Eur Arch Psychiatry Clin Neurosci (2004) 254 : 318–325 DOI 10.1007/s00406-004-0505-2

ORIGINAL PAPER

Jose de Leon · Francisco J. Diaz · Richard C. Josiassen · George M. Simpson

# Possible individual and gender differences in the small increases in plasma prolactin levels seen during clozapine treatment

Received: 25 October 2003 / Accepted: 9 March 2004

**Abstract** In vitro, animal studies and acute short-term clinical studies suggest clozapine releases prolactin but the effect is much smaller than that of typical antipsychotics. Repeated early morning trough measures of plasma clozapine and prolactin levels on each subject were studied during the course of a double-blind dose-response clozapine study. After a 4-week 10 mg/day haloperidol trial and a one-week washout, treatment-refractory schizophrenics were successively randomized to 100, 300, or 600 mg/day of clozapine for a 16-week treatment. The statistical analyses included 35 subjects (19 females and 16 males). The within-subject correlation of prolactin levels was 0.32 with clozapine levels and 0.75 with haloperidol levels. An increment of 100 ng/ml in clozapine level yielded an average increment of 0.45 ng/ml of prolactin levels in females and of 0.15 ng/ml in males. An increment of 1 ng/ml in haloperidol level yielded an average increment of 2.6 ng/ml of prolactin levels in females and of 1.5 ng/ml in males. At least one fourth of patients demonstrated a significant and strong ($r > 0.6$) correlation between clozapine and prolactin levels. This study suggests that clozapine has effects on prolactin levels but effects are small and may be more evident in some individuals, particularly females.

**Key words** prolactin · clozapine · haloperidol · schizophrenia · women

F. J. Diaz, Ph.D.
Department of Statistics
Universidad Nacional
Medellin, Colombia

R. C. Josiassen, Ph.D.
The Arthur P. Noyes Research Foundation
Norristown, PA, USA
and the
Department of Psychiatry
University of Pennsylvania Medical School
Philadelphia, PA, USA

G. M. Simpson, M.D.
Department of Psychiatry and Behavioral Sciences
Keck School of Medicine
University of Southern California
Los Angeles, CA, USA

J. de Leon, M.D. (✉)
Mental Health Research Center at Eastern State Hospital
627 West Fourth St.
Lexington, KY 40508
Tel.: +1-859/246-7487
Fax: +1-859/246-7019
E-Mail: jdeleon@uky.edu

This study was conducted in the Clinical Research Center at Norristown State Hospital, Norristown that at that time was affiliated with the Department of Psychiatry at the Medical College of Pennsylvania, Philadelphia, PA.

EAPCN 505

## Introduction

### Clinicians' experience

Clinicians would easily agree that clozapine treatment is not associated with clinically significant increases in prolactin levels (Dickson et al. 2000). Therefore, clozapine is used to treat patients with symptomatic hyperprolactinemia (Uehlinger and Baumann 1991; Bunker et al. 1997). Moreover, the lack of prolactin elevations is usually considered one of the characteristics of clozapine atypicality (Petty 1999).

### In vitro and animal studies

In vitro (Lamberts et al. 1990; Braghiroli et al. 1997) and rat (Meltzer et al. 1979; Guldesky et al. 1989) studies indicated that in effect clozapine stimulates prolactin secretion. However, clozapine effects are rather small. Lamberts et al. (1990) estimated that clozapine effects on prolactin secretion are 5 to 10 times lower than those of typical antipsychotic drugs (Lamberts et al. 1990).

## Clinical studies

It is reasonable to expect that if clozapine produces small releases of prolactin, these small releases may be difficult to detect in research clinical studies and that they may not be present in all individuals. Research clinical studies in patients provide a more complex picture than in vitro studies. The lack of consistency in clinical findings is probably related to the different statistical power of different methodologies and designs to find significant differences. Gender differences have not been appropriately explored, despite gender being a major determinant of prolactin levels.

**Cross-sectional studies comparing effects on trough prolactin levels of typical antipsychotics and clozapine.** Clinical studies consistently agree on one issue: typical antipsychotics (or risperidone) have significantly higher effects on prolactin levels than clozapine (Table 1).

One concern after reviewing these studies is that they are mainly focused on males (Table 1). Only one study provided mean data in both males and females (Meltzer et al. 1979). This study suggested that the effect size when comparing typical antipsychotics and clozapine appeared larger in females, 3.9, versus males, 1.1 (Table 1).

**Cross-sectional studies comparing effects on trough prolactin levels of clozapine versus baseline conditions.** There are 3 studies comparing different subjects: patients taking clozapine versus controls. These three studies do not agree (Table 2). All were conducted in males and found quite lower effect sizes than those studies comparing clozapine versus typical antipsychotics.

Other designs assessed mean trough prolactin measure in the same patient during two conditions: clozapine versus placebo/drug-free conditions. This second type of study has the advantage of controlling for individual effects. There are six studies of this type (Table 2). Most did not test for statistical differences and none provided information so that effect sizes could be calculated. Again, most of these studies were focused on men and only one study provided mean values in males and females (Szymanski et al. 1996).

**Clinical short-term studies using repeated measures and mean peak prolactin levels.** Studies using repeated clozapine measures are more likely to detect the small effects on prolactin levels suggested by in vitro studies since they can control for the variations within the same individual. There are seven such studies measuring mean peak levels but all have small samples and are almost all limited to males (Table 3). A recent male study with a better design (Turrone et al. 2002) demonstrated a significant small peak with return to the baseline after 24 hours. Three of the older studies (Gruen et al. 1978; Meltzer et al. 1979; Ackenheil 1989) also appeared to show a hint of a short-term prolactin peak produced by clozapine. The prolactin peaks associated with acute clozapine administration are clearly smaller than those

**Table 1** Review of previous studies on serum prolactin levels (ng/ml) using only one trough measure: clozapine vs. typicals

| Author | Sample | Doses (mg/day) | Prolactin levels | Effect sizes |
|---|---|---|---|---|
| *Clozapine patients vs other patients in typicals (or risperidone)* | | | | |
| Meltzer et al. 1979 | 6 M patients | 150–800 | 12.9 ± 6.1[a] clozapine | |
| | 13 M patients | 800 chlorpromazine | 22.4 ± 9.2[a] typicals | 1.1* (typicals vs. clozapine) |
| | 7 F patients | 150–800 | 14.2 ± 5.3[a] clozapine | |
| | 7 F patients | 800 chlorpromazine | 73.7 ± 20.8[a] typicals | 3.9* (typical vs. clozapine) |
| Meltzer 1989 | 21 M patients | 200–900 | 4.3 ± 1.7 | |
| | 43 M on typicals | – | 19.2 ± 16.2 typicals | 1.1* (typicals vs. clozapine) |
| Henderson et al. 2001 | 20 patients (2 F) | mean 395 | 8.4 ± 4.2 clozapine | |
| | 20 patients (2 F) | risperidone added | 35.8 ± 17.4 risperidone | 2.2* (risperidone vs. clozapine) |
| Markianos et al. 2001 | 15 M patients | 200–600 | 7.7 ± 3.8 | |
| | 23 M patients | 19 | 34.4 ± 17.3 haloperidol | 1.9* (haloperidol vs. clozapine) |
| *Clozapine vs typicals in same patient*[b] | | | | |
| Pickar et al. 1992 | 21 patients (8 F) | 540 | 42 on typical vs. 9 on clozapine | |
| Brown et al. 1997 | 9 patients[c] | at least 300 for 6 wks. | 44.0 on haloperidol vs. 8.4 on clozapine | $p < 0.08$ |
| Breier et al. 1999 | 14 patients (6 F) | mean 403 for 6 wks. | 53.3 ± 39.6 on fluphenazine vs. 12.2 ± 7.7 on clozapine | |
| Markianos et al. 1999 | 31 M patients | mean 328 | 39.9 ± 26.1 on typicals vs. 8.3 ± 5.0 on clozapine | |

*M* male; *F* female; *wks.* weeks; * $p < 0.05$

[a] Meltzer et al. described that the standard used provided levels that were 3.8 times higher than those found using conventional standards. Therefore, Meltzer et al.'s levels were divided by 3.8

[b] Standard deviations for paired mean differences were not reported, therefore effect sizes could not be calculated

[c] There were 14 (6 F) completers of the trial. No gender data were provided in subsample with prolactin levels

**Table 2** Review of previous studies on serum prolactin levels (ng/ml) using only one trough measure: clozapine vs. placebo/drug free

| Author | Sample | Doses (mg/day) | Prolactin levels | Effect sizes |
|---|---|---|---|---|
| *Clozapine patients vs other patients* | | | | |
| Meltzer 1989 | 21 M patients | 200–900 | 4.3 ± 1.7 | |
| | 69 drug-free M patients | – | 6.6 ± 3.3 | –0.76* (clozapine vs. drug-free) |
| | 20 M controls | – | 5.9 ± 2.8 | –0.69* (clozapine vs. controls) |
| Markianos et al. 1999 | 31 M patients | mean 328 | 8.3 ± 5.0 | |
| | 38 M controls | – | 5.5 ± 1.8 | 0.78* (clozapine vs. controls) |
| Markianos et al. 2001 | 15 M patients | 200–600 | 7.7 ± 3.8 | |
| | 33 drug-free M patients | – | 8.0 ± 3.6 | –0.08 (clozapine vs. drug-free) |
| | 14 M controls | – | 8.3 ± 3.8 | –0.16 (clozapine vs. controls) |
| *Clozapine condition vs other condition in same patient*[a] | | | | |
| Meltzer et al. 1979 | 7 patients | mean 458–579 | 15.0 ± 5.3[a] on clozapine vs. 15.5 ± 4.5[a] placebo | |
| Nair et al. 1979 | 10 M patients | up to 100 for 3 days | 9.5 ± 1.2 on clozapine vs. 8.1 ± 0.9 off meds | Small increase (17 %) but significant |
| Lemus et al. 1991 | 7 patients (2 F) | mean 357 for 2–3 wks | 6.9 ± 2.6 clozapine vs. 8.0 ± 4.0 washout 2–4 wks | No significant differences |
| Pickar et al. 1992 | 21 patients (8 F) | 540 | 9 on clozapine vs. 8 on placebo | |
| Szymanski et al. 1996 | 39 M patients | mean 588 for 6 wks | 9.2 ± 4.1 clozapine 6$^{th}$ week vs. 7.7 ± 5.6 washout | |
| | 14 F patients | mean 620 for 6 wks | 15.2 ± 6.9 clozapine 6$^{th}$ week vs. 11.5 ± 8.6 washout | |
| Brown et al. 1997 | 10 patients[b] | at least 300 for 6 wks | 8.4 on clozapine vs. 7.4 washout for 2–4 weeks | $p = 0.27$ |
| Wudarsky et al. 1999 | 22 ADOL patients | mean 325 for 6 wks | 11.2 ± 4.0 clozapine vs. 9.0 ± 3.4 placebo | |
| | 16 M patients | | 9.9 ± 3.1 clozapine vs. 8.5 ± 3.4 placebo | |
| | 6 F patients | | 14.7 ± 4.5 clozapine vs. 10.3 ± 3.3 placebo | |

*M* male; *F* female; *wks.* weeks; * $p < 0.05$

[a] Standard deviations for paired mean differences were not reported, therefore effect sizes could not be calculated

[b] There were 14 (6 female) completers of the trial. No gender data were provided in subsample with prolactin levels

**Table 3** Review of previous studies on serum prolactin levels (ng/ml) on clozapine treatment using repeated prolactin levels: short-term studies

| Author | Sample | Doses (mg/day) | Measures and times | Changes |
|---|---|---|---|---|
| *Short-term studies (peak in hours)* | | | | |
| Sachar et al. 1976 | 1 control | 1 dose of 12.5 | every 15 min for 3 h | No increase |
| | 2 patients | 100 | | No increase |
| Gruen et al. 1978 | 3 controls | 12.5 intramuscularly | In 3 h | No increase |
| | 1 F patient | 500 maintenance dose | trough and 2 h | From 12 (normal) to 28 (abnormal) after 2 h |
| Meltzer et al. 1979 | 4 patients | 400–600 | every 30 minutes for 4 h | barely exceeded 95 % upper limit of normal |
| Ackenheil 1989 | 4 controls | 1 dose of 100 | every hour for 4 h | small (< 10) significant peak in 2 h |
| Lee et al. 1995 | 10 M controls | 1 dose of 50 | several in 5 h | no significant increase |
| Pretorius et al. 2001 | 12 M controls | 1 dose of 50 | several for 5 h | no changes |
| Turrone et al. 2002 | 6 M patients | 300 | every h for 8 h and trough | small peak in first 8 h and baseline at 24 h |

*M* male; *F* female; *wk.* week; *h* hour; *Trough* early AM before administering meds and approximately 12 hours after last clozapine night dose

from typical antipsychotics (Meltzer et al. 1979) and return to normal by the next morning.

■ **Lack of studies looking at individual differences on the effects of clozapine on prolactin levels.** All prior clinical studies have looked at mean effects of clozapine on prolactin levels. None of them focused on individual differences, e. g., some subjects may be more sensitive to clozapine effects on prolactin than others. In addition, none of the studies focused on gender differences although females may be more sensitive to the small effects of clozapine on prolactin.

If one wants to explore individual differences, a reasonable approach is to conduct long-term studies to explore whether clozapine doses/levels correlate with prolactin levels in some individuals and not in others. There are five published cases (2 males and 3 gender not described) with repeated measures of trough prolactin levels during several weeks (Table 4). In one of the two male studies followed by Kane et al. (1981), the graphical presentation of clozapine and prolactin levels appeared to suggest a relationship between prolactin and moderate clozapine levels. The relationship seemed to disappear when clozapine levels were high. No single-case statistics were carried out but this case also suggests that long-term studies with repeated measures are needed to rule out whether clozapine and prolactin levels may be correlated in some individuals.

In summary, the literature suggests three facts: 1) in vitro studies suggest that clozapine may cause small releases of prolactin that are lower than those produced by typical antipsychotics, and they may be so small as to have limited clinical significance; 2) clinical studies clearly show that prolactin levels on clozapine are significantly lower than on typical antipsychotics; and 3) clinical studies frequently cannot detect small effects unless they use repeated prolactin measures and look for mean peak values after short-term administration. The literature has focused on mean effects and has not explored the possibility that some subjects may release prolactin after taking clozapine and others may not. Moreover, prior studies have mainly focused on males and have not explored gender differences.

In the present study, the association between plasma clozapine and prolactin levels in male and female patients was explored. In addition, the effects of clozapine and haloperidol on prolactin levels were compared. Repeated trough measures of clozapine, haloperidol, and prolactin levels on each subject were used. The measures were obtained throughout several weeks, during the course of a double-blind dose-response clozapine study (Simpson et al. 1999). The repeated measures allowed control for prolactin variations within subjects. In addition, individualized analyses were performed with the purpose of finding those subjects with a strong clozapine effect on prolactin secretion.

## Methods

### Sample

The study design was previously described in detail (Simpson et al. 1999). Forty DSM-IIIR schizophrenic (or schizoaffective) patients who met criteria for treatment-refractory symptoms (Kane et al. 1988) were included after signing an informed consent. After a 4-week 10 mg/day haloperidol trial and a one-week washout, all subjects were randomized to 100, 300, or 600 mg/day of clozapine for a 16-week double-blind treatment. After this first clozapine trial, non-responders were successively randomized to one or two additional 16-week trials with remaining doses. Four subjects were not treated with haloperidol for 4-weeks due to history of intolerance to haloperidol so they continued under their baseline typical antipsychotic. Thirty nine of the 40 patients had repeated measures of prolactin levels under clozapine, although only 37 completed the first 16-week clozapine trial, 21 patients completed an additional second trial, and 13 also had a third 16-week clozapine double-blind trial. However, all available measures of clozapine and prolactin levels were used in the current study when appropriate, even those from non-completed clozapine trials.

As a comparison between clozapine with haloperidol was planned only those patients with prolactin levels during the haloperidol trial were included in analyses leaving us with 35 subjects (19 females and 16 males). The mean age for females was 49 ± 8 years (range 32–60) and the mean age for males was 42 ± 9 years (range 31–58). Ninety percent (17/19) of females were Caucasian and 10 % (2/19) of females were African-American. Eighty-one percent (13/16) of males were Caucasian and 19 % (3/16) of males were African-American.

### Laboratory assays

Plasma prolactin levels obtained in the haloperidol phase and every other week during the clozapine trials were measured by radioimmunoassay as described previously (Kane et al. 1981). Plasma clozapine and norclozapine levels were measured by gas chromatography (Simpson and Cooper 1978; de Leon et al. 2003). The sum of clozapine and norclozapine levels for each subject was called the total clozapine level. Plasma haloperidol levels were also assayed using radioimmunoassay (Devanad et al. 1998). All laboratory studies were performed in the Nathan Kline Institute, Orangeburg, New York. All levels were collected in the early morning (around 8:00 AM) before medications were administered.

### Statistics

The Statistical Package for Social Sciences (SPSS) was used for statistical analyses (SPSS, Inc 1997). To control for differences among individuals, within-subject correlations between clozapine and prolactin levels were calculated for males, females, and the total sample. This was done by means of repeated measures analyses of covariance (Bland et al. 1995). A within-subject correlation is a measure of the average effect of clozapine on prolactin levels. Each of the analyses of covariance also provided a regression slope for clozapine levels. This slope measures the average increment in prolactin levels (in ng/ml)

**Table 4** Review of previous studies on serum prolactin levels (ng/ml) on clozapine treatment using repeated prolactin levels: long-term studies

| Author | Sample | Doses (mg/day) | Measures and times | Changes |
|---|---|---|---|---|
| *Long-term studies (repeated trough levels for weeks)* | | | | |
| Meltzer et al. 1979 | 3 patients | 400–600 | trough twice a wk for 3–4 wks | no evidence of increase |
| Kane et al. 1981 | 1 M patient | 7-wks up to 900 | several trough | minimally elevated and two peaks during stress |
| | 1 M patient | 15-wks up to 900 | several trough | increased on cloz. levels 329–558; later normalized |

*M* male; *F* female; *wk.* week; *Trough* early AM before administering meds and approximately 12 hours after last clozapine night dose

that is caused by an increment of 100 ng/ml in clozapine level. To establish the relationship between clozapine and prolactin avoiding contamination by haloperidol, those pairs of prolactin and clozapine levels that occurred with detected levels of remaining haloperidol were eliminated.

Within-subject correlations between haloperidol and prolactin levels and corresponding slopes were also computed for comparison purposes. In this case, the slope measures the average increment in prolactin levels (in ng/ml) that is caused by an increment of 1 ng/ml in haloperidol level.

The means of the last prolactin levels measured from the 100, 300 and 600 mg clozapine trials and the haloperidol trial were computed and compared using Friedman and Wilcoxon related-samples tests.

All prior analyses considered patients as a group and determined average effects of clozapine on prolactin in the group of males and females. Additional individualized analyses using subjects with more than 5 prolactin measures not contaminated by residual haloperidol levels were performed. In this case, the effect in each individual was explored by calculating a Pearson correlation between total clozapine and prolactin levels. In 2 subjects, the total clozapine levels appeared to correlate with prolactin levels better than clozapine or norclozapine alone. In most subjects, the correlations using total clozapine and clozapine levels were very similar. Only large correlations with p values $< 0.05$ are described. It must be remembered that sample size has a major influence on the significance of Pearson correlations and the number of repeated measures in the subjects considered for individualized analysis ranged from 6 to 23.

## Results

### Within-subject correlations between clozapine and prolactin levels

The within-subject correlation between clozapine and prolactin levels was low, 0.32 but significant ($p < 0.001$; 400 repeated measures on the 35 subjects were used in computations). The correlation was also significant in each gender, 0.35 in females ($p < 0.001$), and 0.25 in males ($p < 0.001$). The correlations between total clozapine and prolactin levels were essentially the same as correlations between clozapine and prolactin. An increment of 100 ng/ml in clozapine level yielded an average increment of 0.45 ng/ml of prolactin levels in females and of 0.15 ng/ml in males.

### Within-subject correlations between haloperidol and prolactin levels

The within-subject correlation between haloperidol and prolactin levels was higher, 0.75 ($p < 0.001$; 137 repeated measures on the 35 subjects were used). The within-subject correlation for females was 0.76 ($p < 0.001$) and for males 0.86 ($p < 0.001$). An increment of 1 ng/ml in haloperidol level yielded an average increment of 2.6 ng/ml of prolactin levels in females and of 1.5 ng/ml in males.

### Means of last prolactin measures

Among females, the means of the last prolactin levels on 100, 300 and 600 mg/day of clozapine were respectively 9.4 (SD 5.1), 7.7 (SD 2.4) and 11.8 (SD 8.4) ng/ml and were significantly different (Friedman $\chi^2 = 7.4$, $df = 2$, $p = 0.02$). Prolactin levels on 600 mg/day doses were significantly different from prolactin levels on 300 mg/day (Wilcoxon $p = 0.02$) and from prolactin levels on 100 mg/day ($p = 0.05$). However, the 100 and 300 mg/day doses were not significantly different from each other. In females, the mean last prolactin measure under haloperidol treatment, 29.9 (SD 22.9) ng/ml was significantly higher than under 600 mg/day of clozapine (Wilcoxon $p < 0.001$).

Among males, the means of last prolactin levels on 100, 300 and 600 mg/day of clozapine were respectively 5.9 (SD 2.6), 6.3 (SD 2.3) and 6.8 (SD 2.8) ng/ml but were not significantly different ($\chi^2 = 0.8$, $df = 2$, $p = 0.7$). The mean last prolactin measure on haloperidol treatment, 10.2 (SD 3.4) ng/ml was significantly higher than on 600 mg/day of clozapine ($p < 0.046$).

### The effect of clozapine levels on prolactin within individuals

There were 16 females with at least 5 appropriate paired levels of prolactin and total clozapine. Three of them had very high Pearson correlations between total clozapine and prolactin levels. The values were $r = 0.88$ ($p < 0.001$, $N = 15$), $r = 0.76$ ($p < 0.001$, $N = 17$), $r = 0.71$ ($p = 0.001$, $N = 17$). There is little doubt that in these 3 females a significant relationship between total clozapine and prolactin levels occurred. The woman with $r = 0.88$ is described in Fig. 1. To interpret this figure it must be remembered that prolactin oscillations associated with total clozapine oscillations are small. Moreover, prolactin levels under haloperidol (around 100 ng/ml) were clearly larger than under clozapine (a maximum of 58 ng/ml for 600 mg/day and around 20 ng/ml for 100 mg/day). Another three females had correlations with p-values $< 0.05$ ($r = 0.78$, $p = 0.02$, $N = 8$; $r = 0.66$, $p = 0.03$, $N = 11$; and $r = 0.60$, $p = 0.01$, $N = 17$).

There were 15 males with at least 5 paired levels of prolactin and total clozapine. There were 3 males with very high Pearson correlations. The values were $r = 0.96$

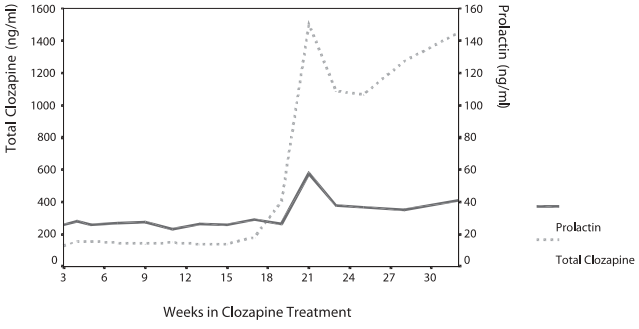


**Fig. 1** Total clozapine and prolactin levels from a female subject ($r = 0.88$, $p < 0.001$, $N = 15$). The first two weeks during clozapine treatment had remaining haloperidol levels; therefore they were not included in the graph

($p < 0.001$, $N = 13$), $r = 0.78$ ($p = 0.01$, $N = 9$), $r = 0.75$ ($p = 0.005$ $N = 12$). No other males had correlations significant at the 0.05 level.

This suggests that when studied longitudinally with repeated levels at least 38% (6/16) of females and 20% (3/15) of males clearly demonstrated a relationship between total clozapine and prolactin levels. In some subjects, it cannot be ruled out that the number of paired measures was too small to detect significance.

## Discussion

### Comparison of the average prolactin effects of clozapine and haloperidol levels

As can be seen from the within-subject correlations, which corrected for individual effects, clozapine levels correlated significantly with prolactin levels. However, these correlations suggest a medium effect size of clozapine on prolactin levels (0.32 for the combined sample). In contrast, haloperidol levels had a large effect size on prolactin levels (0.75).

Comparing the regression slopes from the analysis of covariance can also provide evidence of the larger haloperidol effects on prolactin levels. There is no definitive way of comparing the haloperidol and clozapine levels. To make this comparison possible, an assumption can be made that haloperidol levels are approximately 100 times lower than clozapine levels (haloperidol levels usually ranged 0–10 ng/ml while clozapine levels usually ranged 0–1000 ng/ml). This assumption appeared to be reasonable at the end of the second trial since median clozapine levels were 98 times higher than median haloperidol levels at the end of the haloperidol trial (respectively 366 ng/ml and 3.7 ng/ml). In the other trials, the median clozapine levels were 87 times higher (respectively 321 ng/ml and 3.7 ng/ml) and 55 times higher (respectively 205 ng/ml and 3.7 ng/ml). In summary, at the end of the trials of this study, median clozapine levels ranged approximately 55 to 98 times higher than median haloperidol levels. Therefore, for descriptive purposes, haloperidol levels may be considered to be approximately 50 to 100 times lower than clozapine. In females, the average increases in prolactin levels were 2.6 ng/ml per 1 ng/ml increase in haloperidol levels and 0.45 ng/ml per 100 ng/ml increase in clozapine levels (clozapine-induced prolactin increase was 5.8 to 11.6 lower than the haloperidol-induced prolactin increase). In males prolactin increases were respectively 1.5 ng/ml and 0.15 ng/ml (clozapine-induced prolactin increase was 10 to 20 lower than the haloperidol-induced prolactin increase). In summary, these analyses suggest a small but significant effect of clozapine on prolactin levels. Curiously, an in vitro study suggests that clozapine effects on prolactin secretion are 5 to 10 lower than those of a typical antipsychotic (Lamberts et al. 1990).

### Means of prolactin levels on different clozapine doses

As a way to explore the effect of clozapine dosing on prolactin levels the means of the last prolactin levels measured from the three different clozapine trials were compared. The effect was significant in females but not in males.

Assuming that 100 or 300 mg/day doses of clozapine have non-significant effects on prolactin, the prolactin baseline in female patients was approximately 8–9 ng/ml. Therefore, there was an observed increment of 2–3 ng/ml (from 8–9 to 11.8) in female prolactin levels from 100 or 300 mg/day trial compared to the 600 mg/day clozapine trial. These differences were significant but very small when compared with the increment seen in the haloperidol trial, approximately 20 ng/ml (from 8–9 to 29.9).

In contrast, the variations of male prolactin levels under different clozapine doses were non-significant with this sample size. They were small ranging from a mean prolactin level of 5.9 ng/ml on 100 mg/day to 6.8 ng/ml on 600 mg/day.

Clozapine levels were much better predictors of prolactin variations than clozapine doses. The within-subject correlation between clozapine dose and prolactin levels in males was particularly low, $r = 0.12$ and not significant ($p = 0.10$). Nonetheless, female and male prolactin variations as a function of clozapine levels were small. Large samples with longitudinal follow-ups and statistical analyses correcting for the effect of within individual variations may therefore be needed to study the effect of clozapine on prolactin. Moreover, samples must include females taking high doses of clozapine (at least 600 mg/day) if a significant effect is to be detected. It is likely that longitudinal studies using clozapine doses instead of clozapine levels in calculations will not detect a relationship between clozapine and prolactin.

### Effects of clozapine on individual patients

Our analysis of Pearson correlations in individual patients followed longitudinally clearly suggests that in some individuals (at least one fourth of males and one third of females), when clozapine doses are changed from 100 to 600 mg/day, clozapine levels are powerful predictors of prolactin levels. Again, the variations are significant but are relatively small.

In approximately two thirds of the females and three quarters of the males, there was no significant relationship between total clozapine levels and prolactin levels. It is possible that in some cases this lack of significance was due to the lack of power from insufficient paired clozapine and prolactin levels not contaminated by haloperidol.

Therefore the data can be summarized by saying that the strong correlation between haloperidol levels and prolactin levels cannot be missed by studies focusing on average effects. However, the relationship between

clozapine levels and prolactin levels is smaller and may not be present in some subjects. It is less obvious in males than females. Thus, this relationship may be missed by studies focusing on mean effects.

## Clinical meaning of prolactin changes during clozapine treatment

None of the prior published studies have explored the clinical significance of the small prolactin level increases associated with clozapine treatment. Our study has the same weakness. Unfortunately, when this study was conducted, we did not expect that clozapine will produce significant changes in prolactin levels, therefore no symptoms associated to hyperprolactinemia (particularly changes in female menstrual cycle) were recorded. As these patients had been treated with typical antipsychotics previously and had high prolactin levels before starting clozapine, we are uncertain that it would have been easy to detect clinically significant prolactin changes in some females associated with increases from low to high clozapine doses (600 mg/day). The ideal design to try to establish whether the small prolactin levels have significant clinical implications will require following-up women who are not treated with antipsychotics and then are titrated to relatively high clozapine doses.

The effects of clozapine on prolactin may be of more interest for researchers focused on the mechanism of action of antipsychotics than for clinicians. To demonstrate the effects of clozapine on prolactin, researchers need to conduct in vitro studies or complex clinical studies. The typical cross-sectional studies using only one prolactin trough measure and calculating average effects may not show clozapine effects on prolactin (particularly if females in high doses are excluded). Only longitudinal studies looking for a peak a few hours after the administration of clozapine (Table 3) or large longitudinal follow-ups of repeated trough prolactin levels using wide ranges of clozapine doses and measuring clozapine levels may show the small effects of clozapine on prolactin.

With regard to providing summarized information for the clinician, one can conclude that although clozapine has effects on prolactin levels, the effects are small and probably not meaningful from a clinical point of view. However, it can not be ruled out that in some very sensitive females with very high clozapine levels, clozapine may be associated with clinically significant increases on prolactin. However, these increases on prolactin levels would be much lower than those seen in the same patient taking typical antipsychotics.

To conclude, this longitudinal study of clozapine and prolactin levels in the context of a double-blind dose-response clozapine study suggested that, in spite of the limitations and missing data, an effect of clozapine on trough prolactin levels can be demonstrated in some subjects, at least in one fourth of treatment-refractory schizophrenic patients and perhaps more obviously in females. It is not possible to know if these results can be extrapolated to non-refractory patients but clozapine tends to be mainly used in refractory patients. Acute longitudinal studies can show small short peaks of prolactin after administration of low clozapine doses not only in patients (Meltzer et al. 1978; Turrone et al. 2002) but also in controls (Ackenheil et al. 1989), despite the fact that these studies only included males (Table 3).

**Acknowledgements** This study was supported by the grant from the US National Institute of Health MH-47162 to GMS and RCJ. The Sandoz Research Institute provided free medication. The authors are grateful to Albert R. Di Dario, Superintendent of Norristown State Hospital for administrative support and to Robert W. Ehlers, R. N. for his help in the study particularly with blood samples.

## References

1. Ackenheil M (1989) Clozapine-pharmacokinetic investigations and biochemical effects in man. Psychopharmacology 99: S32–S37
2. Bland JM, Altman DG (1995) Calculating correlation coefficients with repeated observations: Part 1-correlations within subjects. BMJ 310:446
3. Braghiroli L, Ponzianelli A, Fiani M, De Feo G, Cheng CY, Mazzanti G (1997) Effect of clozapine on dopamine-induced inhibition of prolactin release from cultured rat pituitary cells. Pharmacol Res 35:173–175
4. Breier AF, Malhotra AK, Su TP, Pinals DA, Elman I, Adler CM, Lafargue RT, Clifton A, Pickar D (1999) Clozapine and risperidone in chronic schizophrenia: effects on symptoms, parkinsonian side effects, and neuroendocrine response. Am J Psychiatry 156: 294–298
5. Brown AS, Gewirtz G, Harkavy-Friedman J, Cooper T, Brebion G, Amador XF, Malaspina D, Gorman JM (1997) Effects of clozapine on plasma catecholamines and relation to treatment response in schizophrenia: a within-subject comparison with haloperidol. Neuropsychopharmacology 17:317–325
6. Bunker MT, Marken PA, Schneiderhan ME, Ruehter VL (1997) Attenuation of antipsychotic-induced hyperprolactinemia with clozapine. J Child Adolesc Psychopharmacol 7:65–69
7. de Leon J, Odom-White A, Josiassen RC, Diaz FJ, Cooper TB, Simpson GM (2003) Serum muscarinic activity during clozapine treatment. J Clin Psychopharmacol 23:336–341
8. Devanad DP, Marder K, Michaels KS, Sackeim HA, Bell K, Sullivan MA, Cooper TB, Pelton GH, Mayeux R (1998) A randomized, placebo-controlled dose-comparison trial of haloperidol for psychosis and disruptive behaviors in Alzheimer's disease. Am J Psychiatry 155:1512–1520
9. Dickson RA, Seeman MV, Corenblum B (2000) Hormonal side effects in women: typical versus atypical antipsychotic treatment. J Clin Psychiatry 61(suppl 3):10–15
10. Gruen PH, Sachar EJ, Langer G, Altman N, Leifer M, Frantz A, Halpern FS (1978) Prolactin responses to neuroleptics in normal and schizophrenia subjects. Arch Gen Psychiatry 35:108–116
11. Guldesky GA, Nash JF, Berry SA, Meltzer HY (1989) Basic biology of clozapine: electrophysiological and neuroendocrinological studies. Psychopharmacology 99:S13–S17
12. Henderson DC, Goff DC, Connolly CE, Borba CP, Hayden D (2001) Risperidone added to clozapine: impact on serum prolactin levels. J Clin Psychiatry 62:605–608
13. Kane JM, Cooper TB, Sachar EJ, Halpern FS, Bailine S (1981) Clozapine: plasma levels and prolactin response. Psychopharmacology 73:184–187
14. Kane JM, Honigfeld G, Singer J, Meltzer H (1988) Clozapine for the treatment-resistant schizophrenic. A double-blind comparison with chlorpromazine. Arch Gen Psychiatry 45:789–796

15. Lamberts SWJ, van Koetsveld PM, Hofland LJ (1990) The effect of clozapine on prolactin secretion at the level of the lactotroph. Life Sci 46:1013–1019
16. Lee HS, Kim CH, Song DH, Choi NK, Yoo KJ (1995) Clozapine does not elevate serum prolactin levels in healthy men. Biol Psychiatry 38:762–764
17. Lemus CZ, Lieberman JA, Johns CA, Mayerhoff DI, Pollack S, Cooper TB, Novacenko H (1991) Hormonal response to fenfluramine challenges in clozapine-treated schizophrenic patients. Biol Psychiatry 29:691–694
18. Markianos M, Hatzimanolis J, Lykouras L (1999) Switch from neuroleptics to clozapine does not influence pituitary-gonadal axis hormone levels in male schizophrenic patients. Eur Neuropsychopharmacol 9:533–536
19. Markianos M, Hatzimanolis J, Lykouras L (2001) Neuroendocrine responsivities of the pituitary dopamine system in male schizophrenic patients during treatment with clozapine, olanzapine, risperidone, sulpiride, or haloperidol. Eur Arch Psychiatry Clin Neurosci 251:141–146
20. Meltzer HY (1989) Clinical studies on the mechanism of action of clozapine: the dopamine-serotonin hypothesis of schizophrenia. Psychopharmacology 99:S18–27
21. Meltzer HY, Daniels S, Fang VS (1975) Clozapine increases rat serum prolactin levels. Life Sci 17:339–342
22. Meltzer HY, Goode DJ, Schyve PM, Young M, Victor SF (1979) Effect of clozapine on human serum prolactin levels. Am J Psychiatry 136:1550–1555
23. Nair NPV, Cervantes R, Yassa R, Guyda H (1979) Effect of clozapine on apomorphine-induced growth hormone secretion and serum prolactin concentration in schizophrenia. Neuropsychobiology 5:136–142
24. Petty RG (1999) Prolactin and antipsychotic medications: mechanism of action. Schizophr Res 35:S67–S73
25. Pickar D, Owen RR, Litman RE, Konicki E, Gutierrez R, Rapaport MH (1992) Clinical and biologic response to clozapine in patients with schizophrenia. Crossover comparison with fluphenazine. Arch Gen Psychiatry 49:345–353
26. Pretorius JL, Phillips M, Langley RW, Szabadi E, Bradshaw CM (2001) Comparison of clozapine and haloperidol on some autonomic and psychomotor functions, and on serum prolactin concentration, in healthy subjects. Br J Clin Pharmacol 52:322–326
27. Sachar EJ, Gruen PH, Altman N, Halpern FS, Frantz AG (1976) Use of neuroendocrine techniques in psychopharmacology research. In: Sachar EJ (ed) Hormones, Behavior and Psychopathology. Raven Press, New York, pp 161–176
28. Simpson GM, Cooper TA (1978) Clozapine plasma levels and convulsions. Am J Psychiatry 135:99–100
29. Simpson GM, Josiassen RC, Stanilla JK, de Leon J, Nair C, Abraham G, Odom-White A, Turner RM (1999) Double-blind study of clozapine dose response in chronic schizophrenia. Am J Psychiatry 156:1744–1750
30. SPSS Inc (1997) SPSS Advanced Statistics 7.5. Chicago, SPSS Inc
31. Szymanski S, Lieberman J, Pollack S, Kane JM, Safferman A, Munne R, Umbricht D, Woerner M, Masiar S, Kronig M (1996) Gender differences in neuroleptic nonresponsive clozapine-treated schizophrenics. Biol Psychiatry 39:249–254
32. Turrone P, Kapur S, Seeman MV, Flint AJ (2002) Elevation of prolactin levels by atypical antipsychotics. Am J Psychiatry 159: 133–135
33. Uehlinger C, Baumann P (1991) Clozapine as an alternative treatment for neuroleptic-induced gynecomastia (letter). Am J Psychiatry 148:392–393
34. Wudarsky M, Nicolson R, Hamburger SD, Spechler L, Gochman P, Bedwell J, Lenane MC, Rapoport JL (1999) Elevated prolactin in pediatric patients on typical and atypical antipsychotics. J Child Adolesc Psychopharmacol 9:239–245