The use of intravenous catheterisation with a rest period is useful for determination of plasma cortisol levels but not plasma prolactin levels

La pose d’un cathéter intraveineux associée à une période de repos est utile pour le dosage du cortisol mais ne l’est pas pour le dosage de la prolactine

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Abstract

Objective. – The use of an intravenous catheter with a rest period has been recommended to avoid false-positive results for hyperprolactinemia and false-negative results for hypocortisolaemia. We tested the relevance of this recommendation.

Design. – Plasma cortisol and prolactin levels were determined before (T-15) and after a 15-min rest period (T0) in 119 patients, 38 males (M) and 81 females (F). 52 of the 119 patients were known (K; 30 females and 22 males) and 67 unknown (UK; 49 females and 18 males) to the unit.

Results. – Prolactin was lower after rest in women (12.3 ± 22.7 ng/l vs 11.7 ± 22.5 ng/ml, \( P = 0.03 \)) but not in men (6.2 ± 4.5 ng/ml at T-15 vs 5.8 ± 3.2 ng/ml at T0, \( P = 0.09 \)), in the UK subgroup (10.6 ± 20.7 ng/ml at T-15 vs 10.1 ± 20.9 ng/ml at T0, \( P = 0.06 \)) and in the K subgroup (10.1 ± 16.7 ng/ml at T-15 vs 9.7 ± 15.8 ng/ml at T0, \( P = 0.08 \)). None of the patients with prolactin levels higher than 20 ng/ml at T-15 diminished its prolactin value below this cut-off value. Plasma cortisol levels were lower after rest in women (17.9 ± 5.9 μg/dl at T-15 vs 16.5 ± 6.1 μg/dl at T0, \( P < 0.0001 \)) in the UK subgroup (18 ± 6.1 μg/dl at T-15 vs 16.6 ± 6.4 μg/dl at T0, \( P = 0.0003 \)) but not in men (18 ± 4.4 μg/dl at T-15 vs 17.5 ± 5.8 μg/dl at T0, \( P = 0.47 \)) and in the K subgroup (17.8 ± 4.6 μg/dl at T-15 vs 17 ± 5.4 μg/dl at T0, \( P = 0.13 \)). At T0, 3.3% and 15% of patients presented values below the cut-off value of 10 μg/dl (276 nmol/l) and 17 μg/dl (470 nmol/l), respectively.

Conclusion. – These results don’t justify intravenous catheterisation with a rest period for plasma prolactin determination in contrast with plasma cortisol determination.

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Résumé

Objectif. – L’utilisation d’un cathéter intraveineux associée à une période de repos est recommandée afin d’éviter les faux positifs de l’hyperprolactinémie et les faux négatifs de l’hypocortisolaémie. Nous avons testé la validité de cette recommandation.

Méthodes. – Le dosage de la prolactine et du cortisol ont été réalisés avant (T-15) et après une période de 15 mn de repos (T0) chez 119 patients, 38 hommes (M) et 81 femmes (F), 52 étaient connus du service (K = known ; 30 femmes et 22 hommes), 67 y venaient pour la première fois (UK = unknown ; 49 femmes et 18 hommes).

Résultats. – Les taux de prolactine étaient plus bas à T0 chez les femmes (12.3 ± 22.7 ng/l vs 11.7 ± 22.5 ng/ml, \( p = 0.03 \)) mais pas chez les hommes (6.2 ± 4.5 ng/ml à T-15 vs 5.8 ± 3.2 ng/ml à T0, \( p = 0.09 \)), chez les patients venant pour la première fois (10.6 ± 20.7 ng/ml à T-15 vs 10.1 ± 20.9 ng/ml à T0, \( p = 0.06 \)) et chez les patients connus du service (10.1 ± 16.7 ng/ml à T-15 vs 9.7 ± 15.8 ng/ml à T0, \( p = 0.08 \)). Cependant, aucun des patients n’a baissé sa prolactine sous le seuil de 20 ng/ml. Les taux de cortisol étaient plus bas à T0 chez les femmes (17.9 ± 5.9 μg/dl vs 16.5 ± 6.1 μg/dl, \( p < 0.0001 \)), chez les patient venant pour la première fois (18 ± 6.1 μg/dl vs 16.6 ± 6.4 μg/dl, \( p = 0.0003 \)) mais pas chez les hommes (18 ± 4.4 μg/dl vs 17.5 ± 5.8 μg/dl, \( p = 0.47 \)) et chez les patients connus (17.8 ± 4.6 μg/dl vs 17 ± 5.4 μg/dl, \( p = 0.13 \)). À T0, 3.3 et 15 % des patients ont baissé leur cortisol sous le seuil de 10 μg/dl (276 nmol/l) et de 17 μg/dl (470 nmol/l), respectivement.

Conclusion. – Ces résultats ne permettent pas de justifier l’utilisation d’un cathéter intraveineux associé à une période de repos pour le dosage de prolactine qui semble en revanche justifié pour le dosage du cortisol.

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1. Introduction

During the 1970s, stress was identified as a stimulating factor for prolactin and cortisol secretion [5,20,22]. Direct venipuncture is considered to be a stress for patient and insertion of an intravenous catheter before sampling is supposed to diminish the stressful impact on patients and prevent false-positive results for hyperprolactinaemia, leading to unnecessary investigations, and false-negative results for hypocortisolæmia, leading to inappropriate therapy. The use of an intravenous catheter for determination of the plasma levels of these hormones was subsequently recommended. However, studies on hormonal changes after exposure to stress have reported discordant results: changes depend on the type of stress [16,23], and/or the patient’s personality [15,20]. In order to clarify this issue, we conducted a prospective study to determine the relevance of intravenous catheterisation with a rest period in our clinical practice.

2. Patients and methods

2.1. Patients

Plasma cortisol and prolactin levels were determined at the time of intravenous catheterisation (T-15) and after a 15-minute rest period (T0) in 119 patients, 38 males (M), 81 females (F) with a mean age of 46.7 ± 16.9 years. 52 of the 119 patients (30 females and 22 males) were known (K) to the unit and 67 (49 females and 18 males) were unknown (UK) to the unit. Description of patient’s diagnosis is reported in Table 1. Blood samples were drawn between 8 h00 and 10 h00 in the morning. Patients were referred to our unit for endocrinological assessment between June and November 2005. None of these patients were taking treatment known to interfere with plasma prolactin and cortisol levels and patients treated for hyperprolactinaemia or hypocortisolæmia were excluded.

2.2. Methods

On the patient’s arrival in the unit, an intravenous catheter was inserted and a first venous blood sample was drawn (T-15). A second sample was drawn after resting for 15 minutes (T0).

Plasma prolactin levels were measured by an immunoradiometric assay (IRMA, Immunotech, Beckman Coulter). The detection limit is 0.5 ng/ml (15.15 mIU/l). The coefficient of variation intra-assay is below or equal to 6.7% and the coefficient of variation inter-assay below or equal to 7.9%.

For each patient, prolactin and cortisol were measured in the same assay run. Prolactin cut-off value for normality was 20 ng/ml as recommended in [1]. Two cut-off values for cortisol normality were chosen: 10 μg/dl (276 nmol/l) which is predictive of a normal response to CRF test [24] and 17 μg/dl (470 nmol/l) which is predictive of a normal response to insulin hypoglycemia test [10].

2.3. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 4 software for Windows (GraphPad Software, San Diego, California, USA). Variations of plasma prolactin and plasma cortisol levels before and after rest were compared using the Student t-test for paired samples. The male/female ratio was compared in the K and UK subgroups using the chi-square test. Results are shown as means plus/minus standard deviations.

3. Results

3.1. Plasma prolactin variations

Plasma prolactin levels (ng/ml) was significantly lower after rest (10.4 ± 19 ng/ml at T-15 vs 10 ± 19 ng/ml at T0, P = 0.01) in the overall population. On subgroup analysis, this difference was only significant in women (12.3 ± 22.7 ng/ml at T-15 vs 11.7 ± 22.5 ng/ml at T0, P = 0.03), almost significant in the UK subgroup (10.6 ± 20.7 ng/ml at T-15 vs 10.1 ± 20.9 ng/ml at T0, P = 0.06), but not significant in men (6.2 ± 4.5 ng/ml at T-15 vs 5.8 ± 3.2 ng/ml at T0, P = 0.09) and in the K subgroup (10.1 ± 16.7 ng/ml at T-15 vs 9.7 ± 15.8 ng/ml at T0, P = 0.08). The sex ratio was not different between the K and UK subgroups (P = 0.08). Plasma prolactin levels were lower after rest in 83% of patients, remained stable in 9% of patients and were higher in 8% of patients. There were no clinical consequences for plasma prolactin variations, as only one woman presented a plasma prolactin level greater than the cut-off value of 20 ng/ml (29 ng/ml) at T0 and lower than 20 ng/ml (15 ng/ml) at T-15; she had known fluctuating plasma prolactin levels due to disconnection hyperprolactinaemia. Conversely, none of the plasma prolactin levels greater than 20 ng/ml at T-15 (n=9) decreased below the cut-off value at T0 (Fig. 1).

3.2. Plasma cortisol variations

Plasma cortisol (μg/dl) was significantly lower after rest (17.9 ± 5.4 μg/dl at T-15 vs 16.8 ± 6 μg/dl at T0, P = 0.0002) in the overall population. On subgroup analysis, the difference
was significant in women (17.9 ± 5.9 μg/dl at T-15 vs 16.5 ± 6.1 μg/dl at T0, \(p<0.0001\)), in the UK subgroup (18 ± 6.1 μg/dl at T-15 vs 16.6 ± 6.4 μg/dl at T0, \(P = 0.0003\)), but was not significant in men (18 ± 4.4 μg/dl at T-15 vs 17.5 ± 5.8 μg/dl at T0, \(P = 0.47\)) and in the K subgroup (17.8 ± 4.6 μg/dl at T-15 vs 17 ± 5.4 μg/dl at T0, \(P = 0.13\)). The sex ratio was not statistically different between the K and UK subgroups (\(P = 0.08\)). Plasma cortisol levels were lower after rest in 75% of patients, remained stable in 12.4% of patients and were higher in 12.6% of patients. Clinical consequences depended on the cut-off value adopted for the suspicion of hypocortisolaemia: 3.3% (4 women) of patients presented a value below the cut-off value of 10 μg/dl (276 nmol/l) after rest (Fig. 2) and 15% (11 women and 7 men) presented a value below the cut-off value of 17 μg/dl (470 nmol/l) (Fig. 3). Results are shown as means plus/minus standard deviations.

### 4. Discussion

Direct venipuncture is considered to be a stress for patient and insertion of an intravenous catheter before sampling is supposed to diminish the stressful impact on patients and prevent false-positive results for hyperprolactinaemia. Normalisation of hyperprolactinaemia under resting conditions has been reported [9,14] and the concept of stress-related hyperprolactinaemia is logical considering the reported effects of stress on prolactin [3]. Collection of repeated blood samples under resting conditions has therefore been recommended to distinguish stress-

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### Table 1

- **Description of patient’s diagnosis**
- **Tableau 1**
- **Motifs de recours des patients**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>K = known of the unit (n = 52)</th>
<th>UK = unknown of the unit (n = 67)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 women</td>
<td>49 women</td>
</tr>
<tr>
<td>Pituitary disease</td>
<td>22 men</td>
<td>18 men</td>
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<tr>
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<td>1</td>
</tr>
<tr>
<td>Cushing disease</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Non functioning adenoma</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Acromegaly</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Prolactinoma</td>
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<td>2</td>
</tr>
<tr>
<td>Hypophysitis</td>
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<td>1</td>
</tr>
<tr>
<td>Sheehan’s syndrome</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Thyrotropic insufficiency</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Meningioma</td>
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<td>1</td>
</tr>
<tr>
<td>MEN 1</td>
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<td>1</td>
</tr>
<tr>
<td>Diabetes</td>
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<tr>
<td>Thyroid disease</td>
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<td>1</td>
</tr>
<tr>
<td>Hypoparathyroidism</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Adrenal pathology</td>
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<tr>
<td>Conn’s syndrome</td>
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<tr>
<td>Incidentaloma</td>
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<td>2</td>
</tr>
<tr>
<td>PCOS</td>
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<tr>
<td>Gynecomastia</td>
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</tr>
<tr>
<td>Sexual impotence</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Suspension of endocrine disease not confirmed</td>
<td>7</td>
<td>18</td>
</tr>
</tbody>
</table>

PCOS: polycystic ovarian syndrome; MEN 1: type 1 multiple endocrine neoplasia.

PCOS : syndrome des ovaires polykystiques ; MEN 1 : néoplasie endocriniennes multiples de type 1.
related hyperprolactinaemia from organic hyperprolactinaemia [2,8,21], especially for the aetiological diagnosis of infertility related hyperprolactinaemia from organic hyperprolactinaemia [2,8,21]. This recommendation is followed: a survey showed that 30% of the 38 French Endocrinology Units surveyed systematically performed serial sampling for prolactin determination [11]. However, only a limited number of studies have been published. During in vitro fertilisation and embryo transfer in 40 women, an elevation of plasma prolactin and cortisol levels was observed before oocyte retrieval (reflecting emotional and physical stress), whereas an isolated plasma cortisol elevation was observed before embryo transfer (which is a less intense physical stress) [7]. On the other hand, plasma prolactin levels were not correlated with anxiety in 109 patients on the day preceding a surgical operation [4] and a retrospective study did not find any statistical differences in 1,251 determinations of plasma prolactin levels on 5 consecutive venous samplings [11]. In our study, plasma prolactin levels were significantly lower after rest in the overall population. However, we did not observe any stress-related cases of hyperprolactinaemia due to venous sampling. What is the explanation for these differences with reported cases of stress-related hyperprolactinaemia? These differences could possibly be related to the assay kit, as many prolactin assay kits exhibit variable degrees of reactivity with macroprolactin. The assay kit used in our study provides results very close to those of prolactin chromatography with only minor reactivity with macroprolactinaemia [26]. However, stress-related effects on macroprolactinaemia have not been described. Another question concerns the duration of the rest period. Is a 15-minute rest period sufficient? Plasma ACTH and prolactin levels rise 7 min after starting an arithmetic test and public speaking and an elevation of plasma cortisol levels (from 2 to 4 times baseline levels) is observed 15 minutes after ACTH elevation. These values return to normal when the subject returns to the waiting room [17,19]. During parachute jumping, prolactin and cortisol elevation is observed 10 to 20 min after the jump and values return to normal over the following hour for plasma prolactin, while plasma cortisol levels remain elevated [23]. However, it is difficult to extrapolate these results to clinical practice as these stressors are intense. In a French study, prolactin levels were determined over an interval of 20 min to 90 min with no changes [11]. So the rest period duration doesn’t change results. Then, we conclude that our results don’t justify the use of an intravenous catheter and/or a rest period regarding that there are no consequences on determination of plasma prolactin levels in clinical practice.

Concerning plasma cortisol levels, the essential difficulty concerns the absence of a clearly defined cut-off value for the diagnosis of hypocortisolaemia. Various studies have provided discordant results and do not allow the definition of a reliable cut-off value: a plasma cortisol level higher than 17 μg/dl (470 nmol/l) is predictive of a normal response to insulin hypoglycaemia test [10,18], a value of 18 μg/dl (500 nmol/l) is predictive of a normal response to the short synacthen test [18], and a value of 10 μg/dl (285 nmol/l) is predictive of a normal response to the CRF test [24]. In our study, 3.3% (4 women) of patients diminished their plasma cortisol levels below the cut-off value of 10 μg/dl (276 nmol/l) and 15% (11 women and 7 men) below the cut-off value of 17 μg/dl (470 nmol/l) after rest. Then, we conclude that the use of an intravenous catheter and/or a rest period has clinical consequences on plasma cortisol levels, therefore the diagnosis of hypocortisolaemia might be missed.

In our study, plasma cortisol and prolactin levels were significantly lower after rest in the overall population. But, on subgroup analysis, the difference was significant in women but was not significant in men. The fact that results were not significant in men is perhaps a matter of statistical power because only 38 males were tested. However, five men diminished their plasma cortisol levels below the cut-off value of 12 μg/dl (331 nmol/l). Is there a real difference between men and women? Most studies in the 1970s were performed in men because of the supposed identical responses between men and women [3]. The first studies including men and women studied elevation of plasma prolactin levels during the mirror drawing test. An elevation of plasma prolactin levels was observed in neurotic women, but not in neurotic men and “normal” subjects [20]. However, the influence of personality was not subsequently confirmed, as extraverted, neurotic and psychotic patients presented similar hormonal profiles [25]. Differences between men and women are then not clearly defined.

In our study, plasma cortisol and prolactin levels were significantly lower after rest in the overall population. But, on subgroup analysis, the difference was only significant in the UK group for cortisol but was not significant in the K group. These results raise another issue which is habituation to stress. The stress induced by tests (Stroop Colour Word Interference task, public speaking and mental arithmetic in front of an audience) repeated after 7 days does not always have the same effects. In some patients, the elevation of plasma cortisol levels is less marked at the second test: these patients are called low-responders by the authors. In other patients, cortisol elevation remains the same on both tests and these patients are called
high-responders. This difference is not observed for plasma prolactin levels and a decrease is observed in both groups at the second test [13]. These data could explain the observed differences for plasma cortisol levels between the UK and K subgroups and the absence of differences for plasma prolactin levels. Hypothalamus-pituitary-adrenal axis stimulation is an anticipatory cognitive appraisal process and is observed during the hour before the stressful event [12]. The anticipatory process may be lost in the K subgroup already familiar with the test conditions.

5. Conclusion

One might assume that placing a catheter may be more stressful for the patient than simple venipuncture. It cannot be concluded that significant differences between measurements are the result of using a catheter instead of venapuncture. Similarly, it cannot be concluded that observed differences are the result of a period of rest. These results should be compared with a group who did not have rest between measurements. However the use of an intravenous catheter with a rest period of 15 min has no clinical consequences in our clinical practice on determination of plasma prolactin levels and is therefore not recommended. In contrast, plasma cortisol levels are significantly influenced and these results suggest that false-negative results for hypocortisolaemia could diminish when an intravenous catheter and/or a rest period is not used.

6. French version

A French version of this article is available at doi: 10.1016/j.andro.2007.03.001.

References