.

In the present patients the total antipsychotic dosage measured in chlorpromazine equivalents reduced nearly to a half after clozapine discontinuation and was not markedly increased until the end of the year. Despite this many patients recovered gradually during follow-up. This may partly support the possible role of dopamine hypersensitivity in the initial acute deterioration.

The present paper, Study I, is the only one, as far the authors know, to address the consequences of abrupt unplanned clozapine withdrawal in an unselected

patient sample. Only one congress abstract has been published on the same issue (Kuha 1977). Both studies included mainly hospitalized, compliant patients whose clozapine was discontinued without any medical reason, adverse events or without non-adherence. In the report by Kuha (1977) it was found that 39% of the patients prescribed clozapine in outpatient care returned to hospital after clozapine withdrawal. This is in line with the present study, where five out of 11 discharged patients (45.5%) were later rehospitalized.

The findings of the present study suggest that anticholinergic drugs (like typical antipsychotics with anticholinergic effects or antiparkinsonian anticholinergics, such as biperiden) may prevent acute deterioration if clozapine has to be discontinued abruptly.

Whenever possible, clozapine should be tapered off gradually. If this is not an option, as in cases of agranulocytosis or myocarditis, adding antipsychotic agent with anticholinergic properties may prevent or reduce psychotic symptoms.

5.2 Clozapine and smoking

Two of the present original papers specifically addressed problems related to clozapine and smoking. The proportion of smokers in both studies was high. In Study II 77% of all patients (83% of males and 61% of females) were smokers. In Study IV 48.2% of the patients were daily smokers (51.1% of males and 44.5% of females), which is also much higher than the proportion of smokers (19%) in general Finnish population (Official Statistics of Finland 2014). This finding concurs with those of studies on the relationship of schizophrenia and smoking, where the proportion of smoking among schizophrenia patients is 2–3 times higher than in general population (de Leon and Diaz 2005).

The numbers of smokers differed between Studies II and IV. In Study II the patients were hospitalized inpatients, most of them with fulminant positive symptoms when abstinence or smoking cessation may be difficult (Dalack and Meador-Woodruff 1996). The other reason for the different smoking frequency may be the time gap – the material of Study II was collected as early as in 1996 and the material of Study III was collected in 2008-9. During the interval smoking decreased from 24% to 19% in general population (Official Statistics of Finland 2014) .

The effect of smoking on clozapine serum concentration was the main issue in Study II and the relation between reported smoking, weight and weight change in Study IV, but the relationship between clozapine concentration and smoking was also supported in Study IV.

5.2.1 Effect of smoking on clozapine serum concentration (II, IV)

The effect of smoking on clozapine serum concentrations was the main focus in Study II, and this topic was further explored in Study IV.

In Study II, smokers had 38% lower clozapine and desmethylclozapine concentrations than did non-smoking patients. In Study IV the smokers had about 25% higher clozapine dose than non-smokers, however, there was no difference in their clozapine+norclozapine concentrations. In Study IV, as expected, the clozapine dose was lower in non-smokers than in smokers, but there was no difference in the serum concentrations. Here, too, clozapine dosages were adjusted according to clinical judgement, and in some cases by using information from clozapine serum concentration measurements (Haring et al. 1989; Tang et al. 2007).

The present results concur with the findings according to which the CYP1A2 enzyme has a principal role in clozapine biotransformation (Koponen et al. 1996; Chetty and Murray 2007). The polycyclic aromatic hydrocarbons of tobacco smoke are potent inducers of many CYP isoenzymes, including CYP1A2 (Kroon 2007).

Several studies have now mentioned the effects of smoking on clozapine concentrations. In an earlier study smokers had a 32% lower mean serum clozapine concentration than non-smokers (Wetzel et al. 1998). In an Austrian study the average dose and weight corrected plasma concentration in smokers was 81.8% of that in non-smokers (Haring et al. 1989). In smoking males, the average plasma concentration was only 67.9% of that in non-smokers, but in women no significant difference was found.

In a Chinese sample of 193 patients, no significant difference was found in plasma clozapine concentration between male smokers and non-smokers although the clozapine dose was significantly higher in the smoking, than in the non-smoking patients (Tang et al. 2007). Accordingly, Meyer et al. found a 72% increase in serum clozapine levels after smoking cessation in relation to hospitalization (Meyer 2001). Hence, all studies have supported at least numerically, although not always statistically significantly, the interaction between smoking and clozapine concentrations

(Hasegawa et al. 1993). Haslemo et al. reported that even relatively low cigarette consumption, i.e. only 7-12 cigarettes/day was sufficient for the maximum induction of CYP1A2 (Haslemo et al. 2006). They recommended an approximately 50% increase in the starting dose of clozapine and olanzapine for smokers. There are also some early studies suggesting that smoking patients with schizophrenia may have higher levels of positive symptoms than non-smokers (Goff et al. 1992; Ziedonis et al. 1994), but the findings are still inconsistent (Patkar et al. 2002; Kotov et al. 2012).

Too low clozapine concentrations may be associated with insufficient treatment response and too high concentrations are likely to increase the frequency of some side effects. The present findings support the need for dose adjustment of clozapine according to patients' smoking status. Additionally, concentration measurements should be recommended at least if there are changes in patients' smoking habits.

5.3 Study strengths and limitations

Due to its unique unselected patient population, Study I contributes extraordinary information on abrupt clozapine cessation in general. The limitation of Study I is that it is retrospective and the data was collected from hospital case records of varying quality and not written for research purposes. No systematic ratings and systematic, detailed description of symptoms after clozapine withdrawal were available. Moreover, at time of the study there were no published papers and only very limited experience of retrospective patient record ratings with CGI-scale. However, special attention was paid to typical cholinergic rebound symptoms such as gastrointestinal symptoms possibly mentioned in case notes. The present patient sample was less treatment resistant than the populations in the later clozapine studies.

There was no difference in outcome between patients receiving anticholinergics or antipsychotics before clozapine discontinuation. The total antipsychotic dosage measured by CPZ equivalents was lower after withdrawal than before clozapine discontinuation. Whether anticholinergic or dopaminergic rebound or both contribute to clozapine withdrawals remains unanswered. In Study II, the sample size was relatively small, especially the number of non-smoking patients. This caused problems with statistical power, e.g. in gender specific comparisons. On the other hand, the group of smokers was moderate in size and representative of the average smoking patients on clozapine treatment.

The data of Studies III and IV is based on self-report questionnaires, including information on weight and height. In general population surveys, however, there has been a tendency towards overestimation of height and underestimation of weight, which is likely also the case in the present sample (Niedhammer et al. 2000; Paccaud et al. 2001). No structured rater based scales for rating adverse effects were performed in the studies. Moreover, the diagnoses of the patients were based solely on the hospital records and not confirmed with structured interviews. More detailed information on patients' current symptomatology would have improved the reliability of the LUNSERS assessment. On the other hand, this procedure might have reduced the sample size and caused a selection bias. The effect of psychiatric symptoms may also have affected to the results (instead of adverse effects of drug treatment) although the patients were expressly instructed that the purpose of the study was to evaluate side effects. Psychotic, depression and anxiety symptoms were not systematically examined and these may have influenced the LUNSERS data. It may also be that more careful evaluation of parkinsonism-like symptoms would be preferable to detect the possibly extrapyramidal effect of antipsychotics other than clozapine. Thus no definitive answer on the character of factors or symptom clusters like "depression-anxiety" and "sympatichotonia-tension" can be proposed in the study. Thus further studies with more concise comparative information are needed to confirm those results.

All weight-related information, like feelings of hunger, weight gain and BMI reported by the patients were, however, in line with each other, which supports the reliability of the study methods.

Of the 237 study participants, 190 patients provided laboratory samples and in 47 patients the data on serum drug concentrations was unavailable. One reason for the number of dropouts here could be logistical, as the patients were living in a wide geographical area comprising several different laboratory settings. The patients may also have had difficulties to follow instructions due to cognitive deficits. Possible adherence problems in some outpatients may also have contributed to the missing data.

6. CONCLUSIONS

The main findings of the study were:

1. The acute psychiatric deterioration after abrupt clozapine withdrawal was frequent and rapid. Medications with anticholinergic properties alleviated clozapine withdrawal symptoms.
2. Smokers had markedly lower clozapine serum concentrations than non-smokers after adjusting for patients' weight and dosage.
3. Combined clozapine and desmethylclozapine concentrations may be associated with depression and anxiety related side effects during clozapine medication.
4. Number of cigarettes smoked was independently associated with body mass index when age and sex were taken into account.

CLINICAL REMARKS

Deterioration after sudden withdrawal of clozapine is common. This is important when sudden withdrawal of clozapine is unavoidable, for instance due to agranulocytosis or myocarditis. An antipsychotic medication with anticholinergic properties or an antipsychotic combined with anticholinergic agent may therefore be the options.

The decreasing effect of smoking on clozapine serum levels is clinically significant. Clozapine dosage adjustment is needed according to patients' smoking status.

Clozapine serum level measurements can be helpful in reducing the risk of adverse events, especially during changes in smoking habits.

Special attention should be paid to depressive and anxiety symptoms in schizophrenic patients taking clozapine. Sympatichotonia-tension related side effects in patients on clozapine combination therapy and sedation-like side effects in younger patients are also common. Polypharmacy should be avoided.

There was no significant weight difference between smoking and non-smoking patients with schizophrenia. However, the most obese patients were also among the heaviest smokers. As smoking and weight are both major risk factors for cardiovascular disease, special attention should be paid to encourage smoking cessation and weight reduction in this patient group.

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Clozapine Serum Concentrations are Lower in Smoking than in Non-Smoking Schizophrenic Patients

Niko H. Seppälä[1,3], Esa V. J. Leinonen[2,3], Maija-Liisa Lehtonen[1] and Kari T. Kivistö[4]

[1]Department of Clinical Psychiatry and [2]Department of Psychogeriatrics, Tampere University Hospital, Tampere, [3]Department of Clinical Medicine, University of Tampere, Tampere and [4]Department of Clinical Pharmacology, University of Helsinki, Helsinki, Finland

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Abstract: Serum concentrations of clozapine and its main metabolite demethylclozapine were measured in 44 schizophrenic inpatients, of whom ten were non-smokers and 34 smokers. When comparing their clozapine dose and body weight-related serum drug levels, we found that clozapine and demethylclozapine concentrations were about 40% lower in the smoking than in the non-smoking group, probably due to an inducing effect of smoking on the cytochrome P450 (CYP) 1A2, which is involved in the metabolism of clozapine. We conclude that dosage adjustment may be necessary in clozapine-treated smokers.

Clozapine, an atypical antipsychotic agent, is a well-established alternative in the treatment of treatment-refractory or neuroleptic-intolerant schizophrenic patients (Byerly & DeVane 1996).

Clozapine is metabolised to demethylclozapine (norclozapine) and clozapine N-oxide in the liver. The pharmacological activity of these metabolites has been reported to be much lower than that of the parent drug (Jann *et al.* 1993). CYP1A2 and CYP3A4 are the primary enzymes involved in biotransformation of clozapine *in vitro* (Eiermann *et al.* 1997; Fang *et al.* 1998). In a recent study, the CYP1A2 inhibitor fluvoxamine considerably increased serum clozapine concentrations in schizophrenic patients (Wetzel *et al.* 1998). In contrast, the potent CYP3A4 inhibitor itraconazole did not affect the pharmacokinetics of clozapine in healthy volunteers (Raaska & Neuvonen 1998), suggesting that CYP1A2 is the principal CYP enzyme mediating the biotransformation of clozapine in humans (Bertilsson *et al.* 1994). On the other hand, serum clozapine levels can be decreased by drugs inducing CYP enzymes such as carbamazepine (Raitasuo *et al.* 1993) and phenobarbital (Facciola *et al.* 1998).

Cigarette smoking induces CYP1A2 (Carrillo & Benitez 1994), and there are several studies on the effects of smoking on clozapine concentrations in plasma or serum (Haring *et al.* 1989; Hasegawa *et al.* 1993; Wetzel *et al.* 1998). Haring *et al.* (1989) and Wetzel *et al.* (1998) found that clozapine concentrations were lower in smokers than in non-smokers. In contrast, Hasegawa *et al.* (1993) did not find any significant difference in clozapine or demethylclozapine concentrations between smokers and non-smokers.

The aim of the present study was to compare dose- and weight-related serum clozapine and demethylclozapine concentrations between smoking and non-smoking schizophrenic patients in Finland.

Materials and Methods

Subjects. Steady-state serum concentrations of clozapine and demethylclozapine were measured in 44 schizophrenic inpatients (31 men and 13 women) in the Tampere University Hospital. The diagnosis was established clinically by two experienced psychiatrists. All patients had received a fixed dosage of clozapine for at least two weeks prior to the study. Patients using drugs possibly affecting serum clozapine concentrations (e.g. omeprazole, selective serotonin reuptake inhibitors (SSRI) and inducers of CYP enzymes such as carbamazepine) were not included (Centorrino *et al.* 1996; Spina *et al.* 1998). Thirty-four patients (26 men and 8 women) were smokers and 10 (5 men and 5 women) were non-smokers (see table 1 for the characteristics of these two groups). The smokers consumed 10–40 cigarettes/day (average 18.0 cigarettes/day). The number of cigarettes smoked was counted by the nursing staff.

Medication. Clozapine dosage was adjusted according to the therapeutic response and possible side effects. The individual dosage ranged from 150 to 800 mg/day and averaged 520±150 mg/day. The non-smoking patients received lower clozapine doses than the smokers ($P<0.05$); this difference remained statistically significant when the drug dosage was related to the body weight.

Serum drug concentration measurements. Blood samples for measurement of serum clozapine and demethylclozapine were taken 12 hr after the previous dose. The clozapine dosage of each patient was stable for at least two weeks before the sample was taken.

Serum clozapine and demethylclozapine concentrations were determined by high-performance liquid chromatography with ultraviolet detection, using protriptyline as an internal standard (Haring *et al.* 1988; Lovdahl *et al.* 1991). The limit of quantitation was 50

Author for correspondence: Niko Seppälä, Tampere Community Mental Health Center, Rautatienkatu 10, FIN-33100 Tampere, Finland (fax +358 3 247 7919, e-mail niko.seppala@uta.fi)

Table 1.

The characteristics of smoking and non-smoking patients.

	Non-smokers	Smokers	P value
Age (years)	36.6±7.2	38.8±11.8	NS
Weight (kg)	76.4±12.4	75.6±12.2	NS
Clozapine dosage (mg/day)	410±131	553±154	0.010
Clozapine dosage per body weight (mg/kg/day)	5.4±1.4	7.5±2.4	0.012
Duration of hospitalisation (months; median)	13.1	16.7	NS
Duration of clozapine therapy (days; median)	212	267	NS

Data are mean values±S.D. unless otherwise stated. NS, not statistically significant.

Table 2.

Mean±S.D. of serum concentration of clozapine and demethylclozapine (nmol/l per mg/kg). The number of patients is given in parentheses.

	Non-smokers	Smokers	P value
Clozapine			
All patients	298±127 (10)	184±97 (34)	0.021
Females	352±159 (5)	159±97 (8)	0.019
Males	245±59 (5)	191±92 (26)	0.254
Demethylclozapine			
All patients	260±150 (10)	165±89 (34)	0.016
Females	289±177 (5)	169±137 (8)	0.198
Males	231±131 (5)	164±72 (26)	0.106

nmol/l for clozapine and 100 nmol/l for demethylclozapine. The interassay coefficient of variation at relevant concentrations was 5–7% for clozapine and 10–13% for demethylclozapine.

Statistical analysis. The two-sample t-test (two-tailed) was used to compare results between smokers and non-smokers. For statistical comparison, the obtained serum clozapine and demethylclozapine concentrations were divided by the daily dose (mg) per body weight (kg). Results are expressed as mean±S.D. and $P<0.05$ was considered statistically significant.

Results

The smokers had significantly lower mean dose- and weight-related serum clozapine and demethylclozapine concentrations than the non-smokers (table 2). A significant difference in clozapine concentration was found between smoking and non-smoking female patients, but not between smoking and non-smoking men. Generally, the clozapine-demethylclozapine concentration ratio was not affected by smoking.

There were no significant differences in dosage- and weight-related clozapine or demethylclozapine concentration between genders among non-smokers or smokers. In smokers, drug concentrations were not related to the number of cigarettes consumed daily.

Discussion

Both serum clozapine and demethylclozapine concentrations of the patients in this study were on an average about 40% lower in smokers than in non-smokers when they were related to the daily clozapine dose and body weight. Our results are in line with the finding that smoking can induce CYP1A2 (Carrillo & Benitez 1994), the principal CYP enzyme involved in clozapine biotransformation (Eiermann *et al.* 1997; Fang *et al.* 1998). The lower demethylclozapine concentrations in the smoking patients suggest that the metabolism of demethylclozapine may also be induced by smoking.

Previously, Haring *et al.* (1989) studied the effect of smoking on plasma clozapine levels in 148 patients. In this study, the average dose- and weight-related plasma concentration in smokers was 81.8% of that in non-smokers. In smoking male patients, the average plasma clozapine concentration was only 67.9% of that in non-smokers, while a statistically significant difference between female smokers and non-smokers was not observed. Wetzel *et al.* (1998) reported in their study of 30 patients that smokers had a 32% lower mean serum clozapine concentration than non-smokers.

On the other hand, we found a significant difference in clozapine levels between smoking and non-smoking females only, although there was a non-significant trend towards a lower clozapine concentration in male smokers. However, it should be emphasised that, due to small sample sizes, the statistical power of our study was probably too low for analysis of sex differences in the effects of smoking on clozapine pharmacokinetics.

In previous studies, the mean daily dose of clozapine varied between 202 mg/day (Wetzel *et al.* 1998) and 431 mg/day (Hasegawa *et al.* 1993), while the average dosage in our patients was higher (520 mg/day). Despite the different dosages, most of the previous studies as well as the present study found a 30–40% reduction in clozapine concentrations in smokers.

In contrast to the results mentioned above, Hasegawa *et al.* (1993) did not find significant differences in plasma clozapine or demethylclozapine concentrations between smokers and non-smokers among 59 patients. However, concentrations of both clozapine and demethylclozapine tended to be lower in smokers.

Several factors such as differences in study design (e.g. criterion for smoking) and the possible effect of gender on CYP1A2 activity may have contributed to these apparently conflicting results. In a recent study, smoking appeared to increase the metabolism of theophylline, a substrate of CYP1A2, to a greater extent in men than in women (Jennings *et al.* 1993). There is a large between-subject variation in the expression of CYP1A2 (Bertilsson *et al.* 1994), and we speculate whether the inducing effect of smoking on CYP1A2 might depend on the baseline activity of this enzyme. The available data on the effect of sex on CYP1A2 activity are conflicting and no definite conclusions on this point can be made (Harris *et al.* 1995).

In this study, dose- and weight-related concentrations

were used for analysis because the smoking patients received larger clozapine doses than the non-smokers. The dosage of clozapine was adjusted individually according to therapeutic response and possible side-effects. Thus, it is likely that the higher dosages needed in smokers resulted from their lower serum drug concentrations causing insufficient therapeutic response. On the other hand, some evidence suggests that schizophrenic patients who smoke may have higher positive symptom levels than non-smokers (Goff *et al.* 1992; Ziedonis *et al.* 1994). This point could not be addressed in the present study, because clinical responses to clozapine therapy were not assessed regularly.

The threshold of a therapeutic serum clozapine concentration appears to be about 1200 nmol/l, and concentrations above 3000 nmol/l increase the risk of CNS adverse effects (Freeman & Oyewumi 1997). A case has been reported in which clozapine induced seizures in a man who had stopped smoking (McCarthy 1994). Since our data and those from other studies (Haring *et al.* 1989; Wetzel *et al.* 1998) suggest that plasma clozapine levels are lower in smokers than in non-smokers, we suggest periodic measurement of serum clozapine levels in smoking patients, and appropriate dose adjustment to ensure that clozapine concentrations are within the therapeutic range.

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